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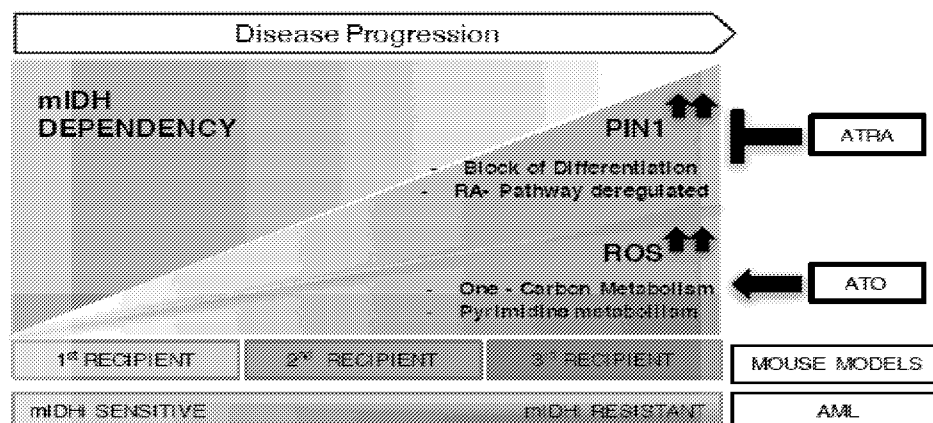
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(54) Title: ARSENIC TRIOXIDE AND RETINOIC ACID COMPOUNDS FOR TREATMENT OF IDH2-ASSOCIATED DISORDERS

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(57) Abstract: The present invention features methods and kits relating to the treatment of cancer by administering arsenic trioxide, a retinoic acid compound, or a combination of an arsenic trioxide and a retinoic acid compound.

ARSENIC TRIOXIDE AND RETINOIC ACID COMPOUNDS FOR TREATMENT OF IDH2-ASSOCIATED DISORDERS

RELATED APPLICATIONS

5 The present application claims priority to U.S. Provisional Patent Application No. 62/593,761 filed December 1, 2017, the entire contents of which are hereby incorporated by reference for all purposes.

BACKGROUND

Isocitrate dehydrogenase enzymes (IDH1 and IDH2) are key metabolic enzymes.
10 Mutations to IDH2 have been identified as important early events in a variety of tumor types. For example, IDH enzymes are mutated in approximately 20% of human acute myeloid leukemias (AMLs). Pathogenic mutants of IDH enzymes have been identified that give rise to 2-hydroxyglutarate (2-HG), an oncometabolite that contributes to the oncogenic phenotype. Accordingly, 2-HG is a predictive biomarker in cancers having a pathogenic IDH2 allele.
15 Specific inhibitors targeting IDH mutants have been identified. For example, the mutant IDH2-targeting Enasidenib (AG-221) has been approved by the FDA for the treatment of relapsed or refractory AML with a mutant form of IDH2 (mIDH2).
However, targeting strong oncogenic drivers often leads to resistance. In particular, little is known about how mIDH2 leukemias may adjust and evolve in response to treatment.
20 Therefore, there is a need to identify common vulnerabilities in mIDH2 leukemias, and to develop rational therapies capable of preventing or overcoming resistance that arises in response to therapy.

SUMMARY

The present invention is based on the discovery of common vulnerabilities in mIDH2
25 leukemia. The inventors have shown that mIDH2 leukemia exhibits sensitivity to reactive oxygen species (ROS)-producing compounds, such as arsenic trioxide (ATO). The inventors have also shown that mIDH2 leukemia exhibits sensitivity to retinoic acid compound-induced differentiation (e.g., ATRA-induce differentiation). The inventors have further shown that treatment of leukemia with the combination of a ROS promoting compound (e.g., arsenic
30 trioxide) and a compound that promotes differentiation (e.g., a Pin1 inhibitor, such as ATRA) provides a synergistic, powerful, and well-tolerated targeted therapy in both mouse and human models of AML.

The present invention therefore features methods of treating cancer by contacting the cells of the cancer with a pharmaceutical compound. The pharmaceutical compound can be an

arsenic trioxide compound (e.g., ATO), a retinoic acid compound (e.g., ATRA), or a combination of arsenic trioxide and a retinoic acid compound (ATO and ATRA).

The cancer can be a leukemia (e.g., mIDH2 leukemia) or a solid tumor (e.g., an IDH2-associated solid tumor). Examples of the solid tumors include, but are not limited to, glioma, paraganglioma, astroglioma, colorectal carcinoma, melanoma, cholangiocarcinoma, chondrosarcoma, thyroid carcinomas, prostate cancers, and non-small cell lung cancer.

In one aspect, the invention features a method of treating leukemia in a subject. The method includes contacting the cells of said leukemia with (e.g., by administering to said subject) an effective amount of a pharmaceutical compound, the pharmaceutical compound being arsenic trioxide, a retinoic acid compound, or a combination thereof, wherein one or more cells of the leukemia has a pathogenic IDH2 allele or has elevated 2-hydroxyglutarate (2-HG) levels; and wherein contacting said cells of said leukemia with said pharmaceutical compound treats said leukemia in said subject.

In another aspect, the invention features a method of treating leukemia in a subject, wherein one or more cells of the leukemia has a pathogenic IDH2 allele or has elevated 2-HG levels. The method includes contacting the cells of said leukemia with (e.g., by administering to said subject) an effective amount of a pharmaceutical compound, the pharmaceutical compound being arsenic trioxide, a retinoic acid compound, or a combination thereof, wherein contacting said cells of said leukemia with said pharmaceutical compound treats said leukemia in said subject.

Elevated 2-HG levels may be considered to include detection of any 2-HG at all. In some assays where background 2-HG is detected, a pathological level of 2-HG associated with an IDH2 cancer may be determined by levels of 2-HG at least about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 500%, 1000%, or greater than the 2-HG levels measured in a normal (e.g., wild-type and/or disease free) subject, tissue, or cell. Elevated 2-HG levels can be determined via (i) the detection of D-2-HG in biological fluids detected by LC-MS/MS; (ii) the detection of total 2-HG (including both L-2-HG and D-2-HG) in the serum above 2 μ M; or (iii) the detection of the presence of mutant variants of IDH2 gene.

In some embodiments, contacting said cells of said leukemia with the arsenic trioxide and the retinoic acid compound results in the remission of the leukemia in the subject (e.g., the symptoms of the leukemia are reduced). In some embodiments, contacting said cells of said leukemia with the arsenic trioxide and the retinoic acid compound results in the complete remission of the leukemia (e.g., all signs and symptoms of the leukemia are absent). In some

embodiments, contacting said cells of said leukemia with the arsenic trioxide and the retinoic acid compound cures the leukemia in the subject (e.g., all signs and symptoms of the leukemia are absent for 1 year or more, for 2 years or more, for 3 years or more, for 4 years or more, or for 5 years or more).

5 In some embodiments, the arsenic trioxide and the retinoic acid compound operate synergistically to treat said leukemia. In some embodiments, the arsenic trioxide and the retinoic acid compound is more effective for treating said leukemia than the same quantities of either said arsenic trioxide or said retinoic acid compound alone.

10 In some embodiments, contacting said cells of said leukemia with of the arsenic trioxide and the retinoic acid compound increases the production of reactive oxygen species (ROS) in one or more cells of said leukemia, e.g., increases the production of ROS by at least about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 500%, 1000%, or greater relative to production of ROS prior to treatment with arsenic trioxide and the retinoic acid compound. Preferably, contacting said
15 cells of said leukemia with of the arsenic trioxide and the retinoic acid compound increases the production of ROS by at least about 10%.

 In some embodiments, contacting said cells of said leukemia with of the arsenic trioxide and the retinoic acid compound promotes differentiation of one or more cells of said leukemia.

20 In some embodiments, contacting said cells of said leukemia with of the combination of arsenic trioxide and a retinoic acid compound is sufficient to inhibit and/or degrade Pin1 in the subject. In some embodiments, this may include an increase in degradation of Pin1 of at least about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 500%, 1000%, or greater relative to Pin1 activity prior to
25 treatment with arsenic trioxide and the retinoic acid compound. Preferably, contacting said cells of said leukemia with of the arsenic trioxide and the retinoic acid compound increases the degradation of Pin1 of at least about 10%.

 In some embodiments, this may include a reduction in Pin1 activity of at least about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 40%, 50%, 60%, 70%,
30 80%, 90%, 100%, 200%, 500%, 1000%, or greater relative to Pin1 activity prior to treatment with arsenic trioxide and the retinoic acid compound. In some embodiments, the contacting said cells of said leukemia with of arsenic trioxide and a retinoic acid compound is more effective for inhibiting and/or degrading Pin1 in the subject than administration of the same quantities of either arsenic trioxide or the retinoic acid compound alone. In some embodiments,

contacting said cells of said leukemia with the arsenic trioxide and the retinoic acid compound is more effective for treating the leukemia than contacting said cells of said leukemia with of the same quantities of either the arsenic trioxide or the retinoic acid compound alone.

In some embodiments, arsenic trioxide and the retinoic acid compound may be
5 administered concurrently (e.g., within about 1min, 2min, 5min, 10min, 20min, 30min, or 60min) or separately. The arsenic trioxide may be administered either prior to or after the retinoic acid compound.

In some embodiments, the invention features a method of treating leukemia in a subject. The method includes contacting the cells of the leukemia with an effective amount of
10 arsenic trioxide to said subject, wherein one or more cells of the leukemia have a pathogenic IDH2 allele or have been previously determined to have elevated 2-hydroxyglutarate (2-HG) levels; and wherein contacting said cells of said leukemia with the arsenic trioxide treats the leukemia in the subject. Elevated 2-HG levels may be considered to include levels of 2-HG at least about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 40%, 50%,
15 60%, 70%, 80%, 90%, 100%, 200%, 500%, 1000%, or greater than the 2-HG levels measured in a normal (e.g., wild-type and/or disease free) subject, tissue, or cell.

In some embodiments, contacting said cells of said leukemia with the arsenic trioxide increases the production of reactive oxygen species (ROS) in one or more cells of said leukemia (e.g., increases the production of ROS by at least about 1%, 2%, 3%, 4%, 5%, 6%,
20 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 500%, 1000%, or greater relative to production of ROS prior to treatment with arsenic trioxide).

In some embodiments, contacting said cells of said leukemia with the arsenic trioxide is sufficient to inhibit and/or degrade Pin1 in the subject. In some embodiments, this may include
25 an increase in degradation of Pin1 of at least about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 500%, 1000%, or greater relative to Pin1 activity prior to treatment with arsenic trioxide. In some embodiments, this may include a reduction in Pin1 activity of at least about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%,
30 200%, 500%, 1000%, or greater relative to Pin1 activity prior to treatment with arsenic trioxide. Preferably, contacting said cells of said leukemia with of the arsenic trioxide increases the degradation of Pin1 of at least about 5%.

In some embodiments, the invention features a method of treating leukemia in a subject. The method includes contacting the cells of the leukemia with an effective amount of a

retinoic acid compound to the subject, wherein the subject has a pathogenic IDH2 allele or wherein one or more cells of the leukemia have been previously determined to have elevated 2-hydroxyglutarate (2-HG) levels; and wherein contacting said cells of said leukemia with the retinoic acid compound treats the leukemia in the subject. Elevated 2-HG levels may be considered to include levels of 2-HG at least about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 500%, 1000%, or greater than the 2-HG levels measured in a normal (e.g., wild-type and/or disease free) subject, tissue, or cell.

In some embodiments, contacting said cells of said leukemia with the retinoic acid compound promotes differentiation of one or more cells of the leukemia.

In some embodiments, contacting said cells of said leukemia with the retinoic acid compound is sufficient to inhibit and/or degrade Pin1 in the subject. In some embodiments, this may include an increase in degradation of Pin1 of at least about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 500%, 1000%, or greater relative to Pin1 activity prior to treatment with retinoic acid compound. In some embodiments, this may include a reduction in Pin1 activity of at least about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 500%, 1000%, or greater relative to Pin1 activity prior to treatment with the retinoic acid compound. Preferably, contacting said cells of said leukemia with the retinoic acid compound increases the degradation of Pin1 of at least about 5%.

In some embodiments, the subject has elevated levels of Pin1 activity (e.g., has previously been determined to have elevated levels of Pin1 activity in one or more cells of the leukemia). Levels of Pin1 activity in the subject may be determined by measuring the levels of at least one Pin1 marker, wherein elevated levels of the Pin1 marker is indicative of elevated Pin1 activity. Non-limiting examples of Pin1 markers include nucleic acid molecules (e.g., mRNA, DNA) that correspond to some or all of a Pin1 gene, peptide sequences (e.g., amino acid sequences) that correspond to some or all of a Pin1 protein, nucleic acid sequences which are homologous to Pin1 gene sequences, peptide sequences which are homologous to Pin1 peptide sequences, alteration of Pin1 protein, antibodies to Pin1 protein, substrates of Pin1 protein, binding partners of Pin1 protein, alteration of Pin1 binding partners, and activity of Pin1. In some embodiments, alteration of a Pin1 protein may include a post-translational modification (e.g., phosphorylation, acetylation, methylation, lipidation, or any other post-translational modification known in the art) of Pin1. In some embodiments, a Pin1 marker is the level of Pin expression (e.g., Pin1 protein expression levels and/or Pin1 mRNA expression

levels) in a subject. Elevated levels of a Pin1 marker include, for example, levels at least about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 500%, 1000%, or greater than the marker levels measured in a normal (e.g., wild-type and/or disease free) subject, tissue, or cell. Preferably, elevated levels of a Pin1 marker include levels at least about 3% or greater than the marker levels measured in a normal subject, tissue, or cell.

In some embodiments, the method further comprises contacting said cells of said leukemia with a compound that inhibits Pin1 activity (e.g., in combination with the retinoic acid and the arsenic trioxide).

In some embodiments, the subject has decreased levels of lysine-specific demethylase (LSD1) activity. Levels of LSD1 activity can be determined by methods known to one of skill in the art, including determining the levels of lysine histone methylation (e.g., H3K4me2 and/or H3K9me2). Decreased levels of LSD1 activity include, for example, a reduction of LSD1 activity of about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 500%, 1000%, or greater than the marker levels measured in a normal (e.g., wild-type and/or disease free) subject, tissue, or cell. Preferably, decreased levels of LSD1 activity include a reduction of LSD1 activity of about 20% or greater than the marker levels measured in a normal subject, tissue, or cell.

In some embodiments, the method further comprises contacting said cells of said leukemia with a compound that inhibits LSD1 activity (e.g., reduced LSD1 activity by about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 500%, 1000%, or greater relative to LSD1 activity prior to treatment).

In some embodiments, the method further comprises contacting said cells of said leukemia with an inhibitor of IDH2. The inhibitor of IDH2 may reduce IDH2 activity (e.g., as measured by a reduction in 2-HG levels) by about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 500%, 1000%, or greater relative to IDH2 activity prior to treatment. The inhibitor of IDH2 may be specific for a pathogenic mutant form of IDH2. When such an inhibitor targets a mutant form of IDH2, it may reduce the aberrant activity of the enzyme by about 20%.

In some embodiments, the pathogenic IDH2 allele is IDH2^{R140Q}.

In some embodiments, the method further includes contacting said cells of said leukemia with an effective amount of dexamethasone.

In a further aspect, the invention features a kit for treating leukemia in a subject,

wherein the kit includes: (a) an effective amount of arsenic trioxide, (b) an effective amount of a retinoic acid compound, and (c) instructions for the use of the arsenic trioxide in combination with the retinoic acid compound for treating the leukemia in the subject, wherein one or more cells of said leukemia has a pathogenic IDH2 allele or wherein the subject has been previously
5 determined to have elevated 2-hydroxyglutarate (2-HG) levels. Elevated 2-HG levels may be considered to include levels of 2-HG at least about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 500%, 1000%, or greater than the 2-HG levels measured in a normal (e.g., wild-type and/or disease free) subject, tissue, or cell.

10 In some embodiments, the kit further includes an effective amount of a compound that inhibits Pin1 activity and instructions for the use of the compound that inhibits Pin1 activity in combination with the arsenic trioxide and the retinoic acid compound.

In some embodiments, the kit further includes an effective amount of a compound that inhibits LSD1 activity and instructions for the use of the compound that inhibits LSD1 activity
15 in combination with the arsenic trioxide and said retinoic acid compound.

In some embodiments, the kit further includes an effective amount of a compound that inhibits Pin1 activity, an effective amount of a compound that inhibits LSD1 activity, and instructions for the use of the compound that inhibits Pin1 activity and the compound that inhibits LSD1 activity in combination with the arsenic trioxide and the retinoic acid compound.

20 In some embodiments, the retinoic acid compound is administered in a low dose such as about 5 mg/kg body weight or less (e.g., about 0.1, 0.2, 0.5, 0.75, 1, 2, 3, 4, or 5 mg/kg body weight or less), 1.5 ug/g body weight or less (e.g., about 0.1, 0.2, 0.5, 0.75, 1, or 1.5 ug/g body weight or less), less than about 25 mg/m² (e.g., less than about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 mg/m²), or between 25 mg/m² and
25 45 mg/m² (e.g., 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, or 45 mg/m²). In some embodiments, the low dose of the retinoic acid compound is a nontoxic dose of the retinoic acid compound. In some embodiments, the low dose of the retinoic acid compound is administered in combination with a low dose of arsenic trioxide. In some embodiments the low dose of the retinoic acid compound and the low dose of the arsenic
30 trioxide are nontoxic.

In some embodiments, a low dose of a retinoic acid compound related to *in vivo* treatments (e.g., in mice treatments) is 1.5 µg/g/day or lower. In some embodiments, a low dose of the retinoic acid compound comprises a dose of about 30 mg/m² body surface area or less. For example, a low dose of a retinoic acid compound related to treating a human with

leukemia is 10-22.5 mg/m² (PO administration, BID). In some embodiments, a low dose of the retinoic acid compound comprises a dose of about 10 mg/m² body surface area or less.

In some embodiments, the arsenic trioxide is administered in a low dose, such as 2 mg/kg body weight or less (e.g., about 0.01, 0.02, 0.03, 0.032, 0.04, 0.05, 0.06, 0.07, 0.08, 5 0.09, 0.1, 0.15, 0.16, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.25, 1.5, 1.75, or 2 mg/kg body weight or less), between about 0.5 mg/kg and about 12 mg/kg body weight (e.g., about 0.5, 0.6, 0.7, 0.8, 0.9, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 mg/kg body weight), or less than about 6 ug/g body weight (e.g., less than about 0.1, 0.2, 0.5, 0.75, 1, 2, 3, 4, 5, or 6 ug/g body weight). In some embodiments, a low dose of arsenic trioxide is about 0.15, about 0.16, or about 0.032 10 mg/kg body weight. In some embodiments, the low dose of the arsenic trioxide is a nontoxic dose of the arsenic trioxide.

In some embodiments, a low dose of arsenic trioxide related to *in vitro* treatments of leukemia cells is 0.25 µM or lower. In some embodiments, a low dose of arsenic trioxide related to *in vivo* treatments (e.g., in mice treatments) is 2.5 µg/g/day or lower. In some 15 embodiments, a low dose of arsenic trioxide comprises a dose of about 2.5 mg/kg body weight or less (e.g., a dose of between about 0.05 mg/kg body weight and about 2.5 mg/kg body weight). For example, a low dose of arsenic trioxide related to treating a human with leukemia is 0.075-0.15 mg/kg (IV administration, QD). In some embodiments, a low dose of arsenic trioxide comprises a dose of about 0.05 mg/kg body weight or less.

20 In some embodiments, the low dose of arsenic trioxide is administered in combination with a low dose of a retinoic acid compound. In some embodiments the low dose of arsenic trioxide and the low dose of retinoic acid compound are nontoxic.

In some embodiments, the retinoic acid compound is all-trans retinoic acid (ATRA), 13-cis-retinoic acid, retinol, retinyl acetate, retinal, or AC-55640, or is a compound structurally 25 similar to retinoic acid. In some embodiments, the retinoic acid compound is a compound selected from Table 1 or Table 2. In a preferred embodiment, the retinoic acid compound is ATRA.

In some embodiments, the leukemia is acute myeloid leukemia (AML). The acute myeloid leukemia may be either relapsed acute myeloid leukemia or refractory acute myeloid 30 leukemia. In some embodiments, the leukemia is an IDH2 independent (e.g., resistant) leukemia. An IDH2 independent leukemia includes, for example, a leukemia having a pathogenic IDH2 allele which has developed resistance to IDH2-targeting therapies (e.g., Enasidenib).

The leukemia described above can be acute promyelocytic leukemia (APL). In some

embodiments, the leukemia is not acute promyelocytic leukemia APL.

In some embodiments, one or more cells of said leukemia has a pathogenic IDH2 allele and elevated 2-hydroxyglutarate (2-HG) levels.

Referring to the method or kit described above, in some embodiments, one or more
 5 cells of the leukemias comprise a pathogenic IDH2 allele in combination with one or more
 other mutants. Examples of the other mutants include, but are not limited to, FLT3, NPM1,
 DNMT3A, RUNX1, TET2, IDH2, CEBPA, TP53, IDH1, NRAS, RARA, PML, TTN, WT1,
 MYH11, BPIFC, CBFB, KIT, KRAS, KMT2A, PTPN11, MUC16, SMC1A, RUNX1T1,
 MPO, U2AF1, ABCA6, DMXL2, DNAH3, KLK3, FBXO7, SMC3, MXRA5, MUC17,
 10 SF3B1, HPS3, PHF6, ASXL1, AHNAK, SENP6, MYCBP2, NF1, PCLO, CSMD3, LRP1B,
 MED12, RAD21, AHNAK2, GDI2, PARP14, KLHL7, BCORL1, SPEN, BRWD1, UBR4,
 STAG2, LNX1, FREM2, MLLT10, DNAH11, SLIT2, DNAH9, BRINP3, HMCN1, SYNE1,
 GABRG3, NPAS3, CSMD1, GATA2, ZCRB1, MTFR2, SLC43A1, SLA2, APOB, PRDM1,
 BTBD10, ABCA5, EP300, TNC, ADGRG4, BSN, IFT74, COL12A1, NUP98, CREBBP,
 15 EPG5, KIF1B, ABL1, SLC16A7, RALGPS2, CHL1, SCLT1, LRP2, VWA3B, FRYL,
 PRUNE2, PLCE1, GRIK4, HIVEP3, RYR3, ANK2, ATRNL1, VPS13B, GPR179, FCGBP,
 KIF15, and DNAH10. Two exemplary mutants are NPMc+ and FLT3-ITD.

In some embodiments of the above method or kit, one or more cells of the leukemia
 comprise a pathogenic IDH2 allele in combination with NPMc+, FLT3-ITD, or both. An
 20 exemplary pathogenic IDH2 allele is IDH2^{R140Q}.

In some embodiments of the above method or kit, the effective amount of arsenic
 trioxide is a low dose of about 2.5 mg/kg body weight or less, the effective amount of the
 retinoic acid compound is a low dose of about 30 mg/m² body surface area or less, and the
 retinoic acid compound is selected from the group consisting of all-trans retinoic acid (ATRA),
 25 13-cis-retinoic acid, retinol, retinyl acetate, retinal, and AC-55640.

In some embodiments of the above method or kit, the effective amount of arsenic
 trioxide is a low dose of between about 0.05 mg/kg body weight and about 2.5 mg/kg body
 weight, the effective amount of the retinoic acid compound is a low dose of about 10 mg/m²
 body surface area or less, and the retinoic acid compound is ATRA.

30 In some embodiments of the above method or kit, one or more cells of the leukemia
 comprise a pathogenic IDH2 allele in combination with NPMc+, FLT3-ITD, or both, in which
 said pathogenic IDH2 allele is IDH2^{R140Q}.

BRIEF DESCRIPTION OF THE DRAWINGS

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

5 Fig. 1A is an image depicting the approach used to model in vivo independence from mIDH2 (IDH2R140Q) in AML. Mouse model is based on serial transplantation of leukemia cells derived from Tg(M2rt-TA) transgenic mice and overexpressing HoxA9 and Meis1a oncoproteins. Red boxes: leukemia. Blue boxes: healthy state.

Fig. 1B is a series of images depicting Flow cytometry plots of bone marrow samples.
10 X- axis, fluorescence Intensity (0 – 105), Y-axis, cell counts.

Fig. 1C is a graph depicting the percentage of Leukemic cells in the bone marrow (BM) of 2nd and 3rd recipients maintained on DOX ON or DOX OFF diet. Quantitation is expressed as percentage of leukemic cells showing positivity for GFP and YFP fluorescence.

Fig. 1D is a series of images showing May-Grünwald-Giemsa staining of peripheral
15 blood (PB) smears at euthanization (6- 9 weeks after transplant).

Fig. 1E is a graph depicting the percentage of blasts in peripheral blood (PB) in of 2nd and 3rd recipients maintained on DOX ON or DOX OFF diet.

Fig. 1F is a graph depicting the LC- MS quantification of the 2-HG peripheral blood (PB) in of 2nd and 3rd recipients maintained on DOX ON or DOX OFF diet.

20 Fig. 1G is a graph depicting real time quantitative PCR analyses for the expression of mIDH2 (IDH2^{R140Q}) in leukemia cells.

Fig. 1H is a graph depicting real time quantitative PCR analyses for the expression of HoxA9 in leukemia cells.

Fig. 1I is a graph depicting real time quantitative PCR analyses for the expression of
25 Meis1A in leukemia cells.

Fig. 1J is a graph depicting the number of methylcellulose colonies generated at 3rd plating by leukemia cells (GFP+/YFP+) isolated from n=3 bone marrow samples. IDH2R140Q inhibitor (IDHi): AGI-6780, 1μM, vehicle: DMSO. Data are means ± SD of n=3 replicates for each sample.

30 Fig. 1K is a series of images depicting representative magnification of methylcellulose colonies generated by blasts at 3rd plating.

Fig. 1L is a graph depicting the relative LC- MS quantification of the 2-HG from colonies pooled and extracted for metabolites at 3rd-plating, vehicle: DMSO, IDHi: AGI-6780, 1μM.

Fig. 2A is an image depicting Hierarchical clustering of leukemia cells' metabolites isolated from Dox+ cohorts. Rows: metabolites; columns: samples; color key indicates metabolite abundance level (blue: lowest; red: highest). Clustering generated by MetaboAnalyst's annotation tool.

5 Fig. 2B is an image depicting the Pathway enrichment analysis of altered metabolic pathways between sensitive (2nd RECIPIENT – derived cells) and resistant (3rd RECIPIENT – derived) leukemia cells.

Fig. 2C is an image depicting the bar chart showing metabolic pathways enriched in resistant leukemia cells isolated from 3rd RECIPIENTS.

10 Fig. 2D is a graph depicting Fold changes of Cysteine (CYS) and Glutathione (GSH) metabolites in resistant leukemia cells compared with sensitive leukemia cells.

Fig. 2E is a graph depicting Fold change of the NAD⁺/NADH and NADP⁺/NADPH ratio in resistant leukemia cells compared with sensitive leukemia cells. NAD⁺: nicotinamide adenine dinucleotide oxidized, NADP⁺: nicotinamide adenine dinucleotide phosphate oxidized, NADH: nicotinamide adenine dinucleotide reduced, NADPH: nicotinamide adenine
15 dinucleotide phosphate reduced.

Fig. 2F is an image depicting flow cytometry plots for fluorescence intensity of sensitive (n=4) and resistant (n=5) leukemia cells incubated with the redox sensitive probe, MitoSox. X- axis: Fluorescence intensity levels; Y-axis: percentage of cell counts.

20 Fig. 2G is a graph depicting the percentage of cells showing high levels of ROS (ROSHi) in sensitive (derived from 2nd RECIPIENTS) and resistant (derived from 3rd RECIPIENT) cells shown in (2F).

Fig. 2H is a series of images depicting Immunofluorescence for the DNA damage marker γ H2A.X (red) and DNA (blue) and relative quantitation of positive nuclei. Data are
25 mean $\pm$ SD of n=3 different fields of n=3 different evaluated samples.

Fig. 2I is a series of images depicting IHC for γ H2A.X in mouse spleen sections and percentage of infiltrating blasts showing positivity for the γ H2A.X marker.

Fig. 2J(a-e): a series of graphs showing the transcriptional rewiring of mIDH leukaemia at early (sensitive) stage, characterized by altered MAPK and ATRA associated pathways. **a.**
30 Bar chart showing the most significantly up-regulated KEGG pathways in HoxA9/Meis1a/mIDH2 cells. The pathways are ranked on the basis of the gene set size indicating activation. **b-c.** Gene Set Enrichment (GSE) plots of Tretinoin related Signature in HoxA9/Meis1a/mIDH2 vs HoxA9/Meis1a. **d-e.** Gene Set Enrichment (GSE) plots of LSD1 Signature in HoxA9/Meis1a/mIDH2 vs HoxA9/Meis1a.

Fig. 3A is an image depicting the heat map of differentially expressed genes ($P < 0.05$) in sensitive and resistant samples or Hoxa9/Meis1a leukemia, not primed by mIDH2. The columns represent the samples and the rows represent the genes. Gene expression is shown with pseudocolor scale (-3 to 3) with upregulated genes shown as a shade of blue and
 5 downregulated genes as a shade of red.

Fig. 3B is an image depicting the bar chart showing the most significantly up-regulated pathways (multiple test corrected p-value < 0.05) in resistant leukemia cells. The pathways are ranked on the basis of Z score indicating activation.

Fig. 3C is an image depicting Gene Set Enrichment (GSE) plots of MAPK, $\text{TNF}\alpha$, and
 10 ATRA related signature in resistant (3rd RECIPIENT) vs sensitive (2nd RECIPIENT) leukemia cells (left panel) and the associated Heat maps (right panels).

Fig. 3D is an image depicting the Venn Plot showing shared genes between mouse mIDH2 leukemia and Schenken et al.; 2013 gene sets.

Fig. 3E is an image depicting the Heat map showing shared genes shown in (3D) are
 15 regulated concordantly in Resistant and Sensitive clone with respect to ATRA+TCP gene sets reported by Schenken et al.; 2013.

Fig. 3F is a series of graphs and images depicting the quantifications of morphological screening of murine leukemia cells isolated from the bone marrow of sensitive (2nd RECIPIENTS, n=2) or resistant (3rd RECIPIENTS, n=3) and treated with physiological (10^{-9}M , 10^{-8}M) or pharmacological (10^{-7}M , 10^{-6}M) concentrations of ATRA. Hoxa9/Meis1a leukemia cells (n=1) were included as the control. For each sample n=100 cells were counted n=3 times for statistical analysis. The figure legend (right panel) describes representative images of leukemia cells at different stages of differentiation (blasts, intermediate, differentiated).
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Fig. 3G is a graph depicting relative LC-MS/MS quantitation of 2-HG in TF1 cells overexpressing IDH2R140Q or respective controls.
 25

Fig. 3H is a series of images and graphs depicting Cytospin images and quantitation of human TF1 cell line stably overexpressing the mutant variant R140Q of IDH2 (mIDH2) to the respective controls (CTRL) that have been treated with pharmacological concentrations of
 30 ATRA (10^{-6}M) or vehicle (DMSO).

Fig. 3I is a graph depicting Cell proliferation assay on TF1 cell line, stably overexpressing the mIDH2 or the empty vector (CTRL). Each cell line was treated with ATRA (10^{-6}M) or vehicle (DMSO) for n=4 days.

Fig. 3J is a series of images depicting different culture media color of TF1 cell line

stably overexpressing mIDH2 or respective control (CTRL). Each cell line was treated with ATRA (10⁻⁶M) or vehicle (DMSO) for n=6 days in presence or absence of EPO. The asterisks (yellow) indicate mIDH2-TF1 leukemia cells treated with ATRA (10⁻⁶M). 1-4 flasks show cultured TF1 cell line (CTRL), 5-8 flasks show cultured TF1 cell line overexpressing the mutant variant R140Q of IDH2 (mIDH2). 1, 5: TF1 treated with DMSO; 2,6: TF1 treated with ATRA; 3,7: TF1 treated with DMSO in media supplemented with pro-differentiating stimuli (EPO); 4,8: TF1 treated with ATRA in media supplemented with pro-differentiating stimuli (EPO).

Fig. 3K(a-e): a series of graphs showing mouse HoxA9/Meis1a/ mIDH2 leukaemia at early (sensitive) stage, marked by switching to One-Carbon metabolism and increased oxidative stress. **a.** Hierarchical clustering of leukaemia cells' metabolites isolated from Dox+ cohorts. Rows: metabolites; columns: samples; colour key indicates metabolite abundance level (blue: lowest; red: highest). Clustering generated by MetaboAnalyst's annotation tool. **b.** Pathway enrichment analysis shows altered metabolic pathways between HoxA9/Meis1a/mIDH2 and respective HoxA9/Meis1a control cells. **c.** LC-MS/MS fold change levels of precursors for One-Carbon metabolism isolated from HoxA9/Meis1a/mIDH2 and respective HoxA9/Meis1a control cells. Data are mean $\pm$ SD. **d.** LC-MS/MS fold change levels of precursors for pyrimidine metabolism. OMP: orotidine -5'-monophosphate, UDP: uridine diphosphate. Data are mean $\pm$ SD. **e.** LC-MS/MS fold change levels of both reduced glutathione (GSH) and oxidized glutathione (GSSH) and their ratio. Data are mean $\pm$ SD.

Fig. 4A is an image depicting Western blot analysis for ATRA targets (Pin1), ATRA receptors (RAR α , RXR α), and factors associated with ATRA – related differentiation pathway in hematopoietic cells (c/EBP α , c/EBP ϵ). The samples are protein extracts from different (n=2) sensitive or resistant leukemia cells. Protein samples isolated from Hoxa9/Meis1a leukemic cells (n=2) were included as control.

Fig. 4B is an image depicting the Western blot analysis for factors associated with ATRA-related pathway in TF1 cell line, stably overexpressing the mIDH2) or the empty vector (CTRL).

Fig. 4C is an image depicting the Western blot analysis for factors associated with ATRA-related pathway in U937 cell line, stably overexpressing the mIDH2) or the empty vector (CTRL).

Fig. 4D is a graph depicting Percentage of the transferrin receptor (CD71+) positive TF1 cells overexpressing the mIDH2 or respective control (CTRL). Each cell line was treated with the Pin1 Inhibitor (Juglone) or vehicle (DMSO) for 3 days.

Fig. 4E is a graph depicting the Colony forming assay of TF1 cells overexpressing the mIDH2 or respective control (CTRL) treated with Juglone or vehicle. Colonies were quantitated after 7 days from plating.

Fig. 4F is a graph depicting the quantification of the relative transcriptional expression levels of Hemoglobin (HBG) in TF1 cells silenced for Pin1 and overexpressing the mIDH2 or respective controls (CTRL).

Fig. 4G is a graph depicting the quantification of the relative transcriptional expression levels of KLF1 in TF1 cells silenced for Pin1 and overexpressing the mIDH2 or respective controls (CTRL).

Fig. 4H is a graph depicting the quantification of the relative transcriptional expression levels of GATA2 in TF1 cells silenced for Pin1 and overexpressing the mIDH2 or respective controls (CTRL).

Fig. 4I is a graph depicting the Fold Induction of CD71 in TF1 cells overexpressing mutant IDH2 alone (mIDH2) or in combination with PIN1 (mIDH2 PIN1++) and induced to differentiation after treatment with the IDH inhibitor AG-221 or respective vehicle (DMSO).

Fig. 4J is a series of graphs depicting the Quantification of the relative transcriptional expression levels of Hemoglobin (HBG), Kruppel like factor 1 (KLF1), in TF1 cells silenced for Pin1 and overexpressing the mIDH2 or respective controls (CTRL).

Fig. 4K is an image depicting the scheme of ATO/ ATRA treatments performed in vivo on BL6J recipient mice transplanted with resistant leukemia

Fig. 4L is an image deciphering the percent of survival of recipient mice transplanted with resistant leukemia cells and treated as shown in 4K. N=10 / arm.

Fig. 4M is an image deciphering the inhibition of LSD1 contributes to increase intracellular levels of oxidative stress. Histogram reporting the intracellular oxidative stress of TF1 cells treated for 3 weeks with the LSD1 inhibitor (LSD1i; GSK2879552, 1 μ M) or DMSO (VHL) as respective control. Measurements were performed by flow cytometry analyses after incubation of TF1 cells with the CellROX molecular probe. Data are mean $\pm$ SD.

Fig. 5A is a series of images and graphs depicting methylcellulose colony forming assay quantification of human primary AML blasts treated *in vitro* with pharmacological concentration of ATRA, ATO, a combination of both, or vehicles as control. Treatments were performed in either the presence of mIDH2 or absence of the mutation (CTRL). Data are means $\pm$ SD of duplicates.

Fig. 5B is an image depicting the Experimental approach to generate PDX for human AML harboring the mutation R140Q in the IDH2 gene

Fig. 5C is an image depicting the Scheme of the pharmacological therapy for PDX resembles therapeutic regimen of human APL patients. Treatment regimen was performed in cycles for a total of 65 days resembling APL0406 protocol (Lo-Coco et al; NEJM 2013).

Fig. 5D is an image depicting the , Kaplan-Meier curve showing the percentage of
5 disease free survival related to ATO /ATRA the pre-clinical study on mIDH2 PDX (n=5 PDX/
cohort). Data analyzed by Log-Rank (Mantel-Cox) test.

Fig. 5E is a series of images depicting the cytopins of cells isolated from BM of PDX mice treated with ATRA and ATO.

Fig. 5F is a graph depicting the percentage of morphologically screened human cells
10 isolated from BM of PDX generated by using IDH2R140Q AML.

Fig. 5G is an image depicting the scheme of leukemia evolution from mIDH dependent to mIDH2 independent states (e.g. acquisition of resistance to mIDH inhibition). Multiple alterations including genetic mutations, metabolic reprogramming, and transcriptional rewiring are acquired during progression and co-exist in the mIDH2 independent stage. In this
15 multifactorial setting, we unveil Pin1 and high ROS levels as key factors of upregulated pro-survival and proliferation programs (MAPK/PI3K pathways), loss of functional ATRA signaling, and altered metabolic state (One-Carbon metabolism and increased nucleic acid synthesis). These features represent targetable vulnerabilities of leukemia cells and highlight the potential for using ATO (pro-oxidant) and ATRA (pro-differentiating) drugs. Most
20 importantly, sensitivity to ATO and ATRA is a vulnerability already present at an early stage in mIDH2 dependent leukemia, which is maintained in later stages of the disease progression.

Fig. 6A is a graph depicting Kaplan-Meier survival curves for 3rd RECIPIENTS (Dox+ = mIDH2 ON; Dox- = mIDH2 OFF).

Fig. 6B is a series of images depicting Haematoxylin and Eosin (H&E) staining of
25 internal organs (spleen, liver, and kidney) from 3rd RECIPIENTS showing infiltrating blasts. Enlarged images (40x) show perivascular accumulation of infiltrating blasts in spleen, liver, and kidney.

Fig. 6C is a series of images depicting H&E and immunohistochemistry (IHC) staining of kidney sections derived from 3rd RECIPIENTS. The IHC staining shows infiltrating blasts
30 are positive for the antigen Ki-67, a cellular marker for proliferation.

Fig. 6D is a series of images depicting H&E staining of internal organs (spleen, liver, and kidney) from 2nd RECIPIENTS. High resolution images show absence or limited infiltrating blasts.

Fig. 6E is a series of images depicting H&E and IHC staining of kidney sections derived from 2nd RECIPIENTS. The IHC staining for the antigen Ki-67 is mostly negative across the examined tissue.

Fig. 6F is a graph depicting the quantification of the IHC staining for the antigen Ki-67, in blasts infiltrating internal organs (spleen, liver, and kidney). Sections of the internal organs from 2nd RECIPIENTS (n=3) and 3rd RECIPIENTS (n=3) were scored for n=3 different fields.

Fig. 6G is a series of graphs depicting the growth curves of primary sensitive and resistant murine leukemia cell lines, respectively generated from bone marrow of 2nd or 3rd RECIPIENTS. Variability of cell growth rate represents heterogeneous samples. Data are reported for n=3 primary murine leukemia cell lines generated by n=3 different recipients.

Fig. 6H is a graph depicting a methylcellulose colony forming assay of in vitro cultured blasts, derived from 2nd and 3rd RECIPIENTS. Primary murine leukemia cells derived from different 2nd and 3rd RECIPIENTS (n=2). For each sample 5000 cells from each line were probed for the ability to generate colonies after 5, 15, 30, and 60 days of culture. Colonies were scored at 7 days after 1st plating. The number of generated colonies is maintained without significant decrease to 30 days of culture. Data are means $\pm$ SD of n=2 samples. mIDH2: IDH2R140Q.

Fig. 7A is an image depicting the scheme of the experiment for metabolites' analysis

Fig. 7B is an image depicting the One-Carbon metabolism pathway. The image is from Locasale J.W. Nature Reviews Cancer13, 572-583 (2013).

Fig. 7C is a graph depicting Fold change of key metabolites in the Methionine pathway in resistant leukemia cells compared to sensitive cells. MET: Methionine; SAM: S-adenosylmethionine; SAH, Sadenosylhomomocysteine; hCYS, homocysteine. d, Representative scheme of the De-novo synthesis of Purine, (Adenine and Guanine) and Pyrimidine (Thymine, Cytosine and Uracil).

Fig. 7D is an image depicting the Representative scheme of the De-novo synthesis of Purine (Adenine and Guanine) and Pyrimidine (Thymine, Cytosine and Uracil).

Fig. 7E is an image depicting the Fold change of the precursors for Purine biosynthesis in resistant leukemia cells compared with sensitive leukemia cell. IMP: inosine monophosphate; AMP: adenosine monophosphate; GMP: guanosine monophosphate.

Fig. 7F is an image depicting the Fold change of the precursors for Pyrimidine biosynthesis in resistant leukemia cells compared with sensitive leukemia cells. Orotate: orotic acid; OMP: orotidine monophosphate; UMP: uridine monophosphate; UDP: uridine diphosphate.

Fig. 7G is a series of images and graphs depicting Flow cytometry plots and quantitation of the percentage of cells showing high levels of mitochondrial ROS (ROSHi) in blast cells overexpressing HoxA9/Meis1 alone (HoxA9/Meis1a mIDH2 OFF) or in combination with mutant IDH2 (HoxA9/Meis1a mIDH2 ON).

Fig. 8A is a scheme depicting samples processed for the Whole Exome Sequencing (WES) analysis. Genomic DNA was isolated from resistant leukemia cells (3rd_08, 3rd_09, 3rd_11) or their common parental sensitive cells (2nd_07).

Fig. 8B is a pie-chart showing frequencies of transversion (2nd_07). Resistant leukemia cells were from different 3rd recipients (n=3), maintained on normal diet (mIDH2 OFF) or Dox+ diet (mIDH2 ON). Both resistant and sensitive cells were sorted from bone marrow for purity of the GFP+/YFP+ cell population. mIDH2: IDH2R140Q or transition mutations responsible for non-synonymous mutations in resistant genomes. Pairs of nucleotide indicate different possible transversion (G>T, C>A, A>C, T>G, G>C, C>G, A>T, T>A) and transition mutations targeting T or A (A>G, T>C) and C or G (G>A, C>T).

Fig. 8C is a principal component analysis (PCA) plot showing clustering of WES samples using single nucleotide coverage of sensitive and resistant cells.

Fig. 8D is a venn diagram showing the number of mutated genes overlapping between resistant leukemia cells (3rd_08, 3rd_09, 3rd_11). Mutations in Sp140 gene are shared between all analyzed resistant clones, but not found in the parental sensitive leukemia.

Fig. 8E is a series of circular plots of the expression of CNV and fusion genes for sensitive (2nd_07) and resistant (3rd_08, 3rd_09, 3rd_11) leukemia cells. Circular tracks from inside to outside: Track 1: genome positions by chromosomes (black lines are cytobands) that are arranged circularly end to end. Track 2: lines plot (red: positive; blue: negative) showing gene expression data represented as normalized read counts. Track 3: Barplot showing segmented data (CNVs). Track 4: Barplot with positive (gain: blue) and negative values (purple: loss) of called CNVs. Amplifications or deletion of relevant oncogenes such as Flt3, Dok genes, and Trp53 are highlighted. Track 5: Interchromosomal translocations (fusion genes) are plotted with lines connecting chromosome segments.

Fig. 9A is a scheme depicting the samples processed through RNA-Sequencing analysis to characterize the transcriptional changes associated with leukemia progression. Resistant leukemia cells 3rd_09 and 3rd_13 were generated from the parental sensitive 2nd_9A. Resistant leukemia cells 3rd_11, 3rd_12, 3rd_08, and 3rd_10 were generated from the parental sensitive 2nd_7A. mIDH2: IDH2R140Q.

Fig. 9B is a principal component analysis (PCA) plot showing the genes that are the sources with the majority of the variance in resistant leukemia cells, compared with sensitive leukemia cells. The genes are represented by black dots. Mpo: myeloperoxidase, Lyz2: lysozyme 2, Rn45s: 45s-pre-ribosomal RNA, Ngp: neutrophilic granule protein, Psap: prosaposin, Ftl1: ferritin light polypeptide 1, Zfp36: zinc finger protein 36, Hspa8: heat shock protein 8, Vim: vimentin, Fosb: FBJ osteosarcoma oncogene B, Fos: FBJ osteosarcoma oncogene, Jun: jun proto-oncogene.

Fig. 9C is a table showing the variance and associated statistical significance of major relevant genes for the Principal Component Analysis (PCA) plot shown in Fig. 9B.

Fig. 9D is an image showing the top altered networks in resistant cells generated by Ingenuity Pathways Analysis tool (www.ingenuity.com). The intensity of the node color indicates the degree of up-regulation (red) and down-regulation (green), while white nodes indicate non-modified genes that may be affected in a non-transcriptional manner. All networks shown were significantly affected, with a score ≥ 29 .

Fig. 9E is a series of graph showing the quantification of intracellular flow staining for p-P44/42 (T201, Y204) and p-Akt (T308) in mouse leukemia cells.

Fig. 9F is a graph showing an increase in 2-HG levels in a human leukemia U937 cell line stably overexpressing the mutant variant R140Q of IDH2 (mIDH2) compared to a U937 control cell line (CTRL).

Fig. 9G is a series of images showing the representative cytopins images of blasts, intermediates, and differentiated cells used as reference for the screening.

Fig. 9H is a graph showing the quantification of human leukemia U937 cell line stably overexpressing the mutant variant R140Q of IDH2 (mIDH2) to the respective U937 controls (CTRL).

Fig. 9I is a graph showing the quantification of flow cytometry analysis for the percentage of CD14 positive cells (CD14+).

Fig. 9J is a graph showing the quantification of flow cytometry analysis for the percentage of CD11b positive cells (CD11b+). Data are means $\pm$ SD of n=3 measurements.

Fig. 10A is a series of images depicting a western blot assay on TF1 cells overexpressing mutant IDH2 (TF1 IDH2R140Q) or respective control vector (TF1 CTRL) stably silenced for PIN1 expression (shPIN1). Non targeting (shSCR) was used as control for non-specific silencing effects.

Fig. 10B is a series of images depicting a western blot assay on TF1 cells overexpressing mutant IDH2 (TF1 IDH2R140Q) or respective control vector (TF1 CTRL)

treated with the Pin1 Inhibitor Juglone for 72h.

Fig. 10C is a series of images depicting a western blot assay on TF1 cells stably overexpressing mutant IDH2 (TF1 IDH2R140Q) and Pin1 or respective control vector vectors (TF1 CTRL, CTRL).

5 Fig. 11A is a series of images depicting a methylcellulose colony forming assay and colony quantifications of mouse leukemia cells derived from 2nd RECIPIENTS or leukemia cells overexpressing Hoxa9/Meis1a.

Fig. 11B is a series of images depicting a methylcellulose colony forming assay and colony quantifications of mouse leukemia cells derived from 3rd RECIPIENTS or leukemia
10 cells overexpressing Hoxa9/Meis1a.

Fig. 11C is a scheme of the *in vivo* approach to evaluate combination arsenic trioxide (ATO) and ATRA treatment on mIDH2 leukemic cells in C57BL/6J mice.

Fig. 11D is a graph depicting Kaplan-Meier survival curves derived from the experiment shown in Fig. 11C.

15 Fig. 11E is a series of images depicting May-Grunwald-Giemsa staining of BM blasts sorted from recipients treated with ATO and ATRA as shown in Fig. 11C.

Fig. 11F is a series of images depicting H&E staining of lung tissues. Arrowheads indicate perivascular leukemia cells infiltrates.

Fig. 11G is a series of images depicting IHC of lung tissues for myeloperoxidase (MPO), a marker of myeloid cell differentiation. Arrowheads indicate strongly MPO positive
20 cells in perivascular infiltrates.

Fig. 12A is a scheme of the *in vivo* experimental approach to evaluate combination ATO and ATRA treatment on mIDH2 U937 human leukemic cell lines.

Fig. 12B is a series of graphs depicting Kaplan- Meier survival curves of NSG mice
25 transplanted with mIDH2-U937 or CTRL-U937 leukemia cells and treated with ATO, ATRA or a combination of both.

Fig. 12C is a series of graphs showing the percentage of leukemia cells in bone marrow (BM) of NSG mice. Data are means $\pm$ SD of samples isolated from different mice.

Fig. 12D is a series of images showing morphological screening analysis of U937 cells
30 isolated as GFP+ cells from NSG mice treated with retinoic acid (ATRA), Arsenic Trioxide (ATO), a combination of both, or respective vehicle solution. Arrowheads show cells displaying differentiated morphology

Fig. 12E is a series of graphs and images depicting cytopins of mIDH2-U937 or CTRL-U937 leukemia cells isolated as GFP+ cells from BM of NSG mice.

Fig. 12F is a series of flow cytometry plots showing CD11b expression levels in mIDH2-U937 or CTRLU937 leukemia cells.

Fig. 12G is a series of graphs showing the quantification of flow cytometry analysis for the percentage of CD11b positive cells (CD11b+). U937 cell line stably overexpressing the mutant variant R140Q of IDH2 (mIDH2) show about 30% reduced percentage of CD11b+ cells compared to the control (CTRL). Data are means $\pm$ SD of n=3 measurements.

Fig. 12H is a series of graphs showing the quantification of spleen weight dissected from NSG mice transplanted with mIDH2-U937 or CTRL-U937 leukemia cells and treated with ATO, ATRA or a combination of both.

Fig. 13a-13k: a series of graphs showing the sensitivity of mIDH2 leukaemia to ATRA and ATO. **a**, Quantifications of morphological screening of murine leukaemia cells isolated from the bone marrow of 2nd RECIPIENTS (n=2) or 3rd RECIPIENTS (n=3) and treated with physiological (10^{-9} M, 10^{-8} M) or pharmacological (10^{-7} M, 10^{-6} M) concentrations of ATRA. Hoxa9/Meis1a leukaemia cells (n=1) were included as the control. For each sample n=100 cells were counted n=3 times for statistical analysis. **b-c**, Cytospin images and quantitation of human TF1 cell line stably overexpressing the mutant variant R140Q of IDH2 (mIDH2) to the respective controls (CTRL) that have been treated with pharmacological concentrations of ATRA (10^{-6} M) or vehicle (DMSO). **d**, Cell proliferation assay on TF1 cell line, stably overexpressing the mIDH2 or the empty vector (CTRL). Each cell line was treated with ATRA (10^{-6} M) or vehicle (DMSO) for n=4 days. Data are means of n=3 replicates for each condition and time point. **e**, Cell proliferation assay on U937 cell line, stably overexpressing the mIDH2 or the empty vector (CTRL). Each cell line was treated with ATRA (10^{-6} M) or vehicle (DMSO) for n=4 days. Data are means of n=3 replicates for each condition and time point. **f**, Colony forming assay of mouse leukaemia cells harbouring mutant or wildtype IDH2 expression and treated with the PIN1 inhibitor (Juglone, 1 μ M) or respective vehicle (VHL). Colonies were quantitated after 12 days from plating. **g**, Colony forming assay of TF1 cells overexpressing the mIDH2 or respective control (CTRL) and treated with the PIN1 inhibitor (Juglone, 1 μ M) or respective vehicle (VHL). Colonies were quantitated after 7 days from plating. **h**, Fold induction of cell death (Annexin V/7AAD positive cells) in mouse leukaemia cells isolated from 2nd or 3rd recipients and *in vitro* treated with ATO (0.5 μ M) for 96h. **i**, Fold induction of cell death (Annexin V/7AAD positive cells) in TF1 leukaemia cells overexpressing IDH2^{R140Q} and treated with ATO (0.5 μ M) for 96h. **j**, Representative images of methylcellulose colony forming assay and colonies quantification of mouse leukaemia cells isolated from 2nd recipients (early stage) treated *in vitro* with pharmacological concentrations

of ATRA (1 μ M), ATO (0.5 μ M), a combination of both (ATRA+ATO), or vehicles (VHL) as control. Treatments were performed in either the presence of mIDH2 or absence of the mutation (CTRL). Data are means $\pm$ SD of duplicates. **k**, Quantification of colony forming ability of mouse leukaemia cells harbouring mutations in IDH2 (IDH2^{R140Q}) or IDH1 (IDH1^{R132C}) in association with different genetic mutant backgrounds such as NPMc+ or FLT3ITD and treated with pharmacological concentrations of ATRA (1 μ M), ATO (0.5 μ M), a combination of both (ATRA+ATO), or vehicles (VHL) as control. Non mutant IDH2 cells such as MLL-AF9 or NPMc+/ FLT3ITD were also included.

Fig. 14a-14h: a series of graphs showing the combination of ATO and ATRA targeting mIDH2 human leukaemia cells. **a**, Scheme of the in vivo experimental approach. **b**, Kaplan-Meier survival curves of NSG mice transplanted with mIDH2-U937 or CTRL-U937 leukaemia cells and treated with ATO, ATRA or a combination of both. **c**, Percentage of leukaemia cells in bone marrow (BM) of NSG mice. Data are means $\pm$ SD of samples isolated from different mice. **d**, Morphological screening analysis of U937 cells isolated as GFP+ cells from NSG mice treated with retinoic acid (ATRA), Arsenic Trioxide (ATO), a combination of both, or respective vehicle solution. **e**, Cytospins showing mIDH2-U937 or CTRL – U937 leukaemia cells isolated as GFP+ cells from BM of NSG mice. Arrowheads show cells displaying differentiated morphology. **f**, Flow cytometry plots showing CD11b expression levels in mIDH2-U937 or CTRL-U937 leukaemia cells. **g**, Quantification of flow cytometry analysis for the percentage of CD11b positive cells (CD11b+). U937 cell line stably overexpressing the mutant variant R140Q of IDH2 (mIDH2) show about 30% reduced percentage of CD11b+ cells compared to the control (CTRL). Data are means $\pm$ SD of n=3 measurements. **h**, Quantification of spleen weight dissected from NSG mice transplanted with mIDH2-U937 or CTRL-U937 leukaemia cells and treated with ATO, ATRA or a combination of both.

Fig. 15a-15g: a series of graphs showing combined ATRA and ATO treatment eradicating mIDH2 leukaemia. **a**, Methylcellulose colony forming assay quantification of human primary mIDH2 AML blasts treated in vitro with pharmacological concentrations of ATRA (1 μ M), ATO (0.5 μ M), a combination of both ATRA and ATO, or vehicles (VHL) as control. Treatments were performed in either the presence of mIDH2 or absence of the mutation (CTRL). Data are means $\pm$ SD of duplicates. **b** and **d**, Kaplan-Meier curve showing the percentage of disease free survival related to ATO/ATRA pre-clinical study on mIDH2 PDX. N=2 different mIDH2 AML were isolated from bone marrow at diagnosis. Data analysed by Log-Rank (Mantel-Cox) test. #DS: mice involved by differentiation syndrome (DS) (aka. ATRA syndrome).

c and **e**, Representative images of cytopins showing cells isolated from BM of PDX mice treated with ATRA and ATO. n.d: not detected as mice were not euthanized. **f-g**, Representative images of IHC for the differentiation marker human myeloperoxidase (MPO) on lung tissues isolated from PDX mice treated with ATRA and ATO or VEHICLE solutions.

5 Accumulation of human MPO positive cells is a major characteristic of DS.

Fig. 15h-15j: a series of graphs showing the effect of ATO and ATRA combination in limiting leukemia aggressiveness in vivo in a pre-clinical study on PDX generated with AML harboring wild-type IDH1 and IDH2 genes. **h**, Scheme of the experiment: NSG mice were transplanted with human AML blasts harboring mutations in WT1 and KIT genes and

10 chromosomal alterations (inv16), but wild-type IDH1 and IDH2 genes. **i**, Scheme of the ATO and ATRA treatments. **j**, Survival curve shows no significant survival extension for mice treated with ATO, ATRA, or combination of both ATRA and ATO, as compared with the respective VHL treated mice.

Fig. 16: a schematic depiction of ATO and ATRA targeting mIDH leukaemia. Scheme

15 of leukaemia evolution from mIDH dependent to mIDH2 independent states (e.g. acquisition of resistance to mIDH inhibition). Multiple alterations including metabolic reprogramming, and transcriptional rewiring are acquired during progression and co-exist in the mIDH2 independent stage. In this multifactorial setting, we unveil PIN1, deregulated *LSD1* signaling and high ROS levels as key factors of upregulated pro-survival and proliferation programs

20 (MAPK/PI3K pathways), alterations to functional ATRA signalling, and altered metabolic state (One-Carbon metabolism and increased nucleic acid synthesis). These features represent targetable vulnerabilities of leukaemia cells and highlight the potential for using ATO (pro-oxidant) and ATRA (pro-differentiating) drugs. Most importantly, sensitivity to ATO and ATRA is a vulnerability already present at an early stage in mIDH2 dependent leukaemia, which is

25 maintained in later stages of the disease progression. mIDH2: IDH2^{R140Q}; BM: bone marrow; ATRA: Retinoic acid; ATO: Arsenic Trioxide.

DEFINITIONS

To facilitate the understanding of this invention, a number of terms are defined below.

30 Terms defined herein have meanings as commonly understood by a person of ordinary skill in the areas relevant to the present invention. Terms such as "a", "an," and "the" are not intended to refer to only a singular entity, but include the general class of which a specific example may be used for illustration. The terminology herein is used to describe specific embodiments of the invention, but their usage does not delimit the invention, except as outlined in the claims.

As used herein, the term “about” refers to a value that is within 10% above or below the value being described.

As used here, any values provided in a range of values include both the upper and lower bounds, and any values contained within the upper and lower bounds.

5 As used interchangeably herein, the terms “arsenic trioxide” and “an arsenic trioxide compound” refer to a compound having the formula As_2O_3 and derivatives thereof. Arsenic trioxide generally has the following structure:



Derivatives of arsenic trioxide may include, for example, arsenic ores, such as, e.g.,
 10 arsenopyrite (grey arsenic; FeAsS), realgar (also known as sandarach or red arsenic; AsS),
 orpiment (yellow arsenic; As_2S_3), and arsenolite, an oxidation product of arsenic sulphides
 (white arsenic; As_2O_3). Arsenic trioxide exhibits high toxicity in mammals, such as humans. In
 some instances, arsenic trioxide ingestion can result in severe side effects, including vomiting,
 abdominal pain, diarrhea, bleeding, convulsions, cardiovascular disorders, inflammation of the
 15 liver and kidneys, abnormal blood coagulation, hair loss, and death. In certain instances,
 arsenic trioxide poisoning may rapidly lead to death. Chronic exposure to even low levels of
 arsenic trioxide can result in arsenicosis and skin cancer. Arsenic trioxide is therefore desirably
 administered to a subject at low enough doses to minimize toxicity. As described herein,
 arsenic trioxide and derivatives thereof may be effective at treating leukemia. In certain
 20 instances, administration of arsenic trioxide to a subject having leukemia may increase the
 production of reactive oxygen species in one or more leukemic cells of said subject. In certain
 instances, organic arsenic compounds are converted to inorganic compounds when absorbed in
 a biological system (see, e.g., Frith, *J. Military Vet. Health* 21(4): 11-17, 2013). Arsenic
 derivatives and uses thereof are described, for example, in Waxman et al. (*Oncologist* 6: 3-10,
 25 2001).

By the term “retinoic acid compound” is meant a compound that is either (a) a
 diterpene retinoic acid, or a derivative thereof, or (b) a compound having the structure $\text{R}^1\text{-Ar}^1\text{-}$
 $\text{L}^1\text{Ar}^2\text{-L}^2\text{-C(=O)R}^3$ (Formula I). Exemplary retinoic acid compounds described herein
 (including derivatives thereof) include, without limitation, all-trans retinoic acid (ATRA), 13-
 30 cis retinoic acid (13cRA), and retinoic acid compounds, and derivatives thereof, e.g., as
 described herein. Examples of retinoic acid compounds include those shown in Tables 1 and 2
 below.

Table 1. Exemplary Retinoic Acid Compounds

CID	IUPAC	Other names
444795	(2E,4E,6E,8E)-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid	Retinoic acid; tretinoin; Vitamin A acid
25145416	(2Z,4E,6Z,8Z)-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid	
23275881	(2Z,4Z,6E,8Z)-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid	
12358678	(2E,4E,6E,8Z)-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid	CHEMBL44478; CHEBI:168407; AC-540
10881132	(2Z,4Z,6E,8E)-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid	
10638113	(2E,4E,6E,8E)-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid	
9861147	(2E,4Z,6Z,8E)-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid	
9796370	(2E,4Z,6E,8E)-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid	11-tyr; (11Z)-retinoic acid; 11-cis-Retinoic acid
6603983	(2E,4Z,6E,8Z)-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid	Tocris-0695; Lopac-R-2625; Lopac-R-3255
6419708	(2Z,4E,6Z,8E)-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid	9,13-di-cis-RA; 9,13-Di-cis-retinoic acid; 9-cis,13-cis-Retinoic acid
5282379	(2Z,4E,6E,8E)-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid	Isotretinoin; 13-cis-Retinoic acid; Accutan
449171	(2E,4E,6Z,8E)-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid	Alitretinoin; Panretin; 9-CIS-RETINOIC ACID
5538	3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid	Spectrum_001676; SpecPlus_000696; AC1L1KKH
54305566	2,4-dideuterio-7-methyl-3-(trideuteriomethyl)-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid	
54305565	9-[3,3-dideuterio-6,6-dimethyl-2-(trideuteriomethyl)cyclohexen-1-yl]-3,7-dimethylnona-2,4,6,8-tetraenoic acid	
10566385	(2E,4E,6Z,8E)-7-methyl-3-(trideuteriomethyl)-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid	
10518761	(2E,4E,6Z,8E)-7-methyl-9-(2,6,6-trimethylcyclohexen-1-yl)-3-(tritritiomethyl)nona-2,4,6,8-tetraenoic acid	

10470200	(2E,4Z,6Z,8E)-4,5-dideuterio-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid	
10425032	(2E,4E,6Z,8E)-4,5-dideuterio-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid	
10357701	(2E,4E,6Z,8E)-3,7-dimethyl-9-(2,6,6-trimethyl-4,5-ditritiocyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid	
10267048	(2E,4E,6Z,8E)-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)-4,5-ditritionona-2,4,6,8-tetraenoic acid	
10086398	(2Z,4Z,6Z,8E)-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)-4,5-ditritionona-2,4,6,8-tetraenoic acid	
10086397	(2E,4E,6Z,8E)-3,7-dimethyl-9-(2,6,6-trimethyl-3,4-ditritiocyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid	
10063649	(2E,4E,6Z,8E)-9-[2,6-dimethyl-6-(trideuteriomethyl)cyclohexen-1-yl]-3,7-dimethylnona-2,4,6,8-tetraenoic acid	
10040620	(2E,4E,6Z,8E)-9-(4,5-dideuterio-2,6,6-trimethylcyclohexen-1-yl)-3,7-dimethylnona-2,4,6,8-tetraenoic acid	
10017935	(2Z,4E,6E,8E)-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)-4,5-ditritionona-2,4,6,8-tetraenoic acid	
10017822	(2E,4E,6Z,8E)-9-(3,4-dideuterio-2,6,6-trimethylcyclohexen-1-yl)-3,7-dimethylnona-2,4,6,8-tetraenoic acid	
9995220	(2E,4Z,6Z,8E)-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)-4,5-ditritionona-2,4,6,8-tetraenoic acid	
9972327	(2Z,4Z,6E,8E)-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)-4,5-ditritionona-2,4,6,8-tetraenoic acid	
9972326	(2E,4Z,6E,8E)-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)-4,5-ditritionona-2,4,6,8-tetraenoic acid	
9839397	(2E,4E,6Z,8E)-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)-5-trititionona-2,4,6,8-tetraenoic acid	
6913160	(2Z,4E,6Z,8E)-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)-5-trititionona-2,4,6,8-tetraenoic acid	Retinoic-11-t acid; AC1OC7MJ; all-trans-(11-3H)-Retinoic acid
6913136	(2Z,4E,6Z,8E)-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)-4,5-ditritionona-2,4,6,8-tetraenoic acid	AC1OC7KP; Retinoic-11,12-t2 acid; 11,12-3H-Retinoic acid
6913131	(2Z,4E,6Z,8E)-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)-5,6-ditritionona-2,4,6,8-tetraenoic acid	AC1OC7KA; Retinoic-10,11-t2 acid; all-trans-(10,11-3H2)-Retinoic acid
6439661	(2Z,4E,6Z,8E)-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid	
134262	3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid	SHGAZHPCJJPHS C-SPLUINJESA-N;

		FDEFF7D13961B7 66CC9FE8A74062 3243
56684147	(2E,6Z,8E)-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,6,8-trienoic acid	
54219808	3,6,7-trimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid	
53936974	3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,6,8-trienoic acid	
53740187	3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6-trienoic acid	
44725022	(Z)-3-[(E)-2-(2,6,6-trimethylcyclohexen-1-yl)ethenyl]hept-2-enoic acid	AC1Q2V68; (2Z)-3-[(E)-2-(2,6,6-trimethylcyclohex-1-en-1-yl)ethenyl]hept-2-enoic acid
21590819	(2Z,4E,8E)-3-methyl-7-methylidene-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,8-trienoic acid	CHEMBL182393
11738545	(2E,4E,6E,8E)-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)deca-2,4,6,8-tetraenoic acid	
10518336	(2E,4E,8E)-3-methyl-7-methylidene-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,8-trienoic acid	CHEMBL426963
10380944	(2E,4E,6Z,8E)-3-ethyl-7-methyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid	
10335106	(2E,4E,6E)-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6-trienoic acid	CHEMBL487208
10286439	(2E,4E,6Z,8E)-7-ethyl-3-methyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid	
10149682	(2E,4E,6Z,8E)-3,6,7-trimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid	
10041353	(2E,4E,6E,8E)-3-ethyl-7-methyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid	
6439749	(2E,4E,6E,8E)-9-(2-ethyl-6,6-dimethylcyclohexen-1-yl)-3,7-dimethylnona-2,4,6,8-tetraenoic acid	SRI 2712-24; 2,4,6,8-Nonatetracenoic acid,
5496917	(2E,4Z,6E)-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6-trienoic acid	AC1NUZ8L
5326825	(2Z,4Z,6E)-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6-trienoic acid	AC1NS159
4136524	3-[2-(2,6,6-trimethylcyclohexen-1-yl)ethenyl]hept-2-enoic acid	AC1N4YDA
135317	9-(2-ethyl-6,6-dimethylcyclohexen-1-yl)-3,7-dimethylnona-2,4,6,8-tetraenoic acid	
54525370	13-(2,6,6-trimethylcyclohexen-1-yl)trideca-2,4,6,8,10,12-	

	hexaenoic acid	
54472611	4,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid	
54398880	3-methyl-5-[2-[2-(2,6,6-trimethylcyclohexen-1-yl)ethenyl]cyclopenten-1-yl]penta-2,4-dienoic acid	
54044750	11-(2,6,6-trimethylcyclohexen-1-yl)undeca-2,4,6,8,10-pentaenoic acid	
53876852	3,7-dimethyl-9-(2,4,6,6-tetramethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid	
53790569	9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid	
53743104	5,9-dimethyl-11-(2,6,6-trimethylcyclohexen-1-yl)undeca-2,4,6,8,10-pentaenoic acid	
44579060	(2E,4E,6Z,8E)-9-(2-butyl-6,6-dimethylcyclohexen-1-yl)-3,7-dimethylnona-2,4,6,8-tetraenoic acid	CHEMBL518436
44393163	(2Z,4E,8E)-7-methylidene-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,8-trienoic acid	
25141345	(2E,4E,6E,8E)-9-(2-butyl-6,6-dimethylcyclohexen-1-yl)-3,7-dimethylnona-2,4,6,8-tetraenoic acid	
19609253	(2E,4E)-3-methyl-5-[2-[(E)-2-(2,6,6-trimethylcyclohexen-1-yl)ethenyl]cyclopenten-1-yl]penta-2,4-dienoic acid	
14731990	(2E,4E,6E,8E)-7-methyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid	
11141121	(2E,4E,6E,8E)-4,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid	
10712359	(2E,4E,6Z)-3-methyl-7-[(E)-2-(2,6,6-trimethylcyclohexen-1-yl)ethenyl]undeca-2,4,6-trienoic acid	
10474100	(2E,4E,6E,8E,10E,12E)-3,7,11-trimethyl-13-(2,6,6-trimethylcyclohexen-1-yl)trideca-2,4,6,8,10,12-hexanoic acid	
10426543	(E,4E)-3-methyl-4-[3-[(E)-2-(2,6,6-trimethylcyclohexen-1-yl)ethenyl]cyclohex-2-en-1-ylidene]but-2-enoic acid	
10358907	(Z,4E)-3-methyl-4-[(4E)-3-methyl-4-[(2,6,6-trimethylcyclohexen-1-yl)methylidene]cyclohexa-2,5-dien-1-yl)methylidene)	
10314319	(2E,4E,6E,8E,10E)-5,9-dimethyl-11-(2,6,6-trimethylcyclohexen-1-yl)undeca-2,4,6,8,10-pentaenoic acid	CHEMBL225948
10286753	(2E,4E,6Z,8E)-7-tert-butyl-3-methyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid	
10266931	(2E,4E,6Z)-3-methyl-7-[(E)-2-(2,6,6-trimethylcyclohexen-1-yl)ethenyl]deca-2,4,6-trienoic acid	CHEMBL507779
10125803	(2E,4E,6Z)-3-methyl-7-[(E)-2-(2,6,6-trimethylcyclohexen-1-yl)ethenyl]deca-2,4,6-trienoic acid	
10087786	(Z,4E)-3-methyl-4-[3-[(E)-2-(2,6,6-trimethylcyclohexen-1-yl)ethenyl]cyclohex-2-en-1-ylidene]but-2-enoic acid	
10015486	(2E,4E,6E)-5-methyl-7-(2,6,6-trimethylcyclohexen-1-yl)hepta-2,4,6-trienoic acid	

9929074	(2E,4E,6E,8E)-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid	
9860303	(2E,4E,6E,8E)-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid	
5355027	(2E,4E)-3-methyl-5-(2,6,6-trimethylcyclohexen-1-yl)penta-2,4-dienoic acid	C15 acid; AC1NS6O9; NSC23978
167095	3-methyl-5-(2,6,6-trimethylcyclohexen-1-yl)penta-2,4-dienoic acid	AC1L4ZB4
56606832	3,7-dimethyl-9-(9,9,11-trimethylspiro[2.5]oct-10-en-10-yl)nona-2,4,6,8-tetraenoic acid	
54548815	3,7,11,11-tetramethyldodeca-2,4-dienoic acid	
54515105	7-methyl-3-[2-(2,6,6-trimethylcyclohexen-1-yl)ethenyl]nona-2,5-dienoic acid	YLWKTERFWUX EBW- UHFFFAOYSA-N; 005B26AC36D10A 0C9DB5EF006864 943F
54358950	3-methyl-5-[2-[2-(2,6,6-trimethylcyclohexen-1-yl)ethenyl]cyclohepten-1-yl]penta-2,4-dienoic acid	
54353726	3,7,11,11-tetramethyltrideca-2,4-dienoic acid	
54193713	3-methyl-5-[2-[2-(2,6,6-trimethylcyclohexen-1-yl)ethenyl]cycloocten-1-yl]penta-2,4-dienoic acid	
53946778	2,3,7-trimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid	
53944823	9-(6,6-dimethylcyclohexen-1-yl)-3,7-dimethylnona-2,4,6,8-tetraenoic acid	JAIGDKSXLVOF MH- UHFFFAOYSA-N; F42136BEED6C5A 3745B9BA23356D 7830
53921377	3-methyl-5-[2-[2-(2,6,6-trimethylcyclohexen-1-yl)ethenyl]cyclohexen-1-yl]penta-2,4-dienoic acid	
44579100	(2E,4E,6Z,8E)-9-[6,6-dimethyl-2-(2-methylpropyl)cyclohexen-1-yl]-3,7-dimethylnona-2,4,6,8-tetraenoic acid	CHEMBL476773
44579056	(2E,4E,6E,8E)-9-[6,6-dimethyl-2-(2-methylpropyl)cyclohexen-1-yl]-3,7-dimethylnona-2,4,6,8-tetraenoic acid	CHEMBL476348
44314230	(2Z,5E)-7-methyl-3-[(E)-2-(2,6,6-trimethylcyclohexen-1-yl)ethenyl]nona-2,5-dienoic acid	CHEMBL75548; CHEBI:220121
25011742	(2E,8E)-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,8-dienoic acid	
22646220	(2E,4E,6E,8E)-2,3-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid	
20830941	(2E,4E,6E,8E)-2,3-dimethyl-9-(2,6,6-trimethylcyclohexen-1-	

	yl)nona-2,4,6,8-tetraenoic acid	
19609240	(2E,4E)-3-methyl-5-[(1Z)-2-[(E)-2-(2,6,6-trimethylcyclohexen-1-yl)ethenyl]cycloocten-1-yl]penta-2,4-dienoic acid	
18977383	(2E,4E,6E,8E)-3,7-dimethyl-9-(2,5,6,6-tetramethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid	
15125883	(2Z,4E,6E,8E)-2,3,7-trimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid	
15125882	(2E,4E,6E,8E)-2,3,7-trimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid	CHEMBL153895; 14-methyl-all-trans-retinoic acid; LMPR01090034
11266097	(2Z,4E,8E)-3-methyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,8-trien-6-ynoic acid	
11000660	(2E,4E,6Z,8E)-9-(6,6-dimethylcyclohexen-1-yl)-3,7-dimethylnona-2,4,6,8-tetraenoic acid	
10733921	(2E,4E,6Z)-7-(8,8-dimethyl-4,5,6,7-tetrahydro-3H-naphthalen-2-yl)-3-methylocta-2,4,6-trienoic acid	
10636975	(2E,4E,6E,8E)-9-(6,6-dimethylcyclohexen-1-yl)-3,7-dimethylnona-2,4,6,8-tetraenoic acid	
10591236	(2E,4E,6Z)-7-(4a,8-dimethyl-4,5,6,7-tetrahydro-3H-naphthalen-2-yl)-3-methylocta-2,4,6-trienoic acid	
10404132	(Z,4E)-3-methyl-4-[(4E)-3-methyl-4-[(2,6,6-trimethylcyclohexen-1-yl)methylidene]cyclohex-2-en-1-ylidene]but-2-enoic acid	
10314318	(E,4E)-3-methyl-4-[(4E)-3-methyl-4-[(2,6,6-trimethylcyclohexen-1-yl)methylidene]cyclohex-2-en-1-ylidene]but-2-enoic acid	
10215224	(2E,4E,6Z,8E)-3-methyl-7-propan-2-yl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid	
10193246	(2E,4E)-3-methyl-6-[1-[(E)-2-(2,6,6-trimethylcyclohexen-1-yl)ethenyl]cyclopropyl]hexa-2,4-dienoic acid	
9841547	(2E,4E)-3-methyl-5-[2-[(E)-2-(2,6,6-trimethylcyclohexen-1-yl)ethenyl]cyclohepten-1-yl]penta-2,4-dienoic acid	
9830767	(2Z,4E,6Z,8E)-9-(6,6-dimethylcyclohexen-1-yl)-3,7-dimethylnona-2,4,6,8-tetraenoic acid	
9819335	(2E,4E)-3-methyl-5-[2-[(E)-2-(2,6,6-trimethylcyclohexen-1-yl)ethenyl]cyclohexen-1-yl]penta-2,4-dienoic acid	Ro 25-6603; 173792-73-9
56667667	(2E,4E,6Z,8E)-3,7-dimethyl-9-(6-methyl-3-prop-1-en-2-ylcyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid	CHEMBL455993; CHEMBL455994
54758572	(2Z,4E,6Z,8E)-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoate	9-cis-Retinoate; CPD-13549
54426679	2,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid	
54325149	6-chloro-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid	
53702687	6-iodo-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid	

29986894	(2E,4Z,6E,8E)-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoate	ZINC22066351
29927144	(2E,4E,6E,8Z)-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoate	ZINC21992287
24916820	(2E,4E,6E)-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6-trienoate	2g78
24771817	3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoate	CHEBI:15036
21917290	(2E,4E,6E,8E)-9-(5-tert-butyl-2,6,6-trimethylcyclohexen-1-yl)-3,7-dimethylnona-2,4,6,8-tetraenoic acid	
19609245	(2E,4E,6E,8E)-6-chloro-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid	
19609224	(2E,4E,6E,8E)-6-iodo-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid	
10924150	(2E,4E,6Z,8E)-9-(2,6-dimethylcyclohexen-1-yl)-3,7-dimethylnona-2,4,6,8-tetraenoic acid	
10613228	(2E,4E,6E,8E)-9-(2,6-dimethylcyclohexen-1-yl)-3,7-dimethylnona-2,4,6,8-tetraenoic acid	
10469989	(2E,6Z,8E)-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,6,8-trien-4-ynoic acid	
10334998	(2E,4E)-3-methyl-5-[2-[(E)-2-(2,6,6-trimethylcyclohexen-1-yl)ethenyl]cyclopropyl]penta-2,4-dienoic acid	
9904356	(2Z,4E,6Z)-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6-trien-8-ynoic acid	
7364357	(2E,4E,6Z,8E)-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoate	AC1OKKW8; ZINC12661824;
7048538	(2Z,4E,6E,8E)-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoate	13-cis-retinoate; ZINC03792789
6440565	2E,4E,6E)-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6-trien-8-ynoic acid	7,8-Dehydroretinoic acid; 7,8-Didehydroretinoic acid
6419707	(2E,4E,6E,8E)-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoate	Retinoate; all-trans-Retinoate; Tretinoine
5771658	(Z)-3-(2,6,6-trimethylcyclohexen-1-yl)prop-2-enoic acid	NSC-202789; AC1NY9IQ; NCGC00014560
5383969	(E)-3-(2,6,6-trimethylcyclohexen-1-yl)prop-2-enoic acid	NSC202789; NSC-20278
5353358	(2Z,4E)-3-methyl-6-(2,7,7-trimethyl-3-methylidene-1,4,5,6-tetrahydroinden-2-yl)hexa-2,4-dienoic acid	AC1NS43Q
5289278	(2E,4E)-3-methyl-6-[(2R)-2,7,7-trimethyl-3-methylidene-1,4,5,6-tetrahydroinden-2-yl]hexa-2,4-dienoic acid	NSC202789; 3-(2,6,6-trimethyl-1-cyclohexen-1-

		yl)acrylic acid; AC1L77HZ
305742	3-(2,6,6-trimethylcyclohexen-1-yl)prop-2-enoic acid	NSC202789; 3-(2,6,6-trimethyl-1-cyclohexen-1-yl)acrylic acid; AC1L77HZ
1851	3-methyl-6-(2,7,7-trimethyl-3-methylidene-1,4,5,6-tetrahydroinden-2-yl)hexa-2,4-dienoic acid	AC1L1CDO
54399542	6-bromo-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid	
54233476	3,7-dimethyl-5-oxo-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,6,8-trienoic acid	
54033110	2,5,9-trimethyl-11-(2,6,6-trimethylcyclohexen-1-yl)undeca-2,4,6,8,10-pentaenoic acid	
53936708	3-methyl-5-[2-[2-(2,6,6-trimethylcyclohexen-1-yl)ethynyl]cyclopenten-1-yl]penta-2,4-dienoic acid	
44314320	(2Z,4E)-3-methyl-5-[2-[(E)-2-(3,3,6,6-tetramethylcyclohexen-1-yl)ethenyl]cyclopropyl]penta-2,4-dienoic acid	CHEMBL73973; CHEBI:220303
44314319	(2E,4E)-3-methyl-5-[2-[(E)-2-(3,3,6,6-tetramethylcyclohexen-1-yl)ethenyl]cyclopropyl]penta-2,4-dienoic acid	CHEMBL74331; CHEBI:220301
22373193	(2E,4E)-3-methyl-5-[2-[2-(2,6,6-trimethylcyclohexen-1-yl)ethynyl]cyclopenten-1-yl]penta-2,4-dienoic acid	
21145248	(2Z,4E,6E,8E)-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid	
20151571	(2E,4E,6E,8E)-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid	
19609231	(2E,4E,6E,8E)-6-bromo-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid	
16727824	(2E,4E,6Z,8E)-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid	All-trans-Retinoic acid & 9-cis-Retinoic Acid
11015604	(2E,4E,6E,8E,10E,12E,14E,16E)-2,6,11,15-tetramethyl-17-(2,6,6-trimethylcyclohexen-1-yl)-3-tritioheptadeca-2,4,6,8-trimethylcyclohexen-1-yl)-3-tritioheptadeca-2,4,6,8,10,12,14,16-octaenoic acid	
10406618	(2E,4Z,6E,8E,10E,12E)-2,7,11-trimethyl-13-(2,6,6-trimethylcyclohexen-1-yl)trideca-2,4,6,8,10,12-hexanoic acid	
9976193	(2E,4E,6E,8E,10E,12E)-2,7,11-trimethyl-13-(2,6,6-trimethylcyclohexen-1-yl)trideca-2,4,6,8,10,12-hexanoic acid	
9843074	(2E,4E,6E)-3-methyl-7-(4,4,7,7-tetramethyl-2-pentyl-1,3,5,6-tetrahydroinden-2-yl)hepta-2,4,6-trienoic acid	

6439881	(2Z,4E,6Z,8E)-9-(3,3-difluoro-2,6,6-trimethylcyclohexen-1-yl)-3,7-dimethylnona-2,4,6,8-tetraenoic acid	DFRA; 4,4-Difluororetinoic acid; AC1O5SM
6436320	(2E,4E,6Z,8E,10E,12E,14E,16E)-2,6,11,15-tetramethyl-17-(2,6,6-trimethylcyclohexen-1-yl)heptadeca-2,4,6,8,10,12,14,16-octaenoic acid	AC1O5LFK; beta-apo-8'-Carotenoic acid; 8'-Apo-beta,psi-carotenoic acid
5387557	(2Z)-2-[5-(2,6,6-trimethylcyclohexen-1-yl)-3-[(E)-2-(2,6,6-trimethylcyclohexen-1-yl)ethenyl]cyclohexanoic acid	NSC624510; AC1INTSHG; AC1Q5T6Y
5366642	(2E,4E,6E,8E)-9-(3,3-difluoro-2,6,6-trimethylcyclohexen-1-yl)-3,7-dimethylnona-2,4,6,8-tetraenoic acid	4,4-Difluororetinoic acid; AC1NSNWF; 4,4-Difluororetinoic acid (all-trans)
361473	2-[5-(2,6,6-trimethylcyclohexen-1-yl)-3-[2-(2,6,6-trimethylcyclohexen-1-yl)ethenyl]cyclohex-2-en-1-yl]heptadeca-2,4,6,8,10,12,14,16-octaenoic acid	AC1L7IQC; NCI60_007432; 2-[5-(2,6,6-trimethylcyclohexen-1-yl)-3-[2-(2,6,6-trimethylcyclohexen-1-yl)ethenyl]cyclohex-2-en-1-ylidene]acetic acid
146218	9-(3,3-difluoro-2,6,6-trimethylcyclohexen-1-yl)-3,7-dimethylnona-2,4,6,8-tetraenoic acid	
56660872	(2E,4E,6Z,8E)-3,7-dimethyl-9-(2-methyl-5-prop-1-en-2-ylcyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid	CHEMBL457645; CHEMBL513434
54587023	(2E,4E,6Z,8E)-3,7-dimethyl-9-[(3S,6R)-3-methyl-6-prop-1-en-2-ylcyclohexen-1-yl]nona-2,4,6,8-tetraenoic acid	CHEMBL1773351
54586043	(2E,4E,6Z)-3-methyl-7-[(3R,6S)-3-methyl-6-propan-2-ylcyclohexen-1-yl]octa-2,4,6-trienoic acid	CHEMBL1773361
54310202	7-ethyl-3,11-dimethyltrideca-2,4-dienoic acid	
54177995	8-(3-ethyl-2-propan-2-ylcyclohex-2-en-1-ylidene)-3,7-dimethylocta-2,4,6-trienoic acid	OZUIXDDSOLQK NK-UHFFFAOYSA-N; 982DADEA9DC55 79A132BDF2AD7 FA647A
54012267	3,8,12-trimethyltrideca-2,4-dienoic acid	
53787191	3,8,13-trimethyltetradeca-2,4-dienoic acid	
53743194	4-methyl-6-(2,6,6-trimethylcyclohexen-1-yl)hex-2-enoic acid	
53710521	3,7,13-trimethyltetradeca-2,4-dienoic acid	
53707670	3,7-dimethyl-8-(3-methyl-2-propan-2-ylcyclohex-2-en-1-ylidene)octa-2,4,6-trienoic acid	BYHSFJNWVLBC

		IM- UHFFFAOYSA-N; 14B10A34153F37 A66327788679FA C42F
53666154	3,7,11-trimethyltrideca-2,4-dienoic acid	
53438161	3,7,11-trimethyltetradeca-2,4-dienoic acid	
53427754	7,7-dimethylcosa-2,4-dienoic acid	
52952998	(2E,4E,6Z,8E)-3,7-dimethyl-9-[(3R,6S)-3-methyl-6-prop-1-en-2-ylcyclohexen-1-yl]nona-2,4,6,8-tetraenoic acid	CHEMBL1773352
44631433	(2Z,4E)-3-methyl-5-(2,2,4-trimethylcyclohex-3-en-1-yl)penta-2,4-dienoic acid	FZFFLFPGBIXCK I-STRRHFTISA-
44291210	(2Z,4Z,6Z,8E)-8-(3-ethyl-2-propan-2-ylcyclohex-2-en-1-ylidene)-3,7-dimethylocta-2,4,6-trienoic acid	CHEMBL43954
44290946	(2E,4Z,6Z,8E)-8-(3-ethyl-2-propan-2-ylcyclohex-2-en-1-ylidene)-3,7-dimethylocta-2,4,6-trienoic acid	CHEMBL43833; CHEBI:167938
24845989	sodium (2Z,4E,6Z,8E)-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoate	LS-143475
23670222	potassium (2E,4E,6E,8E)-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoate	
23665641	sodium (2E,4E,6E,8E)-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoate	Sodium retinoate; Retinoic acid, sodium salt; Vitamin A acid sodium sal
23265304	(2E,4E)-3-methyl-5-(2,2,4-trimethylcyclohex-3-en-1-yl)penta-2,4-dienoic acid	
21437585	(2E,4E)-3,8,12-trimethyltrideca-2,4-dienoic acid	
21437539	(2E,4E)-3,8,13-trimethyltetradeca-2,4-dienoic acid	
21437504	(2E,4E)-3,7,13-trimethyltetradeca-2,4-dienoic acid	
21158960	(2E,4E)-7,7-dimethylcosa-2,4-dienoic acid	
20270951	(6E,8E)-2,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,3,6,8-tetraenoic acid	
19609232	(2E,4E)-3-methyl-5-[2-[(E)-2-(2,6,6-trimethylcyclohexen-1-yl)ethenyl]cyclohexen-1-yl]penta-2,4-dienoic acid	
11130378	(2E,4E,6E,8E)-3,7-dimethyl-9-(2,6,6-trimethylcyclohex-2-en-1-yl)nona-2,4,6,8-tetraenoic acid	
11066537	(2Z,4E,6E,8E)-3,7-dimethyl-9-(2,6,6-trimethylcyclohex-2-en-1-yl)nona-2,4,6,8-tetraenoic acid	
10470917	(2Z,4E,6Z,8E)-8-(3-ethyl-2-propan-2-ylcyclohex-2-en-1-ylidene)-3,7-dimethylocta-2,4,6-trienoic acid	
10402558	(2Z,4E,6E,8E)-3,7-dimethyl-8-(3-methyl-2-propan-2-ylcyclohex-2-en-1-ylidene)octa-2,4,6-trienoic acid	
10357464	(2E,4E,6Z,8E)-3,7-dimethyl-8-(3-methyl-2-propan-2-ylcyclohex-2-en-1-ylidene)octa-2,4,6-trienoic acid	
10086191	(2E,4E,6E,8E)-3,7-dimethyl-8-(3-methyl-2-propan-2-	CHEMBL333032;

	ylcyclohex-2-en-1-ylidene)octa-2,4,6-trienoic acid	CHEBI:299410
10086189	(2Z,4E,6Z,8E)-3,7-dimethyl-8-(3-methyl-2-propan-2-ylcyclohex-2-en-1-ylidene)octa-2,4,6-trienoic acid	
9972952	(2Z,4E,6E,8E)-8-(3-ethyl-2-propan-2-ylcyclohex-2-en-1-ylidene)-3,7-dimethylocta-2,4,6-trienoic acid	CHEMBL44582; CHEBI:168408
9972949	(2E,4E,6Z,8E)-8-(3-ethyl-2-propan-2-ylcyclohex-2-en-1-ylidene)-3,7-dimethylocta-2,4,6-trienoic acid	
9883342	(2E,4E,6E,8E)-8-(3-ethyl-2-propan-2-ylcyclohex-2-en-1-ylidene)-3,7-dimethylocta-2,4,6-trienoic acid	CHEMBL46398; CHEBI:168441
5372326	(E)-3-methyl-5-(2,6,6-trimethylcyclohexen-1-yl)pent-2-enoic acid	AC1NSY3I; 2-Pentenoic acid, 3-methyl-5-(2,6,6-trimethyl-1-cyclohexenyl); (E)-3-methyl-5-(2,6,6-trimethylcyclohexen-1-yl)pent-2-enoic acid
445560	(2E,4E,6Z,8E)-3,7-dimethyl-9-(2,6,6-trimethylcyclohex-2-en-1-yl)nona-2,4,6,8-tetraenoic acid	AC1L9I79
56667221	(2E,4E,6Z,8E)-3,7-dimethyl-9-(3-methyl-6-propan-2-ylcyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid	CHEMBL508378
54585066	(2E,4E,6Z,8E)-3,7-dimethyl-9-[(1S,4R,5R)-4,6,6-trimethyl-3-bicyclo[3.1.1]hept-2-enyl]nona-2,4,6,8-tetraenoic acid	CHEMBL1773358
54585064	(2E,4E,6Z,8E)-3,7-dimethyl-9-[(3R)-3-methyl-6-propan-2-ylidenecyclohexen-1-yl]nona-2,4,6,8-tetraenoic acid	CHEMBL1773355
54582176	(2E,4E,6Z,8E)-3,7-dimethyl-9-[(3S)-3-methyl-6-propan-2-ylidenecyclohexen-1-yl]nona-2,4,6,8-tetraenoic acid	CHEMBL1773354
54581148	(2E,4E,6Z,8E)-3,7-dimethyl-9-[(1R,2R,5S)-2-methyl-5-propan-2-yl-3-bicyclo[3.1.0]hex-3-enyl]nona-2,4,6,8-tetraenoic acid	CHEMBL1773360
54542310	3,4,4-trimethyltetradec-2-enoic acid	
54521054	3,4,4-trimethyloctadec-2-enoic acid	
54518673	3,7-dimethyl-9-(2,6,6-trimethyl-5-oxocyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid	
54348687	3,7,10,11-tetramethyldodeca-2,4-dienoic acid	
54325421	3,4,4-trimethylheptadec-2-enoic acid	
54316493	3,4,4-trimethylpentadec-2-enoic acid	
54305044	2-ethyl-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid	
54265680	3,7,11,15-tetramethylhexadeca-2,4-dienoic acid	
54194359	3,7-dimethyl-9-(2,6,6-trimethyl-4-oxocyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid	
54170467	3,7,11,15-tetramethylhexadeca-2,4,6,14-tetraenoic acid	
54167172	3,4,4-trimethylhexadec-2-enoic acid	
54105865	3,7,7,11,11-pentamethyldodec-2-enoic acid	

54064253	2-ethyl-5,9-dimethyl-3-(2,6,6-trimethylcyclohexen-1-yl)undeca-2,4,6,8,10-pentaenoic acid	
53961371	3,7,11-trimethyldodeca-2,4,11-trienoic acid	
53936602	9-[5-(2-cyclohexylethyl)-2,6,6-trimethylcyclohexen-1-yl]-3,7-dimethylnona-2,4,6,8-tetraenoic acid	
53825233	3,7,11,15,19-pentamethylcosa-2,4,6,10,18-pentaenoic acid	
53801569	3-methyl-5-[2-[2-(2,6,6-trimethylcyclohexen-1-yl)ethynyl]cyclohepten-1-yl]penta-2,4-dienoic acid	
53725805	3,7-dimethyldodeca-2,4-dienoic acid	
53700416	3,7,11,15-tetramethylhexadeca-2,4,6-trienoic acid	
52953080	(2E,4E,6Z,8E)-3,7-dimethyl-9-[(3S,6R)-3-methyl-6-propan-2-ylcyclohexen-1-yl]nona-2,4,6,8-tetraenoic acid	CHEMBL1773353
52952997	(2E,4E,6Z,8E)-3,7-dimethyl-9-[(1R,4S,5S)-4,6,6-trimethyl-3-bicyclo[3.1.1]hept-2-enyl]nona-2,4,6,8-tetraenoic acid	CHEMBL1773357
52921782	(2E,5R,10E,12E)-3,5,15-trimethyl-7-methylidenehexadeca-2,10,12-trienoic acid	LMFA01020367; 16:3(2E,10E,12E)(3 Me,5Me[R],7My,15Me)
46178652	(2E,4E)-5-[(1R)-2,2-dimethyl-6-methylidenecyclohexyl]-3-methylpenta-2,4-dienoic acid	
44579059	(2E,4E,6Z,8E)-3,7-dimethyl-9-(2,2,6-trimethylcyclohexyl)nona-2,4,6,8-tetraenoic acid	CHEMBL451158
25147656	(2E,4E,6Z,8E)-3,7-dimethyl-9-[(3R,6S)-3-methyl-6-propan-2-ylcyclohexen-1-yl]nona-2,4,6,8-tetraenoic acid	CHEMBL508378
22168242	(2E,4E,6E,10E)-3,7,11,15,19-pentamethylcosa-2,4,6,10,18-pentaenoic acid	
22168239	(2E,4E,6E)-3,7,11,15-tetramethylhexadeca-2,4,6-trienoic acid	
22168234	(2E,4E,6E)-3,7,11,15-tetramethylhexadeca-2,4,6,14-tetraenoic acid	
21764469	(2E,4E)-3-methyl-5-[(1R)-2,6,6-trimethylcyclohex-2-en-1-yl]penta-2,4-dienoic acid	
21650797	acetyl (2E,4E,6E,8E)-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraeneperoxoate	
21525820	(2E,4E)-7,11,11-trimethyldodeca-2,4-dienoic acid	
21525806	(2E,4E)-3,7-dimethyldodeca-2,4-dienoic acid	
21291068	(E)-3,4,4-trimethylhexadec-2-enoic acid	
21291063	(E)-3,4,4-trimethyltetradec-2-enoic acid	
21291060	(E)-3,4,4-trimethylpentadec-2-enoic acid	
21291047	(E)-3,4,4-trimethylheptadec-2-enoic acid	
21291045	(E)-3,4,4-trimethyloctadec-2-enoic acid	
20830940	(2E,4E,6Z,8E)-3,7-dimethyl-9-(2,5,6,6-tetramethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoate	
20306860	(2E,4E)-3,7,11-trimethyldodeca-2,4,11-trienoic acid	
20027300	azanium (2E,4E,6E,8E)-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoate	

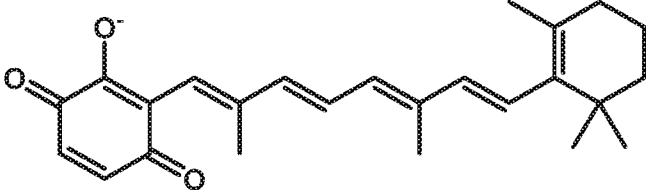
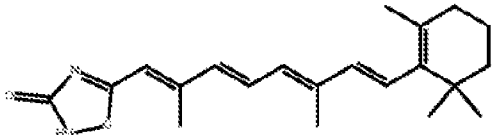
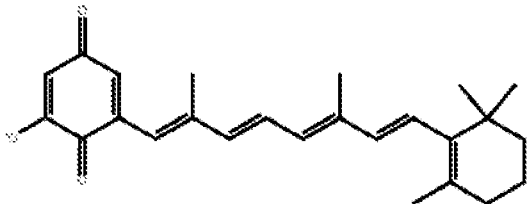
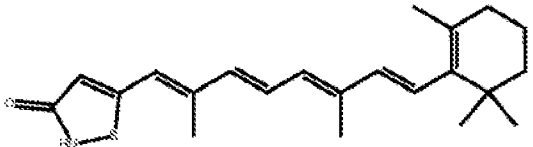
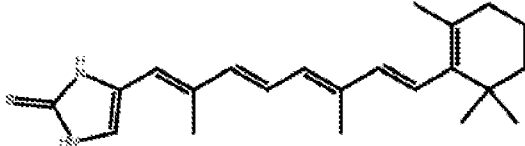
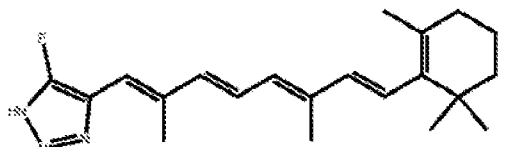
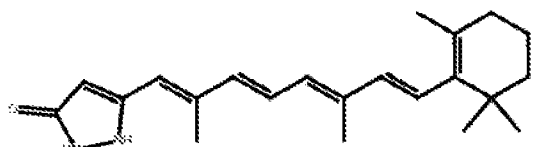
19609235	(2E,4E)-2-iodo-3-methyl-5-(2,6,6-trimethylcyclohexen-1-yl)penta-2,4-dienoic acid	
19606927	(2E,4E,6E,8E)-3,7-dimethyl-9-(2,6,6-trimethyl-4-oxocyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid	
18977382	(2E,4E,6E,8E)-3,7-dimethyl-9-(2,5,6,6-tetramethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoate	
16061319	(2Z,4E,6Z,8E)-7-(hydroxymethyl)-3-methyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid	19-Hydroxy-13-cis-retinoic acid; LMPR01090029
16061318	(2E,4E,6Z,8E)-7-(hydroxymethyl)-3-methyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid	19-Hydroxy-all-trans-retinoic acid; LMPR01090028
15125888	(2E,4E,6E,8E)-2-ethyl-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid	CHEMBL154239
11747707	(2E,4E,6Z,8E)-3,7-dimethyl-9-(6-methylcyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid	
11602784	(2E,4E)-3-methyl-5-[2-[2-(2,6,6-trimethylcyclohexen-1-yl)ethynyl]cyclohepten-1-yl]penta-2,4-dienoic acid	
10516342	(2E,4E,6E,8E)-3,7-dimethyl-9-(6-methylcyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid	
10354668	(Z,4E)-4-(3-ethyl-2-propan-2-ylcyclohex-2-en-1-ylidene)-3-methylbut-2-enoic acid	
10053647	(2Z,4Z,6E,8E,10E,12E,14E,16E,18E,20E,22E,24E)-2,6,10,14,19,23-hexamethyl-25-(2,6,6-trimethylcyclohexen-1-yl)pentacos-2,4,6,8,10,12,14,16,18,20,22,24-dodecaenoic acid	
9995780	(2Z,4E,6E,8E)-3,7-dimethyl-9-(2,6,6-trimethyl-5-oxocyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid	Oxo-13-cis-retinoate; 4-keto-13-cis-retinoate
9949957	(2E,4E,6E,8E)-3,7-dimethyl-8-[3-(2-methylpropyl)-2-propan-2-ylcyclohex-2-en-1-ylidene]octa-2,4,6-trienoic acid	
9948768	(2E,4E,6Z,8E)-3,7-dimethyl-9-(2,6,6-trimethyl-5-oxocyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid	
9829386	(2E,4Z,6E,8E,10E,12E,14E,16E,18E,20E,22E,24E)-2,6,10,14,19,23-hexamethyl-25-(2,6,6-trimethylcyclohexen-1-yl)pentacos-2,4,6,8,10,12,14,16,18,20,22,24-dodecaenoic acid	
6477090	(2Z,4Z,6Z,8E,10Z,12Z,14E,16Z,18Z,20E,22Z,24E)-2,6,10,14,19,23-hexamethyl-25-(2,6,6-trimethylcyclohexen-1-yl)pentacos-2,4,6,8,10,12,14,16,18,20,22,24-dodecaenoic acid	AC1053P5; 3',4'-Didehydro-,psi.-caroten-16'-oic acid
6439734	(2Z,4E,6Z,8E)-3,7-dimethyl-9-(2,2,6-trimethylcyclohexyl)nona-2,4,6,8-tetraenoic acid	7,8-Dihydroretinoic acid
6437018	(2Z,4E)-3,7,11-trimethyldodeca-2,4-dienoic acid	AC105MUO; EINECS 258-354-9
6437016	(2E,4E)-3,7,11-trimethyldodeca-2,4-dienoic acid	AC105MUI;

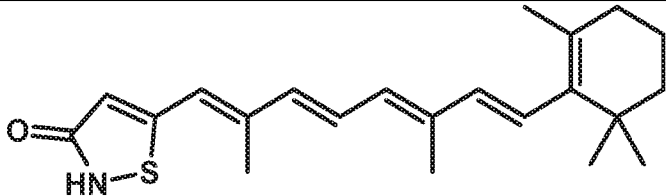
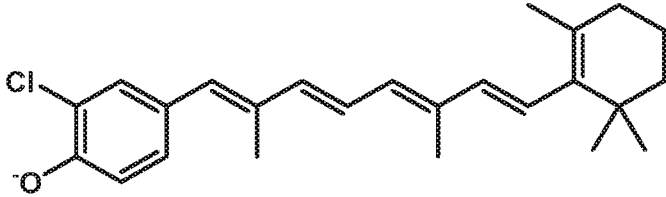
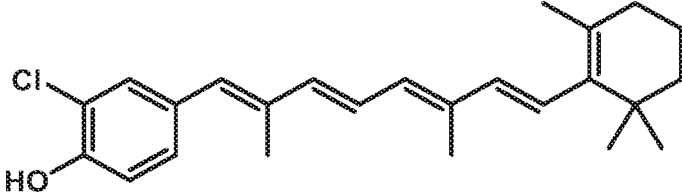
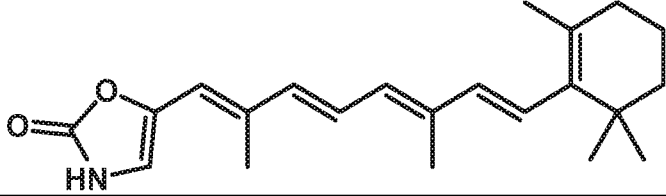
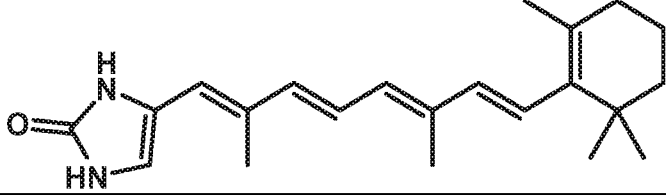
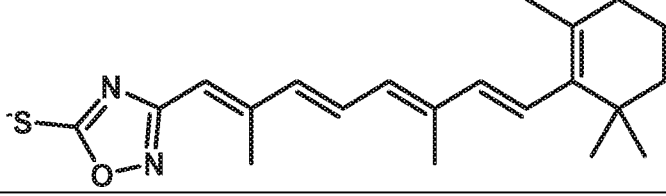
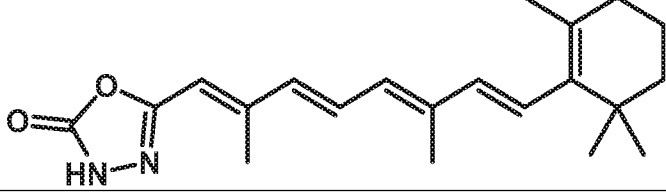
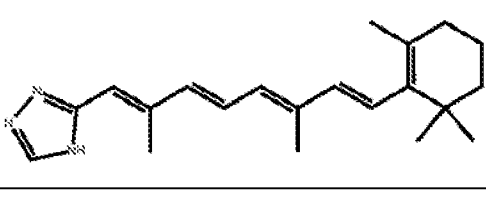
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5476505	(2E,4E)-3-methyl-5-(2,6,6-trimethylcyclohex-2-en-1-yl)penta-2,4-dienoic acid	AC1O5MUI; CHEMBL37590
5460164	(2E,4E,6E,8E)-3,7-dimethyl-9-(2,2,6-trimethylcyclohexyl)nona-2,4,6,8-tetraenoic acid	Retinyl ester; all-trans-Retinyl ester
5281248	(2E,4E,6E,8E,10E,12E,14E,16E,18E,20E,22E,24E)-2,6,10,14,19,23-hexamethyl-25-(2,6,6-trimethylcyclohexanoic acid	NSC635690; Torularhodin; AC1NQY9
637039	2E,4E,6E,8E,10E,12E,14E,16E,18E,20E)-2,6,10,15,19-pentamethyl-21-(2,6,6-trimethylcyclohexen-1-yl)hexanoic acid	Neurosporaxanthin; all-trans-Neurosporaxanthin
428485	3-methyl-5-(2,6,6-trimethylcyclohex-2-en-1-yl)penta-2,4-dienoic acid	AC1L8LML; 3-methyl-5-(2,6,6-trimethylcyclohex-2-en-1-yl)penta-2,4-dienoic acid
103723	3,7,11-trimethyldodeca-2,4-dienoic acid	
94165	2,6,10,14,19,23-hexamethyl-25-(2,6,6-trimethylcyclohexen-1-yl)pentacos-2,4,6,8,10,12,14,16,18,20,22, 24-dodecanenoic acid	AC1L3RN8; NCI60_011910
56661049	(2E,4E,6Z,8E)-3,7-dimethyl-9-(4,4,6,6-tetramethyl-2-bicyclo[3.1.1]hept-2-enyl)nona-2,4,6,8-tetraenoic acid	CHEMBL455992
54581147	(2E,4E,6Z,8E)-9-[(1S,5R)-6,6-dimethyl-4-bicyclo[3.1.1]hept-3-enyl]-3,7-dimethylnona-2,4,6,8-tetraenoic acid	CHEMBL1773359
54478024	3,4,4-trimethylnon-2-enoic acid	
54476971	3,4,4-trimethylundec-2-enoic acid	
54287870	3-formyl-7-methyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid	RVKZSGIKOAA JJ-UHFFFAOYSA-N; 293564D2B64FAC 5F524A1B691CBF 7C6B
54116397	3,7-dimethyl-2-propan-2-yl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid	NKQIYDSGIYJXS A-UHFFFAOYSA-N; 5597749F477D668 D55E163C44DA1F 3EB
54073647	3,4,4-trimethyldec-2-enoic acid	
53995964	3-methyl-5-[2-[2-(2,6,6-trimethylcyclohexen-1-yl)ethenyl]cyclohexyl]penta-2,4-dienoic acid	
53919798	3,4,4-trimethyldodec-2-enoic acid	
53889922	3,7-dimethyl-9-(2,4,4,6,6-pentamethyl-3-oxocyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid	
53887460	4-(hydroxymethyl)-3,7-dimethyl-9-(2,6,6-	

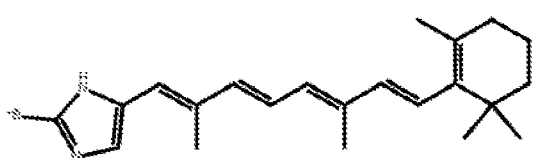
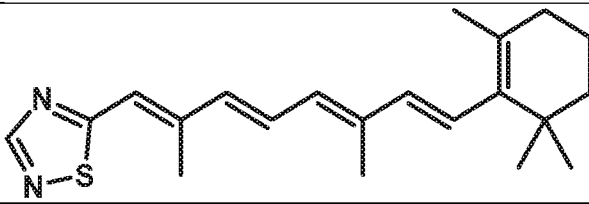
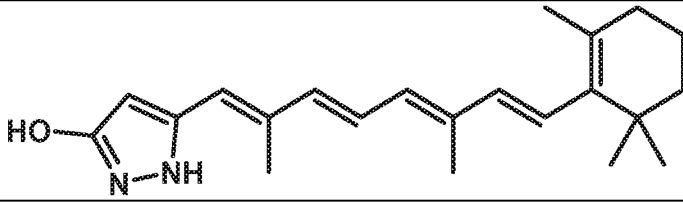
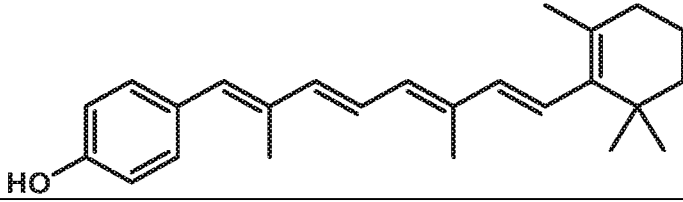
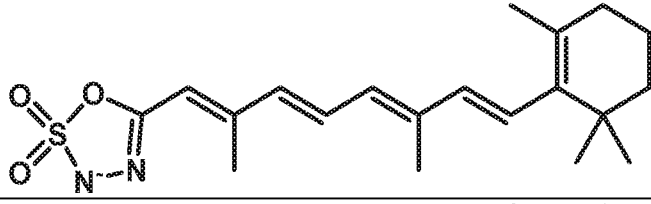
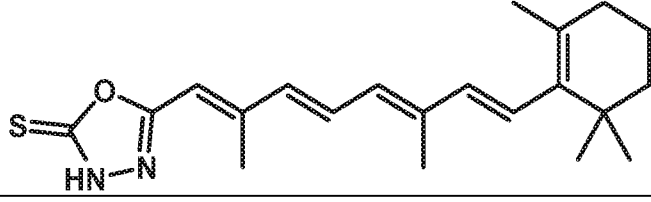
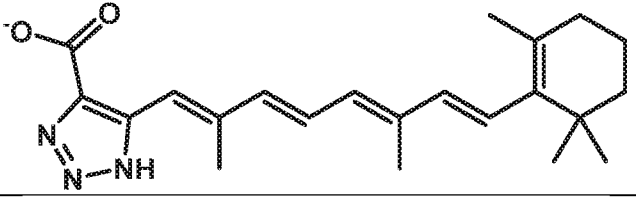
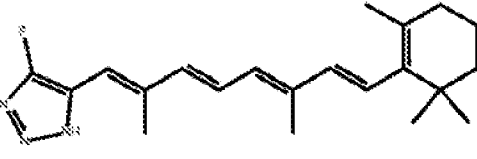
	trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid	
53854796	3-methyl-6-(3,3,7,7-tetramethyl-3a,4,5,6-tetrahydroinden-2-ylidene)hexa-2,4-dienoic acid	
53754609	2-ethyl-5,9-dimethyl-11-(2,6,6-trimethylcyclohexen-1-yl)undeca-2,4,6,8,10-pentaenoic acid	
50925583	(2E,4E,6E,8E)-9-[(1R,2R,4aS,8aR)-1,6-dimethyl-2-propyl-4a,5,8,8a-tetrahydro-2H-naphthalen-1-yl]-8-methylnona-2,4,6,8-tetraenoic acid	
45039634	(2E,4E,6E,8E)-9-[6,6-dimethyl-3-oxo-2-(trideuteriomethyl)cyclohexen-1-yl]-3,7-dimethylnona-2,4,6,8-tetraenoic acid	
21291081	(E)-3,4,4-trimethyldec-2-enoic acid	
21291044	(E)-3,4,4-trimethyldodec-2-enoic acid	
21291042	(E)-3,4,4-trimethylnon-2-enoic acid	
21291032	(E)-3,4,4-trimethylundec-2-enoic acid	
19384872	(E)-4-[(2E,4E,6E,8E)-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoyl]oxy-4-oxobut-2-enoic acid	
16061321	(2Z,4E,6Z,8E)-7-formyl-3-methyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid	19-Oxo-9-cis-retinoic acid; LMPR01090031
16061320	(2E,4E,6Z,8E)-7-formyl-3-methyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid	19-Oxo-all-trans-retinoic acid; LMPR01090030
15125894	(2E,4E,6E,8E)-3,7-dimethyl-2-propan-2-yl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid	CHEMBL153894
10043037	(2E,4E,6E,8E)-3,7-dimethyl-9-(2,4,4,6,6-pentamethyl-3-oxocyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid	CHEMBL103068
9972939	(2E,4E,6Z,8E)-3,7-dimethyl-9-(2,6,6-trimethyl-3-oxocyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid	
9906064	(2E,4E)-3-methyl-5-[(1R)-2-[(E)-2-(2,6,6-trimethylcyclohexen-1-yl)ethenyl]cyclohexyl]penta-2,4-dienoic acid	
9902057	(2Z,4E,6Z,8E)-4-(hydroxymethyl)-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid	
6437087	(2Z,4E,6E,8E)-3,7-dimethyl-9-(2,6,6-trimethyl-3-oxocyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid	Oxoretinoic acid; 4-Oxo-isotretinoin
6437063	(2E,4E,6E,8E)-3,7-dimethyl-9-(2,6,6-trimethyl-3-oxocyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid	4-Oxoretinoic acid; 4-Ketoretinoic acid
447276	(2E,4E,6E,8E)-3,7-dimethyl-9-(2,6,6-trimethylcyclohexa-1,3-dien-1-yl)nona-2,4,6,8-tetraenoic acid	Vitamin A2 acid; 3,4-Didehydroretinoic acid
104857	3,7-dimethyl-9-(2,6,6-trimethyl-3-oxocyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid	

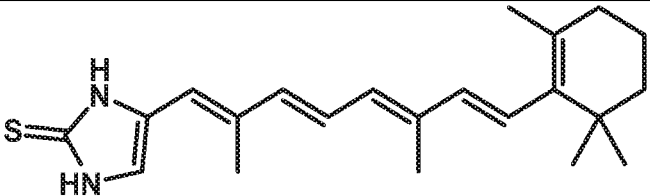
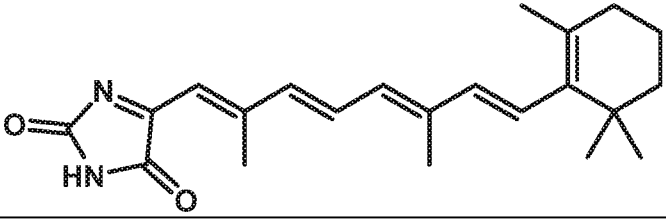
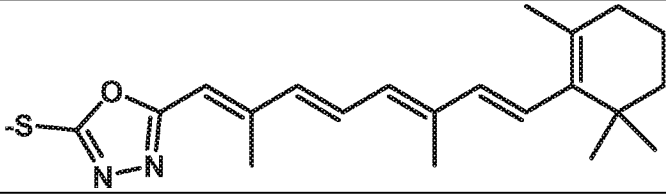
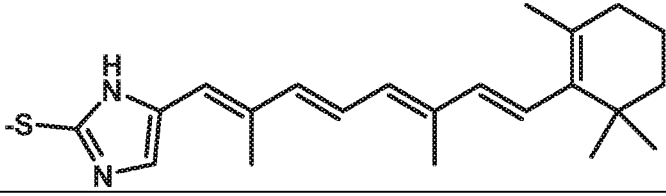
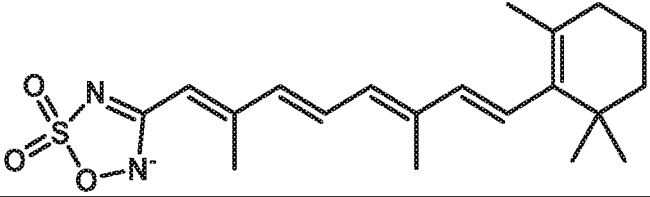
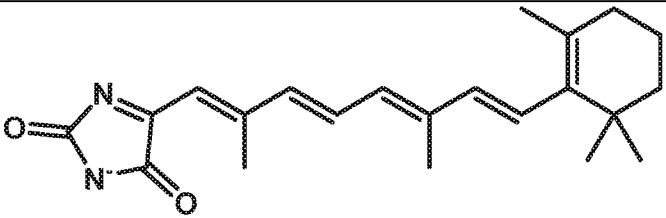
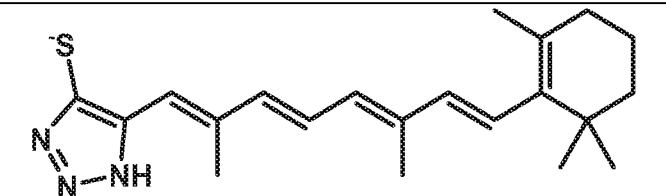
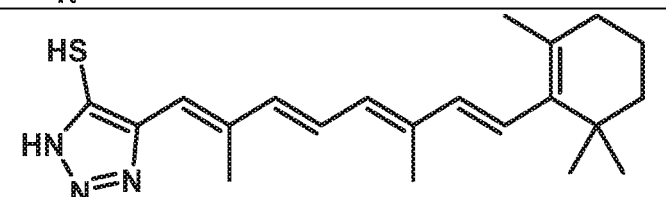
Pubchem Compound Identifiers (CIDs) in Table 1 refer to the compound identification number for pubchem.ncbi.nlm.nih.gov

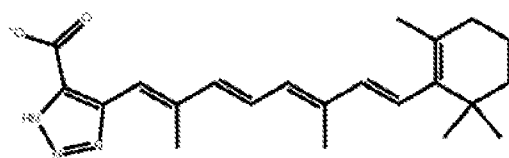
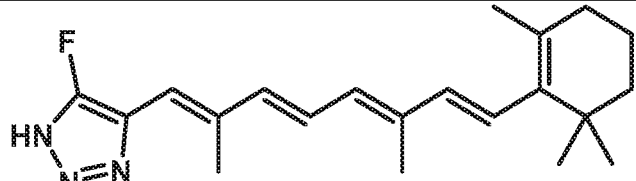
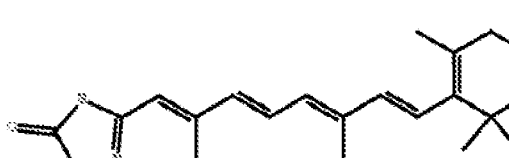
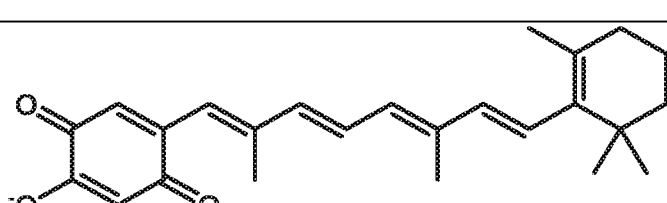
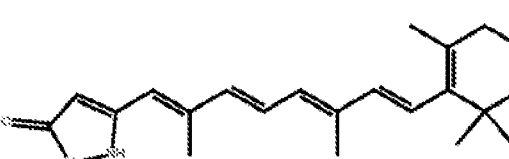
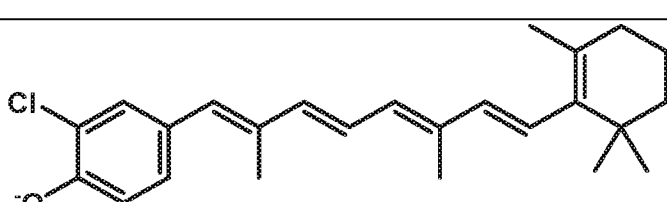
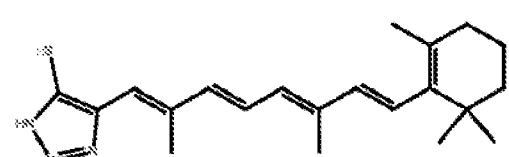
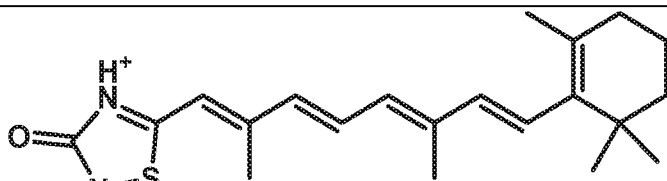
Table 2. Additional retinoic acid compounds

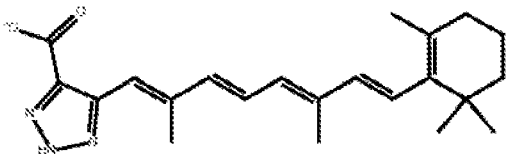
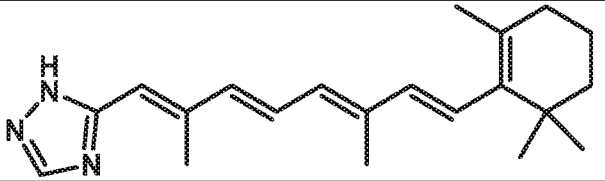
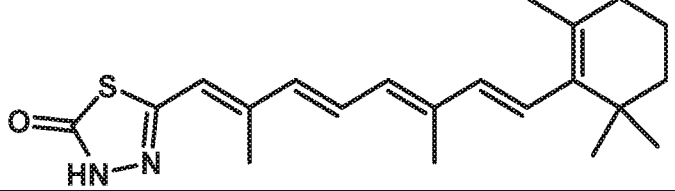
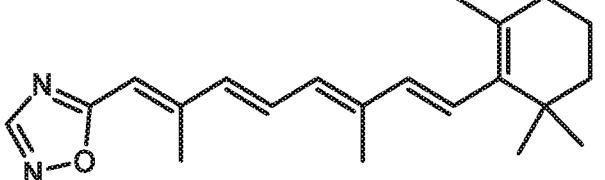
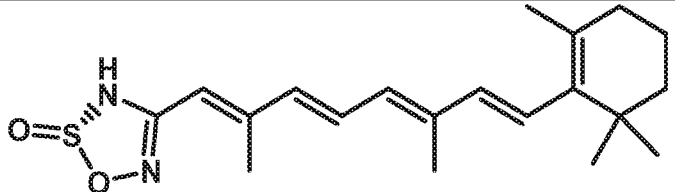
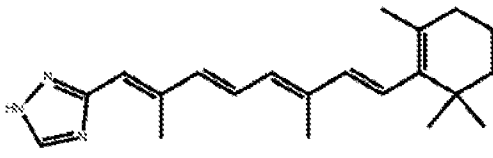
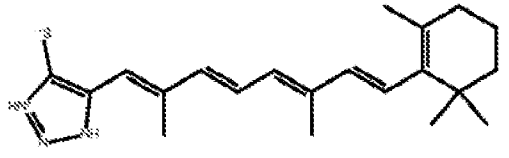
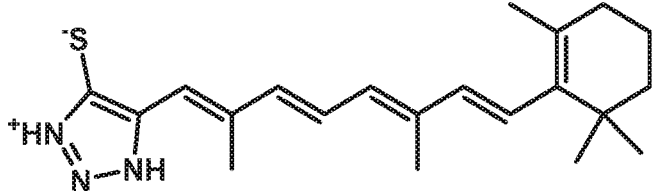
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5		-40.365
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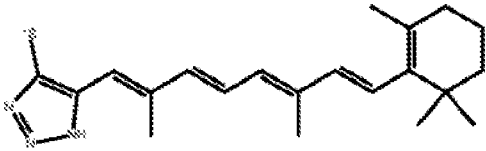
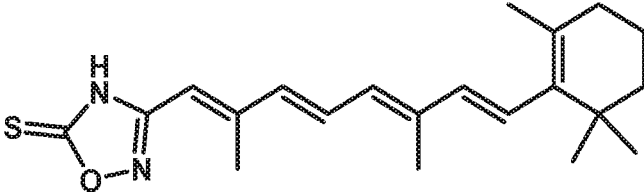
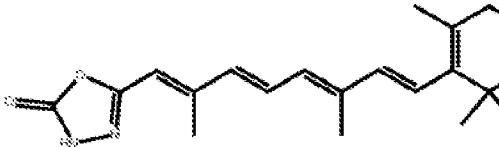
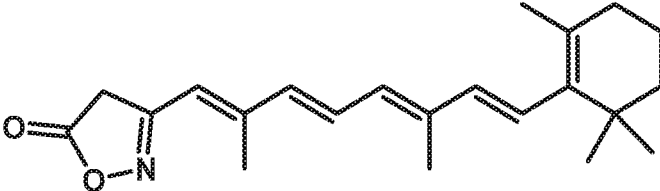
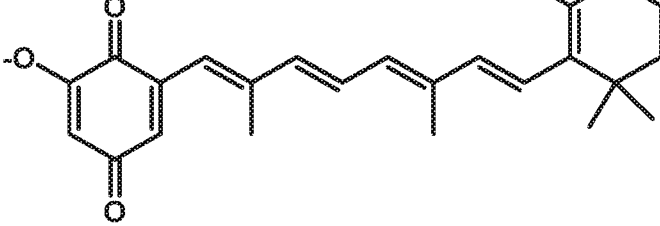
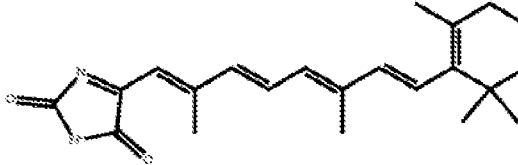
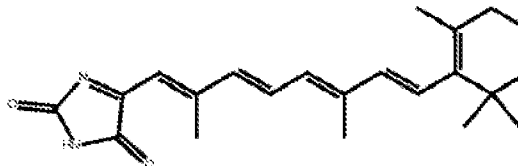
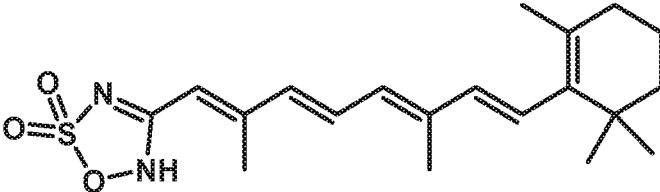
8		-39.417
9		-39.232
10		-39.050
11		-38.984
12		-38.958
13		-38.818
14		-38.817
15		-38.742

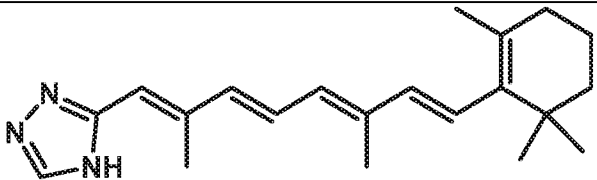
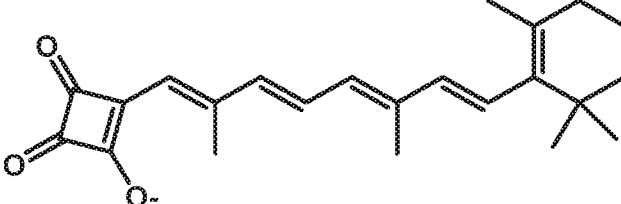
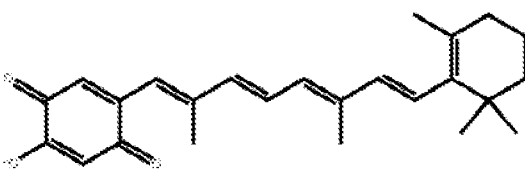
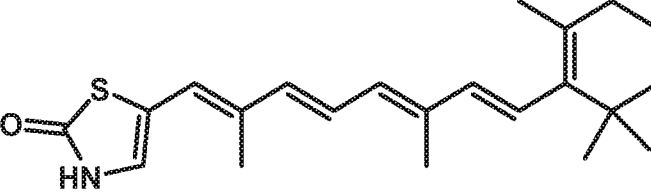
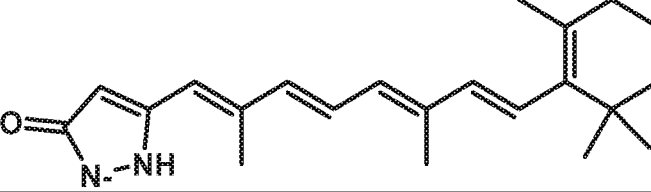
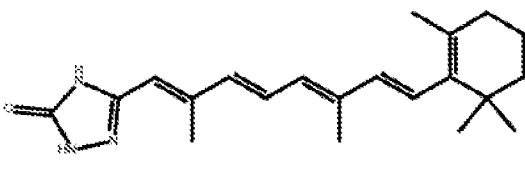
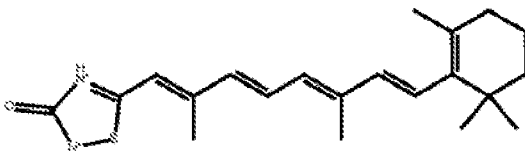
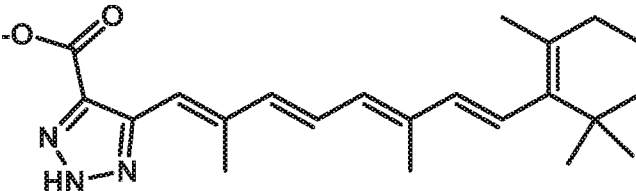
16		-38.627
17		-38.309
18		-38.247
19		-38.124
20		-37.847
21		-37.846
22		-37.804
23		-37.628

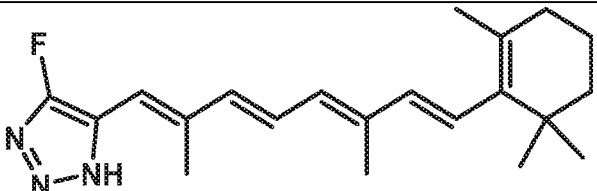
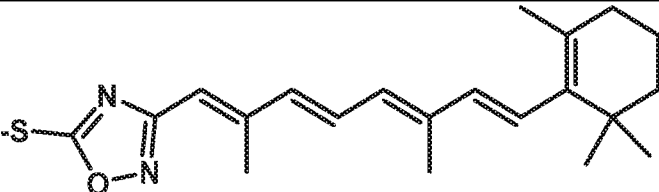
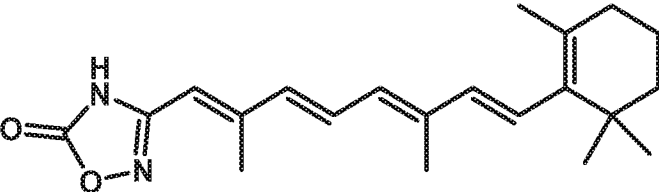
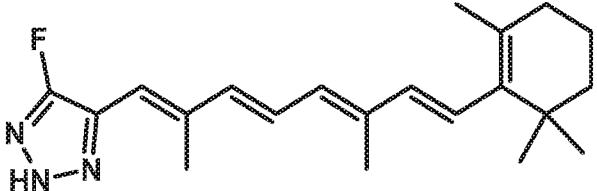
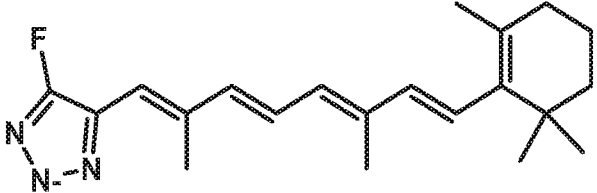
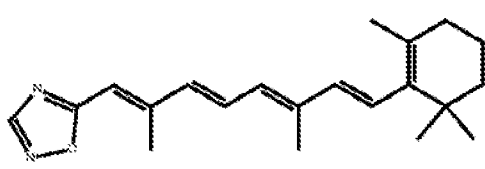
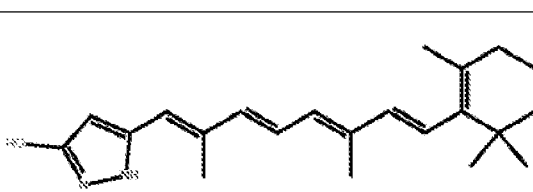
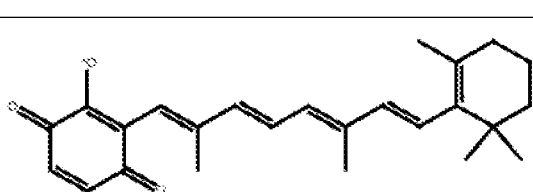
24		-37.601
25		-37.585
26		-37.568
27		-37.558
28		-37.542
29		-37.485
30		-37.460
31		-37.390

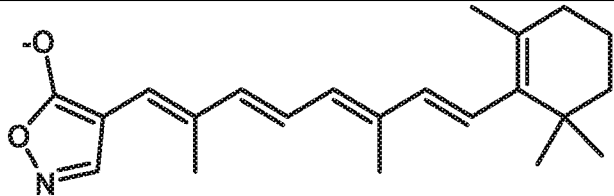
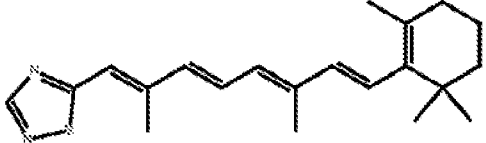
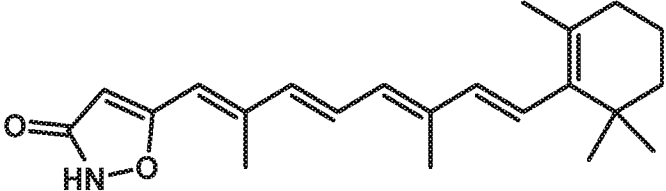
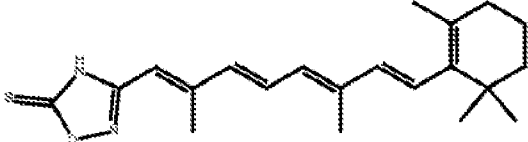
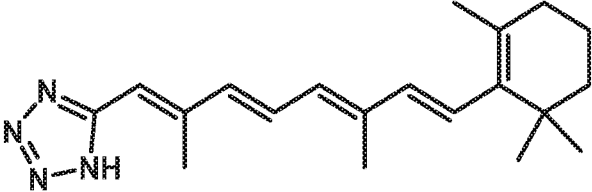
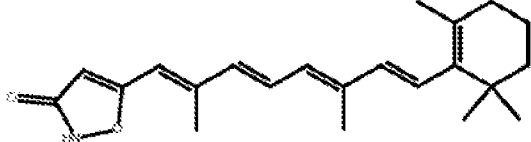
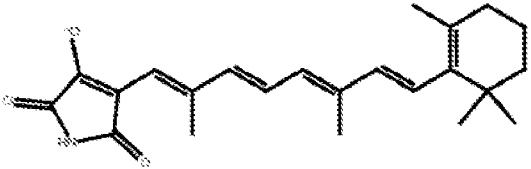
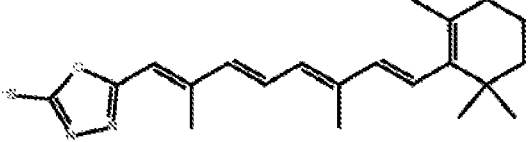
32		-37.361
33		-37.135
34		-36.909
35		-36.848
36		-36.903
37		-36.761
38		-36.718
39		-36.588

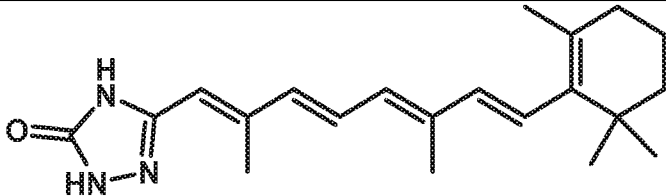
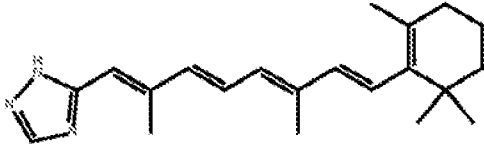
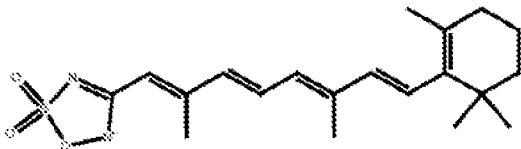
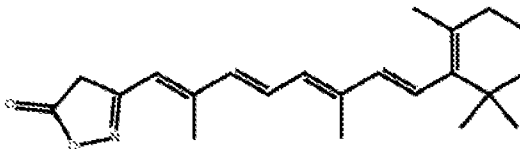
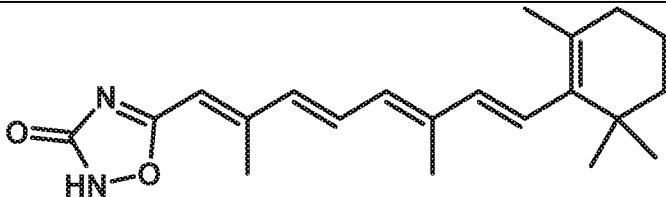
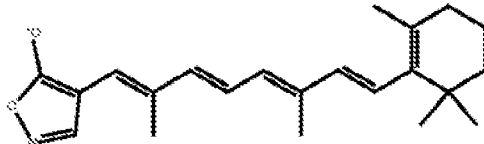
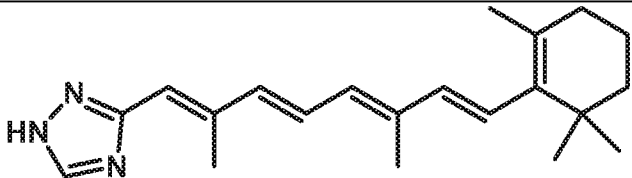
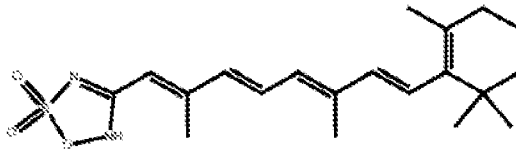
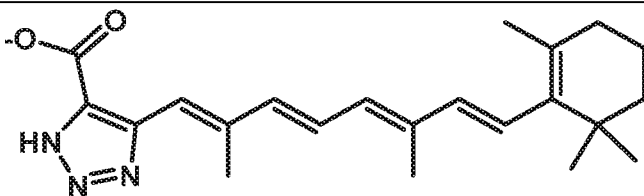
40		-36.555
41		-36.527
42		-36.516
43		-36.418
44		-36.392
45		-36.388
46		-36.384
47		-36.256

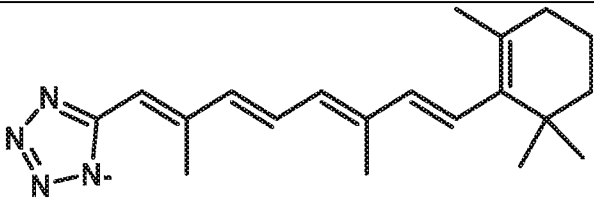
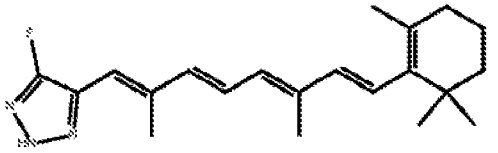
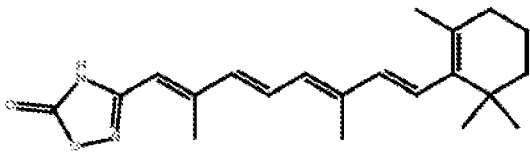
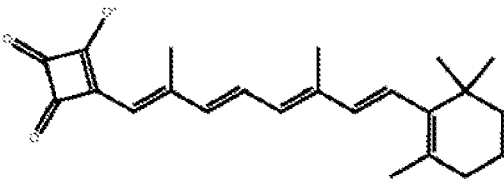
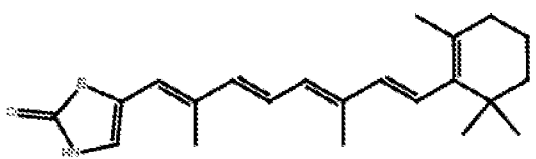
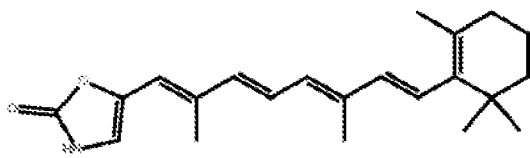
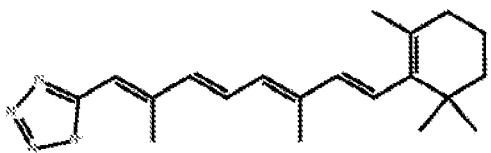
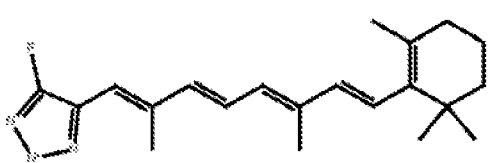
48		-36.247
49		-36.061
50		-35.965
51		-35.875
52		-35.849
53		-35.784
54		-35.682
55		-35.677

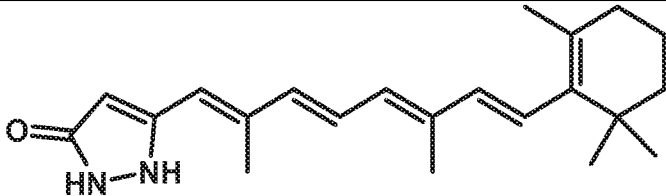
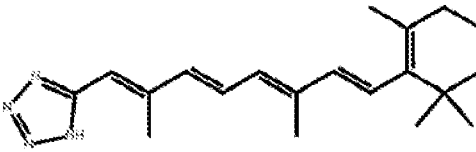
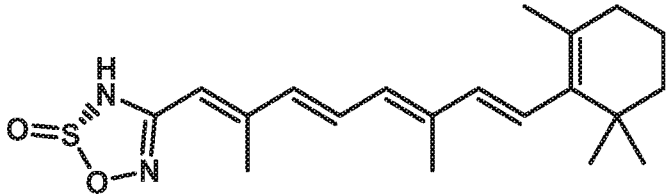
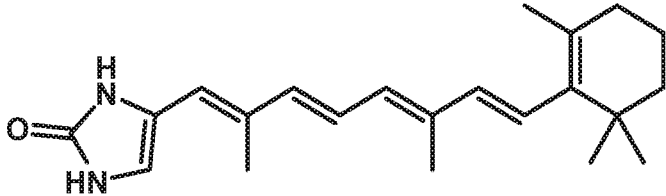
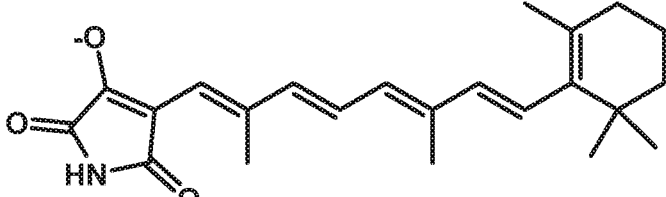
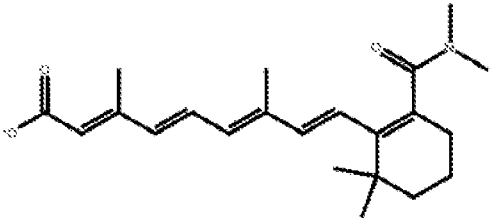
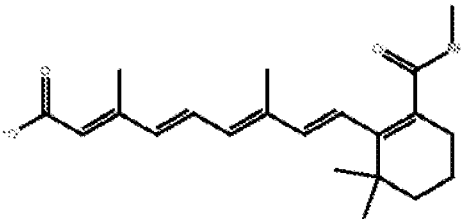
56		-35.622
57		-35.513
58		-35.493
59		-35.321
60		-35.277
61		-35.303
62		-35.186
63		-35.164

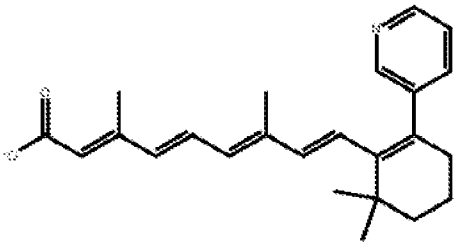
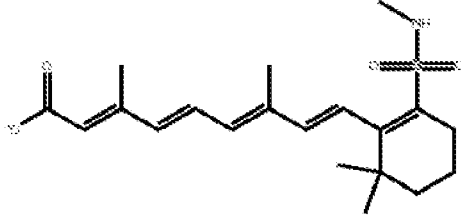
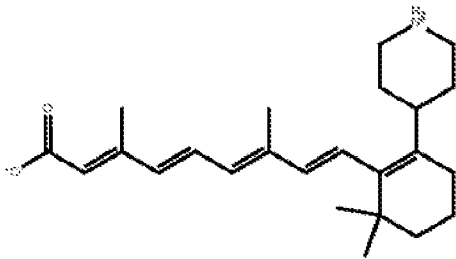
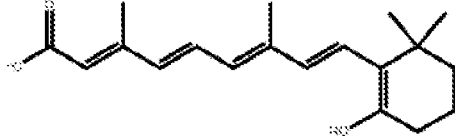
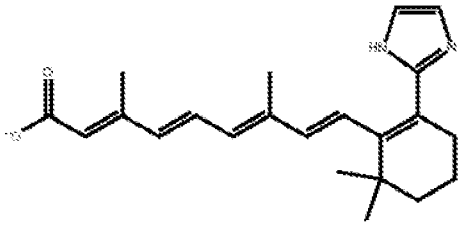
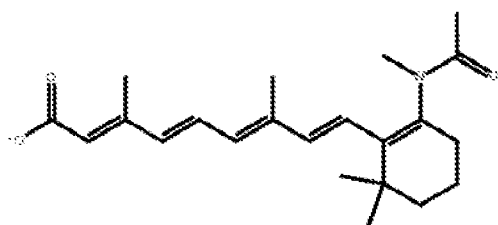
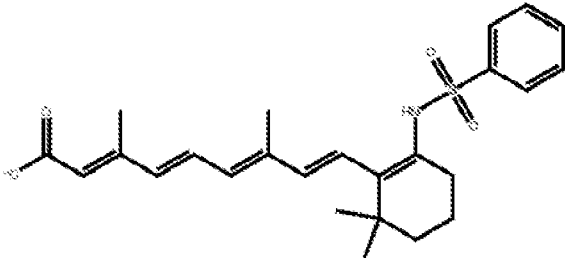
64		-35.152
65		-35.142
66		-34.986
67		-34.949
68		-34.940
69		-34.843
70		-34.823
71		-34.762

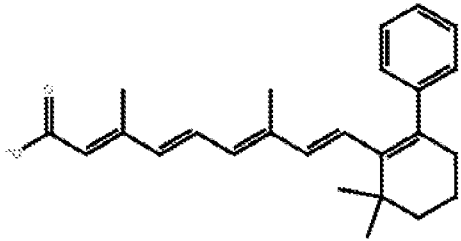
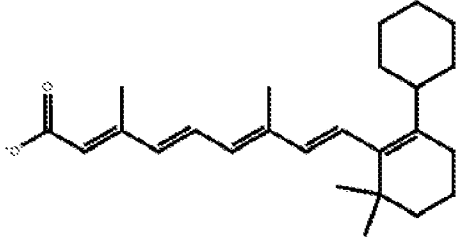
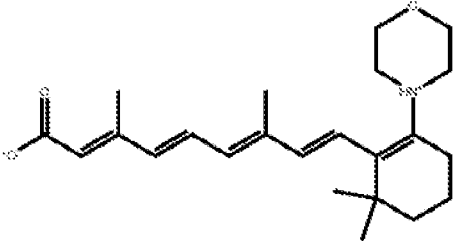
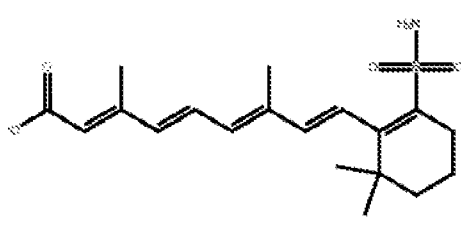
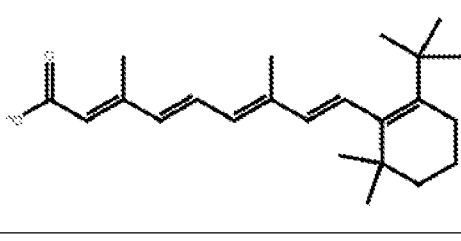
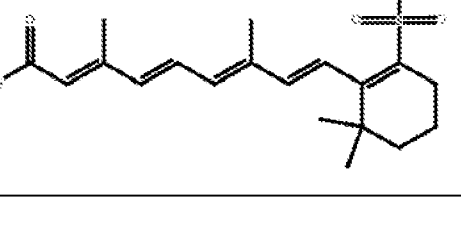
72		-34.648
73		-34.522
74		-34.465
75		-34.303
76		-34.217
77		-34.173
78		-34.134
79		-34.042

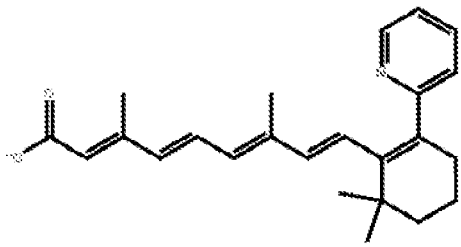
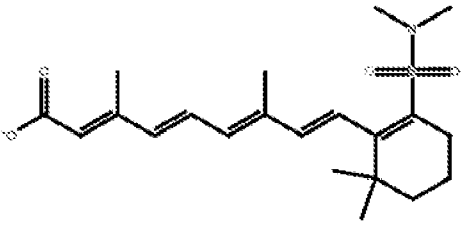
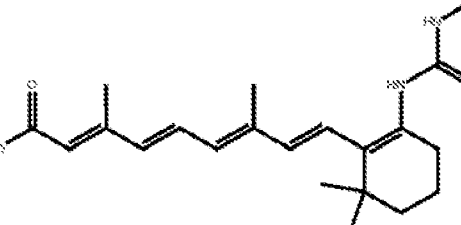
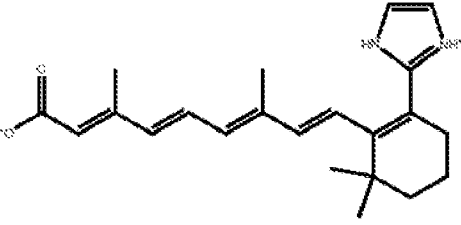
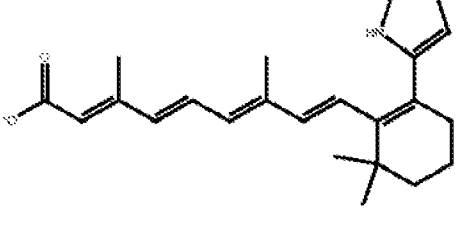
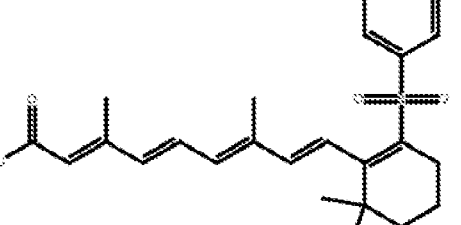
80		-34.010
81		-33.982
82		-33.971
83		-33.948
84		-33.573
85		-33.498
86		-32.867
87		-32.431
88		-32.106

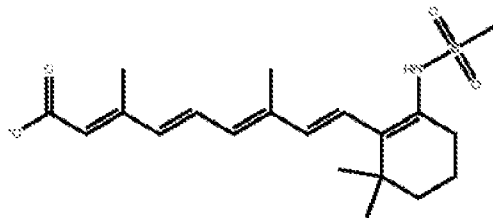
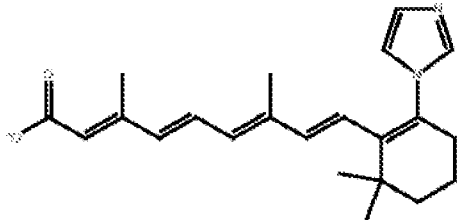
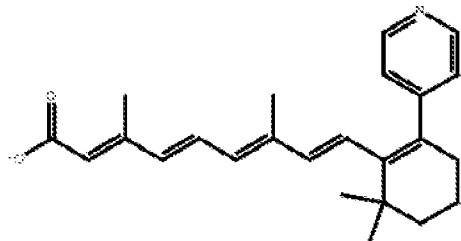
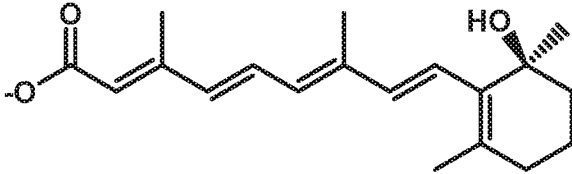
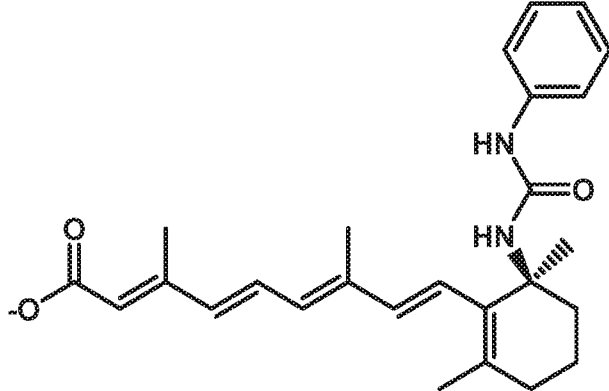
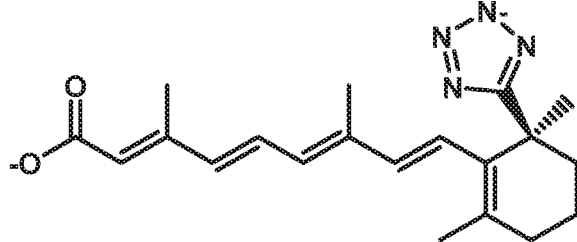
89		-32.103
90		-31.917
91		-31.769
92		-31.700
93		-31.628
94		-31.591
95		-31.533
96		-31.228

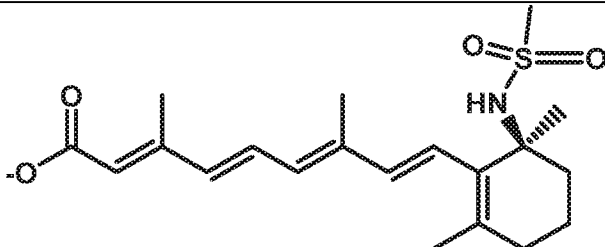
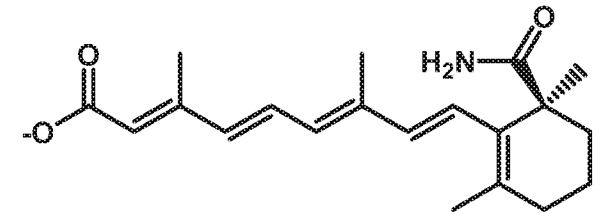
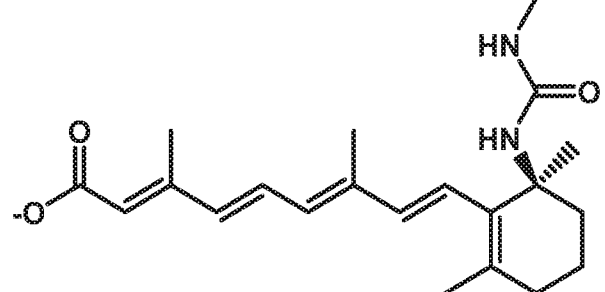
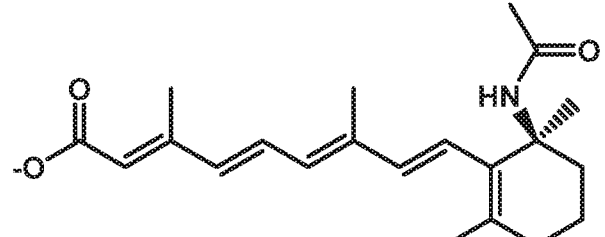
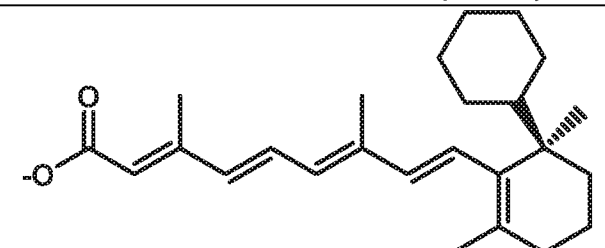
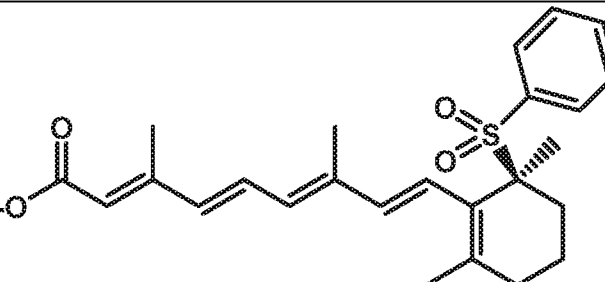
97		-30.921
98		-30.851
99		-30.640
100		-30.511
101		-30.252
102		-41.258
103		-40.318

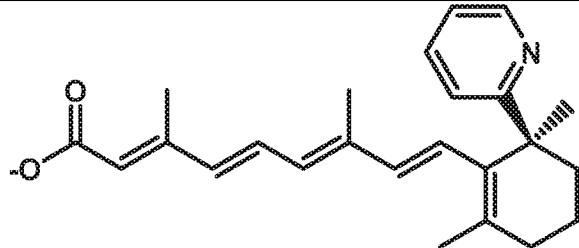
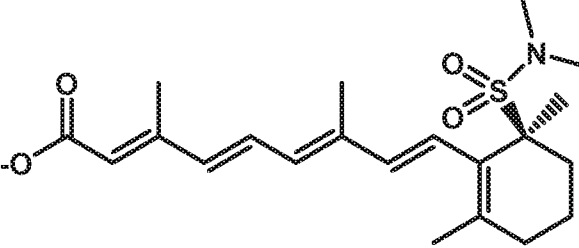
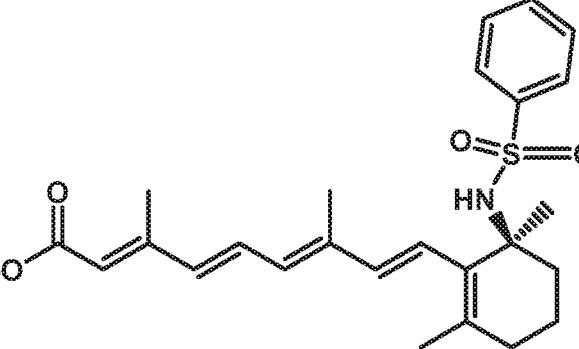
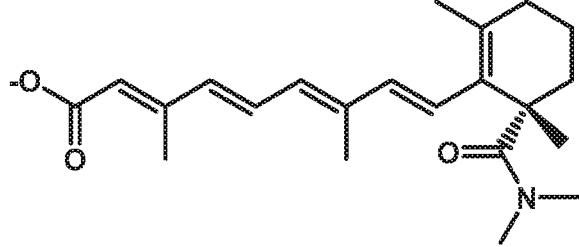
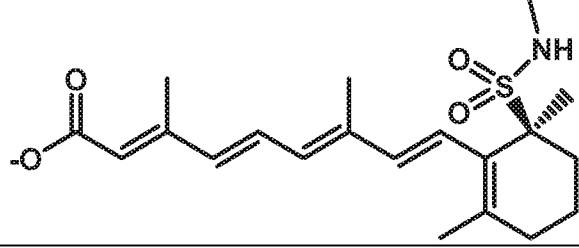
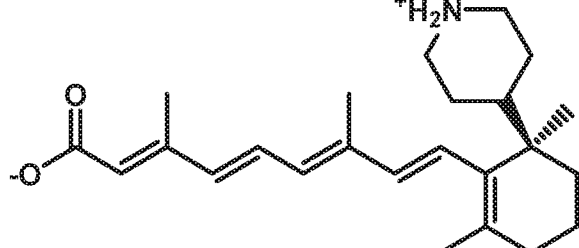
104		-37.914
105		-37.502
106		-37.178
107		-37.014
108		-36.647
109		-36.455
110		-36.050

111		-35.993
112		-35.993
113		-34.396
114		-33.971
115		-33.970
116		-33.501

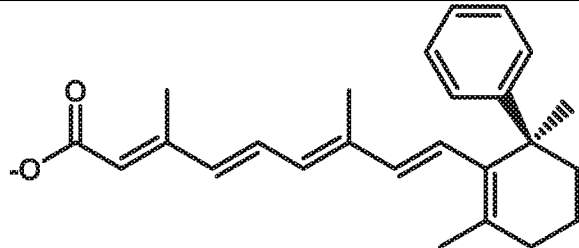
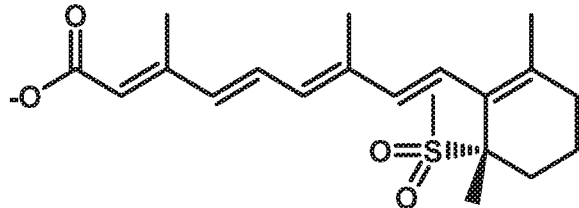
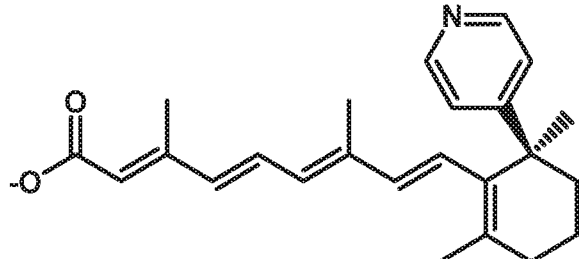
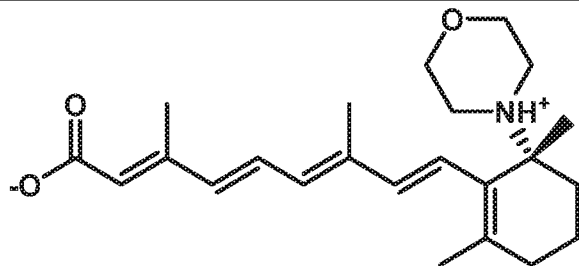
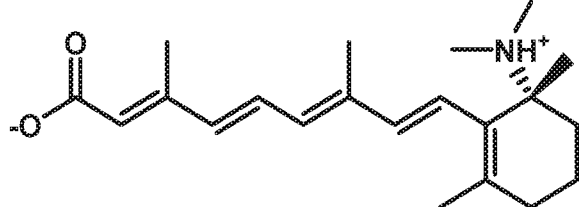
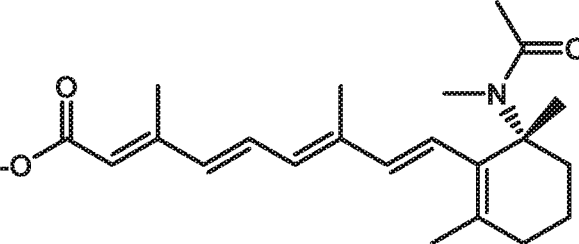
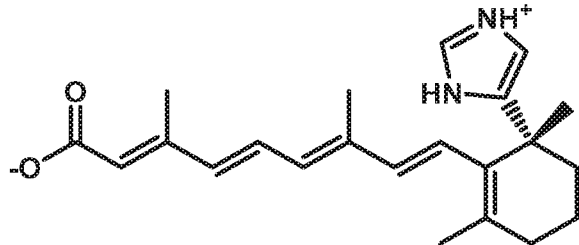
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118		-33.298
119		-32.812
120		-32.087
121		-30.415
122		-30.228

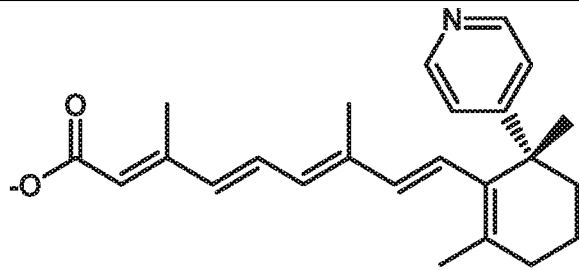
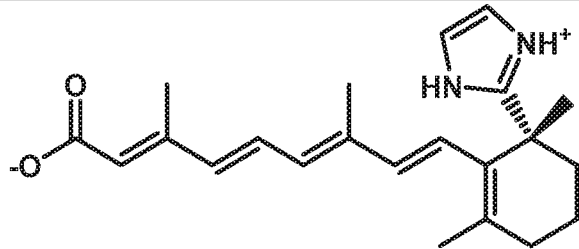
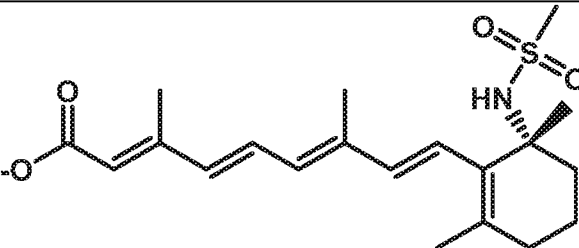
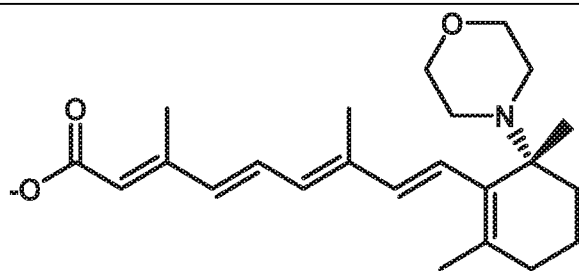
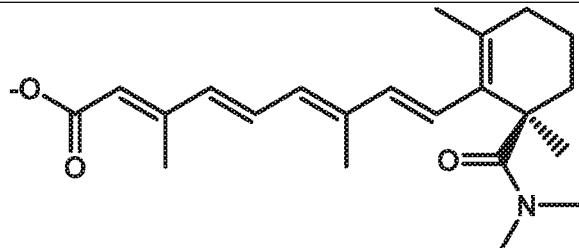
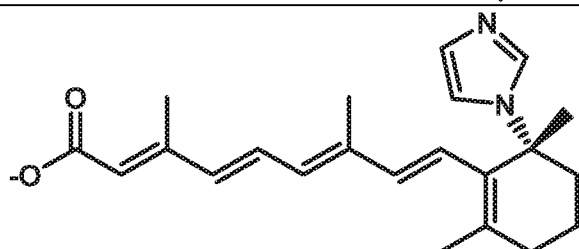
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124		-30.137
125		-30.052
126		-38.710
127		-38.225
128		-37.131

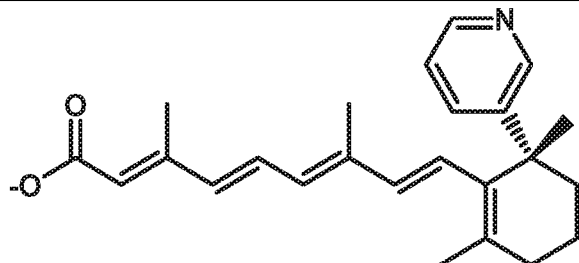
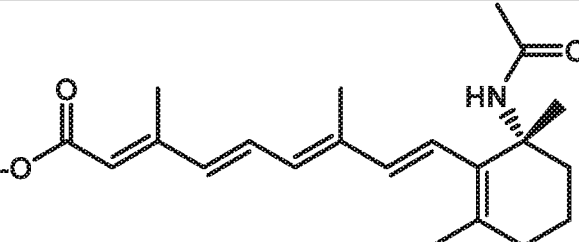
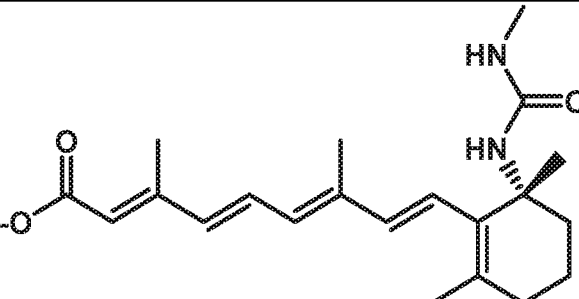
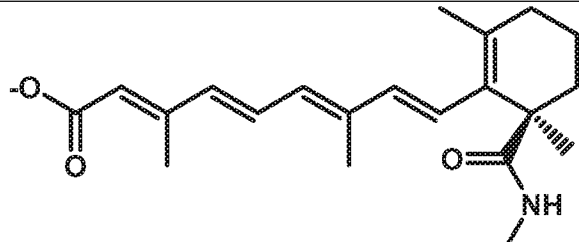
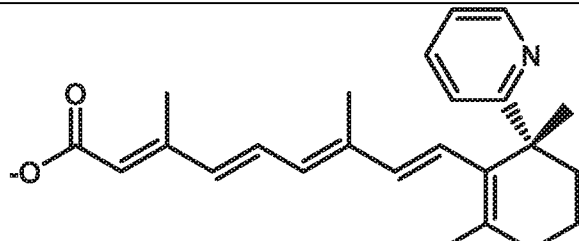
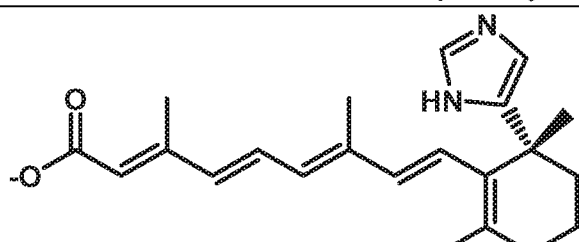
129		-36.785
130		-36.314
131		-35.906
132		-35.695
133		-35.669
134		-35.419

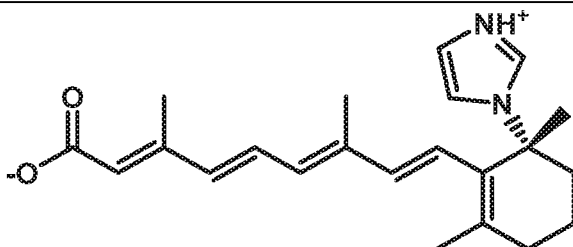
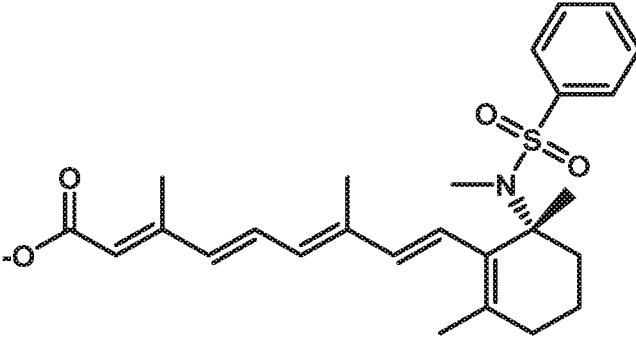
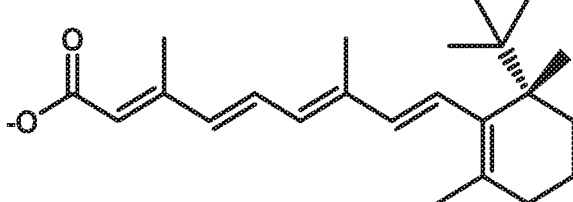
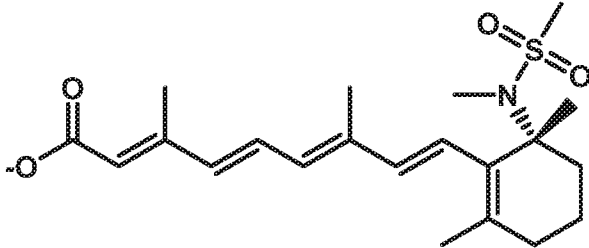
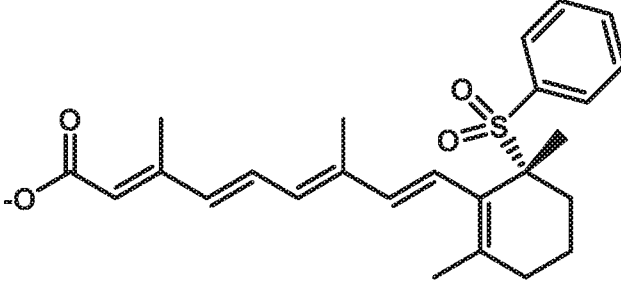
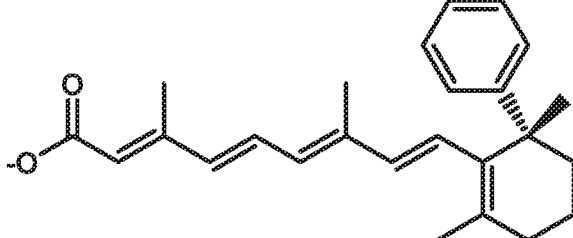
135		-35.284
136		-34.966
137		-34.466
138		-34.411
139		-34.268
140		-34.256

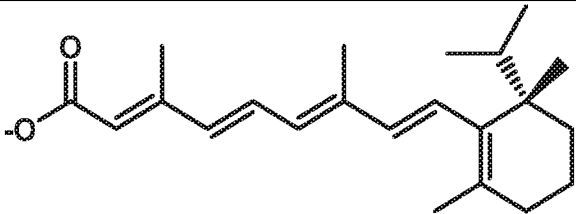
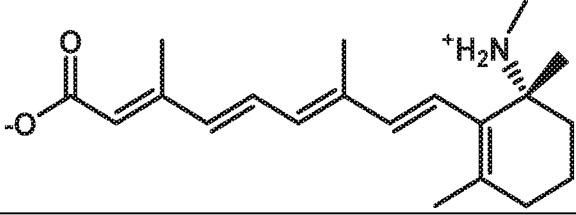
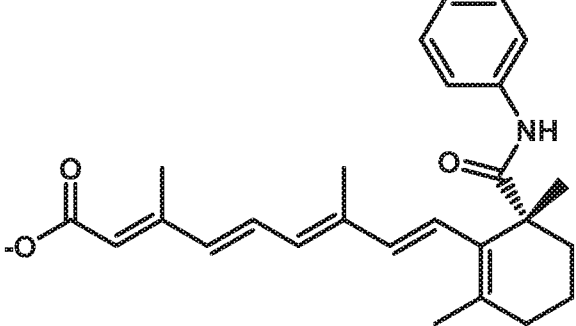
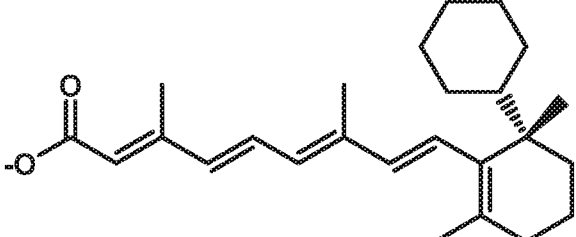
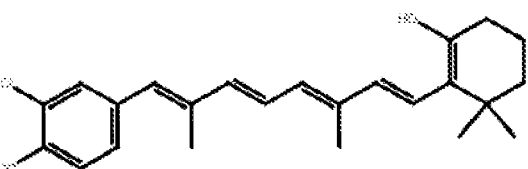
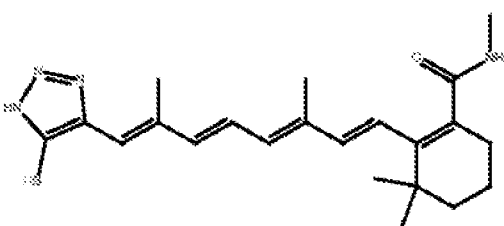
141		-33.085
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143		-32.080
144		-31.664
145		-30.991
146		-30.819
147		-30.766

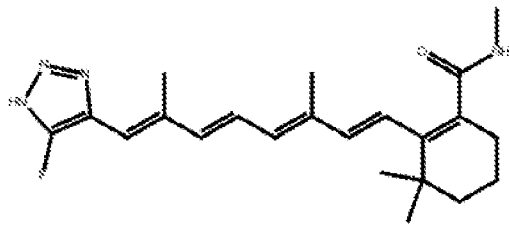
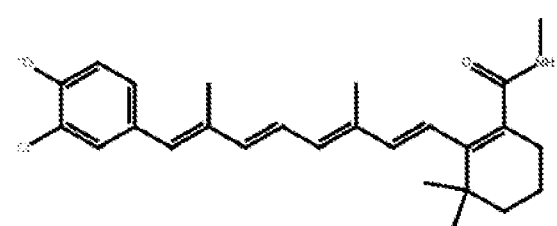
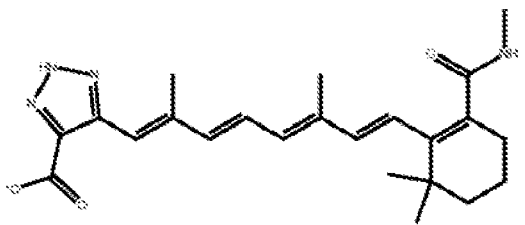
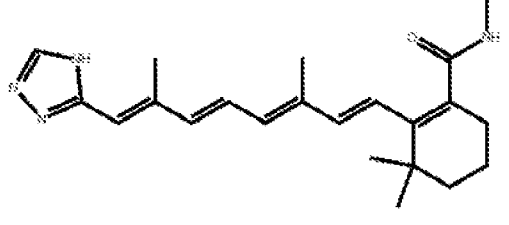
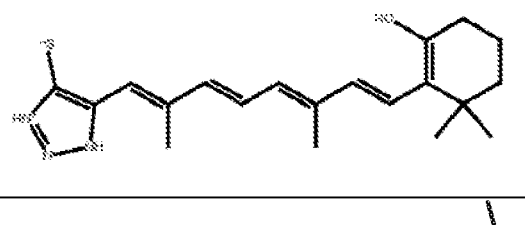
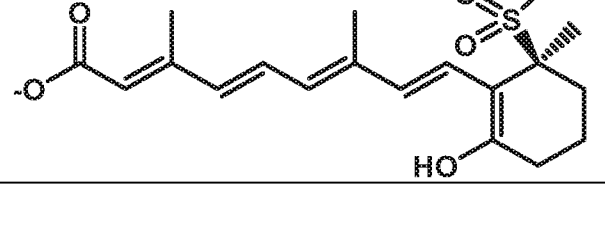
148		-30.370
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150		-30.111
151		-43.497
152		-41.832
153		-40.871
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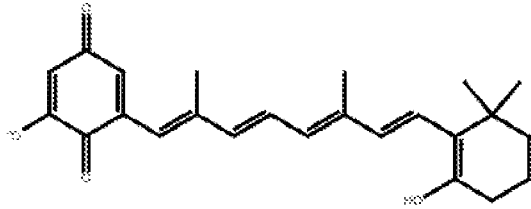
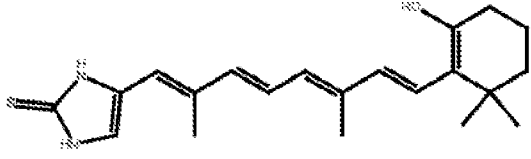
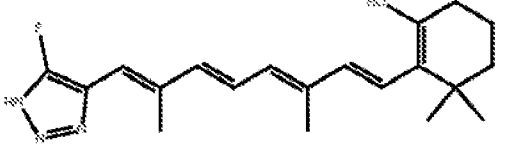
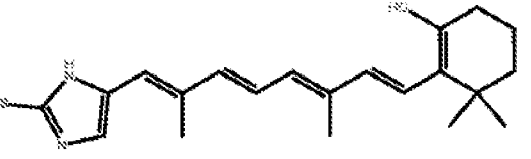
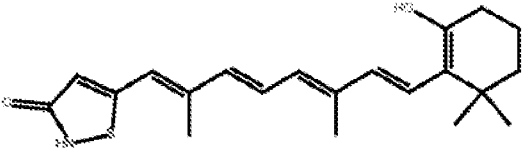
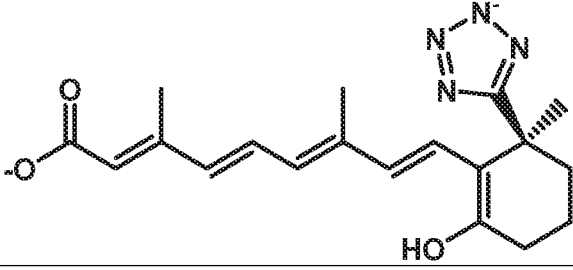
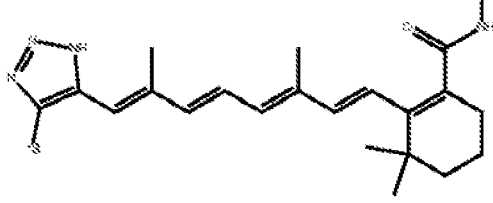
155		-38.360
156		-38.167
157		-37.593
158		-37.523
159		-37.214
160		-36.927

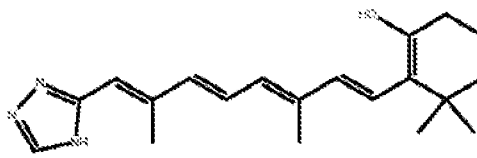
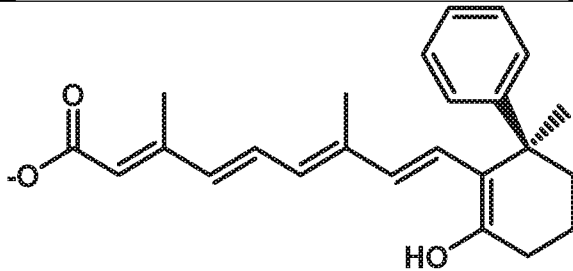
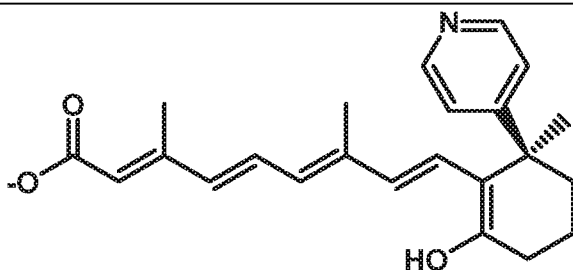
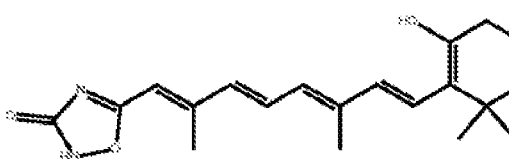
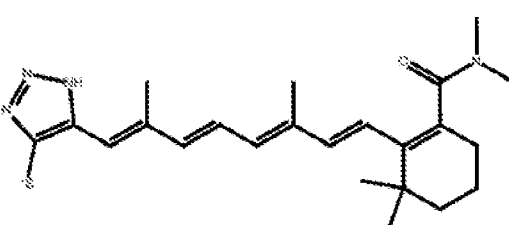
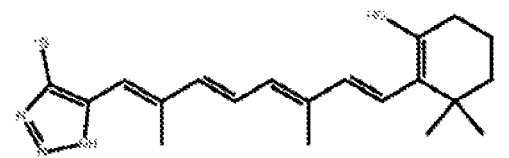
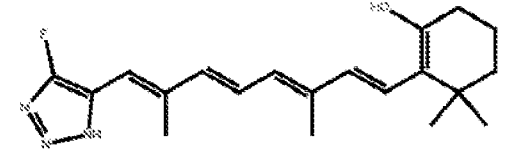
161		-36.711
162		-36.274
163		-35.940
164		-35.824
165		-35.720
166		-35.322

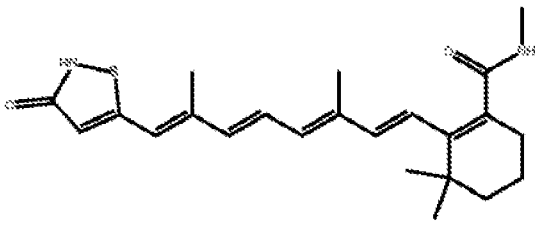
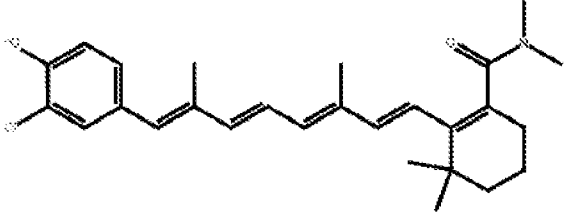
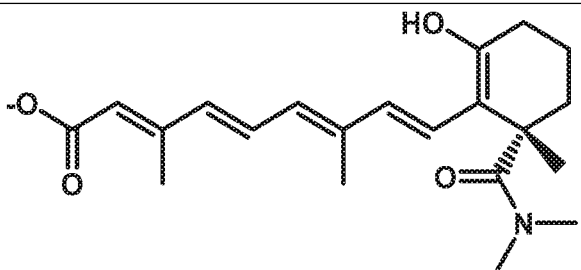
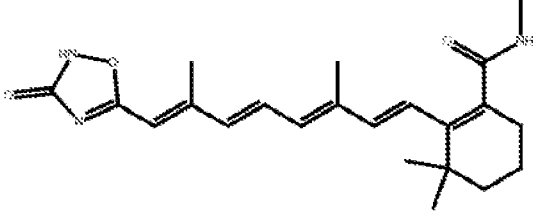
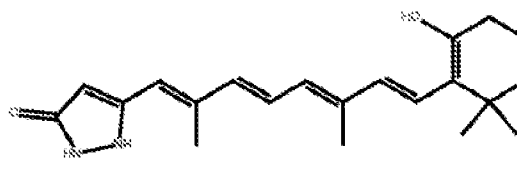
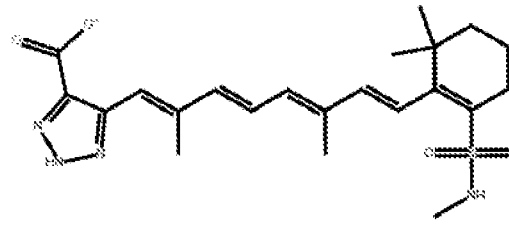
167		-34.656
168		-34.565
169		-33.968
170		-32.483
171		-31.386
172		-31.339

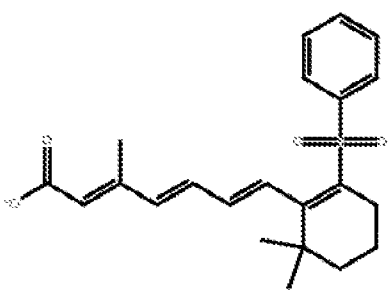
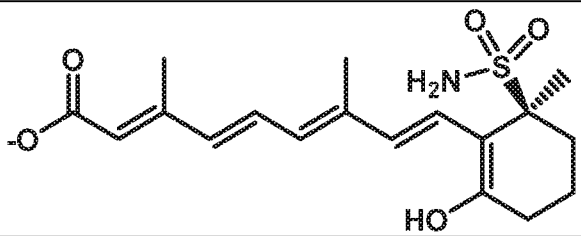
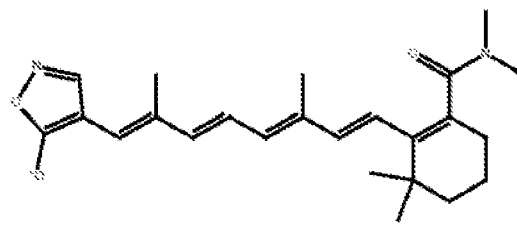
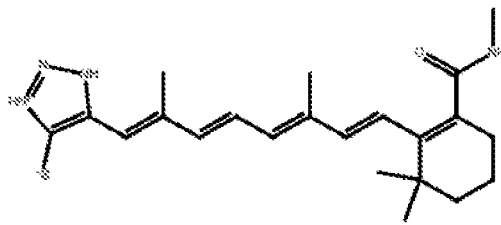
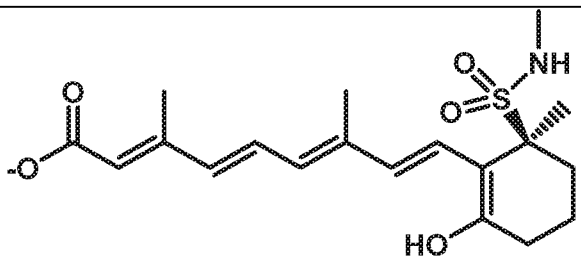
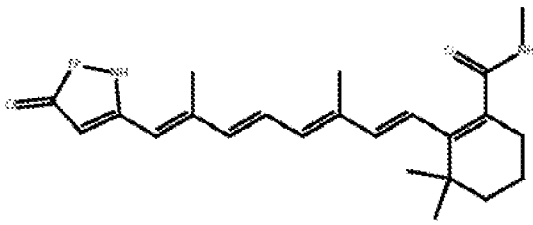
173		-31.060
174		-30.951
175		-30.670
176		-30.553
177		-48.677
178		-48.019

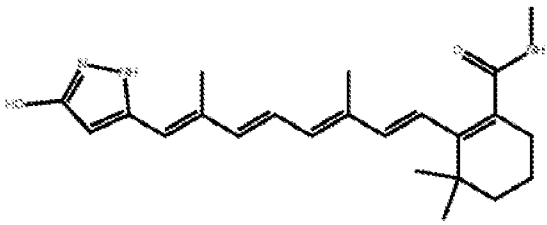
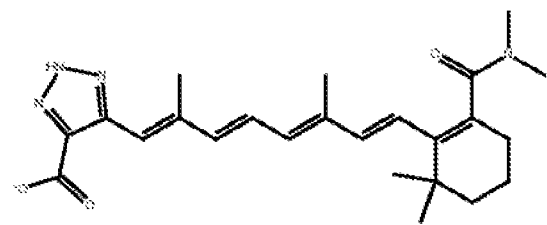
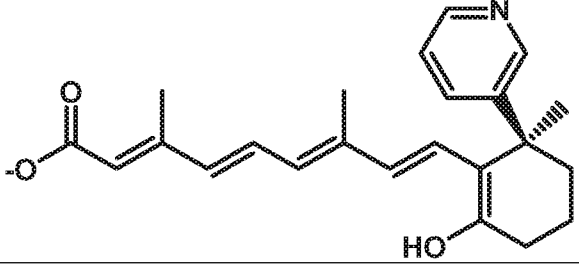
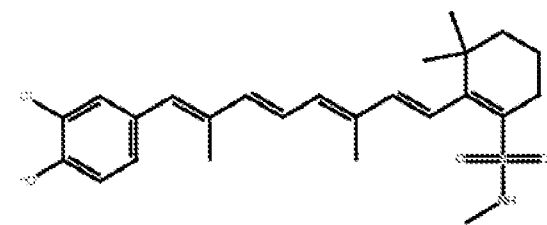
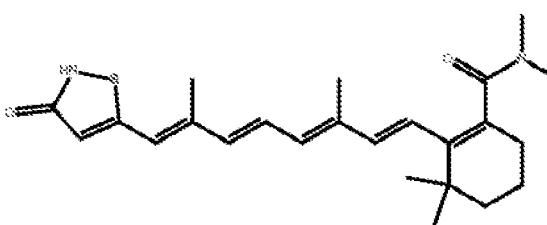
179		-47.880
180		-47.752
181		-47.697
182		-47.360
183		-47.269
184		-46.786

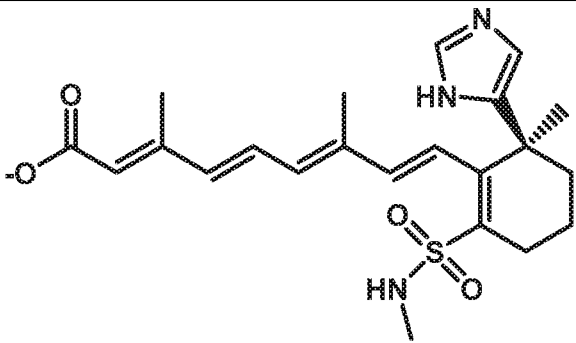
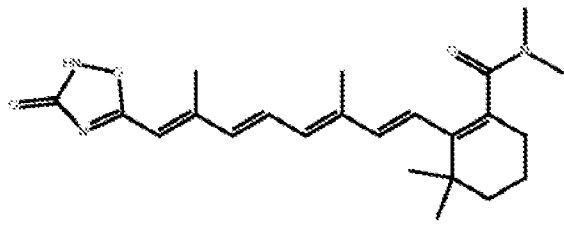
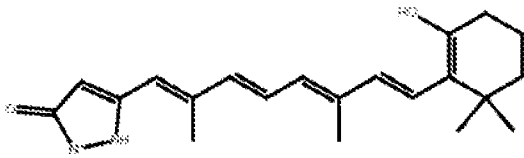
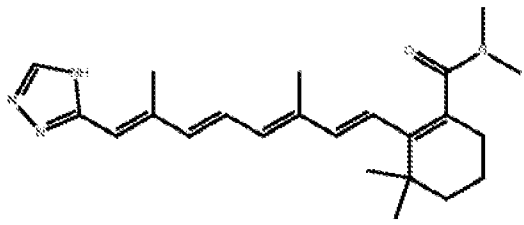
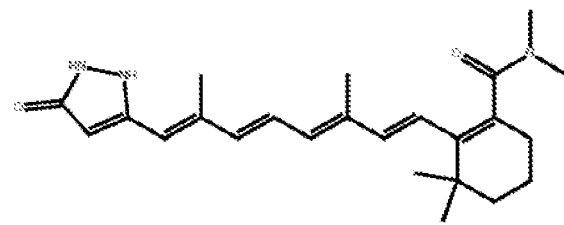
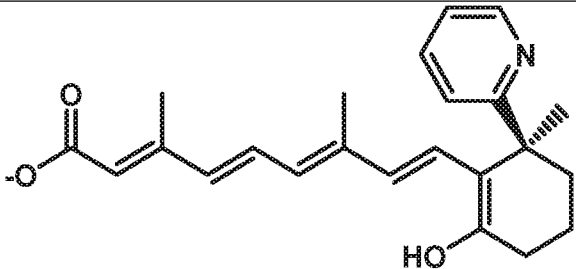
185		-46.761
186		-46.392
187		-45.617
188		-45.455
189		-45.187
190		-44.921
191		-44.915

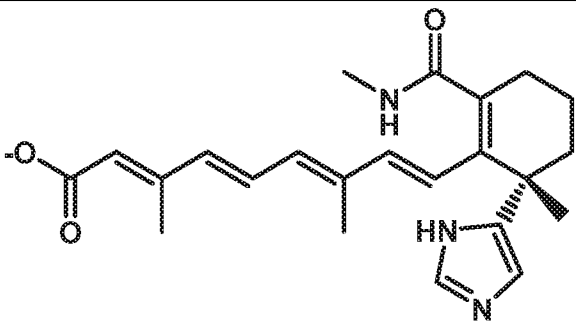
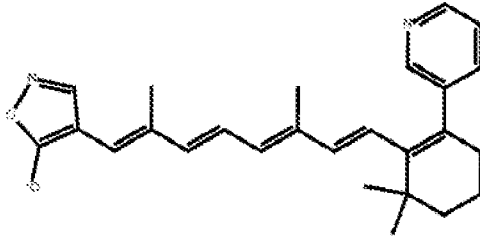
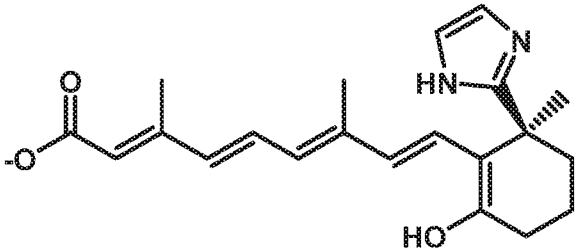
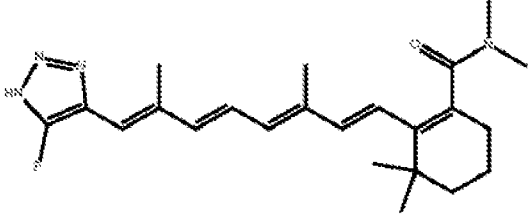
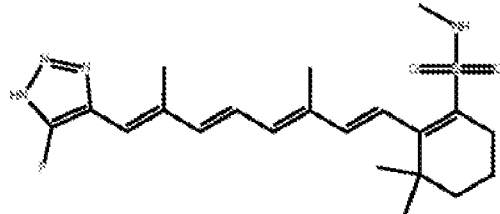
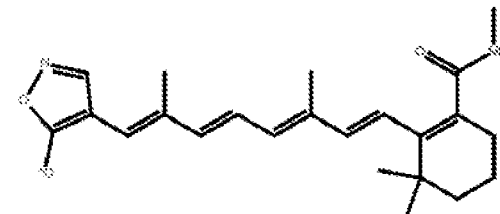
192		-44.866
193		-44.729
194		-44.709
195		-44.707
196		-44.652
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198		-44.505

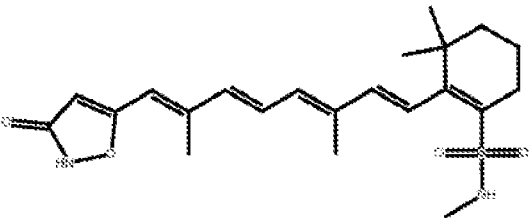
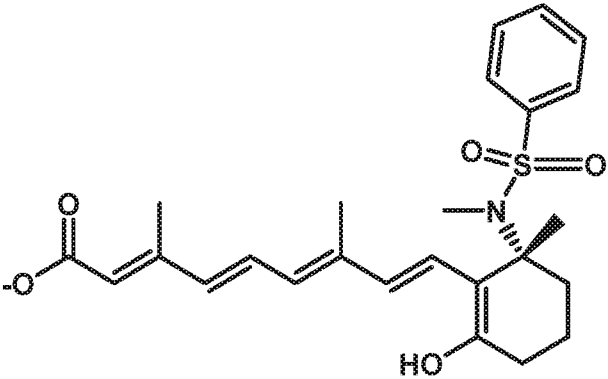
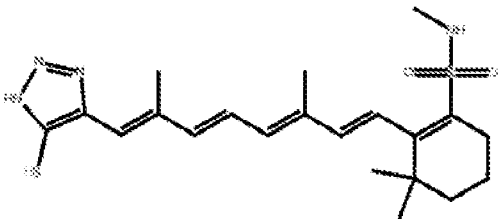
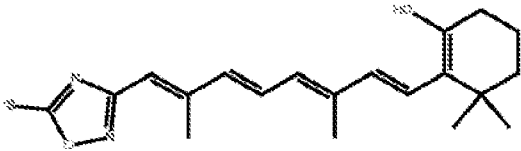
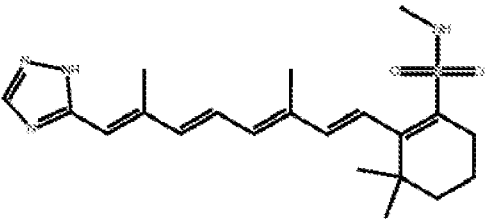
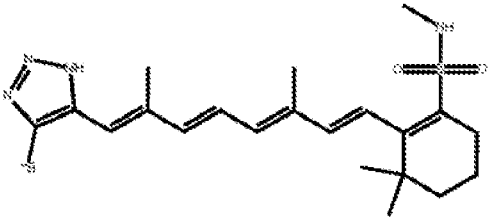
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203		-44.021
204		-43.946

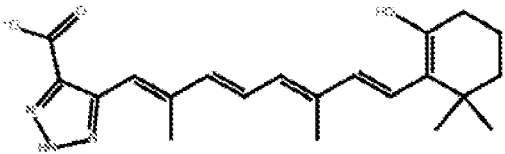
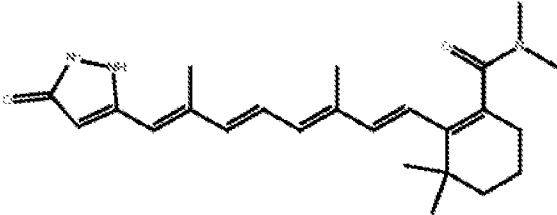
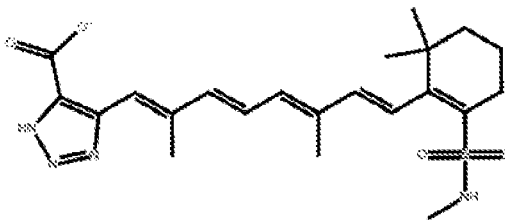
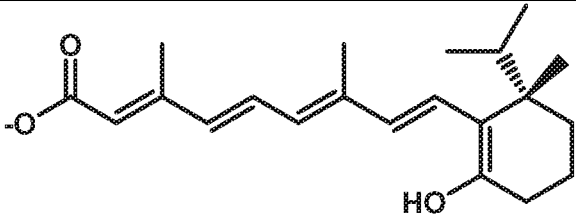
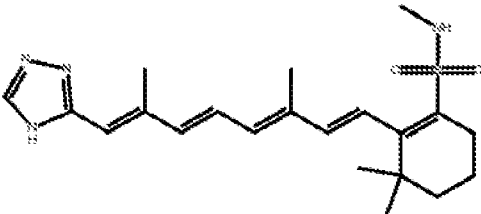
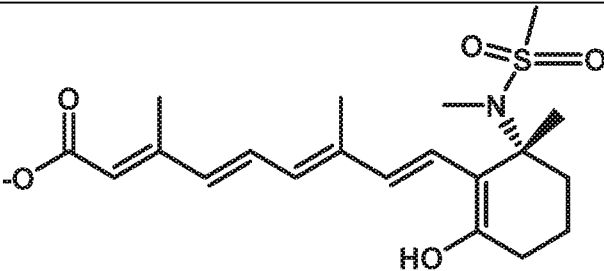
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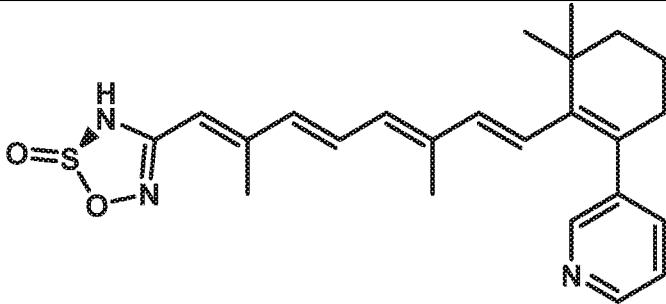
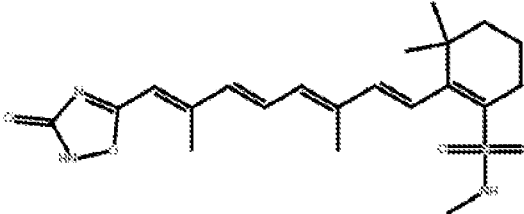
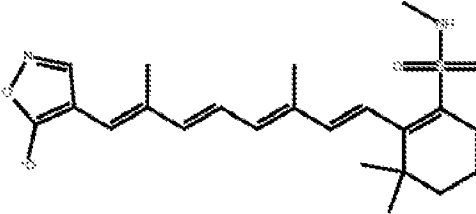
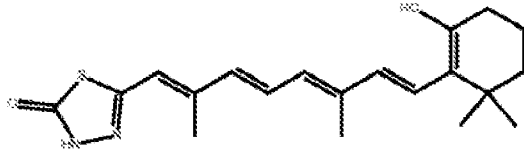
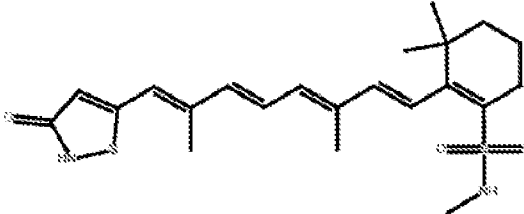
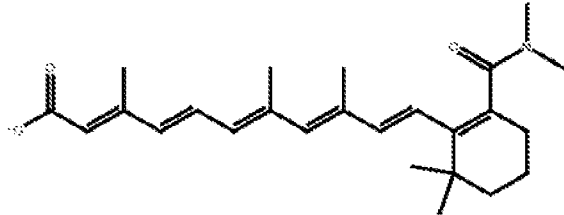
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214		-42.595
215		-42.420

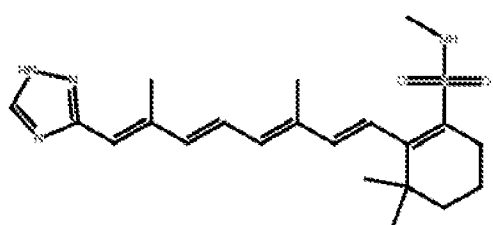
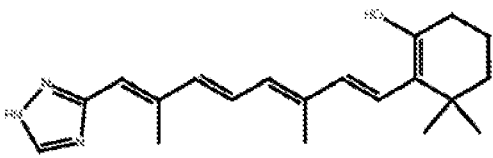
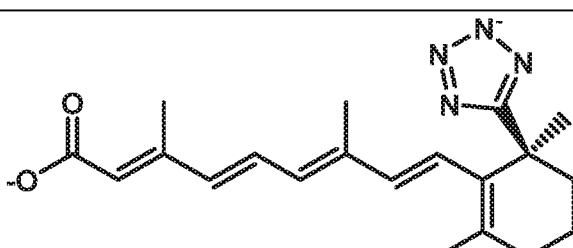
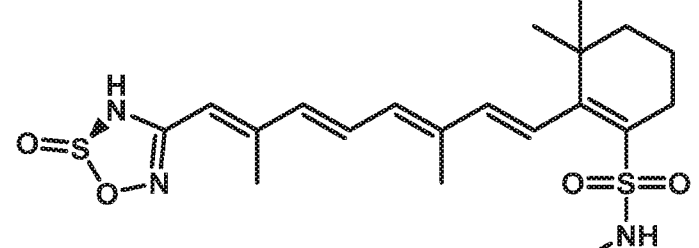
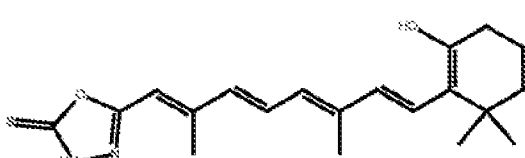
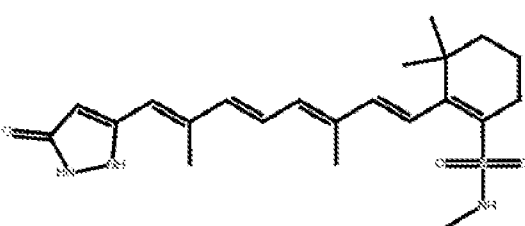
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217		-42.289
218		-42.244
219		-41.962
220		-41.884
221		-41.661

222		-41.517
223		-41.502
224		-40.866
225		-40.861
226		-40.804
227		-40.611

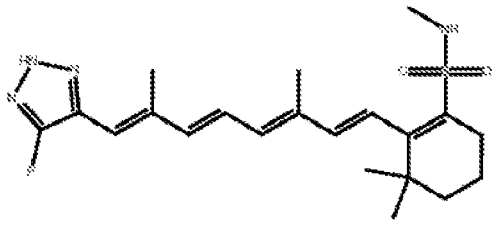
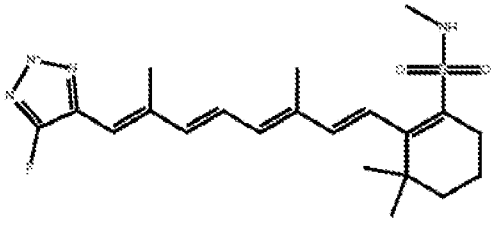
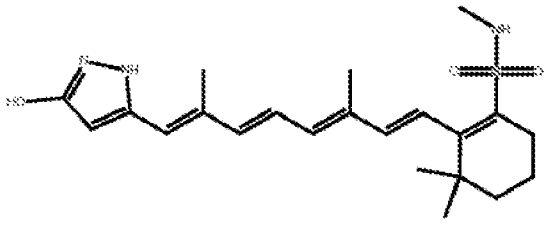
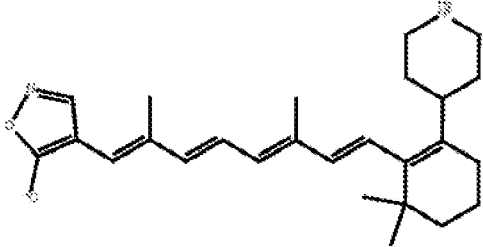
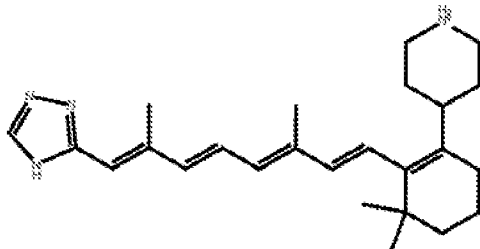
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230		-39.895
231		-39.785
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233		-39.520

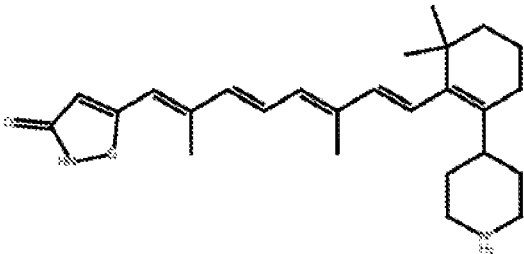
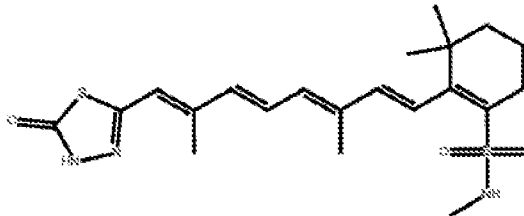
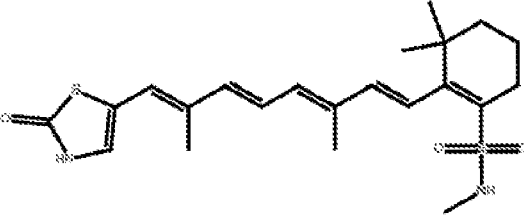
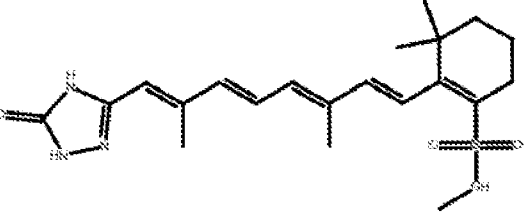
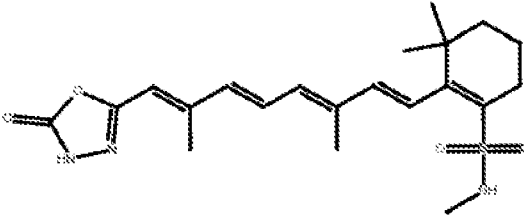
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235		-39.169
236		-39.122
237		-38.985
238		-38.949
239		-38.918

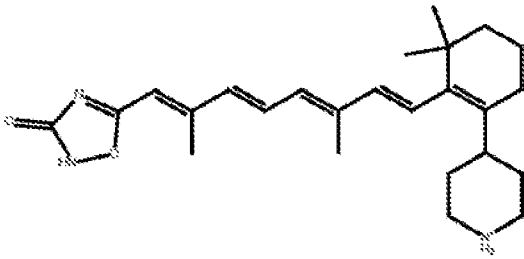
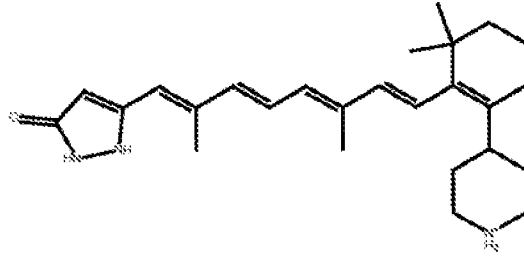
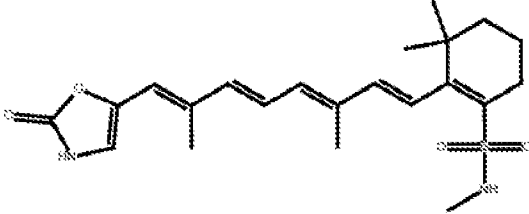
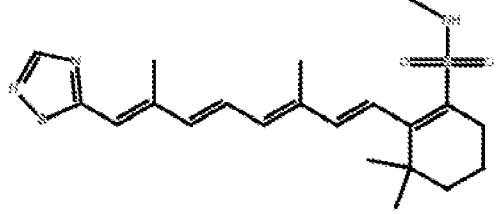
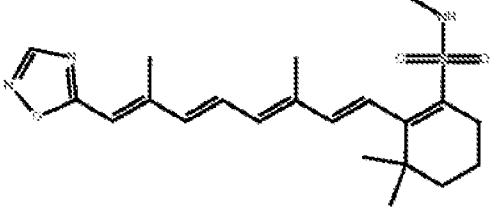
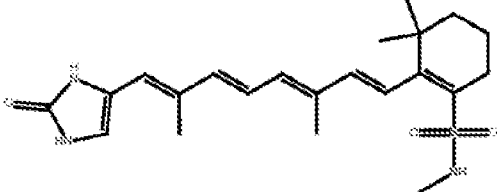
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241		-38.478
242		-38.423
243		-38.203
244		-38.198
245		-37.907

246		-37.747
247		-37.674
248		-37.617
249		-37.559
250		-37.499
251		-37.379

252		-37.159
253		-36.667
254		-36.467
255		-35.928
256		-35.741

257		-35.403
258		-35.039
259		-34.906
260		-34.492
261		-32.922

262		-32.805
263		-32.786
264		-32.382
265		-31.879
266		-31.566

267		-31.378
268		-31.366
269		-31.288
270		-30.991
271		-30.938
272		-30.652

Further examples of retinoic acid compounds include any retinoic acid compounds, or derivatives thereof, known in the art, including those described in PCT Publication Nos. WO 2013/185055, WO 2015/143190, and WO 2016/145186, each of which is incorporated herein with respect to the compounds described therein.

5 The term “diterpene retinoic acid” encompasses any stereoisomer of retinoic acid (e.g., the retinoic acid may be in the all-trans configuration (ATRA) or one or more of the double bonds may be in the *cis* configuration, for example, 13cRA. Derivatives of the diterpene retinoic acid include reduced forms such as retinal, retinol, and retinyl acetate. In Formula I, each of Ar¹ and Ar² is, independently, optionally substituted aryl or an optionally substituted
10 heteroaryl; R¹ is H, an optionally substituted alkyl group, an optionally substituted alkenyl group, or an optionally substituted alkynyl group; each of L¹ and L² is selected, independently from a covalent bond, an optionally substituted C₁₋₁₀ alkylene, an optionally substituted C₂₋₁₀ alkenylene (e.g., -CH=CH-, -COCH=CH-, -CH=CHCO-, a dienyl group, or a trienyl group), optionally substituted C₂₋₁₀ alkynylene (e.g., -C≡C-), or -(CHR⁴)_nCONR⁵-, -NR⁵CO-, where n is
15 0 or 1, R⁴ is H or OH, and R⁵ is H or optionally substituted alkyl; and R³ is H, OR⁴ or N(R⁴)², where each R⁴ is selected, independently, from H, optionally substituted alkyl, or optionally substituted heteroalkyl.

The term “pharmaceutical compound” herein refers to a pharmaceutical product that fits a unique need of a patient. It can be a single therapeutic agent or a combination of multiple
20 therapeutic agents. For example, a pharmaceutical compound used in this invention can be arsenic trioxide, a retinoic acid compound described above, or a combination thereof.

As used herein, the term “C1-C6 alkoxy” represents a chemical substituent of formula –OR, where R is an optionally substituted C1-C6 alkyl group, unless otherwise specified. In some embodiments, the alkyl group can be substituted, e.g., the alkoxy group can have 1, 2, 3,
25 4, 5 or 6 substituent groups as defined herein.

As used herein, the term “C1-C6 acyl” refers to a C1-C6 alkyl group that includes a C(=O) moiety and which may be further substituted as described herein.

As used herein, the term “alkyl,” “alkenyl” and “alkynyl” include straight-chain, branched-chain and cyclic monovalent substituents, as well as combinations of these,
30 containing only C and H when unsubstituted. Examples include methyl, ethyl, isobutyl, cyclohexyl, cyclopentylethyl, 2 propenyl, 3 butynyl, and the like. The term “cycloalkyl,” as used herein, represents a monovalent saturated or unsaturated non-aromatic cyclic alkyl group having between three to nine carbons (e.g., a C3-C9 cycloalkyl), unless otherwise specified, and is exemplified by cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl,

bicyclo[2.2.1]heptyl, and the like. In some embodiments, the cycloalkyl is a polycyclic (e.g., adamantyl). Cycloalkyl groups may be unsubstituted or substituted with, e.g., 1, 2, 3, or 4 substituent groups as defined herein. When the cycloalkyl group includes one carbon-carbon double bond, the cycloalkyl group can be referred to as a “cycloalkenyl” group. Exemplary
5 cycloalkenyl groups include cyclopentenyl, cyclohexenyl, and the like.

Typically, the alkyl, alkenyl and alkynyl groups contain 1-12 carbons (e.g., C1-C12 alkyl) or 2-12 carbons (e.g., C2-C12 alkenyl or C2-C12 alkynyl). In some embodiments, the alkyl groups are C1-C8, C1-C6, C1-C4, C1-C3, or C1-C2 alkyl groups; or C2-C8, C2-C6, C2-C4, or C2-C3 alkenyl or alkynyl groups. Further, any hydrogen atom on one of these groups
10 can be replaced with a substituent as described herein.

The term “aryl,” as used herein, represents a mono- or bicyclic C₆-C₁₄ group with $[4n + 2]$ π electrons in conjugation and where n is 1, 2, or 3. Aryl groups also include ring systems where the ring system having $[4n + 2]$ π electrons is fused to a non-aromatic cycloalkyl or a non-aromatic heterocyclyl. Phenyl is an aryl group where n is 1. Aryl groups may be
15 unsubstituted or substituted with, e.g., 1, 2, 3, or 4 substituent groups as defined herein. Still other exemplary aryl groups include, but are not limited to, naphthyl, 1,2-dihydronaphthyl, 1,2,3,4-tetrahydronaphthyl, fluorenyl, indanyl, and indenyl.

The term “heteroaryl,” as used herein, represents an aromatic (i.e., containing $4n+2$ pi electrons within the ring system) 5- or 6-membered ring containing one, two, three, or four
20 heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur, as well as bicyclic, tricyclic, and tetracyclic groups in which any of the aromatic ring is fused to one, two, or three heterocyclic or carbocyclic rings (e.g., an aryl ring). Exemplary heteroaryls include, but are not limited to, furan, thiophene, pyrrole, thiadiazole (e.g., 1,2,3-thiadiazole or 1,2,4-thiadiazole), oxadiazole (e.g., 1,2,3-oxadiazole or 1,2,5-oxadiazole),
25 oxazole, isoxazole, isothiazole, pyrazole, thiazole, triazole (e.g., 1,2,4-triazole or 1,2,3-triazole), pyridine, pyrimidine, pyrazine, triazine (e.g., 1,2,3-triazine, 1,2,4-triazine, or 1,3,5-triazine), 1,2,4,5-tetrazine, indolyl, quinoliny, isoquinoliny, benzimidazolyl, benzothiazolyl, and benzoxazolyl. Heteroaryls may be unsubstituted or substituted with, e.g., 1, 2, 3, or 4 substituents groups as defined herein.

30 The term “heterocyclyl,” as used herein represents a non-aromatic 5-, 6- or 7-membered ring, unless otherwise specified, containing one, two, three, or four heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur. Heterocyclyl groups may be unsubstituted or substituted with, e.g., 1, 2, 3, or 4 substituent groups as defined herein.

As used herein, the term “aryloxy” refers to aromatic or heteroaromatic systems which are coupled to another residue through an oxygen atom. A typical example of an O-aryl is phenoxy. Similarly, “thioaryloxy” refers to aromatic or heteroaromatic systems which are coupled to another residue through a sulfur atom.

5 As used herein, a halogen is selected from F, Cl, Br, and I, and more particularly it is fluoro or chloro.

Where a group is substituted, the group may be substituted with 1, 2, 3, 4, 5, or 6 substituent groups. Optional substituent groups include, but are not limited to: C₁₋₆ alkyl, C₂₋₆ alkenyl, C₂₋₆ alkynyl, aryl, heteroaryl, cycloalkyl, heterocyclyl, halogen (-F, -Cl, -Br, or -I),
 10 azido(-N₃), nitro (-NO₂), cyano (-CN), acyloxy(-OC(=O)R'), acyl (-C(=O)R'), alkoxy (-OR'), amido (-NR'C(=O)R'' or -C(=O)NRR'), amino (-NRR'), carboxylic acid (-CO₂H), carboxylic ester (-CO₂R'), carbamoyl (-OC(=O)NR'R'' or -NRC(=O)OR'), hydroxy (-OH), oxo (=O), isocyano (-NC), sulfonate (-S(=O)₂OR), sulfonamide (-S(=O)₂NRR' or -NRS(=O)₂R'), or sulfonyl (-S(=O)₂R), where each R or R' is selected, independently, from H, C₁₋₆ alkyl, C₂₋₆
 15 alkenyl, C₂₋₆ alkynyl, aryl, or heteroaryl.

In general, a substituent group (e.g., alkyl, alkenyl, alkynyl, or aryl (including all heteroforms defined above) may itself optionally be substituted by additional substituents. The nature of these substituents is similar to those recited with regard to the substituents on the basic structures above. Thus, where an embodiment of a substituent is alkyl, this alkyl may
 20 optionally be substituted by the remaining substituents listed as substituents where this makes chemical sense, and where this does not undermine the size limit of alkyl per se; e.g., alkyl substituted by alkyl or by alkenyl would simply extend the upper limit of carbon atoms for these embodiments, and is not included. For example, where a group is substituted, the group may be substituted with 1, 2, 3, 4, 5, or 6 substituents. Optional substituents include, but are
 25 not limited to: C₁₋₆ alkyl or heteroaryl, C₂₋₆ alkenyl or heteroalkenyl, C₂₋₆ alkynyl or heteroalkynyl, halogen; aryl, heteroaryl, azido(-N₃), nitro (-NO₂), cyano (-CN), acyloxy(OC(=O)R'), acyl (-C(=O)R'), alkoxy (-OR'), amido (-NR'C(=O)R'' or -C(=O)NRR'), amino (NRR'), carboxylic acid (-CO₂H), carboxylic ester (-CO₂R'), carbamoyl (OC(=O)NR'R'' or -NRC(=O)OR'), hydroxy (OH), isocyano (-NC), sulfonate (S(=O)₂OR), sulfonamide (S(=O)₂NRR' or -NRS(=O)₂R'), or sulfonyl (S(=O)₂R), where each R or R' is selected,
 30 independently, from H, C₁₋₆ alkyl or heteroaryl, C₂₋₆ alkenyl or heteroalkenyl, C₂₋₆ alkynyl or heteroalkynyl, aryl, or heteroaryl. A substituted group may have, for example, 1, 2, 3, 4, 5, 6, 7, 8, or 9 substituents.

Typical optional substituents on aromatic or heteroaromatic groups include independently halo, CN, NO₂, CF₃, OCF₃, COOR', CONR'², OR', SR', SOR', SO₂R', NR'², NR'(CO)R', NR'C(O)OR', NR'C(O)NR'², NR'SO₂NR'², or NR'SO₂R', wherein each R' is independently H or an optionally substituted group selected from alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, heteroaryl, and aryl (all as defined above); or the substituent may be an optionally substituted group selected from alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, aryl, heteroaryl, O-aryl, O-heteroaryl and arylalkyl.

Optional substituents on a non-aromatic group (e.g., alkyl, alkenyl, and alkynyl groups), are typically selected from the same list of substituents suitable for aromatic or heteroaromatic groups, except as noted otherwise herein. A non-aromatic group may also include a substituent selected from =O and =NOR' where R' is H or an optionally substituted group selected from alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, heteroaryl, and aryl (all as defined above).

As used herein, the term “cancer” refers to a proliferative disease in a subject (e.g., a human) having a pathogenic IDH2 allele or having elevated 2-hydroxyglutarate (2-HG) levels. Such cancer includes IDH2-associated leukemias and IDH2-associated solid tumors. An IDH2-associated leukemia can contain one or more mutants selected from FLT3, NPM1, DNMT3A, RUNX1, TET2, IDH2, CEBPA, TP53, IDH1, NRAS, RARA, PML, TTN, WT1, MYH11, BPIFC, CBFB, KIT, KRAS, KMT2A, PTPN11, MUC16, SMC1A, RUNX1T1, MPO, U2AF1, ABCA6, DMXL2, DNAH3, KLK3, FBXO7, SMC3, MXRA5, MUC17, SF3B1, HPS3, PHF6, ASXL1, AHNK, SENP6, MYCBP2, NF1, PCLO, CSMD3, LRP1B, MED12, RAD21, AHNK2, GDI2, PARP14, KLHL7, BCORL1, SPEN, BRWD1, UBR4, STAG2, LNX1, FREM2, MLLT10, DNAH11, SLIT2, DNAH9, BRINP3, HMCN1, SYNE1, GABRG3, NPAS3, CSMD1, GATA2, ZCRB1, MTFR2, SLC43A1, SLA2, APOB, PRDM1, BTBD10, ABCA5, EP300, TNC, ADGRG4, BSN, IFT74, COL12A1, NUP98, CREBBP, EPG5, KIF1B, ABL1, SLC16A7, RALGPS2, CHL1, SCLT1, LRP2, VWA3B, FRYL, PRUNE2, PLCE1, GRIK4, HIVEP3, RYR3, ANK2, ATRNL1, VPS13B, GPR179, FCGBP, KIF15, and DNAH10. An IDH2-associated solid tumor can be glioma, paraganglioma, astrogloma, colorectal carcinoma, melanoma, cholangiocarcinoma, chondrosarcoma, thyroid carcinomas, prostate cancers, or non-small cell lung cancer.

As used herein, the term “pathogenic IDH2 allele” is meant to include any allele encoding an IDH2 protein having a mutation associated with increased cellular proliferation, associated with increased likelihood or severity of cancer, associated with increased likelihood or severity of leukemia, or associated with increased levels of the oncometabolite, 2-

hydroxyglutarate (2-HG) compared with a wild-type cell, tissue, or subject. Pathogenic alleles of IDH2 may encode, for example, any of the following mutations in the corresponding IDH2 protein: R140Q, R140W, R172K, R172M, R172G, or R172W.

As used herein, the term “IDH2 inhibitor” is meant to include any compound that
 5 reduces the level of IDH2 activity or expression in a cell, tissue, or subject. A reduction in IDH2 expression or activity may be measured by methods known to one of skill in the art, including the reduction in corresponding IDH2 mRNA or protein levels in a cell (e.g., a reduction of about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 500%, 1000%, or greater) or the reduction of 2-HG
 10 levels in a cell (e.g., a reduction of about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 500%, 1000%, or greater). In some embodiments, the IDH2 inhibitor may be specific for a pathogenic mutant form of IDH2 (e.g., Enasidenib).

As used herein, “Pin1 activity” refers to binding of the protein Pin1 to a substrate (e.g.,
 15 a substrate protein) and Pin1-catalyzed isomerization of the substrate. Pin1 generally acts as a peptidyl-prolyl isomerase (PPIase) that catalyzes prolyl isomerization of the substrate (e.g., conversion of a peptidyl-prolyl group on the substrate from a *trans* conformation to a *cis* conformation, or vice versa). “Elevated Pin1 activity” or “elevated levels of Pin1 activity,” as used herein, generally refer to an increase in Pin1-catalyzed isomerization of one or more Pin1
 20 substrates, for example, relative to a reference level of Pin1 activity. In some embodiments, the reference level of Pin1 activity is the level of Pin1 activity in a wild-type cell (e.g., a wild-type cell of the same cell type as a cell of interest). In some embodiments, the reference level of Pin1 activity is the level of Pin1 activity in a wild-type subject (e.g., a subject not having leukemia), such that an increase in Pin1 activity in a subject of interest relative to a wild-type
 25 subject indicates that the subject of interest has elevated Pin1 activity. In some embodiments, alteration in Pin1 activity can be assessed by determining the levels of a Pin1 marker in a cell and/or a subject of interest, relative to a reference cell or subject (e.g., a wild-type cell or subject). Elevated levels of Pin1 activity include, for example, Pin1 activity levels at least about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 40%, 50%, 60%,
 30 70%, 80%, 90%, 100%, 200%, 500%, 1000%, or greater than the activity level measured in a normal (e.g., wild-type and/or disease free) subject, tissue, or cell. A decrease in Pin1 activity includes a reduction of at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 500%, 1000% in the Pin1 activity of a

subject, tissue, or cell (e.g., after treatment by any of the compositions of methods described herein).

As used herein, the term “Pin1 marker” refers to a marker which is capable of being indicative of Pin1 activity levels (e.g., in a sample obtained from a cell or subject of interest).

5 Non-limiting examples of Pin1 markers include nucleic acid molecules (e.g., mRNA, DNA) that correspond to some or all of a Pin1 gene, peptide sequences (e.g., amino acid sequences) that correspond to some or all of a Pin1 protein, nucleic acid sequences which are homologous to Pin1 gene sequences, peptide sequences which are homologous to Pin1 peptide sequences, alteration of Pin1 protein, antibodies to Pin1 protein, substrates of Pin1 protein, binding
10 partners of Pin1 protein, alteration of Pin1 binding partners, and activity of Pin1. In some instances, alteration of a Pin1 protein may include a post-translational modification (e.g., phosphorylation, acetylation, methylation, lipidation, or any other post-translational modification known in the art) of Pin1. In some instances, a Pin1 marker is the level of Pin expression (e.g., Pin1 protein expression levels and/or Pin1 mRNA expression levels) in a
15 subject. By “elevated levels of a Pin1 marker” is meant a level of Pin1 marker that is altered, which may, in some instances, indicate the presence of elevated Pin1 activity. Elevated levels of a Pin1 marker include, for example, levels at least about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 500%, 1000%, or greater than, or about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%,
20 25%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% less than the marker levels measured in a normal (e.g., wild-type and/or disease free) subject, tissue, or cell.

As used herein, the terms “compound that inhibits LSD1” or “LSD1 inhibitor” are meant to include any compound that reduces the level of LSD1 activity or expression in a cell, tissue, or subject. A reduction in LSD1 expression or activity may be measured by methods
25 known to one of skill in the art, including the reduction in corresponding LSD1 mRNA or protein levels in a cell (e.g., a reduction of about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 500%, 1000%, or greater) or the reduction of LSD1-specific histone methylation, such as H3K4me2 and/or H3K9me2 histone methylation (e.g., a reduction of about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%,
30 9%, 10%, 15%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 500%, 1000%, or greater).

The term “synergy” or “synergistic,” as used herein, refers to an improved effect when two agents are administered that is greater than the additive effects of each of the two agents when administered alone. In one example, administration of an arsenic trioxide and a retinoic

acid compound (e.g., ATRA) to a subject (e.g., a subject having leukemia) may result in a greater than additive effect on the subject than administration of either arsenic trioxide or the retinoic acid compound alone.

By a “low dose” or “low dosage” is meant a dosage of at least 5% less (e.g., at least 5%, 10%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% less) than the standard recommended dosage or lowest standard recommended dosage of a particular agent, e.g., a therapeutic agent formulated for a given route of administration for treatment of any human disease or condition. For example, a low dosage of an agent formulated for oral administration may differ from a low dosage of the agent formulated for intravenous administration. In some instances, a low dosage of an agent may be selected to be a nontoxic dosage of the agent. In some instances, a low dosage may be selected as a dosage that minimizes particular side effects of an agent, but which may still retain some side effects. For example, a dosage may be selected that minimizes or eliminates side effects that can lead to significant mortality or severe illness among subjects while still permitting more tolerable side effects, such as headache. In some instances, a low dose of arsenic trioxide is a dose of about 2 mg/kg body weight or less (e.g., about 0.01, 0.02, 0.03, 0.032, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.1, 0.15, 0.16, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.25, 1.5, 1.75, or 2 mg/kg). In certain instances, a low dose of arsenic trioxide is about 0.15, about 0.16, or about 0.032 mg/kg body weight. In other instances, a low dose of arsenic trioxide is a dose between about 0.5 mg/kg and about 12 mg/kg body weight (e.g., about 0.5, 0.6, 0.7, 0.8, 0.9, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 mg/kg). In some instances, a low dose of a retinoic acid compound is a dose of about 5 mg/kg body weight or less (e.g., about 0.1, 0.2, 0.5, 0.75, 1, 2, 3, 4, or 5 mg/kg). In some instances, a low dose of a retinoic acid compound is a dose of about 25 mg/m² or less (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 mg/m²). In other instances, the low dose of the retinoic acid compound is a dose of between 25 mg/m² and 45 mg/m² (e.g., 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, or 45 mg/m²).

A “nontoxic” dose of an agent (e.g., arsenic trioxide and/or a retinoic acid compound) is a dosage low enough to minimize or eliminate toxic side effects of the agent on the subject to which the agent is administered. A nontoxic dosage may be achieved by reducing the quantity of the agent administered per dose and/or increasing the length of time between deliveries of individual doses.

The term “effective amount” or an amount “sufficient to” as used interchangeably herein, refers to a quantity of an agent that, when administered alone or with one or more

additional therapeutic agents, induces a desired response or confers a therapeutic effect on the treated subject. The desired response may be a therapeutic response. In one example, the desired response is decreasing the signs or symptoms of a disorder described herein (e.g., leukemia). In another example, the desired response is decreasing the risk of developing or decreasing the risk of recurrence of a disorder described herein (e.g., leukemia). An effective amount of an agent may desirably provide a therapeutic effect without causing substantial toxicity in the subject. In general, an effective amount of a composition administered to a human subject will vary depending upon a number of factors associated with that subject, for example, the overall health of the subject, the condition to be treated, and/or the severity of the condition. An effective amount of a composition can be determined by varying the dosage of the product and measuring the resulting therapeutic response. The effective amount can be dependent, for example, on the source applied, the subject being treated, the severity and type of the condition being treated, and the manner of administration.

The term “treat”, “treating”, or “treatment” refers to application or administration of a pharmaceutical compound by any route, e.g., orally, topically, or by inhalation to a subject with the purpose to cure, alleviate, relieve, alter, remedy, improve, or affect the disease, the symptom, or the predisposition. The compound can be administered alone or in combination with one or more additional compounds. Treatments may be sequential, with a compound being administered before or after the administration of other agents. Alternatively, compounds may be administered concurrently. The subject, e.g., a patient, can be one having a disorder (e.g., a leukemia), a symptom of a disorder, or a predisposition toward a disorder. Treatment is not limited to curing or complete healing, but can result in one or more of alleviating, relieving, altering, partially remedying, ameliorating, improving or affecting the disorder, reducing one or more symptoms of the disorder or the predisposition toward the disorder. In an embodiment the treatment (at least partially) alleviates or relieves symptoms related to a fibrotic disease. In an embodiment the treatment (at least partially) alleviates or relieves symptoms related to an inflammatory disease. In one embodiment, the treatment reduces at least one symptom of the disorder or delays onset of at least one symptom of the disorder. The effect is beyond what is seen in the absence of treatment. Treatment of leukemia may be considered to include administration of any of the compounds described herein resulting in the remission of the leukemia in the subject (e.g., the symptoms of the leukemia are reduced). Treatment of the leukemia may further be considered to include administration of any of the compounds described herein resulting in the complete remission of the leukemia (e.g., all signs and symptoms of the leukemia are absent). Treatment of the leukemia may further be considered to

include administration of any of the compounds described herein, wherein the treatment cures the leukemia in the subject (e.g., all signs and symptoms of the leukemia are absent for 1 year or more, for 2 years or more, for 3 years or more, for 4 years or more, or for 5 years or more).

The term “subject,” as used herein, refers to any organism or portion thereof to be administered a composition as described herein (e.g., arsenic trioxide, a retinoic acid compound, and combinations or derivatives thereof). A subject may be an animal, such as a mammal (e.g., a human, mouse, rat, rabbit, dog, cat, goat, pig, and horse). Preferably, the subject is human.

As used herein, the term “administering” may also be considered to include contacting. For example, wherein a compound is administered to a cell, it may be considered to be equivalent to contacting the cell with the compound.

As used herein, the term “contacting one or more cells” with a compound of the invention is meant to include administering a compound of the invention to a subject, such that the compound contacts one or more cells of the subject.

DETAILED DESCRIPTION

There is a critical need to develop rational combinations to treat disease and overcome resistance that arises in response to therapy. The present invention is based on the discovery of common vulnerabilities in mIDH2 leukemia. The inventors have shown that mIDH2 leukemia exhibits sensitivity to reactive oxygen species (ROS)-producing compounds, such as arsenic trioxide (ATO). The inventors have also shown that mIDH2 leukemia exhibits sensitivity to retinoic acid compound-induced differentiation (e.g., ATRA-induce differentiation). The inventors have further shown that treatment of leukemia with the combination of a ROS-promoting compound (e.g., arsenic trioxide) and a compound that promotes differentiation (e.g., a Pin1 inhibitor, such as ATRA) provides a synergistic, powerful, and well-tolerated targeted therapy in both mouse and human models of AML.

The present invention therefore features methods, compositions, and kits relating to the treatment of mIDH2 leukemia by administering an arsenic trioxide compound (e.g., ATO), a retinoic acid compound (e.g., ATRA), or a combination of an arsenic trioxide and a retinoic acid compound (ATO and ATRA).

Disorders of the invention

Isocitrate dehydrogenases are enzymes (IDH enzymes) are metabolic enzymes that catalyze the oxidative decarboxylation of isocitrate to 2-oxoglutarate. The protein encoded by the IDH2 gene is the NADP(+)-dependent isocitrate dehydrogenase found in the mitochondria.

It plays a role in intermediary metabolism and energy production.

Mutations to IDH enzymes have been identified as important early events in a variety of tumor types. For example, IDH enzymes are mutated in approximately 20% of human acute myeloid leukemias (AMLs). Pathogenic mutants of IDH2 enzymes have been identified that
5 give rise to 2-hydroxyglutarate (2-HG), an oncometabolite that contributes to the oncogenic phenotype. Accordingly, 2-HG is a predictive biomarker in cancers having a pathogenic IDH2 allele.

In some embodiments of the invention, the methods described herein are used to treat a subject having a disorder associated with a mutation in the IDH2 enzyme (mIDH2), thereby
10 giving rise to a pathogenic phenotype (e.g., leukemia). In some embodiments, the mIDH2 disorder is associated with a pathogenic IDH2 allele, wherein a pathogenic IDH2 allele is meant to include any allele encoding an IDH2 protein having a mutation associated with increased cellular proliferation, associated with increased likelihood or severity of cancer, associated with increased likelihood or severity of leukemia, or associated with increased
15 levels of the oncometabolite, 2-hydroxyglutarate (2-HG) compared with a wild-type cell, tissue, or subject. Pathogenic alleles of IDH2 may encode, for example, any of the following mutations in the corresponding IDH2 protein: R140Q, R140W, R172K, R172M, R172G, or R172W.

mIDH2-Associated leukemia

20 In some embodiments of the invention, the methods described herein are used to a subject having a leukemia, wherein the leukemia is associated with a mutation in the IDH2 enzyme. In some embodiments the subject having a leukemia has a pathogenic IDH2 allele and/or has elevated 2-hydroxyglutarate (2-HG) levels (e.g., in one or more cells of the leukemia). In some embodiments, the leukemia is an IDH2 sensitive leukemia (e.g., a leukemia
25 that is responsive to IDH2 inhibitors). In some embodiments, the leukemia is an IDH2 independent or resistant leukemia (e.g., leukemia resistant to known IDH2 inhibitors, such as, Enasidenib).

Compounds of the Invention

The present invention features methods of treating leukemia (e.g., mIDH2-associated
30 leukemia) using arsenic trioxide and/or a retinoic acid compound, and derivatives thereof. In some instances, subject is treated with arsenic trioxide in combination with a retinoic acid compound (e.g., as described herein).

Arsenic Trioxide

Arsenic trioxide generally has the following structure:



Arsenic trioxide exhibits high toxicity in subjects of the invention, including mammals (e.g., humans). For example, arsenic trioxide ingestion can result in severe side effects, including vomiting, abdominal pain, diarrhea, bleeding, convulsions, cardiovascular disorders, inflammation of the liver and kidneys, abnormal blood coagulation, hair loss, and death. Chronic exposure to even low levels of arsenic trioxide can result in arsenicosis and skin cancer. Arsenic trioxide is therefore desirably administered to a subject at low enough doses to minimize toxicity. Arsenic trioxide and derivatives thereof (e.g., as described herein) may be effective at increasing the production of reactive oxygen species in a cell, tissue, or subject. Arsenic trioxide and derivatives thereof may also be effective in reducing Pin1 activity in a cell, tissue, or subject. In some instances, arsenic trioxide may operate synergistically with a retinoic acid compound to treat a disorder described herein. In certain instances, the combination of arsenic trioxide and the retinoic acid compound are administered in amounts that result in minimal toxicity.

Retinoic Acid Compounds

Retinoic acid compounds are generally derivatives of the diterpene retinoic acid (e.g., as described herein). Retinoic acid compounds may be effective in promoting differentiation in a cell (e.g., a leukemic cell). Retinoic acid compounds may also be effective in reducing Pin1 activity in a cell, tissue, or subject. Exemplary retinoic acid compounds of the invention include all-trans retinoic acid (ATRA), 13-cis retinoic acid (13cRA), and retinoic acid compounds, and derivatives thereof, e.g., as described herein. Retinoic acid compounds of the invention may be a y compound selected from Table 1 or Table 2. In some instances, a retinoic acid compound is administered in combination with arsenic trioxide. In certain instances, the combination of arsenic trioxide and the retinoic acid compound are administered in amounts that result in minimal toxicity.

Certain embodiments of the invention feature a deuterated retinoic acid compound that is made by replacing some or all hydrogen with deuterium using state of the art techniques (e.g., as described herein and at www.concertpharma.com).

Combination Therapies

The arsenic trioxide and/or retinoic acid compound(s) of the invention may be further combined with additional therapeutic agents for treatment of any of the disorders described herein (e.g., leukemia).

5 In some instances, such compounds may act synergistically with arsenic trioxide and/or a retinoic acid compound to treat the disorder (e.g., leukemia). Additionally, co-administration with arsenic trioxide and/or a retinoic acid compound may result in the efficacy of the additional therapeutic agent at lower and safer doses (e.g., at least 5% less, for example, at least 10%, 20%, 50%, 80%, 90%, or even 95% less) than when the additional therapeutic agent is
10 administered alone.

Anti-proliferative agents

In some instances, the arsenic trioxide and/or retinoic acid compounds may be combined with anti-proliferative and other anti-cancer compounds (e.g., anti-angiogenic compounds) for treating a disorder (e.g., leukemia). Anti-proliferative agents that can be used
15 in combination with a retinoic acid compound include, without limitation, microtubule inhibitors, topoisomerase inhibitors, platins, alkylating agents, and anti-metabolites. Exemplary anti-proliferative agents that are useful in the methods and compositions of the invention include, without limitation, paclitaxel, gemcitabine, doxorubicin, vinblastine, etoposide, 5-fluorouracil, carboplatin, altretamine, aminoglutethimide, amsacrine, anastrozole, azacitidine,
20 bleomycin, busulfan, carmustine, chlorambucil, 2-chlorodeoxyadenosine, cisplatin, colchicine, cyclophosphamide, cytarabine, cytoxan, dacarbazine, dactinomycin, daunorubicin, docetaxel, estramustine phosphate, floxuridine, fludarabine, gentuzumab, hexamethylmelamine, hydroxyurea, ifosfamide, imatinib, interferon, irinotecan, lomustine, mechlorethamine, melphalen, 6-mercaptopurine, methotrexate, mitomycin, mitotane, mitoxantrone, pentostatin,
25 procarbazine, rituximab, streptozocin, tamoxifen, temozolomide, teniposide, 6-thioguanine, topotecan, trastuzumab, vincristine, vindesine, and vinorelbine. The ability of a compound to inhibit the growth of a neoplasm can be assessed using known animal models.

Pin1 inhibitors

Pin1 is an enzyme that catalyzed the cis-trans isomerization of phosphorylated Ser/Thr-
30 Pro motifs and which has been shown to be involved in an increasing number of diseases. Elevated Pin1 activity has been associated with the development and progression of cancer. For example, Pin1 is overexpressed in some human cancer samples and the levels of Pin1 are correlated with the aggressiveness of tumors. Moreover, inhibition of Pin1 by various approaches, including the Pin1 inhibitor, Pin1 antisense polynucleotides, or genetic depletion,

kills human and yeast dividing cells by inducing premature mitotic entry and apoptosis. Thus, upon phosphorylation, Pin1 latches onto phosphoproteins and twists the peptide bond next to the proline, which regulates the function of phosphoproteins and participates in controlling the timing of mitotic progression. In addition, Pin1 has been shown to regulate the expression and/or activity of a diverse array of proteins associated with cancer progression. For example, known Pin1 substrates include, without limitation, Her2, PKM2, FAK, Raf-1, AKT, β -catenin, c-Myc, p53, and numerous other proteins known to play roles in cancer progression. In some instances, the arsenic trioxide and/or retinoic acid compounds may be combined with a compound that inhibits Pin1 activity or expression. In some instances, the arsenic trioxide and/or retinoic acid compounds may be combined with a compound known to interact with other proteins implicated in Pin1 signaling pathways (see, e.g., the targets and compounds in Table 3).

Table 3. Exemplary Additional Therapeutic Agents

Target	Target Class	Representative Antagonist
AKT	Kinase	MK-2206
Cyclin D1	Cyclin	ON 013105
HER2/Neu (ErbB-2)	Kinase	Herceptin
NF- κ F	Transcription Factor	RTA 402
Plk	Kinase	BI 2536
Raf-1	Kinase	Sorafenib
Stat3	Transcription Factor	ISIS-STAT3Rx
ISIS-STAT	Adhesion	Nucleic Acid-Based Rx in Enzon Program at Santaris

LSD1 inhibitors

Lysine-specific histone demethylase 1 (LSD1) is a flavin-dependent monoamine oxidase, which can demethylate mono- and di-methylated lysines, specifically H3K4 and H3K9). The LSD1 enzyme has roles critical in embryogenesis and tissue-specific differentiation, as well as oocyte growth. The inventors have also identified a shared set of ATRA responsive genes previously reported to be regulated by inhibition of the demethylase LSD1 and associated with ATRA sensitivity. In some instances, the arsenic trioxide and/or retinoic acid compounds may be combined with a compound that inhibits LSD1 activity or expression.

Methods of Treatment

Therapy according to the invention may be performed alone or in conjunction with another therapy and may be provided at home, the doctor's office, a clinic, a hospital's outpatient department, or a hospital. Treatment optionally begins at a hospital so that the doctor
5 can observe the therapy's effects closely and make any adjustments that are needed, or it may begin on an outpatient basis. The duration of the therapy depends on the type of disease or disorder being treated, the age and condition of the patient, the stage and type of the patient's disease, and how the patient responds to the treatment.

Routes of administration for the various embodiments include, but are not limited to,
10 topical, transdermal, nasal, and systemic administration (such as, intravenous, intramuscular, subcutaneous, inhalation, rectal, buccal, vaginal, intraperitoneal, intraarticular, ophthalmic, otic, or oral administration). As used herein, "systemic administration" refers to all nondermal routes of administration, and specifically excludes topical and transdermal routes of administration.

15 In combination therapy (e.g., arsenic trioxide and/or a retinoic acid compound in combination with an additional therapeutic agent), the dosage and frequency of administration of each component of the combination can be controlled independently. For example, one or more of the compounds may be administered three times per day, while another compound or compounds may be administered once per day. Alternatively, one compound may be
20 administered earlier and another compound may be administered later. Combination therapy may be given in on-and-off cycles that include rest periods so that the patient's body has a chance to recover from any as yet unforeseen side effects. The compounds may also be formulated together such that one administration delivers both compounds.

Each compound of the combination may be formulated in a variety of ways that are
25 known in the art. For example, a plurality of therapeutic agents (e.g., arsenic trioxide, a retinoic acid compound, and/or an additional therapeutic agent, as described herein) may be formulated together or separately. In some instances, multiple agents are formulated together for the simultaneous or near simultaneous administration of the agents. Such co-formulated compositions can include the drugs together in the same pill, ointment, cream, foam, capsule,
30 liquid, etc. It is to be understood that, when referring to the formulation of combinations of the invention, the formulation technology employed is also useful for the formulation of the individual agents of the combination, as well as other combinations of the invention. By using different formulation strategies for different agents, the pharmacokinetic profiles for each agent can be suitably matched.

Certain embodiments of the invention feature formulations of arsenic trioxide and/or a retinoic acid compound for, e.g., controlled or extended release. Many strategies can be pursued to obtain controlled and/or extended release in which the rate of release outweighs the rate of metabolism of the therapeutic compound. For example, controlled release can be obtained by the appropriate selection of formulation parameters and ingredients (e.g., appropriate controlled release compositions and coatings). Examples include single or multiple unit tablet or capsule compositions, oil solutions, suspensions, emulsions, microcapsules, microspheres, nanoparticles, patches, and liposomes. The release mechanism can be controlled such that the arsenic trioxide and/or retinoic acid compound is released at period intervals, the release could be simultaneous, or a delayed release of one of the agents of the combination can be affected, when the early release of one particular agent is preferred over the other.

The individually or separately formulated agents can be packaged together as a kit. Non-limiting examples include kits that contain, e.g., a plurality of pills (e.g., two pills or three pills), a pill and a powder, a suppository and a liquid in a vial, two topical creams, ointments, foams etc. The kit can include optional components that aid in the administration of the unit dose to patients, such as vials for reconstituting powder forms, syringes for injection, customized IV delivery systems, inhalers, etc. Additionally, the unit dose kit can contain instructions for preparation and administration of the compositions. The kit may be manufactured as a single use unit dose for one patient, multiple uses for a particular patient (at a constant dose or in which the individual compounds may vary in potency as therapy progresses), or the kit may contain multiple doses suitable for administration to multiple patients ("bulk packaging"). The kit components may be assembled in cartons, blister packs, bottles, tubes, and the like.

Examples

Without further elaboration, it is believed that one skilled in the art can, based on the above description, utilize the present invention to its fullest extent. The following specific examples, i.e., Examples 1-9, are therefore to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever. All publications cited herein are incorporated by reference in their entirety.

Materials and methods

Murine Leukaemia animal model.

The Hoxa9-Meis1-IDH2R140Q model of murine leukaemia cells was generated by the retroviral transduction of KSL (c-Kit⁺, Sca-1⁺, Lin⁻) cells as reported in Quek et al., *Nat. Med.*, 2018, 24:1167-1177. The generation of murine leukaemia cells showing independence

by the mutant IDH2 was casually generated after three serial bone marrow transplantations of murine leukaemia cells. Serial bone marrow transplantation experiments were started by harvesting leukemic bone marrow cells from a single donor mouse and transplanting 5-10 * 10⁴ cells into 8-10 recipients. The recipients were age-matched, female C57BL/6J (6-8 weeks old).

In brief, hematopoietic stem cells LSK were isolated from the bone marrow of a single Tg (M2rt/TA, IDH2R140Q) donor (3-5 months old) maintained on doxycycline diet. For LSK isolation, bones from hind limbs and hips were collected and crushed in PBS1X using a mortar and pestle. PBS medium containing bone marrow extracts was filtered and purified from red blood cells by incubation on ACK Lysing Buffer (Gibco). Lineage negative cells were immunostained using Lineage Cell Detection Cocktail-Biotin, mouse (Miltenyi Biotec) and streptavidin – APC-Cy7.

While anti-cKit-APC and anti-Sca-1-PE antibodies were used to stain cKit and Sca-1 positive populations. LSK cells (Lineage-, cKit+, Sca-1+) were sorted on a BD FACSAria II high speed cell sorters (Becton Dickinson) at the Hematologic Neoplasia and Jimmy Fund Cores (Dana Farber) and collected on StemSpan medium (Stem Cell Technologies) supplemented with cytokines (PeproTech): IL3 (25ng/ml), IL6 (25ng/ml), TPO (50 ng/ml), SCF (50ng/ml), FLT3Ligand (50ng/ml). Sorted LSK were transduced by spinoculation with Hoxa9-IRES-GFP and Meis1a-IRES-YFP retroviruses and maintained in culture for no more than 72 hours before transplantation on a primary host (1st RECIPIENT, C57BL/6J females).

After a period of incubation (6-8 weeks), the leukemic cells were harvested from the bone marrow of a single donor, sorted as GFP+/YFP+ population and transplanted into a cohort of mice (2nd RECIPIENTS, C57BL/6J females). After 72 hours, the transplanted 2nd RECIPIENTS were divided into two groups and fed with doxycycline food to maintain the expression of the mutant enzyme (Dox+ = IDH2^{R140Q} ON) or normal diet to avoid the expression of mutant IDH2 (Dox- = IDH2^{R140Q} OFF). Leukaemia cells were collected from the Dox+ = IDH2^{R140Q} ON group after 6-8 weeks and transplanted into a new cohort of mice (3rd RECIPIENTS, C57BL/6J females). At this time point both Dox+ = IDH2^{R140Q} ON and Dox- = IDH2^{R140Q} OFF cohorts were euthanized to evaluate disease state. As before, leukaemia cells were allowed to engraft for 72 hours and mice divided into two cohorts (Dox+ = IDH2^{R140Q} ON and Dox- = IDH2^{R140Q} OFF). After 6-9 weeks, both Dox+ = IDH2^{R140Q} ON and Dox- = IDH2^{R140Q} OFF cohorts were euthanized and bone marrow, peripheral blood or internal organs were harvested to evaluate disease penetrance. Age-matched C57BL/6J recipient female mice (age 8-10 weeks, Jackson Laboratories) were injected intravenously (tail vein) or retro-

orbitally with $5-10 \times 10^5$ leukaemia cells in a volume of 200 μ l of sterile PBS. All recipient animals received 650 cGy of radiation 24 hours prior to injection of leukemic cells. Leukemic bone marrow cells harvested from 2nd and 3rd RECIPIENTS were *ex vivo* expanded in RPMI media (Gibco) supplemented with murine SCF, FLT3 Ligand, IL-3, and IL-6 (2.5 ng/mL) for subsequent studies.

Intravenous Xenograft Leukaemia model.

Female NSG (NOD.CgPrkdc^{scid} Il2^{rgtm1Wjl}/SzJ) recipient mice 6-8 weeks of age were used for the U937 *in vivo* model. Cells (25×10^4) were introduced intravenously by tail vein injection. Mice were treated with ATRA (10mg/mouse) by subcutaneous implantation of ATRA pellets (5mg/pellet /21 days release), Arsenic trioxide (2.5 mg/Kg/day), or respective vehicle solutions via intraperitoneal injection. The experiment, as well as all treatments, were stopped after 21 days following the exhaustion of ATRA pellets.

AML patient - derived xenograft (PDX) model.

NSG mice engrafted with human AML blasts were purchased from Jackson Laboratories (NOD *scid* gamma, NOD-*scid* IL2Rg^{null}, NOD3 *scid* IL2Rgamma^{null}). Female NSG mice (age: 4-5 weeks old) were irradiated (400 cGy) 24 hours prior to the retro-orbital transplantation of sorted human AML blasts. Engraftment and disease progression was detected as evidence of human CD45- positive cells in the peripheral blood. Mice were randomly divided into groups before starting of the treatments.

Preclinical Testing of ATO and ATRA.

All *in vivo* treatments started after assessment of leukaemia engraftment and detection of blasts in PB of transplanted NSG or C57BL/6J. The Scheme of the pharmacological therapy resembles therapeutic regimen of human APL patients. Treatment regimen was performed in cycles for a total of 65 days resembling APL0406 protocol (Lo-Coco et al; NEJM 2013).

Accordingly, each cycle (65-71 days) was as follows: 15-21 days combination of ATO and ATRA, 10 days no therapy administered (off period) followed by two rounds of single drug administration: 10 days of ATO therapy before 10 days of ATRA therapy. Before the starting of a new cycle, no therapy was administered for 10 days. ATO (2.5 μ g/g) and ATRA (1.5 μ g/g) were administered by intraperitoneal injection as previously established methods (Rego, E. M., He, L.-Z., Warrell, R. P., Jr, Wang, Z.-G., & Pandolfi, P. P. (2016). Retinoic acid (RA) and As2O3 treatment in transgenic models of acute promyelocytic leukemia (APL) unravel the distinct nature of the leukemogenic process induced by the PML-RAR and PLZF-RAR oncoproteins).

In vitro culture of murine primary leukaemia cells.

The Hoxa9/Meis1a/IDH2^{R140Q} murine primary leukaemia cells were cultured and expanded in RPMI media supplemented with murine SCF (2.4 ng/mL), FLT3 Ligand (5ng/mL), IL-3 (2 ng / mL), and IL6 (2.5 ng/mL) (PeproTech). When needed, doxycycline
 5 (Sigma) was added to the media to maintain the expression of the IDH2^{R140Q} allele *in vitro*. Conditioned media (1:2) was used for serial re-plating.

Methylcellulose colony assay.

Colony assay and re-plating of mouse or human leukaemia cells, respectively, were performed with Methocult M3434 medium or MethoCult H4434 Classic (Stem Cell
 10 Technologies). According to specific conditions, methylcellulose medium was supplemented with Doxycycline (Gibco), Arsenic Trioxide (Sigma), Tretinoin (Sigma), AGI-6780 or AG-221 (Cayman Chemical), Juglone (Cayman Chemical) or respective vehicle solutions. Single colonies were picked up 1- 2 weeks after culture and then processed by cytopsin, assayed for cell viability, flow cytometry analysis, and/ or harvested for nucleic acids extraction. Mouse
 15 leukaemia cells were isolated from bone marrow as GFP+ cells and plated at 5000 cells / dish. Then, 2500 cells/dish were used for serial plating. Human AML blasts (1.5 -2.5 *10⁴ cells/dish) were plated in 35-mm Petri dishes and incubated in a humidified CO₂ incubator. Colonies were counted as units of at least 30 cells were generated after plating (2 – 4 weeks).

Differentiation Assay.

20 Human TF1 cells were pre-treated for 12 days with AG-221 (1 μM) or vehicle and washed with PBS to remove residual GM-CSF. Cells were then induced to differentiate using EPO (2 unit/ml, StemCell Technologies) and Hemin (10 μM, Sigma). Induction continued for 12 days and the cell pellets were collected and subjected to real-time qPCR to detect fetal haemoglobin (HBG) and KLF-1 gene expression or flow cytometry to quantify CD71.

Analysis of intracellular polar metabolites extraction and 2-HG measurements.

25 Metabolites extractions and 2-HG measurements were performed as reported in Pinton et al., *Science* 2007, 315:659-663. In brief, viable mouse or human cells (1*10⁵ - 3*10⁶ viable cells) or peripheral blood (200 μl) were collected and extracted for polar metabolites. Dried metabolites were suspended into 100μl of deionized water or methanol, and analysed by liquid
 30 chromatography–mass spectrometry. Calculations of 2-HG were subsequently normalized based on the number of cells or volumes extracted.

Real-time qPCR analysis of leukaemia cell lines and primary blasts.

Total RNA was extracted using Trizol Reagent (Invitrogen) and RNA Isolation Kit (Ambion). cDNA synthesis and PCR amplification were performed using SuperScript™ One-

Step RT-PCR System with Platinum™ Taq DNA Polymerase (Invitrogen). Universal SYBR Green quantitative PCR assay (Sigma) was used to quantify gene expression by using the following specific oligos: mouse HoxA9 (fw: 5'-TCCTCCAGTTGATAGAGAAAAACA-3',
 rw: 5'-GCGAGCATGTAGCCAGTTG-3'); Meis1A (fw: 5'-

- 5 GGGGATAACAGCAGTGAGCA -3', rw: 5'-CCACGCTTTTTGTGA CGCTT-3'); Hprt (fw: 5'-GGTGGAGATGATCTCTCAACTT-3', rw:5'-CCAGCA AGCTTGCAACCTTAAC-3'); Tbp (fw:5'-GAAGAACAATCCAGACTAGCAGCA-3', rw:5'-CCTTATAGGGAAGTTCACATCACAG-3'); IDH2 (fw:5'-GCAGAGCC TCAGGCTCGCG-3', rw: 5'-GGCTGCTCTTGCGAGGT-3'), RPL0 (fw:5'-GCTTCC
 10 TGGAGGGTGTCC-3', rw:5'-GGACTCGTTTGTACCCGTTG-3'). Human HBG, KLF1, PIN1, ITGAM, LILRA5, RARA, PRAM1 and GAPDH gene expression were measured using the Taqman assay (Invitrogen) according to manufacturer's instruction.

2HG treatments.

- Human TF1 cells TF-1 cells were treated with vehicle (0.1% EtoH) or 0.1 mM (2R)-
 15 Octyl-2-HG and harvested at indicated time for Western blot analyses.

Cell Proliferation and viability Assay. Cells (TF1 or U937, 8-10 *10³ cells) were plated in complete medium on multiwell plates and treated with ATRA and/or ATO for 4 days. Cells were assayed every 24h for cell viability or proliferation by using CellTiter Glo, MTS Assay (Promega) according with manufacturer protocols.

- 20 *FACS Analysis of mouse and human leukaemia cells.*

- FACS analyses on human and mouse leukaemia cells were performed according to standard protocols. See, e.g., Pinton et al., *Science* 2007, 315:659-663. In brief, mice were euthanized and single-cell suspensions from the bone marrow were generated by bone crushing in PBS supplemented with 2% FBS. Cell suspensions were passed through 100µM cell
 25 strainers, centrifuged, and then re-suspended in 1-2 ml ACK red cell lysis buffer (GIBCO). Red blood cells were lysed on ice for 1min. Cell suspension were then washed in 2%FBS/PBS, centrifuged, and then re-suspended in 1ml of 2%FBS in PBS1X. Cells were subsequently processed for GFP+/YFP+ sorting, KLS isolation, or intracellular flow staining. Staining with specific antibodies (1:100) or Annexin V/ 7AAD was performed for 15 - 30 minutes at room
 30 temperature in the dark, following the protocol reported in Carracedo et al., *Nat Rev Cancer* 2013, 13:227-232. For intracellular phosphoflow cytometry on mouse leukaemia blasts, the cells were fixed in 4% PFA at 37°C, 5% CO2 for 10 minutes and permeabilized with 90% ice-cold methanol and processed as reported in Pandolfi et al., *Cancer Cell* 2017, 32:552-560. Flow cytometry data were collected on a J LSR II (VF415) or LSR J-Fortessa (JF415) flow

cytometer (Becton Dickinson). Resulting profiles were further processed and analysed using the FlowLogic or FloJo software. FACS Cell sorting was performed on a JF Aria II SORP or Sony SH800z Cell Sorter, with the support of the Dana-Farber Cancer Institute Flow Cytometry Core Facility.

5 The following antibodies were used for Flow Cytometry analyses:

Antibodies	SOURCE	IDENTIFIER
Rabbit mAb P44/42 MAPK (Erk1/2) (Alexa® 647)	Cell Signaling Technology	Catalog #5376S
Rabbit mAb P-Akt (S473) (D9E) XP® (Alexa® 647Conj)	Cell Signaling Technology	Catalog #4075S
Rabbit mAb Akt (pan) (C67E7) (PE conjugate)	Cell Signaling Technology	Catalog #8790S
Rabbit Ab Rabbit IgG Isotype (Alexa® 647Conj)	Cell Signaling Technology	Catalog #3452S
Rabbit (DAE1E) mAb IgG XP® Isotype Control (PE Conjugate)	Cell Signaling Technology	Catalog #5742S
Rabbit mAb (PE) P-Akt (T308) (D25E6) XP®	Cell Signaling Technology	Catalog #13842S
Rabbit mAb (Pac Blue) P-p44/42 MAPK(Erk1/2) (T202/Y204) (197G2)	Cell Signaling Technology	Catalog #14196S
Rabbit (DA1E) mAb IgG XP Isotype Control (Pacific Blue™ Conj)	Cell Signaling Technology	Catalog #9078S
Rabbit mAb Phospho-Histone H2A.X (Ser139) (20E3)	Cell Signaling Technology	Catalog #9718
Mouse (G3A1) mAb IgG1 Isotype Control (PE Conjugate)	Cell Signaling Technology	Catalog #6899S
Rabbit mAb IDH2 (D7H6Q)	Cell Signaling Technology	Catalog #12652S
PE/Cy Mouse IgG1, κ isotype Ctrl. Clone: MOPC-21	Biologend	Catalog #400125
PE Mouse IgG1, κ isotype Ctrl. Clone: MOPC-21	Biologend	Catalog #400111
7-AAD Viability Staining solution	Biologend	Catalog #420403
Pacific Blue™ Mouse IgG1, κ isotype Ctrl. Clone: MOPC-21	Biologend	Catalog #400131
PE/Cy7 anti-human CD11b (activated). Clone: CBRM1/5	Biologend	Catalog #301412
PE/Cy anti-human CD14. Clone: 63D3	Biologend	Catalog #367112
Pacific Blue™ anti-human CD15 (SSEA-1). Clone: W6D3	Biologend	Catalog #323022
Purified mouse monoclonal IgG anti-hCEBP ε. Clone: 695345	R&D Systems	Catalog #MAB6726
Goat anti-Rabbit IgG (H+L) Secondary Antibody, Alexa Fluor 546	Thermo Fisher Scientific	Catalog #A-11035
Biotinylated Anti-rabbit IgG (H+L)	Vector Laboratories	Catalog #BA-1000
Rabbit polyclonal IgG RARα (C-20)	Santa Cruz Biotechnology	Catalog #sc-551
Anti-Human CD14 PE. Clone: 61D3	eBioscience	Catalog #12-0149-41

Intracellular Flowcytometry on patient's samples.

Human AML blasts ($2-5 \times 10^4$ cells) were fixed in 4%PFA for 5 minutes at 37 °C, washed twice with cold PBS and permeabilized with 90% methanol (ice-cold). Pellets were washed twice in ice cold PBS and resuspended in Staining buffer (0.5%BSA in PBS1X) and
 5 incubated (at 4 °C) with anti – PIN1 antibody (Rb mAb to PIN1, ab76309, abcam) or respective IgG control (Rb IgG monoclonal isotype control, ab172730, abcam). Cells were washed twice and incubated with Donkey anti – rabbit Alexa Fluor 647 for 1 hour at room temperature. Pellets were washed three times before flow analysis.

Cytospins and May-Grunwald –Giemsa Staining.

10 Blood smears and bone marrow cytospin slides were prepared according to the methods reported in Mason et al., *Am J Hematol* 2018, 93:504-510. Slides were fixed in 100% methanol for 10 minutes and air dried completely before staining. Slides were stained in May-Grünwald solution (Sigma; 1:20 dilution in dH₂O) for 5 minutes, washed twice in dH₂O, and then stained with the Giemsa solution (Sigma; 1:20 dilution in dH₂O) for 15
 15 minutes. Finally, slides were washed in dH₂O and air dried. Slides were sealed with Cytoseal and then analysed on a Nikon Eclipse 50i microscope or Olympus BX41 equipped with an Olympus Q Color 5 camera.

Immunohistochemistry of mouse tissues.

Tissues were fixed in 4% paraformaldehyde overnight, paraffin embedded, and then
 20 sectioned at 5µm. After deparaffinization and rehydration, antigen retrieval was performed in a pressure cooker with sodium citrate buffer at 95 °C for 25 minutes. Sections were incubated in a 0.3% H₂O₂ solution in 1x PBS, and then a 10% serum solution in 1x PBS for 30 minutes each solution was used to block endogenous peroxidase and background from the secondary antibody, respectively. The sections were stained with the primary antibodies: Ki67 (1:200,
 25 Thermo Fisher Scientific, #MA5-14520) or Anti-Myeloperoxidase antibody (1:50, abcam #ab9535), or Phospho-Histone H2A.X (1:200, Cell Signalling #9718S) and incubated in a biotinylated secondary antibody in 1x PBS (1:500-1:1000) at room temperature for 30 minutes. The Vectastain ABC Elite kit was used to enhance specific staining, and the staining was visualized using a 3'-diaminobenzidine (DAB) substrate. Stained sections were
 30 counterstained using hematoxylin and dehydrated before they were sealed with a coverslip with Richard-Allan Scientific® Cytoseal™ XYL Mounting Medium.

Immunofluorescence.

Cells were fixed at room temperature in 4% paraformaldehyde for 20 minutes and permeabilized for 10 minutes using a 0.5% Triton X-100 solution in 1x PBS. Blocking was

performed for one hour using a 5% bovine serum albumin solution in 1x PBS. The sections were stained with the P-Histone H2A.X primary antibody (1:200) in a 2% bovine serum albumin and 1x PBS solution at 4 °C overnight, and then incubated with a goat anti-rabbit IgG Alexa Fluor 546 conjugate (1:1000) in PBS1X at room temperature for 1 hour before
 5 staining with DAPI (1:1000) in 1x PBS for 10 minutes. Cells were washed with 1x PBS and milli-Q water before being sealed with a coverslip with Fluorescence Mounting Medium.

Transcriptome profiling using RNA quantification sequencing.

RNA derived from resistant and sensitive leukaemia cells was subjected to next-generation sequencing (NGS) to generate deep coverage RNASeq data. For each treatment
 10 and control group, sequencing was performed on several biological samples (mIDH2-DEPENDENT leukaemia n=7, mIDH2-INDEPENDENT leukaemia n=6, Hoxa9/Meis1a leukaemia n=3). Sequencing libraries of Poly A selected mRNA were generated from the double-stranded cDNA, using the Illumina TruSeq kit according to the manufacturer's protocol. Library quality control was checked using the Agilent DNA High Sensitivity Chip
 15 and qRT-PCR. High quality libraries were sequenced on an Illumina HiSeq 4000. To achieve comprehensive coverage for each sample, we generated about 30-35 million single end reads.

RNA-Seq / Gene expression analysis.

The raw sequencing data was processed to remove any adaptors, PCR primers, and low quality transcripts using FASTQC and fastx. These provided a very comprehensive
 20 estimate of sample quality on the basis of read quality, read length, GC content, uncalled bases, ratio of bases called, sequence duplication, adaptors, and PCR primer contamination. These high quality, cleaned reads were aligned against the mouse transcriptome (mm10) using bowtie1 with parameters: p 12 -q -n 2 -m 1 -S -best. Gene expression measurement was performed from aligned reads by counting the unique reads using htseq-count (v0.6.1)
 25 with parameters: -a 10 -m intersection_strict. The read count based gene expression data was normalized and analyzed using the “DESeq2 R package”. The differentially expressed genes were identified on the basis of FDR value and fold change. Genes were considered significantly differentially expressed if the multiple hypothesis test-t corrected p-value was <0.05. The resulting gene expression matrix was subsequently subjected to gene set
 30 enrichment analysis (F-GSEA, <https://bioconductor.org/packages/release/bioc/html/fgsea.html>) and then visualized through the R pheatmap package.

WES sequencing and read alignment.

Sequencing reads from each sample were aligned to the C57BL/6J reference genome

(GRCm38) using BWA, and then merged into a single BAM file using Picard tools (v. 1.119). To improve SNP and indel calling, the Genome Analysis Toolkit (GATK) ‘IndelRealigner’ (www.broadinstitute.org/gatk/) was used to realign reads near indels from the Mouse Genomes Project (v5). The BAM files were then re-sorted and quality scores were
 5 recalibrated using GATK ‘Table Recalibration’ and PCR duplicates were marked using Picard ‘MarkDuplicates’.

Variant Calling.

SNPs and indels were identified using the SAMtools mpileup function and variants were called using VarScan suite (v.2, varscan.sourceforge.net). Variants were filtered using
 10 an in-house Perl script, to account for SNVs and indels not included in the control sample (wild type), for mapping quality, number of mismatches, read depth, and known variants (Mouse genome project, mmp v.3). Finally, variants were annotated using Annovar (<http://annovar.openbioinformatics.org>, v.20150322)

Copy Number analysis.

15 Copy number variations (CNVs) were called using the binary segmentation algorithm implemented in the “DNACopy R package”. The default parameters and segmented data were merged using mergeSegments.pl from VarScan suite.

Example 1. Development of cell autonomous resistance to mIDH2 targeting by serial transplantation of mouse AML

20 A previously generated mIDH2 (IDH2^{R140Q}) dependent AML model was used to predict mechanisms promoting resistance to mIDH2 inhibitors in patients. Using a serial transplantation protocol (Fig. 1A) mIDH2 dependency was manipulated in a model of mouse bone marrow (BM) cells overexpressing mIDH2 transduced with Hoxa9-GFP and Meis1a-IRES-YFP retroviruses transplanted in a primary host in the presence or absence of
 25 doxycycline to induce mIDH2 expression. Dependence on mIDH2 in secondary transplanted disease was confirmed (Figs. 1B-D, 2nd Recipients), and mIDH2 independent (resistant) disease was observed upon transplant of blast cells from Dox+ 2nd recipient mice (Figs. 1B-D, 3rd Recipients). Importantly, no differences were observed in the survival of mIDH2 ON or mIDH2 OFF AMLs (Fig. 6A), and 3rd recipients demonstrated an extensive proliferative
 30 infiltration of extra-medullary tissues including spleen, liver and kidney (Figs. 6B and 6C). As expected, it was observed that 3rd recipients had much higher levels of disease infiltration as compared with 2nd recipient mice (Fig. 6D) and a higher proliferative index in the infiltrates (Figs. 6E and 6F).

2-HG was strongly reduced in the mIDH2 OFF setting compared to respective

mIDH2 ON 19 condition (Fig. 1F), and IDH2R140Q expression was appropriately repressed in the mIDH2 OFF setting for 2nd and 3rd recipient mice (Fig. 1G), with stable expression of both Hoxa9 (Fig. 1H) and Meis1a (Fig. 1I) in these leukemias.

To further evaluate if the independence from mIDH2 in vivo represents a cell autonomous process, in vitro culture systems were established to assay stemness and the proliferative potential of these AMLs (Figs. 6G and 6H). In these conditions, AMLs from both 2nd and 3rd recipient mice demonstrated a similar capacity to generate colonies in methylcellulose (Fig. 1J, vehicle treated). Targeted inhibition of mIDH2 activity in vitro decreased the colony forming capacity of AMLs isolated from 2nd recipients, but not of those cells isolated from 3rd recipient (Figs. 1J and 1K), though 2-HG levels were reduced (Fig. 1I). All together these data demonstrate the independence from mIDH2 expression corresponds with resistance to mIDH2 inhibition and occur cell autonomously during time.

Example 2. De novo resistance is associated with a distinct metabolic switch to one-carbon metabolism and altered redox balance

Since the expression of mIDH2 directly impacts cellular metabolism, polar metabolites were extracted from sensitive and resistant AML cells from both 2nd and 3rd transplanted mice (Fig. 7A). Comparison of metabolite abundance established multiple metabolic processes altered between the mIDH2 leukemic states (Fig. 2A and 2B). Resistant cells were significantly enriched in glycerophospholipid, pyrimidine, purine, cysteine and methionine metabolism pathways (Fig. 2B). These pathways can be fueled by one-carbon metabolism, which consists of both the folate and the methionine cycles (Figs. 7B and 7C). Importantly, one-carbon metabolism is a main cellular engine contributing to nucleotide metabolism and global methylation, as well as cellular redox status. Similar to recent findings for purine metabolism in cancer, a general increase in purine precursors in 3rd compared to 2nd recipient AMLs was observed (Figs. 7D-F).

Finally, one-carbon metabolism also contributes to redox balance through the biosynthesis of glutathione (GSH). Glutathione metabolism was the most significant and highly enriched metabolic pathway in resistant AML cells (Fig. 2C). 3rd recipient AML cells demonstrated high levels of glutathione and cysteine, glutathione's major precursor (Fig. 2D), suggesting that these cells may have an altered redox balance. Indeed, altered NADPH/NADP⁺ and NADH/NAD⁺ ratios confirm this oxidative stress (Fig. 2E), and excessive ROS levels were visualized using MitoSOX™ Red staining (Figs. 2F-G), especially relative to Hoxa9/Meis1a driven leukemias lacking mIDH2 (Fig. 7G).

In this connection, it was observed that mouse early stage HoxA9/Meis1a/mIDH2

leukemia at early (sensitive) stage were marked by the same features of late (resistant) leukemia. See Figs. 2J(a-e) and 3K(a-e). This evidenced that the mutant IDH leukemia at early stage show the same characteristics/features of the late stage leukemia, when using ATO combined with ATRA, providing a rational for the utilization of ATO and ATRA not only in mutant IDH2 leukemia at late sage (when cells became resistant to the inhibitor), but also at early stage (when cells are sensitive to the inhibitor).

To evaluate genotoxic stress, staining for γ -H2A.X was carried out on mouse bone marrow leukemia cells (Fig. 2H). Similarly, staining for γ -H2A.X was carried out on infiltrated spleens from both 2nd and 3rd transplanted mice (Fig. 2I). Evidence of increased DNA damage suggests that altered redox balance may represent a vulnerability within mIDH2 leukemias that could be targeted for therapy.

Example 3. Translocations of hematopoietic genes are associated with mIDH2 resistant leukemias

To determine if such genotoxic stress may contribute to AML evolution through targeted mutation whole exome sequencing (WES) of a 2nd recipient and three of its derived 3rd recipient 'daughter' leukemias was performed (Fig. 8A). Non-synonymous base substitutions and both G>A:C>T and A>G:T>C transitions accounted for two-thirds of the single base substitutions (SBS) observed, as is frequently found in cancers without biased substitution (Fig. 8B). Additionally, principal component analysis (PCA) established that these leukemias did not appear to cluster (Fig. 8C). Although a variety of mutations were observed in progression from 2nd to 3rd recipient leukemias sequenced the majority of these mutations identified were non-synonymous single nucleotide variants (SNV), and included a number of indel mutations to a variety of genes. Only *Sp140* appeared to be commonly mutated across all samples (Fig. 8D). Each of these leukemias harbored a unique point mutation in *Sp140* (*Sp140*^{Y5D} in 3rd_11; *Sp140*^{R94Q} in 3rd_08; *Sp140*^{C203G} in 3rd_09) (Fig. 8D). *Sp140* is a component of the PML nuclear body, however, no obvious or consistent alteration to Sp140 or PML localization was observed for these mutant variants.

Global changes across these leukemia genomes identified a distinct pattern of both genomic amplification and deletion in each AMLs (Fig. 8E). Several leukemia-associated genes were found to be affected, including amplification of the *Flt3* proto-oncogene (3rd_08) and deletion of the tumor suppressors *Dok1* and *Trp53* (3rd_09) and *Dok3* and *Dok4* (3rd_11) (Fig. 8E). Interestingly, a number of translocations shared amongst 3rd recipient leukemias may be particularly relevant in the context of this disease. These translocations involve known hematopoietic genes including *Rae1* (*Rae1*; *Pappa*; t(4;2)), *Meis1* (*Eel1*; *Meis1*;

t(11;10)), and both *Ppp1r13b* (*Aspp1*) and *Cadps2* (*Ppp1r13b:Cadps2*; t(6;12)) (Fig. 8E). The translocation involving *Ppp1r13b* and *Cadps2* was of particular interest since loss of function for both of these genes has been implicated in hematopoietic stem cell maintenance. The *Ppp1r13b* gene is also implicated in leukemia development, coordinating tumor
 5 suppression with the *TP53*. While the *Ppp1r13b:Cadps2* translocation was only identified in two out of three of the 3rd recipient AMLs (3rd_08 and 3rd_11), the third leukemia showed genomic loss of *Trp53*, suggesting that targeting of *Trp53* may contribute to progression.

Example 4. Aberrant ATRA signaling characterizes resistance to mIDH2 withdrawal

RNAseq analysis was performed to create a global picture of leukemia progression
 10 from a state of mIDH2 dependence to independence (Fig. 9A). Primary leukemias derived from Hoxa9;Meis1 overexpression without mIDH2 was used for comparison. PCA analysis identified distinct clusters of 2nd and 3rd recipient leukemias (Fig. 9B). Differential gene expression patterns highlighted clear differences between sensitive and resistant leukemias (Fig. 3A), suggestive of common pathways leading to independence from mIDH2. A gene-
 15 centric PCA identified genes associated with the Jun/Fos AP-1 transcription factor family (*Jun*, *Fos*, *Fosb*), granulopoiesis and myeloid differentiation (*Myo*, *Lyz2*, *Ngp*), and inflammation (*Zfp36*) as differential (Fig. 9C). Altered expression status of myeloid granule components also suggested that 2nd and 3rd recipient leukemias may represent different stages of myeloid development (Fig. 9C).

Furthermore, significant enrichment of cancer associated signaling pathways
 20 including MAPK, Erk, PI3K and Akt was observed in resistant leukemias, as well as an enrichment in inflammation and tumor microenvironment (IL-1, IL-6, ILK, MIF, TNFR2, etc.) (Fig. 3B). Gene set enrichment analysis (GSEA) identified similar pathways altered in resistant AMLs, including enrichment of Kras, MAPK, TNF signaling and response to
 25 Tretinoin (aka. all-trans retinoic acid; ATRA) (Fig. 3C), while an interactive network of all differentially regulated genes highlighted the central role of Erk/MAPK signaling in defining the signatures associated with resistant disease (Fig. 8C) consistent with previously published data, suggesting that the present model recapitulates mechanisms relevant to human AML. Indeed, cancer signaling pathway activation was confirmed by flow cytometry analysis (Fig.
 30 8E).

Intriguingly, ATRA-responsive genes were highlighted by GSEA
 (Tretinoin_Response_DOWN gene cluster), suggesting a more undifferentiated state as mIDH2 AML evolves to resistance (Fig. 3D). Indeed, further analysis highlighted a shared set of ATRA responsive genes previously reported to be regulated by inhibition of the

demethylase LSD1 and associated with ATRA sensitivity (Fig. 3E). This prompted us to hypothesize mIDH2 leukemia might harbor sensitivity to ATRA. Indeed, both pharmacological and sub-pharmacological doses of ATRA demonstrated profound differentiation effects on mIDH2 expressing AMLs (Fig. 3F). To determine if this is also the case in human hematopoietic cells expressing mIDH2, TF1 cell lines overexpressing the mIDH2 allele and increased 2-HG levels were generated (Fig. 3G). Treatment of mIDH2 overexpressing TF1 with ATRA (10^{-6} M) resulted in increased differentiation as compared to controls (Fig. 3H), with a concomitant decrease in cell growth (Figs. 3I and 3J). In this connection, it was observed that continued inhibition of LSD1 impacted the intracellular levels of oxidative stress in TF1 cells (Fig. 4M).

Furthermore, these data were replicated in U937 cells overexpressing mIDH2 treated with ATRA in the presence of vitamin D (Figs. 8G-J).

Example 5. PIN1 upregulation is a common hallmark of the resistant state

The mechanism by which mIDH2 cells conferred sensitivity to the differentiation effects of ATRA was evaluated. ATRA has been recently established as a specific inhibitor of the PIN1 proto-oncogene. PIN1 has been reported to be upregulated by C/EBP α -p30, and can promote activation of MAPK. Similarly, it promotes PI3K activity and regulates AKT's stability and phosphorylation. While ATRA is a specific inhibitor of PIN1, it is also important to note that PIN1 itself can be a negative regulator of ATRA, and there is an expanding role for PIN1 as a negative regulator of hematopoietic differentiation.

It was therefore hypothesized that PIN1 may contribute to proliferative signaling (MAPK and PI3K dependent), increased survival to ROS-mediated apoptosis, and the ATRA mediated differentiation block observed. Indeed, a marked upregulation of Pin1 protein in both Dox ON and OFF mIDH2 leukemias at late stages of progression (Fig. 4A, Lanes 5-8). This suggested that 3rd recipient leukemias may be sensitized to ATRA and that targeting PIN1 may relieve both the oncogenic signaling and the differentiation block in these cells.

Next, the status of RAR genes in differentiation was examined and strong downregulation of both Rar α and Rxr α in 3rd recipient AML was observed (Fig. 4A, Lanes 5-8 compared with Lanes 1 and 2), in line with Pin1 degradation of Rar α . Additionally downregulation of the tumor suppressive p42 form of C/EBP α , and decreased expression of C/EPB ϵ were observed, which may also contribute to a primitive differentiation state of these cells (Fig. 4A).

Surprisingly, ATRA-sensitive retinoic acid receptors were upregulated in mIDH2 2nd recipient AML, as well as increased C/EBP α and C/EBP ϵ (Fig. 4A, Lanes 3 and 4). However,

PIN1 itself, the negative regulator of the pathway, was also concomitantly induced (Fig. 4A, Lanes 3 and 4). However, validated Rara/Rxra responsive genes were not transcriptionally activated, suggesting that RARs are transcriptionally inactive.

mIDH2 overexpressing TF-1 and U937 cell lines were used to understand if the same was true for human leukemia. Indeed, both TF-1 (Fig. 4B) and U937 (Fig. 4C) lines demonstrated upregulation of PIN1 in the presence of mIDH2, along with retinoic acid receptors and C/EBP α . Inhibition of PIN1 by using a specific inhibitor (Juglone) clearly demonstrated PIN1 to be required for blocking differentiation driven by erythropoietin (EPO) (Fig. 4D) and reduced their clonogenic ability (Figs. 4E, 10A, and 10B). In addition, knockdown of PIN1 resulted in up-regulation of the differentiation associated HGB, KLF1, and GATA2 genes (Fig. 4F-H). Similarly, PIN1 overexpression promoted expression of stem markers CD34 and CD117 in mIDH2 expressing cells (Figs. 4I, 4J, and 10C).

Furthermore, this was also observed to be the case for TF-1 cells overexpressing mutant IDH1. Importantly, comparison of a cohort of primary AML patients with or without mIDH2 mutation identified increased levels in PIN1 mRNA levels in these patients. Although the Cancer Genome Atlas (TCGA) AML dataset represents a cohort of primary AML patients at early diagnosis, concomitant analysis demonstrated variable PIN1 expression, and patients harbouring mIDH mutations had a trend towards higher PIN1 expression. Indeed, a similar trend was observed in PIN1 protein levels assessed by intracellular flow cytometry from primary AML patients.

Example 6. ATRA and ATO combination for treatment of mIDH2 AML

Given ATRA sensitivity and extensive ROS in mIDH2 AML, it was hypothesized that the combination of the pro-oxidant ATO together with ATRA may be utilized to treat mIDH2 AML. While Hoxa9/Meis1a leukemias alone showed some sensitivity to only the combination, 2nd recipient leukemias demonstrated sensitivity to both single agents and a synergistic response to the combination (Fig. 11A). Late stage disease (e.g., 3rd recipient, mIDH2-independent leukemias in both the presence and absence of mIDH2) demonstrated a similar level of response to both single agent and combination treatments disease both in the presence and absence of the mIDH2 oncogene (Fig. 11B), indicating efficacy in both mIDH2 sensitive and resistant disease. To confirm these findings in vivo a mIDH2 independent (e.g., resistant) mouse AML was transplanted into BL6 (Fig. 11C) and placed mice on a treatment regime as outlined in Fig. 4K. Both single agent and combination treatments demonstrated efficacy as measured by increase in mean survival of mice treated (Fig. 11D). Strikingly, a number of mice treated with the ATO/ATRA combination demonstrated a strong

differentiation response in the leukemic blasts associated with an excessive infiltration of mature myeloid cells in the lung (Fig. 11E-G). Thus, steroid dexamethasone was also administered. As outlined in Figs. 4K-L, dexamethasone treatment successfully overcame the differentiation syndrome, and enabled extensive survival benefits upon treatment with the ATO/ATRA combination.

Example 7. Differentiation therapy for the treatment of human mIDH2 AML

An *in vivo* pre-clinical study was performed using the U937 cell line in immunocompromised NSG host mice (Fig. 12A). 21 days after initiating treatment all control mice had succumbed to disease, while only mIDH2 overexpressing U937 treated with the ATO/ATRA combination remained alive (Fig 12B). Bone marrow (BM) analysis demonstrated a significant decrease in the percentage of GFP+ leukemic blasts, and increased differentiation, in both the ATRA and ATO/ATRA treated cohorts of mice transplanted with mIDH2 overexpressing cells (Figs. 12C-E). Importantly, only mIDH2 expressing U937 cells treated with the combination therapy demonstrated the mature myeloid CD11b marker amongst all the cohorts analyzed (Figs. 12F and 12G). Additionally, reduced spleen weights were observed in mIDH2 expressing U937 cells treated with ATO/ATRA, demonstrating the power of this treatment combination to impact the severity of disease (Fig. 12H).

Next, a panel of human primary AMLs was treated with ATO/ATRA to evaluate their potential for clinical efficacy, as measured by colony formation in methylcellulose.

Importantly, only AML harboring mIDH2 showed a significantly reduced number of colonies upon treatment, and strong synergy was observed in combination treatments (Fig. 5A). NSG mice transplanted with human primary mIDH2 positive AML (Fig. 5B) were subjected to the treatment protocol as outlined in Fig. 5C to evaluate *in vivo* efficacy. To minimize onset of DS a metronomic dosing schedule was utilized. Importantly, a notable survival advantage for both ATO and ATRA treated mice was observed, while combination treated mice appeared to demonstrate a durable remission in most cases (Fig. 5D). Interestingly, the single agent ATO and ATRA treated cohorts demonstrated a significant increase in differentiating blasts in the bone marrow as evaluated by morphology (Figs. 5E and 5F).

Example 8. Therapeutic vulnerabilities driven by mIDH

Based on the above results, it was hypothesized that PIN1, ATRA sensitivity and high ROS levels represented therapeutic vulnerabilities for intervention in mIDH leukemia. Importantly, ATRA can inhibit the enzymatic activity of its negative regulator PIN1 through direct binding, as reported in Wei et al., *Nat. Med.* 2015, 21:457-466. To test this, the ability of ATRA was evaluated to promote differentiation of leukemic blasts in a murine model of

AML. Indeed, both pharmacological and sub-pharmacological doses of ATRA demonstrated striking differentiation effects on murine mIDH2 expressing AMLs, both in the mIDH2 dependent and independent phases of disease evolution (Fig. 13a).

To further determine whether the sensitivity to ATRA is indeed mIDH2-dependent and whether this would also be the case in human hematopoietic cells expressing mIDH2, studies were conducted with TF1 cell lines overexpressing the mIDH2 allele. Treatment of mIDH2 overexpressing TF1 with a pharmacological ATRA dose (10^{-6} M) resulted in a dramatic difference in cellular differentiation as compared to controls (Figs. 13b and 13c), with a concomitant decrease in cell growth (Fig. 13d). Furthermore, as pointed out above, a study was performed to generate U937 cells overexpressing the mutant isoform R140Q of IDH2. This cell line has been extensively utilized to study how the fusion genes of APL (e.g. PML-RAR α) cause a block in cellular differentiation. Once again, treatment of mIDH2 - overexpressing U937 with pharmacological doses of ATRA (10^{-6} M) in the presence of vitamin D, resulted in a dramatic difference in cellular differentiation as compared to controls and a concomitant decrease in proliferation (Fig. 13e), while treatment of TF1 cell line overexpressing mIDH1 also demonstrated a similar decreased proliferative response. To determine if this sensitivity was a dependency on PIN1 activity, a study was conducted to assess whether PIN1 could act as a critical mediator of the block of differentiation observed in mIDH2 leukaemia through both pharmacological and genetic targeting. Inhibition of PIN1 by using a specific inhibitor (Juglone) clearly demonstrated Pin1 to be differentially required in mouse AML harbouring mIDH2 which showed a significantly decreased colony forming capacity upon Pin1 targeting (Fig. 13f), while mIDH2 TF1 cells also demonstrated a similar sensitivity as demonstrated by reduced clonogenic capacity upon specific targeting of PIN1 by Juglone (Fig. 13g). Similarly, dependence on *Pin1* in mIDH2 independent AML cells was demonstrated by genetic silencing, which significantly attenuated their colony forming capacity. Congruent with this behaviour, mIDH2- overexpression in TF1 cells inhibited the induction of the differentiation marker CD71 upon differentiation driven by erythropoietin (EPO), while specific targeting of PIN1 with Juglone restored the upregulation of this marker. Also similarly, these findings were mimicked by genetic targeting of PIN1 in mIDH2- overexpressing TF1 cells, with up-regulation of the CD71 and CD44 markers detected by flow cytometry and the differentiation associated *HGB* and *KLF1* genes demonstrating the increased sensitivity to differentiation upon loss of PIN1. Critically, the overexpression of PIN1 favoured the maintenance of the distinctive undifferentiated state of mIDH2 cells, even upon treatment with the specific mutant IDH2 inhibitor AG-221,

rendering them resistant, highlighting the dependence on Pin1 and underscoring the relationship between mIDH2 and PIN1.

In the same fashion, the therapeutic opportunity afforded by ROS induction was evaluated in this model through the use of arsenic trioxide, hypothesizing that the pro-oxidant activity of ATO could cause genotoxic stress and cell death. Indeed, the murine AML model demonstrated increased apoptosis in both a sensitive mIDH2 dependent and resistant mIDH2 independent setting (Fig. 13h), while the expression of mIDH2 in TF1 cells conferred sensitivity to induction of apoptosis upon treatment with ATO that was not observed in control infected cells (Fig. 13i). Interestingly, we also demonstrated the ability of mIDH2 and PIN1 overexpression to facilitate the induction of ROS in TF1 cells, while TF1 cells overexpressing mIDH1 were also found to respond to treatment with ATO.

Thus, it was hypothesized that these agents may function as targeted therapies towards mIDH AML, especially as both ATRA and ATO have also been shown to be able to directly and indirectly target PIN1 at multiple levels consistent with our data. Combining these agents *in vitro* against AML cells isolated from Hoxa9/Meis1a and Hoxa9/Meis1a/mIDH2 murine leukaemias, only mIDH2-expressing AML cells demonstrated enhanced sensitivity to the combination as measured by reduced colony forming capacity. Indeed, 2nd recipient leukaemias demonstrated sensitivity to both single agents and a synergistic response to combination (Fig. 13j). Late stage disease (i.e. 3rd recipient, mIDH2-independent leukaemias in both the presence and absence of mIDH2) demonstrated a similar pattern of responses, showing sensitivity to single agents and synergistic effect of combination treatments, indicating efficacy in both mIDH2 dependent and independent disease and consistent with the hypothesis based on the AML RNAseq data (Fig. 4c-f). Finally, to ensure that the efficacy conferred by treatment of mIDH2 AML with the ATRA/ATO combination was not due simply to oncogenic interaction with HOXA9 and Meis1a, we evaluated the ability of ATRA and ATO to effectively target mIDH AMLs derived from a number of distinct genetic contexts. In particular we analysed the clonogenic capacity of murine leukaemia cells derived from AML with mIDH2 in combination with FLT3-ITD, and mIDH1 in combination with NPMc+ or with FLT3-ITD/NPMc+ (Fig. 13k). Each of the AMLs harbouring mIDH demonstrated exquisite sensitivity to the ATRA/ATO combination as compared to AML derived from MLL-AF9 overexpression with wild type IDH alleles, as negative controls. Murine AML arising from the combination of NPMc+/FLT3-ITD alone were used as positive control as this genotype was previously shown to confer sensitivity to ATRA/ATO. However, mIDH conferred superior sensitivity

even when compared with this genetic make-up (Fig. 13k).

Example 9. ATRA and ATO combination for treatment of mIDH AML

To evaluate the efficacy of this treatment in a human setting, an *in vivo* pre-clinical study was carried out using our U937 cell line in immunocompromised NSG host mice (Fig. 14a). 21 days after initiating treatment mice engrafted with mIDH2-overexpressing U937 treated with the ATO/ATRA combination remained alive, while all control mice had succumbed to disease (Fig. 14b). Bone marrow (BM) analysis demonstrated a significant decrease in the percentage of human leukemic blasts, and increased differentiation, in both the ATRA and ATO/ATRA treated cohorts of mice transplanted with mIDH2-overexpressing cells (Fig. 14c-14e). Importantly, only mIDH2 expressing U937 cells treated with the combination therapy demonstrated the mature myeloid CD11b marker amongst all the cohorts analysed (Fig. 14f and 14g). Reduced spleen weights were observed in mice transplanted with mIDH2-expressing U937 cells treated with ATO/ATRA, demonstrating the power of this treatment combination to impact the severity of disease (Fig. 14h).

We next treated a panel of human primary AMLs, including eleven harbouring an IDH2 mutation, 3 harbouring an IDH1 mutation, and seven AMLs, with wild type IDH, with ATO/ATRA to evaluate their potential for clinical efficacy, as measured by colony formation in methylcellulose. Importantly, only AML harbouring mIDH2 showed significantly reduced number of colonies upon treatment (Fig. 15a), and strong synergy was observed in combination treatments (Fig. 15a). Interestingly, in the case of patient #AML_2, colonies arising from single agent ATRA or ATO treatment gave primarily blast-like morphology, while the combination treatment resulted in almost complete loss of this morphology. Moreover, we treated one mIDH2 AML displaying *de-novo* resistance to mIDH2 inhibitor (AG-221). Once again, this sample displayed PIN1 elevation and remarkable sensitivity to these agents.

Given the promising efficacy associated with this treatment *in vitro*, we next determined the impact of treatment *in vivo*, using GEMM and human AML PDX models. Using 3rd transplanted mIDH2 independent murine leukaemia cells, we established a pre-clinical study to evaluate the efficacy of the ATRA/ATO combination *in vivo*. For each of the single treatments we observed a significant enhancement in survival of these mice, associated with reduction in bone marrow associated blast-like phenotype. Importantly, while the combination treatment also demonstrated an increased survival capacity over vehicle-treated mice, several of the mice treated with ATRA/ATO died not as a result of frank AML, but rather succumbed to differentiation syndrome (DS) characterized by infiltration of lungs with

mature myeloid cells demonstrating strong myeloperoxidase (MPO) expression, a feature also associated with APL patients treated with ATRA. To further evaluate the efficacy of this treatment combination in AML harbouring mIDH2, we carried out two human PDX trials with two independent human primary AMLs (Figs. 15b and 15d). NSG mice transplanted with human primary mIDH2 positive AML were subjected to the treatment protocol, and to minimize onset of differentiation syndrome (DS), we utilized a metronomic dosing schedule, as for APL patients' treatment protocols. Importantly, for one PDX trial we observed a notable survival advantage for both ATO and ATRA treated mice, while combination treated mice appeared to demonstrate a durable remission (Fig. 15b), and single agent ATO and ATRA treated cohorts demonstrated a significant increase in differentiating blasts in the bone marrow as evaluated by morphology (Fig. 15c). Our second PDX trial, also demonstrated efficacy of ATO and the combination of ATRA and ATO with extensive differentiation of human blasts observed in the bone marrow at the time of euthanasia (Fig. 15e). Importantly, for both PDX trials, and in particular the second case, aggressive DS was observed (Figs. 15f and 15g), highlighting the sensitivity of mIDH AML to combination of ATRA and ATO and a potential need for caution when treating human patients.

Thus, these data indicate that mIDH1- or mIDH2 -driven leukaemias are highly responsive to the classic APL treatment protocol, and differentiation therapy may represent a unique and complementary therapeutic approach for combination with mIDH targeting agents.

Clearly, the above studies identified vulnerabilities associated with the expression of mIDH2 and the leukemia evolution of a mIDH2 independent state (Fig. 16). Further, we extended our analysis and generalized our conclusions taking advantage of a number of mIDH1/2 mouse models of various genotypes, human mIDH1/2 cell lines, as well as primary mIDH1/2 AMLs.

Common outputs identified in the progression of mIDH2 AML include altered redox balance with ROS accumulation, genotoxic stress, genetic mutation and translocation, MAPK/PI3K hyper-activation, and an aberrant ATRA transcriptional program (Fig. 16). Indeed, the relevance of MAPK dependence and relevance of differentiation in therapy has recently been highlighted for patients treated with Enasidenib, thereby validating the molecular underpinnings of the pre-clinical model used in the above studies. See, e.g., Amatangelo et al., *Blood*, 2017, 130:732-741.

It has been shown that mIDH2-dependent molecular and metabolic rewiring can drive ROS accumulation, and a dependence on PIN1 with the prolyl isomerase acting as a critical

node in mediating a differentiation block in spite of the increase ATRA sensitization triggered by mIDH. While PIN1 is proto-oncogenic in its action triggering the activation of MAPK/PI3K signaling, it is also known to trigger ROS production through mitochondrial activation. As leukemic blasts are known to be particularly sensitive to ROS levels, this in turn creates an inherent vulnerability, which negatively impacts stem cell maintenance and facilitates exhaustion. Indeed, the above data illustrate how natural selection towards a mIDH2-independent state further exacerbates a PIN1-ATRA block and the altered cellular balance towards extreme levels of oxidation.

On this basis, we identify the classical targeted combination therapy of APL, ATRA and ATO, as a therapeutic strategy for the treatment of AML harboring mutation in mIDH1/2, independent of multiple and diverse additional genetic events. This is highlighted on the one hand by the exquisite sensitivity to this combination therapy of our multiple leukaemic models harbouring mIDH along with other established driver mutations (e.g., NPMc+ and FLT3-ITD), and on the other hand by the fact that ATRA/ATO are an effective combination also against mIDH AML that has drifted to independence and has accumulated additional genetic mutation and translocation events. Such an approach is of utmost relevance in view of multiple resistance mechanisms driven by a diverse mutational landscape might be at play to restore the block in differentiation, highlighting the need for additional therapeutic approaches to overcome such resistance.

In APL, the ATRA/ATO combination selectively targets the PIN1-PML-RAR α oncogenic node, while in mIDH-leukemia, the ATRA/ATO combination treatment selectively targets the oncogenic mIDH-PIN1-RAR α node. In addition, using a cohort of primary human AML cells that harbor either mIDH1 or mIDH2, we have been able to clearly demonstrate the potential efficacy of the ATRA/ATO combination to treat this subset of AML. Our analysis points to the ability of these oncogenes to sensitize to the effects of ATRA plus ATO. Thus, the combination of these agents with specific mIDH targeting agents may in turn potentiate and extend the efficacy of these inhibitors. Indeed, the fact that mIDH blocks myeloid differentiation and the ATRA response similarly to PML-RAR α , also suggests that rare atypical APL cases lacking the usual PML/RAR α translocations may actually harbor mutation in IDH enzymes. Intriguingly, in keeping with this notion, out of two atypical APL we analyzed, one displayed mutations in IDH2 enzyme. Furthermore, mutations in IDH1/2 have been described for AMLs displaying an acute promyelocytic leukemia-like (APL-like) immunophenotype by flow cytometry, reinforcing the idea that APL and AMLs harboring mIDH2 harbor common pathways of evolution and vulnerabilities

despite distinct genetic drivers.

Taken together, the above studies provide critical understanding of the mechanisms underlying mIDH-driven AML pathogenesis, and underscore the value and insights that can be gained from modeling cancer dependencies and vulnerabilities in appropriate model
5 systems. Critically, these data point towards the development of a rationale-based therapeutic strategy for the targeted and effective treatment of leukemia.

Other Embodiments

While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is
10 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 invention 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 within the claims.

CLAIMS

What is claimed is:

1. A method of treating cancer in a subject having a pathogenic IDH2 allele or having elevated 2-hydroxyglutarate (2-HG) levels, said method comprising contacting the cells of said cancer with an effective amount of a pharmaceutical compound, said pharmaceutical compound being arsenic trioxide, a retinoic acid compound, or a combination thereof,

wherein contacting said cells of said cancer with said pharmaceutical compound treats said cancer in said subject.

2. The method of claim 1, wherein said cancer is a leukemia.

3. The method of claim 1, wherein said cancer is a solid tumor.

4. The method of claim 1, wherein said pathogenic IDH2 allele is IDH2^{R140Q}.

5. The method of claim 1, said method comprising contacting the cells of said leukemia with an effective amount of arsenic trioxide.

6. The method of claim 1, said method comprising contacting the cells of said leukemia with an effective amount of a retinoic acid compound.

7. The method of claim 1, said method comprising contacting the cells of said leukemia with an effective amount of arsenic trioxide and an effective amount of a retinoic acid compound.

8. The method of claim 7, wherein contacting said cells of said leukemia with said arsenic trioxide and said retinoic acid compound cures said leukemia in said subject.

9. The method of claim 7, wherein said arsenic trioxide and said retinoic acid compound operate synergistically to treat said leukemia.

10. The method of claim 7, wherein contacting said cells of said leukemia with said arsenic trioxide and said retinoic acid compound is more effective for treating said leukemia

than administering the same quantities of either said arsenic trioxide or said retinoic acid compound alone.

11. The method of claim 7, wherein contacting said cells of said leukemia with said arsenic trioxide and said retinoic acid compound increases the production of reactive oxygen species in one or more cells of said leukemia.

12. The method of claim 7, wherein contacting said cells of said leukemia with said arsenic trioxide and said retinoic acid compound promotes differentiation of one or more cells of said leukemia.

13. The method of claim 7, wherein contacting said cells of said leukemia with said arsenic trioxide and said retinoic acid decreases Pin1 activity in one or more cells of said leukemia.

14. The method of claim 7, wherein said arsenic trioxide and said retinoic acid compound are administered to said subject, and wherein said administration of said arsenic trioxide and said retinoic acid compound occurs concurrently.

15. The method of claim 7, wherein said arsenic trioxide and said retinoic acid compound are administered to said subject, and wherein said administration of said arsenic trioxide and said retinoic acid compound occurs separately.

16. The method of claim 15, wherein said arsenic trioxide and said retinoic acid compound are administered to said subject, and wherein said arsenic trioxide is administered prior to said retinoic acid compound.

17. The method of claim 15, wherein said arsenic trioxide and said retinoic acid compound are administered to said subject, and wherein said arsenic trioxide is administered after said retinoic acid compound.

18. The method of claim 7, wherein said effective amount of arsenic trioxide is a low dose of arsenic trioxide.

19. The method of claim 18, wherein said low dose of arsenic trioxide is a nontoxic dose of arsenic trioxide.

20. The method of claim 18, wherein said low dose of said arsenic trioxide comprises a dose of about 2.5 mg/kg body weight or less.

21. The method of claim 20, wherein said low dose of said arsenic trioxide comprises a dose of between about 0.05 mg/kg body weight and about 2.5 mg/kg body weight.

22. The method of claim 20, wherein said low dose of said arsenic trioxide comprises a dose of about 0.05 mg/kg body weight or less.

23. The method of claim 7, wherein said effective amount of said retinoic acid compound is a low dose of said retinoic acid compound.

24. The method of claim 23, wherein said low dose of said retinoic acid compound is a nontoxic dose of said retinoic acid compound.

25. The method of claim 23, wherein said low dose of said retinoic acid compound comprises a dose of about 30 mg/m² body surface area or less.

26. The method of claim 25, wherein said low dose of said retinoic acid compound comprises a dose of about 10 mg/m² body surface area or less.

27. The method of claim 7, wherein said retinoic acid compound is selected from the group consisting of all-trans retinoic acid (ATRA), 13-cis-retinoic acid, retinol, retinyl acetate, retinal, and AC-55640.

28. The method of claim 27, wherein said retinoic acid compound is ATRA.

29. The method of claim 5, wherein contacting said cells of said leukemia with said arsenic trioxide increases the production of reactive oxygen species in one or more cells of said leukemia.

30. The method of claim 6, wherein contacting said cells of said leukemia with said retinoic acid compound promotes differentiation of one or more cells of said leukemia.

31. The method of claim 1, wherein said subject has elevated levels of Pin1 activity.

32. The method of claim 31, wherein said elevated levels of Pin1 activity were previously determined by measuring the levels of a Pin1 marker, wherein elevated levels of said Pin1 marker is indicative of elevated Pin1 activity.

33. The method of claim 32, wherein said Pin1 marker is Pin1 mRNA expression level, PIN1 protein expression level, or expression of a downstream effector of Pin1.

34. The method of claim 1, wherein said method further comprises contacting said cells of said leukemia with an inhibitor of Pin1.

35. The method of claim 34, wherein said subject has decreased levels of LSD1 activity.

36. The method of claim 1, wherein said method further comprises contacting said cells of said leukemia with an inhibitor of LSD1.

37. The method of claim 1, wherein said method further comprises contacting said cells of said leukemia with dexamethasone.

38. The method of claim 1, wherein said leukemia is acute myeloid leukemia.

39. The method of claim 38, wherein said acute myeloid leukemia is relapsed or refractory acute myeloid leukemia.

40. The method of claim 38, wherein said acute myeloid leukemia is resistant to treatment with an IDH2 inhibitor.

41. The method of claim 7, wherein one or more cells of said leukemia comprise a pathogenic IDH2 allele and have elevated 2-hydroxyglutarate (2-HG) levels.

42. The method of claim 7, wherein one or more cells of said leukemia comprise a pathogenic IDH2 allele in combination with NPMc+, FLT3-ITD, or both.

43. The method of claim 42, wherein one or more cells of said leukemia comprise a pathogenic IDH2 allele in combination with both NPMc+ and FLT3-ITD.

44. The method of claim 42, wherein said pathogenic IDH2 allele is IDH2^{R140Q}.

45. The method of claim 7, wherein said effective amount of arsenic trioxide is a low dose of about 2.5 mg/kg body weight or less, said effective amount of said retinoic acid compound is a low dose of about 30 mg/m² body surface area or less, and said retinoic acid compound is selected from the group consisting of all-trans retinoic acid (ATRA), 13-cis-retinoic acid, retinol, retinyl acetate, retinal, and AC-55640.

46. The method of claim 45, wherein said effective amount of arsenic trioxide is a low dose of between about 0.05 mg/kg body weight and about 2.5 mg/kg body weight, said effective amount of said retinoic acid compound is a low dose of about 10 mg/m² body surface area or less, and said retinoic acid compound is ATRA.

47. The method of claim 46, wherein one or more cells of said leukemia comprise a pathogenic IDH2 allele in combination with NPMc+, FLT3-ITD, or both, in which said pathogenic IDH2 allele is IDH2^{R140Q}.

48. A kit for treating leukemia in a subject, said kit comprising:

(a) an effective amount of arsenic trioxide,

(b) an effective amount of a retinoic acid compound, and

(c) instructions for the use of said arsenic trioxide in combination with said retinoic acid compound for treating said leukemia in said subject,

wherein one or more cells of said leukemia comprise a pathogenic IDH2 allele or have elevated 2-hydroxyglutarate (2-HG) levels.

49. The kit of claim 48, wherein said kit further comprises an effective amount of a compound that inhibits Pin1 activity and instructions for the use of said compound that inhibits Pin1 activity in combination with said arsenic trioxide and said retinoic acid compound.

50. The kit of claim 48, wherein said kit further comprises an effective amount of a compound that inhibits LSD1 activity and instructions for the use of said compound that inhibits LSD1 activity in combination with said arsenic trioxide and said retinoic acid compound.

51. The kit of claim 48, wherein said kit further comprises an effective amount of a compound that inhibits Pin1 activity, an effective amount of a compound that inhibits LSD1 activity, and instructions for the use of said compound that inhibits Pin1 activity and said compound that inhibits LSD1 activity in combination with said arsenic trioxide and said retinoic acid compound.

52. The kit of claim 48, wherein said effective amount of arsenic trioxide is a low dose of arsenic trioxide.

53. The kit of claim 52, wherein said low dose of arsenic trioxide is a nontoxic dose of arsenic trioxide.

54. The kit of claim 52, wherein said low dose of said arsenic trioxide comprises a dose of about 2.5 mg/kg body weight or less.

55. The kit of claim 54, wherein said low dose of said arsenic trioxide comprises a dose of between about 0.05 mg/kg body weight and about 2.5 mg/kg body weight.

56. The kit of claim 54, wherein said low dose of said arsenic trioxide comprises a dose of about 0.05 mg/kg body weight or less.

57. The kit of claim 48, wherein said effective amount of said retinoic acid compound comprises a low dose of said retinoic acid compound.

58. The kit of claim 57, wherein said low dose of said retinoic acid compound is a nontoxic dose of said retinoic acid compound.

59. The kit of claim 57, wherein said low dose of said retinoic acid compound comprises a dose of about 30 mg/m² body surface area or less.

60. The kit of claim 59, wherein said low dose of said retinoic acid compound comprises a dose of about 10 mg/m² body surface area or less.

61. The kit of claim 48, wherein said retinoic acid compound is selected from the group consisting of all-trans retinoic acid (ATRA), 13-cis-retinoic acid, retinol, retinyl acetate, retinal, and AC-55640.

62. The kit of claim 61, wherein said retinoic acid compound is ATRA.

63. The kit of any one of claims 48-62, wherein said leukemia is acute myeloid leukemia.

64. The kit of claim 63, wherein said acute myeloid leukemia is relapsed or refractory acute myeloid leukemia.

65. The kit of claim 63, wherein said acute myeloid leukemia is resistant to treatment with an IDH2 inhibitor.

66. The kit of claim 48, wherein one or more cells of said leukemia comprise a pathogenic IDH2 allele and have elevated 2-hydroxyglutarate (2-HG) levels.

67. The kit of claim 48, wherein one or more cells of said leukemia comprise a pathogenic IDH2 allele in combination with NPMc+, FLT3-ITD, or both.

68. The kit of claim 67, wherein one or more cells of said leukemia comprise a pathogenic IDH2 allele in combination with both NPMc+ and FLT3-ITD.

69. The kit of claim 67, wherein said pathogenic IDH2 allele is IDH2^{R140Q}.

70. The kit of claim 48, wherein said effective amount of arsenic trioxide is a low dose of about 2.5 mg/kg body weight or less, said effective amount of said retinoic acid compound is a low dose of about 30 mg/m² body surface area or less, and said retinoic acid compound is selected from the group consisting of all-trans retinoic acid (ATRA), 13-cis-retinoic acid, retinol, retinyl acetate, retinal, and AC-55640.

71. The kit of claim 70, wherein said effective amount of arsenic trioxide is a low dose of between about 0.05 mg/kg body weight and about 2.5 mg/kg body weight, said effective amount of said retinoic acid compound is a low dose of about 10 mg/m² body surface area or less, and said retinoic acid compound is ATRA.

72. The kit of claim 71, wherein one or more cells of said leukemia comprise a pathogenic IDH2 allele in combination with NPMc+, FLT3-ITD, or both, in which said pathogenic IDH2 allele is IDH2^{R140Q}.

1A

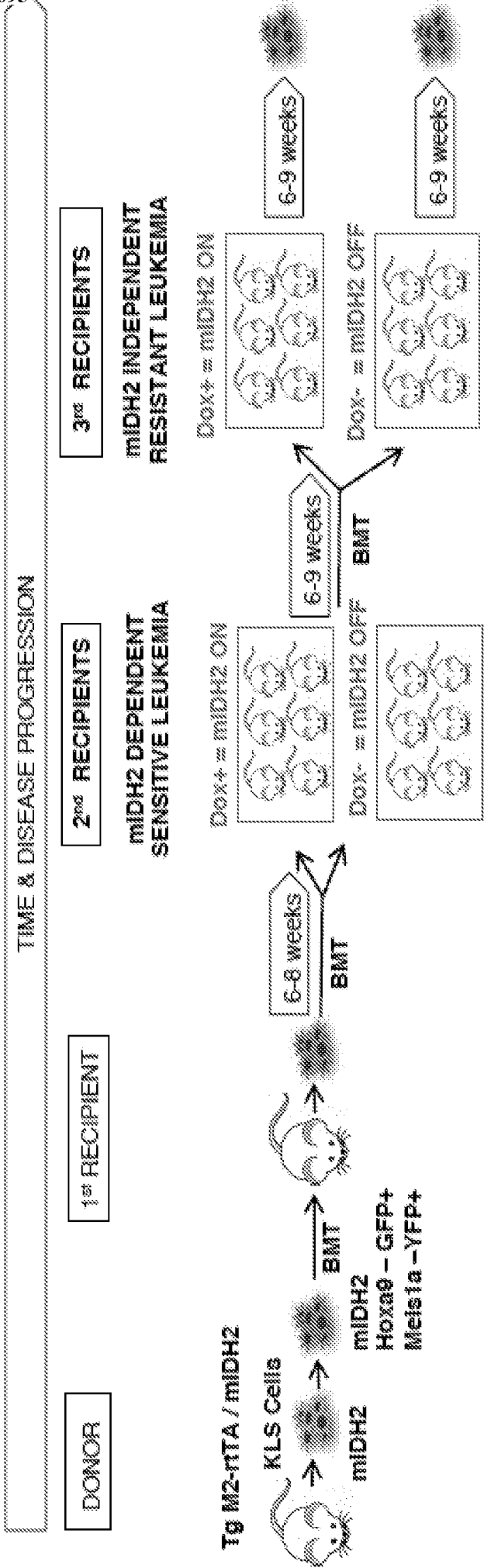


FIG. 1A

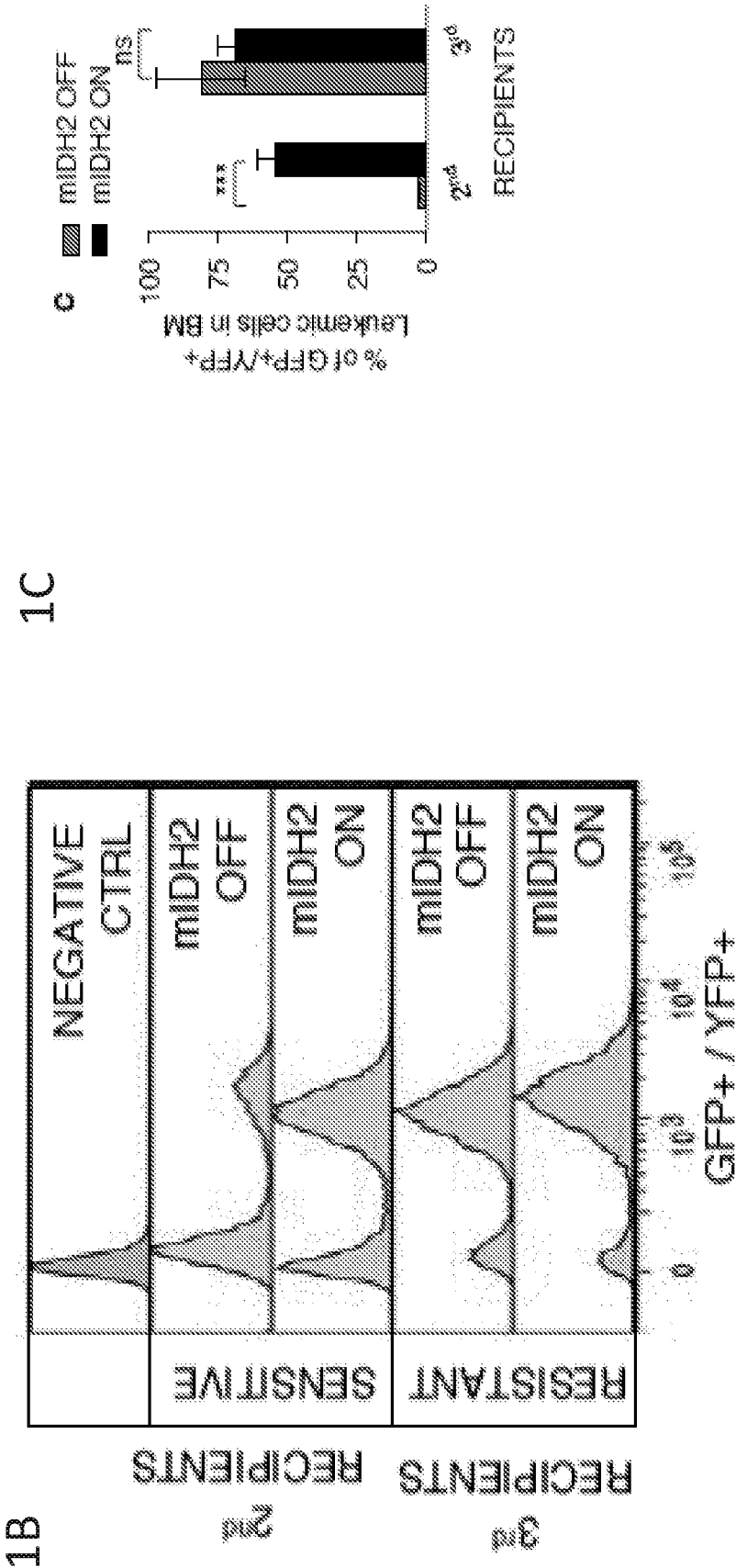


FIG. 1B-C

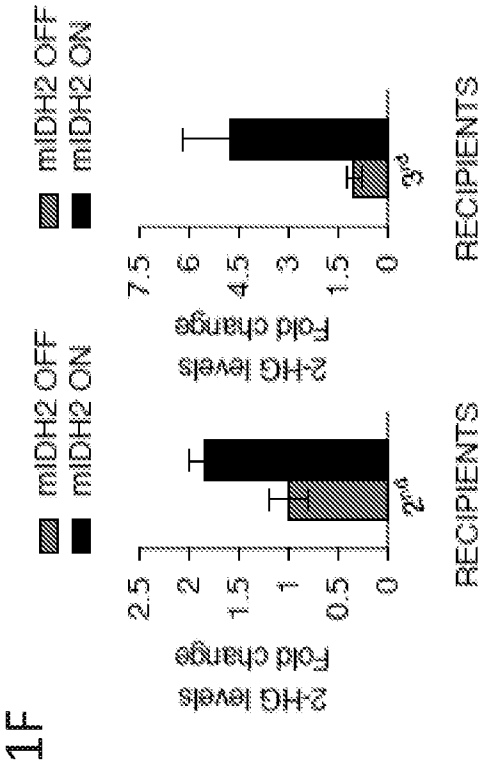
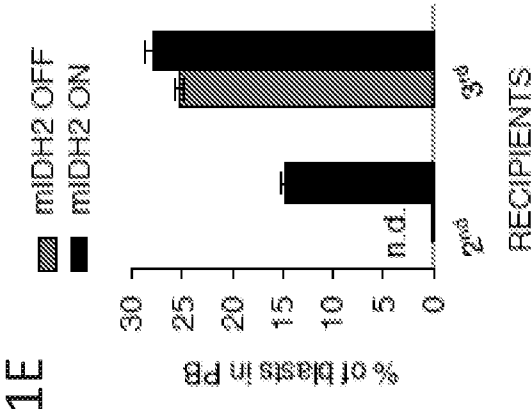
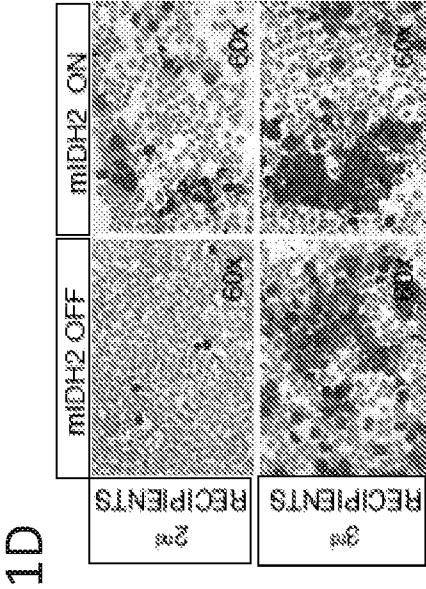


FIG. 1D-F

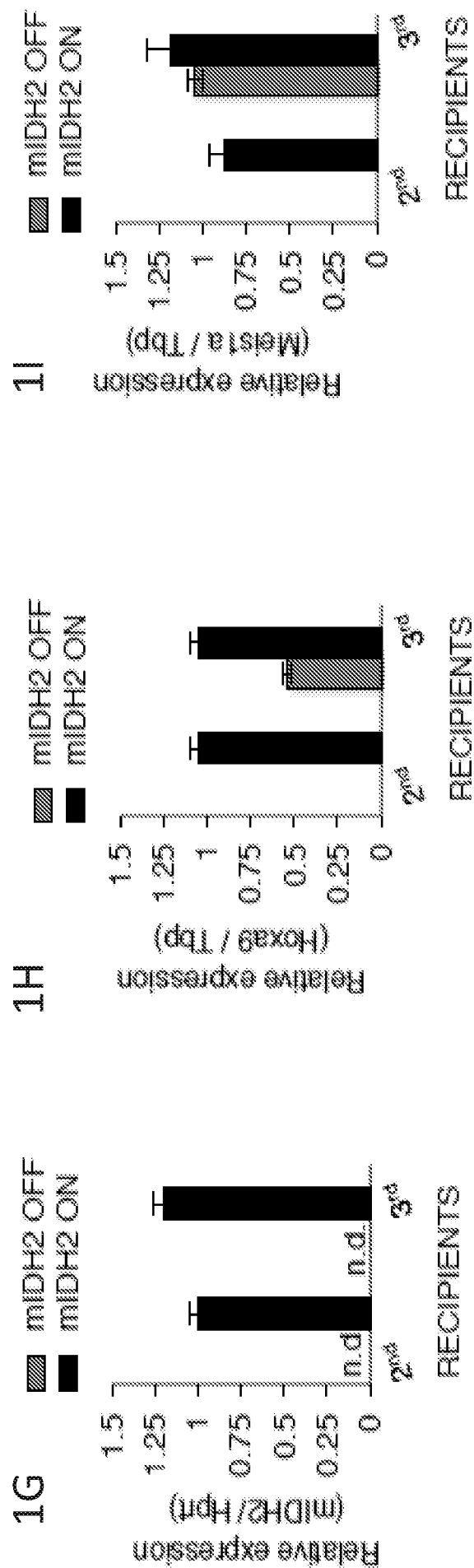


FIG. 1G-I

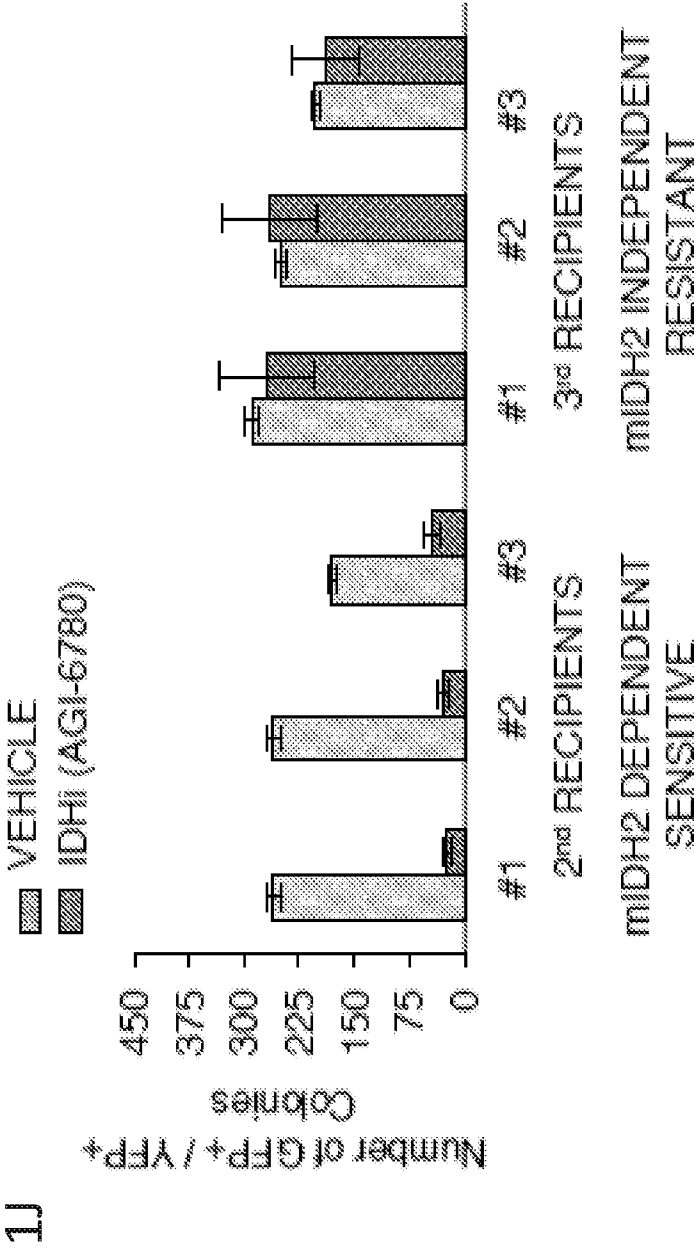


FIG. 1J

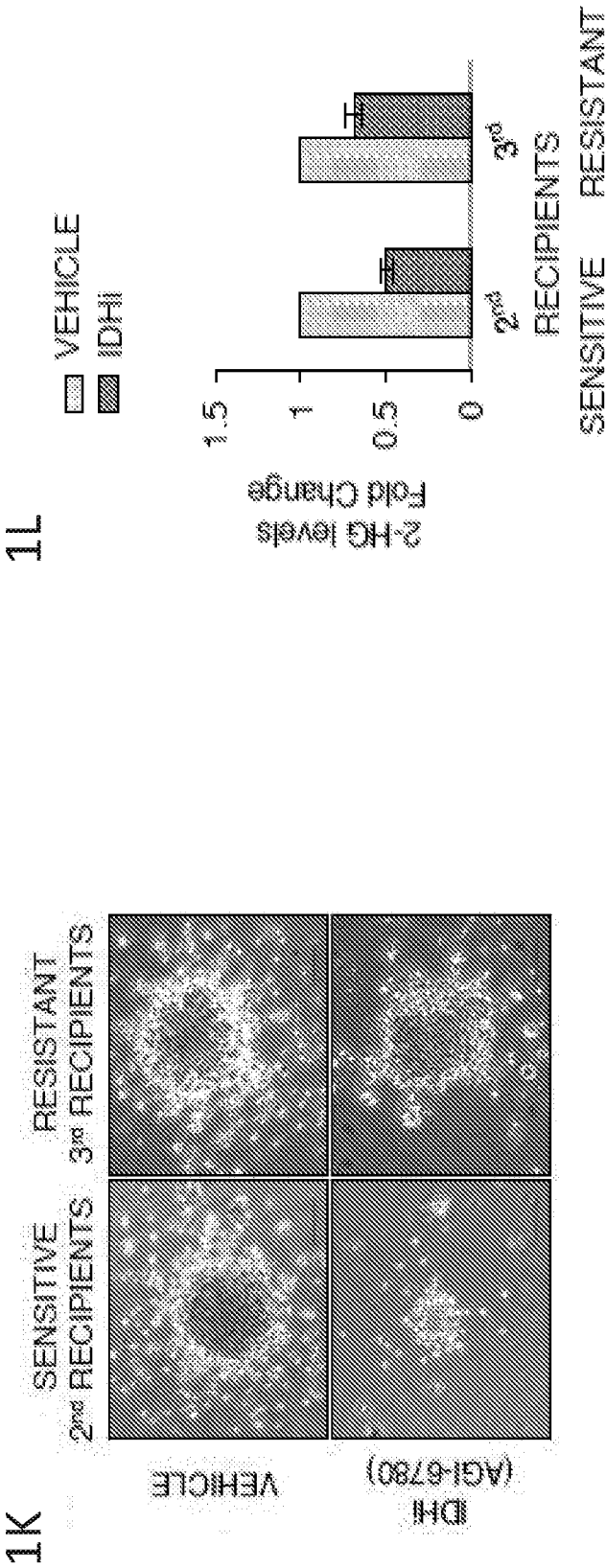
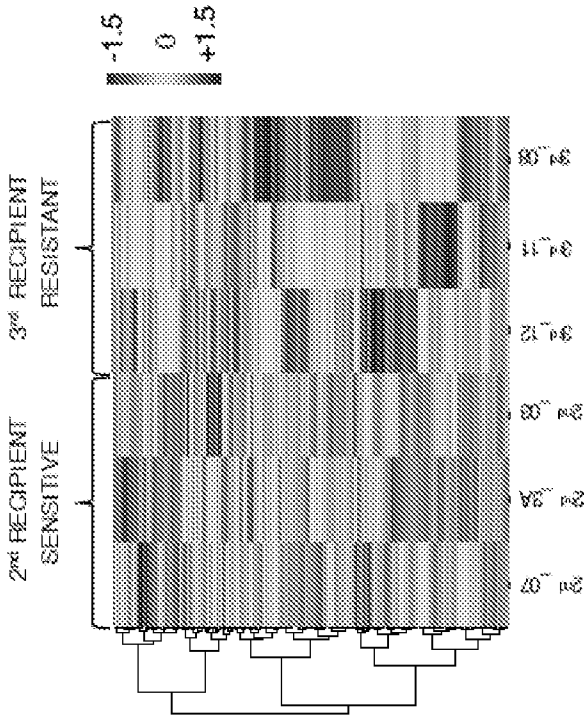


FIG. 1K-L

2A



2B

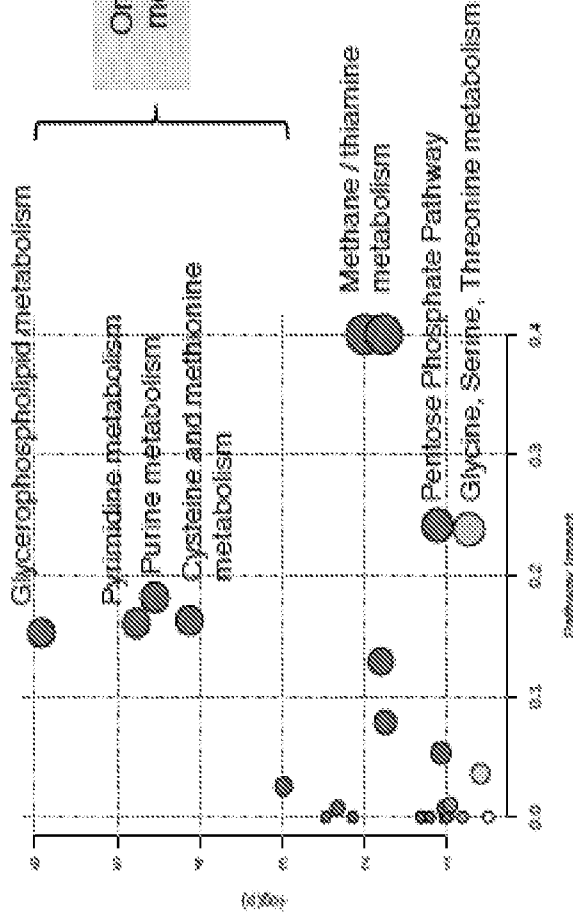


FIG. 2A-B

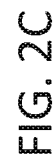


FIG. 2C

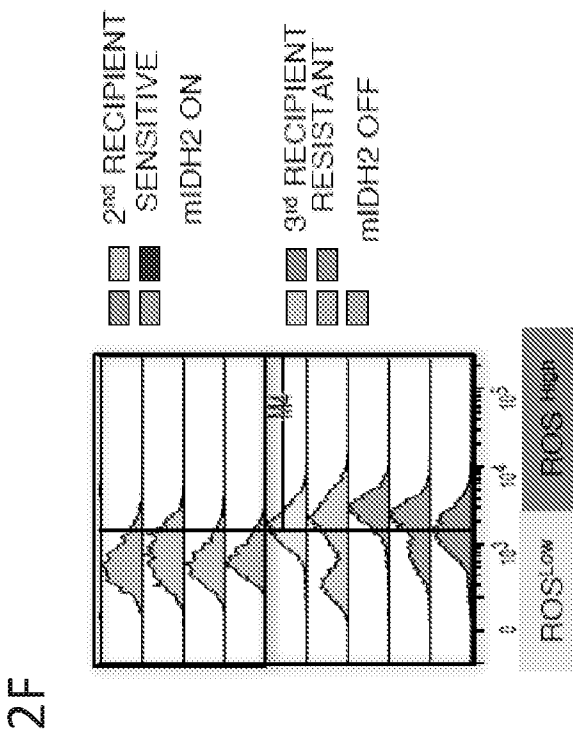
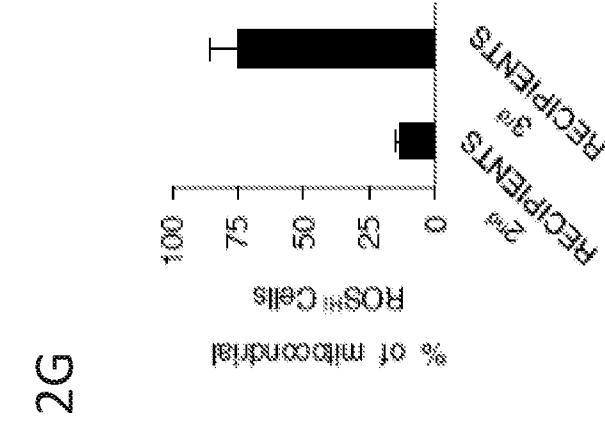
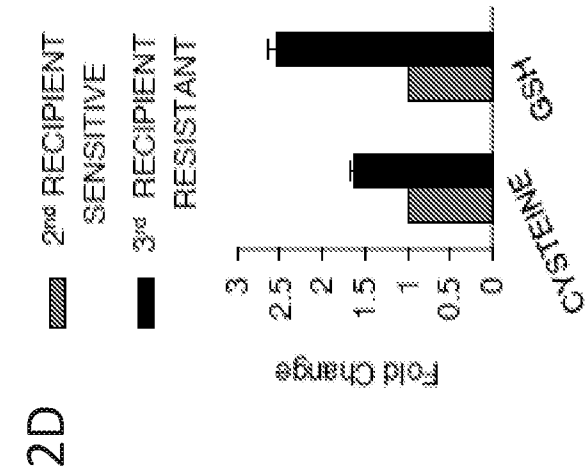
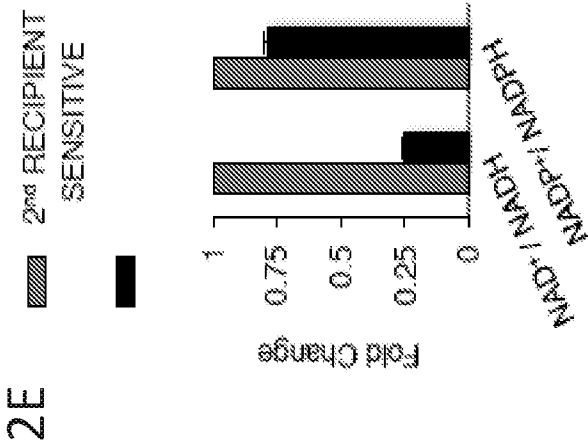
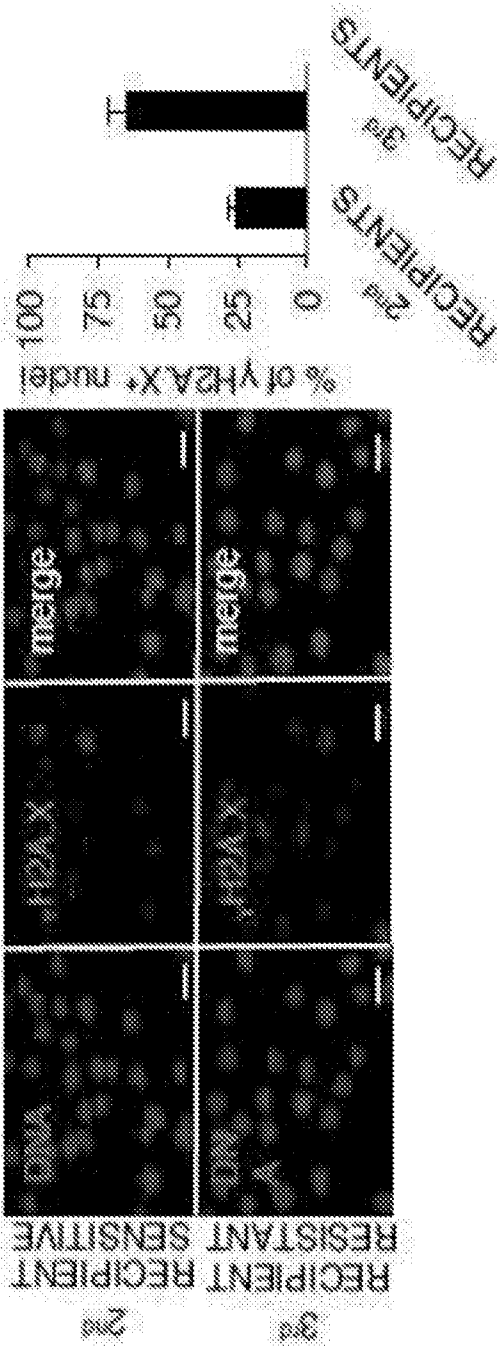
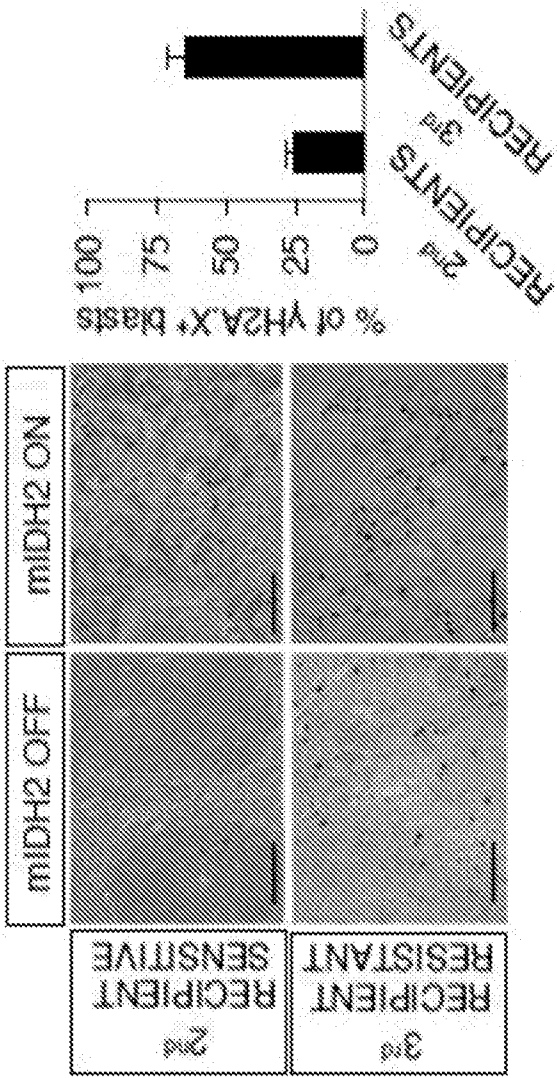


FIG. 2D-G



2H



2I

FIG. 2H-I

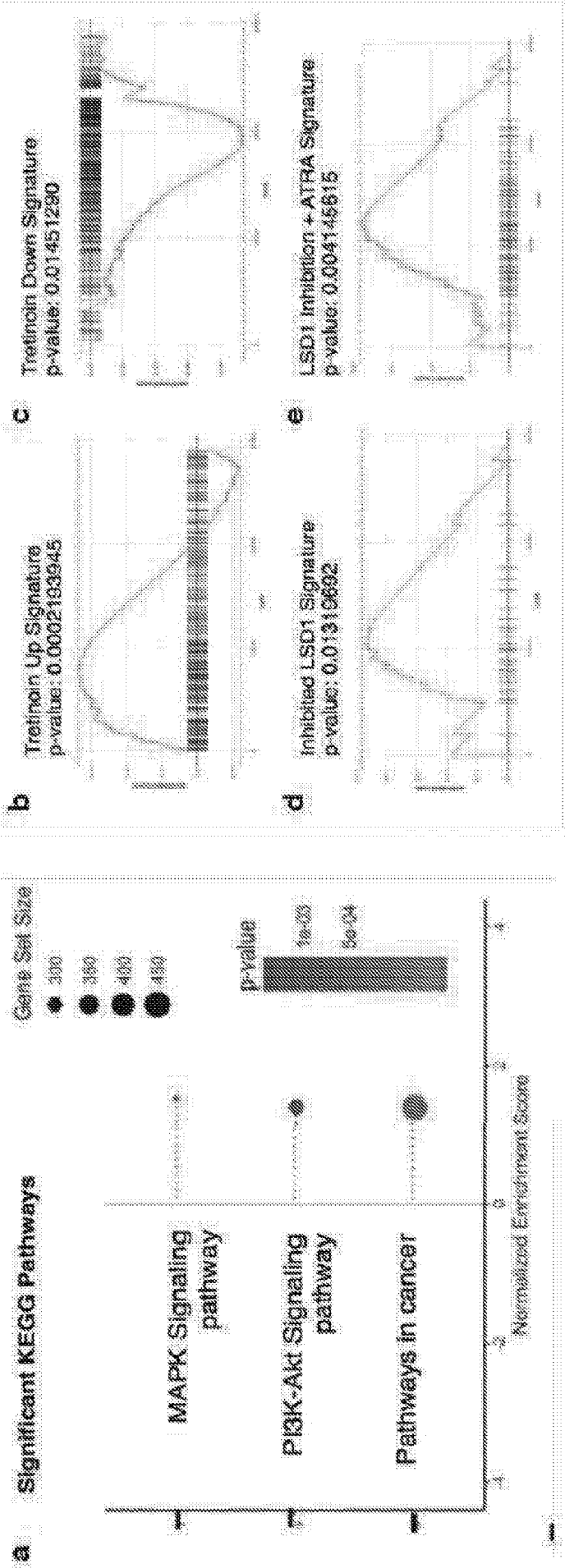
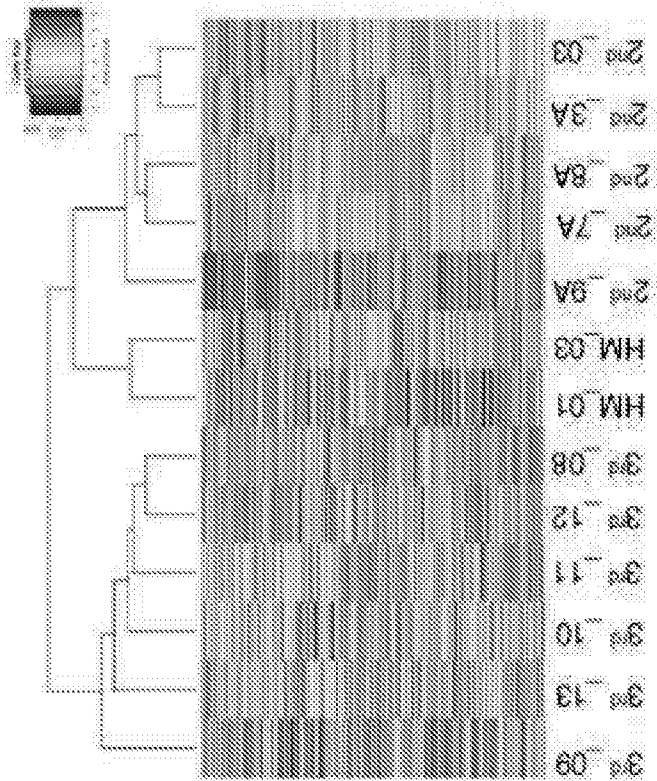


FIG. 2J

3A



3B

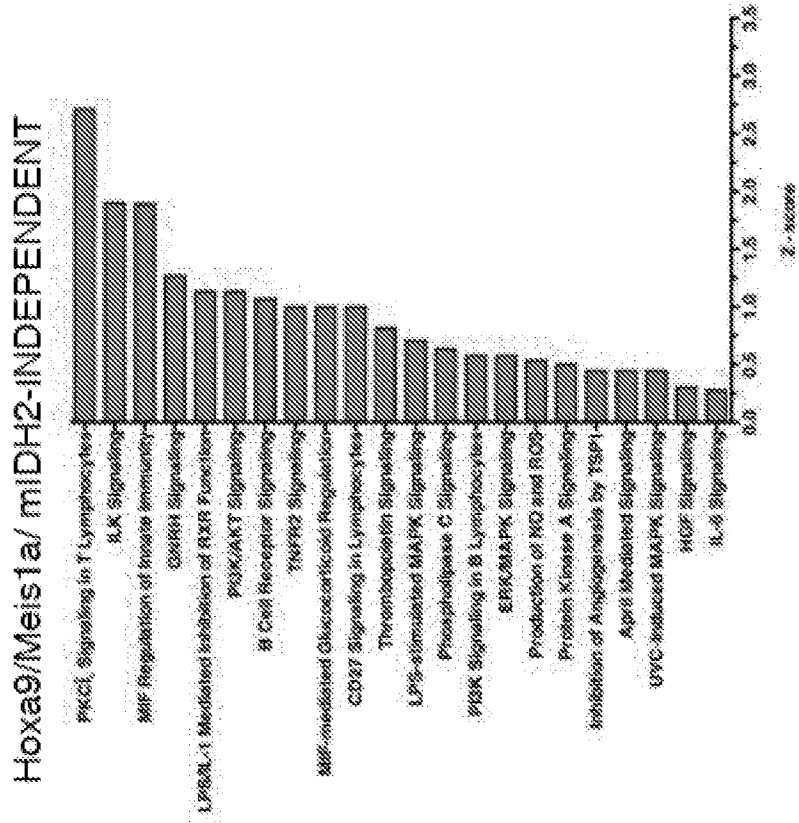


FIG. 3A-B

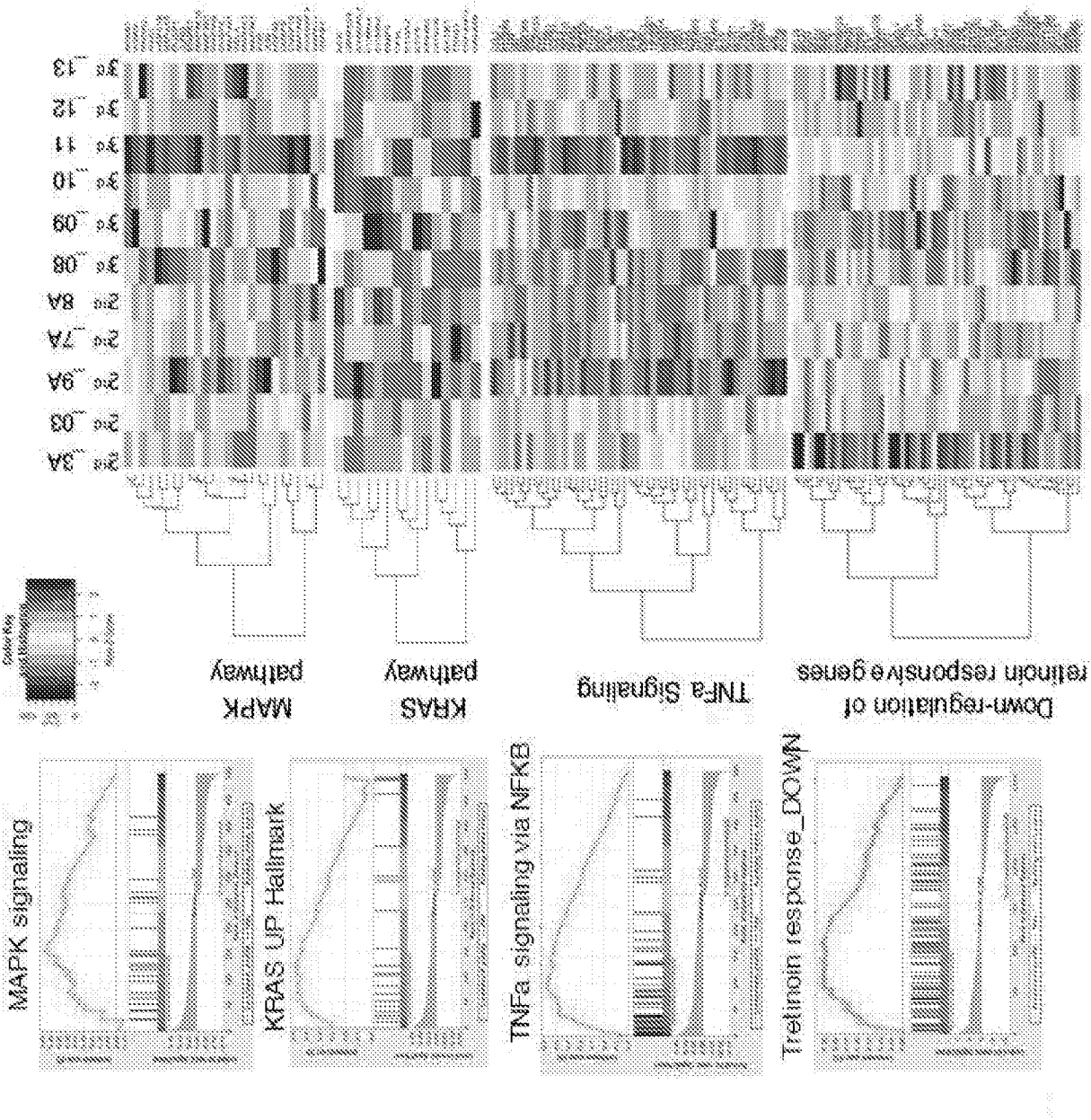


FIG. 3C

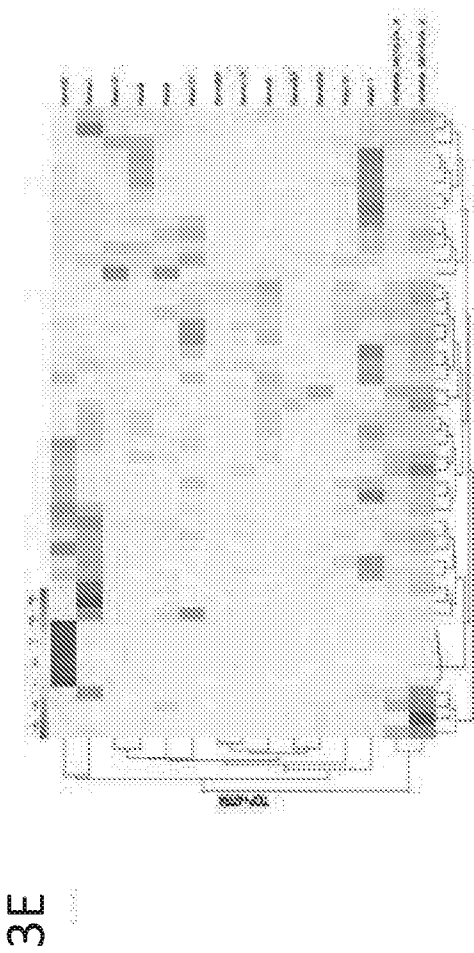
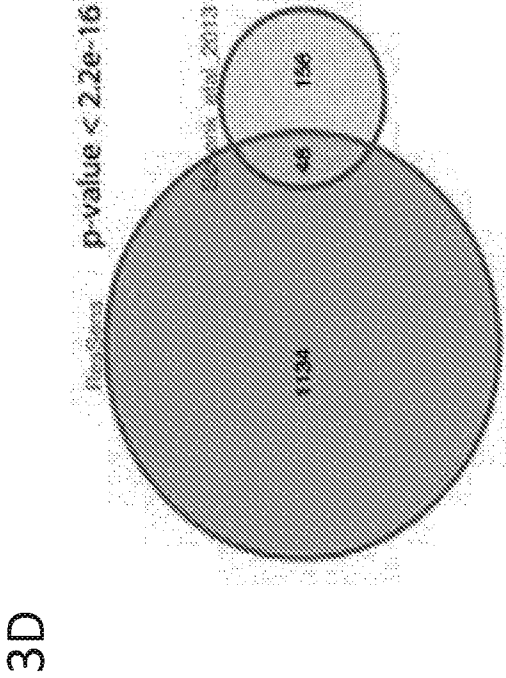


FIG. 3D-E

3F

MOUSE LEUKEMIA

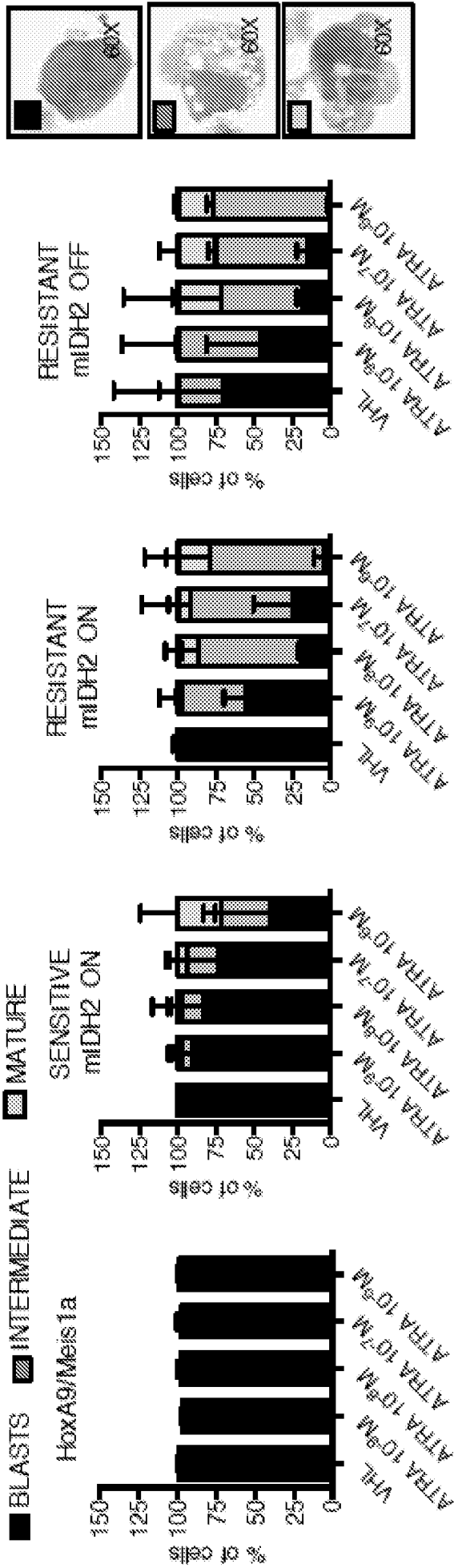


FIG. 3F

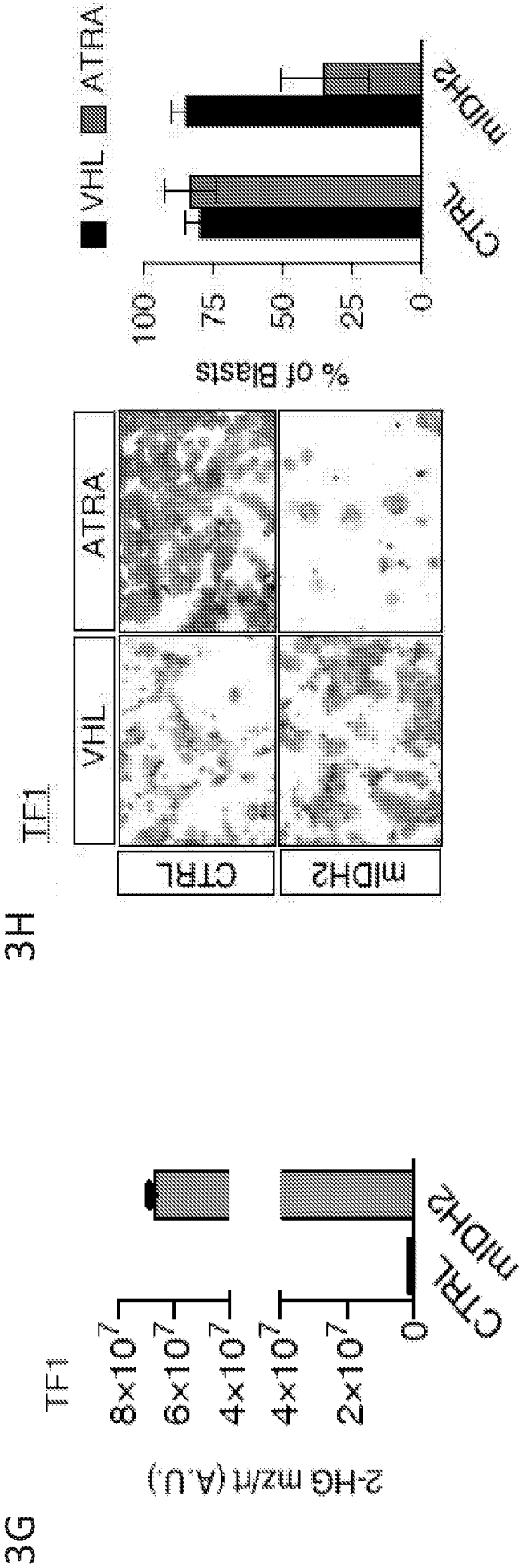


FIG. 3G-H

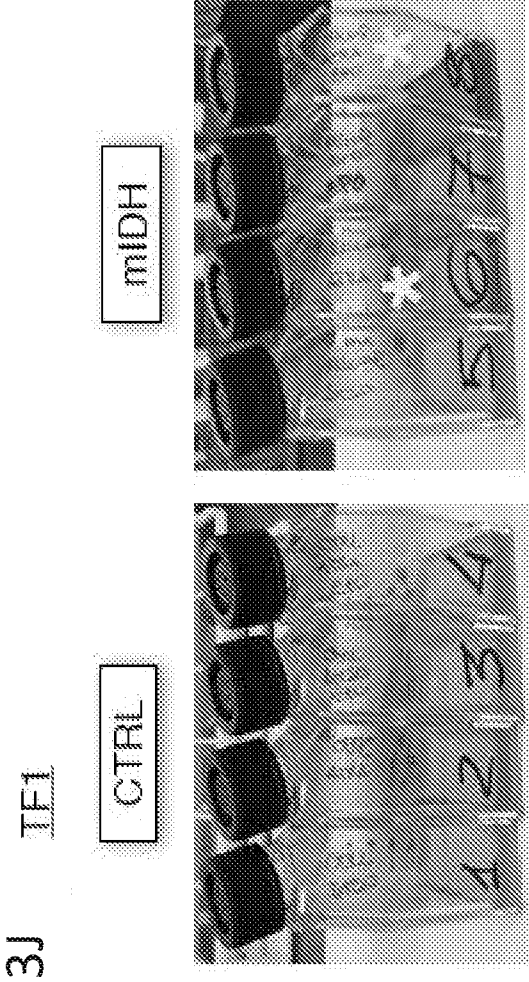
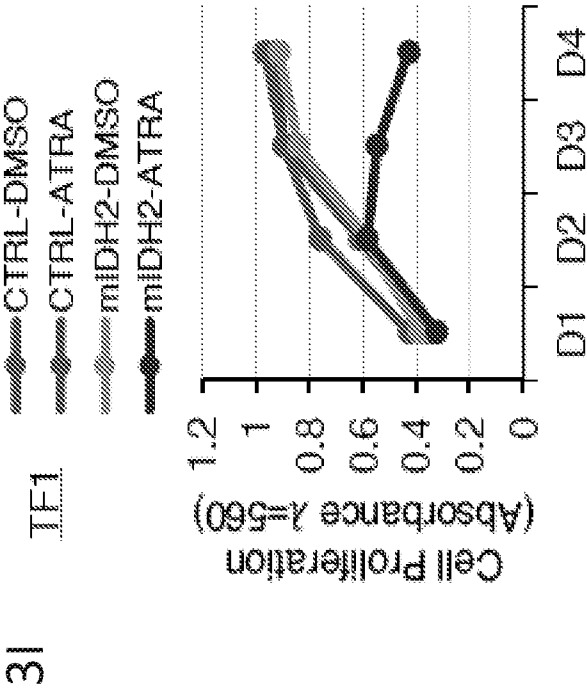


FIG. 3I-J

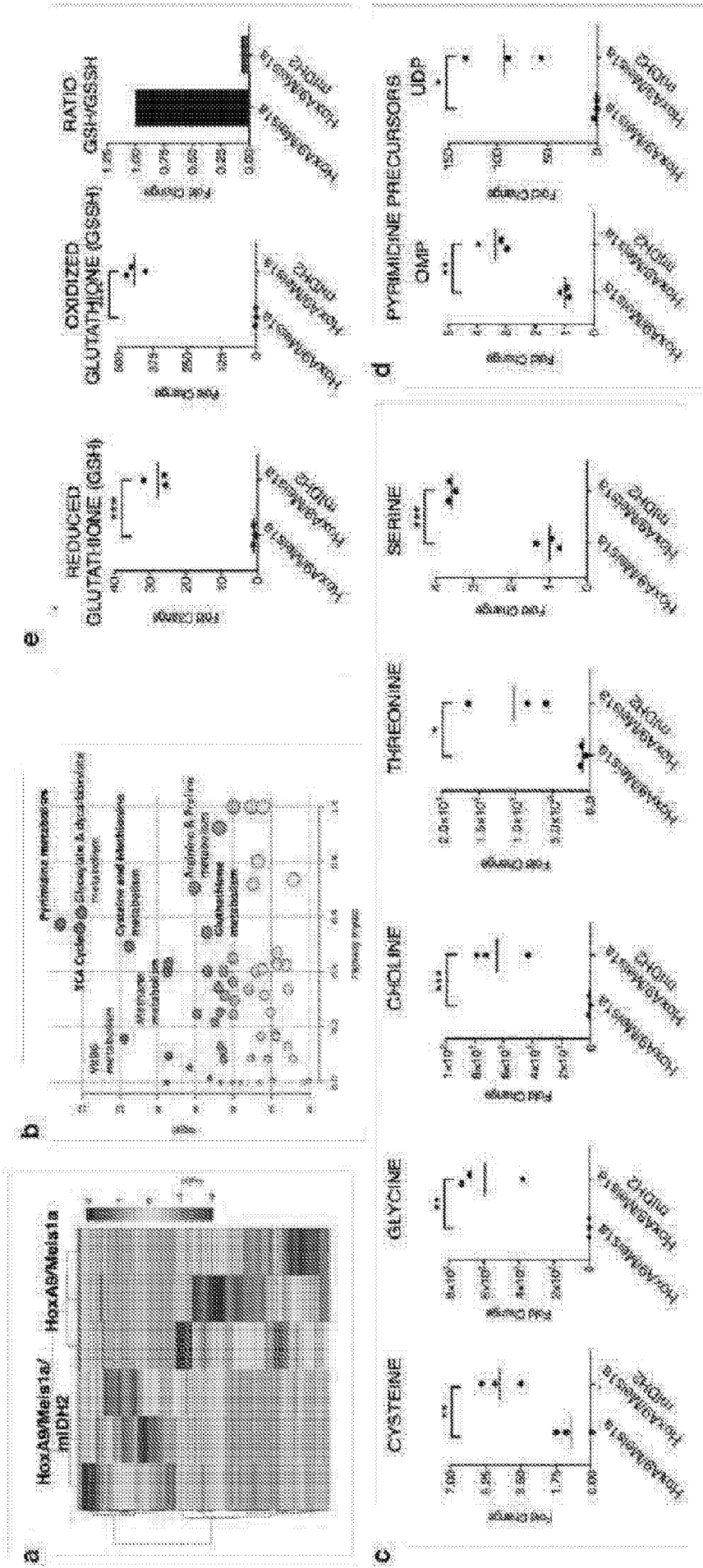


FIG. 3K

4A

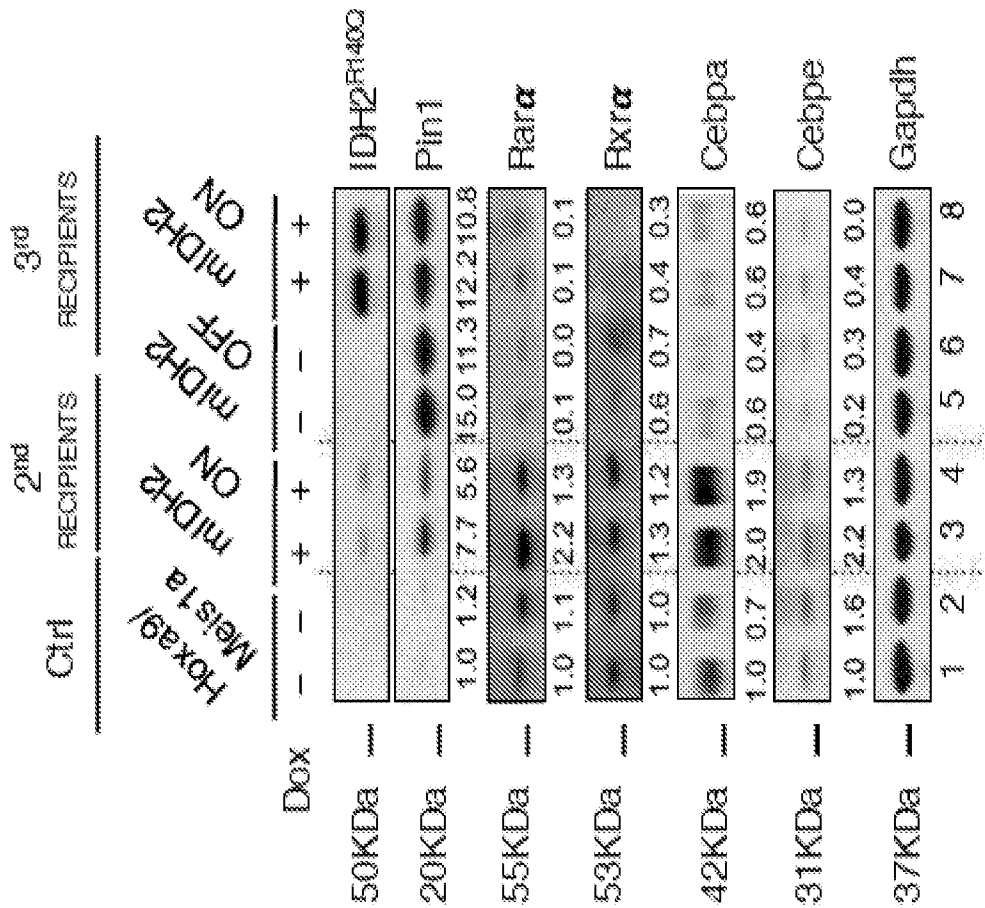
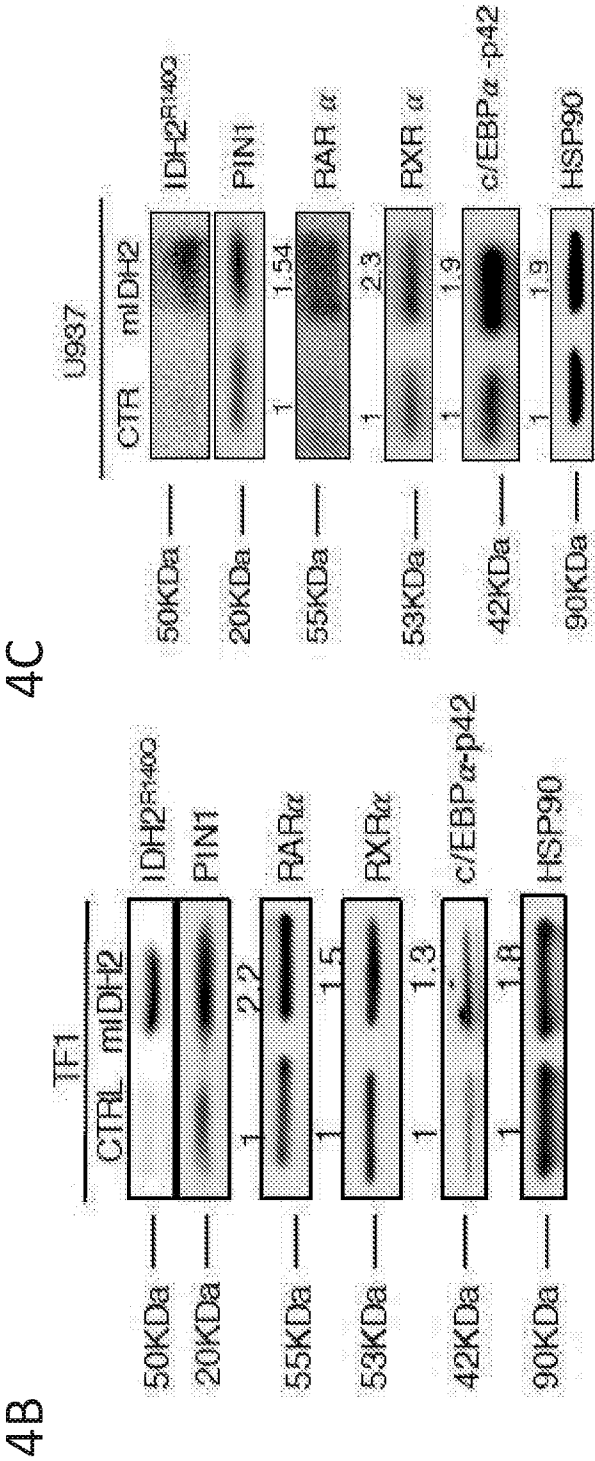


FIG. 4A



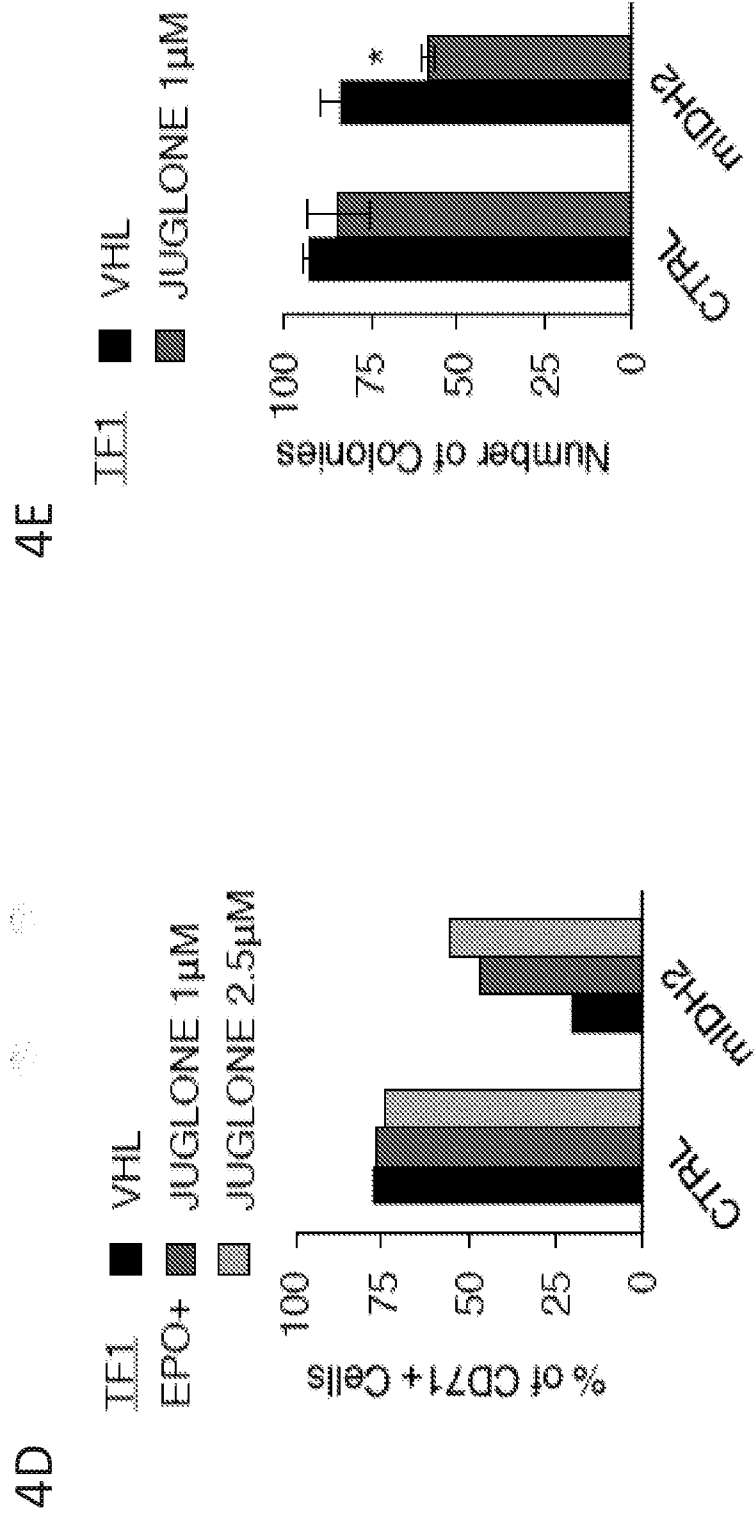


FIG. 4D-E

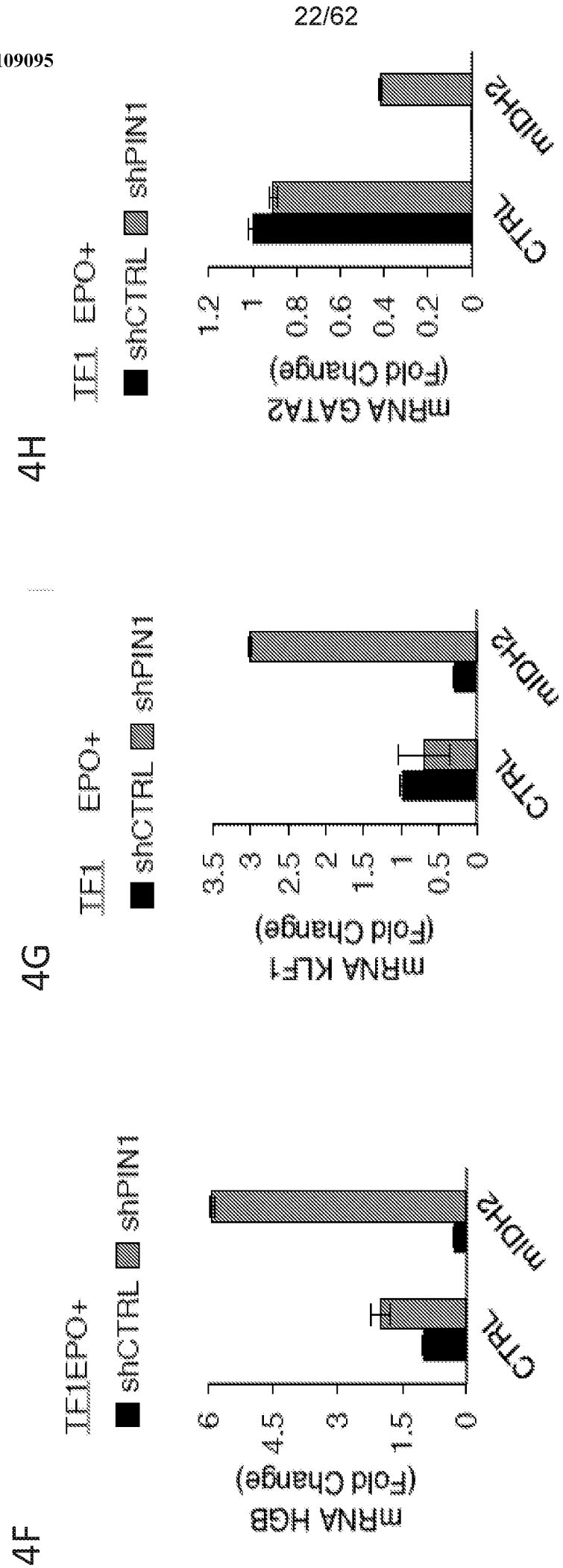


FIG. 4F-H

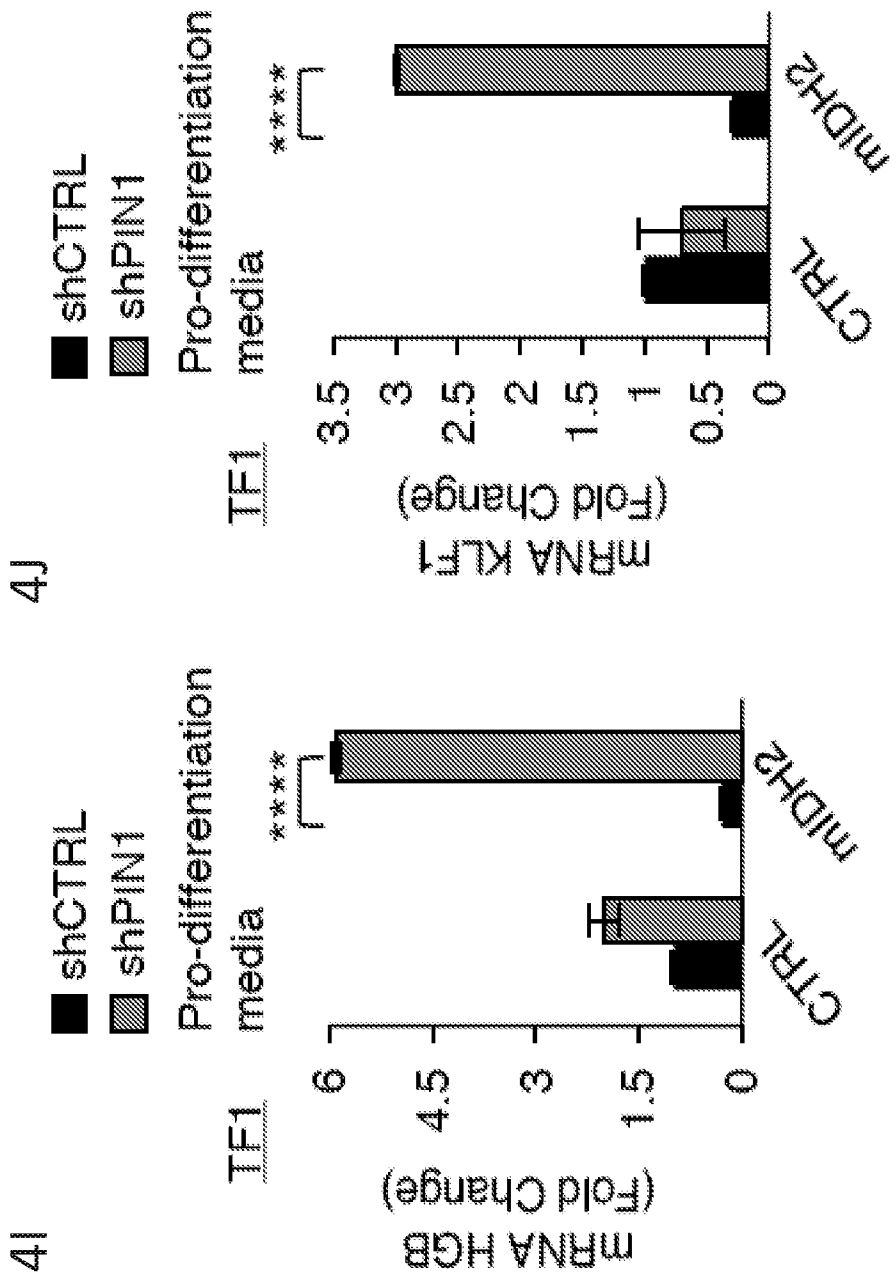


FIG. 4I-J

4K

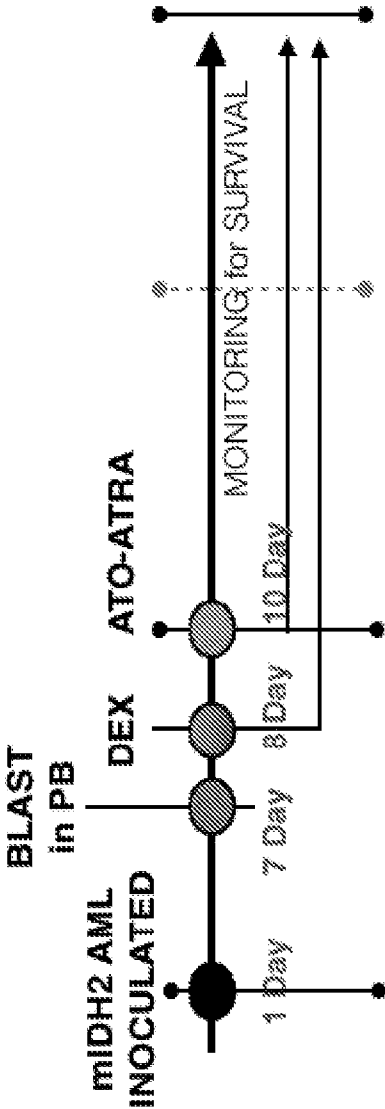


FIG. 4K

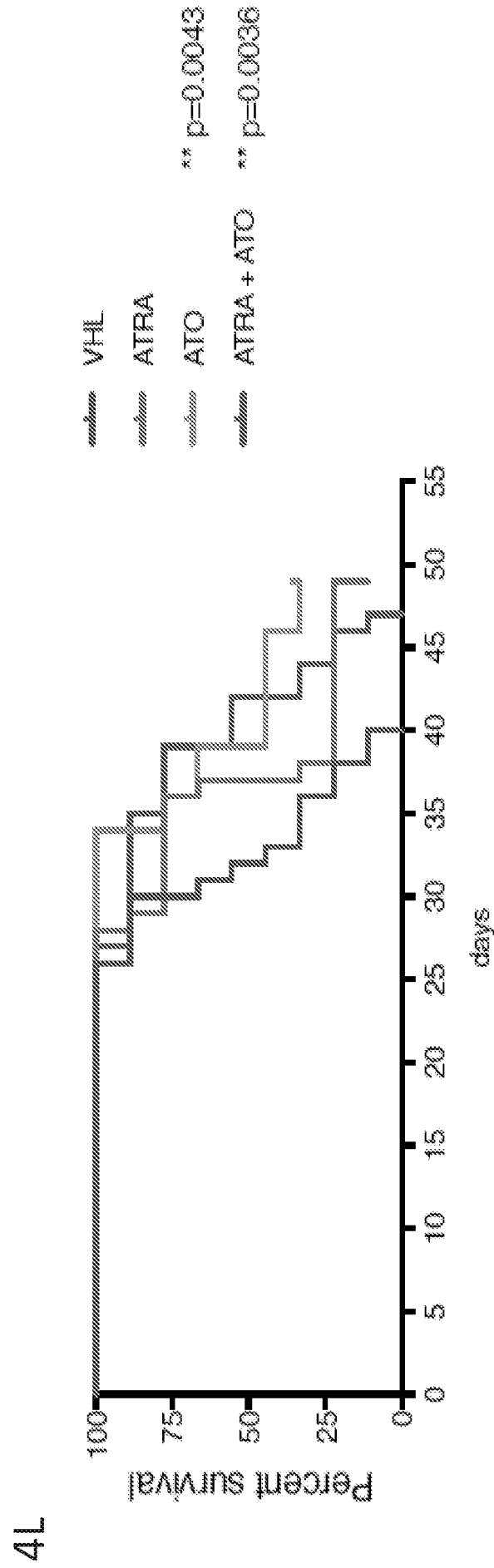


FIG. 4L

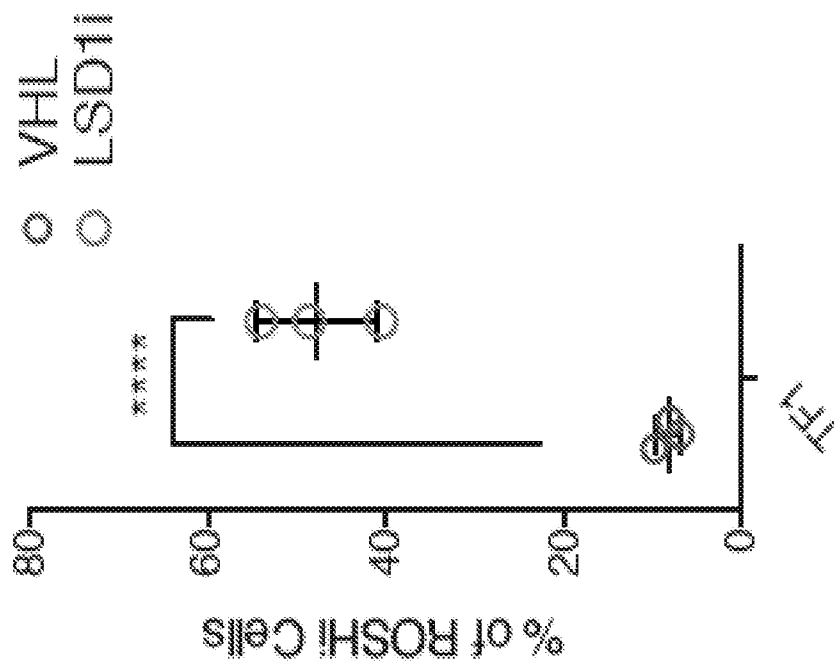


FIG. 4M

5A

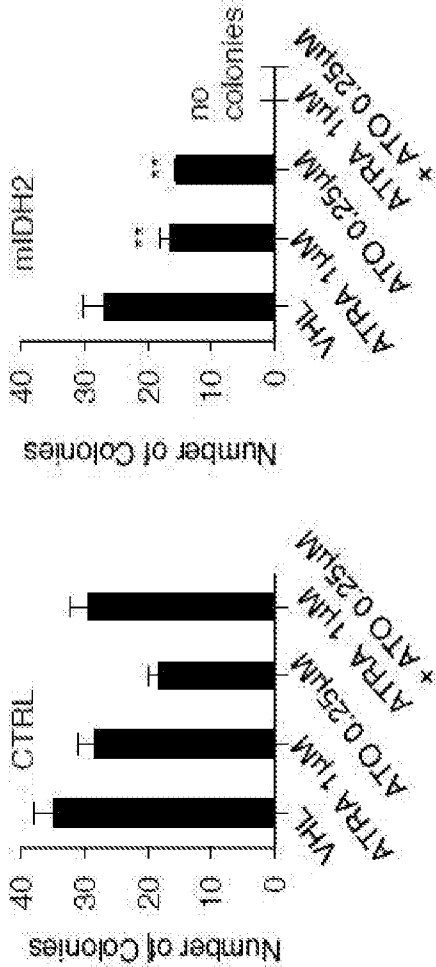
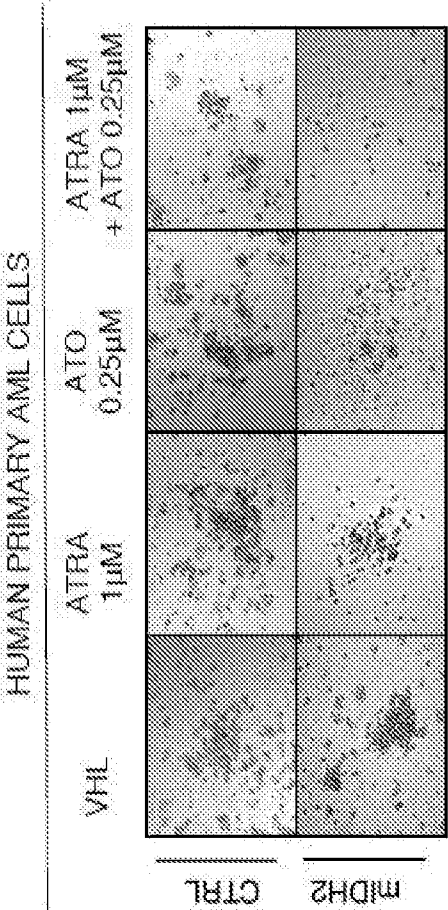


FIG. 5A

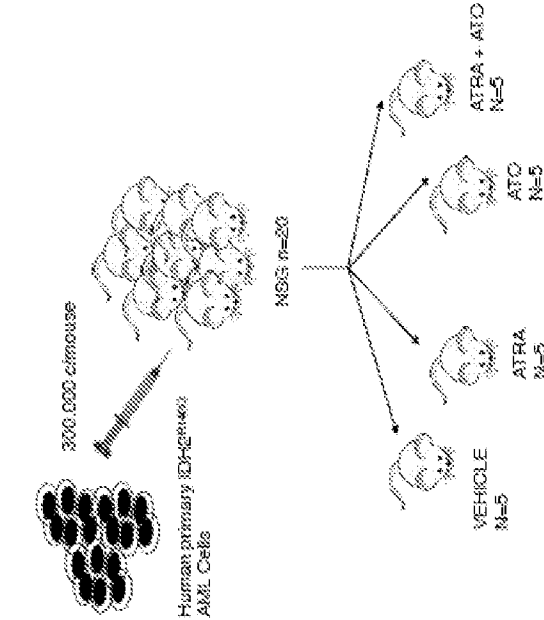
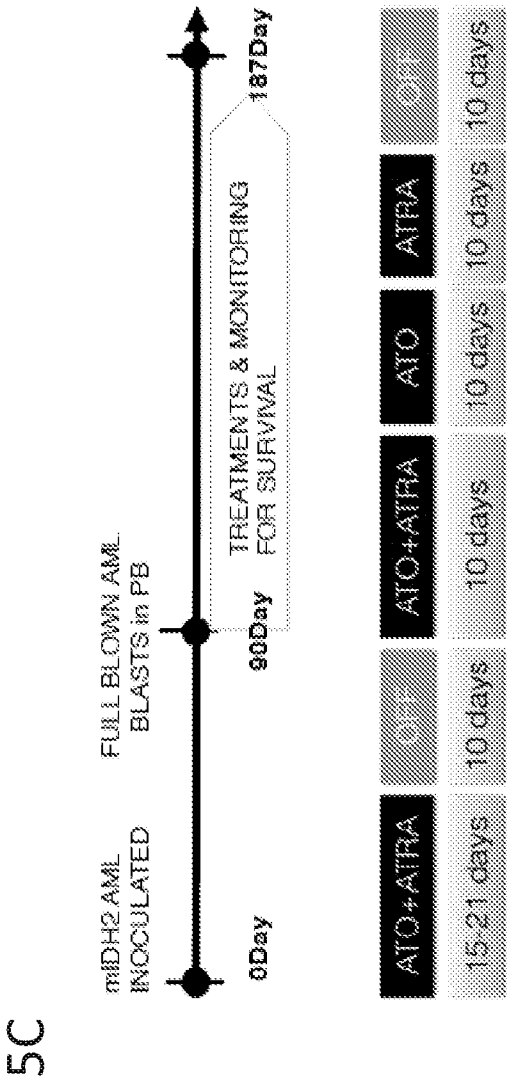
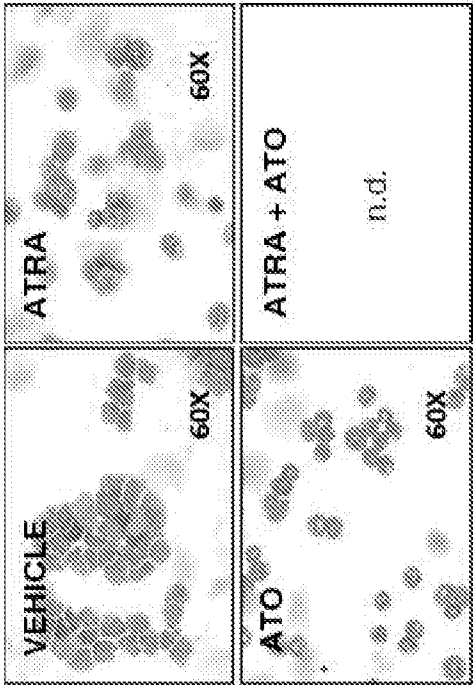


FIG. 5B-C

5E

PDX IDH2R140Q AML (BM)



5D

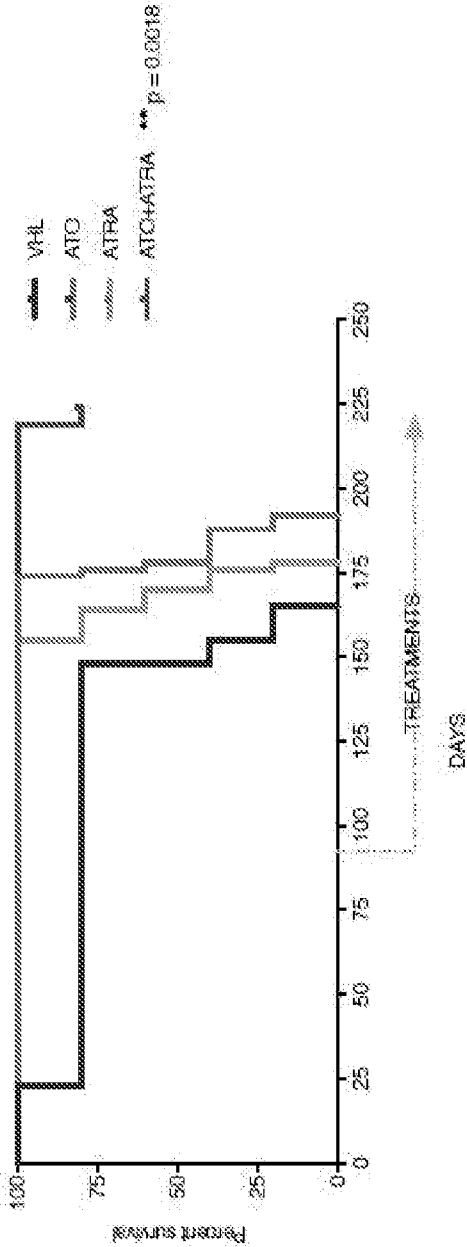
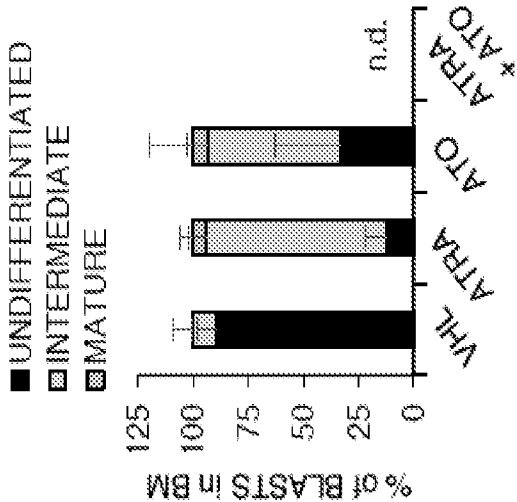


FIG. 5D-E

5F

PDX IDH2^{R150Q} AML _ BM



5G

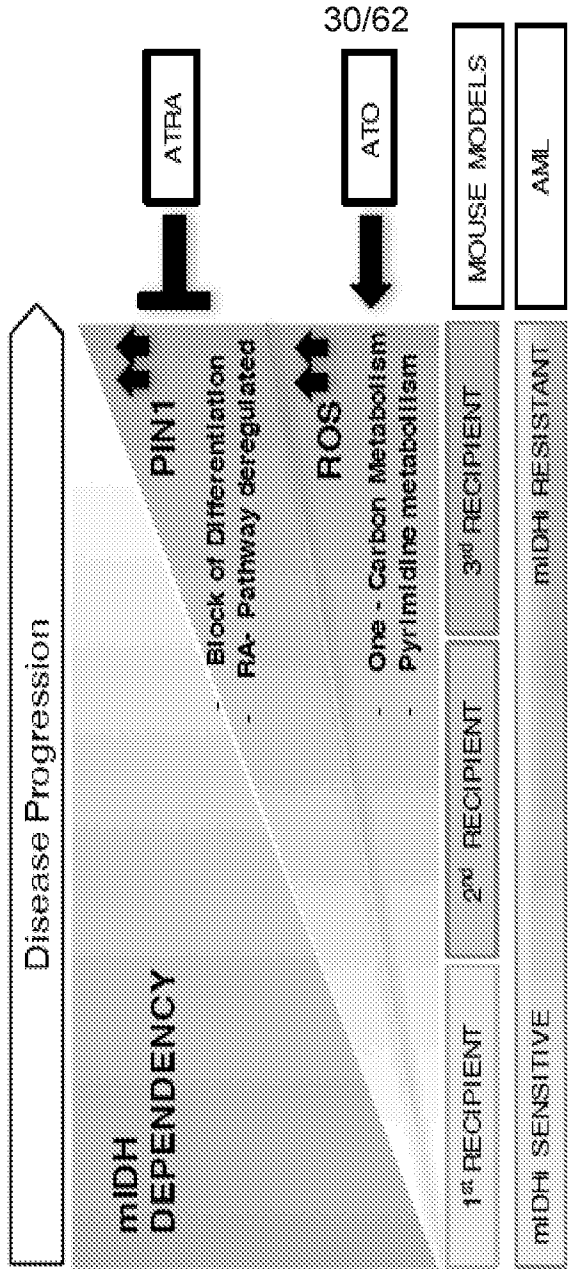


FIG. 5F-G

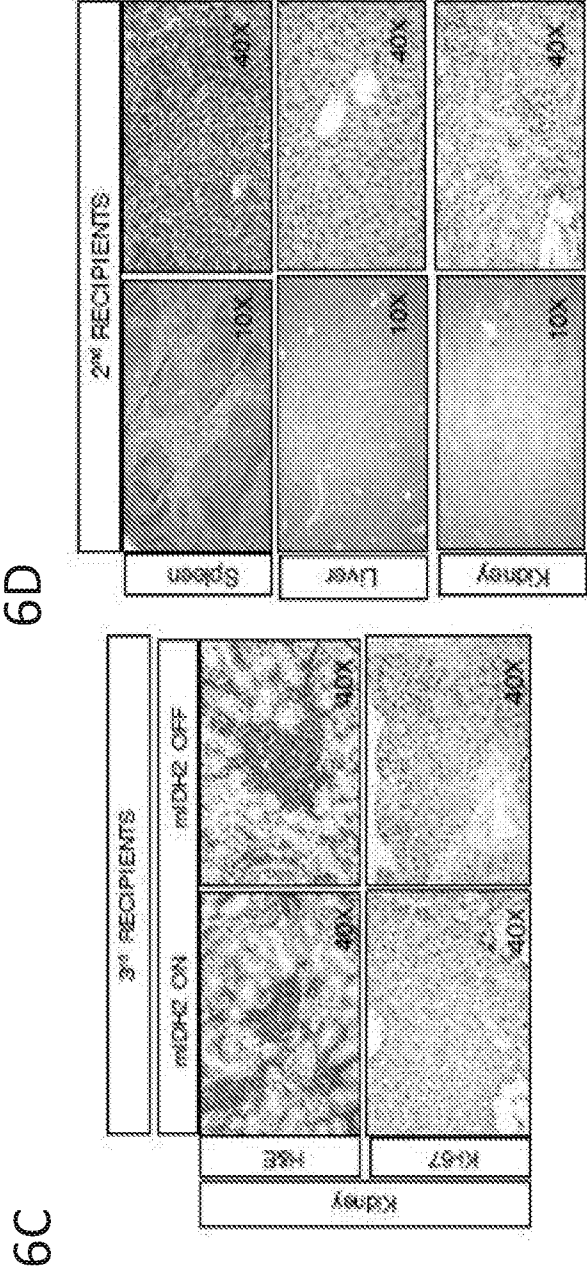
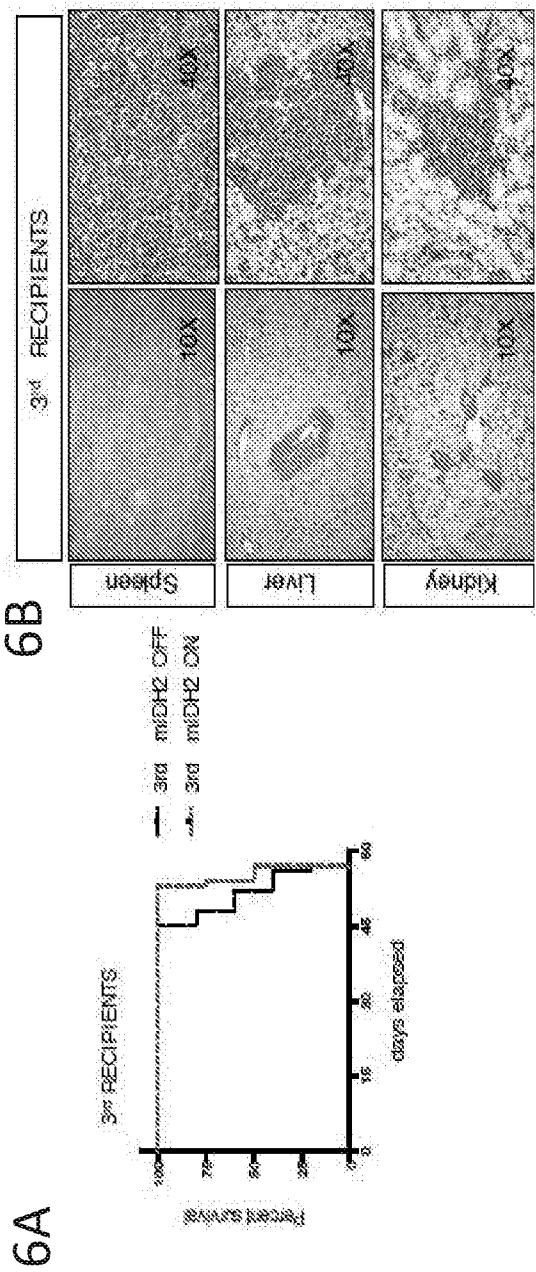


FIG. 6A-D

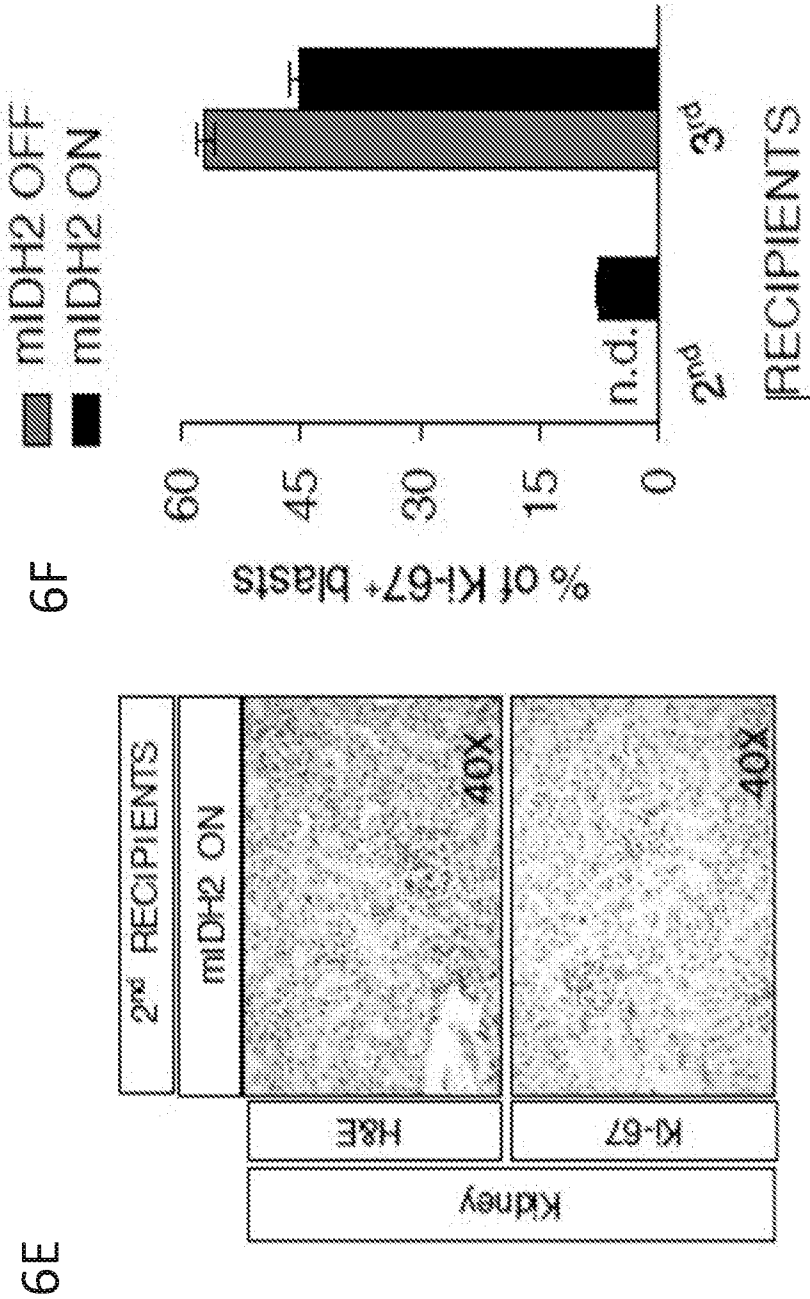


FIG. 6E-F

6G

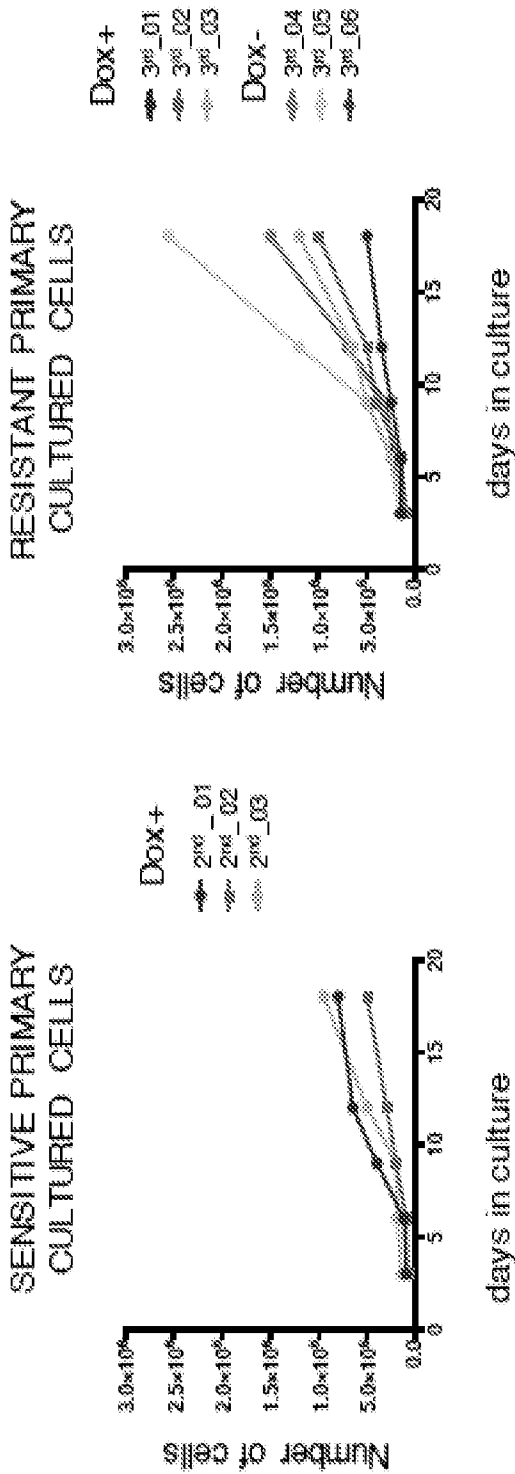


FIG. 6G

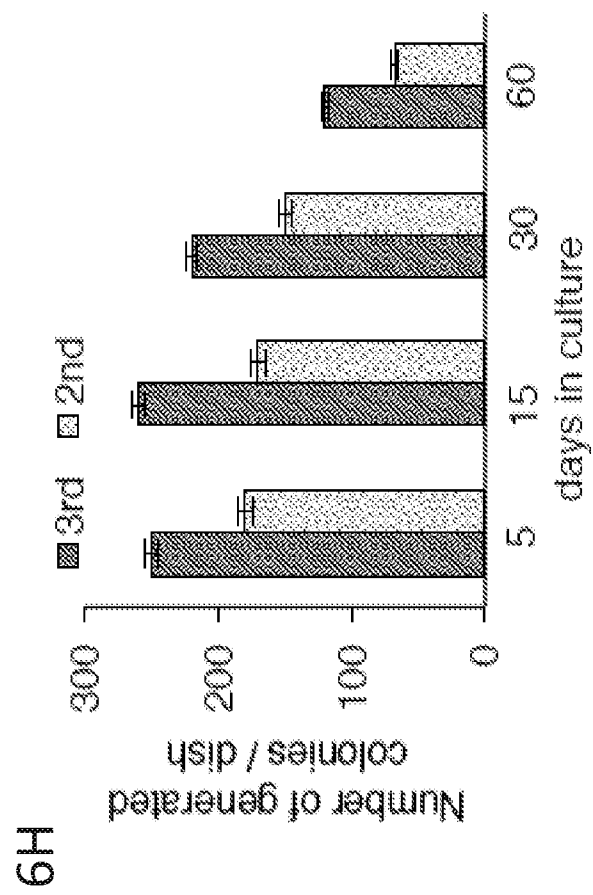


FIG. 6H

7A

2nd RECIPIENT
SENSITIVE
mIDH2 ON

3rd RECIPIENT
RESISTANT
mIDH2 ON

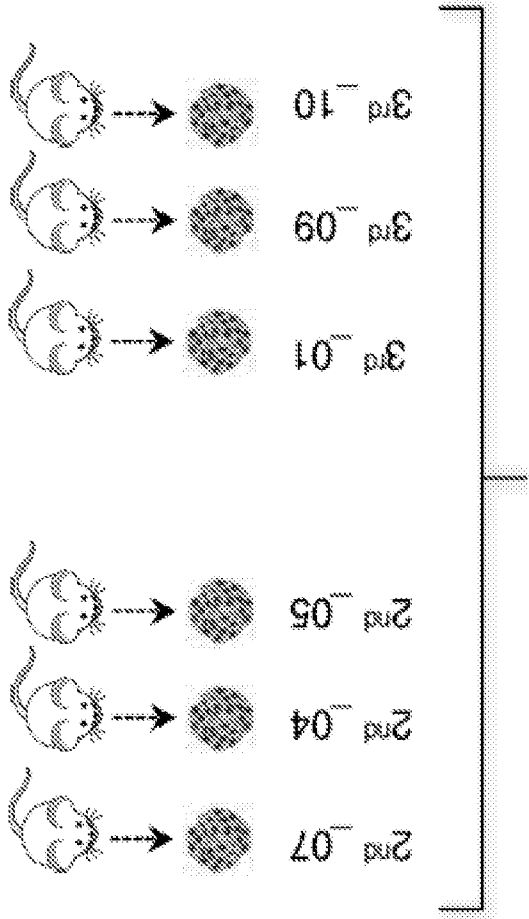
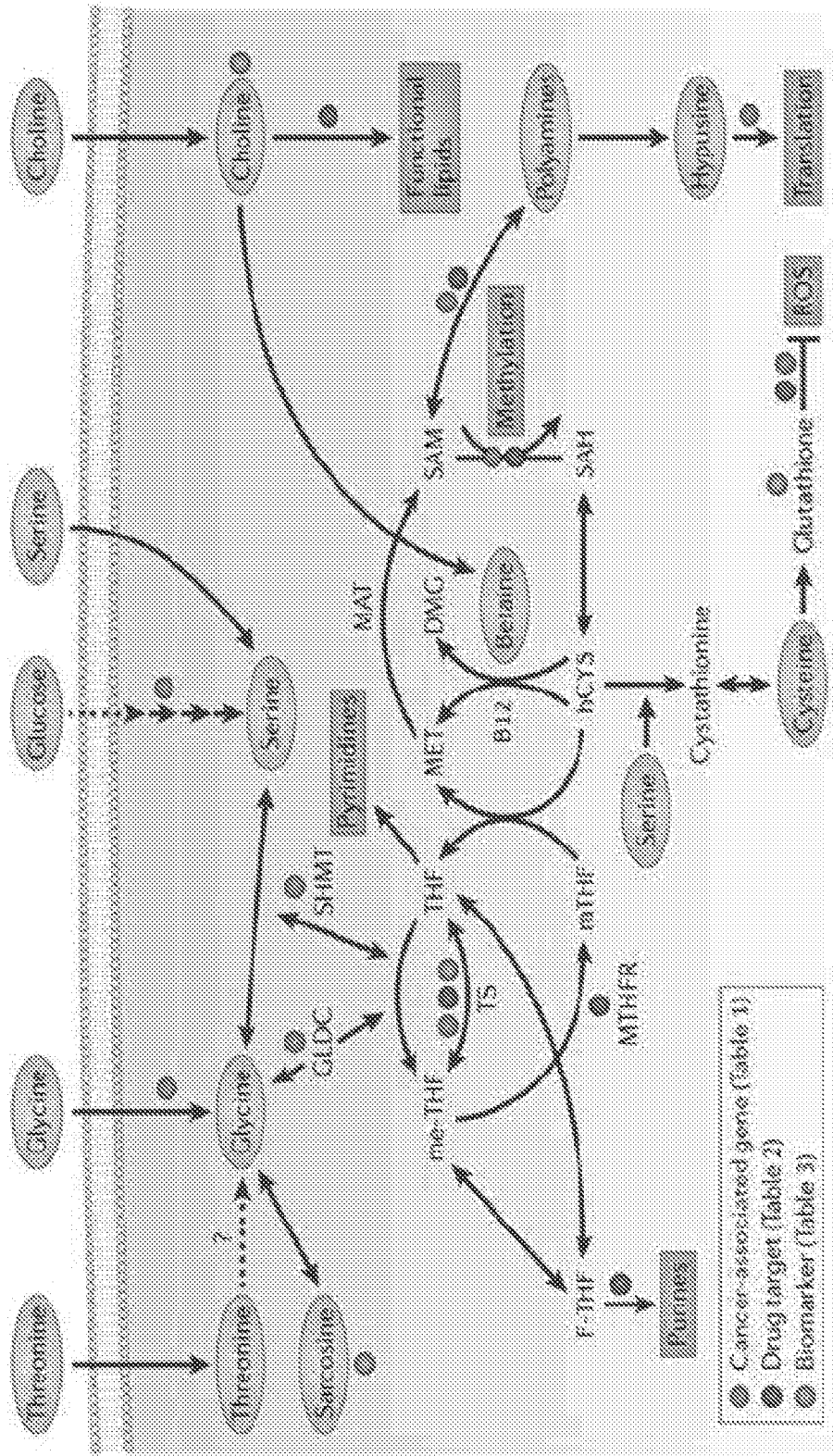


FIG. 7A

One-carbon metabolism



Locasale J.W. *Nature Reviews Cancer* 13, 572–583 (2013)

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FIG. 7B

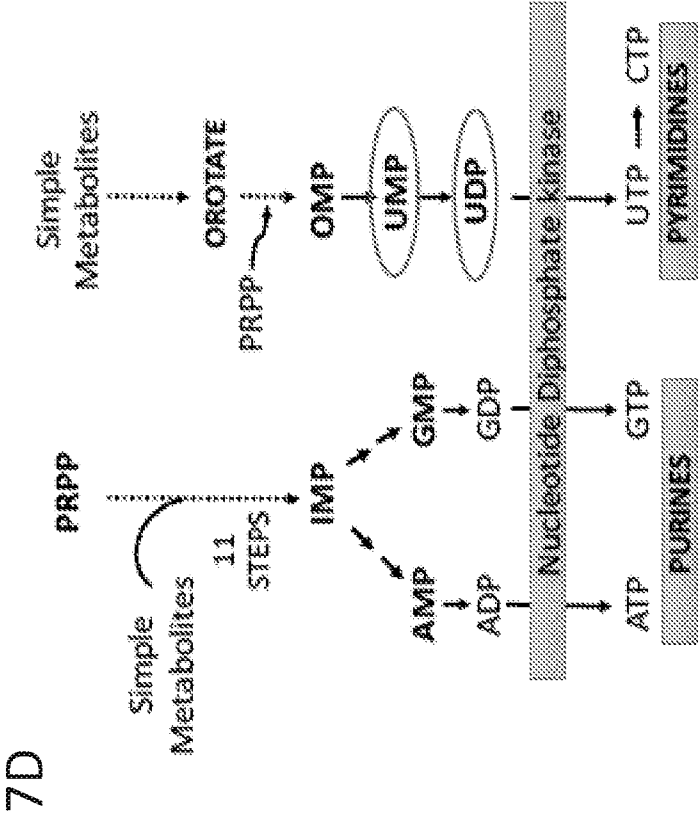
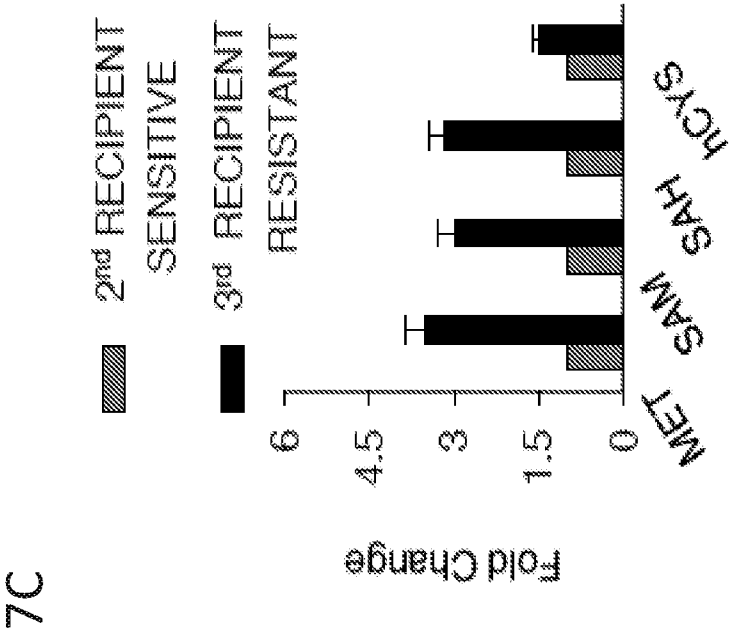


FIG. 7C-D

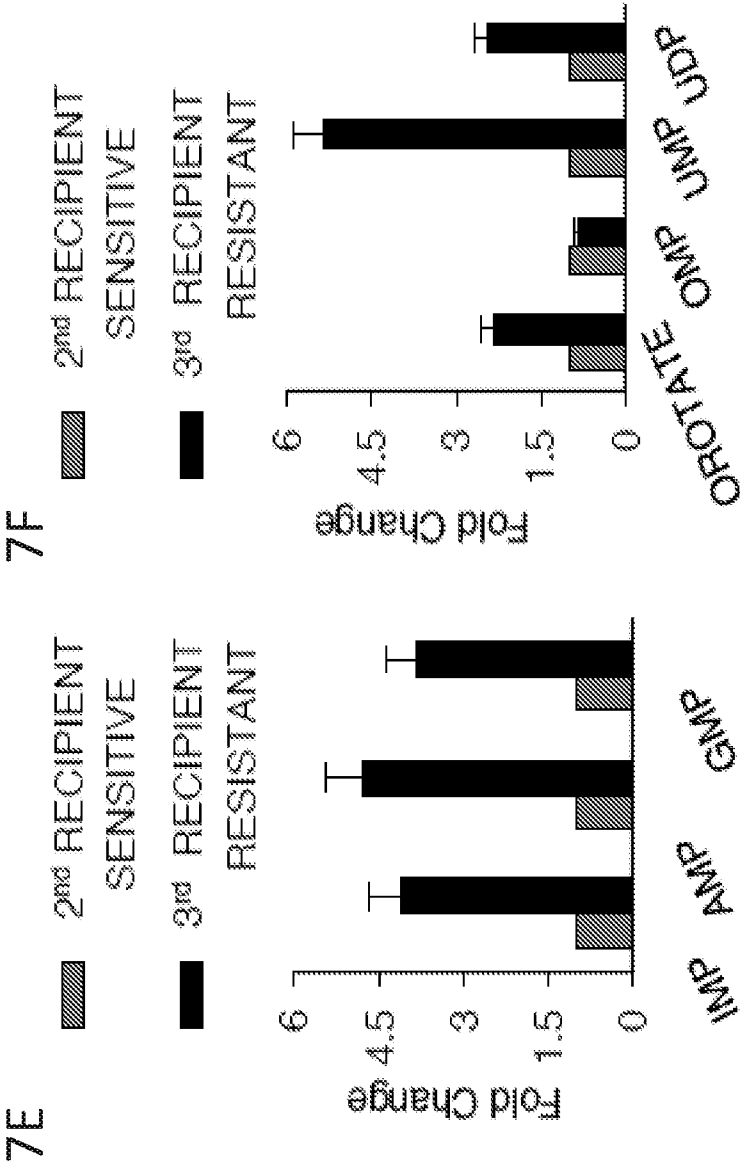


FIG. 7E-F

7G

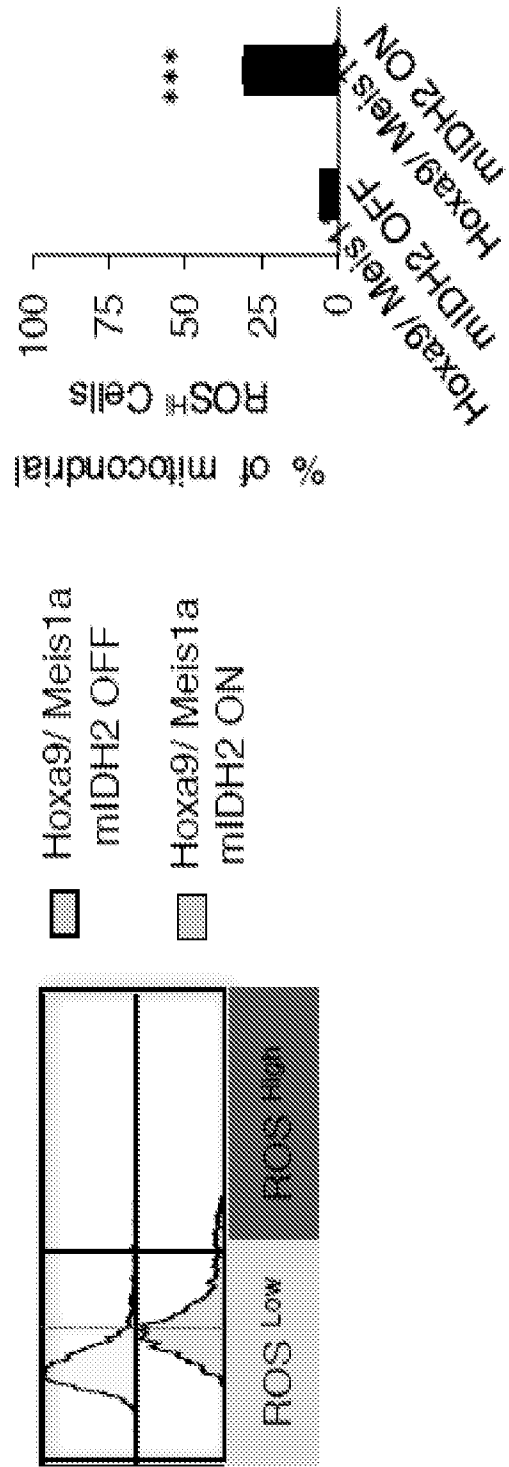
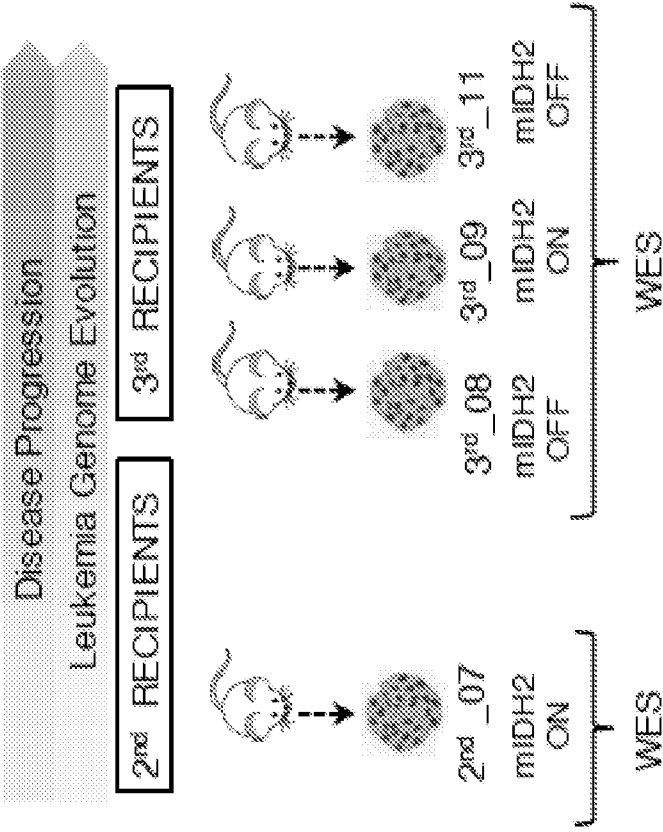


FIG. 7G

8A



8B

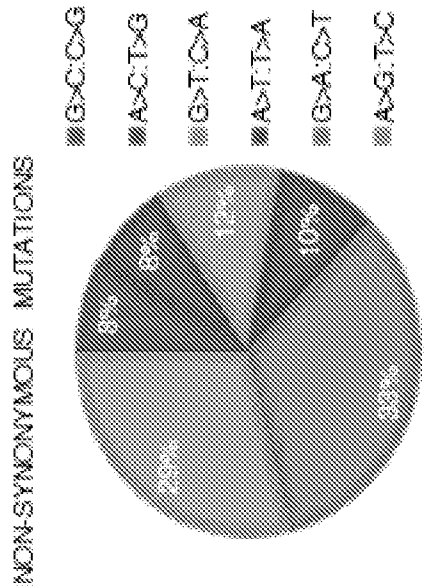


FIG. 8A-B

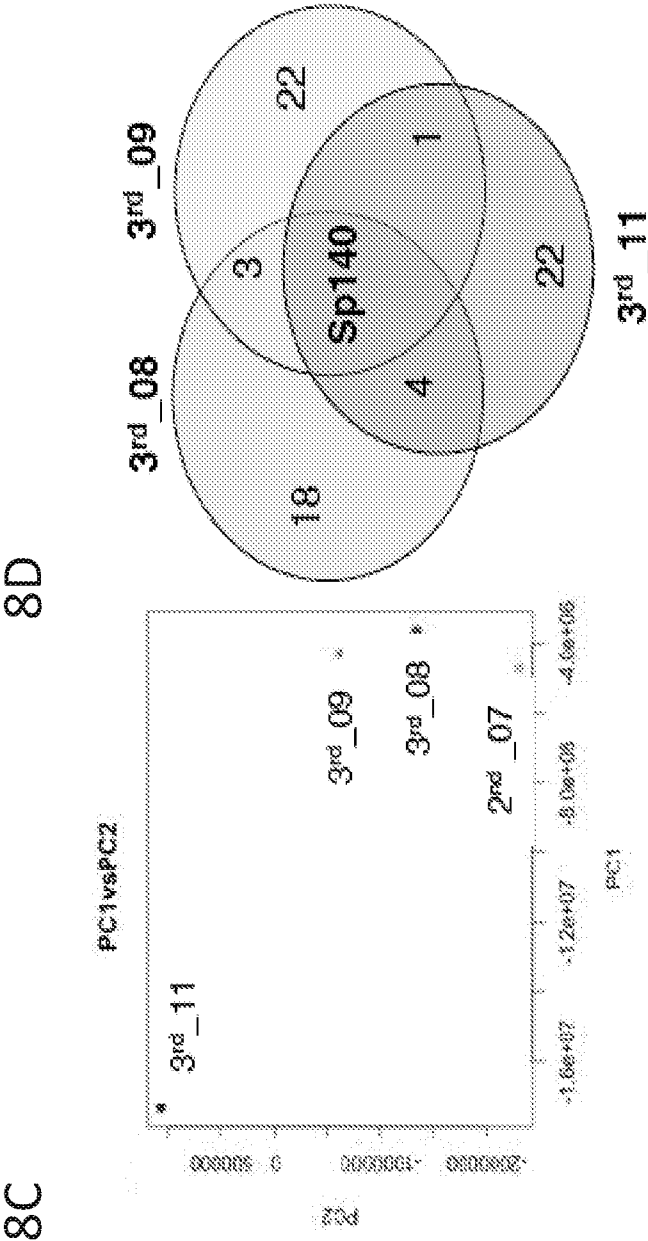


FIG. 8C-D

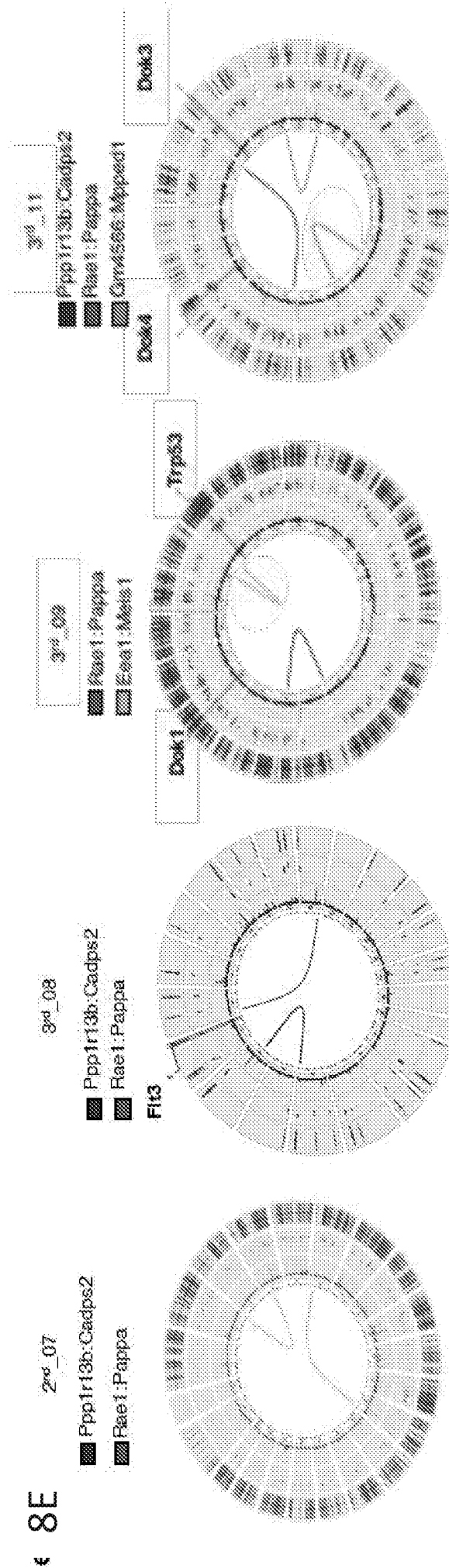
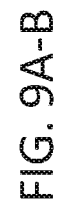
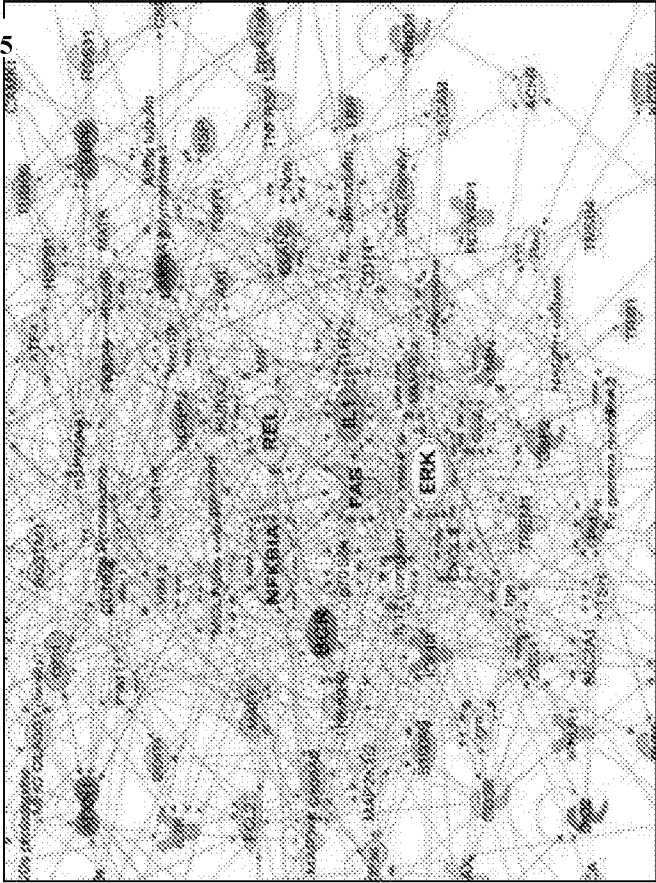


FIG. 8E



9D



9C

Gene	BaseMean	log2FC	padj
Ngp	6.27E+04	-2.7757528	3.38E-03
Zfp36	36063.75002	2.07595526	0.00033816
Hspa8	88925.13	0.9740221	0.002786943
Vim	61314.79	1.106799	0.000158346
Fos	65655.34437	2.2715723	0.000462202
Fosb	40527.62178	3.0482871	0.001009326
Junb	16056.97	1.267215	8.99E-02
Mpo	77471.367	-1.059484	0.2112287
Lyz2	165970.1	-0.8405201	0.1491053
Peap	178719.0279	-0.7551721	0.147382
Rn45s	81210	0.7815114	0.1478973
Ftl1	535.035	0.5313958	0.5778416

FIG. 9C-D

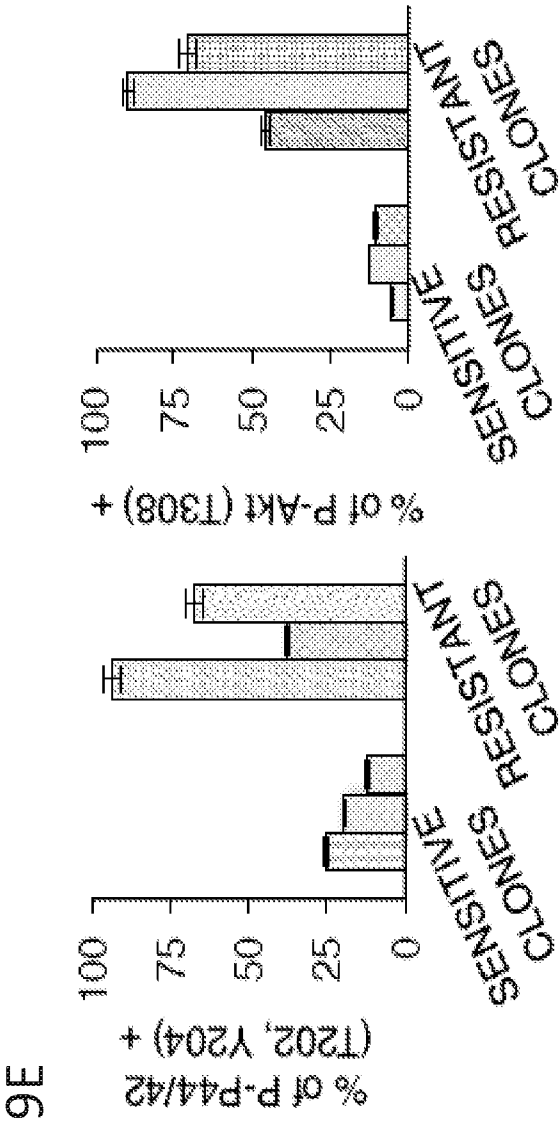
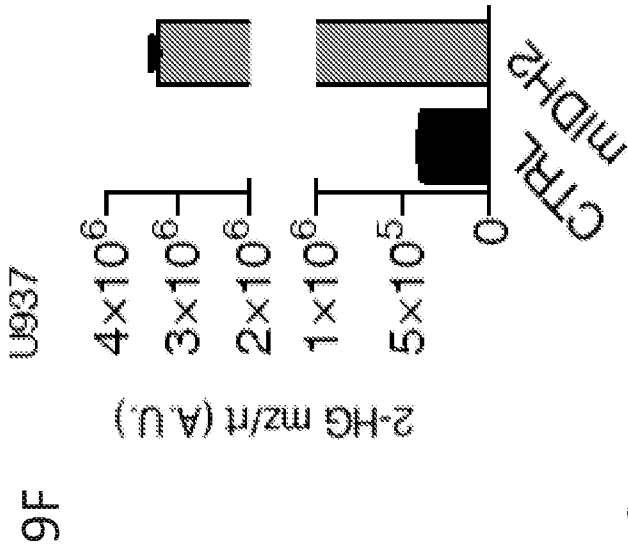


FIG. 9E-F

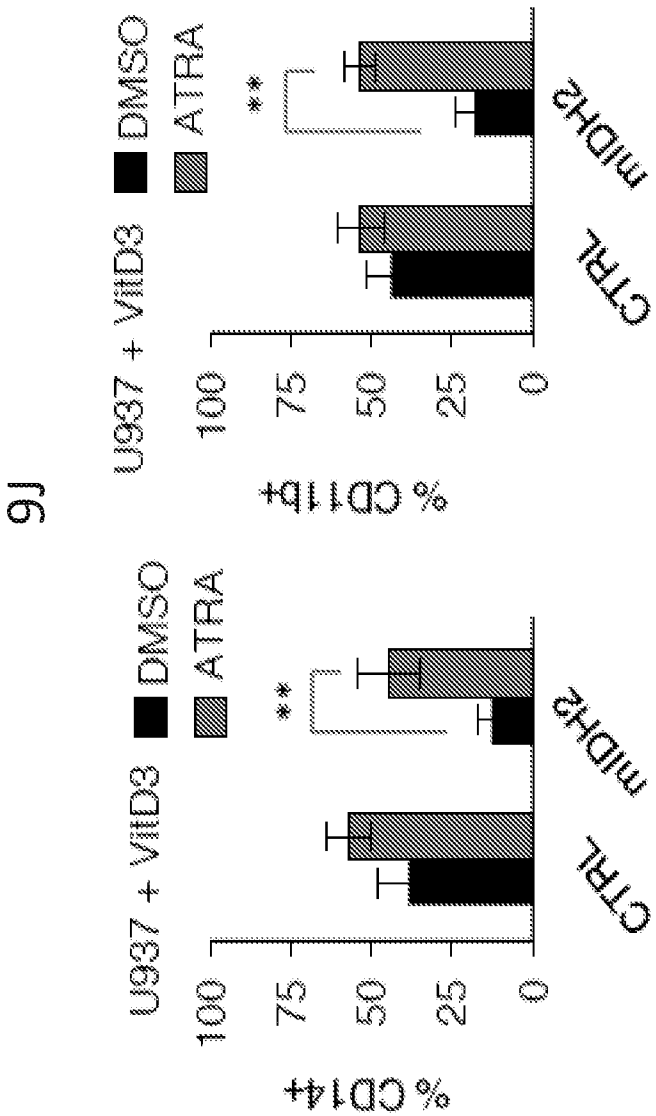
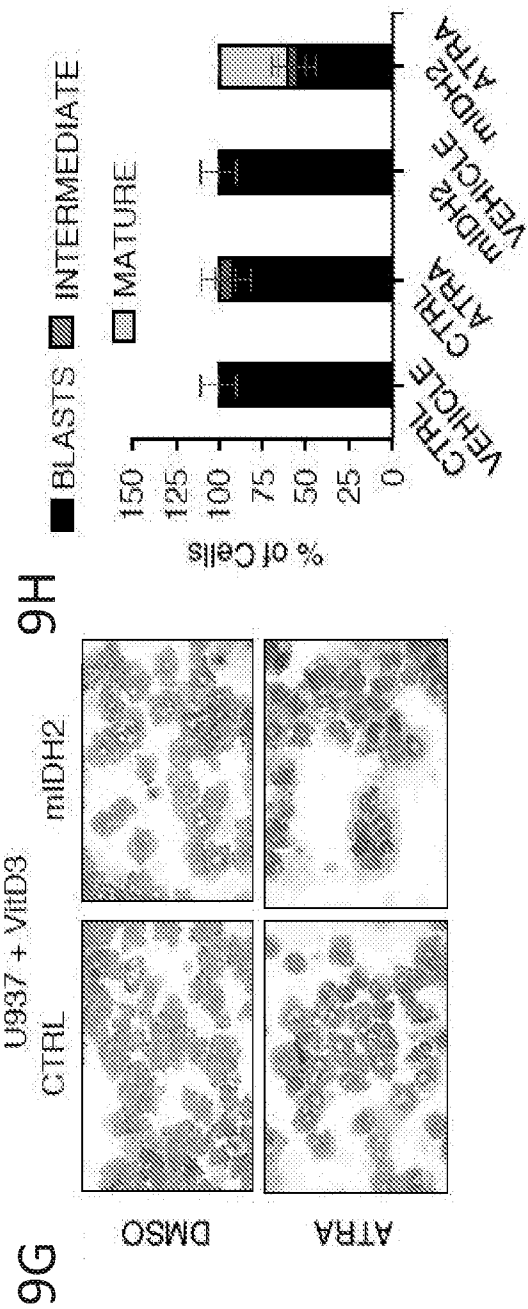


FIG. 9G-J

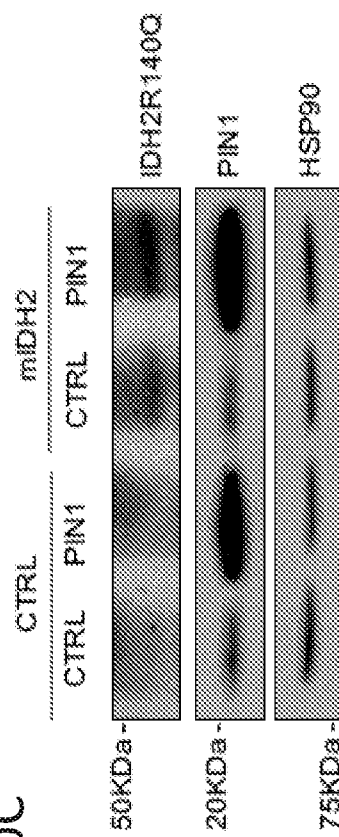
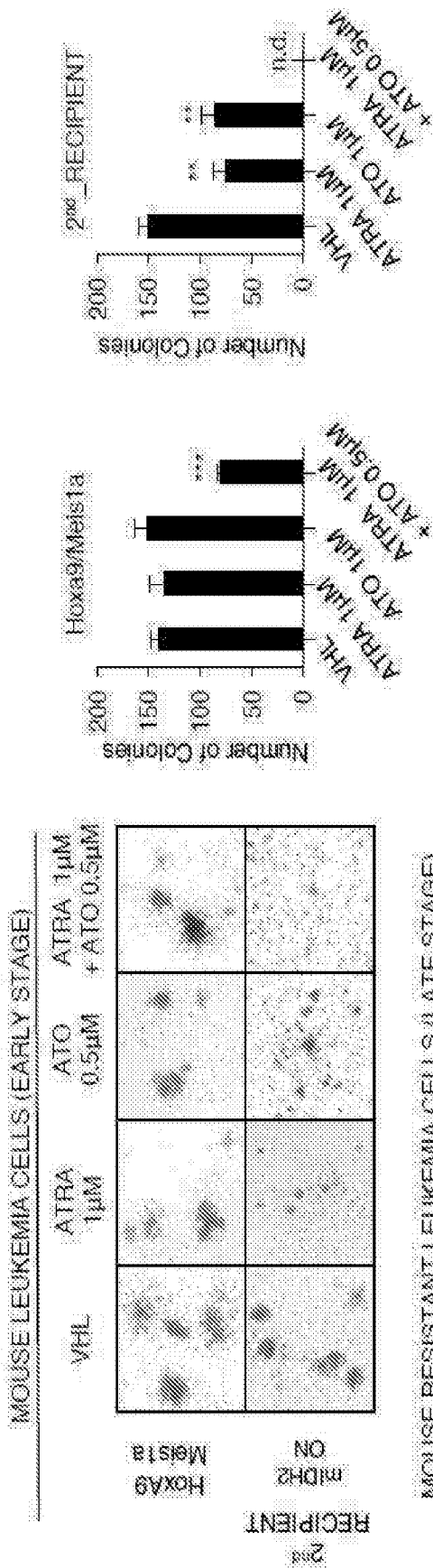


FIG. 10A-C

11A



11B

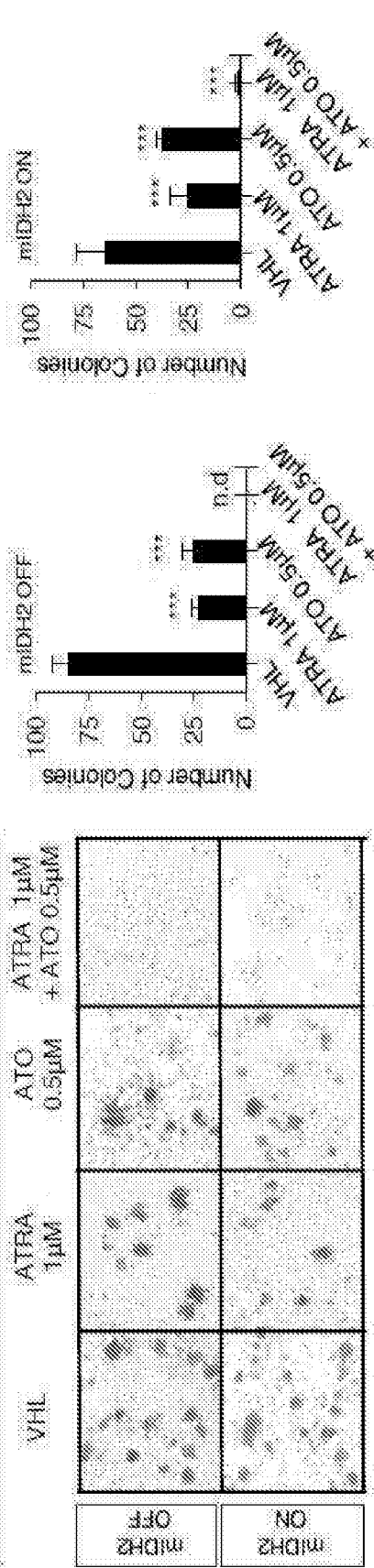
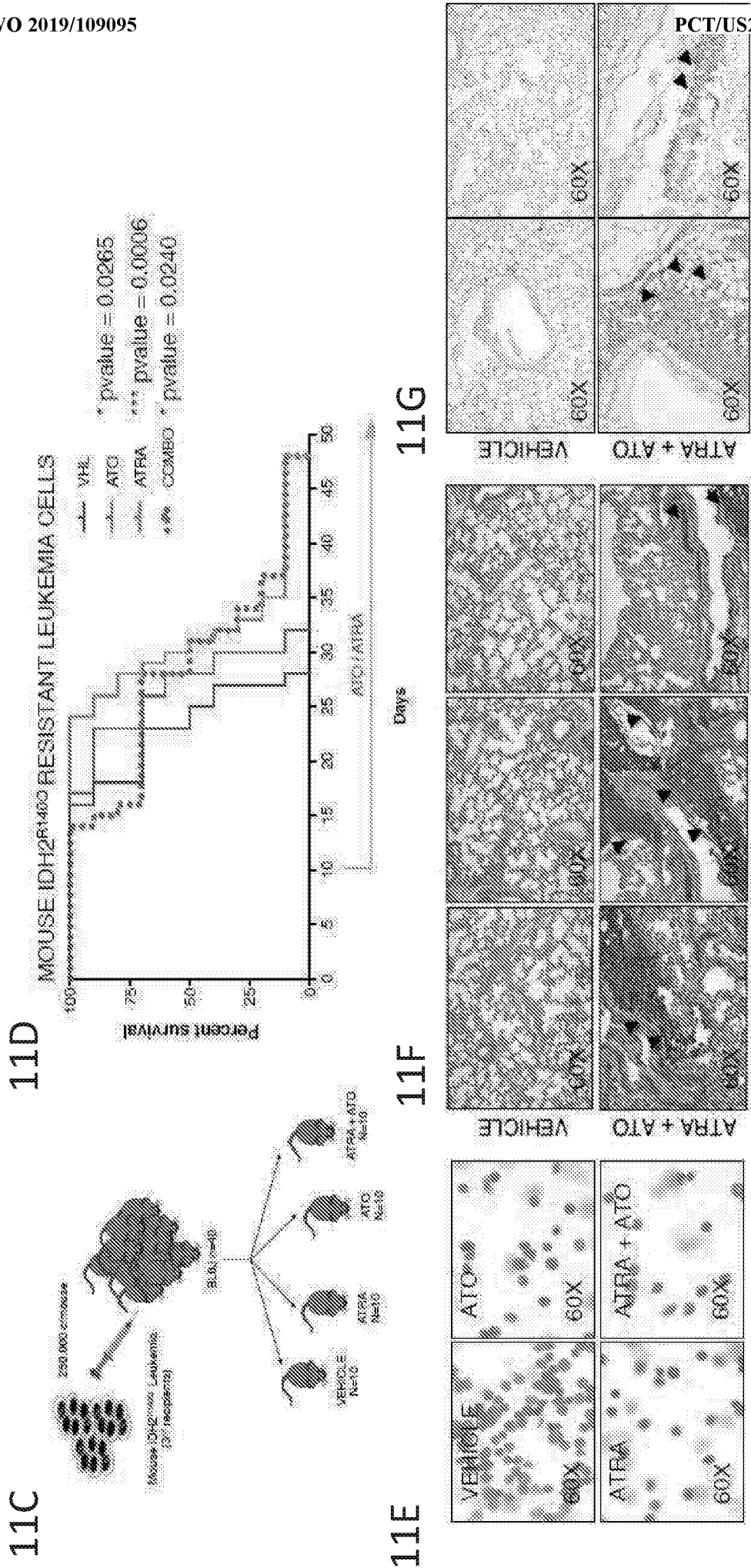
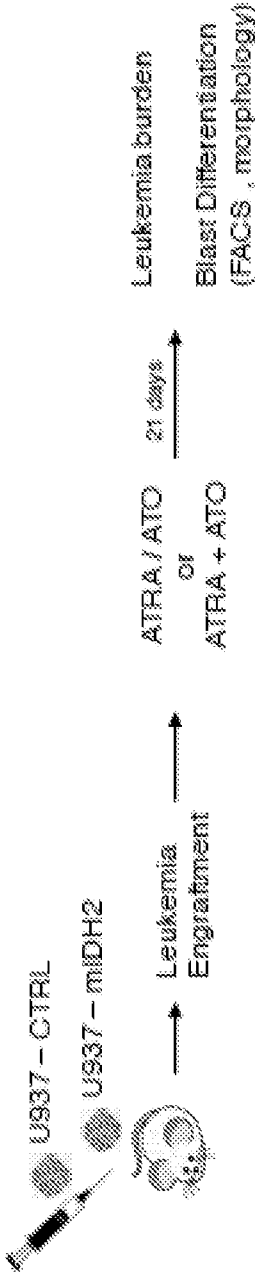


FIG. 11A-B



12A



12B

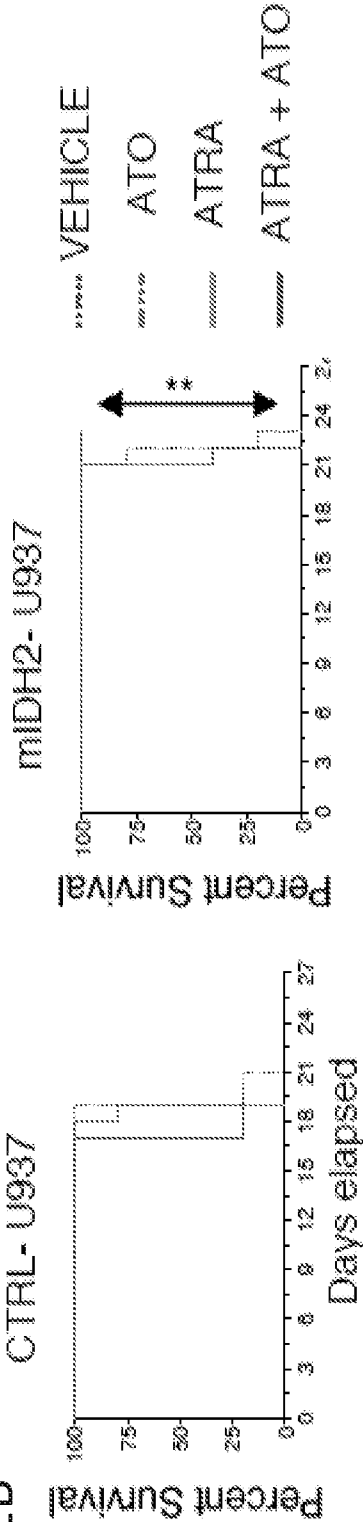
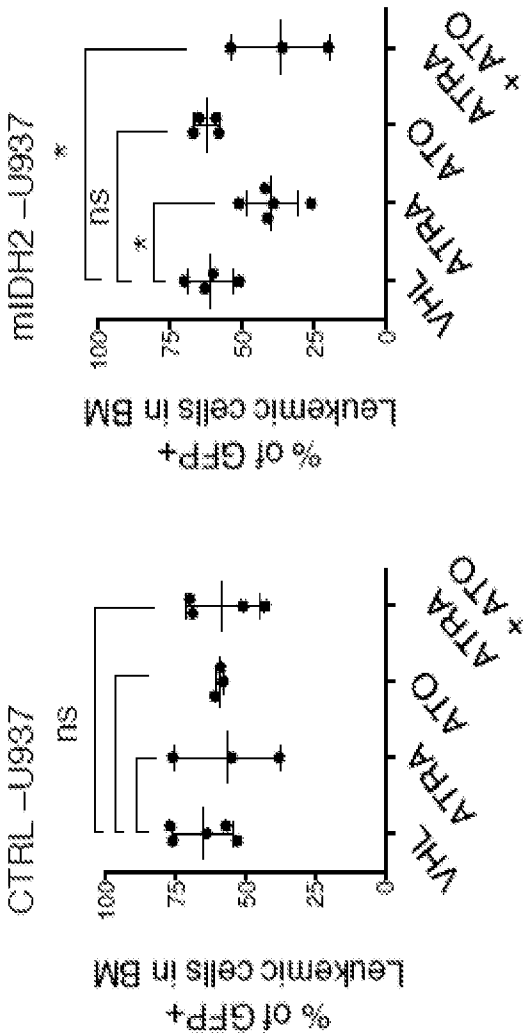


FIG. 12A-B

12C



12D

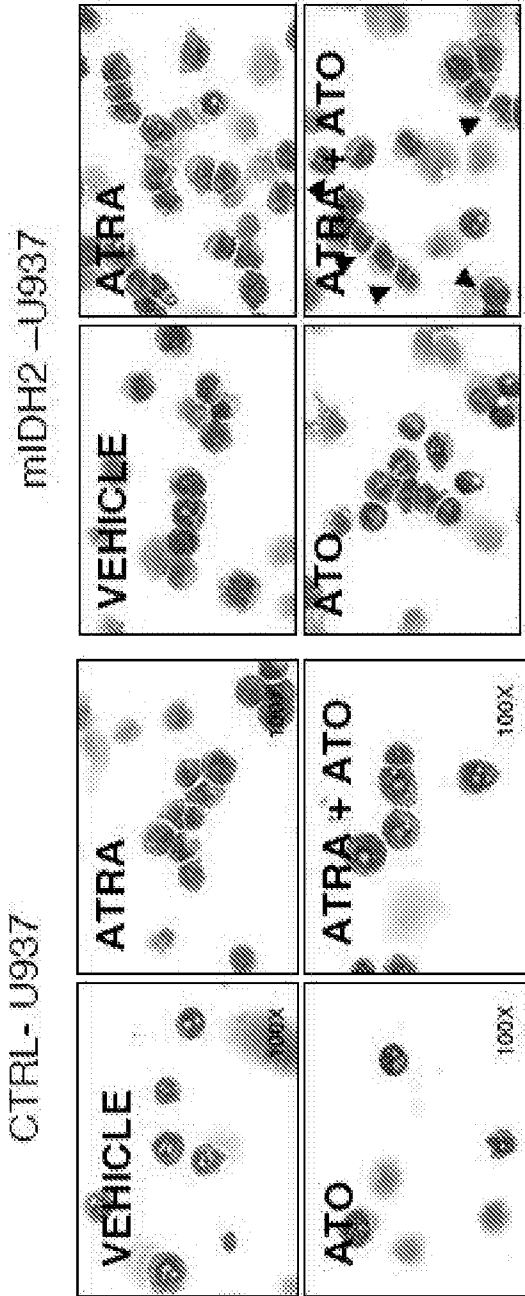


FIG. 12C-D

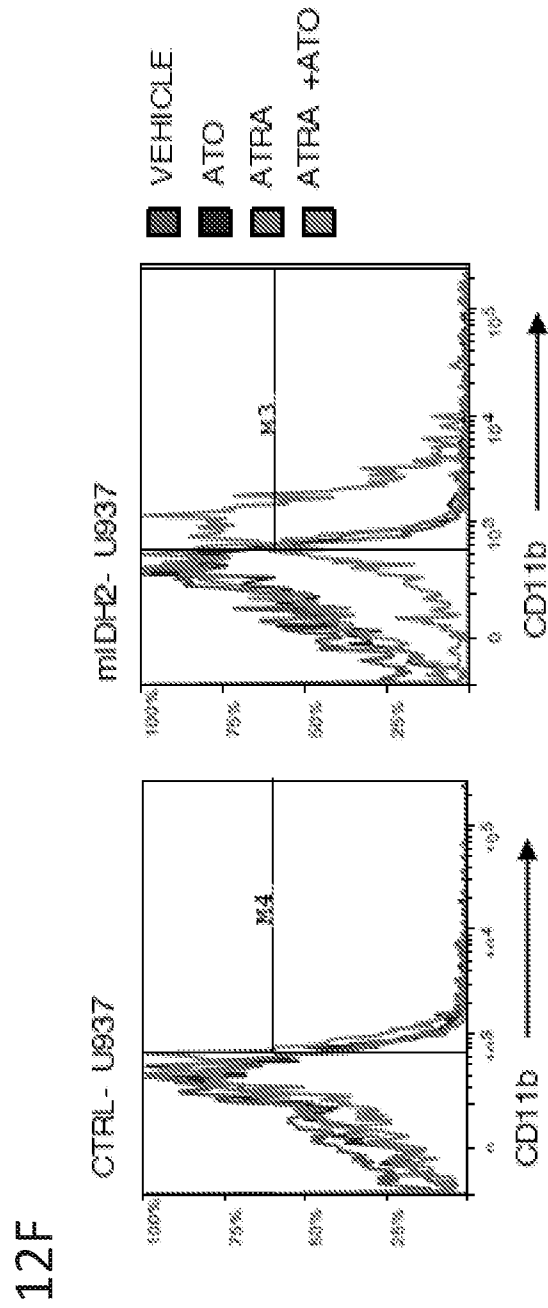
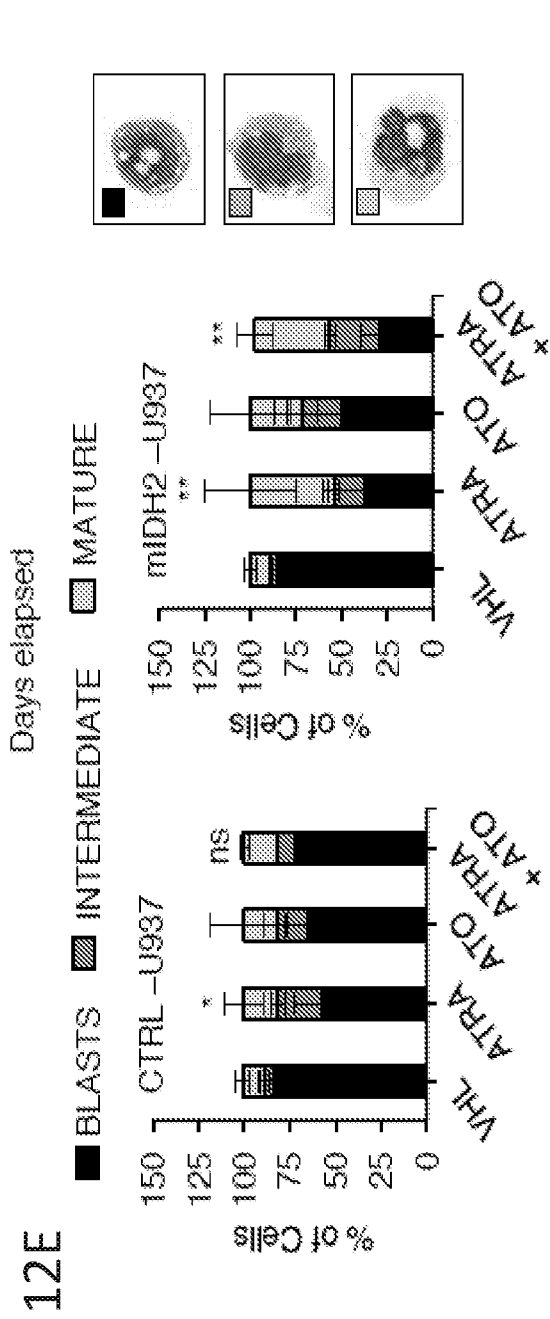
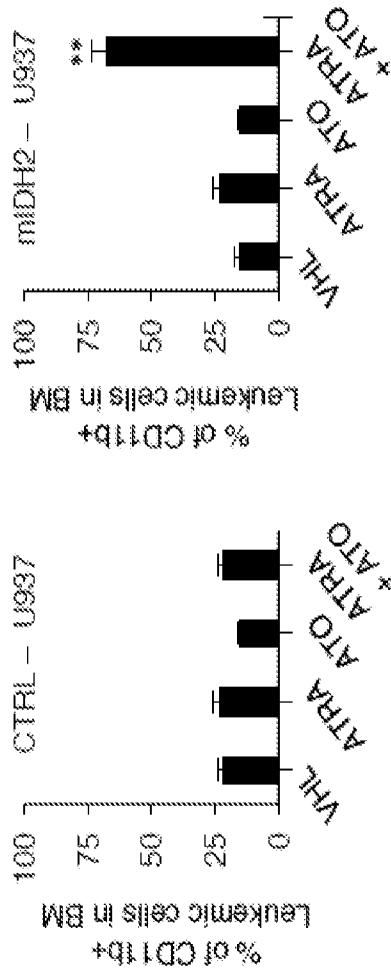


FIG. 12E-F

12G



12H

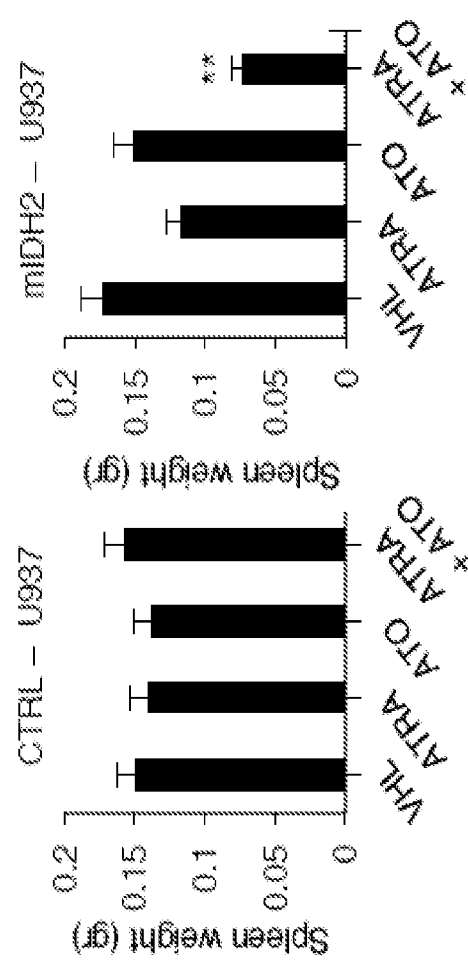
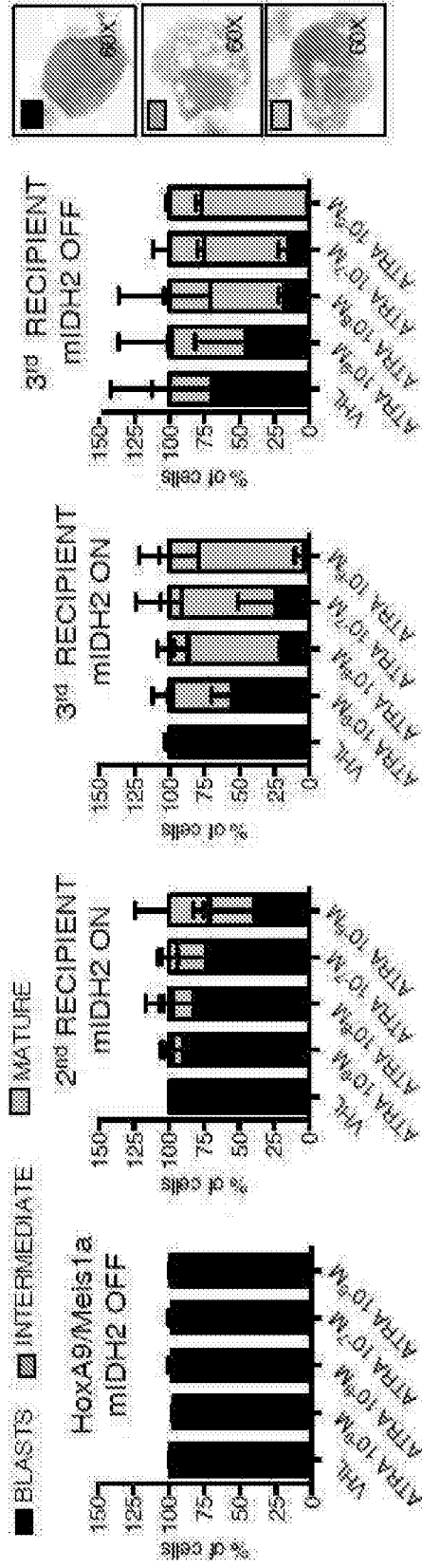
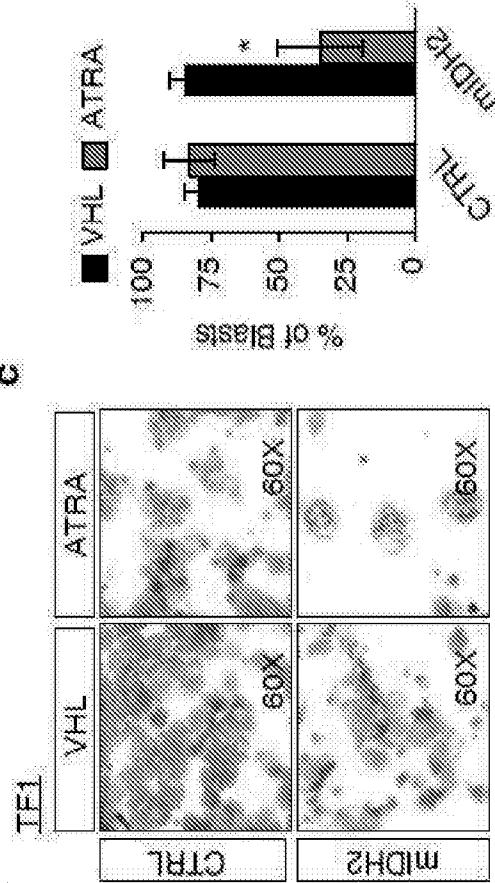


FIG. 12G-H

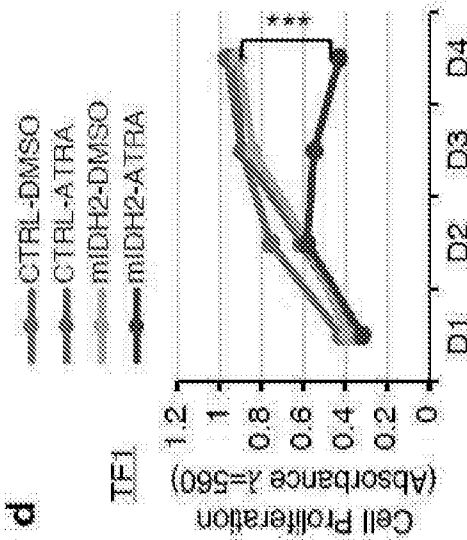
a MOUSE LEUKEMIA



b



d



e

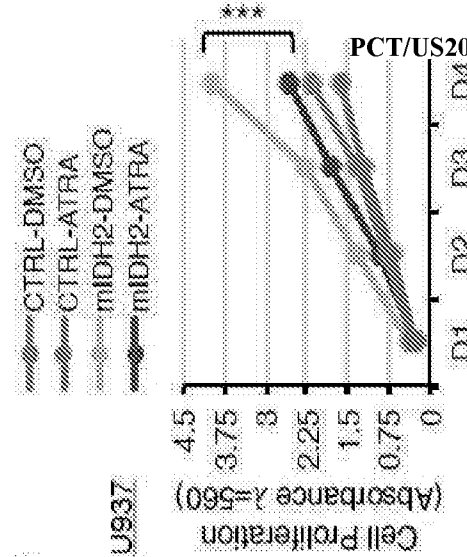


FIG. 13a-e

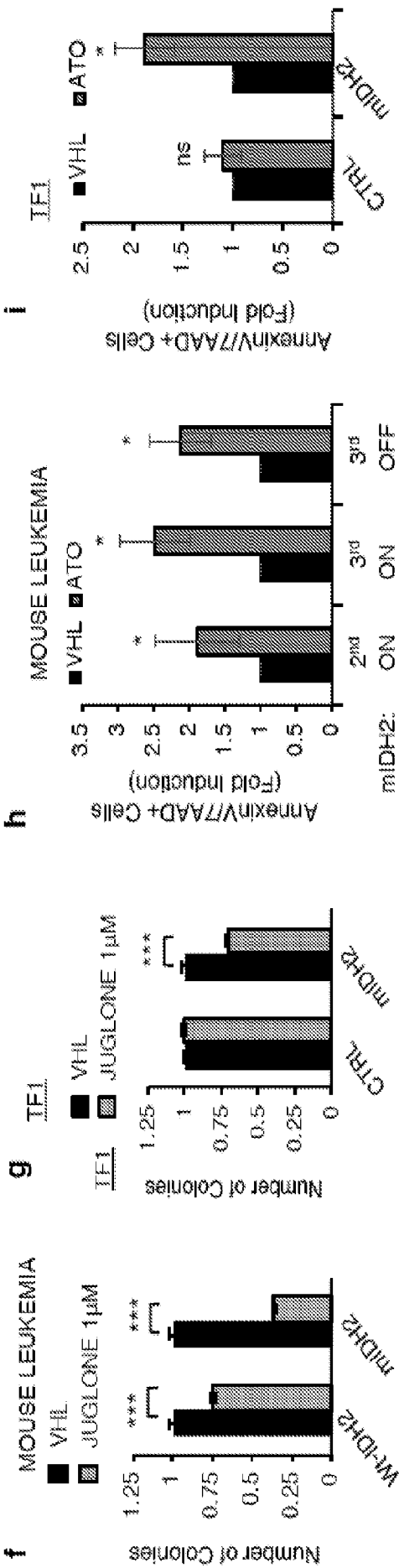


FIG. 13f-i

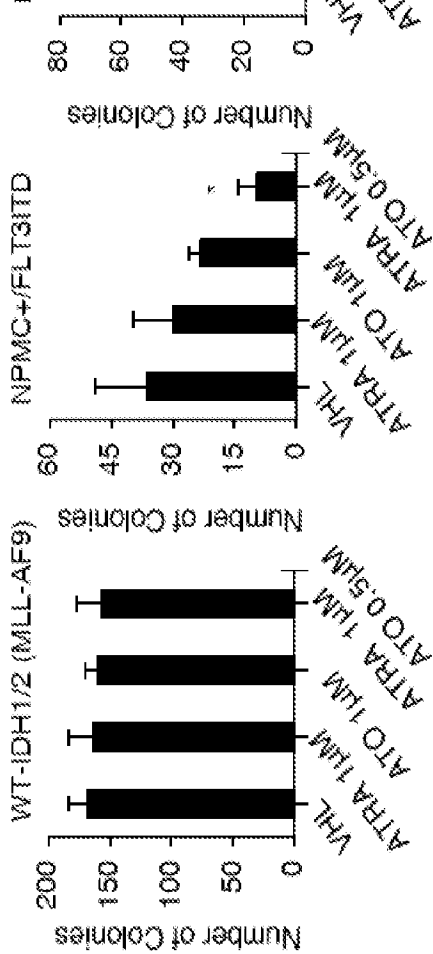
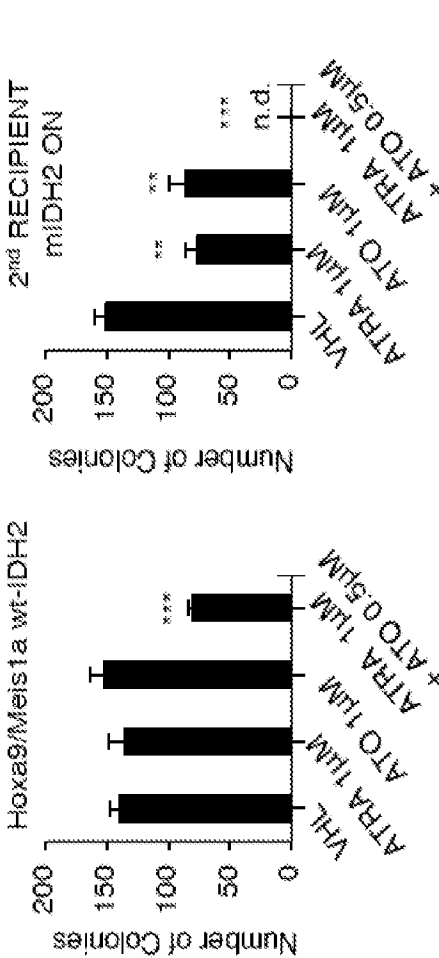
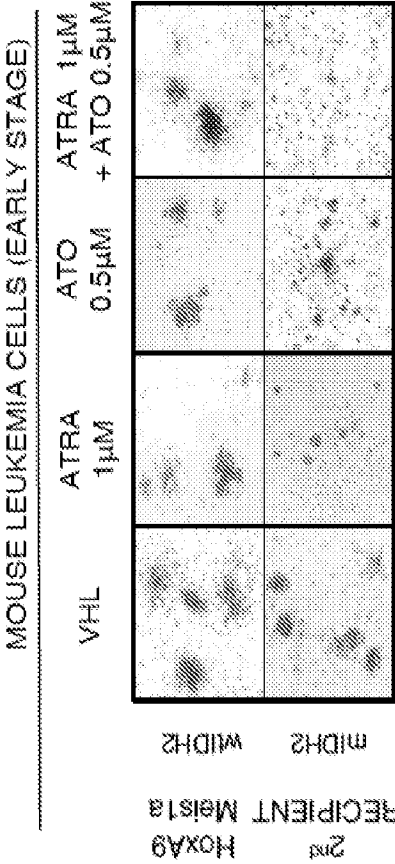


FIG. 13j-k

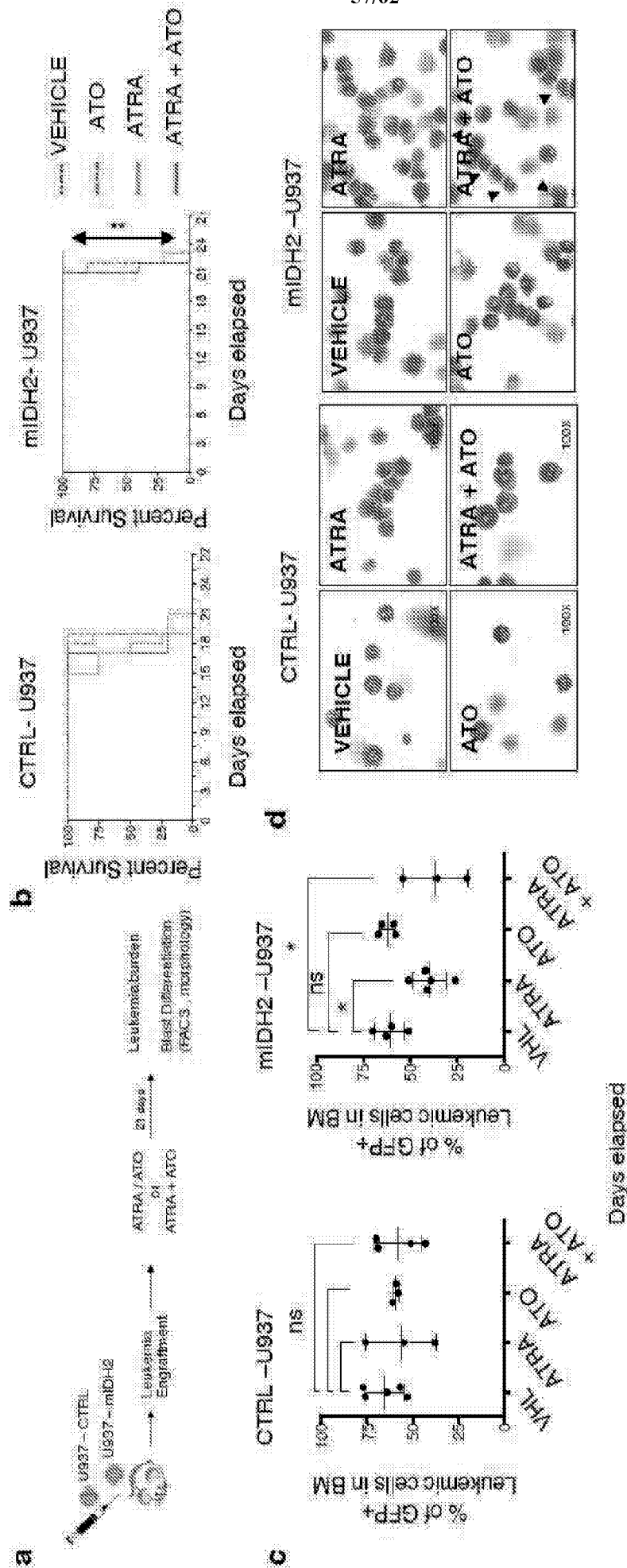
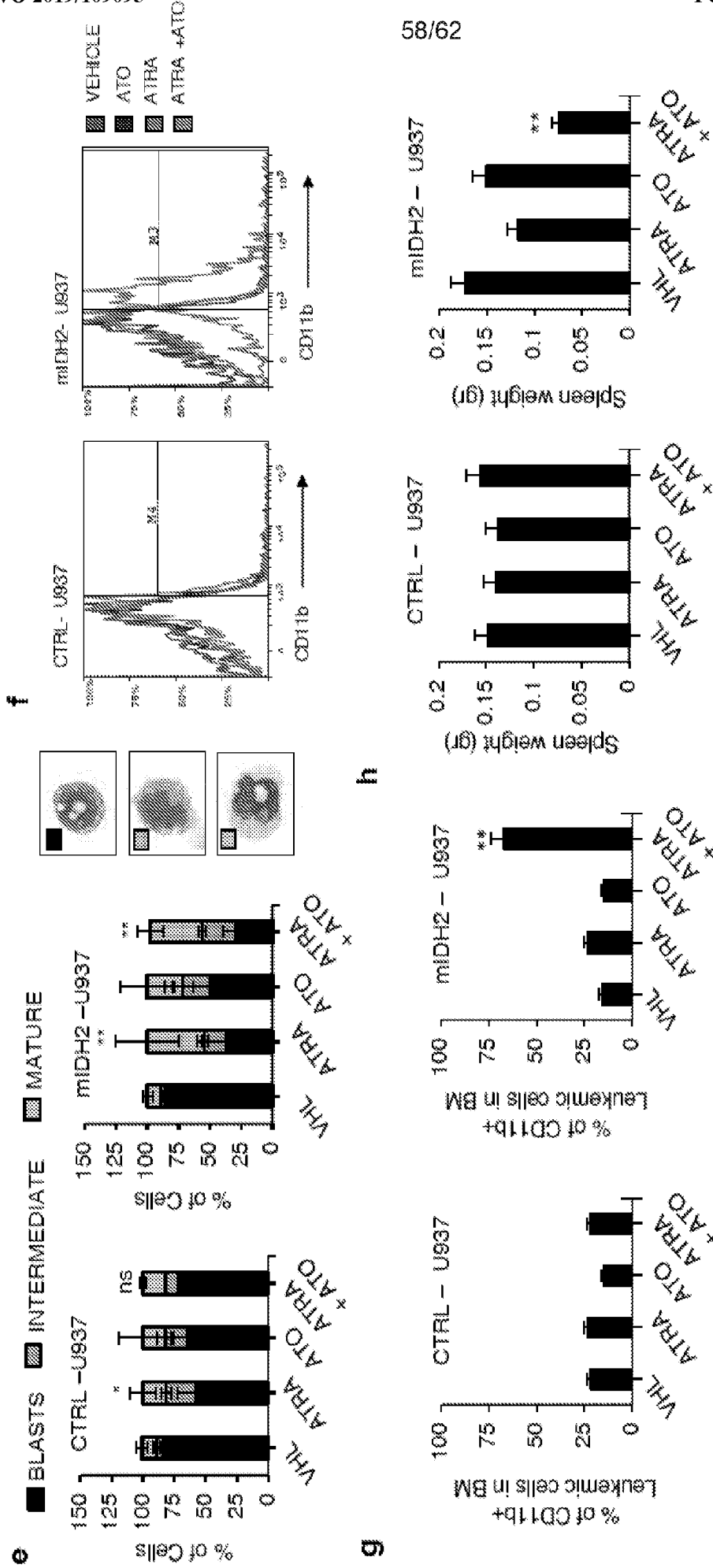


FIG. 14a-d



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FIG. 14e-h

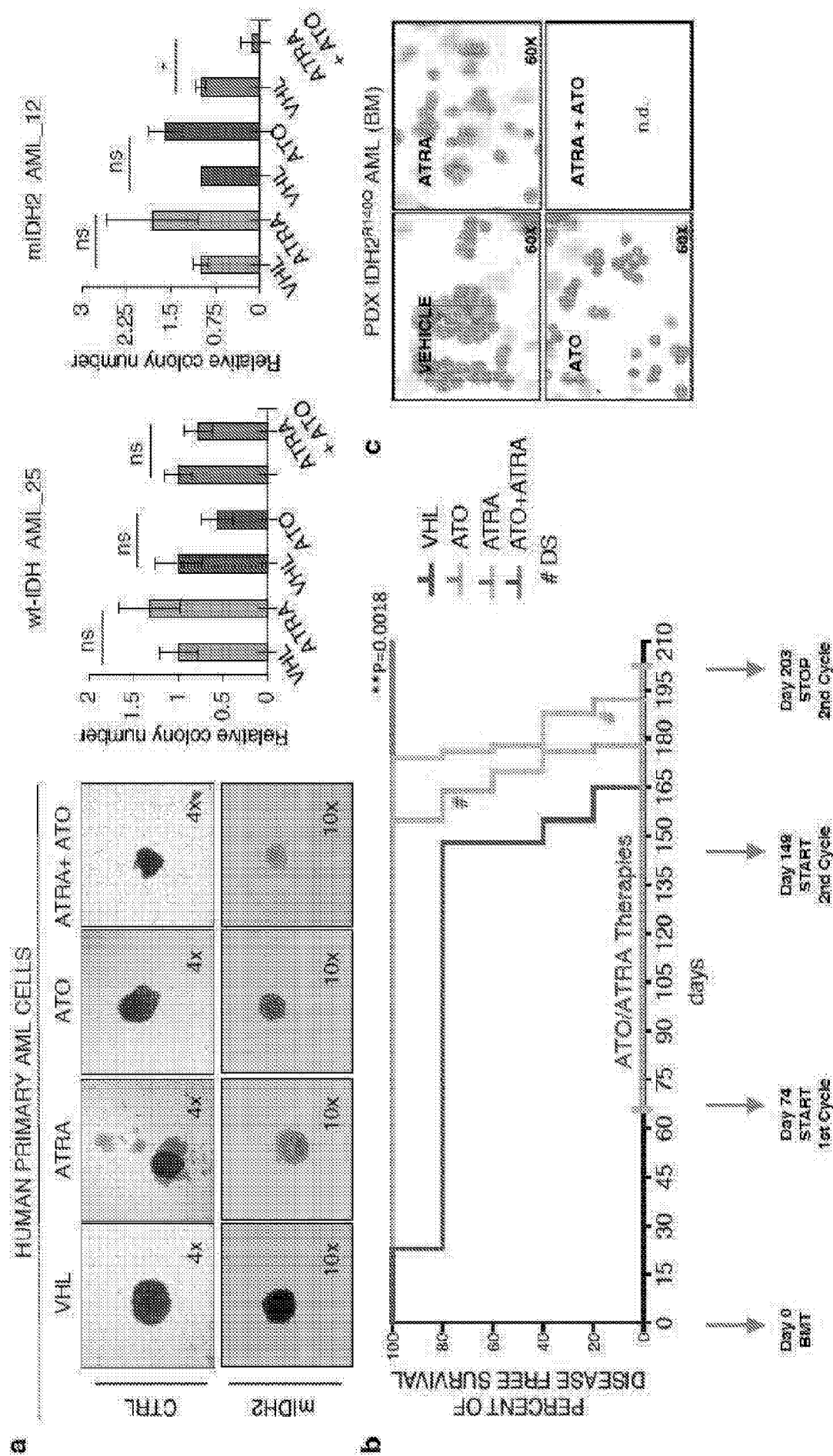


FIG. 15a-c

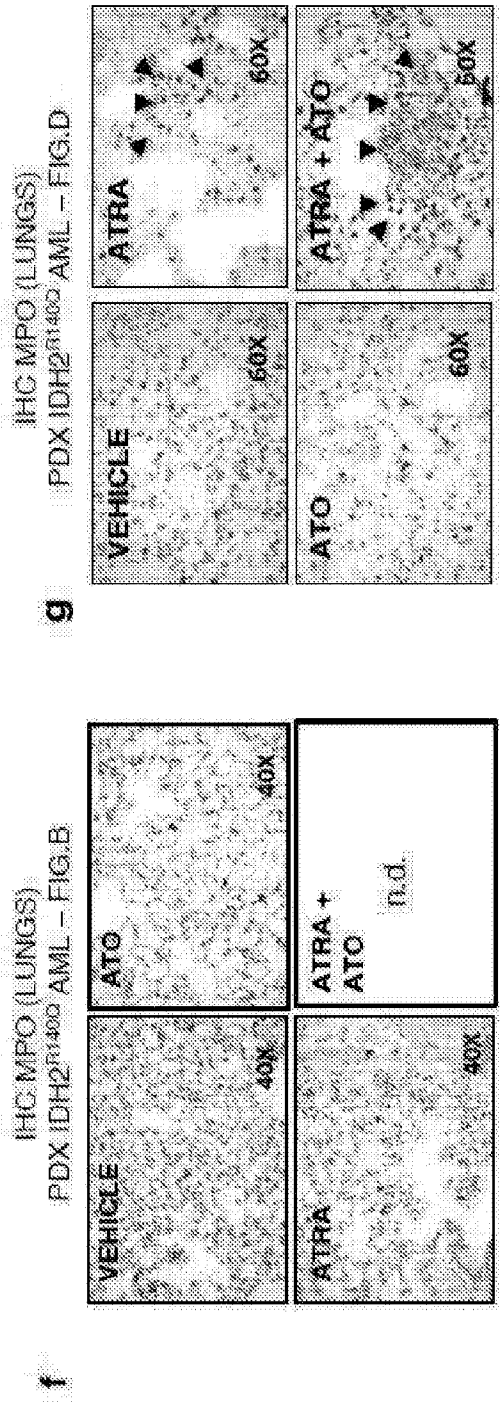
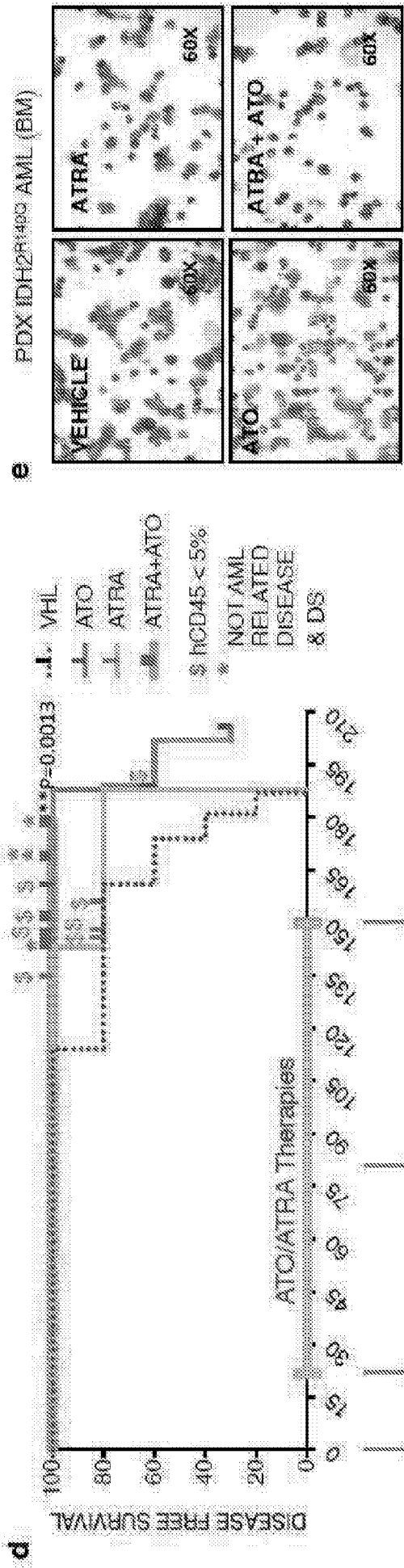


FIG. 15d-g

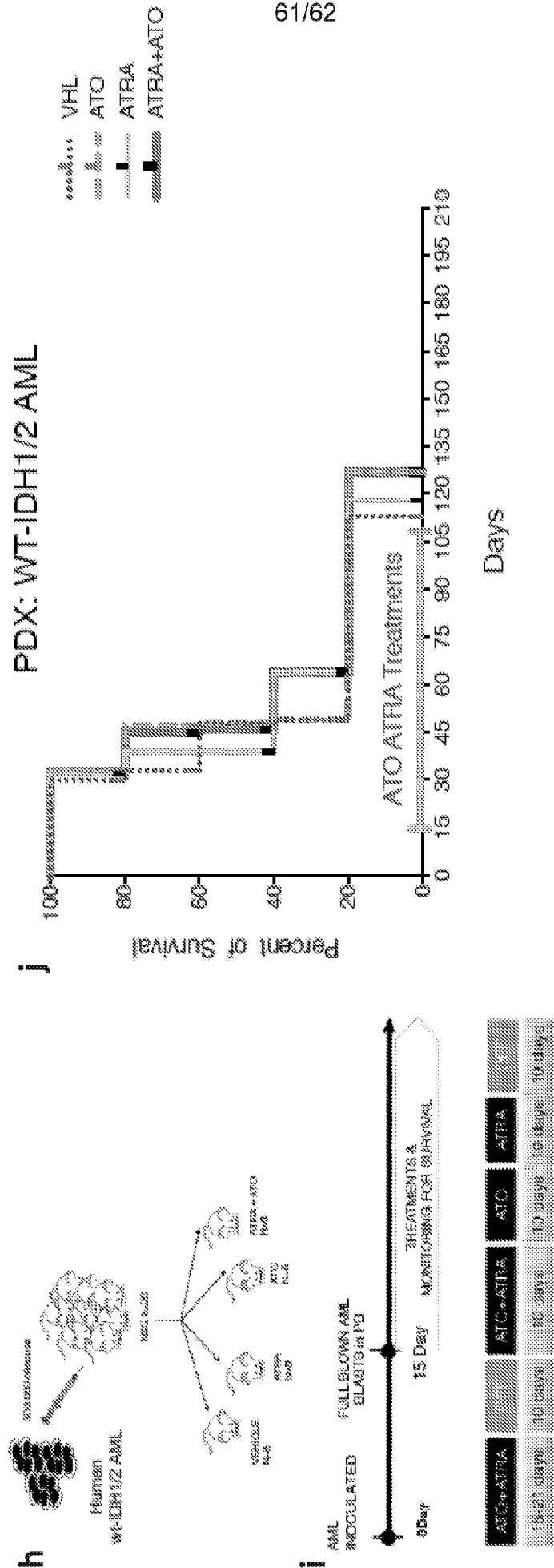


FIG. 15h-j

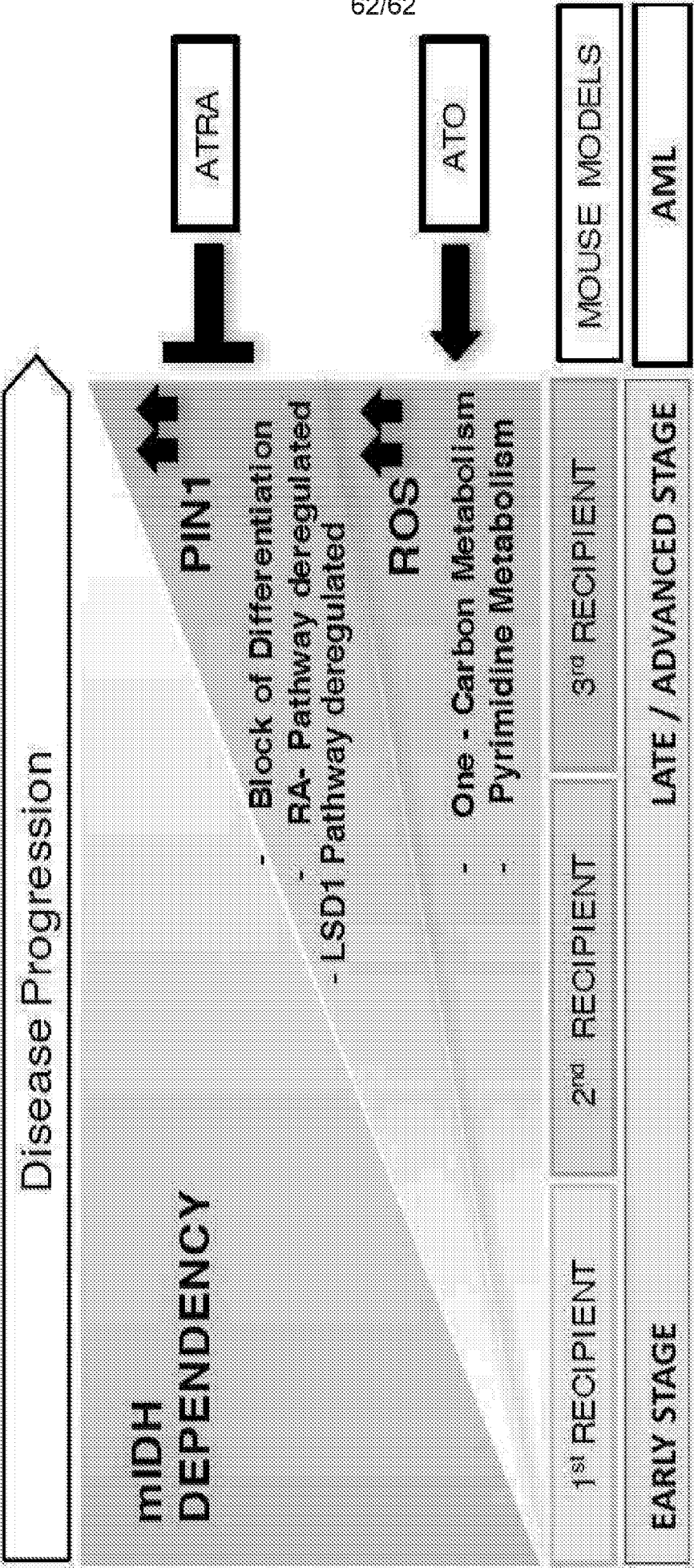


FIG. 16

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US18/63666

A. CLASSIFICATION OF SUBJECT MATTER

IPC - A61K 31/704, 31/706, 31/7068, 39/395, 45/06; A61N 5/10; C07K 16/28 (2019.01)

CPC - A61K 31/704, 31/706, 31/7068, 39/395, 39/39558, 45/06; A61N 5/1027, 5/1045, 5/1084; C07K 16/28, 16/2896

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.
X ----- Y	US 2016/0222106 A1 (JANSSEN BIOTECH INC.) 04 August 2016; abstract; paragraphs [0044], [0102]-[0104], [0118], [0126], [0240], [0248], [0298]	1-7, 12, 23-31, 34, 38-39, 42-44 ----- 8-11, 13-22, 32-33, 35-37, 40-41, 45-62, 63/48, 63/48, 63/52-62, 64/63/48, 64/63/52-62, 66-72
Y	US 2004/0146575 A1 (WARRELL et al.) 29 July 2004; abstract; paragraphs [0016], [0020], [0025], [0035], [0041], [0056], [0069], [0092], [0101], [0125]; claim 1	8-22, 30, 37, 41, 45-62, 63/48, 63/48, 63/52-62, 64/63/48, 64/63/52-62, 65/63/48 65/63/52-62, 66-72
Y	US 2017/0202799 A1 (BETH ISRAEL DEACONESS MEDICAL CENTER INC.) 20 July 2017; paragraph [0012]	13, 31-35, 49
Y	US 2014/0371176 A1 (GLAXOSMITHKLINE INTELLECTUAL PROPERTY (NO 2) LTD) 18 December 2014; abstract; paragraphs [0245], [0302]	35-36, 50-51
Y	US 2017/0246174 A1 (CELGENE CORPORATION et al.) 31 August 2017; paragraph [0150]	40, 65/63/48, 65/63/52-62
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☒ Further documents are listed in the continuation of Box C.☐ See patent family annex.

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29 January 2019 (29.01.2019)

Date of mailing of the international search report

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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2008/0119559 A1 (WEISSBACH et al.) 22 May 2008; paragraph [0009]	11, 29