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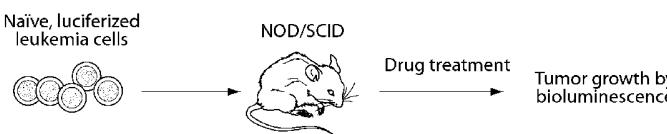
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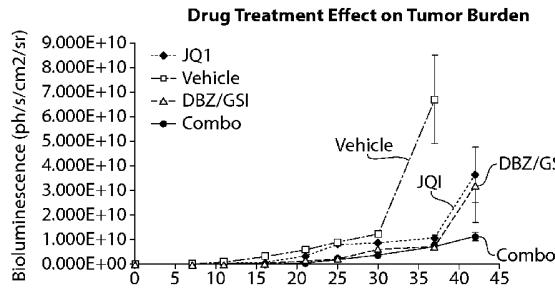
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(54) Title: DIAGNOSTIC AND TREATMENT METHODS IN SUBJECTS HAVING OR AT RISK OF DEVELOPING RESISTANCE TO CANCER THERAPY



Drug Treatment Effect on Tumor Burden



Treatment (days)	Vehicle (ph/s/cm²/sr)	JQ1 (ph/s/cm²/sr)	DBZ/GSI (ph/s/cm²/sr)	Combo (ph/s/cm²/sr)
0	0.000E+00	0.000E+00	0.000E+00	0.000E+00
5	0.000E+00	0.000E+00	0.000E+00	0.000E+00
10	0.000E+00	0.000E+00	0.000E+00	0.000E+00
15	0.000E+00	0.000E+00	0.000E+00	0.000E+00
20	0.000E+00	0.000E+00	0.000E+00	0.000E+00
25	0.000E+00	0.000E+00	0.000E+00	0.000E+00
30	~1.0E+10	~1.0E+10	~1.0E+10	~1.0E+10
35	~7.5E+10	~2.0E+10	~2.0E+10	~1.0E+10
40	~7.5E+10	~3.5E+10	~3.5E+10	~1.5E+10
45	~7.5E+10	~3.5E+10	~3.5E+10	~1.5E+10

(57) Abstract: The invention relates to methods of treatment and diagnosis of subjects with cancer. In some aspects, the invention relates to methods of treatment and diagnosis of subjects with cancer, wherein the cancer is characterized by a Notch pathway activation mutation or by resistance to a Notch pathway inhibitor.

Fig. 10

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**DIAGNOSTIC AND TREATMENT METHODS IN SUBJECTS HAVING OR AT RISK
OF DEVELOPING RESISTANCE TO CANCER THERAPY**

FIELD OF INVENTION

5 The invention relates to treatment of certain cancers, including cancers that are resistant to the standard of care, and to methods of diagnosing the resistance phenotype.

GOVERNMENT SUPPORT

This invention was made with government support under 5U01ES017155 awarded by
10 the National Institutes of Health. The Government has certain rights in the invention.

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 61/661,772, filed June 19, 2012, U.S. Provisional Application No. 61/661,884, filed June 20, 2012, and U.S. 15 Provisional Application No. 61/780,665, filed March 13, 2013. The entire contents of each of these referenced provisional applications are incorporated by reference herein.

BACKGROUND OF INVENTION

T-cell acute lymphoblastic leukemia (T-ALL) is a devastating form of cancer
20 characterized by malignant, immature white blood cells that continuously multiply and are overproduced in the bone marrow. T-ALL leads to pain and damage throughout the body and eventually death due to the crowding out of normal cells in the bone marrow and the spreading of tumor cells to other organs. 50% of T-ALL cases harbor a mutation in the Notch signaling pathway. As a result, gamma secretase inhibitors (GSI) that inhibit cleavage of the activated
25 form of Notch have been developed. Unfortunately, early relapse and refractory disease are common in T-ALL cases due to the transient response of the cancer to a GSI. Additionally, GSI treatment is also associated with toxicity, especially damage to the gastrointestinal tract.

SUMMARY OF INVENTION

30 The invention provides new therapies to treat certain cancers, including those having Notch pathway activation mutations, those having observed resistance to Notch inhibitors, and

those likely to manifest resistance to Notch inhibitors. The invention also provides methods for identifying subjects to be treated with the new therapies provided herein.

Thus, in one aspect, the invention provides a method comprising administering to a subject having cancer (i) a bromodomain inhibitor and/or (ii) a Bcl-2 inhibitor, and a Notch pathway inhibitor, in an effective amount to treat the cancer.

In some embodiments, the cancer is resistant to a previously-administered Notch pathway inhibitor. In some embodiments, the cancer is characterized by the presence of a Notch pathway activation mutation. In some embodiments, the cancer is characterized by abnormal increased HPI-alpha, HPI-beta, and/or HPI-gamma level. In some embodiments, the cancer is characterized by a chromatin compactness or a marker thereof.

In some embodiments, the bromodomain inhibitor and the Notch pathway inhibitor are administered concurrently or sequentially. In some embodiments, the Bcl-2 inhibitor and the Notch pathway inhibitor are administered concurrently or sequentially. In some embodiments, the bromodomain inhibitor, the Bcl-2 inhibitor and the Notch pathway inhibitor are administered. In some embodiments, the bromodomain inhibitor, the Bcl-2 inhibitor and the Notch pathway inhibitor are administered concurrently or sequentially.

In another aspect, the invention provides a method comprising: administering to a subject having cancer (i) a bromodomain inhibitor and/or (ii) a Bcl-2 inhibitor, in an effective amount to treat the cancer, wherein the cancer is characterized by the presence of a Notch pathway activation mutation.

In some embodiments, the method further comprises identifying the subject having cancer characterized by the presence of a Notch pathway activation mutation. In some embodiments, the method further comprises administering to the subject a Notch pathway inhibitor in an effective amount to treat the cancer.

In some embodiments, the bromodomain inhibitor and the Notch pathway inhibitor are administered concurrently or sequentially. In some embodiments, the Bcl-2 inhibitor and the Notch pathway inhibitor are administered concurrently or sequentially. In some embodiments, the bromodomain inhibitor, the Bcl-2 inhibitor, and the Notch pathway inhibitor are administered. In some embodiments, the bromodomain inhibitor, the Bcl-2 inhibitor, and the Notch pathway inhibitor are administered concurrently or sequentially.

In another aspect, the invention provides a method comprising: administering to a subject having cancer (i) a bromodomain inhibitor and/or (ii) a Bcl-2 inhibitor in an effective amount to treat the cancer, wherein the cancer is resistant to treatment with a Notch pathway inhibitor.

In some embodiments, the method further comprises administering to the subject a Notch pathway inhibitor in an effective amount to treat the cancer. In some embodiments, the bromodomain inhibitor and the Notch pathway inhibitor are administered concurrently or sequentially. In some embodiments, the Bcl-2 inhibitor and the Notch pathway inhibitor are 5 administered concurrently or sequentially. In some embodiments, the bromodomain inhibitor, the Bcl-2 inhibitor, and the Notch pathway inhibitor are administered. In some embodiments, the bromodomain inhibitor, the Bcl-2 inhibitor, and the Notch pathway inhibitor are administered concurrently or sequentially.

In another aspect, the invention provides a method comprising: administering to a subject 10 having cancer (i) a bromodomain inhibitor and/or (ii) a Bcl-2 inhibitor in an effective amount to treat the cancer, wherein the cancer is characterized by abnormal increased HPI level, wherein the HPI level is HPI-alpha level, HPI-beta level and/or HPI-gamma level.

In some embodiments, the method further comprises identifying a subject having cancer characterized by the abnormal increased HPI level. In some embodiments, the method further 15 comprises administering to the subject a Notch pathway inhibitor in an effective amount to treat the cancer.

In some embodiments, the bromodomain inhibitor and the Notch pathway inhibitor are administered concurrently or sequentially. In some embodiments, the Bcl-2 inhibitor and the Notch pathway inhibitor are administered concurrently or sequentially. In some embodiments, 20 the bromodomain inhibitor, the Bcl-2 inhibitor, and the Notch pathway inhibitor are administered. In some embodiments, the bromodomain inhibitor, the Bcl-2 inhibitor and the Notch pathway inhibitor are administered concurrently or sequentially.

In embodiments of any of the aspects of the invention, the bromodomain inhibitor, the Bcl-2 inhibitor and/or the Notch pathway inhibitor is a small compound or inhibitory nucleic acid molecule. In some embodiments, the inhibitory nucleic acid molecule is an siRNA, shRNA, or antisense nucleic acid molecule. In some embodiments, the bromodomain inhibitor is a BET inhibitor. In some embodiments, the bromodomain inhibitor is JQ1 or a derivative thereof including those compounds described in WO 2011/143660. In some embodiments, the Bcl-2 inhibitor is G3139, GX15-070, ABT-737 or ABT-199, or a derivative thereof. In some 25 embodiments, the Notch pathway inhibitor is a gamma secretase inhibitor.

In another aspect, the invention provides a method comprising: administering to a subject having cancer an inhibitor of ARID3B, EZH2, PRMT2, SND1, BRD1, SUV39H1, PRMT5,

SS18, BRD4, KDM5D, PRMT7, STAG3L1, CD2BP2, MLL5, SUDS3, CHD1, MINA, CHD8, MORF4L1, or CHRAC1, wherein the cancer is resistant to a Notch pathway inhibitor.

In some embodiments, the method further comprises administering to the subject a Notch pathway inhibitor. In some embodiments, the method further comprises administering to the subject a bromodomain inhibitor and/or a Bcl-2 inhibitor in an effective amount to treat the cancer. In some embodiments, the inhibitor is an shRNA, an siRNA, or an antisense nucleic acid molecule.

In another aspect, the invention provides a method comprising: (a) measuring nucleus size in a tumor sample from a subject; and (b) comparing nucleus size in the tumor sample to a control, wherein a decreased nucleus size in the tumor sample compared to the control identifies a subject to be treated with a bromodomain inhibitor and/or Bcl-2 inhibitor. In some embodiments, the nucleus size is nucleus diameter or nucleus volume.

In another aspect, the invention provides a method comprising: (a) measuring HPI level in a tumor sample from a subject; and (b) comparing the HPI level in the tumor sample to a control, wherein an increased HPI level in the tumor sample compared to the control identifies a subject to be treated with a bromodomain inhibitor and/or Bcl-2 inhibitor, wherein the HPI level is HPI-alpha level, HPI-beta level, and/or HPI-gamma level.

In another aspect, the invention provides a method comprising: (a) measuring level of a chromatin state biomarker (CSB) in a tumor sample from a subject, the CSB selected from: (i) a first CSB group consisting of NPM1, NARG1, RCC1, SSRP1, PRMT3, SAP30, CBX6, CHMP2B, UBE2M, WDR77, HMGB1, CARM1, USP13, HDAC4, COQ3, SET, GATAD2A, PRMT6, HMG20B, DNMT1, ADA, SS18, UBE3A, ZMYND11, and NOC2LL (“Group I CSB”); and (ii) a second CSB group consisting of UTX, SIN3A, SAP30L, FLJ20309, RCOR2, ARID5A, UBE2Q2, TRIM24, BAZ2B, SMYD3, EZH2, PHF1, PHF2, BCR, SMARCD3, BMI1, CHD6, FBXL11, SIRT7, ASF1A, RCOR3, CBX4, EPC1, BRD1, and BNF11 (“Group II CSB”); (b) comparing the Group I and/or Group II CSB level with a control, wherein a Group I CSB level that is reduced in the tumor sample compared to a control and/or a Group II CSB level that is elevated in the tumor sample compared to a control identifies a subject to be treated with a bromodomain inhibitor and/or Bcl-2 inhibitor.

In another aspect, the invention provides a method comprising: (a) measuring a level of a biomarker in a tumor sample from a subject, the biomarker selected from DTX1, HES4, CD1d, ETS1, ETV6, Runx1, Bcl-2, MYC and CD52; and (b) comparing the biomarker level with a control, wherein a level of DTX1, HES4, or CD1d that is reduced in the tumor sample compared

to the control and/or a level of ETS1, ETV6, Runx1, CD52, MYC or Bcl-2 that is elevated in the tumor sample compared to the control identifies a subject to be treated with a bromodomain inhibitor and/or Bcl-2 inhibitor.

In another aspect, the invention provides a method comprising: (a) measuring a level of histone modification in a tumor sample from a subject, the histone modification selected from H3K27me3 and H3K9me3; and (b) comparing the histone modification level with a control, wherein a level of histone modification that is elevated in the tumor sample compared to the control identifies a subject to be treated with a bromodomain inhibitor and/or Bcl-2 inhibitor.

In another aspect, the invention provides a method comprising: (a) measuring a level of H3K27Ac histone modification in a tumor sample from a subject; and (b) comparing the H3K27Ac histone modification level with a control, wherein a level of H3K27Ac histone modification that is reduced in the tumor sample compared to the control identifies a subject to be treated with a bromodomain inhibitor and/or Bcl-2 inhibitor.

In another aspect, the invention provides a method comprising: (a) measuring a level of H3K4me1 histone modification at a site of elevated H3K27Ac histone modification; and (b) comparing the H3K4me1 histone modification level with a control, wherein a level of H3K4me1 histone modification at a site of elevated H3K27Ac histone modification that is elevated in the tumor sample compared to the control identifies a subject to be treated with a bromodomain inhibitor and/or Bcl-2 inhibitor.

In some embodiments of the foregoing methods, the level is an mRNA level or a protein level. In some embodiments, the control is a nucleus size, an HPI-alpha level, an HPI-beta level, an HPI-gamma level, a CSB level, a histone modification level or a biomarker level selected from DTX1, HES4, CD1d, ETS1, ETV6, Runx1, Bcl-2, MYC and CD52 in a non-tumor sample. In some embodiments, the control is a predetermined threshold. In some embodiments, the methods further comprise identifying the subject to be treated with a bromodomain inhibitor and/or Bcl-2 inhibitor. In some embodiments, the methods further comprise administering to the identified subject a bromodomain inhibitor and/or Bcl-2 inhibitor in an effective amount. In some embodiments, the methods further comprise further comprising administering to the identified subject a Notch pathway inhibitor in an effective amount. In some embodiments, the cancer or tumor is a T-ALL. In some embodiments, the T-ALL is resistant to a Notch pathway inhibitor.

BRIEF DESCRIPTION OF DRAWINGS

FIG. 1 is a line graph depicting Gene Set Enrichment Analysis (GSEA) for leukemia cells resistant to a gamma secretase inhibitor (GSI) and naïve leukemia cells (not treated with a GSI).

5 FIG. 2 is a bar graph depicting the ratio of lactate production to glucose consumption and the ratio of glutamate production to glutamine consumption in resistant leukemia cells versus naïve leukemia cells.

10 FIG. 3 depicts cell and nucleus size changes in resistant leukemia cells compared to naïve leukemia cells. FIG. 3A is a line graph depicting the forward scatter of naïve leukemia cells and resistant leukemia cells. FIG. 3B is a photograph of a nuclear stain (DAPI) and a cytoplasmic stain (Actin) in naïve leukemia cells and resistant leukemia cells.

15 FIG. 4 is an analysis of the chromatin state of naïve leukemia cells and resistant leukemia cells. FIG. 4A is a Western blot showing the levels of HP1-gamma in naïve, short-term GSI treated, and resistant leukemia cells. FIG. 4B is a DNA gel showing partial MNase digestion in naïve and resistant leukemia cells.

FIG. 5 depicts the chromatin compaction state in naïve and resistant leukemia cells. FIG. 5A is a bar graph depicting the levels of chromatin marks H3K27me3 and H3K9me3 in naïve and resistant cells. FIG. 5B is a heat map of expression levels of chromatin state biomarkers in naïve and resistant leukemia cells.

20 FIG. 6 is a Western blot depicting the protein levels of BRD4 in naïve, short-term GSI treated, and resistant leukemia cells.

FIG. 7 is two line graphs depicting proliferation of naïve and resistant leukemia cells after 7 days in specific concentrations of JQ1 (left) and apoptosis of naïve and resistant leukemia cells after 4 days in specific concentrations of JQ1 (right).

25 FIG. 8 is an example ChIP-SEQ readout of a genomic location where BRD4, H3K4me1, and H3K27ac all bind in the same place. Peaks indicate potential binding locations. Pie charts depict the number of “active” enhancers occupied by BRD4 in naïve and resistant leukemia cells.

30 FIG. 9 depicts the presence of BRD4 near the Bcl-2 gene. FIG. 9A is a ChIP-Seq readout of the location of BRD4, H3K4me1, and H3K27ac near the Bcl-2 gene. Peaks indicate potential binding locations. FIG. 9B is a Western blot depicting the protein levels of Bcl-2 in naïve and resistant cells treated with JQ1.

FIG. 10 is a line graph depicting the bioluminescence of luciferized leukemia cells, which is a readout of tumor burden, in mice treated with vehicle, DBZ (a GSI), JQ1, and JQ1 + DBZ.

5 FIG. 11 is a photograph of Periodic acid-Schiff (PAS) staining of a section of the gastrointestinal tract from a mouse treated with vehicle, DBZ, JQ1, or JQ1 + DBZ.

FIG. 12 is an analysis of Notch signaling in naïve (N), short-term treated (ST, 5 days), persister (P), reversed (Rev) and re-treated (Rev tx) cells. FIG. 12A is a pair of line graphs showing DTX1 and HES4 mRNA expression. FIG. 12B is a photograph of a western blot showing expression of activated intracellular NOTCH1 (ICN) and MYC.

10 FIG. 13 is a line graph depicting cell proliferative response to the AKT inhibitor MK-2206 in naïve and persister cells.

FIG. 14 shows a western blot of Phospho-mTOR (p2481), a marker of activated mTOR signaling, total mTOR and Tubulin for naïve (N), short-term treated (ST) and persister (R) cells and a line graph depicting cell proliferative response to Rapamycin in naïve and persister cells.

15 FIG. 15 is a graphical depiction of the shRNA screen for chromatin regulators preferentially required for naïve or persister cell survival. Top hits for each cell state are shown in boxes (left box and right box, naïve and persister cells, respectively).

20 FIG. 16 shows that ETS and Runx transcription factors are targets of BRD4. FIG. 16A shows the motifs enriched at BRD4 binding sites. FIG. 16B shows mRNA levels of ETS1, ETV6, and Runx1 in naïve and persister cells.

FIG. 17A is two photographs of western blots show MYC expression in naïve and persister cells after 3 day treatment with 2 µM AKT inhibitor, MK-2206, or 10 nM mTOR inhibitor, Rapamycin. FIG. 17B depicts graphs showing BRD4 binding at the *MYC* and *BCL2* promoters.

25 FIG. 18A is a line graph showing the proliferative response of persister cells after 6 days of treatment with the Bcl-2 inhibitor ABT-737. FIG. 18B is a bar graph depicting the proliferative response of persister cells transfected with Bcl-2 expression vector (or empty vector control) after 6 days of treatment with JQ1.

30 FIG. 19A is a series of photographs depicting hematoxylin and eosin (H&E) stains and immunohistochemistry for activated Notch (ICN) for bone marrow from leukemic mice treated with Notch inhibitor DBZ for 5 days (ST) or 3 weeks (LT) or treated with vehicle (Veh). FIG. 19B is a series of bar graphs showing levels of HES4, DTX1, HP1γ, and Bcl-2 for leukemia cells sorted from spleens of vehicle (Veh) or long-term (LT) treated mice.

FIG. 20 is a pair of graphs depicting the level of CD1d in *in vitro* naïve and persister cells. The left graph shows expression of CD1d in naïve or persister populations. The right graph depicts quantification of CD1d expression for clones originating from single naïve cells that proliferated in the presence of GSI (n=7) or control conditions (n=7)(p<0.002).

5 FIG. 21 is a pair of graphs depicting data from primary human T-ALL cells. The left graph shows the proliferative response of primary human T-ALL cells from 6 different patients after 5 days of treatment with 1 micromolar GSI and the indicated JQ1 doses, relative to no JQ1 control. The right graphs shows the relative expression of intracellular BCL2 measured by flow cytometry in primary T-ALL cells after 3 days of treatment with 1 micromolar GSI and 0.5
10 micromolar JQ1, normalized to untreated.

It is to be understood that the Figures are not required for enablement of the invention.

DETAILED DESCRIPTION OF INVENTION

The invention provides methods to diagnose and treat subjects using single or
15 combination therapy. Certain methods of the invention relate to treatment of cancers that are resistant to therapy with a Notch pathway inhibitor or that are likely to become resistant to therapy with a Notch pathway inhibitor.

It has been found, in accordance with the invention, that a Notch inhibitor resistant phenotype (also referred to herein as a resister or persister phenotype) correlated with changes in
20 chromatin structure of the cancer cells, resulting in more compact chromatin and smaller cell size, and with changes in expression of heterochromatin markers such as HPI-alpha, beta and gamma and various chromatin regulatory markers, such as histone modifications, or chromatin state biomarkers such as but not limited to BRD4. It was also found that changes in biomarkers selected from DTX1, HES4, CD1d, ETS1, ETV6, Runx1, Bcl-2, MYC and CD52 correlated
25 with resistance to a Notch pathway inhibitor. Significantly, it has also been found, in accordance with the invention, that the resistance to the Notch pathway inhibitor could be reversed by using the Notch pathway inhibitor with a bromodomain inhibitor such as a BRD4 inhibitor. Without intending to be bound by any particular mechanism or theory, it is believed that the bromodomain inhibitor reverses the observed changes in chromatin structure, thereby
30 reversing the resistance to the Notch pathway inhibitor. The invention further contemplates that a bromodomain inhibitor may be administered before resistance to a Notch pathway inhibitor is even manifest, thereby preventing the resistant phenotype from developing altogether or delaying its onset. The invention therefore contemplates treating subjects having certain cancers

with a Notch pathway inhibitor and a bromodomain inhibitor. It has also been found, in accordance with the invention, that Notch pathway inhibitor resistant cells were sensitive to treatment with an mTOR or AKT inhibitor. The invention therefore contemplates treating subjects having certain cancers with a Notch pathway inhibitor and an mTOR and/or AKT inhibitor. The inhibitors may be administered before or after resistance to the Notch pathway inhibitor is observed. The cancers to be treated in this manner may be those that would be treated with a Notch pathway inhibitor, such as for example T-ALL, and those that are characterized by the presence of a Notch pathway activation mutation.

The invention further contemplates use of a bromodomain inhibitor alone or in combination with other anti-cancer agents in the treatment of cancers characterized by chromatin compaction or abnormal expression of chromatin regulatory markers or chromatin state biomarkers. Such cancers may manifest a reduced nucleus or cell size (or diameter) relative to normal, non-cancerous cells and/or they may have an increased expression level of HPI-alpha, beta and/or gamma mRNA and/or proteins relative to normal, non-cancerous cells, inter alia. In some embodiments, the cancer is (a) characterized by a compact nucleus or smaller cell size compared to a normal control, (b) characterized by abnormal increased (mRNA or protein) levels of HPI-alpha, beta and/or gamma, (c) characterized by abnormal increased (mRNA or protein) levels of certain chromatin regulatory proteins or chromatin state biomarkers such as but not limited to BRD4 and/or abnormal decreased (mRNA or protein) levels of other chromatin regulatory proteins or chromatin state biomarkers, (d) characterized by decreased expression of DTX1, HES4, and/or CD1d and/or by increased expression of ETS1, ETV6, Runx1, CD52, MYC or Bcl-2, (e) characterized by increased levels of repressive chromatin markers such as H3K27me3, or H3K9me2/3 and/or decreased levels of other chromatin markers such as H3K27Ac, and/or (f) characterized by resistance to a previously administered anti-cancer agent such as a Notch pathway inhibitor.

It has also been found, in accordance with the invention, that bromodomain inhibition, such as BRD4 inhibition, inhibits expression of Bcl-2, a known anti-apoptotic mediator. It is therefore contemplated by the invention that bromodomain inhibition may mediate its effects via Bcl-2 inhibition. This suggests that Bcl-2 inhibitors may be used instead of bromodomain inhibitors in certain methods of the invention, including those that involve combination therapy with a Notch pathway inhibitor.

In addition to treatment methods, the invention also provides methods of identifying subjects to be treated with bromodomain inhibitors alone or in combination with another anti-

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cancer such as but not limited to a Notch pathway inhibitor. Such subjects will be identified based on the presence of a cancer that is (a) characterized by the presence of a Notch pathway activation mutation, (b) characterized by a compact nucleus or smaller cell size compared to a normal control, (c) characterized by abnormal increased (mRNA or protein) levels of HPI-alpha, 5 beta and/or gamma, (d) characterized by abnormal increased (mRNA or protein) levels of certain chromatin regulatory proteins or chromatin state biomarkers such as but not limited to BRD4 and/or abnormal decreased (mRNA or protein) levels of other chromatin regulatory proteins or chromatin state biomarkers, (e) characterized by decreased expression of DTX1, HES4, and/or CD1d and/or by increased expression of ETS1, ETV6, Runx1, CD52, MYC or Bcl-2, (f) 10 characterized by increased levels of repressive chromatin markers such as H3K27me3, or H3K9me2/3 and/or decreased levels of other chromatin markers such as H3K27Ac. and/or (g) characterized by resistance to a previously administered anti-cancer agent such as a Notch pathway inhibitor.

In still other aspects, the invention provides methods for treating a subject having a 15 cancer that is resistant to a Notch pathway inhibitor using inhibitors of a number of chromatin regulatory proteins such as ARID3B, EZH2, PRMT2, SND1, BRD1, SUV39H1, PRMT5, SS18, BRD4, KDM5D, PRMT7, STAG3L1, CD2BP2, MLL5, SUDS3, CHD1, MINA, CHD8, MORF4L1, or CHRAC1.

As shown in the Examples, shRNA based knock-down of any of these chromatin 20 regulatory proteins caused cell death in cell lines that were resistant to Notch pathway inhibition.

These and other aspects of the invention will be described in greater detail herein.

Bromodomain-Containing Proteins

As described herein, various methods of the invention involve the use of a bromodomain 25 inhibitor. Bromodomain inhibitors are compounds that inhibit the activity of bromodomain-containing proteins. Bromodomain-containing proteins, as their name implies, are proteins that comprise a bromodomain. Bromodomains (BRDs) function by detecting lysine acetylation (i.e., detecting ϵ -N-acetyl lysine, also known as Kac) on other proteins. Lysine acetylation neutralizes charge and can therefore alter protein conformation and protein-protein interactions. 30 Lysine acetylation involves histone acetyltransferases (or HATs) and lysine deacetylation involves histone deactylases (or HDACs).

Bromodomains (BRDs) are a diverse family of evolutionarily conserved protein-interaction modules. One family of bromodomain-containing proteins, the BET (bromodomain and extra-terminal) family, is represented by six members in humans (BRD1, BRD2, BRD3, BRD4, BRD7 and the testis-specific isoform BRDT), with each containing two N-terminal 5 BRDs. BRD4 and BRD2 mediate transcriptional elongation by recruiting the positive transcription elongation factor complex (P-TEFb). The P-TEFb core complex is composed of cyclin-dependent kinase-9 (CDK9) and its activator cyclin T. CDK9 phosphorylates the RNA polymerase II (RNAPII) C-terminal domain. RNAPII undergoes sequential phosphorylation at Ser5 during promoter clearance and at Ser2 by P-TEFb at the start of elongation. It has been 10 shown that BRD4 couples P-TEFb to acetylated chromatin through its BRDs.

Examples of BRD-containing proteins include, but are not limited to, ASH1L, ATAD2A/B, BAZ1A/B, BAZ2A/B, BRD1, BRD2, BRD3, BRD4, BRDT, BRD7, BRD8A/B, BRD9, BRPF1A/B, BRPF3A, BRWD3, CECR2, CREBBP, EP300, FALZ, GCN5L2, MLL, PB1, PCAF, PHIP, PRKCBP1, SMARCA2A/B, SMARCA4, SP100/SP110/SP140, 15 TAF1/TAF1L, TRIM24/TRIM28/TRIM33/TRIM66, WDR9, and ZMYND11.

As described herein, a novel role for BRD-containing proteins and other chromatin regulatory proteins in cancer has been elucidated in accordance with the invention. Tumor cells resistant to Notch pathway inhibitor treatment were found to have globally compact chromatin and altered expression of certain chromatin regulatory proteins with some having increased 20 expression levels and some having decreased expression levels compared to a normal control. Knock-down of several chromatin regulatory proteins, including BRD4, in Notch pathway inhibitor-resistant tumor cells, using shRNA, resulted in decreased proliferation and increased cell death. Accordingly, aspects of the invention provide methods comprising administering to a subject having cancer a bromodomain inhibitor in an effective amount to treat the cancer, 25 wherein the cancer is characterized by a Notch Activation mutation or by resistance to a Notch pathway inhibitor.

As described herein, use of a BRD inhibitor in combination with a Notch pathway inhibitor was found to result in reduction of tumor burden in a mouse model that was greater than treatment with the BRD inhibitor or the Notch pathway inhibitor alone. Additionally, use of 30 the BRD inhibitor in combination with the Notch pathway inhibitor reduces the side effects associated with treatment using either agent alone. In particular, the side effects observed with the BRD inhibitor, JQ1, are reduced when the inhibitor is used in combination with a Notch pathway inhibitor. Such side effects include without limitation gastrointestinal side effects. The

invention therefore contemplates a combination therapy that is associated with a lower frequency of side effects and/or less severe side effects. Accordingly, aspects of the invention provide methods comprising administering to a subject having cancer a BRD inhibitor and a Notch pathway inhibitor in an effective amount to treat the cancer.

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Bromodomain Inhibitors

Bromodomain inhibitors are known in the art. A bromodomain inhibitor is any molecule or compound that can prevent or inhibit, in part or in whole, the binding of at least one bromodomain to acetyl-lysine residues of proteins (e.g., to the acetyl-lysine residues of histones). The bromodomain inhibitor may be any molecule or compound that inhibits a bromodomain as described above, including nucleic acids such as DNA and RNA aptamers, antisense oligonucleotides, siRNA and shRNA, small peptides, antibodies or antibody fragments, and small molecules such as small chemical compounds. It is to be understood that the bromodomain inhibitor may inhibit only one bromo-domain-containing protein or it may inhibit more than one or all bromodomain-containing proteins.

Examples of bromodomain inhibitors are described in JP 2009028043, JP 2009183291, WO 2011054843, WO 2011054848, WO2009/084693A1, WO2009084693, WO 2011054844, WO 2011054846, US 2012028912, Filippakopoulos et al. Bioorg Med Chem. 20(6): 1878-1886, 2012; Chung et al. J Med Chem. 54(11):3827-38, 2011; and Chung et al. J Biomol Screen. 16(10):1170-85, 2011, which are incorporated herein by reference.

In some embodiments, the bromodomain inhibitor is 1-[2-(1 /-/benzimidazol-2-ylthio)ethyl]-1 ,3-dihydro-3-methyl-2H-benzimidazole-2-thione (JP2008-156311), Alprazolam (Sigma-Aldrich), Midazolam (Sigma-Aldrich, GW841819X (BZD, GlaxoSmithKline), a compound in Table 1 (WO 2011054843), or any other bromodomain inhibitor compound described herein.

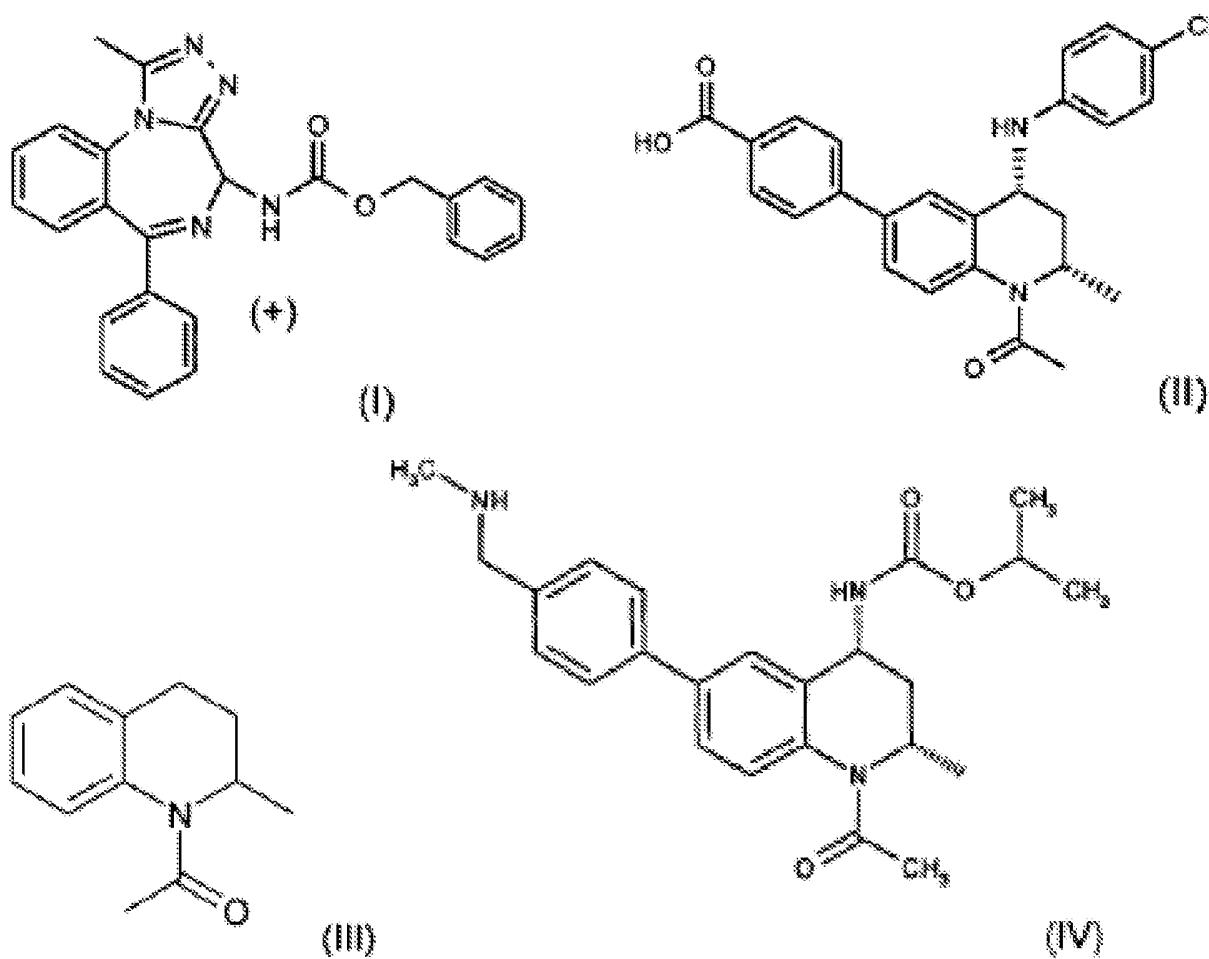
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	Name	Structure
Example 1	1-methylethyl ((2 <i>S</i> ,4 <i>R</i>)-1-acetyl-2-methyl-6-{4-[(methylamino)methyl]phenyl}-1,2,3,4-tetrahydro-4-quinolinyl)carbamate	
Example 2	2-[(4 <i>S</i>)-6-(4-Chlorophenyl)-1-methyl-8-(methyoxy)-4 <i>H</i> -[1,2,4]triazolo[4,3-a][1,4]benzodiazepin-4-yl]-N-ethylacetamide	
Example 3	7-(3,5-dimethyl-4-isoxazolyl)-8-(methoxy)-1-[(1 <i>R</i>)-1-(2-pyridinyl)ethyl]-1,3-dihydro-2 <i>H</i> -imidazo[4,5-c]quinolin-2-one	
Example 4	7-(3,5-dimethyl-4-isoxazolyl)-8-(methoxy)-1-[(1 <i>R</i>)-1-phenylethyl]-2-(tetrahydro-2 <i>H</i> -pyran-4-yl)-1 <i>H</i> -imidazo[4,5-c]quinoline	
Example 5	4-{(2 <i>S</i> ,4 <i>R</i>)-1-acetyl-4-[(4-chlorophenyl)amino]-2-methyl-1,2,3,4-tetrahydro-6-quinolinyl}benzoic acid	
Example 6	N-{1-methyl-7-[4-(1-piperidinylmethyl)phenyl][1,2,4]triazolo[4,3-a]quinolin-4-yl}urea	

Table 1: Examples of Bromodomain inhibitors

In some embodiments, the bromodomain inhibitor is a BET inhibitor. A BET inhibitor is any molecule or compound that can prevent or inhibit the binding of the bromodomain of at least one BET family member to acetyl-lysine residues of proteins. The BET inhibitor may be any molecule or compound that inhibits a BET as described above, including nucleic acids such as DNA and RNA aptamers, antisense oligonucleotides, siRNA and shRNA, small peptides, antibodies or antibody fragments, and small molecules such as small chemical compounds.

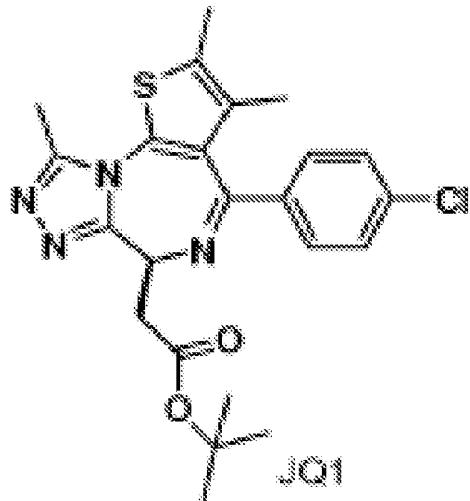
Examples of BET inhibitors are described in US 2011143651, WO2009/084693A1, WO 2011143669, WO 2011143660, WO 2011054851, and JP 2008156311, which are incorporated herein by reference. It is to be understood that a BET inhibitor may inhibit only one BET family member or it may inhibit more than one or all BET family members. Examples of BET inhibitors known in the art include, but are not limited to, RVX-208 (Resverlogix), PFI-1 (Structural Genomics Consortium), OTX015 (Mitsubishi Tanabe Pharma Corporation), BzT-7, GSK525762A (iBET, GlaxoSmithKline), and the compounds below (WO 2011054851, GlaxoSmithKline):



In some embodiments, the BET inhibitor is a small molecule compound (e.g., JQ1 or derivatives thereof and compounds of formulas I-XXII or any other compound described herein) that binds to the binding pocket of the first bromodomain of a BET family member (e.g., BRD1, BRD2, BRD3, BRD4, BRD7, BRDT; see WO 2011143669).

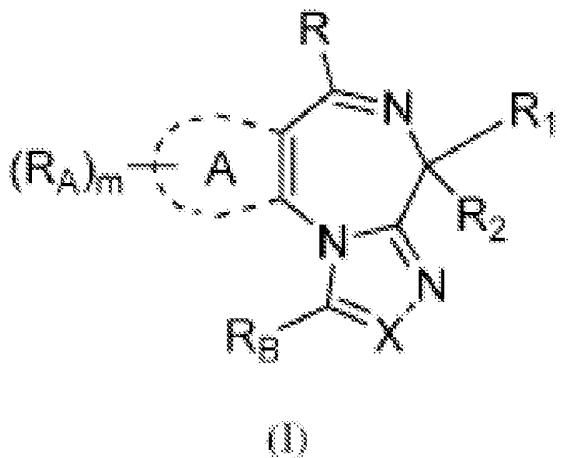
- 15 -

In some important embodiments, the BET inhibitor is JQ1 and has the formula below:



In some embodiments, the BET inhibitor has the structures of Formulas I-XXII or any other compound as described below. These structures are known in the art (WO 2011143660, 5 which is incorporated herein by reference).

In some embodiments, a bromodomain or BET inhibitor is a compound of Formula I:



wherein X is N or CR₅; R₅ is H, alkyl, cycloalkyl, heterocycloalkyl, aryl, or heteroaryl, each of which is optionally substituted; R_B is H, alkyl, hydroxylalkyl, aminoalkyl, alkoxyalkyl, haloalkyl, hydroxy, alkoxy, or -COO-R₃, each of which is optionally substituted; ring A is aryl or heteroaryl; each R_A is independently alkyl, cycloalkyl, heterocycloalkyl, aryl, or heteroaryl, each of which is optionally substituted; or any two R_A together with the atoms to which each is attached, can form a fused aryl or heteroaryl group; R is alkyl, cycloalkyl, heterocycloalkyl, aryl, or heteroaryl; each of which is optionally substituted;

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In certain embodiments, L is H, -COO-R₃, -CO-N(R₃R₄), -S(0)₂-R₃, -S(0)₂-N(R₃R₄), N(R₃R₄), N(R₄)C(0)R₃ or optionally substituted aryl. In certain embodiments, each R₃ is independently selected from the group consisting of: H, -C_i-C₈ alkyl, which is optionally substituted, containing 0, 1, 2, or 3 heteroatoms selected from O, S, or N; or
5 NH₂, N=CR₄R₆.

In certain embodiments, R₂ is H, D, halogen or methyl.

In certain embodiments, R_B is alkyl, hydroxyalkyl, haloalkyl, or alkoxy; each of which is optionally substituted.

In certain embodiments, R_B is methyl, ethyl, hydroxy methyl, methoxymethyl,

10 trifluoromethyl, COOH, COOMe, COOEt, or COOCH₂OC(0)CH₃.

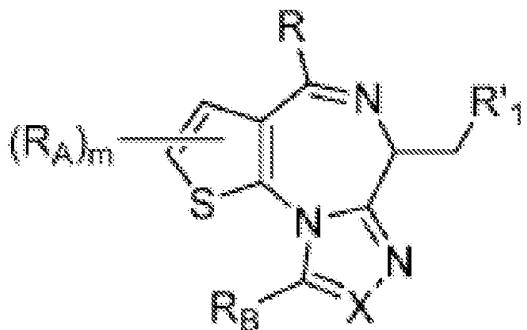
In certain embodiments, ring A is a 5 or 6-membered aryl or heteroaryl. In certain embodiments, ring A is thiofuranyl, phenyl, naphthyl, biphenyl, tetrahydronaphthyl, indanyl, pyridyl, furanyl, indolyl, pyrimidinyl, pyridazinyl, pyrazinyl, imidazolyl, oxazolyl, thieryl, thiazolyl, triazolyl, isoxazolyl, quinolinyl, pyrrolyl, pyrazolyl, or
15 5,6,7,8-tetrahydroisoquinolinyl.

In certain embodiments, ring A is phenyl or thieryl.

In certain embodiments, m is 1 or 2, and at least one occurrence of R_A is methyl.

In certain embodiments, each R_A is independently H, an optionally substituted alkyl, or any two R_A together with the atoms to which each is attached, can form an aryl.

20 In some embodiments, a bromodomain or BET inhibitor is a compound of Formula II:



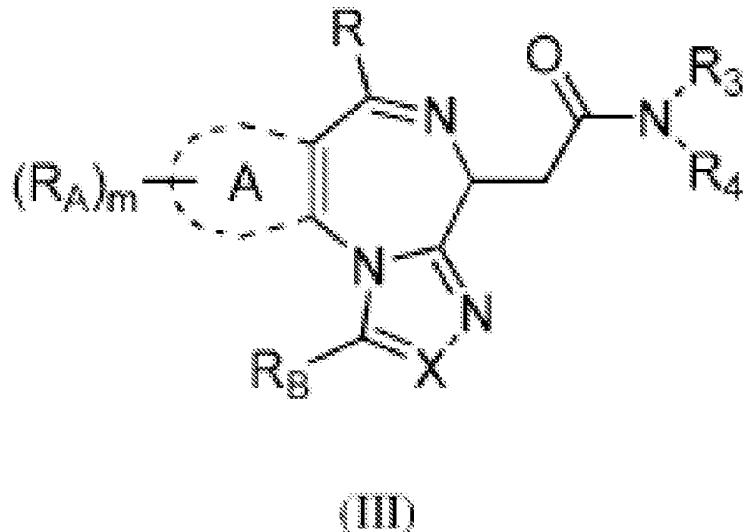
(II)

wherein X is N or CR₅; R₅ is H, alkyl, cycloalkyl, heterocycloalkyl, aryl, or heteroaryl, each of which is optionally substituted; R_B is H, alkyl, hydroxylalkyl, aminoalkyl, alkoxyalkyl, haloalkyl, hydroxy, alkoxy, or -COO-R₃, each of which is optionally substituted;

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In certain embodiments, each R_A is methyl.

In some embodiments, a bromodomain or BET inhibitor is a compound of formula III:



wherein

5 X is N or CR₅; R₅ is H, alkyl, cycloalkyl, heterocycloalkyl, aryl, or heteroaryl, each of which is optionally substituted; RB is H, alkyl, hydroxylalkyl, aminoalkyl, alkoxyalkyl, haloalkyl, hydroxy, alkoxy, or -COO-R₃, each of which is optionally substituted; ring A is aryl or heteroaryl; each R_A is independently alkyl, cycloalkyl, heterocycloalkyl, aryl, or heteroaryl, each of which is optionally substituted; or any two RA together with the atoms to which each is attached, can form a fused aryl or heteroaryl group;

10 R is alkyl, cycloalkyl, heterocycloalkyl, aryl, or heteroaryl, each of which is optionally substituted;

each R₃ is independently selected from the group consisting of:

15 (i) H, aryl, substituted aryl, heteroaryl, or substituted heteroaryl;

(ii) heterocycloalkyl or substituted heterocycloalkyl;

16 (iii) -C₁-C₈ alkyl, -C₂-C₈ alkenyl or -C₂-C₈ alkynyl, each containing 0, 1, 2, or 3 heteroatoms selected from O, S, or N; -C₃-C₁₂ cycloalkyl, substituted -C₃-C₁₂ cycloalkyl, -C₃-C₁₂ cycloalkenyl, or substituted -C₃-C₁₂ cycloalkenyl, each of which may be optionally substituted; and

20 (iv) NH₂, N=CR₄R₆;

each R₄ is independently H, alkyl, alkyl, cycloalkyl, heterocycloalkyl, aryl, or heteroaryl, each of which is optionally substituted; or R₃ and R₄ are taken together with the nitrogen atom to which they are attached to form a 4-10-membered ring;

- 20 -

R₆ is alkyl, alkenyl, cycloalkyl, cycloalkenyl, heterocycloalkyl, aryl, or heteroaryl, each of which is optionally substituted; or R₄ and R₆ are taken together with the carbon atom to which they are attached to form a 4-10- membered ring;

m is 0, 1, 2, or 3;

5 provided that:

(a) if ring A is thienyl, X is N, R is phenyl or substituted phenyl, R_B is methyl, then R₃ and R₄ are not taken together with the nitrogen atom to which they are attached to form a morpholino ring; and

10 (b) if ring A is thienyl, X is N, R is substituted phenyl, R₂ is H, R_B is methyl, and one of R₃ and R₄ is H, then the other of R₃ and R₄ is not methyl, hydroxyethyl, alkoxy, phenyl, substituted phenyl, pyridyl or substituted pyridyl;

or a salt, solvate or hydrate thereof.

In certain embodiments, R is aryl or heteroaryl, each of which is optionally substituted.

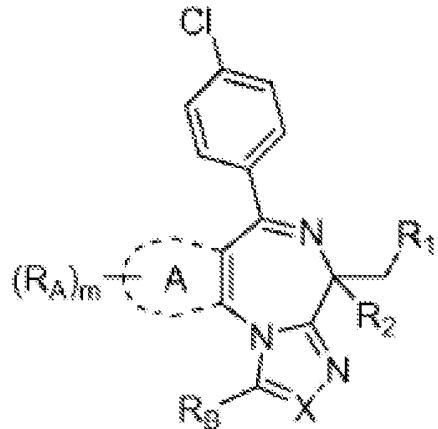
In certain embodiments, R is phenyl or pyridyl, each of which is optionally substituted.

15 In certain embodiments, R is p-Cl-phenyl, o-Cl-phenyl, m-Cl-phenyl, p-F-phenyl, o-F-phenyl, m-F-phenyl or pyridinyl. In certain embodiments, R₃ is H, NH₂, or N=CR₄R₆.

In certain embodiments, each R₄ is independently H, alkyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl; each of which is optionally substituted.

20 In certain embodiments, R₆ is alkyl, alkenyl, cycloalkyl, cycloalkenyl, heterocycloalkyl, aryl, or heteroaryl, each of which is optionally substituted.

In some embodiments, a bromodomain or BET inhibitor is a compound of formula IV:



(IV)

wherein X is N or CR₅; R₅ is H, alkyl, cycloalkyl, heterocycloalkyl, aryl, or heteroaryl, each of which is optionally substituted;

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(c) if ring A is thienyl, X is N, R₂ is H, R_B is methyl, and Ri is -(CH₂)_n-L, in which n is 0 and L is -COO-R₃, then R₃ is not methyl or ethyl; or a salt, solvate or hydrate thereof.

In certain embodiments, Ri is -(CH₂)_n-L, in which n is 0-3 and L is -COO-R₃, optionally substituted aryl, or optionally substituted heteroaryl; and R₃ is -C₁-C₈ alkyl, which contains 0, 1, 2, or 3 heteroatoms selected from O, S, or N, and which may be optionally substituted. In certain embodiments, n is 1 or 2 and L is alkyl or -COO-R₃, and R₃ is methyl, ethyl, propyl, i-propyl, butyl, sec-butyl, or t-butyl; or n is 1 or 2 and L is H or optionally substituted phenyl.

In certain embodiments, R₂ is H or methyl.

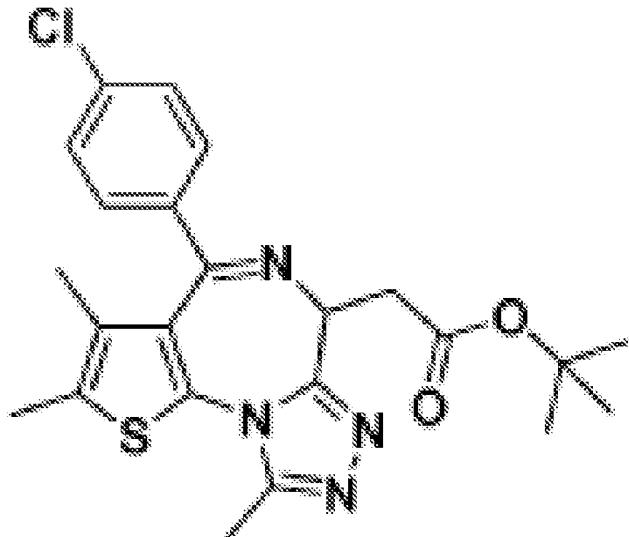
10 In certain embodiments, R_B is methyl, ethyl, hydroxy methyl, methoxymethyl, trifluoromethyl, COOH, COOMe, COOEt, COOCH₂OC(0)CH₃.

In certain embodiments, ring A is phenyl, naphthyl, biphenyl, tetrahydronaphthyl, indanyl, pyridyl, furanyl, indolyl, pyrimidinyl, pyridazinyl, pyrazinyl, imidazolyl, oxazolyl, thienyl, thiazolyl, triazolyl, isoxazolyl, quinolinyl, pyrrolyl, pyrazolyl, or 15 5,6,7,8-tetrahydroisoquinolinyl.

In certain embodiments, each R_A is independently an optionally substituted alkyl, or any two R_A together with the atoms to which each is attached, can form an aryl.

The invention also provides compounds of Formulae V-XXII, and any compound described herein.

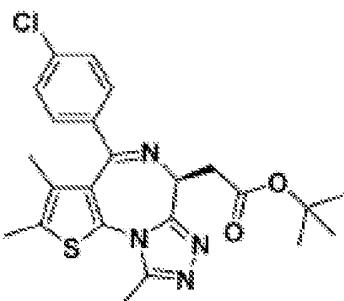
20 In some embodiments, a bromodomain or BET inhibitor is:



a salt, solvate or hydrate thereof.

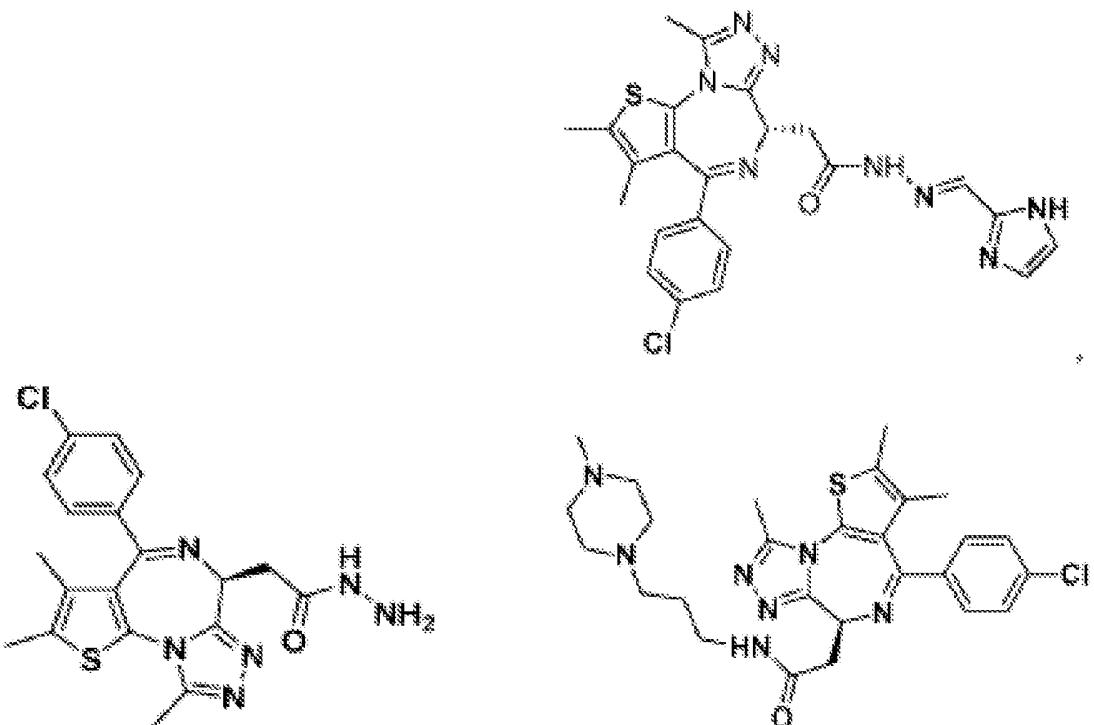
In certain embodiments, the compound is (+)-JQ1:

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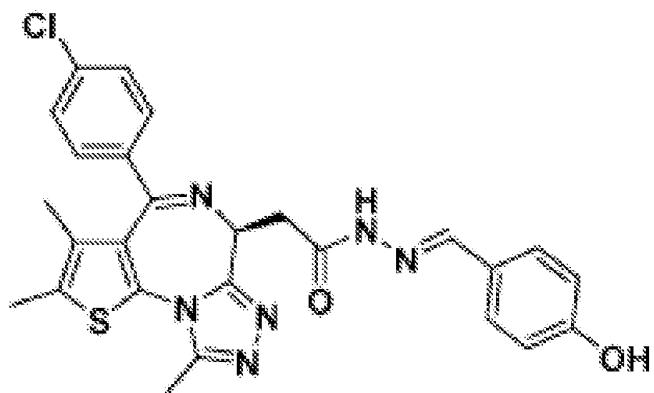
or a salt, solvate or hydrate thereof.

In some embodiments, a bromodomain or BET inhibitor is a compound represented by the formula:



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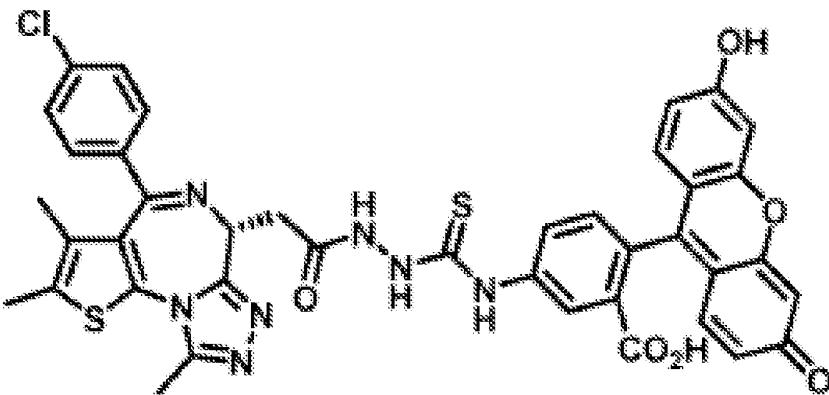
or



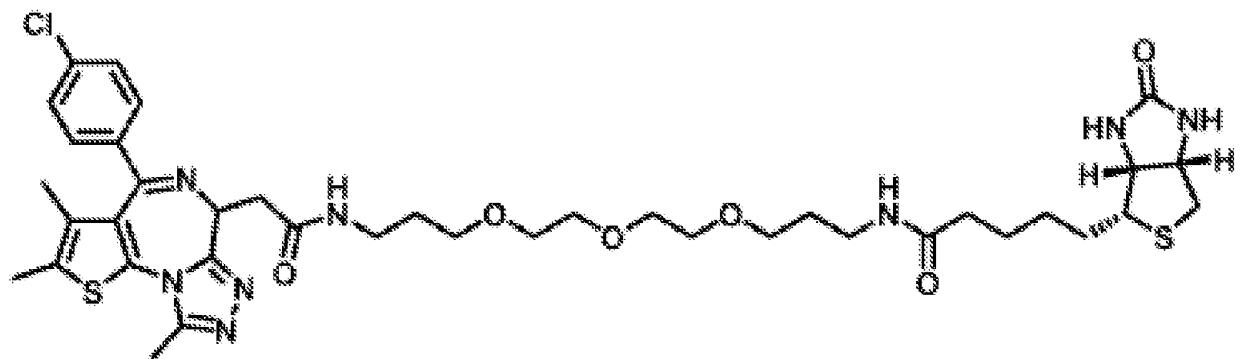
a salt, solvate or hydrate thereof.

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In some embodiments, a bromodomain or BET inhibitor is a compound represented by the formula:



or

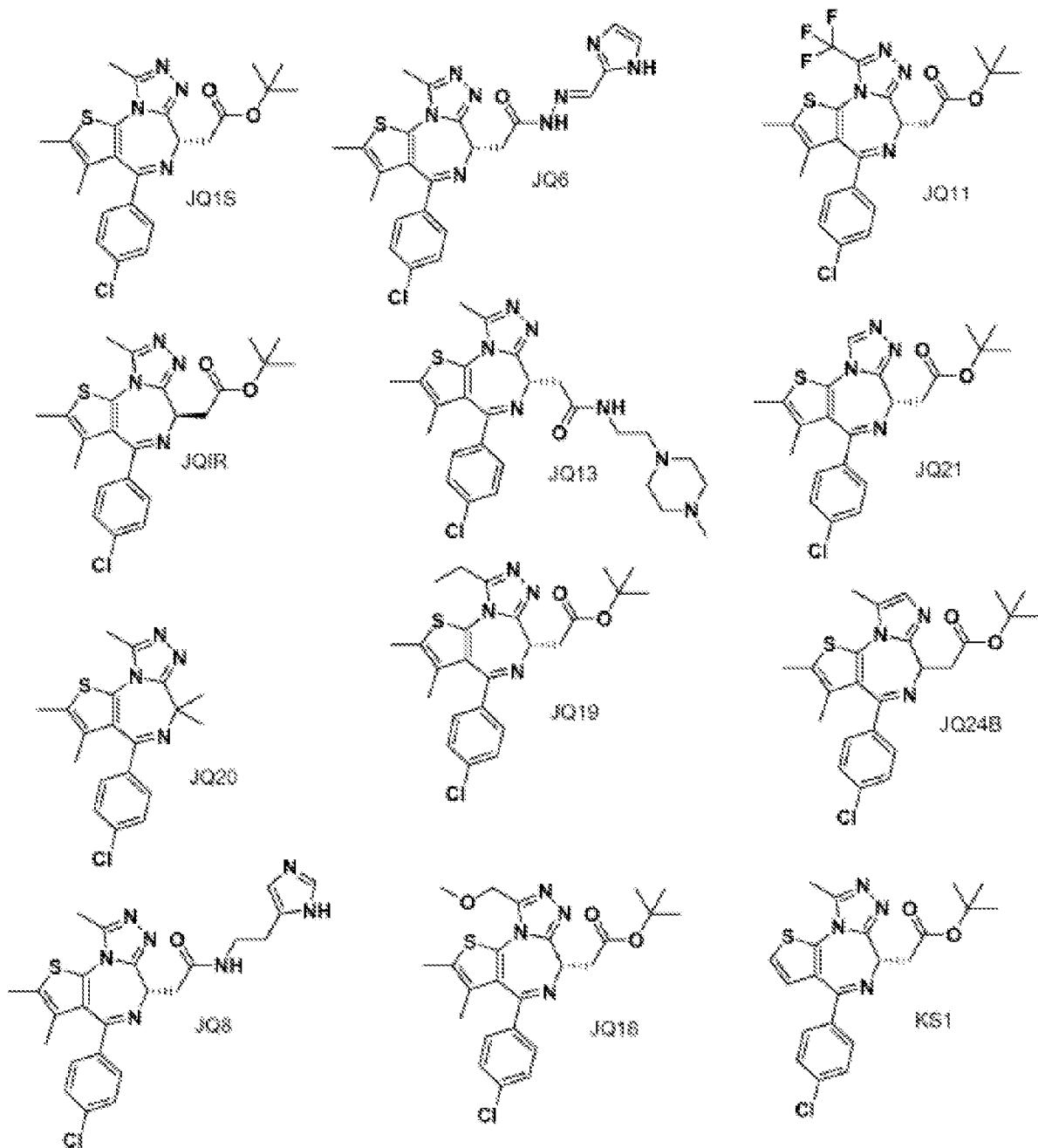


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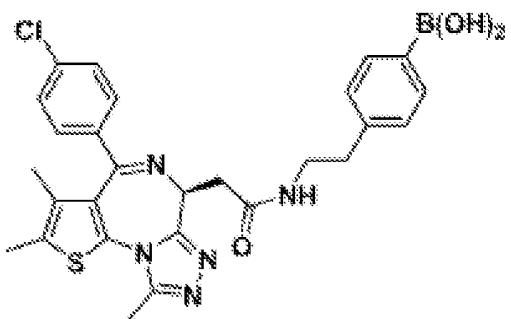
a salt, solvate or hydrate thereof.

In some embodiments, a bromodomain or BET inhibitor is a compound represented by any following formulae:

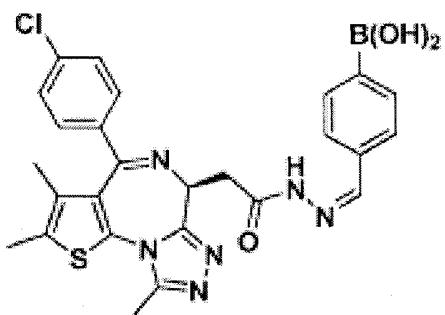
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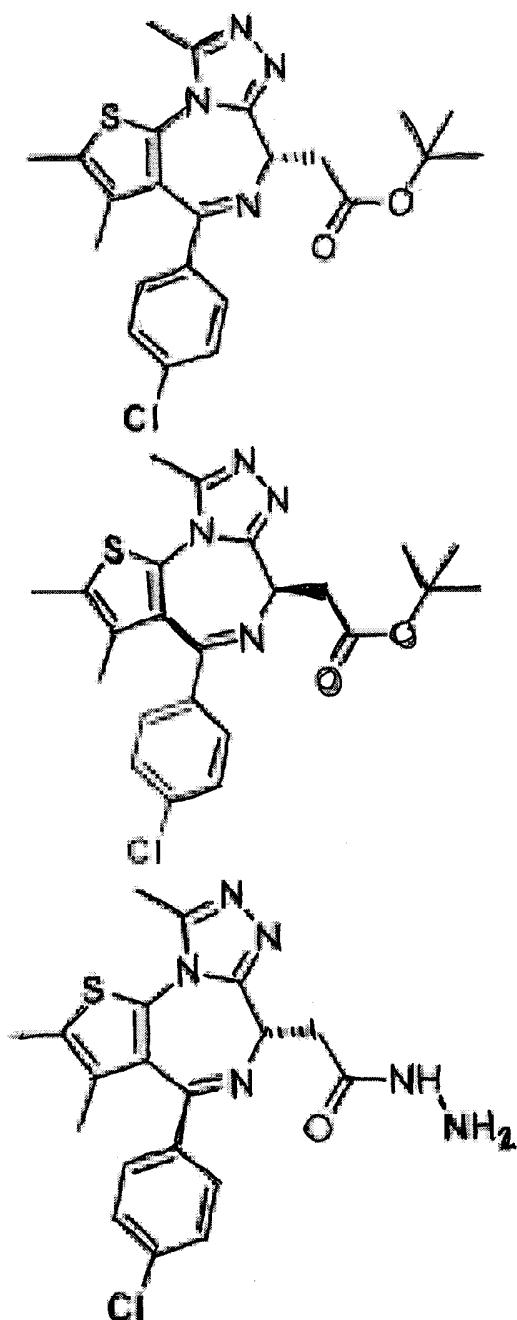
, or a salt, solvate or hydrate thereof. In some embodiments, a bromodomain or BET inhibitor is a compound represented by any one of the following formulae:

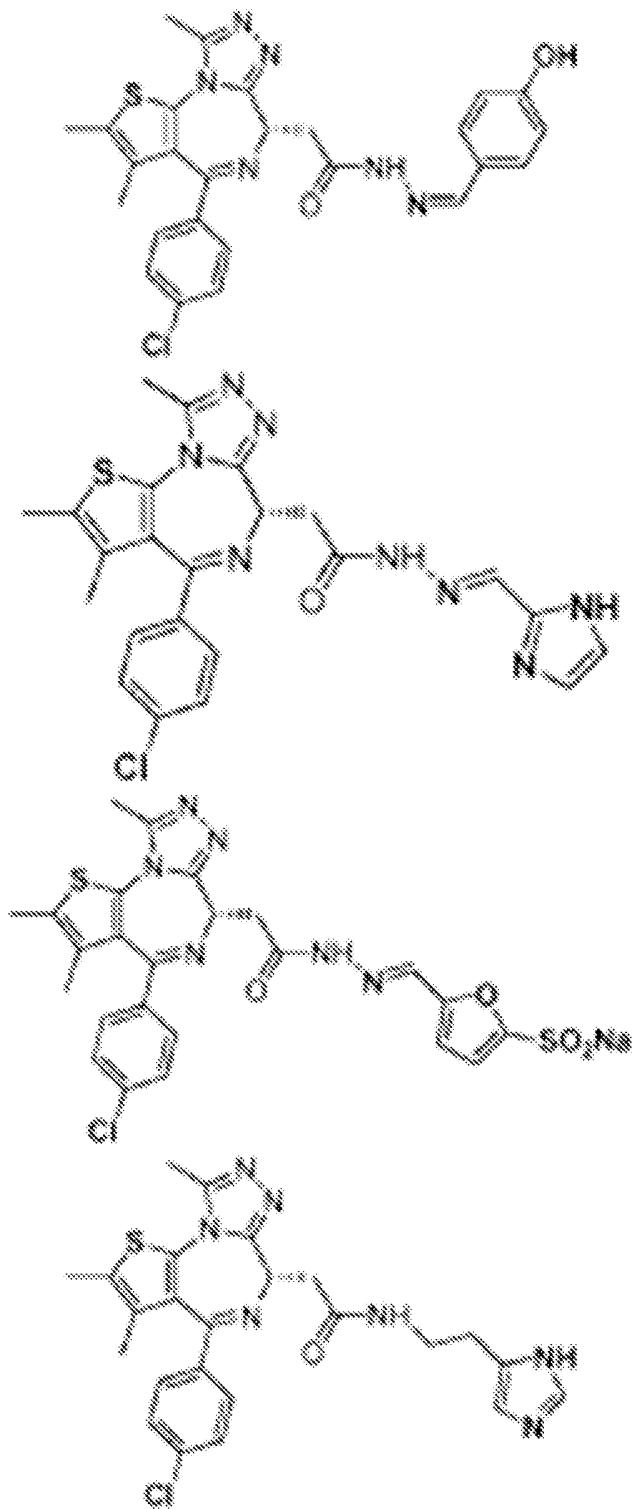


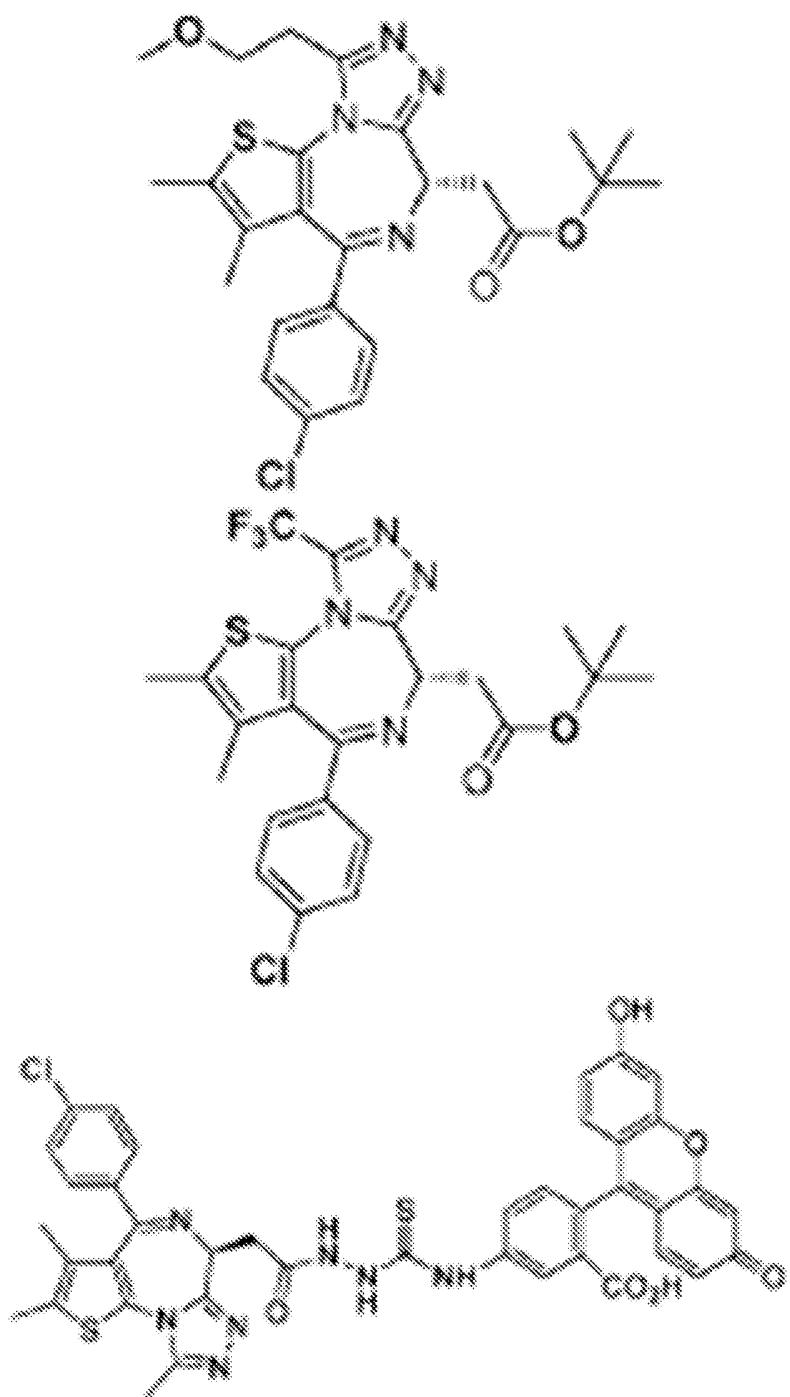
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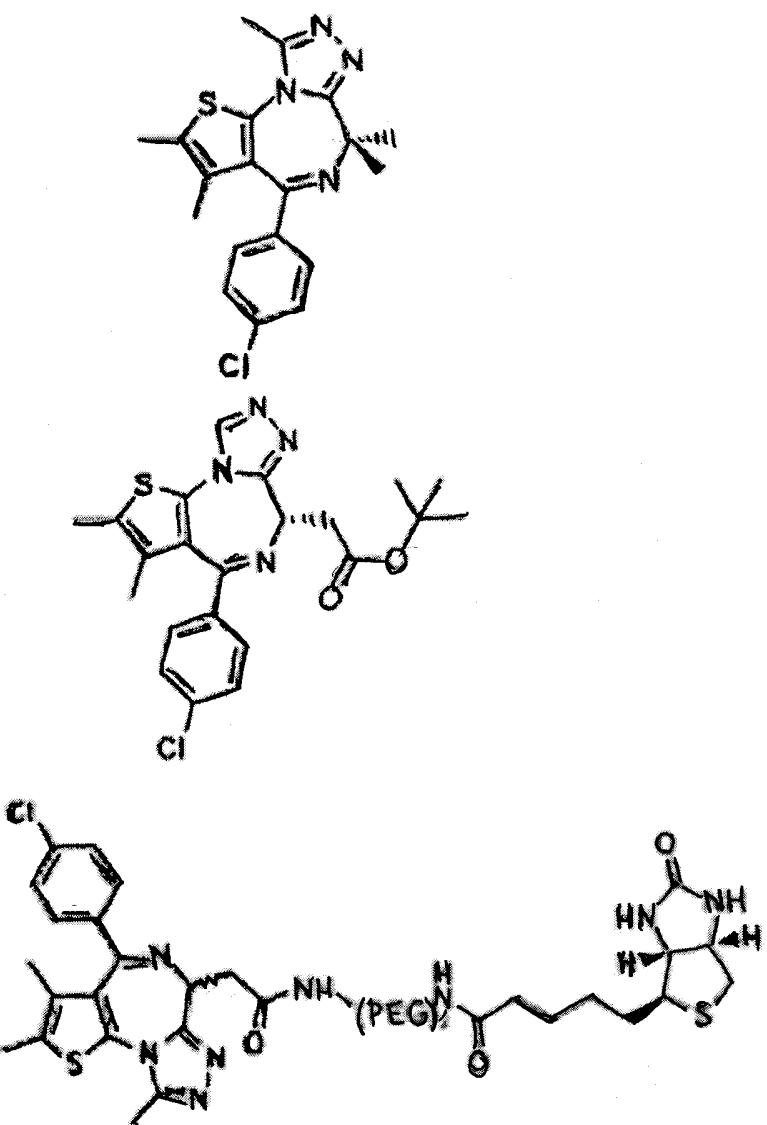
a salt, solvate or hydrate thereof. In some embodiments, a bromodomain or BET inhibitor is a compound represented by any following structures:

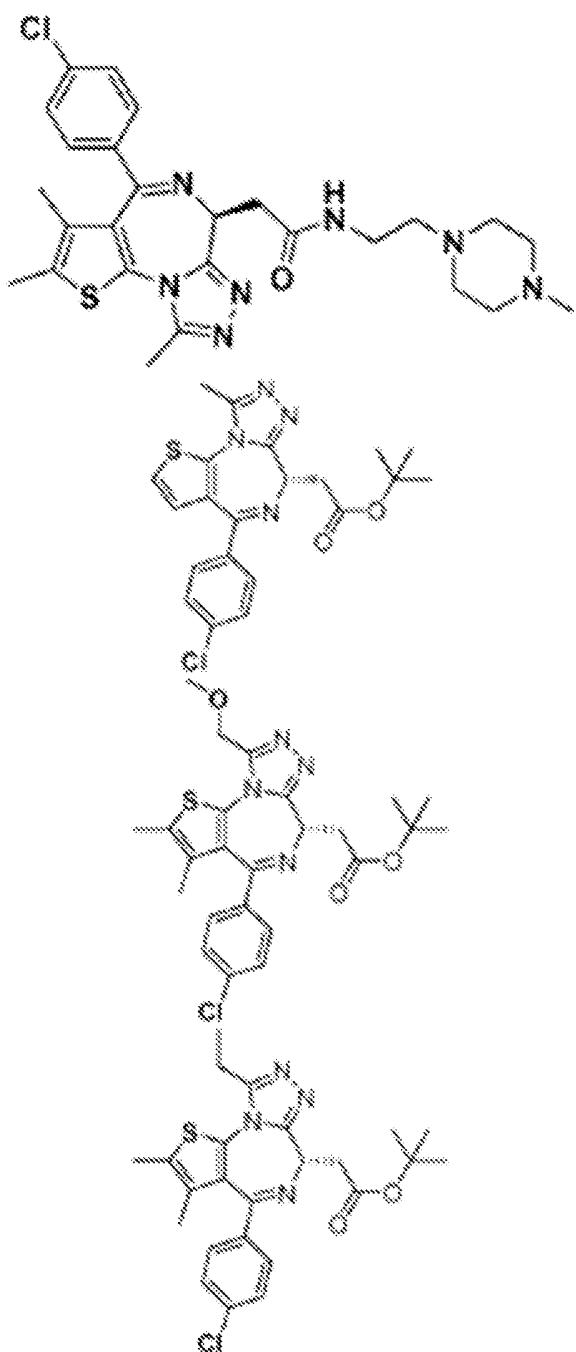


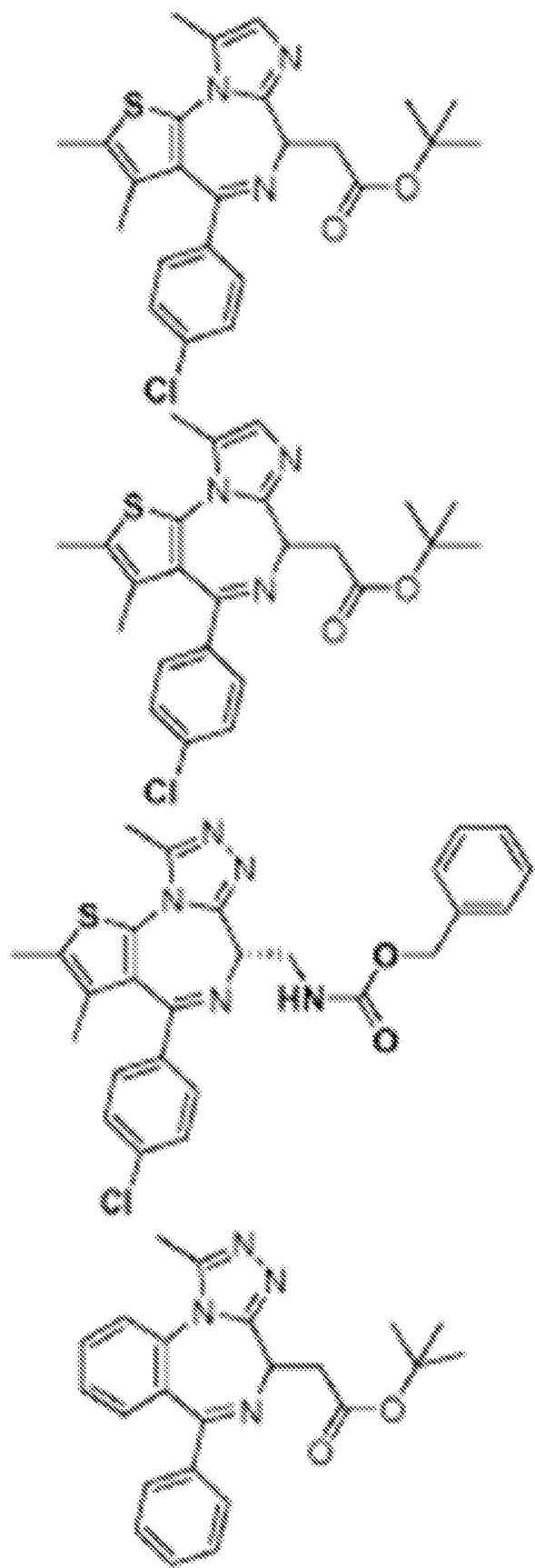


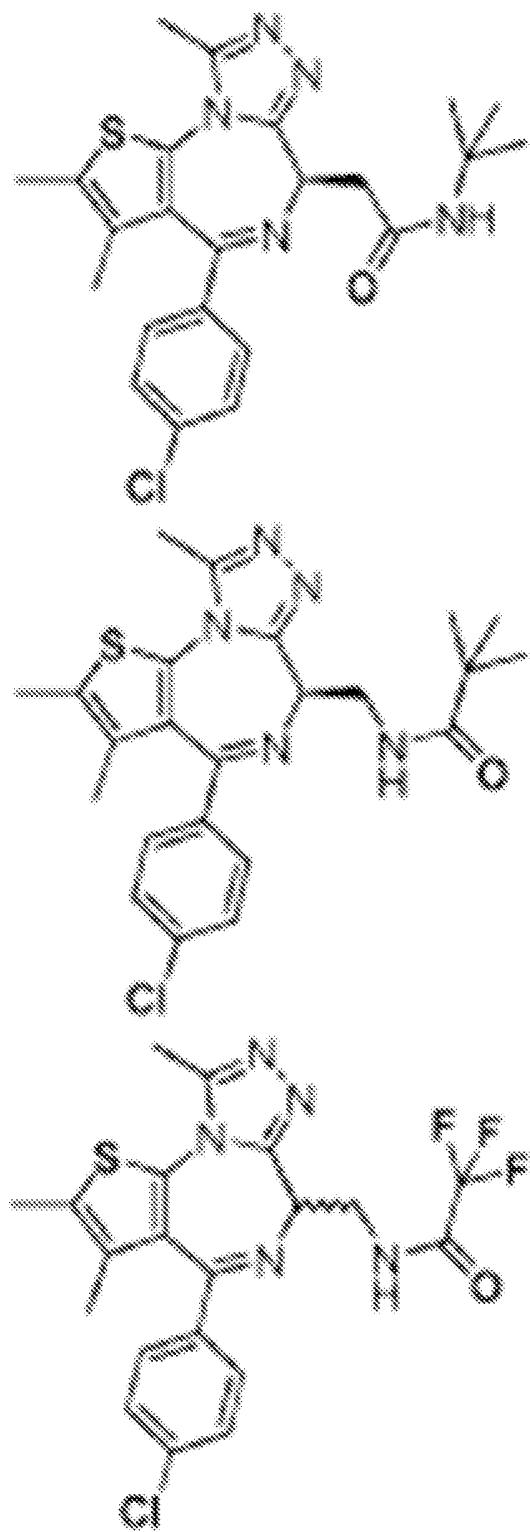


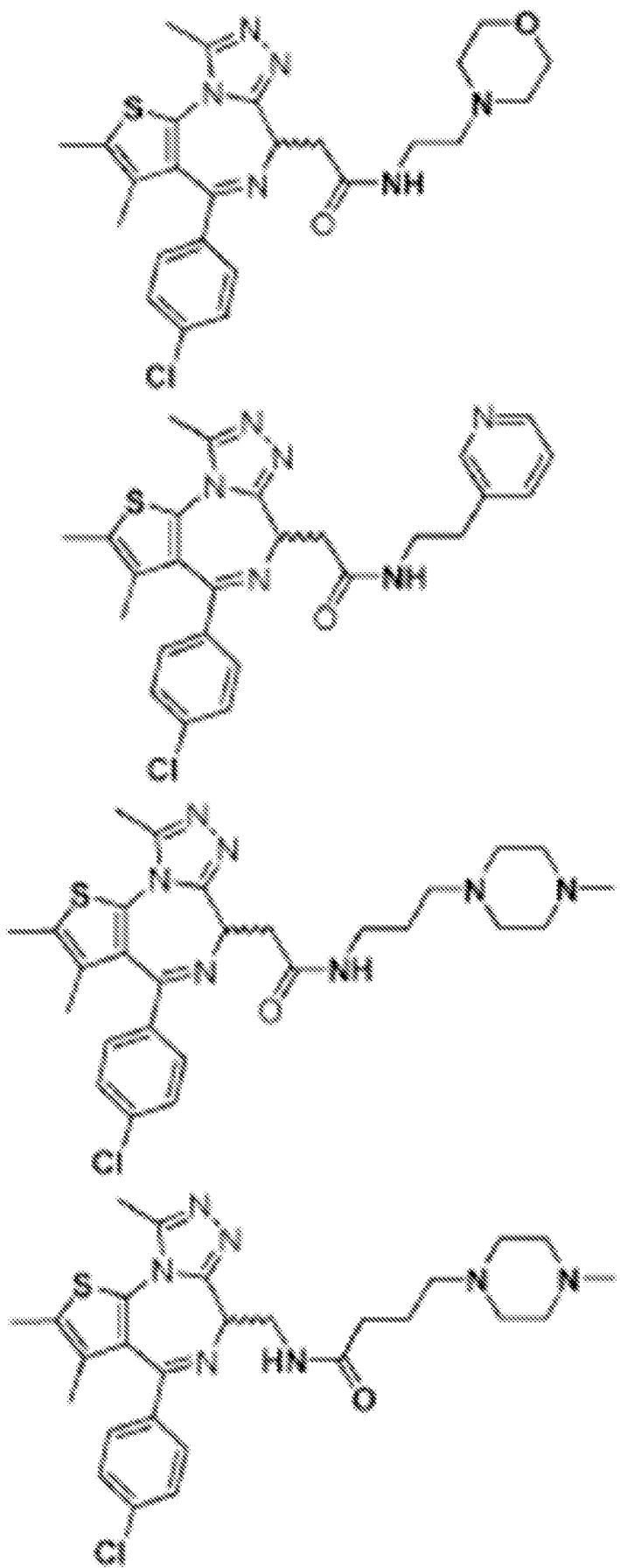
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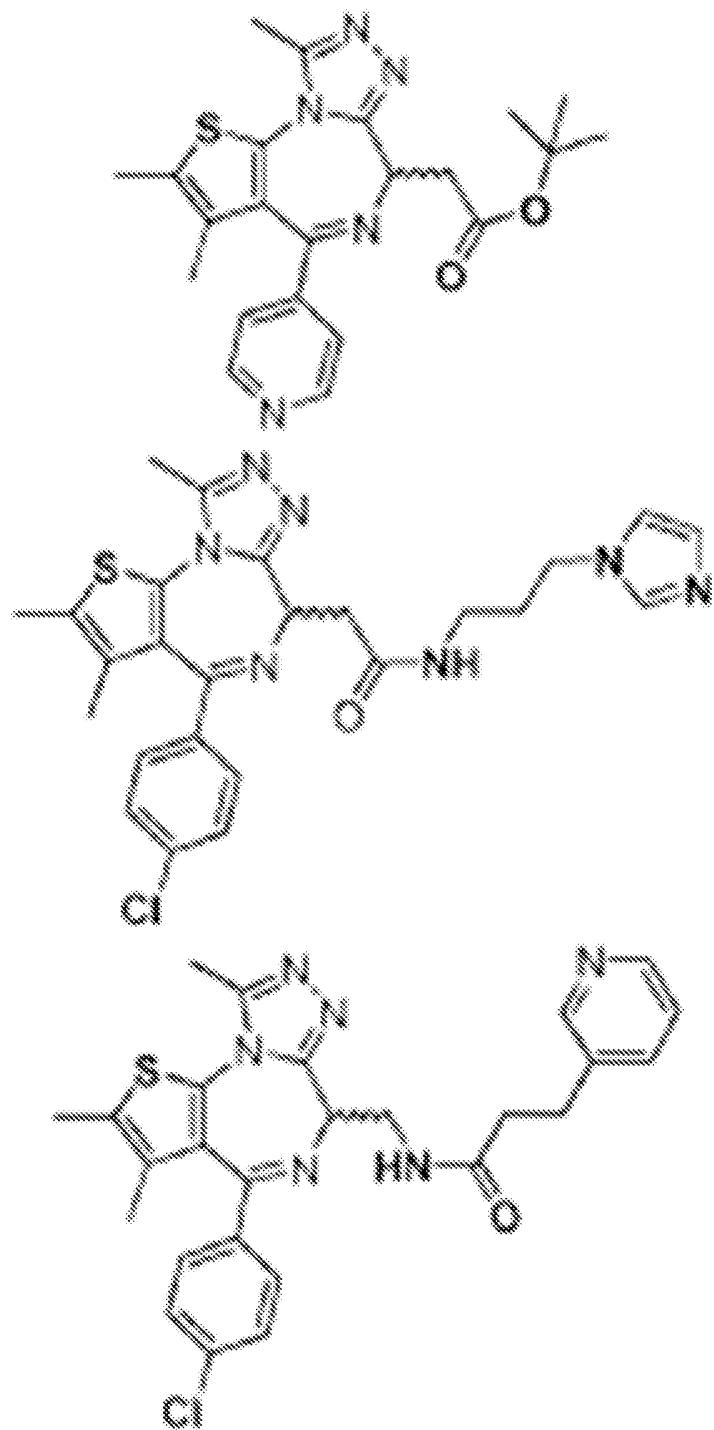


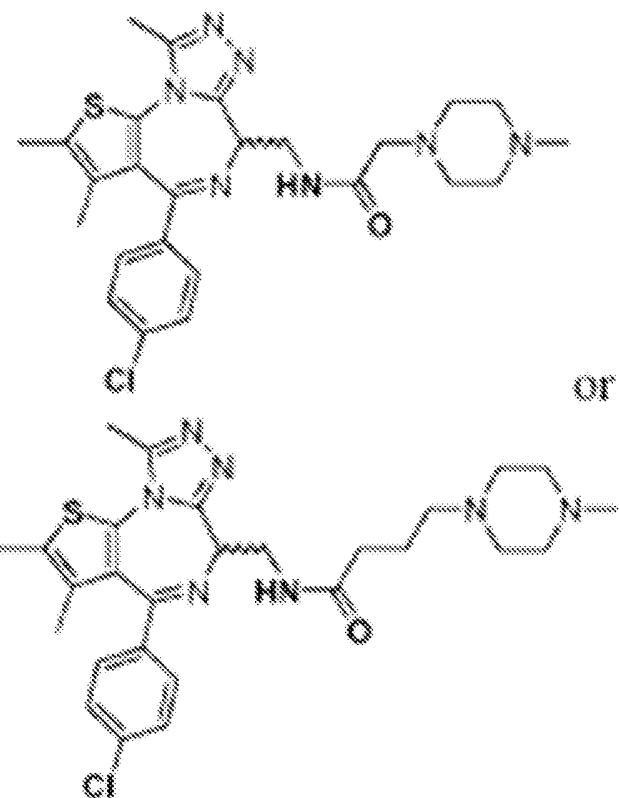




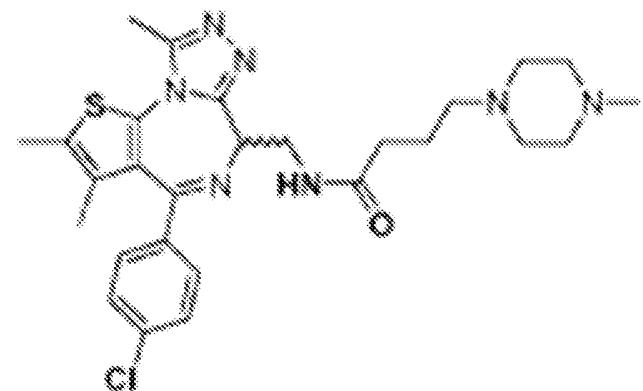




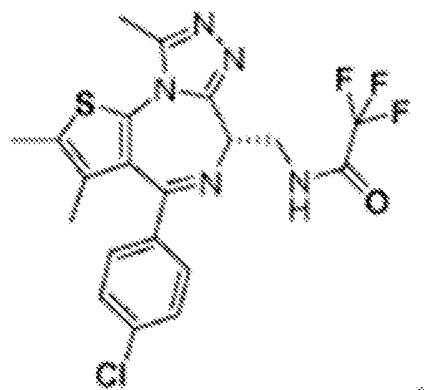




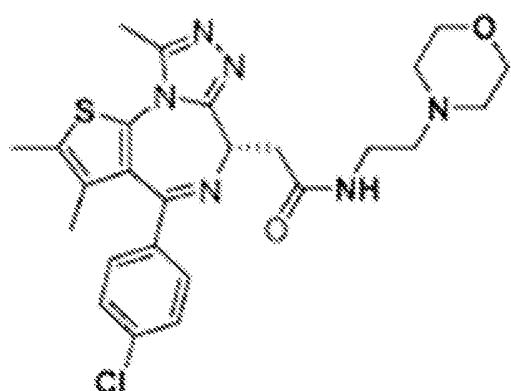
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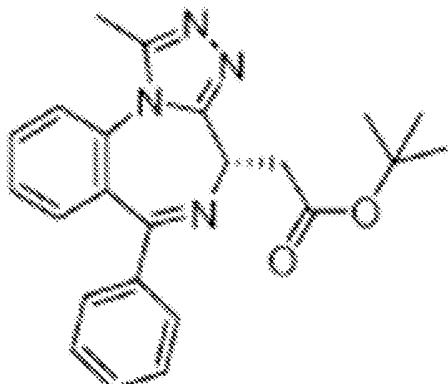
or a salt, solvate or hydrate thereof. In some embodiments, a bromodomain or BET inhibitor can be one of the following structures:



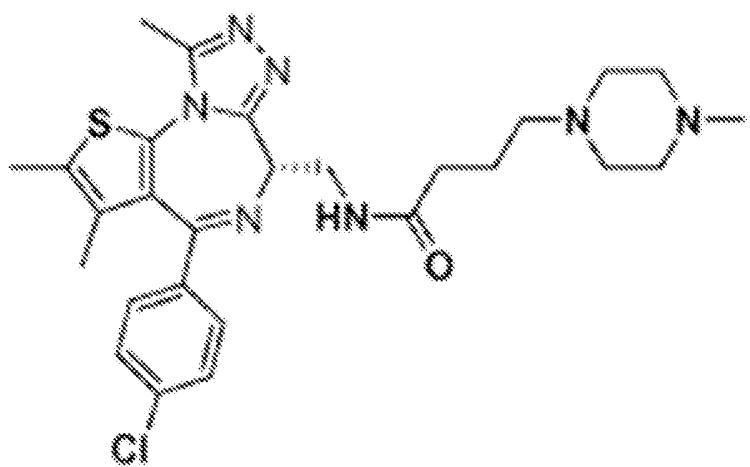
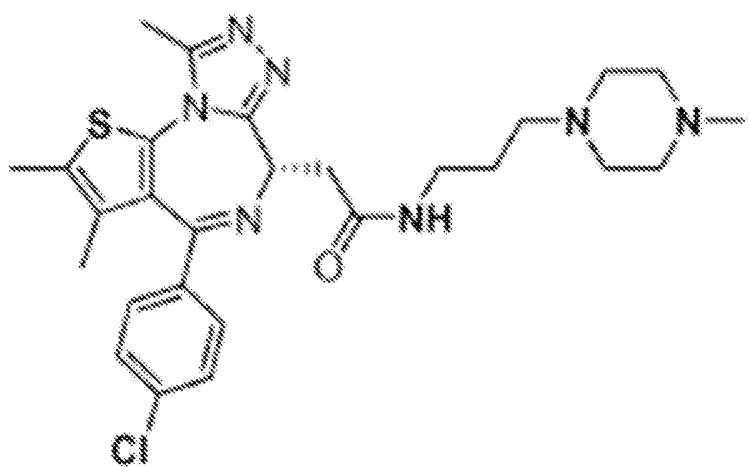
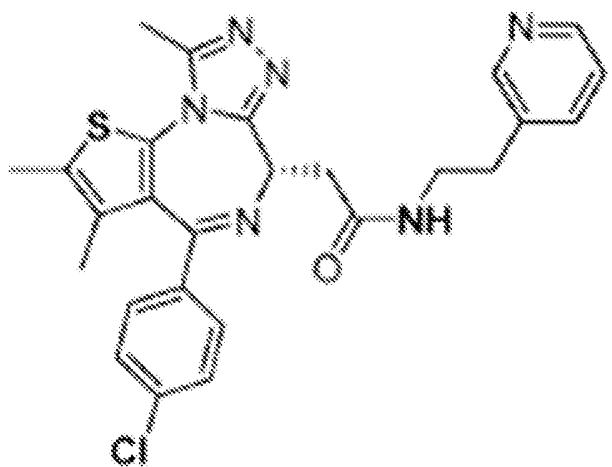
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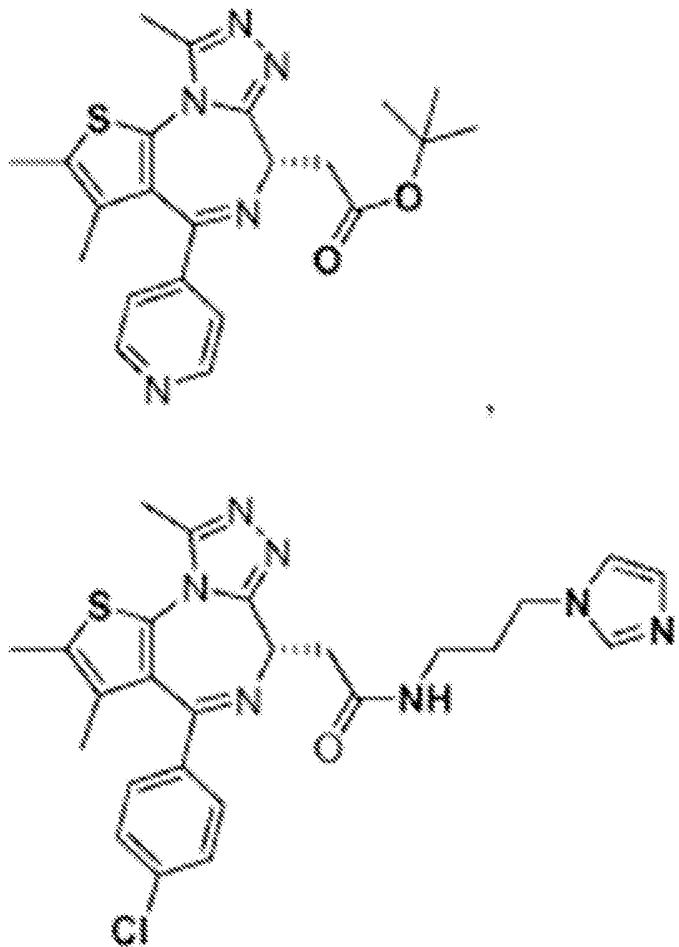


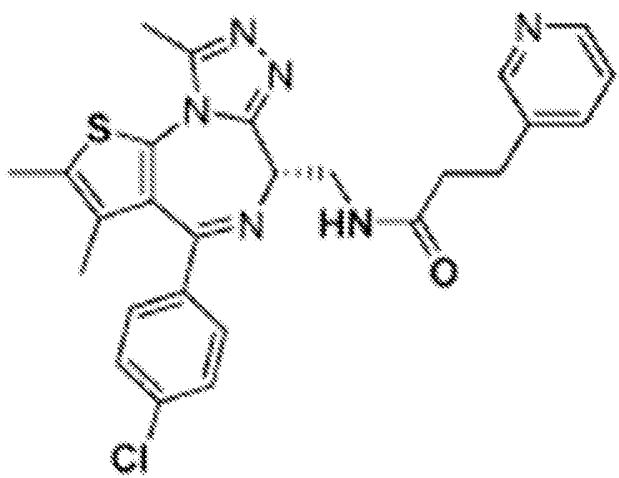
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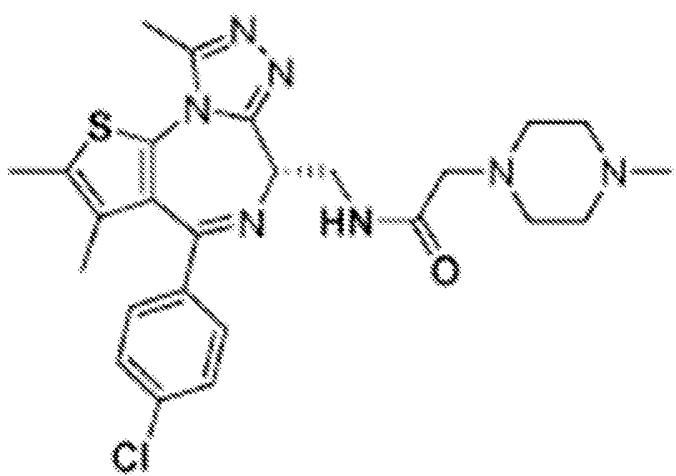
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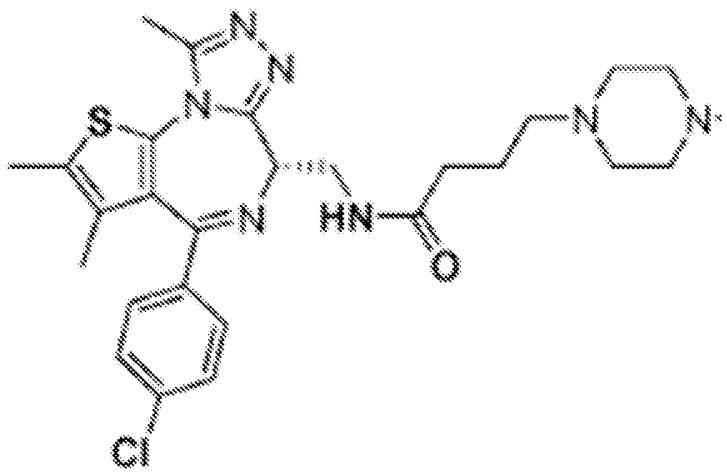


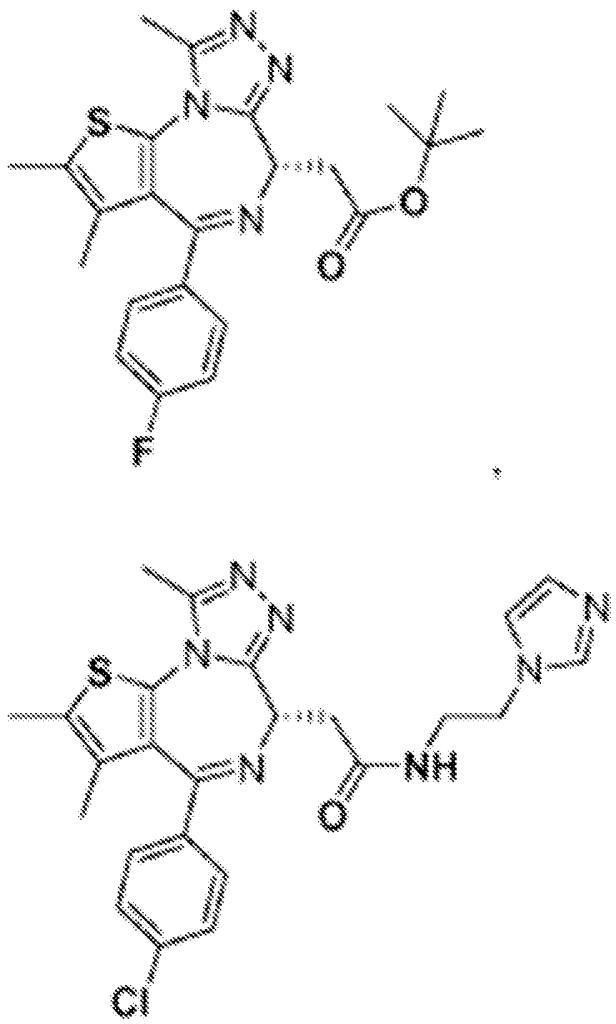


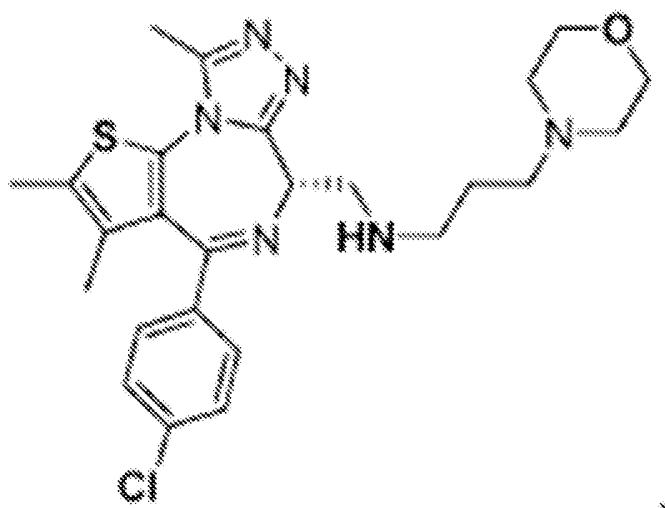
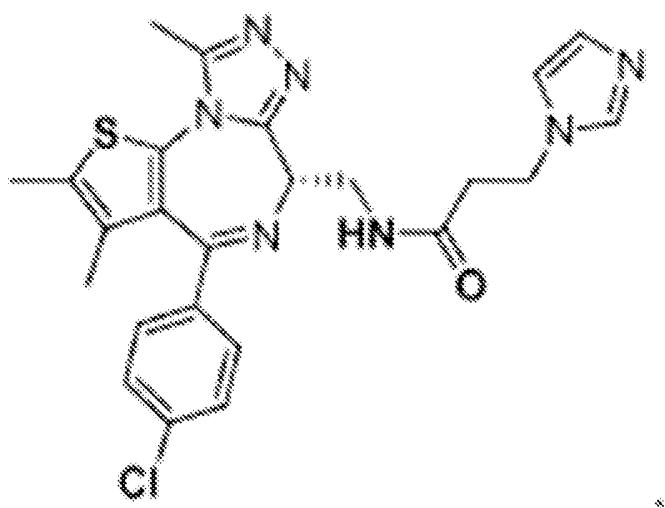
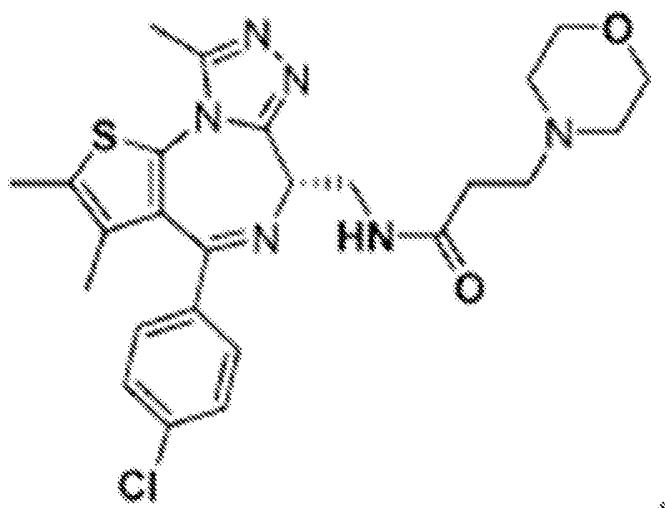
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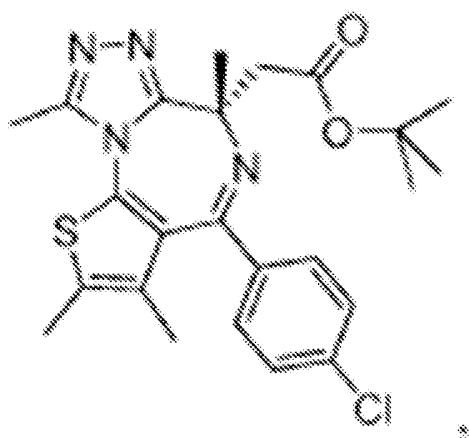
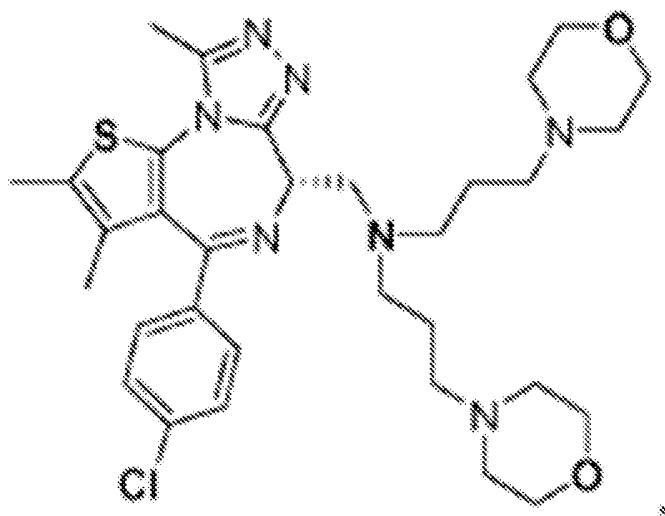


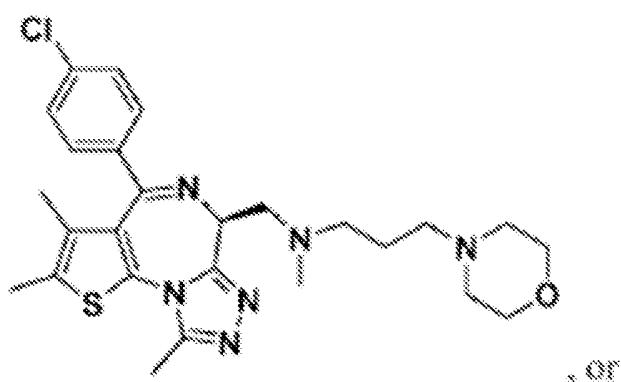
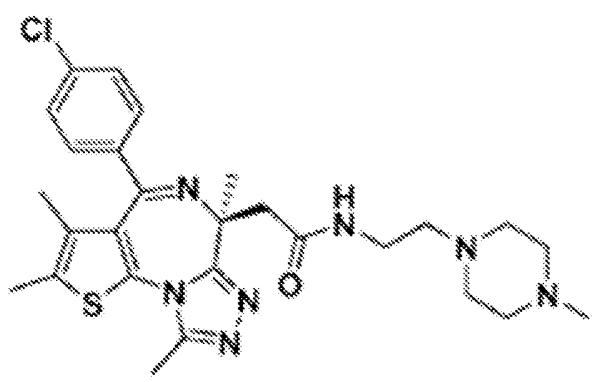
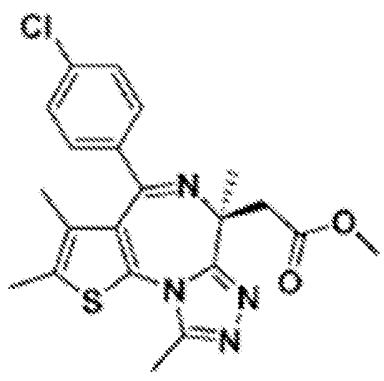
*



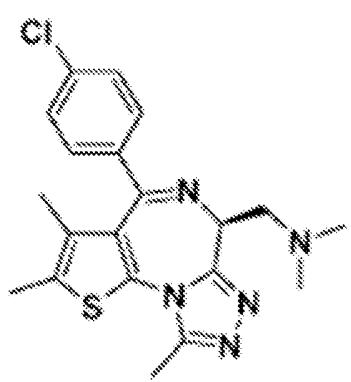








, or

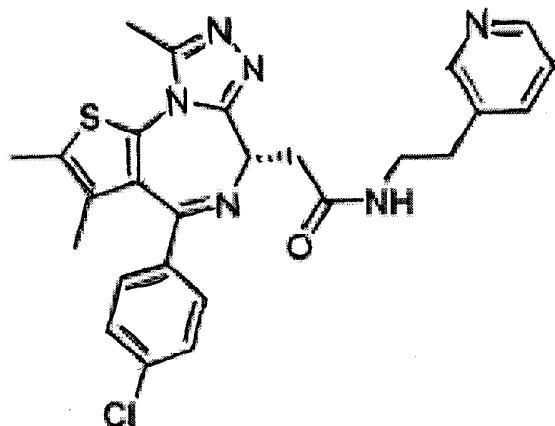


;

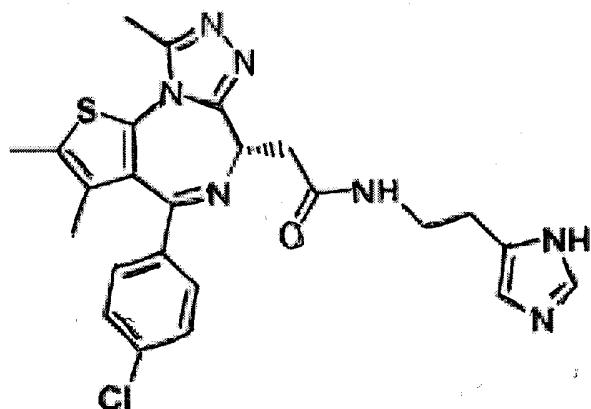
a salt, solvate or hydrate thereof.

- 32 -

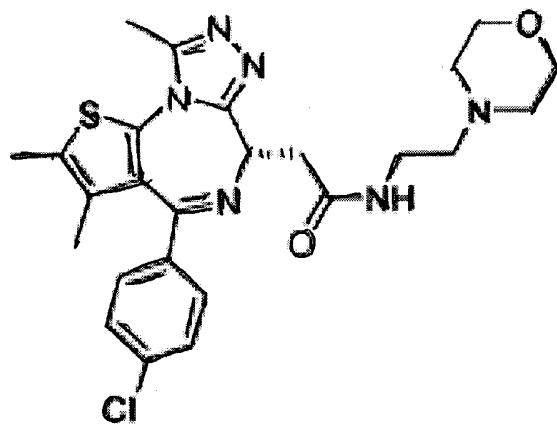
In some embodiments, a bromodomain or BET inhibitor is a compound represented by the following structure:



or a salt, solvate or hydrate thereof. In some embodiments, a bromodomain or BET inhibitor is a compound
5 represented by the following structure:

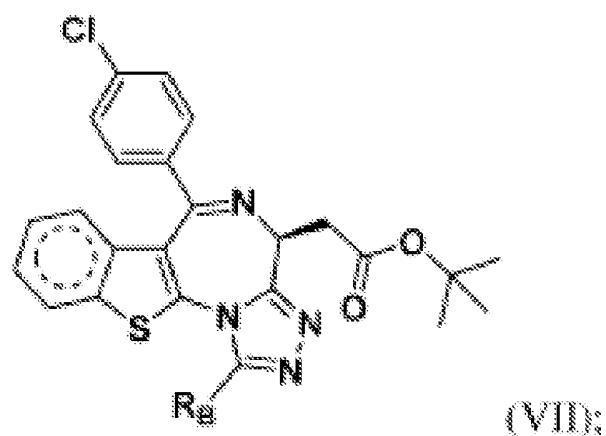
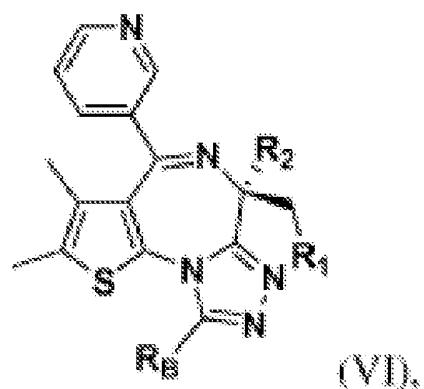
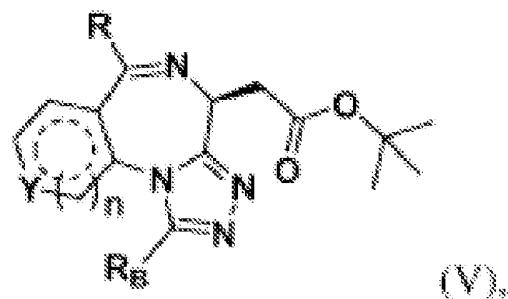


or a salt, solvate or hydrate thereof. In some embodiments, a bromodomain or BET inhibitor is a compound
represented by the following structure:



10 or a salt, solvate or hydrate thereof. In some embodiments, a bromodomain or BET inhibitor is a compound with
the opposite chirality of any compound shown herein. In some embodiments, a bromodomain or BET inhibitor is a
compound represented by Formula (V), (VI), or (VII):

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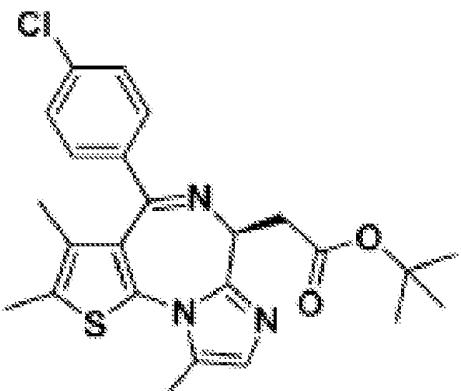


in which R, R₁, and R₂ and R₆ have the same meaning as in Formula (I); Y is O, N, S, or CR₅, in which R₅ has the same meaning as in Formula (I); n is 0 or 1; and the dashed circle in Formula (VII) indicates an aromatic or non-aromatic ring; or a salt, solvate or hydrate thereof.

In certain embodiments of any of the Formulae I- IV and VI (or any formula herein), R₆ represents the non-carbonyl portion of an aldehyde shown in Table A, below (i.e., for an aldehyde of formula R₆CHO, R₆ is the non-carbonyl portion of the aldehyde). In certain embodiments, R₄ and R₆ together represent the non-carbonyl portion of a ketone shown in Table A (i.e., for a ketone of formula R₆C(0)R₄, R₄ and R₆ are the non-carbonyl portion of the ketone).

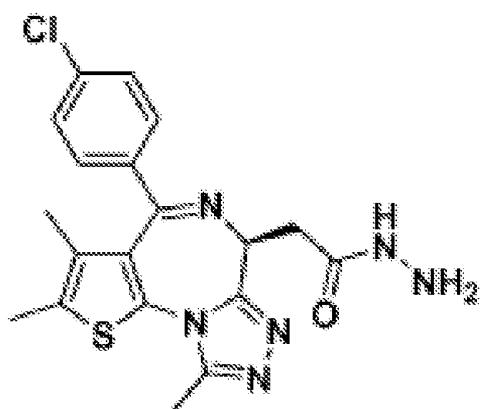
- 50 -

In some embodiments, a bromodomain or BET inhibitor is a compound represented by the formula:



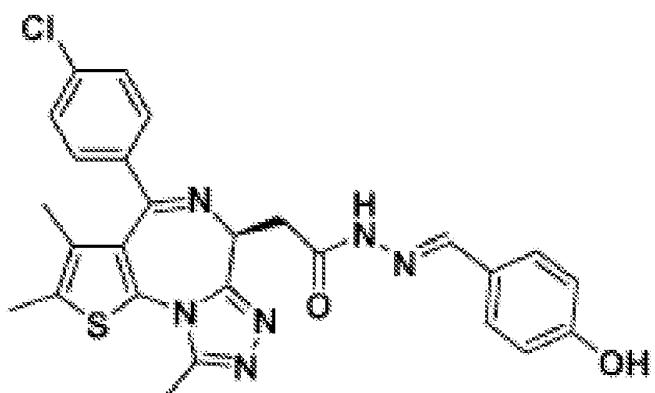
(VIII)

, or a salt, solvate, or hydrate thereof. In some embodiments, a bromodomain or BET inhibitor is (racemic) JQ1; in certain embodiments, the compound is (+)-JQ1. In some embodiments, a bromodomain or BET inhibitor is a compound selected from the group consisting of :



(3)

and



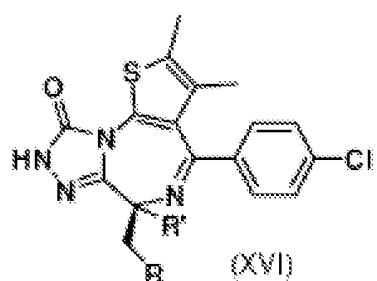
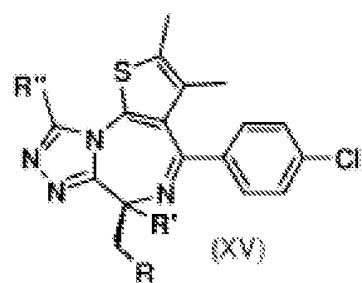
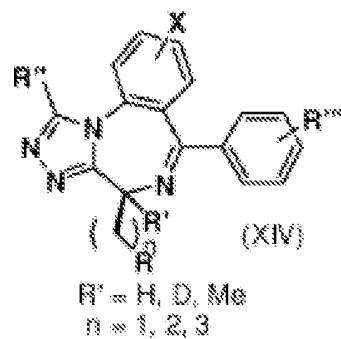
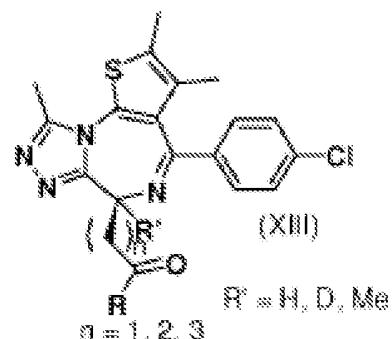
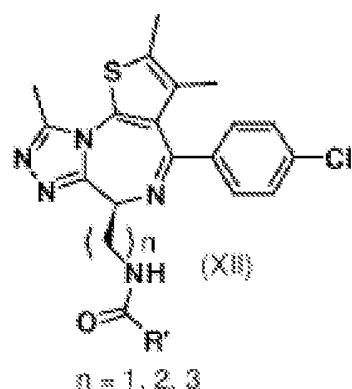
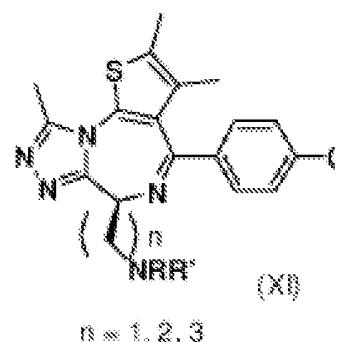
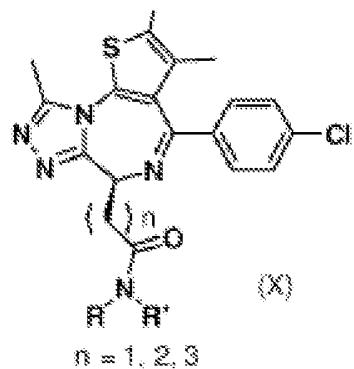
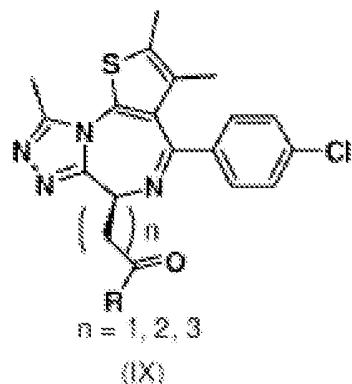
(4)

, or a salt, solvate, or hydrate thereof.

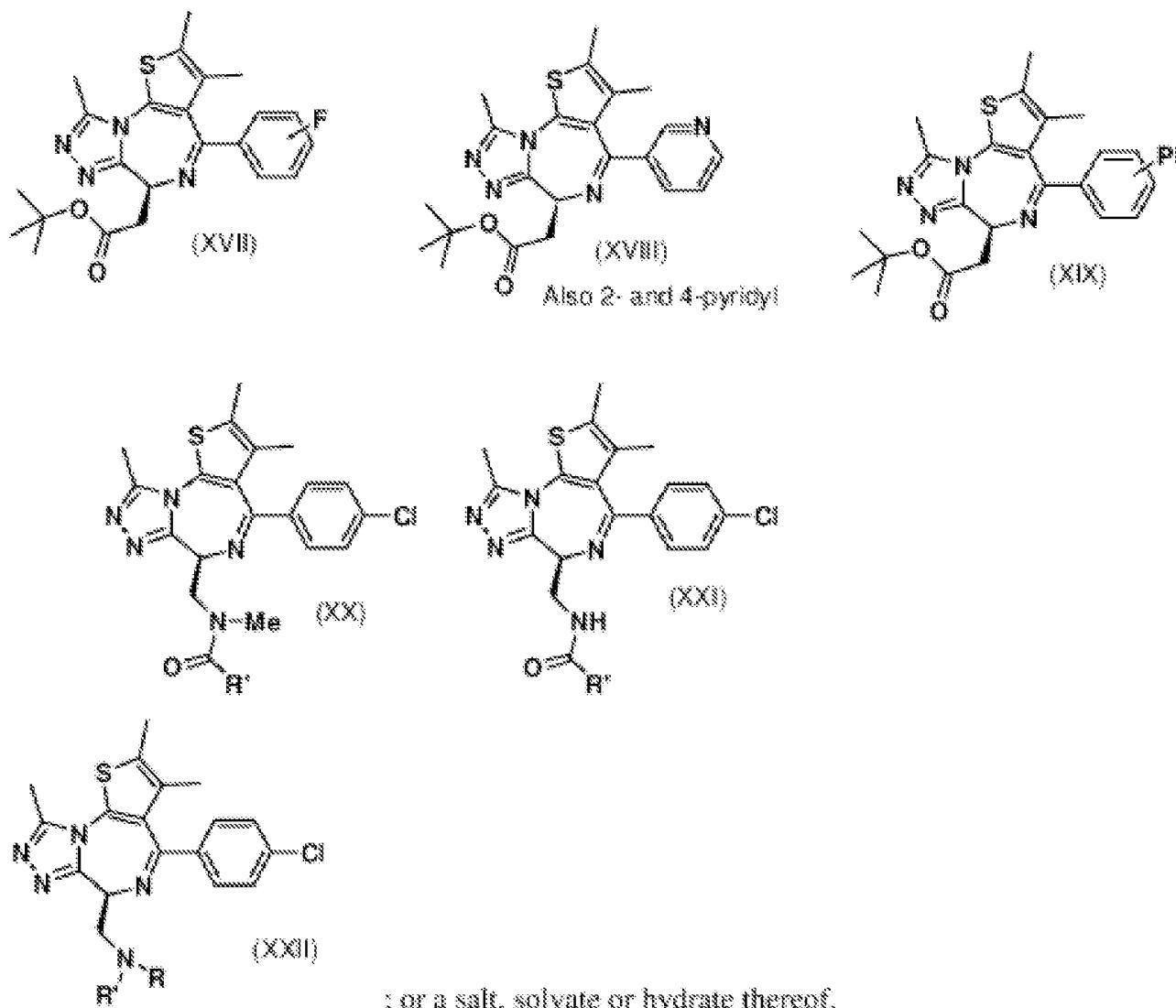
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Additional examples of compounds include compounds according to any of the following formulae:

R



$R' = \text{OMe}, \text{CH}_2\text{OH}, \text{CH}_2\text{NH}_2, \text{CH}_2\text{OMe}$



; or a salt, solvate or hydrate thereof.

In Formulae IX-XXII, R and R' can be, e.g., H, aryl, substituted aryl, heteroaryl, heteroaryl, heterocycloalkyl, -C₁-C₈ alkyl, -C₂-C₈ alkenyl, -C₂-C₈ alkynyl, -C₃-C₁₂ cycloalkyl, substituted -C₃-C₁₂ cycloalkyl, -C₃-C₁₂ cycloalkenyl, or substituted -C₃-C₁₂ cycloalkenyl, each of which may be optionally substituted. In Formulae XIV, X can be any substituent for an aryl group as described herein.

The compounds described in herein can be prepared using methods well known in the prior art (see, e.g., WO 011143669, the entirety which is incorporated by reference herein).

As used herein, the term an "aromatic ring" or "aryl" means a monocyclic or polycyclic-aromatic ring or ring radical comprising carbon and hydrogen atoms. Examples of suitable aryl groups include, but are not limited to, phenyl, tolyl, anthacenyl, fluorenyl, indenyl, azulenyl, and naphthyl, as well as benzo-fused carbocyclic moieties such as 5,6,7,8-tetrahydronaphthyl. An aryl group can be unsubstituted or optionally is substituted with one or more substituents, e.g., substituents as described herein for alkyl groups (including without limitation alkyl (preferably,

lower alkyl or alkyl substituted with one or more halo), hydroxy, alkoxy (preferably, lower alkoxy), alkylthio, cyano, halo, amino, boronic acid (-B(OH)2, and nitro). In certain embodiments, the aryl group is a monocyclic ring, wherein the ring comprises 6 carbon atoms.

As used herein, the term "alkyl" means a saturated straight chain or branched non-cyclic hydrocarbon typically having from 1 to 10 carbon atoms. Representative saturated straight chain alkyls include methyl, ethyl, n-propyl, n-butyl, n-pentyl, n-hexyl, n-heptyl, n-octyl, n-nonyl and n-decyl; while saturated branched alkyls include isopropyl, sec-butyl, isobutyl, tert-butyl, isopentyl, 2-methylbutyl, 3-methylbutyl, 2-methylpentyl, 3-methylpentyl, 4-methylpentyl, 2-methylhexyl, 3-methylhexyl, 4-methylhexyl, 5-methylhexyl, 2,3-dimethylbutyl, 2,3-dimethylpentyl, 2,4-dimethylpentyl, 2,3-dimethylhexyl, 2,4-dimethylhexyl, 2,5-dimethylhexyl, 2,2-dimethylpentyl, 2,2-dimethylhexyl, 3,3-dimethylpentyl, 3,3-dimethylhexyl, 4,4-dimethylhexyl, 2-ethylpentyl, 3-ethylpentyl, 2-ethylhexyl, 3-ethylhexyl, 4-ethylhexyl, 2-methyl-2-ethylpentyl, 2-methyl-3-ethylpentyl, 2-methyl-4-ethylpentyl, 2-methyl-2-ethylhexyl, 2-methyl-3-ethylhexyl, 2-methyl-4-ethylhexyl, 2,2-diethylpentyl, 3,3-diethylhexyl, 2,2-diethylhexyl, 3,3-diethylhexyl and the like. Alkyl groups included in compounds of this invention may be unsubstituted, or optionally substituted with one or more substituents, such as amino, alkylamino, arylamino, heteroaryl amino, alkoxy, alkylthio, oxo, halo, acyl, nitro, hydroxyl, cyano, aryl, heteroaryl, alkylaryl, alkylheteroaryl, aryloxy, heteroaryloxy, arylthio, heteroarylthio, arylamino, heteroaryl amino, carbocyclyl, carbocyclyloxy, carbocyclylthio, carbocyclylamino, heterocyclyl, heterocyclyloxy, heterocyclylamino, heterocyclylthio, and the like. Lower alkyls are typically preferred for the compounds of this invention.

The term "diastereomers" refers to stereoisomers with two or more centers of dissymmetry and whose molecules are not mirror images of one another.

The term "enantiomers" refers to two stereoisomers of a compound which are non-superimposable mirror images of one another. An equimolar mixture of two enantiomers is called a "racemic mixture" or a "racemate."

The term "halogen" designates -F, -Cl, -Br or -I.

The term "haloalkyl" is intended to include alkyl groups as defined above that are mono-, di- or polysubstituted by halogen, e.g., fluoromethyl and trifluoromethyl.

The term "hydroxyl" means -OH.

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

The term "heteroaryl" refers to an aromatic 5-8 membered monocyclic, 8-12 membered bicyclic, or 11-14 membered tricyclic ring system having 1-4 ring heteroatoms if monocyclic, 1-6 heteroatoms if bicyclic, or 1-9 heteroatoms if tricyclic, said heteroatoms selected from O, N, or S, and the remainder ring atoms being carbon. Heteroaryl groups may be optionally substituted
5 with one or more substituents as for aryl groups. Examples of heteroaryl groups include, but are not limited to, pyridyl, furanyl, benzodioxolyl, thienyl, pyrrolyl, oxazolyl, oxadiazolyl, imidazolyl thiazolyl, isoxazolyl, quinolinyl, pyrazolyl, isothiazolyl, pyridazinyl, pyrimidinyl, pyrazinyl, triazinyl, triazolyl, thiadiazolyl, isoquinolinyl, indazolyl, benzoxazolyl, benzofuryl, indolizinyl, imidazopyridyl, tetrazolyl, benzimidazolyl, benzothiazolyl, benzothiadiazolyl,
10 benzoxadiazolyl, and indolyl.

The term "heterocyclic" as used herein, refers to organic compounds that contain at least at least one atom other than carbon (e.g., S, O, N) within a ring structure. The ring structure in these organic compounds can be either aromatic or non-aromatic. Some examples of heterocyclic moieties include, are not limited to, pyridine, pyrimidine, pyrrolidine, furan,
15 tetrahydrofuran, tetrahydrothiophene, and dioxane.

The term "isomers" or "stereoisomers" refers to compounds which have identical chemical constitution, but differ with regard to the arrangement of the atoms or groups in space.

The term "isotopic derivatives" includes derivatives of compounds in which one or more atoms in the compounds are replaced with corresponding isotopes of the atoms. For example, an
20 isotopic derivative of a compound containing a carbon atom (C 112") would be one in which the carbon atom of the compound is replaced with the C 13 isotope.

The term "optical isomers" as used herein includes molecules, also known as chiral molecules, that are exact non-superimposable mirror images of one another.

The terms "polycyclyl" or "polycyclic radical" refer to the radical of two or more cyclic rings (e.g., cycloalkyls, cycloalkenyls, cycloalkynyls, aryls and/or heterocyclyls) in which two or more carbons are common to two adjoining rings, e.g., the rings are "fused rings". Rings that are joined through non-adjacent atoms are termed "bridged" rings. Each of the rings of the polycycle can be substituted with such substituents as described above, as for example, halogen, hydroxyl,
25 alkylcarbonyloxy, arylcarbonyloxy, alkoxy carbonyloxy, aryloxycarbonyloxy, carboxylate, alkylcarbonyl, alkoxy carbonyl, aminocarbonyl, alkylthiocarbonyl, alkoxy, phosphate, phosphonato, phosphinato, cyano, amino (including alkyl amino, dialkylamino, arylamino, diarylamino, and alkylarylamino), acylamino (including alkylcarbonylamino,

arylcarbonylamino, carbamoyl and ureido), amidino, imino, sulfhydryl, alkylthio, arylthio, thiocarboxylate, sulfates, sulfonato, sulfamoyl, sulfonamido, nitro, trifluoromethyl, cyano, azido, heterocyclyl, alkyl, alkylaryl, or an aromatic or heteroaromatic moiety.

The term "sulfhydryl" or "thiol" means -SH.

5 The recitation of a listing of chemical groups in any definition of a variable herein includes definitions of that variable as any single group or combination of listed groups. The recitation of an embodiment for a variable or aspect herein includes that embodiment as any single embodiment or in combination with any other embodiments or portions thereof.

10 In some embodiments, the bromodomain inhibitor is any molecule or compound that reduces or prevents expression of BRD-containing proteins. Examples of such inhibitors include siRNA, shRNA, dsRNA, oligomimics, and proteases that target one or more BRD-containing protein.

Methods for producing inhibitors as described above are well known in the art (See e.g.,
 15 Sambrook, Fritsch and Maniatis, MOLECULAR CLONING: A LABORATORY MANUAL,
 (Current Edition); CURRENT PROTOCOLS IN MOLECULAR BIOLOGY (F. M. Ausubel et
 al. eds., (Current Edition)); the series METHODS IN ENZYMOLOGY (Academic Press, Inc.):
 PCR 2: A PRACTICAL APPROACH (Current Edition) ANTIBODIES, A LABORATORY
 MANUAL and ANIMAL CELL CULTURE (R. I. Freshney, ed. (1987)). DNA Cloning: A
 20 Practical Approach, vol. I & II (D. Glover, ed.); Oligonucleotide Synthesis (N. Gait, ed., Current
 Edition); Nucleic Acid Hybridization (B. Hames & S. Higgins, eds., Current Edition);
 Transcription and Translation (B. Hames & S. Higgins, eds., Current Edition); Fundamental
 Virology, 2nd Edition, vol. I & II (B. N. Fields and D. M. Knipe, eds.).

25 Examples of bromodomain-containing proteins are shown in Table 2.

Gene	Ensembl gene ID	Ensembl transcript ID	Ensembl protein ID
ASH1L	ENSG00000116539	ENST00000548830, ENST00000492987, ENST00000392403, ENST00000368346	ENSP00000449283, ENSP00000448820, ENSP00000376204, ENSP00000357330
ATAD2	ENSG00000156802	ENST00000521903, ENST00000519124, ENST00000517666, ENST00000287394	ENSP00000429213, ENSP00000429617, ENSP00000429331, ENSP00000287394

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BAZ1A/B	ENSG00000198604 (A), ENSG0000009954 (B)	ENST00000555331 (A), ENST00000554865 (A), ENST00000553573 (A), ENST00000543083 (A), ENST00000382422 (A), ENST00000360310 (A), ENST00000358716 (A), ENST00000404251 (B), ENST00000339594 (B)	ENSP00000450902 (A), ENSP00000450923 (A), ENSP00000451896 (A), ENSP00000445562 (A), ENSP00000371859 (A), ENSP00000353458 (A), ENSP00000351555 (A), ENSP00000385442 (B), ENSP00000342434 (B)
BAZ2A/B	ENSG0000076108 (A), ENSG0000123636 (B)	ENST00000551996 (A), ENST00000551812 (A), ENST00000549884 (A), ENST00000549787 (A), ENST00000549506 (A), ENST00000547650 (A), ENST00000547453 (A), ENST00000546695 (A), ENST00000379441 (A), ENST00000179765 (A), ENST00000546335 (B), ENST00000541068 (B), ENST00000441143 (B), ENST00000437839 (B), ENST00000426648 (B), ENST00000392783 (B), ENST00000392782 (B), ENST00000355831 (B), ENST00000343439 (B), ENST00000294905 (B)	ENSP00000447591 (A), ENSP00000446880 (A), ENSP00000447941 (A), ENSP00000448760 (A), ENSP00000447248 (A), ENSP00000449473 (A), ENSP00000447314 (A), ENSP00000449496 (A), ENSP00000368754 (A), ENSP00000179765 (A), ENSP00000437619 (B), ENSP00000441341 (B), ENSP00000393565 (B), ENSP00000415613 (B), ENSP00000400505 (B), ENSP00000376534 (B), ENSP00000376533 (B), ENSP00000348087 (B), ENSP00000339670 (B), ENSP00000294905 (B)
BRD1	ENSG0000100425	ENST00000542442, ENST00000457780, ENST00000438393, ENST00000419212, ENST00000404760, ENST00000404034, ENST00000342989, ENST00000216267	ENSP00000437514, ENSP00000410042, ENSP00000388027, ENSP00000399110, ENSP00000385858, ENSP00000384076, ENSP00000345886, ENSP00000216267
BRD2	ENSG0000234704, ENSG0000236227, ENSG0000230678, ENSG0000204256, ENSG0000234507, ENSG0000235307, ENSG0000215077	ENST0000050598, ENST00000547286, ENST00000546777, ENST00000479699, ENST00000450320, ENST00000449118, ENST00000433783, ENST00000427021, ENST00000414731	ENSP00000447012, ENSP00000448429, ENSP00000449979, ENSP00000434155, ENSP00000413845, ENSP00000399009, ENSP00000416399, ENSP00000400737, ENSP00000391246

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BRD3	ENSG00000169925	ENST00000540795, ENST00000433041, ENST00000371842, ENST00000371834, ENST00000357885, ENST00000303407	ENSP00000442302, ENSP00000406749, ENSP00000360908, ENSP00000360900, ENSP00000350557, ENSP00000305918
BRD4	ENSG00000141867	ENST00000371835, ENST00000360016, ENST00000263377	ENSP00000360901, ENSP00000353112, ENSP00000263377
BRDT	ENSG00000137948	ENST00000552654, ENST00000548992, ENST00000539070, ENST00000457265, ENST00000450792, ENST00000449584, ENST00000448194, ENST00000440509, ENST00000427104, ENST00000426141, ENST00000423434, ENST00000402388, ENST00000399546, ENST00000394530, ENST00000370389, ENST00000362005, ENST00000355011	ENSP00000446599, ENSP00000447394, ENSP00000441862, ENSP00000408138, ENSP00000414349, ENSP00000408625, ENSP00000410587, ENSP00000416714, ENSP00000400002, ENSP00000404969, ENSP00000396351, ENSP00000384051, ENSP00000387822, ENSP00000378038, ENSP00000359416, ENSP00000354568, ENSP00000400199
BRD7	ENSG00000166164	ENST00000569774, ENST00000562383, ENST00000394689, ENST00000394688	ENSP00000461556, ENSP00000458430, ENSP00000378181, ENSP00000378180

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BRD8	ENSG00000112983	ENST00000512140, ENST00000511898, ENST00000506167, ENST00000472478, ENST00000455658, ENST00000454473, ENST00000453824, ENST00000450756, ENST00000441656, ENST00000432618, ENST00000430331, ENST00000428808, ENST00000427976, ENST00000418329, ENST00000411594, ENST00000402931, ENST00000254900, ENST00000239899, ENST00000230901	ENSP00000427475, ENSP00000426385, ENSP00000427126, ENSP00000420884, ENSP00000408396, ENSP00000398067, ENSP00000407129, ENSP00000396487, ENSP00000398084, ENSP00000398676, ENSP00000407414, ENSP00000414625, ENSP00000392646, ENSP00000398873, ENSP00000394330, ENSP00000384845, ENSP00000254900, ENSP00000239899, ENSP00000230901
BRD9	ENSG0000028310	ENST00000523139, ENST00000519112, ENST00000518251, ENST00000518250, ENST00000495265, ENST00000490814, ENST00000489816, ENST00000489093, ENST00000487688, ENST00000483173, ENST00000467963, ENST00000466684, ENST00000435709, ENST00000388890, ENST00000323547, ENST00000323510	ENSP00000430170, ENSP00000429353, ENSP00000428194, ENSP00000430510, ENSP00000420080, ENSP00000417431, ENSP00000419752, ENSP00000420722, ENSP00000420492, ENSP00000419845, ENSP00000419765, ENSP00000420397, ENSP00000402984, ENSP00000373542, ENSP00000325200, ENSP00000323557
BRPF1	ENSG00000156983	ENST00000457855, ENST00000433861, ENST00000426583, ENST00000424362, ENST00000420291, ENST00000383829, ENST00000302054	ENSP00000410210, ENSP00000402485, ENSP00000404235, ENSP00000398863, ENSP00000416728, ENSP00000373340, ENSP00000306297

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BRPF3	ENSG0000096070	ENST00000543502, ENST00000534694, ENST00000534400, ENST00000532330, ENST00000527657, ENST00000454960, ENST00000449261, ENST00000446974, ENST00000443324, ENST00000441730, ENST00000441123, ENST00000394572, ENST00000357641, ENST00000339717	ENSP00000445352, ENSP00000434501, ENSP00000436504, ENSP00000437087, ENSP00000431894, ENSP00000413655, ENSP00000416842, ENSP00000410669, ENSP00000387368, ENSP00000413022, ENSP00000411558, ENSP00000378073, ENSP00000350267, ENSP00000345419
BRWD3	ENSG0000165288	ENST00000373275	ENSP00000362372
CECR2	ENSG0000099954	ENST00000400585, ENST00000400573, ENST00000355219, ENST00000342247, ENST00000262608	ENSP00000383428, ENSP00000383417, ENSP00000347357, ENSP00000341219, ENSP00000262608
CREBBP	ENSG0000005339	ENST00000573517, ENST00000572134, ENST00000571826, ENST00000570939, ENST00000382070, ENST00000323508, ENST00000262367	ENSP00000460474, ENSP00000458254, ENSP00000459490, ENSP00000461002, ENSP00000371502, ENSP00000323550, ENSP00000262367
EP300	ENSG0000100393	ENST00000263253	ENSP00000263253
FALZ	aka BPTF: ENSG0000262858, ENSG0000171634	ENST00000544778, ENST00000544491, ENST00000424123, ENST00000342579, ENST00000335221, ENST00000321892, ENST00000306378, ENST00000576412, ENST00000575874, ENST00000574652, ENST00000574648, ENST00000573838, ENST00000573834, ENST00000571054	ENSP00000440854, ENSP00000443949, ENSP00000388405, ENSP00000343837, ENSP00000334351, ENSP00000315454, ENSP00000307208, ENSP00000461707, ENSP00000459656, ENSP00000459309, ENSP00000459251, ENSP00000458864, ENSP00000461014, ENSP00000460704

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GCN5L2	aka KAT2A: ENSG00000259958, ENSG00000108773	ENST00000564173, ENST00000225916	ENSP00000456712, ENSP00000225916
MLL	ENSG00000118058	ENST00000534358, ENST00000533790, ENST00000532204, ENST00000531904, ENST00000529852, ENST00000527869, ENST00000392873, ENST00000389507, ENST00000389506, ENST00000359313, ENST00000354520, ENST00000328469	ENSP00000436786, ENSP00000436700, ENSP00000434618, ENSP00000432391, ENSP00000436564, ENSP00000432652, ENSP00000376612, ENSP00000374158, ENSP00000374157, ENSP00000352262, ENSP00000346516, ENSP00000333556
PB1	ENSG00000163939	ENST00000458294, ENST00000450271, ENST00000449505, ENST00000446103, ENST00000439181, ENST00000431678, ENST00000424867, ENST00000423351, ENST00000420148, ENST00000412587, ENST00000410007, ENST00000409767, ENST00000409114, ENST00000409057, ENST00000394830, ENST00000356770, ENST00000337303, ENST00000296302	ENSP00000411895, ENSP00000416851, ENSP00000412401, ENSP00000397662, ENSP00000404635, ENSP00000409939, ENSP00000397399, ENSP00000387775, ENSP00000389390, ENSP00000404579, ENSP00000386529, ENSP00000386601, ENSP00000386643, ENSP00000386593, ENSP00000378307, ENSP00000349213, ENSP00000338302, ENSP00000296302
PCAF	ENSG00000114166	ENST00000263754	ENSP00000263754
PHIP	ENSG00000146247	ENST00000355098, ENST00000275034	ENSP00000347215, ENSP00000275034

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PRKCBP1	ENSG00000101040	ENST00000540497, ENST00000536340, ENST00000471951, ENST00000467200, ENST00000461685, ENST00000458360, ENST00000446994, ENST00000446894, ENST00000441977, ENST00000435836, ENST00000396281, ENST00000372023, ENST00000360911, ENST00000355972, ENST00000352431, ENST00000311275, ENST00000262975	ENSP00000443086, ENSP00000439800, ENSP00000420095, ENSP00000418495, ENSP00000418210, ENSP00000392964, ENSP00000396725, ENSP00000394379, ENSP00000393806, ENSP00000413727, ENSP00000379577, ENSP00000361093, ENSP00000354166, ENSP00000348246, ENSP00000335537, ENSP00000312237, ENSP00000262975
SMARCA2	ENSG0000080503	ENST00000457226, ENST00000452193, ENST00000450198, ENST00000439732, ENST00000423555, ENST00000417599, ENST00000416751, ENST00000382203, ENST00000382194, ENST00000382186, ENST00000382185, ENST00000382183, ENST00000382182, ENST00000357248, ENST00000349721, ENST00000324954, ENST00000302401	ENSP00000415218, ENSP00000401096, ENSP00000392081, ENSP00000409398, ENSP00000413057, ENSP00000387486, ENSP00000412242, ENSP00000371638, ENSP00000371629, ENSP00000371621, ENSP00000371620, ENSP00000371618, ENSP00000371617, ENSP00000349788, ENSP00000265773, ENSP00000324770, ENSP00000305411
SMARCA4	ENSG0000127616	ENST00000541122, ENST00000538456, ENST00000450717, ENST00000444061, ENST00000429416, ENST00000421844, ENST00000413806, ENST00000358026, ENST00000344626	ENSP00000445036, ENSP00000443848, ENSP00000397783, ENSP00000392837, ENSP00000395654, ENSP00000403803, ENSP00000414727, ENSP00000350720, ENSP00000343896

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SP100	ENSG0000067066	ENST00000452345, ENST00000432979, ENST00000431952, ENST00000427101, ENST00000414648, ENST00000413284, ENST00000409897, ENST00000409824, ENST00000409341, ENST00000409112, ENST00000341950, ENST00000340126, ENST00000264052	ENSP00000416563, ENSP00000391616, ENSP00000393679, ENSP00000399389, ENSP00000412837, ENSP00000400277, ENSP00000386998, ENSP00000387311, ENSP00000386404, ENSP00000386427, ENSP00000342729, ENSP00000343023, ENSP00000264052
SP110	ENSG00000135899	ENST00000540870, ENST00000455674, ENST00000416610, ENST00000409815, ENST00000392048, ENST00000358662, ENST00000338556, ENST00000258382, ENST00000258381	ENSP00000439558, ENSP00000393992, ENSP00000399978, ENSP00000387172, ENSP00000375902, ENSP00000351488, ENSP00000344049, ENSP00000258382, ENSP00000258381
SP140	ENSG0000079263	ENST00000537563, ENST00000486687, ENST00000420434, ENST00000417495, ENST00000392045, ENST00000392044, ENST00000373645, ENST00000350136, ENST00000343805	ENSP00000445084, ENSP00000440107, ENSP00000398210, ENSP00000393618, ENSP00000375899, ENSP00000375898, ENSP00000362749, ENSP00000345846, ENSP00000342096
TAF1	ENSG00000147133	ENST00000538124, ENST00000483985, ENST00000463163, ENST00000449580, ENST00000437147, ENST00000423759, ENST00000395779, ENST00000373790, ENST00000373775, ENST00000276072	ENSP00000441908, ENSP00000424526, ENSP00000421611, ENSP00000389000, ENSP00000406517, ENSP00000406549, ENSP00000379125, ENSP00000362895, ENSP00000362880, ENSP00000276072
TAF1L	ENSG00000122728	ENST00000242310	ENSP00000418379

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TRIM24	ENSG00000122779	ENST00000536822, ENST00000452999, ENST00000439939, ENST00000415680, ENST00000378381, ENST00000343526	ENSP00000440535, ENSP00000402079, ENSP00000403347, ENSP00000390829, ENSP00000367632, ENSP00000340507
TRIM28	ENSG00000130726	ENST00000341753, ENST00000253024	ENSP00000342232, ENSP00000253024
TRIM33	ENSG00000197323	ENST00000450349, ENST00000448034, ENST00000369543, ENST00000358465	ENSP00000412077, ENSP00000402333, ENSP00000358556, ENSP00000351250
TRIM66	ENSG00000166436	ENST00000530502, ENST00000402157, ENST00000299550	ENSP00000437234, ENSP00000384876, ENSP00000299550
WDR9	ENSG00000185658	ENST00000455867, ENST00000446924, ENST00000445668, ENST00000445245, ENST00000430093, ENST00000424441, ENST00000412604, ENST00000380800, ENST00000380783, ENST00000342449, ENST00000341322, ENST00000333229	ENSP00000389882, ENSP00000391014, ENSP00000395575, ENSP00000390684, ENSP00000393702, ENSP00000415066, ENSP00000398900, ENSP00000370178, ENSP00000370160, ENSP00000344333, ENSP00000342106, ENSP00000330753

ZMYND11	ENSG0000015171, ENSG0000260150	ENST00000545619, ENST00000535374, ENST00000509513, ENST00000439456, ENST00000403354, ENST00000402736, ENST00000397962, ENST00000397959, ENST00000397955, ENST00000381607, ENST00000381604, ENST00000381602, ENST00000381591, ENST00000381584, ENST00000309776, ENST00000568927, ENST00000568174, ENST00000565311, ENST00000564303, ENST00000563851, ENST00000562898, ENST00000562457	ENSP00000438461, ENSP00000439587, ENSP00000424205, ENSP00000397072, ENSP00000385484, ENSP00000386010, ENSP00000381053, ENSP00000381050, ENSP00000381046, ENSP00000371020, ENSP00000371017, ENSP00000371015, ENSP00000371003, ENSP00000370996, ENSP00000309992, ENSP00000458138, ENSP00000457204, ENSP00000457248, ENSP00000456325, ENSP00000456634, ENSP00000454775, ENSP00000455330
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Table 2: Bromodomain-containing proteins

In some embodiments, “inhibit”, “block”, “suppress” or “prevent” means that the activity being inhibited, blocked, suppressed, or prevented is reduced by at least 5%, 10%, 15%, 5 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% as compared to the activity of a control (e.g., activity in the absence of the inhibitor). In some embodiments, “inhibit”, “block”, “suppress” or “prevent” means that the expression of the target of the inhibitor (e.g. a bromodomain-containing protein) is reduced by at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100% as compared to a control (e.g., the expression in the absence of the inhibitor).

Notch Pathway Inhibitors

A Notch pathway inhibitor prevents or inhibits, in part or in whole, the activity of components of the Notch pathway. It is to be understood that the activity of components of the 15 Notch pathway may include one or more activities, such as cell fate specification, differentiation, proliferation, apoptosis, adhesion, epithelial-mesenchymal transition, migration, or angiogenesis. In some embodiments, a Notch pathway inhibitor suppresses or inhibits a Notch pathway activation mutation. Examples of Notch pathway activation mutations are

described below. Notch pathway inhibitors are known in the art. In some embodiments, a Notch pathway inhibitor is a gamma secretase inhibitor (GSI). Gamma secretase is a multi-subunit protease complex that cleaves Notch. This cleavage releases Notch from the cell membrane, allowing Notch to enter the nucleus and modify gene expression. Examples of 5 gamma secretase inhibitors include, but are not limited to, DBZ (Axon Medchem, Cat. No. 1488), BMS-906024 (Bristol-Myers Squibb), RO4929097 (Roche/Genentech), LY450139 (Eli Lilly), BMS-708163 (Bristol-Myers Squibb), MK-0752 (University of Michigan), PF-03084014 (Pfizer), IL-X (also referred to as cbz-IL-CHO, Calbiochem), z-Leu-leu-Nle-CHO (EMD Millipore), N-[N-(3,5-difluorophenacetyl)-L-alanyl]-S-phenylglycine t-butyl ester (DAPT), 10 BH589 (Panobinostat, Novartis), MEDI0639 (MedImmune LLC), Choline magnesium trisalicylate (e.g., Trilisate), and Curcumin (a curcuminoid of turmeric).

Other Notch pathway inhibitors include antibodies and antibody fragments. Examples include monoclonal antibodies against extracellular Notch receptors (developed by Genentech and described by Wu et al. Nature 2010.). Another example is a stapled peptide inhibitor of the 15 intracellular Notch transcriptional complex (SAHM1) described by Moellering et al. Nature, 2009 (being developed by Aileron Therapeutics).

In some embodiments, a Notch pathway inhibitor is any molecule or compound that reduces or prevents (mRNA or protein) expression of any component of the Notch pathway (e.g. Notch, Notch ligands, downstream effectors, and the like). Examples of such inhibitors include 20 siRNA, shRNA, dsRNA, oligomimics, and proteases that target one or more components of the Notch pathway. Components of the Notch pathway include, but are not limited to, those in Table 3.

Gene	Ensembl gene ID	Ensembl transcript ID	Ensembl protein ID
ADAM17	ENSG00000151694	ENST00000310823, ENST00000497134, ENST00000538558, ENST00000478059	ENSP00000309968, ENSP00000418728, ENSP00000439780
AKT1	ENSG00000142208	ENST00000349310,ENS T00000407796,ENST00 000402615,ENST00000 554848,ENST00000554 581,ENST00000555528 ,ENST00000544168,EN ST00000554192,ENST0 0000555380,ENST0000 0555926,ENST0000055	ENSP00000270202,E NSP00000384293,EN SP00000385326,ENS P00000451166,ENSP 00000451828,ENSPO 0000450688,ENSP00 00443897,ENSP000 00450681,ENSP0000 0451290,ENSP00000

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		5458	451824,ENSP000004 51470
APH1A	ENSG00000117362	ENST00000360244,ENS T00000369109,ENST00 00236017,ENST00000 414276	ENSP00000353380,E NSP00000358105,EN SP00000236017,ENS P00000397473
APH1B	ENSG00000138613	ENST00000261879,ENS T00000380343,ENST00 00560353,ENST00000 560890,ENST00000380 340,ENST00000559971	ENSP00000261879,E NSP00000369700,EN SP00000453327,ENS P00000453002,ENSP 00000369697,ENSP0 000453516
CDKN1A	ENSG00000124762	ENST00000244741,ENS T00000373711,ENST00 00405375,ENST00000 448526	ENSP00000244741,E NSP00000362815,EN SP00000384849,ENS P00000409259
CIR1	ENSG00000138433	ENST00000342016,ENS T00000377973,ENST00 00414336,ENST00000 362053,ENST00000425 101	ENSP00000339723,E NSP00000367211,EN SP00000395036,ENS P00000355034,ENSP 00000405693
CUL1	ENSG00000055130	ENST00000409469,ENS T00000325222,ENST00 00433865,ENST00000 543583	ENSP00000387160,E NSP00000326804,EN SP00000396011,ENS P00000441340
DLL1	ENSG00000198719	ENST00000366756	ENSP00000355718
DLL3	ENSG00000090932	ENST00000205143, ENST00000356433	ENSP00000205143, ENSP00000348810
DLL4	ENSG00000128917	ENST00000249749	ENSP00000249749
DTX1	ENSG00000135144	ENST00000257600	ENSP00000257600
EP300	ENSG00000100393	ENST00000263253	ENSP00000263253
FBXW7	ENSG00000109670	ENST00000263981,ENS T00000281708,ENST00 00296555,ENST00000 393956	ENSP00000263981,E NSP00000281708,EN SP00000296555,ENS P00000377528

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FHL1	ENSG0000022267	ENST00000370690,ENS T00000345434,ENST00 00370676,ENST00000 370683,ENST00000420 362,ENST00000370674 ,ENST00000452016,EN ST00000434885,ENST0 0000458357,ENST0000 0456445,ENST0000044 9474,ENST0000039415 3,ENST00000394155,E NST00000456218,ENST 00000535737,ENST000 00536581,ENST000005 39015,ENST000005427 04,ENST00000543669	ENSP00000359724,E NSP0000071281,EN SP00000359710,ENS P00000359717,ENSP 00000391779,ENSP0 0000359708,ENSP00 000408038,ENSP000 00413798,ENSP0000 0389920,ENSP00000 412642,ENSP000004 14604,ENSP0000037 7709,ENSP00000377 710,ENSP000003928 13,ENSP0000044481 5,ENSP00000445335, ENSP00000437673,E NSP00000446441,EN SP00000443333
GATA3	ENSG0000107485	ENST00000379328,ENS T00000346208,ENST00 000544011	ENSP00000368632,E NSP00000341619,EN SP00000439641
GSK3B	ENSG0000082701	ENST00000264235,ENS T00000316626,ENST00 000539838	ENSP00000264235,E NSP00000324806,EN SP00000437981
HAT1	ENSG0000128708	ENST00000264108,ENS T00000392584,ENST00 000412731,ENST00000 457761	ENSP00000264108,E NSP00000376363,EN SP00000407921,ENS P00000403466
HDAC1	ENSG0000116478	ENST00000373548, ENST00000428704, ENST00000373541	ENSP00000362649, ENSP00000407859, ENSP00000362642
HDAC10	ENSG0000100429	ENST00000216271,ENS T00000448072,ENST00 000349505,ENST00000 415993,ENST00000429 374,ENST00000454936	ENSP00000216271,E NSP00000397542,EN SP00000343540,ENS P00000397517,ENSP 00000407640,ENSP0 000406150
HDAC11	ENSG0000163517	ENST00000295757,ENS T00000433119,ENST00 000402259,ENST00000 402271,ENST00000404 040,ENST00000404548 ,ENST00000405025,EN ST00000405478,ENST0 0000458642,ENST0000 0418189,ENST0000043 4848,ENST0000041624 8,ENST00000455904,E NST00000437379,ENST	ENSP00000295757,E NSP00000412514,EN SP00000384706,ENS P00000384123,ENSP 00000385475,ENSP0 000385528,ENSP00 00384019,ENSP000 00385252,ENSP0000 0405403,ENSP00000 411792,ENSP000003 98651,ENSP0000040 2298,ENSP00000396

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		00000522202,ENST0000446613,ENST00000425430	122,ENSP00000395188,ENSP00000429794,ENSP00000401487,ENSP00000399792
HDAC2	ENSG00000196591	ENST00000519065,ENST0000425835,ENST0000368632,ENST00000519108,ENST00000518690,ENST00000523240,ENST00000521610,ENST00000524334,ENST0000520895,ENST00000523628,ENST00000522371,ENST00000521163,ENST00000398283	ENSP00000430432,ENSP00000417026,ENSP00000357621,ENSP00000430008,ENSP00000428653,ENSP0000429236,ENSP0000429901,ENSP0000428989,ENSP0000428861,ENSP00000427861,ENSP00000428599,ENSP00000428024,ENSP00000381331
HDAC3	ENSG00000171720	ENST00000305264,ENST0000523088,ENST00000523353,ENST00000519474	ENSP00000302967,ENSP00000429099,ENSP00000430667,ENSP00000430782
HDAC4	ENSG00000068024	ENST00000345617,ENST00000446876,ENST0000454542,ENST00000445704,ENST00000430200,ENST00000544989,ENST00000393621,ENST00000456922,ENST00000541256,ENST00000543185	ENSP00000264606,ENSP00000392912,ENSP00000405226,ENSP00000391226,ENSP00000410551,ENSP0000438111,ENSP0000377243,ENSP0000406618,ENSP00000443057,ENSP00000440481
HDAC5	ENSG00000108840	ENST00000225983,ENST00000336057,ENST00000393622	ENSP00000225983,ENSP00000337290,ENSP00000377244
HDAC6	ENSG00000094631	ENST00000334136,ENST00000376643,ENST0000426196,ENST00000430858,ENST0000037619,ENST00000423941,ENST00000438518,ENST00000376610,ENST00000441703,ENST00000443563,ENST0000044	ENSP00000334061,ENSP00000365831,ENSP00000402189,ENSP00000397697,ENSP00000365804,ENSP0000392815,ENSP0000403370,ENSP00000365795,ENSP00000393916,ENSP00000

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		0653,ENST0000041316 3,ENST00000436813,E NST00000444343	402751,ENSP000003 94377,ENSP0000039 8801,ENSP00000405 449,ENSP000003985 66
HDAC7	ENSG0000061273	ENST0000080059,ENS T00000354334,ENST00 000417107,ENST00000 450805,ENST00000433 685,ENST00000447463 ,ENST00000427332,EN ST00000434070,ENST0 0000445237,ENST0000 0421231,ENST0000041 7902,ENST0000043067 0,ENST00000440293,E NST00000422254,ENST 00000552960,ENST000 00380610,ENST000005 48080,ENST000005489 38,ENST00000547259,E NST00000425451,ENST 00000485796,ENST000 00551602,ENST000004 77203	ENSP0000080059,E NSP00000351326,EN SP00000387792,ENS P00000397236,ENSP 0000403149,ENSP00 000389501,ENSP00 00404394,ENSP000 00388561,ENSP0000 0390415,ENSP00000 412155,ENSP000004 00811,ENSP0000039 6159,ENSP00000411 058,ENSP000004100 68,ENSP0000044853 2,ENSP00000369984, ENSP00000446538,E NSP00000448305,EN SP00000447191,ENS P00000401872,ENSP 0000448448,ENSP00 000449193,ENSP00 00449171
HDAC8	ENSG0000147099	ENST00000373573,ENS T00000439122,ENST00 000373556,ENST00000 373571,ENST00000373 554,ENST00000373568 ,ENST00000373560,EN ST00000373559,ENST0 000421523,ENST0000 0373583,ENST0000041 5409,ENST0000037356 1,ENST00000373589,E NST00000429103,ENST 00000412342,ENST000 00444609,ENST000004 36675	ENSP00000362674,E NSP00000414486,EN SP00000362657,ENS P00000362672,ENSP 00000362655,ENSP00 000362669,ENSP00 000362661,ENSP000 00362660,ENSP0000 0398997,ENSP00000 362685,ENSP000003 96424,ENSP0000036 2662,ENSP00000362 691,ENSP000003884 59,ENSP0000040018 0,ENSP00000409778, ENSP00000416489

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HDAC9	ENSG0000048052	ENST00000406451,ENS T00000405010,ENST00 00406072,ENST00000 417496,ENST00000433 709,ENST00000413509 ,ENST00000430454,EN ST00000413380,ENST0 0000441986,ENST0000 0456174,ENST0000040 1921,ENST0000044154 2,ENST00000524023,E NST00000432645,ENST 0000428307,ENST000 00262069,ENST000003 41009,ENST000004466 46	ENSP00000384657,E NSP00000384382,EN SP00000384017,ENS P00000401669,ENSP 00000409003,ENSP0 000412497,ENSP00 000411422,ENSP000 00392564,ENSP0000 0404763,ENSP00000 388568,ENSP000003 83912,ENSP0000040 8617,ENSP00000430 036,ENSP000004103 37,ENSP0000039565 5,ENSP00000262069, ENSP00000339165,E NSP00000415095
HES1	ENSG00000114315	ENST00000232424	ENSP00000232424
HES5	ENSG00000197921	ENST00000378453	ENSP00000367714
HES6	ENSG00000144485	ENST00000272937,ENS T00000409002,ENST00 00409160,ENST00000 436051,ENST00000409 574,ENST00000409182 ,ENST00000409356,EN ST00000450098,ENST0 000417803	ENSP00000272937,E NSP00000387155,EN SP00000387215,ENS P00000392596,ENSP 00000387008,ENSP0 000387343,ENSP00 000387107,ENSP000 00390870,ENSP0000 0401797
HEY1	ENSG00000164683	ENST00000354724,ENS T00000523976,ENST00 000518733,ENST00000 337919,ENST00000542 205	ENSP00000346761,E NSP00000429792,EN SP00000429705,ENS P00000338272,ENSP 00000445025
HEY2	ENSG00000135547	ENST00000368364, ENST00000368365	ENSP00000357348, ENSP00000357349
HIF1A	ENSG00000100644	ENST00000337138,ENS T00000323441,ENST00 00394997,ENST00000 557538,ENST00000394 988,ENST00000539097 ,ENST00000539494	ENSP00000338018,E NSP00000323326,EN SP00000378446,ENS P00000451696,ENSP 00000378439,ENSP0 0000437955,ENSP00 000446436
ITCH	ENSG00000078747	ENST00000374864,ENS T00000262650,ENST00 000535650	ENSP00000363998,E NSP00000262650,EN SP00000445608

JAG1	ENSG00000101384	ENST00000254958, ENST00000423891	ENSP00000254958, ENSP00000389519
JAG2	ENSG00000184916	ENST00000331782, ENST00000347004	ENSP00000328169, ENSP00000328566
JAK2	ENSG00000096968	ENST00000381652,ENS T00000539801,ENST00 00544510	ENSP00000371067,E NSP00000440387,EN SP00000443103
LCK	ENSG00000182866	ENST00000336890,ENS T00000482949,ENST00 00333070,ENST00000 495610,ENST00000373 557,ENST00000477031 ,ENST00000461712,EN ST00000373562,ENST0 0000373564,ENST0000 0398345,ENST0000043 6824	ENSP00000337825,E NSP00000431517,EN SP00000328213,ENS P00000435605,ENSP 00000362658,ENSP0 000436554,ENSP00 00434525,ENSP000 0362663,ENSP0000 0362665,ENSP00000 381387,ENSP000004 0092
LFNG	ENSG00000106003	ENST00000222725,ENS T00000359574,ENST00 00402506,ENST00000 402045,ENST00000338 732	ENSP00000222725,E NSP00000352579,EN SP00000385764,ENS P00000384786,ENSP 00000343095
MAGEA1	ENSG00000198681	ENST00000356661	ENSP00000349085
MAML1	ENSG00000161021	ENST00000292599, ENST00000376951	ENSP00000292599, ENSP00000366150
MAML2	ENSG00000184384	ENST00000524717, ENST00000440572	ENSP00000434552, ENSP00000412394
MAML3	ENSG00000196782	ENST00000509479,ENS T00000502696,ENST00 00327122,ENST00000 398940,ENST00000538 400	ENSP00000421180,E NSP00000422783,EN SP00000313316,ENS P00000381913,ENSP 00000444397
MFNG	ENSG00000100060	ENST00000356998,ENS T00000442496,ENST00 00436341,ENST00000 424765,ENST00000454 291,ENST00000416983 ,ENST00000450946,EN ST00000430411,ENST0 000438891	ENSP00000349490,E NSP00000389274,EN SP00000394081,ENS P00000407110,ENSP 00000407094,ENSP0 000413855,ENSP00 00396605,ENSP000 00414342,ENSP0000 0414222
MTOR	ENSG00000198793	ENST00000361445,ENS T00000376838,ENST00 00455339,ENST00000 539766	ENSP00000354558,E NSP00000366034,EN SP00000398745,ENS P00000440730

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MYC	ENSG00000136997	ENST00000377970,ENS T00000259523,ENST00 00517291,ENST00000 524013,ENST00000520 751,ENST00000454617	ENSP00000367207,E NSP00000259523,EN SP00000429441,ENS P00000430235,ENSP 0000430226,ENSP0 000405312
NCOR1	ENSG00000141027	ENST00000268712,ENS T00000436828,ENST00 00395851,ENST00000 395849,ENST00000436 068,ENST00000395848 ,ENST00000411510,EN ST00000430577,ENST0 000395857,ENST0000 0458113	ENSP00000268712,E NSP00000387727,EN SP00000379192,ENS P00000379190,ENSP 0000389839,ENSP0 000379189,ENSP00 00407998,ENSP000 00410784,ENSP0000 0379198,ENSP00000 395091
NCOR2	ENSG00000196498	ENST00000429285,ENS T00000404621,ENST00 00458234,ENST00000 420698,ENST00000405 201,ENST00000448614 ,ENST00000453428,EN ST00000440187,ENST0 000440337,ENST0000 0418829,ENST0000041 3172,ENST0000044800 8,ENST00000443451,E NST00000542927,ENST 00000356219,ENST000 00397355,ENST000004 04121,ENST000004470 11,ENST00000447675	ENSP00000400281,E NSP00000384202,EN SP00000402808,ENS P00000405367,ENSP 0000384018,ENSP0 000408247,ENSP00 00400687,ENSP000 00396044,ENSP0000 0398963,ENSP00000 391389,ENSP000004 07357,ENSP0000040 3034,ENSP00000405 246,ENSP000004436 89,ENSP0000034855 1,ENSP00000380513, ENSP00000385618,E NSP00000396746,EN SP00000401058
NCSTN	ENSG00000162736	ENST00000294785,ENS T00000368063,ENST00 00438008,ENST00000 421914,ENST00000437 169,ENST00000424645 ,ENST00000435149,EN ST00000424754,ENST0 000368065,ENST00000 0368067,ENST0000039 2212,ENST0000053585 7	ENSP00000294785,E NSP00000357042,EN SP00000389370,ENS P00000390409,ENSP 0000415442,ENSP0 000388118,ENSP00 00407849,ENSP000 00410124,ENSP0000 0357044,ENSP00000 357046,ENSP000003 76047,ENSP0000044 2605

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NFKB1	ENSG00000109320	ENST00000226574, ENS T00000394820, ENST00 00505458, ENST00000 507079, ENST00000511 926, ENST00000509165 , ENST00000508584	ENSP00000226574, E NSP00000378297, EN SP00000424790, ENS P00000426147, ENSP 00000420904, ENSP0 0000423877, ENSP00 00424815
NOTCH1	ENSG00000148400	ENST00000277541	ENSP00000277541
NOTCH2	ENSG00000134250	ENST00000256646, ENS T00000369342, ENST00 000401649, ENST00000 538680, ENST00000539 617	ENSP00000256646, E NSP00000358348, EN SP00000384752, ENS P00000439516, ENSP 00000438937
NOTCH3	ENSG00000074181	ENST00000263388, ENST00000539383	ENSP00000263388, ENSP00000446150
NOTCH4	ENSG00000204301	ENST00000375023, ENST00000443903	ENSP00000364163, ENSP00000398123
NUMB	ENSG00000133961	ENST00000554546, ENS T00000555394, ENST00 000557597, ENST00000 555238, ENST00000356 296, ENST00000556772 , ENST00000559312, EN ST00000554521, ENST0 000560335, ENST0000 0555738, ENST0000055 4818, ENST0000055530 7, ENST00000555987, E NST00000555859, ENST 00000554394, ENST000 00326018, ENST000003 55058, ENST000003595 60, ENST00000454166, E NST00000535282, ENST 00000544991	ENSP00000452416, E NSP00000451625, EN SP00000451117, ENS P00000451300, ENSP 00000348644, ENSP0 000451513, ENSP00 00452888, ENSP000 00450817, ENSP0000 0453209, ENSP00000 452069, ENSP0000045 1959, ENSP0000045 2357, ENSP00000451 559, ENSP000004513 26, ENSP0000045137 4, ENSP00000315193, ENSP00000347169, E NSP00000352563, EN SP00000394025, ENS P00000441258, ENSP 00000446001
NUMBL	ENSG00000105245	ENST00000252891, ENST00000540131	ENSP00000252891, ENSP00000442759
PSENEN	ENSG00000205155	ENST00000222266	ENSP00000222266

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PSEN1	ENSG0000080815	ENST00000324501,ENS T00000357710,ENST00 000394164,ENST00000 394157,ENST00000406 768,ENST00000556864 ,ENST00000557037,EN ST00000556533,ENST0 0000556066,ENST0000 0553599,ENST0000055 7356,ENST0000055695 1,ENST00000557293,E NST00000553719,ENST 00000554131,ENST000 00555254,ENST000005 56011,ENST000005575 11,ENST00000560005,E NST00000261970,ENST 00000344094,ENST000 00555386,ENST000005 53855,ENST000005593 61	ENSP00000326366,E NSP00000350342,EN SP00000377719,ENS P00000377712,ENSP 00000385948,ENSP0 000451588,ENSP00 000451347,ENSP000 00452128,ENSP0000 0452267,ENSP00000 452477,ENSP000004 51498,ENSP0000045 0551,ENSP00000451 880,ENSP000004516 74,ENSP0000045191 5,ENSP00000450652, ENSP00000451662,E NSP00000451429,EN SP00000453466,ENS P00000261970,ENSP 00000339523,ENSP0 000450845,ENSP00 00452242,ENSP000 00454156
PSEN2	ENSG0000143801	ENST00000366783,ENS T00000366782,ENST00 000495488,ENST00000 460775,ENST00000472 139,ENST00000422240 ,ENST00000524196,EN ST00000340188,ENST0 0000391872,ENST0000 0496965	ENSP00000355747,E NSP00000355746,EN SP00000429682,ENS P00000427912,ENSP 00000427806,ENSP0 000403737,ENSP00 000429036,ENSP000 00339860,ENSP0000 0375745,ENSP00000 430647
PTCRA	ENSG0000171611	ENST00000304672,ENS T00000418903,ENST00 000441198,ENST00000 446507	ENSP00000304447,E NSP00000407061,EN SP00000409550,ENS P00000392288

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RBPJ	ENSG00000168214	ENST00000345843,ENS T00000361572,ENST00 000342320,ENST00000 512351,ENST00000512 671,ENST00000505958 ,ENST00000507561,EN ST00000504907,ENST0 0000506956,ENST0000 0514807,ENST0000050 9158,ENST0000051473 0,ENST00000507574,E NST00000514675,ENST 0000515573,ENST000 00511546,ENST000005 04938,ENST000005044 23,ENST00000510778,E NST00000348160,ENST 00000342295,ENST000 00355476,ENST000005 13182,,ENST00000510 778,ENST00000348160 ,ENST00000342295,EN ST00000355476,ENST0 0000513182	ENSP00000305815,E NSP00000354528,EN SP00000340124,ENS P00000424789,ENSP 00000423644,ENSP0 0000426872,ENSP00 000423907,ENSP000 00423703,ENSP0000 0425750,ENSP00000 424989,ENSP000004 24804,ENSP0000042 5061,ENSP00000422 617,ENSP000004235 75,ENSP0000042340 6,ENSP00000422838, ENSP00000424459,E NSP00000421804,EN SP00000427170,ENS P00000339699,ENSP 00000345206,ENSP0 000347659,ENSP00 000427344,,ENSP000 00427170,ENSP0000 0339699,ENSP00000 345206,ENSP000003 47659,ENSP0000042 7344
RFNG	ENSG00000169733	ENST00000310496, ENST00000429557	ENSP00000307971, ENSP00000402931
RING1	ENSG00000204227	ENST00000374656	ENSP00000363787
SKP1	ENSG00000113558	ENST00000353411,ENS T00000522552,ENST00 000519321,ENST00000 517625,ENST00000522 855,ENST00000520417 ,ENST00000523359,EN ST00000328392,ENST0 0000521216,ENST0000 0519718,ENST0000052 3966,ENST0000051905 4	ENSP00000231487,E NSP00000429472,EN SP00000429415,ENS P00000429961,ENSP 00000429686,ENSP0 000429996,ENSP00 000428962,ENSP000 00331708,ENSP0000 0431067,ENSP00000 430774,ENSP000004 29995,ENSP0000043 0885
SNW1	ENSG00000100603	ENST00000261531,ENS T00000555761,ENST00 000554775,ENST00000 554324,ENST00000416 259,ENST00000556428	ENSP00000261531,E NSP00000451129,EN SP00000452059,ENS P00000452473,ENSP 00000387847,ENSP0

			0000451741
STAT3	ENSG00000168610	ENST00000264657, ENS T00000404395, ENST00 00389272	ENSP00000264657, E NSP00000384943, EN SP00000373923
TLE1	ENSG00000196781	ENST00000376499, ENS T00000418319, ENST00 000376484, ENST00000 376463, ENST00000355 002, ENST00000376472	ENSP00000365682, E NSP00000391347, EN SP00000365667, ENS P00000365646, ENSP 00000347102, ENSP0 000365655

Table 3: Notch pathway components***Bcl-2 Inhibitors***

As shown in the Examples, Bcl-2 expression is inhibited in the presence of the 5 bromodomain inhibitor (JQ1). Accordingly, certain aspects of the invention contemplate treatment using Bcl-2 inhibitors alone or in combination with Notch pathway inhibitors to treat certain cancers.

Members of the Bcl-2 family control the integrity of the outer mitochondrial membrane (OMM) and thus are involved in determining the susceptibility of cells to apoptosis induced by 10 the intrinsic pathway. The Bcl-2 family comprises anti-apoptotic members, such as Bcl-2, Mcl-1, Bcl-XL, Bcl-w and Bcl-2A1 (Bfl-1/A1), multidomain proapoptotic members, such as Bax and Bak, and proapoptotic BH3-only proteins, including Bad, Bim, Puma, Bid, Bik, Noxa and Bmf.

A Bcl-2 inhibitor is any molecule or compound that can prevent or inhibit the activity, in part or in whole, of Bcl-2 family members (e.g., Bcl-2, Bcl-X, Bcl-w, Mcl-1 or Bcl-2A1). It is 15 to be understood that the activity of Bcl-2 family members may include one or more activities, such as cell survival or apoptosis. Inhibitors of Bcl-2 family members are known in the art.

Examples of Bcl-2 inhibitors include, but are not limited to, HA14-1(Tocris Bioscience), BH3I-1 (Sigma-Aldrich), antimycin A (Sigma-Aldrich), chelerythrine (Fermentek), gossypol (NSC19048, NCI-Developmental Therapeutics Program), apogossypol (NSC736630, NCI-20 Developmental Therapeutics Program), TW-37 (Selleckchem), 4-(3-methoxy-phenylsulfonyl)-7-nitro-benzofurazan-3-oxide (MNB), TM12-06, obatoclax (GX15-070, Cephalon), ABT-737 (Selleckchem) and a related orally-active derivative, ABT-263 (Navitoclax, Genentech), AT-101

(Ascenta Therapeutics), pyrogallol-based molecules (Tang et al. J Med Chem. 2007, 50(8):1723-6), and ABT-199 (Abbott and Genentech).

In some embodiments, a Bcl-2 inhibitor is any molecule or compound that reduces or prevents expression of Bcl-2 family members. Examples of such inhibitors include siRNA, shRNA, dsRNA, oligomimics, and proteases that target one or more Bcl-2 family members. In 5 some embodiments, the Bcl-2 inhibitor is the antisense oligonucleotide drug Genasense (G3139, Genta).

Examples of Bcl-2 family members are shown in Table 4.

Gene	Ensembl gene ID	Ensembl transcript ID	Ensembl protein ID
Bcl-2	ENSG00000171791	ENST00000444484, ENST00000398117, ENST00000333681	ENSP00000404214, ENSP00000381185, ENSP00000329623
Bcl-X	ENSG00000171552	ENST00000456404, ENST00000450273, ENST00000439267, ENST00000434194, ENST00000422920, ENST00000420653, ENST00000420488, ENST00000376062, ENST00000376055, ENST00000307677	ENSP00000395545, ENSP00000406203, ENSP00000389688, ENSP00000401173, ENSP00000411252, ENSP00000405563, ENSP00000390760, ENSP00000365230, ENSP00000365223, ENSP00000302564
Bcl-w	ENSG00000129473	ENST00000557579, ENST00000557236, ENST00000556599, ENST00000554635, ENST00000553824, ENST00000250405	ENSP00000452265, ENSP00000451701, ENSP00000451197, ENSP00000451234, ENSP00000451148, ENSP00000250405
Mcl-1	ENSG00000143384	ENST00000439749, ENST00000369026, ENST00000307940	ENSP00000411395, ENSP00000358022, ENSP00000309973
Bcl-2A1	ENSG00000140379	ENST00000335661, ENST00000267953	ENSP00000335250, ENSP00000267953

Table 4: Examples of Bcl-2 family members

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AKT and mTOR inhibitors

As shown in the Examples, persister cells were found to be sensitive to treatment with an AKT inhibitor or treatment with a mammalian target of rapamycin (mTOR) inhibitor.

Accordingly, certain aspects of the invention contemplate treatment using mTOR or AKT 15 inhibitors alone or in combination with Notch pathway inhibitors to treat certain cancers.

An mTOR inhibitor is any molecule or compound that can prevent or inhibit, in part or in whole, the activity of mTOR. It is to be understood that the activity of mTOR may include one or more activities, such as cell growth, cell proliferation, cell motility, cell survival, protein synthesis, or transcription. mTOR gene, mRNA, and protein sequence identifiers are provided 5 in Table 5. Inhibitors of mTOR are known in the art. Examples of mTOR inhibitors include, but are not limited to, rapamycin (sirolimus, Pfizer), temsirolimus (CCI-779, Wyeth), everolimus (Novartis), deforolimus (AP23573, MK-8669, Merck and ARIAD Pharmaceuticals), NVP-BEZ235 (Novartis), BGT226 (Novartis), SF1126 (Semafore Pharmaceuticals), PKI-587 (Selleckchem), PF-04691502 (Selleckchem), INK128 (Intellikine), 10 AZD8055 (Selleckchem), and AZD2014 (AstraZeneca).

In some embodiments, an mTOR inhibitor is any molecule or compound that reduces or prevents expression of mTOR. Examples of such inhibitors include siRNA, shRNA, dsRNA, and oligomimics with complementarity to mTOR mRNA, and proteases that target mTOR protein.

15 An AKT inhibitor is any molecule or compound that can prevent or inhibit, in part or in whole, the activity of an AKT family member (AKT1, AKT2, or AKT3). It is to be understood that the activity of AKT may include one or more activities, such as glucose metabolism, apoptosis, cell proliferation, transcription and cell migration. AKT family member gene, mRNA, and protein sequence identifiers are provided in Table 5. Inhibitors of AKT are known 20 in the art. Examples of AKT inhibitors include, but are not limited to, MK-2206 (Selleckchem), GDC-0068 (Genetech), Perifosine (Selleckchem), GSK690693 (Selleckchem), AT7867 (Selleckchem), CCT128930 (Selleckchem), PF-04691502 (Selleckchem), INK128 (Selleckchem), RX-0201 (Rexahn Pharmaceuticals), PBI-05204 (Phoenix Biotechnology, Inc.), GSK2141795 (GlaxoSmithKline), Erucylphosphocholine (ErPC, AEterna Zentaris Inc.), and 25 XL-418 (Exelixis).

In some embodiments, an AKT inhibitor is any molecule or compound that reduces or prevents expression of an AKT family member (AKT1, AKT2, or AKT3). Examples of such inhibitors include siRNA, shRNA, dsRNA, oligomimics, and proteases that target AKT.

Gene	Ensembl gene ID	Ensembl transcript ID	Ensembl protein ID
FRAP1 /mTOR	ENSG00000198793	ENST00000361445, ENST00000376838, ENST00000455339,	ENSP00000354558, ENSP00000366034, ENSP00000398745

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		ENST00000495435, ENST00000476768, ENST00000490931, ENST00000473471	
AKT1	ENSG00000142208	ENST00000349310, ENST00000407796, ENST00000402615, ENST00000554848, ENST00000554581, ENST00000555528, ENST00000544168, ENST00000554192, ENST00000555380, ENST00000555926, ENST00000555458, ENST00000553797, ENST00000557494, ENST00000554585, ENST00000557552, ENST00000553506, ENST00000556836, ENST00000554826	ENSP00000270202, ENSP00000384293, ENSP00000385326, ENSP00000451166, ENSP00000451828, ENSP00000450688, ENSP00000443897, ENSP00000450681, ENSP00000451290, ENSP00000451824, ENSP00000451470
AKT2	ENSG00000105221	ENST00000392038, ENST00000452077, ENST00000456441, ENST00000423127, ENST00000416994, ENST00000416362, ENST00000427375, ENST00000441941, ENST00000392037, ENST00000497948, ENST00000579047, ENST00000311278, ENST00000578123, ENST00000583859, ENST00000580747, ENST00000486368, ENST00000578615, ENST00000578310, ENST00000476247, ENST00000358335, ENST00000596634, ENST00000424901, ENST00000492463, ENST00000489375, ENST00000584288, ENST00000391844, ENST00000491778, ENST00000601166,	ENSP00000375892, ENSP00000404083, ENSP00000396532, ENSP00000403842, ENSP00000392458, ENSP00000407999, ENSP00000403890, ENSP00000396968, ENSP00000375891, ENSP00000472382, ENSP00000471369, ENSP00000309428, ENSP00000462022, ENSP00000462715, ENSP00000463806, ENSP00000463686, ENSP00000463262, ENSP00000462919, ENSP00000463368, ENSP00000351095, ENSP00000470604, ENSP00000399532, ENSP00000462776, ENSP00000470822, ENSP00000462469, ENSP00000375719, ENSP00000463086, ENSP00000472371

		ENST00000498350, ENST00000391845, ENST00000486647, ENST00000581582, ENST00000480878, ENST00000476266, ENST00000483166, ENST00000487537, ENST00000537834, ENST00000496089, ENST00000580878, ENST00000579345, ENST00000578282, ENST00000578975	
AKT3	ENSG00000117020	ENST00000366539, ENST00000336199, ENST00000366540, ENST00000552631, ENST00000263826, ENST00000463991, ENST00000490018, ENST00000491219, ENST00000492957, ENST00000550388	ENSP00000355497, ENSP00000336943, ENSP00000355498, ENSP00000447820, ENSP00000263826

Table 5: mTOR and AKT family members***Treatment Methods***

The invention provides methods of treatment of a patient having cancer. In some 5 embodiments, the patient is identified as one who has cancer associated with or characterized by a Notch pathway activation mutation. The methods may comprise administration of one or more BRD inhibitors in the absence of a second therapy.

Other methods of the invention comprise administration of a first inhibitor and a second inhibitor. The designation of “first” and “second” inhibitors is used to distinguish between the 10 two and is not intended to refer to a temporal order of administration of the inhibitors.

The first inhibitor may be a bromodomain inhibitor. The bromodomain inhibitor may target the gene, mRNA expression, protein expression, and/or activity of any member of the bromodomain family, in all instances reducing the level and/or activity of one or more bromodomain-containing proteins (e.g., Brd1, Brd2, Brd3, Brd4, Brd7, or BrdT). Inhibitors may 15 be nucleic acids such as DNA and RNA aptamers, antisense oligonucleotides, siRNA and shRNA, small peptides, antibodies or antibody fragments, and small molecules such as small

chemical compounds. The bromodomain inhibitor may be a pan-bromodomain inhibitor or a selective bromodomain inhibitor.

In some embodiments, the first inhibitor may be a BET inhibitor. The BET inhibitor may target the gene, mRNA expression, protein expression, and/or activity of any member of the 5 BET family, in all instances reducing the level and/or activity of one or more BET (e.g., Brd1, Brd2, Brd3, Brd4, Brd7, or BrdT.). Inhibitors may be nucleic acids such as DNA and RNA aptamers, antisense oligonucleotides, siRNA and shRNA, small peptides, antibodies or antibody fragments, and small molecules such as small chemical compounds.

The BET inhibitor may be a pan-BET inhibitor or a selective BET inhibitor. BET 10 inhibitors include but are not limited to, RVX-208, PFI-1, OTX015, GSK525762A, JQ1 and Formulas I-XXII and any other compounds as outlined in herein. In some embodiments, the BET inhibitor is JQ1.

The first inhibitor may be a Bcl-2 inhibitor. The Bcl-2 inhibitor may target the gene, mRNA expression, protein expression, and/or activity of any member of the Bcl-2 family, in all 15 instances reducing the level and/or activity of one or more Bcl-2 family member (e.g. Bcl-2, Bcl-X, Bcl-w, Mcl-1 or Bcl-2A1). Inhibitors may be nucleic acids such as DNA and RNA aptamers, antisense oligonucleotides, siRNA and shRNA, small peptides, antibodies or antibody fragments, and small molecules such as small chemical compounds.

The Bcl-2 inhibitor may be a pan- Bcl-2 inhibitor or a selective Bcl-2 inhibitor. In some 20 embodiments, the Bcl-2 inhibitor selectively inhibits one or more of: Bcl-2, Bcl-X, Bcl-w, Mcl-1 or Bcl-2A1. Examples of pan- and selective-Bcl-2 inhibitors include but are not limited to HA14-1, BH3I-1, antimycin A, chelerythrine, gossypol (NSC19048), apogossypol (NSC736630), TW-37, 4-(3-methoxy-phenylsulfonyl)-7-nitro-benzofurazan-3-oxide (MNB), TM12-06, obatoclax (GX15-070), ABT-737, ABT-263, AT-101, pyrogallol-based molecules, 25 ABT-199, and Genasense (G3139).

In some embodiments, the first inhibitor may be an AKT or mTOR inhibitor. In some embodiments, an AKT inhibitor and mTOR inhibitor may be administered together.

The second inhibitor may be an inhibitor of the Notch signaling pathway. It is to be understood that the inhibitor can act on any part of the Notch signaling pathway as described 30 herein. The Notch pathway inhibitor may target a gene, mRNA expression, protein expression, and/or activity in the Notch pathway or associated with the Notch pathway, in all instances reducing the level and/or activity of the Notch signaling pathway. Inhibitors may be nucleic acids such as DNA and RNA aptamers, antisense oligonucleotides, siRNA and shRNA, small

peptides, antibodies or antibody fragments, and small molecules such as small chemical compounds.

In some embodiments, the Notch pathway inhibitor is a gamma secretase inhibitor (GSI). In some embodiments the GSI is DBZ, BMS-906024, RO4929097, LY450139, BMS-708163, 5 MK-0752, PF-03084014, IL-X, z-Leu-leu-Nle-CHO, or N-[N-(3,5-difluorophenacetyl)-L-alanyl]-Sphenylglycine t-butyl ester (DAPT). Other Notch pathway inhibitors are provided herein such as in but not limited to LBH589 (Panobinostat), MEDI0639, Choline magnesium trisalicylate (e.g., Trilisate), or Curcumin (a curcuminoid of turmeric).

In some embodiments, the invention contemplates the use of a BRD inhibitor, a Notch 10 pathway inhibitor, and a Bcl-2 inhibitor.

Other aspects of the invention provide methods for treating subjects with cancer with an inhibitor of one or more chromatin regulatory proteins selected from ARID3B, EZH2, PRMT2, SND1, BRD1, SUV39H1, PRMT5, SS18, BRD4, KDM5D, PRMT7, STAG3L1, CD2BP2, MLL5, SUDS3, CHD1, MINA, CHD8, MORF4L1, or CHRAC1. In some embodiments, the 15 one or more chromatin regulatory proteins are selected from BRD4 or PRMT7.

When two or more inhibitors or agents are administered to a subject, these can be administered simultaneously (e.g., where they are pre-mixed and administered together), substantially simultaneously (e.g., where they are administered one after another in the time it would take a medical practitioner to administer two agents to a subject), or sequentially with a 20 period of time lapsing between the inhibitor administrations. The two or more inhibitors can also be administered by the same route or by a different route. For example, the inhibitors may be all administered by injection (e.g., intravenous injection) or orally. As another example, one inhibitor may be administered by injection and another may be administered orally.

The term “treat”, “treated,” “treating” or “treatment” is used herein to mean to relieve, 25 reduce or alleviate at least one symptom of a disease such as cancer in a subject. For example, treatment can be diminishment of one or several symptoms of a disorder or complete eradication of a disorder, such as cancer. Within the meaning of the present invention, the term “treat” also denotes to arrest, delay the onset (i.e., the period prior to clinical manifestation of a disease) and/or reduce the risk of developing or worsening a disease. The term “protect” is used herein 30 to mean prevent delay or treat, or all, as appropriate, development or continuance or aggravation of a disease in a subject. Within the meaning of the present invention, the disease is typically a cancer.

The term "effective amount" is used herein to mean the amount of an agent or inhibitor required to ameliorate the symptoms of a disease relative to an untreated patient. The effective amount of active compound(s) used to practice the present invention for therapeutic treatment of a disease varies depending upon the manner of administration, the age, body weight, and general 5 health of the subject. Ultimately, the attending physician or veterinarian will decide the appropriate amount and dosage regimen. Such amount is referred to as an "effective" amount.

Where two or more inhibitors are administered to the subject, the effective amount may be a combined effective amount. The effective amount of a first inhibitor may be different when it is used with a second and optionally a third inhibitor. When two more inhibitors are used 10 together, the effective amounts of each may be the same as when they are used alone.

Alternatively, the effective amounts of each may be less than the effective amounts when they are used alone because the desired effect is achieved at lower doses. Alternatively, again, the effective amount of each may be greater than the effective amounts when they are used alone because the subject is better able to tolerate one or more of the inhibitors which can then be 15 administered at a higher dose provided such higher dose provides more therapeutic benefit.

Subjects

The term "subject" or "patient" is intended to include humans and animals that are capable of suffering from or afflicted with a cancer or any disorder involving, directly or 20 indirectly, a cancer. Examples of subjects include mammals, e.g., humans, dogs, cows, horses, pigs, sheep, goats, cats, mice, rabbits, rats, and transgenic non-human animals. In some embodiments, subjects include companion animals, e.g. dogs, cats, rabbits, and rats. In some embodiments, subjects include livestock, e.g., cows, pigs, sheep, goats, and rabbits. In some embodiments, subjects include Thoroughbred or show animals, e.g. horses, pigs, cows, and 25 rabbits. In important embodiments, the subject is a human, e.g., a human having, at risk of having, or potentially capable of having cancer.

Cancer

The term "cancer" is used herein to mean malignant solid tumors as well as 30 hematological malignancies. Examples of cancer include but are not limited to leukemias, lymphomas, myelomas, carcinomas, metastatic carcinomas, sarcomas, adenomas, nervous system cancers and genito-urinary cancers. In certain embodiments, the cancer is acute lymphoblastic leukemia. In other embodiments, the foregoing methods are useful in treating

adult and pediatric acute lymphoblastic leukemia, acute myeloid leukemia, adrenocortical carcinoma, AIDS-related cancers, anal cancer, cancer of the appendix, astrocytoma, basal cell carcinoma, bile duct cancer, bladder cancer, bone cancer, osteosarcoma, fibrous histiocytoma, brain cancer, brain stem glioma, cerebellar astrocytoma, malignant glioma, ependymoma, 5 medulloblastoma, supratentorial primitive neuroectodermal tumors, hypothalamic glioma, breast cancer, male breast cancer, bronchial adenomas, Burkitt lymphoma, carcinoid tumor, carcinoma of unknown origin, central nervous system lymphoma, cerebellar astrocytoma, malignant glioma, cervical cancer, childhood cancers, chronic lymphocytic leukemia, chronic myelogenous leukemia, chronic myeloproliferative disorders, colorectal cancer, cutaneous T-cell lymphoma, 10 endometrial cancer, ependymoma, esophageal cancer, Ewing family tumors, extracranial germ cell tumor, extragonadal germ cell tumor, extrahepatic bile duct cancer, intraocular melanoma, retinoblastoma, gallbladder cancer, gastric cancer, gastrointestinal stromal tumor, extracranial germ cell tumor, extragonadal germ cell tumor, ovarian germ cell tumor, gestational trophoblastic tumor, glioma, hairy cell leukemia, head and neck cancer, hepatocellular cancer, 15 Hodgkin lymphoma, non-Hodgkin lymphoma, hypopharyngeal cancer, hypothalamic and visual pathway glioma, intraocular melanoma, islet cell tumors, Kaposi sarcoma, kidney cancer, renal cell cancer, laryngeal cancer, lip and oral cavity cancer, small cell lung cancer, non-small cell lung cancer, primary central nervous system lymphoma, Waldenstrom macroglobulinemia, malignant fibrous histiocytoma, medulloblastoma, melanoma, Merkel cell carcinoma, malignant 20 mesothelioma, squamous neck cancer, multiple endocrine neoplasia syndrome, multiple myeloma, mycosis fungoides, myelodysplastic syndromes, myeloproliferative disorders, chronic myeloproliferative disorders, nasal cavity and paranasal sinus cancer, nasopharyngeal cancer, neuroblastoma, oropharyngeal cancer, ovarian cancer, pancreatic cancer, parathyroid cancer, penile cancer, pharyngeal cancer, pheochromocytoma, pineoblastoma and supratentorial 25 primitive neuroectodermal tumors, pituitary cancer, plasma cell neoplasms, pleuropulmonary blastoma, prostate cancer, rectal cancer, rhabdomyosarcoma, salivary gland cancer, soft tissue sarcoma, uterine sarcoma, Sezary syndrome, non-melanoma skin cancer, small intestine cancer, squamous cell carcinoma, squamous neck cancer, supratentorial primitive neuroectodermal tumors, testicular cancer, throat cancer, thymoma and thymic carcinoma, thyroid cancer, 30 transitional cell cancer, trophoblastic tumors, urethral cancer, uterine cancer, uterine sarcoma, vaginal cancer, vulvar cancer, and Wilms tumor.

In some embodiments, the cancer is a resistant to a Notch pathway inhibitor. A cancer that is resistant to a Notch pathway inhibitor means that the cancer does not respond to such

inhibitor, for example as evidenced by continued proliferation and increasing tumor growth and burden. In some instances, the cancer may have initially responded to treatment with such inhibitor (referred to herein as a previously administered therapy) but may have grown resistant after a time. In some instances, the cancer may have never responded to treatment with such 5 inhibitor at all.

Notch Pathway Activation Mutations

In some embodiments, the cancer is associated with or is characterized by a Notch pathway activation mutation. A cancer that is associated with or is characterized by a Notch 10 pathway activation mutation is a cancer that carries such a mutation, as detected by any number of diagnostic assays and methods including fluorescence in situ hybridization (FISH), genomic sequencing, whole exome sequencing, whole genome sequencing, and the like. For example, a mutation in the Notch1 gene can be detected by Notch1 exon sequencing which can be done using a variety of methods as described above including, for example Sanger sequencing. In 15 some embodiments, tumorigenesis, tumor progression, and/or metastasis is increased or enhanced by a Notch pathway activation mutation. Such tumorigenesis, tumor progression and/or metastasis processes that may be increased or enhanced by a Notch pathway activation mutation include, but is not limited to: the epithelial-to-mesenchymal transition (EMT), angiogenesis, and bone metastasis (Sethi et al. British Journal of Cancer, 105; 1805-1810, 2011). 20 In some embodiments, the cancer is sensitive to a Notch pathway inhibitor.

The term “Notch pathway” as used herein encompasses Notch, Notch ligands, and upstream and downstream effectors of the Notch signaling pathway. The term “activation mutation” as used herein refers to mutations (e.g. a mutation in the coding region of a 25 downstream effector of the Notch pathway or chromosomal translocation, point mutations, and chromosomal amplification at the Notch receptor loci) or epigenetic modifications within Notch, Notch ligands, upstream and/or downstream effectors of the Notch pathway.

Examples of cancers that are associated with a Notch pathway activation mutation, are characterized by a Notch pathway activation mutation, or are sensitive to a Notch pathway inhibitor include, but are not limited to: hematological tumors, T-cell acute lymphoblastic 30 leukemia, B-cell malignancies, breast cancer, gut cancer, skin cancer, keratinocyte-derived carcinoma, melanocyte-derived carcinoma, primary melanoma, basal cell carcinoma, squamous cell carcinoma, cervical cancer, prostate cancer, non-small cell lung adenocarcinoma, ovarian carcinoma, medulloblastoma, Kaposi’s sarcoma, pancreatic cancer, colorectal cancer, and

glioma (see, e.g., Bolós et. al., Notch Signaling in Development and Cancer, Endocrine Reviews, 28(3):339-363, 2007; Sethi et al., British Journal of Cancer, 105; 1805-1810, 2011). In some embodiments, the cancer that has or is characterized by a Notch pathway activation mutation is acute lymphoblastic leukemia. In certain embodiments, the cancer that has or is 5 characterized by a Notch pathway activation mutation is T-cell acute lymphoblastic leukemia.

Other cancers associated with or are characterized by a Notch pathway activation mutation may be identified by detecting mutations or epigenetic modifications within Notch, Notch ligands, upstream and/or downstream effectors of the Notch pathway using methods well known in the art (e.g., genomic and/or proteomic means to identify regions or factors that affect 10 the expression of any gene within the Notch pathway).

Pharmaceutical Formulations, Administration and Dosages

Provided herein are pharmaceutical formulations comprising single agents, such as bromodomain inhibitors and/or pharmacologically active metabolites, salts, solvates and 15 racemates thereof, or a combination of agents which can be, for example, a combination of two types of agents comprising: (1) a bromodomain inhibitor and/or pharmacologically active metabolites, salts, solvates and racemates thereof and (2) Notch pathway inhibitor and/or pharmacologically active metabolites, salts, solvates and racemates thereof.

In another embodiment, the combination of agents comprises (1) a Bcl-2 inhibitor and/or 20 pharmacologically active metabolites, salts, solvates and racemates thereof and (2) Notch pathway inhibitor and/or pharmacologically active metabolites, salts, solvates and racemates thereof. In still another embodiment, the combination of agents comprises (1) a bromodomain inhibitor and/or pharmacologically active metabolites, salts, solvates and racemates thereof, (2) a Bcl-2 inhibitor and/or pharmacologically active metabolites, salts, solvates and racemates 25 thereof and (3) Notch pathway inhibitor and/or pharmacologically active metabolites, salts, solvates and racemates thereof.

In another embodiment, the combination of agents comprises (1) an MTOR inhibitor and/or pharmacologically active metabolites, salts, solvates and racemates thereof and (2) a Notch pathway inhibitor and/or pharmacologically active metabolites, salts, solvates and 30 racemates thereof.

In another embodiment, the combination of agents comprises (1) an AKT inhibitor and/or pharmacologically active metabolites, salts, solvates and racemates thereof and (2) Notch

pathway inhibitor and/or pharmacologically active metabolites, salts, solvates and racemates thereof.

For therapeutic uses, the inhibitors described herein may be administered systemically, for example, formulated in a pharmaceutically-acceptable buffer such as physiological saline.

5 Preferable routes of administration include, for example, subcutaneous, intravenous, intraperitoneally, intramuscular, or intradermal injections that provide continuous, sustained levels of the drug in the subject.

Treatment of human patients or other animals will be carried out using a therapeutically effective amount of a therapeutic identified herein in a physiologically-acceptable carrier.

10 Suitable carriers and their formulation are described, for example, in Remington's Pharmaceutical Sciences by E. W. Martin. The amount of the therapeutic agent to be administered varies depending upon the manner of administration, the age and body weight of the patient, and with the clinical symptoms of the cancer. Generally, amounts will be in the range of those used for other agents used in the treatment of other diseases associated with such 15 diseases or states, although in certain instances lower amounts will be needed because of the increased specificity of the compound. A compound is administered at a dosage that is cytotoxic to a neoplastic cell, that reduces the biological activity of a bromodomain, Notch pathway, or Bcl-2 family member, or that reduces the proliferation, survival, or invasiveness of a neoplastic cell as determined by a method known to one skilled in the art

20 Human dosage amounts can initially be determined by extrapolating from the amount of compound used in mice, as a skilled artisan recognizes it is routine in the art to modify the dosage for humans compared to animal models. In certain embodiments it is envisioned that the dosage may vary from between about 1 µg compound/Kg body weight to about 5000 mg compound/Kg body weight; or from about 5 mg/Kg body weight to about 4000 mg/Kg body weight or from about 10 mg/Kg body weight to about 3000 mg/Kg body weight; or from about 25 50 mg/Kg body weight to about 2000 mg/Kg body weight; or from about 100 mg/Kg body weight to about 1000 mg/Kg body weight; or from about 150 mg/Kg body weight to about 500 mg/Kg body weight. In other embodiments this dose may be about 1, 5, 10, 25, 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050, 30 1100, 1150, 1200, 1250, 1300, 1350, 1400, 1450, 1500, 1600, 1700, 1800, 1900, 2000, 2500, 3000, 3500, 4000, 4500, or 5000 mg/Kg body weight. In other embodiments, it is envisaged that doses may be in the range of about 5 mg compound/Kg body to about 20 mg compound/Kg body. In other embodiments the doses may be about 8, 10, 12, 14, 16 or 18 mg/Kg body weight.

Of course, this dosage amount may be adjusted upward or downward, as is routinely done in such treatment protocols, depending on the results of the initial clinical trials and the needs of a particular patient.

The formulation of a compound for the treatment of a cancer may be by any suitable means that results in a concentration of the therapeutic that, combined with other components, is effective in ameliorating, reducing, or stabilizing cancer. The compound may be contained in any appropriate amount in any suitable carrier substance, and is generally present in an amount of 1-95% by weight of the total weight of the composition. The composition may be provided in a dosage form that is suitable for parenteral (e.g., subcutaneously, intravenously, 5 intramuscularly, or intraperitoneally) administration route. The pharmaceutical compositions may be formulated according to conventional pharmaceutical practice (see, e.g., Remington: The Science and Practice of Pharmacy (20th ed.), ed. A. R. Gennaro, Lippincott Williams & Wilkins, 2000 and Encyclopedia of Pharmaceutical Technology, eds. J. Swarbrick and J. C. Boylan, 10 1988-1999, Marcel Dekker, New York).

Pharmaceutical compositions according to the invention may be formulated to release the active compound substantially immediately upon administration or at any predetermined time or time period after administration. The latter types of compositions are generally known as controlled release formulations, which include (i) formulations that create a substantially constant concentration of the drug within the body over an extended period of time; (ii) 15 formulations that after a predetermined lag time create a substantially constant concentration of the drug within the body over an extended period of time; (iii) formulations that sustain action during a predetermined time period by maintaining a relatively, constant, effective level in the body with concomitant minimization of undesirable side effects associated with fluctuations in the plasma level of the active substance (sawtooth kinetic pattern); (iv) formulations that localize 20 action by, e.g., spatial placement of a controlled release composition adjacent to or in contact with the thymus; (v) formulations that allow for convenient dosing, such that doses are administered, for example, once every one or two weeks; and (vi) formulations that target a neoplasia or inflammatory disease by using carriers or chemical derivatives to deliver the therapeutic agent to a particular cell type (e.g., neoplastic cell). For some applications, controlled 25 release formulations obviate the need for frequent dosing during the day to sustain the plasma level at a therapeutic level.

Any of a number of strategies can be pursued in order to obtain controlled release in which the rate of release outweighs the rate of metabolism of the compound in question. In one

example, controlled release is obtained by appropriate selection of various formulation parameters and ingredients, including, e.g., various types of controlled release compositions and coatings. Thus, the therapeutic is formulated with appropriate excipients into a pharmaceutical composition that, upon administration, releases the therapeutic in a controlled manner. Examples 5 include single or multiple unit tablet or capsule compositions, oil solutions, suspensions, emulsions, microcapsules, microspheres, molecular complexes, nanoparticles, patches, and liposomes.

Parenteral Compositions

10 The pharmaceutical composition may be administered parenterally by injection, infusion or implantation (subcutaneous, intravenous, intramuscular, intraperitoneal, or the like) in dosage forms, formulations, or via suitable delivery devices or implants containing conventional, non-toxic pharmaceutically acceptable carriers and adjuvants. The formulation and preparation of such compositions are well known to those skilled in the art of pharmaceutical formulation.

15 Formulations can be found in Remington: The Science and Practice of Pharmacy, supra. Compositions for parenteral use may be provided in unit dosage forms (e.g., in single-dose ampoules), or in vials containing several doses and in which a suitable preservative may be added (see below). The composition may be in the form of a solution, a suspension, an emulsion, an infusion device, or a delivery device for implantation, or it may be presented as a 20 dry powder to be reconstituted with water or another suitable vehicle before use. Apart from the active agent that reduces or ameliorates cancer, the composition may include suitable parenterally acceptable carriers and/or excipients. The active therapeutic agent(s) may be incorporated into microspheres, microcapsules, nanoparticles, liposomes, or the like for controlled release. Furthermore, the composition may include suspending, solubilizing, 25 stabilizing, pH-adjusting agents, tonicity adjusting agents, and/or dispersing, agents.

As indicated above, the pharmaceutical compositions according to the invention may be in the form suitable for sterile injection. To prepare such a composition, the suitable active anticancer therapeutic(s) are dissolved or suspended in a parenterally acceptable liquid vehicle. Among acceptable vehicles and solvents that may be employed are water, water adjusted to a 30 suitable pH by addition of an appropriate amount of hydrochloric acid, sodium hydroxide or a suitable buffer, 1,3-butanediol, Ringer's solution, and isotonic sodium chloride solution and dextrose solution. The aqueous formulation may also contain one or more preservatives (e.g., methyl, ethyl or n-propyl p-hydroxybenzoate). In cases where one of the compounds is only

sparingly or slightly soluble in water, a dissolution enhancing or solubilizing agent can be added, or the solvent may include 10-60% w/w of propylene glycol or the like.

Controlled Release Parenteral Compositions

5 Controlled release parenteral compositions may be in form of aqueous suspensions, microspheres, microcapsules, magnetic microspheres, oil solutions, oil suspensions, or emulsions. Alternatively, the active drug may be incorporated in biocompatible carriers, liposomes, nanoparticles, implants, or infusion devices.

Materials for use in the preparation of microspheres and/or microcapsules are, e.g.,
10 biodegradable/bioerodible polymers such as polygalactia poly-(isobutyl cyanoacrylate), poly(2-hydroxyethyl-L-glutaminine) and, poly(lactic acid).

Biocompatible carriers that may be used when formulating a controlled release parenteral formulation are carbohydrates (e.g., dextrans), proteins (e.g., albumin), lipoproteins, or antibodies. Materials for use in implants can be non-biodegradable (e.g., polydimethyl siloxane)
15 or biodegradable (e.g., poly(caprolactone), poly(lactic acid), poly(glycolic acid) or poly(ortho esters) or combinations thereof).

Solid Dosage Forms For Oral Use

Formulations for oral use include tablets containing the active ingredient(s) in a mixture
20 with non-toxic pharmaceutically acceptable excipients. Such formulations are known to the skilled artisan. Excipients may be, for example, inert diluents or fillers (e.g., sucrose, sorbitol, sugar, mannitol, microcrystalline cellulose, starches including potato starch, calcium carbonate, sodium chloride, lactose, calcium phosphate, calcium sulfate, or sodium phosphate); granulating and disintegrating agents (e.g., cellulose derivatives including microcrystalline cellulose,
25 starches including potato starch, croscarmellose sodium, alginates, or alginic acid); binding agents (e.g., sucrose, glucose, sorbitol, acacia, alginic acid, sodium alginate, gelatin, starch, pregelatinized starch, microcrystalline cellulose, magnesium aluminum silicate, carboxymethylcellulose sodium, methylcellulose, hydroxypropyl methylcellulose, ethylcellulose, polyvinylpyrrolidone, or polyethylene glycol); and lubricating agents, glidants,
30 and antiadhesives (e.g., magnesium stearate, zinc stearate, stearic acid, silicas, hydrogenated vegetable oils, or talc). Other pharmaceutically acceptable excipients can be colorants, flavoring agents, plasticizers, humectants, buffering agents, and the like.

The tablets may be uncoated or they may be coated by known techniques, optionally to delay disintegration and absorption in the gastrointestinal tract and thereby providing a sustained action over a longer period. The coating may be adapted to release the active drug in a predetermined pattern (e.g., in order to achieve a controlled release formulation) or it may be
5 adapted not to release the active drug until after passage of the stomach (enteric coating). The coating may be a sugar coating, a film coating (e.g., based on hydroxypropyl methylcellulose, methylcellulose, methyl hydroxyethylcellulose, hydroxypropylcellulose, carboxymethylcellulose, acrylate copolymers, polyethylene glycols and/or polyvinylpyrrolidone), or an enteric coating (e.g., based on methacrylic acid copolymer,
10 cellulose acetate phthalate, hydroxypropyl methylcellulose phthalate, hydroxypropyl methylcellulose acetate succinate, polyvinyl acetate phthalate, shellac, and/or ethylcellulose). Furthermore, a time delay material, such as, e.g., glycetyl monostearate or glycetyl distearate may be employed.

The solid tablet compositions may include a coating adapted to protect the composition
15 from unwanted chemical changes, (e.g., chemical degradation prior to the release of the active therapeutic substance). The coating may be applied on the solid dosage form in a similar manner as that described in Encyclopedia of Pharmaceutical Technology, supra.

At least two therapeutics may be mixed together in the tablet, or may be partitioned. In one example, the first active anti-neoplasia therapeutic is contained on the inside of the tablet,
20 and the second active therapeutic is on the outside, such that a substantial portion of the second therapeutic is released prior to the release of the first therapeutic.

Formulations for oral use may also be presented as chewable tablets, or as hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent (e.g., potato starch, lactose, microcrystalline cellulose, calcium carbonate, calcium phosphate or kaolin), or as soft
25 gelatin capsules wherein the active ingredient is mixed with water or an oil medium, for example, peanut oil, liquid paraffin, or olive oil. Powders and granulates may be prepared using the ingredients mentioned above under tablets and capsules in a conventional manner using, e.g., a mixer, a fluid bed apparatus or a spray drying equipment.

30 ***Controlled Release Oral Dosage Forms***

Controlled release compositions for oral use may, e.g., be constructed to release the active anti-neoplasia or anti-inflammatory therapeutic by controlling the dissolution and/or the diffusion of the active substance. Dissolution or diffusion controlled release can be achieved by

appropriate coating of a tablet, capsule, pellet, or granulate formulation of compounds, or by incorporating the compound into an appropriate matrix. A controlled release coating may include one or more of the coating substances mentioned above and/or, e.g., shellac, beeswax, glycowax, castor wax, carnauba wax, stearyl alcohol, glycetyl monostearate, glycetyl distearate, 5 glycerol palmitostearate, ethylcellulose, acrylic resins, dl-polylactic acid, cellulose acetate butyrate, polyvinyl chloride, polyvinyl acetate, vinyl pyrrolidone, polyethylene, polymethacrylate, methylmethacrylate, 2-hydroxymethacrylate, methacrylate hydrogels, 1,3 butylene glycol, ethylene glycol methacrylate, and/or polyethylene glycols. In a controlled release matrix formulation, the matrix material may also include, e.g., hydrated methylcellulose, 10 carnauba wax and stearyl alcohol, carbopol 934, silicone, glycetyl tristearate, methyl acrylate-methyl methacrylate, polyvinyl chloride, polyethylene, and/or halogenated fluorocarbon.

A controlled release composition containing one or more therapeutic compounds may also be in the form of a buoyant tablet or capsule (i.e., a tablet or capsule that, upon oral administration, floats on top of the gastric content for a certain period of time). A buoyant tablet 15 formulation of the compound(s) can be prepared by granulating a mixture of the compound(s) with excipients and 20-75% w/w of hydrocolloids, such as hydroxyethylcellulose, hydroxypropylcellulose, or hydroxypropylmethylcellulose. The obtained granules can then be compressed into tablets. On contact with the gastric juice, the tablet forms a substantially water-impermeable gel barrier around its surface. This gel barrier takes part in maintaining a density of 20 less than one, thereby allowing the tablet to remain buoyant in the gastric juice.

Combination Therapies

Optionally, one or more inhibitors of the invention may be administered in combination with any other standard anti-cancer therapy known in the art; such methods are known to the 25 skilled artisan and described in Remington's Pharmaceutical Sciences by E. W. Martin. If desired, agents of the invention (e.g., bromodomain inhibitors, Notch pathway inhibitors, and Bcl-2 inhibitors) are administered in combination with any conventional cancer therapy, including but not limited to, surgery, radiation therapy, or chemotherapy.

In preferred embodiments, a compound of the invention is administered in combination 30 with an epigenetic or transcriptional modulator (e.g., DNA methyltransferase inhibitor, histone deacetylase inhibitor (HDAC inhibitor), lysine methyltransferase inhibitor), with antimitotic drugs (e.g., taxanes, vinca alkaloids), hormone receptor modulators (e.g., estrogen receptor modulators, androgen receptor modulators), cell signaling pathway inhibitors (e.g., tyrosine

kinase inhibitors), modulators of protein stability (proteasome inhibitors), hsp90 inhibitors, conventional therapeutics, glucocorticoids, all-trans retinoic acid or other agents that promote differentiation.

5 ***Diagnostic/Prognostic Methods***

In one aspect, the invention provides methods of detecting the presence of one or more predictive, diagnostic or prognostic markers in a sample (e.g., a biological sample from a cancer patient). A variety of screening methods known to one of skill in the art may be used to detect the presence and the level of the marker in the sample including DNA, RNA and protein detection. The techniques described herein can be used to determine the presence or absence of a target in a sample obtained from a patient.

Identification of one or more markers (including identification of elevated levels of one or more markers) in a patient assists a physician or other medical professional in determining a treatment protocol for the patient. For example, in a patient having one or more markers, the physician may treat the patient with a combination therapy as described in more detail above.

15 ***Detection Methods***

The methods invention may be protein or mRNA based. Examples of protein-based assays include immunoassays (also referred to herein as immune-based assays), Western blots, Western immunoblotting, multiplex bead-based assays, and assays involving aptamers (such as SOMAmer™ technology) and related affinity agents. Examples of mRNA-based assays include Northern analysis, quantitative RT-PCR, microarray hybridization, RNA sequencing, and multiplex bead-based assays. These assays are well known in the art and generally and commonly detect and measure the level of the marker of interest. The level of the marker may then be compared to a control level. The control level may be a level of the same marker in a control tissue, control subject, or a population of control subjects. The “control” may be (or may be derived from) a normal subject (or normal subjects). Normal may refer to a subject that is apparently cancer-free. It is to be understood however that the methods provided herein do not require that a control level be measured every time a subject is tested. Rather, it is contemplated that control levels of markers are obtained and recorded and that any test level is compared to such a pre-determined level. Such pre-determined control levels may also be referred to herein as pre-determined threshold levels.

Chromatin Compaction

In some aspects, the invention relates to methods for diagnosing a subject in need of treatment with a bromodomain inhibitor or a combination of agents as described herein, including, but not limited to, a bromodomain inhibitor and/or a Bcl-2 inhibitor. In some 5 embodiments, the method comprises measuring the global level of chromatin compaction. Chromatin compaction can be measured, for example, (a) by partial micrococcal nuclease (MNase) or DNase digestion, (b) by increased expression of heterochromatin associated proteins, such as HP1 alpha, beta and/or gamma, (c) by measuring nucleus size, (d) by decreased expression of DTX1, HES4, and/or CD1d and/or by increased expression of ETS1, ETV6, 10 Runx1, CD52, MYC or Bcl-2, and/or (d) by measuring increased levels of repressive chromatin markers such as H3K27me3, H3K9me2/3 and/or decreased levels of other chromatin markers such as H3K27Ac.

In some embodiments, the method comprises measuring nucleus size, nucleosomal repeat length, or cell size in a sample. The nucleus or cell size may be the nucleus or cell 15 diameter or the nucleus or cell volume. Methods for measuring nucleus size are known in the art and may involve staining nuclei (e.g. with DAPI), fluorescently labeling nuclei (e.g. with green fluorescent protein), or May-Grunwald giemsa staining (particularly for hematopoietic cells). Methods for measuring cell size are known in the art and may involve forward and side scatter analysis using fluorescent activated cell sorting (FACS). Methods for measuring 20 nucleosomal repeat length are known in the art and may involve a micrococcal nuclease (MNase) digestion assay. In some embodiments, a decreased nucleus size or cell size, or an increased nucleosomal repeat length, in a tumor sample compared to a control identifies a subject to be treated with a bromodomain inhibitor or a combination of agents as described herein, including, but not limited to, a bromodomain inhibitor and/or a Bcl-2 inhibitor.

25 In some embodiments the methods for diagnosing comprise measuring the expression level of HPI-alpha, beta, or gamma. The expression level may be an mRNA level or a protein level. Methods for measuring mRNA and protein levels in a cell population are known in the art. In some embodiments, an increased HPI level in a tumor sample compared to a control identifies a subject to be treated with a bromodomain inhibitor or a combination of agents as 30 described herein, including, but not limited to, a bromodomain inhibitor and/or a Bcl-2 inhibitor.

In some embodiments, the methods for diagnosing comprise measuring expression levels of at least one chromatin state biomarker (CSB). The CSB may be selected from NPM1, NARG1, RCC1, SSRP1, PRMT3, SAP30, CBX6, CHMP2B, UBE2M, WDR77, HMGB1,

CARM1, USP13, HDAC4, COQ3, SET, GATAD2A, PRMT6, HMG20B, DNMT1, ADA, SS18, UBE3A, ZMYND11, NOC2LL, UTX, SIN3A, SAP30L, FLJ20309, RCOR2, ARID5A, UBE2Q2, TRIM24, BAZ2B, SMYD3, EZH2, PHF1, PHF2, BCR, SMARCD3, BMI1, CHD6, FBXL11, SIRT7, ASF1A, RCOR3, CBX4, EPC1, BRD1, and BNF11.

5 In some embodiments, decreased levels in a tumor sample of a CSB from the group consisting of NPM1, NARG1, RCC1, SSRP1, PRMT3, SAP30, CBX6, CHMP2B, UBE2M, WDR77, HMGB1, CARM1, USP13, HDAC4, COQ3, SET, GATAD2A, PRMT6, HMG20B, DNMT1, ADA, SS18, UBE3A, ZMYND11, and/or NOC2LL (“Group I CSB”) compared to a control identifies a subject to be treated with a bromodomain inhibitor or a combination of
10 agents as described herein, including, but not limited to, a bromodomain inhibitor and/or a Bcl-2 inhibitor.

In some embodiments, increased levels in a tumor sample of a CSB from the group consisting of UTX, SIN3A, SAP30L, FLJ20309, RCOR2, ARID5A, UBE2Q2, TRIM24, BAZ2B, SMYD3, EZH2, PHF1, PHF2, BCR, SMARCD3, BMI1, CHD6, FBXL11, SIRT7,
15 ASF1A, RCOR3, CBX4, EPC1, BRD1, and/or BNF11 (“Group II CSB”) compared to a control identifies a subject to be treated with a bromodomain inhibitor or a combination of agents as described herein, including, but not limited to, a bromodomain inhibitor and/or a Bcl-2 inhibitor.

In some embodiments, more than one CSB expression level is measured. The more than one CSB may be chosen from Group I, Group II, or both Group I and Group II.

20 Chromatin state biomarkers of the invention include those in Table 6.

Gene	Ensembl gene ID	Ensembl transcript ID	Ensembl protein ID
NPM1	ENSG00000181163	ENST00000523622, ENST00000521672, ENST00000517671, ENST00000393820, ENST00000351986, ENST00000296930	ENSP00000428647, ENSP00000429485, ENSP00000428755, ENSP00000377408, ENSP00000341168, ENSP00000296930
NARG1	ENSG00000164134	ENST00000544077, ENST00000515576, ENST00000398947, ENST00000296543	ENSP00000443524, ENSP00000421839, ENSP00000381920, ENSP00000296543
RCC1	ENSG00000180198	ENST00000434290, ENST00000430407, ENST00000429051, ENST00000427469, ENST00000419074,	ENSP00000405258, ENSP00000394650, ENSP00000416220, ENSP00000402740, ENSP00000402260,

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		ENST00000411533, ENST00000398958, ENST00000373833, ENST00000373832, ENST00000373831	ENSP00000413644, ENSP00000381931, ENSP00000362939, ENSP00000362938, ENSP00000362937
SSRP1	ENSG00000149136	ENST00000529002, ENST00000526696, ENST00000278412	ENSP00000434546, ENSP00000431154, ENSP00000278412
PRMT3	ENSG00000185238	ENST00000541255, ENST00000526583, ENST00000525188, ENST00000437750, ENST00000331079, ENST00000330796	ENSP00000440367, ENSP00000434260, ENSP00000435151, ENSP00000397766, ENSP00000331879, ENSP00000329586
SAP30	ENSG00000164105	ENST00000296504	ENSP00000296504
CBX6	ENSG00000183741	ENST00000407418, ENST00000216083	ENSP00000384490, ENSP00000216083
CHMP2B	ENSG00000083937	ENST00000494980, ENST00000471660, ENST00000263780	ENSP00000418920, ENSP00000419998, ENSP00000263780
UBE2M	ENSG00000130725	ENST00000253023	ENSP00000253023
WDR77	ENSG00000116455	ENST00000449340, ENST00000411751, ENST00000235090	ENSP00000409300, ENSP00000400321, ENSP00000235090
HMGB1	ENSG00000189403	ENST00000426225, ENST00000405805, ENST00000399494, ENST00000399489, ENST00000398908, ENST00000341423, ENST00000339872, ENST00000326004	ENSP00000411269, ENSP00000384678, ENSP00000382417, ENSP00000382412, ENSP00000410465, ENSP00000345347, ENSP00000343040, ENSP00000369904
CARM1	ENSG00000142453	ENST00000344150, ENST00000327064	ENSP00000340934, ENSP00000325690
USP13	ENSG00000058056	ENST00000497380, ENST00000497155, ENST00000496897, ENST00000263966	ENSP00000418651, ENSP00000420057, ENSP00000417146, ENSP00000263966
HDAC4	ENSG00000068024	ENST00000544989, ENST00000543185, ENST00000541256, ENST00000456922, ENST00000454542, ENST00000446876, ENST00000445704, ENST00000430200, ENST00000393621, ENST00000345617	ENSP00000438111, ENSP00000440481, ENSP00000443057, ENSP00000406618, ENSP00000405226, ENSP00000392912, ENSP00000391226, ENSP00000410551, ENSP00000377243, ENSP00000264606
COQ3	ENSG00000132423	ENST00000369242,	ENSP00000358245,

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		ENST00000369240, ENST00000254759	ENSP00000358243, ENSP00000254759
SET	ENSG00000119335	ENST00000454747, ENST00000409104, ENST00000372692, ENST00000372688, ENST00000372686, ENST00000322030	ENSP00000410806, ENSP00000387321, ENSP00000361777, ENSP00000361773, ENSP00000361771, ENSP00000318012
GATAD2A	ENSG00000167491	ENST00000537887, ENST00000457895, ENST00000448576, ENST00000444839, ENST00000432704, ENST00000429563, ENST00000429242, ENST00000418032, ENST00000417582, ENST00000404158, ENST00000360315, ENST00000358713, ENST00000252577	ENSP00000442588, ENSP00000404212, ENSP00000416452, ENSP00000407293, ENSP00000390495, ENSP00000388416, ENSP00000414252, ENSP00000411869, ENSP00000403703, ENSP00000384899, ENSP00000353463, ENSP00000351552, ENSP00000252577
PRMT6	ENSG00000198890	ENST00000540389, ENST00000370078, ENST00000361318	ENSP00000440829, ENSP00000359095, ENSP00000355145
HMG20B	ENSG00000064961	ENST00000453933, ENST00000435022, ENST00000417382, ENST00000416526, ENST00000402569, ENST00000333651, ENST00000262949	ENSP00000402877, ENSP00000393481, ENSP00000393904, ENSP00000410924, ENSP00000385987, ENSP00000328269, ENSP00000262949
DNMT1	ENSG00000130816	ENST00000541266, ENST00000540357, ENST00000359526, ENST00000340748	ENSP00000437951, ENSP00000440457, ENSP00000352516, ENSP00000345739
ADA	ENSG00000196839	ENST00000539235, ENST00000537820, ENST00000536532, ENST00000372874	ENSP00000446464, ENSP00000441818, ENSP00000440946, ENSP00000361965
SS18	ENSG00000141380	ENST00000545952, ENST00000542743, ENST00000542420, ENST00000539849, ENST00000539244, ENST00000415083, ENST00000269138, ENST00000269137	ENSP00000443097, ENSP00000444551, ENSP00000438066, ENSP00000444647, ENSP00000441760, ENSP00000414516, ENSP00000269138, ENSP00000269137
UBE3A	ENSG00000114062	ENST00000566215, ENST00000438097, ENST00000428984,	ENSP00000457771, ENSP00000411258, ENSP00000401265,

		ENST00000397954, ENST00000356465, ENST00000232165	ENSP00000381045, ENSP00000348850, ENSP00000232165
NOC2L	ENSG00000188976	ENST00000327044	ENSP00000317992
UTX	ENSG00000147050	ENST00000543216, ENST00000542299, ENST00000536777, ENST00000535688, ENST00000451692, ENST00000433797, ENST00000431196, ENST00000414389, ENST00000382899, ENST00000377967, ENST00000334516	ENSP00000443078, ENSP00000444873, ENSP00000437405, ENSP00000444629, ENSP00000399980, ENSP00000398929, ENSP00000408230, ENSP00000405910, ENSP00000372355, ENSP00000367203, ENSP00000334340
SIN3A	ENSG00000169375	ENST00000570115, ENST00000568431, ENST00000568309, ENST00000568190, ENST00000567289, ENST00000565264, ENST00000564778, ENST00000562776, ENST00000394949, ENST00000394947, ENST00000360439	ENSP00000455662, ENSP00000454750, ENSP00000455644, ENSP00000456997, ENSP00000455834, ENSP00000454296, ENSP00000455204, ENSP00000455072, ENSP00000378403, ENSP00000378402, ENSP00000353622
SAP30L	ENSG00000164576	ENST00000440364, ENST00000426761, ENST00000297109	ENSP00000390927, ENSP00000416393, ENSP00000297109
FLJ20309	ENSG00000114933	ENST00000424117, ENST00000414320, ENST00000403263, ENST00000233270	ENSP00000402369, ENSP00000409031, ENSP00000384198, ENSP00000233270
RCOR2	ENSG00000167771	ENST00000301459	ENSP00000301459
ARID5A	ENSG00000196843	ENST00000454558, ENST00000412735, ENST00000359765, ENST00000357485	ENSP00000400785, ENSP00000397286, ENSP00000352808, ENSP00000350078
UBE2Q2	ENSG00000140367	ENST00000569423, ENST00000567921, ENST00000561851, ENST00000561723, ENST00000426727, ENST00000338677, ENST00000267938	ENSP00000456324, ENSP00000454742, ENSP00000456229, ENSP00000458006, ENSP00000400960, ENSP00000340187, ENSP00000267938
TRIM24	ENSG00000122779	ENST00000536822, ENST00000452999, ENST00000439939, ENST00000415680,	ENSP00000440535, ENSP00000402079, ENSP00000403347, ENSP00000390829,

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		ENST00000378381, ENST00000343526	ENSP00000367632, ENSP00000340507
BAZ2B	ENSG00000123636	ENST00000546335, ENST00000541068, ENST00000441143, ENST00000437839, ENST00000426648, ENST00000392783, ENST00000392782, ENST00000355831, ENST00000343439, ENST00000294905	ENSP00000437619, ENSP00000441341, ENSP00000393565, ENSP00000415613, ENSP00000400505, ENSP00000376534, ENSP00000376533, ENSP00000348087, ENSP00000339670, ENSP00000294905
SMYD3	ENSG00000185420	ENST00000544586, ENST00000541742, ENST00000490107, ENST00000455277, ENST00000453676, ENST00000403792, ENST00000391836, ENST00000388985	ENSP00000443400, ENSP00000444184, ENSP00000419184, ENSP00000394281, ENSP00000408122, ENSP00000385380, ENSP00000375712, ENSP00000373637
EZH2	ENSG00000106462	ENST00000541220, ENST00000536783, ENST00000492143, ENST00000483967, ENST00000483012, ENST00000478654, ENST00000476773, ENST00000460911, ENST00000350995, ENST00000320356	ENSP00000443219, ENSP00000439305, ENSP00000417377, ENSP00000419856, ENSP00000417704, ENSP00000417062, ENSP00000419050, ENSP00000419711, ENSP00000223193, ENSP00000320147
PHF1	ENSG00000239756, ENSG00000225553, ENSG00000112511	ENST00000495185, ENST00000475137, ENST00000454914, ENST00000447305, ENST00000427869, ENST00000423868, ENST00000421466, ENST00000419154, ENST00000495509, ENST00000487667, ENST00000428274, ENST00000427826, ENST00000427004, ENST00000374516, ENST00000374512	ENSP00000433516, ENSP00000434774, ENSP00000407295, ENSP00000396023, ENSP00000391901, ENSP00000399226, ENSP00000395839, ENSP00000413510, ENSP00000434347, ENSP00000432419, ENSP00000392697, ENSP00000404788, ENSP00000410494, ENSP00000363640, ENSP00000363636
PHF2	ENSG00000197724	ENST00000375376, ENST00000359246	ENSP00000364525, ENSP00000352185
BCR	ENSG00000186716	ENST00000427791, ENST00000420248, ENST00000398512,	ENSP00000396531, ENSP00000445910, ENSP00000381524,

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		ENST00000359540, ENST00000334149, ENST00000305877, ENST00000292697, ENST00000290956	ENSP00000352535, ENSP00000335450, ENSP00000303507, ENSP00000292697, ENSP00000290956
SMARCD3	ENSG0000082014	ENST00000491651, ENST00000485592, ENST00000469154, ENST00000392811, ENST00000356800, ENST00000347683, ENST00000262188	ENSP00000419886, ENSP00000417145, ENSP00000417908, ENSP00000376558, ENSP00000349254, ENSP00000173385, ENSP00000262188
BMI1	ENSG00000168283	ENST00000456675, ENST00000443519, ENST00000442508, ENST00000417470, ENST00000416820, ENST00000376691, ENST00000376663	ENSP00000401773, ENSP00000390768, ENSP00000397912, ENSP00000398759, ENSP00000399220, ENSP00000365881, ENSP00000365851
CHD6	ENSG00000124177	ENST00000440697, ENST00000440647, ENST00000373233, ENST00000373222, ENST00000309279	ENSP00000404637, ENSP00000392503, ENSP00000362330, ENSP00000362319, ENSP00000308684
FBXL11	ENSG00000173120	ENST00000530342, ENST00000529006, ENST00000446134, ENST00000398645, ENST00000308783	ENSP00000435776, ENSP00000432786, ENSP00000392902, ENSP00000381640, ENSP00000309302
SIRT7	ENSG00000187531	ENST00000576971, ENST00000576004, ENST00000575360, ENST00000572902, ENST00000328666	ENSP00000458897, ENSP00000458737, ENSP00000459524, ENSP00000461044, ENSP00000329466
ASF1A	ENSG00000111875	ENST00000229595	ENSP00000229595
RCOR3	ENSG00000117625	ENST00000534478, ENST00000534460, ENST00000533469, ENST00000529763, ENST00000529572, ENST00000528926, ENST00000485186, ENST00000452621, ENST00000419091, ENST00000367006, ENST00000367005	ENSP00000436057, ENSP00000433441, ENSP00000436838, ENSP00000437048, ENSP00000434605, ENSP00000432779, ENSP00000434181, ENSP00000398558, ENSP00000413929, ENSP00000355973, ENSP00000355972
CBX4	ENSG00000141582	ENST00000495122, ENST00000448310, ENST00000343048, ENST00000269397	ENSP00000461198, ENSP00000415348, ENSP00000345967, ENSP00000269397

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EPC1	ENSG00000120616	ENST00000375110, ENST00000319778, ENST00000263062	ENSP00000364251, ENSP00000318559, ENSP00000263062
BRD1	ENSG00000100425	ENST00000542442, ENST00000457780, ENST00000438393, ENST00000419212, ENST00000404760, ENST00000404034, ENST00000342989, ENST00000216267	ENSP00000437514, ENSP00000410042, ENSP00000388027, ENSP00000399110, ENSP00000385858, ENSP00000384076, ENSP00000345886, ENSP00000216267
BNF11	ENSG0000054938	ENST00000534276, ENST00000529912, ENST00000528789, ENST00000528471, ENST00000525413, ENST00000393519, ENST00000376332, ENST00000376324, ENST00000376323, ENST00000263671	ENSP00000432055, ENSP00000432345, ENSP00000431380, ENSP00000434589, ENSP00000434257, ENSP00000377154, ENSP00000365510, ENSP00000365502, ENSP00000365501, ENSP00000263671
HP1A (CBX5)	ENSG0000094916	ENST00000550489, ENST00000209875, ENST00000550411, ENST00000439541, ENST00000552562	ENSP00000448452, ENSP00000209875, ENSP00000449207, ENSP00000401009, ENSP00000450190
HP1B (CBX1)	ENSG00000108468	ENST00000225603, ENST00000393408, ENST00000444685, ENST00000402583	ENSP00000225603, ENSP00000377060, ENSP00000393179, ENSP00000385413
HP1G (CBX3)	ENSG00000122565	ENST00000337620, ENST00000396386, ENST00000409747, ENST00000456948	ENSP00000336687, ENSP00000379670, ENSP00000387348, ENSP00000408672

Table 6: Chromatin state biomarkers

In some embodiments, the methods for diagnosing comprise measuring expression levels of at least one biomarker selected from DTX1, HES4, CD1d, ETS1, ETV6, Runx1, Bcl-2, MYC and CD52. In some embodiments, decreased levels in a tumor sample of DTX1, HES4, and/or

- 5 CD1d compared to a control identifies a subject to be treated with a bromodomain inhibitor or a combination of agents as described herein, including, but not limited to, a bromodomain inhibitor and/or a Bcl-2 inhibitor. In some embodiments, increased levels in a tumor sample of a biomarker selected from the group consisting of ETS1, ETV6, Runx1, CD52, MYC or Bcl-2 compared to a control identifies a subject to be treated with a bromodomain inhibitor or a combination of agents as described herein, including, but not limited to, a bromodomain
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inhibitor and/or a Bcl-2 inhibitor. Expression levels of biomarkers could be, for example, mRNA and/or protein levels.

In some embodiments, more than one biomarker selected from DTX1, HES4, CD1d, ETS1, ETV6, Runx1, Bcl-2, MYC and CD52 is measured and the diagnosis or identification of 5 a subject is made based on 2 or more biomarkers. These biomarkers are found in Table 7.

Gene	Ensembl gene ID	Ensembl transcript ID	Ensembl protein ID
DTX1	ENSG00000135144	ENST00000257600, ENST00000547974, ENST00000553140, ENST00000547730, ENST00000548759	ENSP00000257600
HES4	ENSG00000188290	ENST00000304952, ENST00000428771, ENST00000484667, ENST00000481869	ENSP00000304595, ENSP00000393198, ENSP00000425085
CD1d	ENSG00000158473	ENST00000368171	ENSP00000357153
ETS1	ENSG00000134954	ENST00000392668, ENST00000319397, ENST00000531611, ENST00000526145, ENST00000345075, ENST00000535549, ENST00000525404, ENST00000527676, ENST00000530924	ENSP00000376436, ENSP00000324578, ENSP00000435666, ENSP00000433500, ENSP00000340485, ENSP00000441430
ETV6	ENSG00000139083	ENST00000396373, ENST00000545027, ENST00000266427, ENST00000544715, ENST00000541426	ENSP00000379658, ENSP00000441463, ENSP00000266427
Runx1	ENSG00000159216	ENST00000344691 ENST00000300305 ENST00000416754 ENST00000358356 ENST00000399240 ENST00000455571 ENST00000399237 ENST00000325074 ENST00000437180 ENST00000486278 ENST00000482318 ENST00000479325 ENST00000467577 ENST00000475045 ENST00000468726	ENSP00000340690, ENSP00000300305, ENSP00000405158, ENSP00000351123, ENSP00000382184, ENSP00000388189, ENSP00000382182, ENSP00000319459, ENSP00000409227, ENSP00000438019

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		ENST00000494829 ENST00000467692 ENST00000460207 ENST00000469087	
Bcl-2	ENSG00000171791	ENST00000398117, ENST00000333681, ENST00000589955, ENST00000444484, ENST00000590515	ENSP00000381185, ENSP00000329623, ENSP00000466417, ENSP00000404214
MYC	ENSG00000136997	ENST00000377970, ENST00000259523, ENST00000517291, ENST00000524013, ENST00000520751	ENSP00000367207, ENSP00000259523, ENSP00000429441, ENSP00000430235, ENSP00000430226
CD52	ENSG00000169442	ENST00000374213, ENST00000470468, ENST00000492808	ENSP00000363330

Table 7. DTX1, HES4, CD1d, ETS1, ETV6, Runx1, Bcl-2, MYC and CD52 Biomarkers

In some embodiments, the methods for diagnosing comprise measuring a level of histone modification. Histone modifications can be detected, e.g., using chromatin immunoprecipitation sequencing (ChIP-Seq). In some embodiments, the histone modification is selected from H3K27me3, H3K9me3, and H3K27Ac. In some embodiments, decreased levels in a tumor sample of H3K27Ac histone modification compared to a control identifies a subject to be treated with a bromodomain inhibitor or a combination of agents as described herein, including, but not limited to, a bromodomain inhibitor and/or a Bcl-2 inhibitor. In some embodiments, increased levels in a tumor sample of a histone modification from the group consisting of H3K27me3 and H3K9me3 compared to a control identifies a subject to be treated with a bromodomain inhibitor and/or a combination of agents as described herein, including, but not limited to, a bromodomain inhibitor or a combination of agents as described herein, including, but not limited to, a bromodomain inhibitor and/or a Bcl-2 inhibitor.

In some embodiments, the histone modification is H3K4me1. In some embodiments, elevated levels of H3K4me1 histone modification at a site of elevated H3K27Ac histone modification in the tumor sample compared to the control identifies a subject to be treated with a bromodomain inhibitor or a combination of agents as described herein, including, but not limited to, a bromodomain inhibitor and/or a Bcl-2 inhibitor.

EXAMPLES

Example 1: Modeling and Characterization of Drug Resistance in T-ALL**Methods:**

5 Notch inhibitor sensitive T-ALL cell lines were cultured in the presence of 1 μ M GSI in vitro. Cells were plated at 0.25×10^6 /ml in 20 mL and split every 3 – 4 days. Cell viability, cell size and cell cycle were measured every 3 – 4 days. GSI treated cells demonstrated decreased proliferation as measured by decreased fold expansion after 7 to 14 days. GSI-treated cells stopped proliferating between day 14 to 30 of culture, but eventually started proliferating again
10 despite the presence of GSI with subsequent growth rates being equal or similar to untreated cells.

For RNA expression profiles, cells were washed once in PBS and then snap frozen. RNA was isolated using the Qiagen RNeasy kit. Expression profiles were obtained with the Affymetrix HU133+2 array.

15 To measure metabolites, cell culture supernatant was taken on day 1, 2, 3, 4, 5 of culture and frozen. Metabolites were measured by mass spectrometry and results were adjusted to input metabolites in media and fold expansion over time.

Cell size was determined by measuring the forward/side scatter on a FACS Calibur (BD Biosciences). To determine nuclear size, cells were spun on slides using a Cytospin centrifuge,
20 then fixed and permeabilized prior to staining with DAPI or anti-Actin antibody. Staining intensity was measured by fluorescent microscopy.

HP1 protein expression was measured by performing Western Blot analysis using the iblot® system (Invitrogen) and enhanced chemiluminescence (ECL) for visualization.

To measure chromatin compaction, naïve or resistant cells were washed once in PBS,
25 lysed and nuclei were isolated using a sucrose gradient. Samples were adjusted for equal nuclear size prior to digestion with MNase. Digests were done for increasing time intervals. DNA was isolated by phenol/chloroform extraction and nucleosomes run on a gel and visualized by SYBR® Safe DNA gel stain (Invitrogen).

Global histone levels were measured by enzyme-linked immunosorbent assay (ELISA)
30 after acid extraction of histones.

For ChIP-Seq, cells were crosslinked in 1% formaldehyde for 15 minutes. Cells were lysed and sonicated with a Branson 250 to a size range of 200 to 700 bp. Chromatin was immunoprecipitated with antibody against K4me1, H3K27ac or BRD4.

Libraries were prepared from ~5 nanograms of ChIP DNA, loaded onto flow cells and sequenced on the Illumina HiSeq Genome Analyzer by standard procedures. Reads were aligned to the reference (hg19) human genome. For data analysis, enrichment profiles were generated for each histone modification. Briefly, aligned reads were extended to 300 bases to approximate 5 the average ChIP fragment. Signal was then estimated at any given position (25 bp resolution) as the number of sequenced ChIP fragments that overlap that position. A sliding window approach was used to identify significantly enriched intervals or ‘peaks’ from each dataset.

Results:

10 A gamma secretase inhibitor (GSI)-resistant leukemia cell population was produced using the methods above. Several biological parameters were compared between the GSI-resistant leukemia cells and naïve leukemia cells (those not treated with a GSI).

15 Firstly, RNA expression profiles of naïve cells and resistant cells were compared using the methods described above. Gene set enrichment analysis (GSEA) was performed and revealed a shift from Myc-dependent signaling to JNK/Map kinase dependent signaling (FIG. 1).

20 Secondly, the metabolic state of resistant versus naïve cells was compared as described above. The ratio of lactate production to glucose consumption was found to be higher in naïve cells than in resistant cells whereas the ratio of glutamate production to glutamine consumption was found to be lower in naïve cells than in resistant cells (FIG. 2).

Thirdly, the nuclear and cellular morphology of resistant versus naïve cells was compared as described above. Forward-scatter FACS analysis revealed that resistant cells were smaller than naïve cells (FIG. 3A). DAPI staining showed that resistant cells contained smaller nuclei than naïve cells (FIG. 3B).

25 The smaller nuclear size of resistant cells prompted an investigation of the chromatin status in naïve versus resistant cells. HP1-gamma, a marker of global chromatin compaction was measured using Western blot analysis as described above. A higher level of HP1-gamma was found in resistant cells and those treated for a short period of time with a GSI indicating a higher level of chromatin compaction compared to naïve cells (FIG. 4A). Additionally, a partial 30 MNase digestion was performed as described above and showed that resistant cells are protected from MNase digestion, indicating a higher level of chromatin compaction compared to naïve cells (FIG. 4B).

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To further analyze the chromatin state in naïve versus resistant cells, the methylation status of certain histone residues that are indicative of the level of chromatin compaction were examined. Tri-methylation of lysine 9 and lysine 27 of histone 3 (H3K9me3 and H3K27me3, respectively) are markers of compacted heterochromatin (a repressed chromatin state). As depicted in FIG. 5A, when assessing global levels of these repressive chromatin marks, both H3K9me3 and H3K27me3 levels were found to be elevated in resistant cells as compared to naïve cells, indicating that resistant had more compacted heterochromatin than naïve cells.

Differences in the chromatin regulatory network between naïve and resistant cells were assessed by determining relative expression levels of chromatin state biomarkers. As depicted in FIG. 5B, expression levels of certain chromatin state biomarkers were increased in resistant cells as compared to naïve cells. The chromatin state biomarkers with increased expression levels were UTX, SIN3A, SAP30L, FLJ20309, RCOR2, ARID5A, UBE2Q2, TRIM24, BAZ2B, SMYD3, EZH2, PHF1, PHF2, BCR, SMARCD3, BMI1, CHD6, FBXL11, SIRT7, ASF1A, RCOR3, CBX4, EPC1, BRD1, and BNF11. Such chromatin state biomarkers may be associated with a repressed heterochromatin state.

Conversely, relative expression levels of certain chromatin state biomarkers were decreased in resistant cells as compared to naïve cells. These chromatin state biomarkers included NPM1, NARG1, RCC1, SSRP1, PRMT3, SAP30, CBX6, CHMP2B, UBE2M, WDR77, HMGB1, CARM1, USP13, HDAC4, COQ3, SET, GATAD2A, PRMT6, HMG20B, DNMT1, ADA, SS18, UBE3A, ZMYND11, and NOC2LL. Such chromatin state biomarkers may be associated with promoting an active, open chromatin state.

Example 2: A BRD inhibitor blocks proliferation of and kills resistant leukemia cells in vitro and in vivo

25 Methods:

Notch inhibitor sensitive and resistant T-ALL cell lines were plated in 96 well plates and treated with vehicle, 1 uM GSI and/or increasing concentrations of the bet inhibitor JQ1. Cell viability was determined by measuring luminescence after CelltiterGlo® (Promega) addition on day 6. Apoptosis was measured using CaspaseGlo® (Promega) on day 4.

30 Luciferized KOPTK1 cells were injected into NOD/SCID mice by tail vein injection. Tumor burden was measured by following the bioluminescence signal in the mouse body. Following KOPTK1 cell engraftment, mice were treated with a GSI called dibenzazepine (DBZ), JQ1, DBZ + JQ1, or a vehicle control.

Balb/c mice were treated with DBZ + JQ1, DBZ, or vehicle alone. Hyperproliferation of globlet cells was measured by Periodic acid-Schiff (PAS) stain.

Results:

5 One gene from Example 3, BRD4, was chosen as an example chromatin regulatory factor for further analysis in vitro and in vivo. BRD4 was found to be upregulated in leukemia cells that were resistant to a GSI and leukemia cells that underwent short-term treatment with a GSI compared to naïve leukemia cells (FIG. 6). Treatment with a bromodomain inhibitor, JQ1, resulted in a decrease in proliferation and an increase in apoptosis in resistant leukemia cells
10 compared to naïve leukemia cells (FIG. 7).

Chromatin Immunoprecipitation (ChIP) with sequencing (Seq) was performed using a BRD4 antibody to identify regions of the genome that were associated with BRD4. BRD4 was found to be associated with enhancers that were also shown to have histone marks H3K4me1 and H3K27ac (FIG. 8). Of interest, BRD4 was found to be associated with the enhancer region
15 of BCL-2, a known anti-apoptotic gene (FIG. 9A). Bcl-2 protein was found to be upregulated in GSI resistant leukemia cells compared to naïve leukemia cells (FIG. 9B). Treatment with the bromodomain inhibitor JQ1 resulted in a decreased level of Bcl-2 protein in resistant cells (FIG.
9B).

Lastly, in vivo animal studies were performed on preclinical model of T-ALL using
20 NOD/SCID mice. Mice were injected via tail-vein with luciferized KOPTK1 cells and engraftment was monitored by bioluminescence. Following leukemia cell engraftment, mice were treated with a GSI dibenzazepine (DBZ), JQ1, DBZ + JQ1, or a vehicle control. As shown in FIG. 10, the combination of DBZ + JQ1 greatly reduced the tumor burden compared to either drug alone or the control. Additionally, side effects normally associated with GSI treatment,
25 namely gastrointestinal damage, were reduced in normal BALB/c mice with the combination of DBZ + JQ1 compared to use of DBZ alone. Mice treated with DBZ showed significant disruption of intestinal villi architecture caused by hyperproliferation of globlet cells, as shown by Periodic acid-Schiff (PAS) stain (FIG. 11). Addition of JQ1 largely ameliorated villi architecture by partially restoring proper enterocyte differentiation.

30 The results indicate that bromodomain inhibitors and Bcl-2 inhibitors are a useful treatment for cancers with a Notch pathway activation mutation or cancers that are resistant to treatment with a Notch pathway inhibitor.

Example 3: An epigenetic mechanism of resistance to targeted therapy in T-cell acute lymphoblastic leukemia

T-ALL is an aggressive malignancy with significant rates of therapy failure that is frequently associated with activating mutations in *NOTCH1*, a critically important oncogene in this disease. Gamma secretase inhibitors (GSIs) that inhibit NOTCH1 cleavage and activation have been tested in clinical trials and mouse models, but responses have been modest and transient (Palomero, T. & Ferrando, A. Therapeutic targeting of NOTCH1 signaling in T-cell acute lymphoblastic leukemia. *Clin Lymphoma Myeloma* 9 Suppl 3, S205-210 (2009)). To understand mechanisms by which T-ALL cells overcome chronic Notch1 inhibition, GSI-resistance was modeled *in vitro* and *in vivo*, and the functional and molecular characteristics of resistant cells were investigated. When Notch-dependent T-ALL cells were treated with GSI *in vitro* a majority of cells stopped proliferating over the course of two weeks, but a fraction persisted and recovered their growth capacity. These ‘persister’ cells tolerated concentrations of GSI (compound E) more than 50-fold higher than the IC₅₀, suggesting that they have become resistant to Notch inhibition. In order to address the mechanism of drug resistance, it was determined whether acquired resistance is fixed or reversible. It was found that, upon removal of GSI, the persisters re-express Notch target genes within a week (FIG. 12). Retreatment of these reversed cells with GSI leads to cell cycle arrest in a pattern similar to that seen for naïve T-ALL cells. Furthermore, the re-treated cells eventually acquired resistance with similar kinetics as the naïve population. The reversibility of acquired GSI tolerance in this model suggests that the resistance phenotype is mediated epigenetically.

To characterize the drug tolerant cells, the active intracellular form of NOTCH1 (ICN) (Guruharsha, K. G., Kankel, M. W. & Artavanis-Tsakonas, S. The Notch signalling system: recent insights into the complexity of a conserved pathway. *Nat Rev Genet* 13, 654-666 (2012)). ICN was examined and found to be present at high levels in naïve T-ALL cells, but is essentially undetectable in the persisters (FIG. 12). The persisters re-expressed ICN when GSI was removed, consistent with their reversible phenotype. Expression of Notch target genes followed a similar pattern: *DTX1* and *HES4* were profoundly down-regulated in the persisters (FIG. 12), but gradually recover after removal of GSI. Induction of MYC is thought to be a major mechanism by which constitutive Notch activation leads to malignancy in T-ALL (Weng, A. P. et al. c-Myc is an important direct target of Notch1 in T-cell acute lymphoblastic leukemia/lymphoma. *Genes Dev* 20, 2096-2109 (2006) and Palomero, T. et al. NOTCH1 directly regulates c-MYC and activates a feed-forward-loop transcriptional network promoting

leukemic cell growth. Proc Natl Acad Sci U S A 103, 18261-18266 (2006)). Although MYC levels are dramatically reduced by short term GSI treatment, expression of this oncoprotein recovers modestly in the persisters (FIG. 12). Nonetheless, gene expression profiles indicated that the persisters had lost the predominating MYC transcriptional signature of naïve T-ALL 5 cells. These data suggest that the persisters had acquired the ability to proliferate in the absence of Notch signaling, but maintained modest MYC activity through alternative means.

In contrast to the reduced MYC signature, gene expression profiles revealed enhanced signatures for MAPK, JNK, PI3K and mTOR signaling in the persisters. Genetic alterations of PTEN resulting in increased AKT pathway activity have been associated with Notch resistance 10 (Gutierrez, A. et al. High frequency of PTEN, PI3K, and AKT abnormalities in T-cell acute lymphoblastic leukemia. Blood 114, 647-650 (2009) and Palomero, T. et al. Mutational loss of PTEN induces resistance to NOTCH1 inhibition in T-cell leukemia. Nat Med 13, 1203-1210 (2007)). Although the T-ALL cell lines modeled here do not harbor PTEN mutations, the persisters have increased levels of phosphorylated PTEN and, accordingly, are more sensitive to 15 treatment with an AKT inhibitor (FIG. 13). mTOR has been demonstrated to be active in leukemia, including Notch-dependent T-ALL (Chan, S. M., Weng, A. P., Tibshirani, R., Aster, J. C. & Utz, P. J. Notch signals positively regulate activity of the mTOR pathway in T-cell acute lymphoblastic leukemia. Blood 110, 278-286 (2007) and Kalaitzidis, D. et al. mTOR complex 1 plays critical roles in hematopoiesis and Pten-loss-evoked leukemogenesis. Cell Stem Cell 11, 20 429-439 (2012)). The persisters showed increased levels of the phosphorylated form p2481 of mTOR and sensitivity to the mTOR inhibitor Rapamycin (FIG. 14). These alterations in cytoplasmic signaling were accompanied by changes in the metabolic profiles indicative of a shift from a predominantly glycolytic state in naïve T-ALL cells to a greater reliance on 25 oxidative phosphorylation in the persisters (FIG. 2). These data suggest that rewired signaling and metabolic programs in the persister cells are critical for proliferation in the absence of Notch signaling.

The persistence phenotype was also notable for morphologic changes, including a profound decrease in cell and nuclear size, which was reversible after removal of GSI (FIGS. 3A-C). It was postulated that these changes might reflect global chromatin compaction 30 associated with exposure and subsequent tolerance to Notch inhibition. In support of this view, it was found that the persisters up-regulate heterochromatin-associated HP1 proteins and have high global levels of repressive chromatin modifications (FIG. 4A). To evaluate global compaction more directly, chromatin was digested from naïve and persister cells with micrococcal nuclease.

It was found that chromatin in the persisters is relatively inaccessible and has a longer average nucleosomal repeat length indicative of linker histone H1 incorporation (FIG. 4B), as is consistent with a more compact chromatin state. Recent links drawn between metabolism and chromatin regulation (Lu, C. & Thompson, C. B. Metabolic regulation of epigenetics. *Cell Metab* 16, 9-17 (2012)) prompted evaluation of the relationships between metabolic changes and chromatin compaction. It was found that glucose restriction led to increased chromatin compaction in naïve T-ALL cells. It was also found that chromatin compaction was an early consequence of Notch inhibition, with rapid up-regulation of HP1 protein expression upon GSI exposure (FIG. 4A). The functional significance of chromatin state changes in T-ALL persister cells was then investigated further.

First, histone modifications were profiled in naïve and persister T-ALL cells by ChIP-seq. Marks associated with promoters (H3K4me3), transcripts (H3K36me3), enhancers (H3K4me1, H3K27ac) and Polycomb-repressed loci (H3K27me3) were surveyed (Zhou, V. W., Goren, A. & Bernstein, B. E. Charting histone modifications and the functional organization of mammalian genomes. *Nat Rev Genet* 12, 7-18 (2011) and Dunham, I. et al. An integrated encyclopedia of DNA elements in the human genome. *Nature* 489, 57-74 (2012)). Striking differences in chromatin state were evident within loci encoding Notch target genes, including CD300A and DELTEX1. Promoter, transcript and enhancer chromatin signals in these loci were markedly reduced in the persisters, consistent with reduced gene expression¹⁷. Conversely, chromatin activity was increased within certain loci, such as MAP3K5, that are induced in the persisters. In addition, the persisters exhibited a modest increase in H3K27me3 levels over euchromatic loci and a reduction in bulk H3K27ac levels (FIG. 5A). Thus, acquisition of GSI tolerance is associated with focal chromatin changes at differentially-regulated loci and a genome-wide reduction of accessible euchromatin.

It was postulated that their altered chromatin state might uncover new susceptibilities in the persister cells that could be targeted by emerging epigenetic therapies. To test this, a lentiviral short-hairpin RNA (shRNA) knockdown screen was designed that targeted ~350 chromatin regulators with an average of 5 independent hairpins per gene (FIG. 15). About 15 genes were identified for which knockdown compromised survival in both naïve and persister cells, and thus presumed to be generally required for T-ALL cells. Riger analysis was then used to identify genes preferentially required for survival of either naïve or persister cells. Naïve cells were found to be preferentially dependent on several histone deactylase (HDACs) enzymes, while the persister cells showed a greater dependence on other genes, including several arginine

methyltransferases (FIG. 15). The chromatin regulatory proteins identified for the persister cells include: ARID3B, EZH2, PRMT2, SND1, BRD1, SUV39H1, PRMT5, SS18, BRD4, KDM5D, PRMT7, STAG3L1, CD2BP2, MLL5, SUDS3, CHD1, MINA, CHD8, MORF4L1, or CHRAC1. The gene name and transcript IDs provided in Table 8 can be used to identify the shRNA sequences used by searching the RNAi Consortium (TRC) Portal (Broad Institute, Cambridge, MA).

GENE NAME	GENE ID	TRANSCRIPT ID	PROTEIN ID
ARID3B	ENSG00000179361	ENST00000346246, ENST00000566147, ENST00000563567, ENST00000569680, ENST00000566468	ENSP00000343126, ENSP00000455668
EZH2	ENSG00000106462	ENST00000483967, ENST00000320356, ENST00000478654, ENST00000476773, ENST00000460911, ENST00000350995, ENST00000536783, ENST00000541220, ENST00000492143, ENST00000483012, ENST00000498186, ENST00000469631	ENSP00000419856, ENSP00000320147, ENSP00000417062, ENSP00000419050, ENSP00000419711, ENSP00000223193, ENSP00000439305, ENSP00000443219, ENSP00000417377, ENSP00000417704
PRMT2	ENSG00000160310	ENST00000355680 ENST00000334494 ENST00000397637 ENST00000397638 ENST00000455177 ENST00000397628 ENST00000440086 ENST00000291705 ENST00000451211 ENST00000458387 ENST00000498151 ENST00000491389 ENST00000482508 ENST00000481861 ENST00000486520	ENSP00000347906 ENSP00000335490 ENSP00000380759 ENSP00000380760 ENSP00000406127 ENSP00000380752 ENSP00000397266 ENSP00000291705 ENSP00000411984 ENSP00000407463
SND1	ENSG00000197157	ENST00000354725, ENST00000486037, ENST00000461056, ENST00000468621, ENST00000483503, ENST00000492772, ENST00000468166, ENST00000465900, ENST00000467238,	ENSP00000346762, ENSP00000419327

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		ENST00000470723, ENST00000470463, ENST00000485871, ENST00000484767, ENST00000492840, ENST00000489417, ENST00000463020	
BRD1	ENSG00000100425	ENST00000542442, ENST00000457780, ENST00000438393, ENST00000419212, ENST00000404760, ENST00000404034, ENST00000342989, ENST00000216267	ENSP00000437514, ENSP00000410042, ENSP00000388027, ENSP00000399110, ENSP00000385858, ENSP00000384076, ENSP00000345886, ENSP00000216267
SUV39H1	ENSG00000101945	ENST00000376687, ENST00000337852, ENST00000453214, ENST00000482260, ENST00000462786	ENSP00000365877, ENSP00000337976, ENSP00000410686
PRMT5	ENSG00000100462	ENST00000324366, ENST00000397440, ENST00000557443, ENST00000397441, ENST00000216350, ENST00000553897, ENST00000553550, ENST00000554910, ENST00000553502, ENST00000556043, ENST00000555530, ENST00000555454, ENST00000454731, ENST00000421938, ENST00000556616, ENST00000554867, ENST00000538452, ENST00000556426, ENST00000553915, ENST00000557415, ENST00000553787, ENST00000476175, ENST00000554716, ENST00000553641, ENST00000557015, ENST00000557758, ENST00000553417, ENST00000556032	ENSP00000319169, ENSP00000380582, ENSP00000452501, ENSP00000380583, ENSP00000216350, ENSP00000452555, ENSP00000450737, ENSP00000452411, ENSP00000450956, ENSP00000452509, ENSP00000452409, ENSP00000451245, ENSP00000387663, ENSP00000409482, ENSP00000450919, ENSP00000452218, ENSP00000444915, ENSP00000451127, ENSP00000450633, ENSP00000452102
SS18	ENSG00000141380	ENST00000269137, ENST00000542420, ENST00000415083, ENST00000581021, ENST00000584083,	ENSP00000269137, ENSP00000438066, ENSP00000414516, ENSP00000463586, ENSP00000463943,

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		ENST00000579061, ENST00000539849, ENST00000542743, ENST00000545952, ENST00000579640, ENST00000585121, ENST00000539244, ENST00000269138, ENST00000582448, ENST00000578700, ENST00000577572, ENST00000578954, ENST00000581570, ENST00000582792, ENST00000580751, ENST00000580642, ENST00000577636, ENST00000577751, ENST00000583595, ENST00000578595, ENST00000585241, ENST00000582092, ENST00000580003, ENST00000580958	ENSP00000462766, ENSP00000444647, ENSP00000444551, ENSP00000443097, ENSP00000462363, ENSP00000462838, ENSP00000441760, ENSP00000269138, ENSP00000464609, ENSP00000464673, ENSP00000463802, ENSP00000464664, ENSP00000464556, ENSP00000463928, ENSP00000464049, ENSP00000462104, ENSP00000463933
BRD4	ENSG00000141867	ENST00000371835, ENST00000360016, ENST00000263377,	ENSP00000360901, ENSP00000353112, ENSP00000263377
KDM5D	ENSG00000012817	ENST00000317961, ENST00000382806, ENST00000447300, ENST00000440077, ENST00000415360, ENST00000535647, ENST00000541639, ENST00000492117, ENST00000469599, ENST00000485154, ENST00000478891	ENSP00000322408, ENSP00000372256, ENSP00000416377, ENSP00000398543, ENSP00000389433, ENSP00000445530, ENSP00000444293
PRMT7	ENSG00000132600	ENST00000339507, ENST00000563562, ENST00000449359, ENST00000565745, ENST00000566657, ENST00000569571, ENST00000569047, ENST00000348497, ENST00000441236, ENST00000568975, ENST00000562050, ENST00000566341, ENST00000562381	ENSP00000343103, ENSP00000455238, ENSP00000414716, ENSP00000456190, ENSP00000454980, ENSP00000455538, ENSP00000456848, ENSP00000345775, ENSP00000409324, ENSP00000454776, ENSP00000457381, ENSP00000455705, ENSP00000456364
STAG3L1	ENSG00000205583	ENST00000404291, ENST00000487154,	

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		ENST00000402225, ENST00000456374, ENST00000338421, ENST00000339898, ENST00000436837	
CD2BP2	ENSG00000169217	ENST00000305596, ENST00000569466, ENST00000564525	ENSP00000304903, ENSP00000456935
MLL5	ENSG00000005483	ENST00000311117, ENST00000476671, ENST00000473063, ENST00000495267, ENST00000478990, ENST00000474203, ENST00000257745, ENST00000334877, ENST00000334914, ENST00000334884, ENST00000482560, ENST00000478079, ENST00000480368, ENST00000485619, ENST00000468607, ENST00000496191, ENST00000479838	ENSP00000312379, ENSP00000417888, ENSP00000417156, ENSP00000420415, ENSP00000419883, ENSP00000420206, ENSP00000257745, ENSP00000335599, ENSP00000333986, ENSP00000335398, ENSP00000417193, ENSP00000419525
SUDS3	ENSG00000111707	ENST00000543473, ENST00000397564, ENST00000541591, ENST00000541280, ENST00000360286	ENSP00000443988, ENSP00000380695
CHD1	ENSG00000153922	ENST00000284049, ENST00000512844, ENST00000505657, ENST00000511067, ENST00000514344, ENST00000512392, ENST00000511628, ENST00000513064, ENST00000508756, ENST00000414220, ENST00000505982	ENSP00000284049, ENSP00000422589, ENSP00000422225
MINA	ENSG00000170854	ENST00000394198, ENST00000333396, ENST00000360258, ENST00000507612, ENST00000503097, ENST00000506099, ENST00000330299, ENST00000514314, ENST00000506682, ENST00000503517	ENSP00000377748, ENSP00000328251, ENSP00000353395, ENSP00000424530, ENSP00000421347, ENSP00000423816, ENSP00000327424, ENSP00000424955
CHD8	ENSG00000100888	ENST00000430710, ENST00000557364,	ENSP00000406288, ENSP00000451601,

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		ENST00000553622, ENST00000555935, ENST00000553283, ENST00000553870, ENST00000399982, ENST00000553651, ENST00000555962, ENST00000556833, ENST00000557727, ENST00000557329, ENST00000554384, ENST00000555301	ENSP00000450957, ENSP00000451442, ENSP00000450860, ENSP00000451071, ENSP00000382863
MORF4L1	ENSG00000185787	ENST00000331268, ENST00000426013, ENST00000559345, ENST00000379535, ENST00000560422, ENST00000559690, ENST00000559158, ENST00000559930, ENST00000559244, ENST00000559751, ENST00000558746, ENST00000558830, ENST00000558502, ENST00000559697, ENST00000558539, ENST00000561171, ENST00000558893, ENST00000558522, ENST00000559258, ENST00000557961, ENST00000559619, ENST00000558923, ENST00000560710	ENSP00000331310, ENSP00000408880, ENSP00000452717, ENSP00000368850, ENSP00000453625, ENSP00000453351, ENSP00000453432, ENSP00000454191, ENSP00000454030, ENSP00000453972, ENSP00000453231, ENSP00000453738, ENSP00000452808
CHRAC1	ENSG00000104472	ENST00000220913, ENST00000519533, ENST00000518971, ENST00000519618, ENST00000523569	ENSP00000220913, ENSP00000428697, ENSP00000430484, ENSP00000430003

Table 8. Chromatin Regulatory Proteins for which the persister cells showed a greater dependence

BRD4 was identified as a top hit in the screen, with 3 shRNAs significantly reducing
5 persister cell proliferation without affecting the naïve population. BRD4 is a member of the BET family of bromodomains that selectively bind acetylated histones and mediate epigenetic gene regulation (Dawson, M. A. & Kouzarides, T. Cancer epigenetics: from mechanism to therapy. Cell 150, 12-27 (2012) and Zhao, R., Nakamura, T., Fu, Y., Lazar, Z. & Spector, D. L.

Gene bookmarking accelerates the kinetics of post-mitotic transcriptional re-activation. *Nat Cell Biol* 13, 1295-1304 (2011)). BRD4 has been implicated in several malignancies, including acute myeloid leukemia and lymphoma (Blobel, G. A., Kalota, A., Sanchez, P. V. & Carroll, M. Short hairpin RNA screen reveals bromodomain proteins as novel targets in acute myeloid leukemia.

- 5 Cancer Cell 20, 287-288 (2011) and Zuber, J. et al. RNAi screen identifies Brd4 as a therapeutic target in acute myeloid leukemia. *Nature* 478, 524-528 (2011)). It was confirmed that the shRNAs silence BRD4 at protein levels and also replicated their selective effect on persister cell survival. The exquisite dependency of GSI tolerant persister cells on BRD4 is of particular interest given the recent development of small molecule BET inhibitors, such as JQ1
- 10 (Filippakopoulos, P. et al. Selective inhibition of BET bromodomains. *Nature* 468, 1067-1073 (2010) and Nicodeme, E. et al. Suppression of inflammation by a synthetic histone mimic. *Nature* 468, 1119-1123 (2010)). Indeed, it was found that the persisters undergo proliferation arrest in response to JQ1 concentrations that are well tolerated by naïve T-ALL cells (FIG. 7), whereas a JQ1 enantiomer had no effect. Thus, chromatin state changes in the persister cells are
- 15 accompanied by markedly increased sensitivity to inhibition of BRD4, a ‘reader’ of acetylated chromatin.

To investigate its regulatory functions, BRD4 was mapped genome-wide in persister cells. BRD4 bound mainly to promoters (~30% of sites) and putative enhancers enriched for H3K4me1 and H3K27ac (~60%), consistent with its biochemical affinity for acetylated histones.

- 20 In contrast, sites of open chromatin marked exclusively by H3K4me1 were rarely bound by BRD4. BRD4-bound enhancers were enriched near genes with functions related to phosphoprotein signaling (539/989 genes; 53%), including many in the AKT and mTOR pathways. The binding sites were also found to be enriched for consensus motifs for ETS family and RUNX transcription factors (FIG. 16). Consistently, RT-PCR revealed increased expression 25 of ETS1, ETV6 and RUNX1 in the persister cells (FIG. 16B). Our analysis suggests that BRD4 mediates gene regulatory programs required for the proliferation of drug tolerant T-ALL cells. The global chromatin compaction in the persister cells may render enhancers and their gene targets particularly reliant on BRD4 for their epigenetic maintenance.

- Next, individual genes that might account for the BRD4 dependency of the persister cells 30 were examined. MYC was focused on first, as it is a known BRD4 target (Filippakopoulos, P. et al. Selective inhibition of BET bromodomains. *Nature* 468, 1067-1073 (2010)), whose expression is sustained in the persister cells, albeit at a lower level compared to the naïve. It was postulated that increased AKT and mTOR signaling might maintain MYC expression in these

cells. Indeed, a small molecule inhibitor of the mTOR pathway markedly reduce MYC expression in persister cells, but have essentially no effect in naïve cells (FIG. 17A). BRD4 bound several putative enhancers in the *MYC* locus (FIG. 17B). JQ1 significantly reduced MYC expression in the persisters at doses that did not alter MYC in naïve cells (FIG. 17A).

5 Thus, altered mechanisms of MYC activation appear to sensitize persister cells to BRD4 inhibition.

The anti-apoptosis regulator *BCL2* is an established BRD4-dependent gene in mixed lineage leukemia (Dawson, M. A. et al. Inhibition of BET recruitment to chromatin as an effective treatment for MLL-fusion leukemia. *Nature* 478, 529-533 (2011)). Intense BRD4 binding is evident throughout the *BCL2* locus (FIG. 9A and 17B), which is highly expressed in the drug tolerant T-ALL cells. JQ1 treatment significantly reduced Bcl-2 expression in these cells, but had little effect on the naïve population (FIG. 9B). It was reasoned that loss of Bcl-2 might account for the apoptosis seen in persisters treated with JQ1 (FIG. 7). In support of this model, the Bcl-2 inhibitor ABT-737 effectively killed persister cells (FIG. 18A). Furthermore, 10 Bcl-2 over-expression partially rescues persister cells from JQ1 treatment (FIG. 18B). Hence, down-regulation of this survival gene is critical for JQ1-induced cell death in drug tolerant T-ALL cells.

To investigate the *in vivo* relevance of the GSI resistance and associated epigenetic changes, KOPT-K1 T-ALL cells with a mcherry marker were injected orthotopically into NOD-SCID mice and the bioluminescence was followed over time. GSI resistance developed rapidly *in vivo* after a short period of slowed tumor growth. ICN levels were drastically reduced in bone marrow of GSI-treated mice and Notch target genes were down-regulated in the corresponding leukemia cells, indicating that resistance is not due to Notch reactivation (FIG. 19A). The '*in vivo* persisters' also share other phenotypic characteristics with their *in vitro* counterparts, 20 including increased HP1 γ , Bcl-2 and CD52 expression and decreased CD1d expression, suggestive of a common mechanism (FIG. 19B). Primary T-ALL cells were also examined for their sensitivity to the combination of GSI and JQ1. Growth arrest was observed in two-thirds of cases, and this was associated with marked reduction in BCL2 expression (FIG. 20). These data support the *in vivo* relevance of GSI drug tolerance and a shared mechanism of BET 25 inhibitor sensitivity in primary T-ALL.

Finally, the population level dynamics of naïve T-ALL cells exposed to GSI were considered. The initial response to drug appears relatively homogeneous, with near complete loss of activated Notch, rapid chromatin compaction and a marked reduction in the size

distribution of the population. However, striking cell-to-cell variability was observed in terms of the efficiency with which naïve cells acquire resistance. Specifically, when single cell clones were isolated and treated with GSI, it was found that ~3% of clones in the starting population could resume growth in the presence of drug. The single cell persister clones shared phenotypic
5 markers with persisters derived by treating naïve T-ALL populations (FIG. 21). The reversibility of the persister phenotype indicates that these clones are distinguished by epigenetic as opposed to genetic alterations. This suggests that while Notch inhibition elicits a relatively uniform response, GSI resistance may depend on a pre-existing population of epigenetically distinct T-ALL cells able to engage requisite resistance mechanisms.

10 Therapeutic resistance plagued early GSI trials in humans (Palomero, T. & Ferrando, A. Therapeutic targeting of NOTCH1 signaling in T-cell acute lymphoblastic leukemia. Clin Lymphoma Myeloma 9 Suppl 3, S205-210 (2009)) and is a major challenge in cancer treatment today, pertinent to conventional chemotherapy and targeted therapy alike (Haber, D. A., Gray, N. S. & Baselga, J. The evolving war on cancer. Cell 145, 19-24 (2011)). As shown herein, T-
15 ALL cells can acquire GSI resistance by a fully reversible epigenetic mechanism reminiscent of a previously established model of drug tolerant lung cancer cells (Sharma, S. V. et al. A chromatin-mediated reversible drug-tolerant state in cancer cell subpopulations. Cell 141, 69-80 (2010)). GSI resistance in T-ALL is mediated through rewired signaling and metabolic pathways, and a dramatic chromatin state transition that uncovers a new therapeutic sensitivity
20 to BET inhibition. The data shown herein suggests that the heightened sensitivity of persister cells to BET inhibition reflects a stringent requirement for BRD4 to sustain the activity of distal gene enhancers, as is consistent with previously established bookmarking functions at promoters (Zhao, R., Nakamura, T., Fu, Y., Lazar, Z. & Spector, D. L. Gene bookmarking accelerates the kinetics of post-mitotic transcriptional re-activation. Nat Cell Biol 13, 1295-1304 (2011)). The
25 study described herein provides a framework for understanding epigenetic alterations that underlie tumor pathogenesis, and suggests the potential of combination therapy that incorporates new classes of epigenetic therapies as a means to avert resistance phenotypes.

Without further elaboration, it is believed that one skilled in the art can, based on the
30 above description, utilize the present invention to its fullest extent. The specific embodiments are, therefore, to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever. All publications, patent application, and patents cited herein are incorporated by reference for the purposes or subject matter referenced herein.

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The indefinite articles “a” and “an,” as used herein in the specification and in the claims, unless clearly indicated to the contrary, should be understood to mean “at least one.”

From the above description, one skilled in the art can easily ascertain the essential characteristics of the present invention, and without departing from the spirit and scope thereof, 5 can make various changes and modifications of the invention to adapt it to various usages and conditions. Thus, other embodiments are also within the claims.

What is claimed is:

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CLAIMS

1. A method comprising:

administering to a subject having cancer

- 5 (i) a bromodomain inhibitor and/or
(ii) a Bcl-2 inhibitor, and

a Notch pathway inhibitor, in an effective amount to treat the cancer.

2. The method of claim 1, wherein the cancer is resistant to a previously-administered

10 Notch pathway inhibitor.

3. The method of claim 1 or 2, wherein the cancer is characterized by the presence of a Notch pathway activation mutation.

15 4. The method of any one of claims 1-3, wherein the cancer is characterized by abnormal increased HPI-alpha, HPI-beta, and/or HPI-gamma level.

5. The method of any one of claims 1-3, wherein the cancer is characterized by a chromatin compactness or a marker thereof.

20

6. The method of any one of claims 1-5, wherein the bromodomain inhibitor and the Notch pathway inhibitor are administered concurrently or sequentially.

25

7. The method of any one of claims 1-5, wherein the Bcl-2 inhibitor and the Notch pathway inhibitor are administered concurrently or sequentially.

8. The method of any one of claims 1-5, wherein the bromodomain inhibitor, the Bcl-2 inhibitor and the Notch pathway inhibitor are administered.

30

9. The method of any one of claims 1-5, wherein the bromodomain inhibitor, the Bcl-2 inhibitor and the Notch pathway inhibitor are administered concurrently or sequentially.

10. A method comprising:

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administering to a subject having cancer

- (i) a bromodomain inhibitor and/or
- (ii) a Bcl-2 inhibitor,

in an effective amount to treat the cancer, wherein the cancer is characterized by the

5 presence of a Notch pathway activation mutation.

11. The method of claim 10, further comprising identifying the subject having cancer characterized by the presence of a Notch pathway activation mutation.

10 12. The method of claim 10 or 11, further comprising administering to the subject a Notch pathway inhibitor in an effective amount to treat the cancer.

13. The method of claim 12, wherein the bromodomain inhibitor and the Notch pathway inhibitor are administered concurrently or sequentially.

15

14. The method of claim 12, wherein the Bcl-2 inhibitor and the Notch pathway inhibitor are administered concurrently or sequentially.

15. The method of claim 12, wherein the bromodomain inhibitor, the Bcl-2 inhibitor, and the
20 Notch pathway inhibitor are administered.

16. The method of claim 12, wherein the bromodomain inhibitor, the Bcl-2 inhibitor, and the Notch pathway inhibitor are administered concurrently or sequentially.

25 17. A method comprising:

administering to a subject having cancer

- (i) a bromodomain inhibitor and/or
- (ii) a Bcl-2 inhibitor

in an effective amount to treat the cancer, wherein the cancer is resistant to treatment

30 with a Notch pathway inhibitor.

18. The method of claim 17, further comprising administering to the subject a Notch pathway inhibitor in an effective amount to treat the cancer.

19. The method of claim 18, wherein the bromodomain inhibitor and the Notch pathway inhibitor are administered concurrently or sequentially.

5 20. The method of claim 17, wherein the Bcl-2 inhibitor and the Notch pathway inhibitor are administered concurrently or sequentially.

21. The method of claim 17, wherein the bromodomain inhibitor, the Bcl-2 inhibitor, and the Notch pathway inhibitor are administered.

10

22. The method of claim 17, wherein the bromodomain inhibitor, the Bcl-2 inhibitor, and the Notch pathway inhibitor are administered concurrently or sequentially.

23. A method comprising:

15 administering to a subject having cancer

- (i) a bromodomain inhibitor and/or
- (ii) a Bcl-2 inhibitor

in an effective amount to treat the cancer, wherein the cancer is characterized by abnormal increased HPI level, wherein the HPI level is HPI-alpha level, HPI-beta level and/or
20 HPI-gamma level.

24. The method of claim 23, further comprising identifying a subject having cancer characterized by the abnormal increased HPI level.

25 25. The method of claim 23 or 24, further comprising administering to the subject a Notch pathway inhibitor in an effective amount to treat the cancer.

26. The method of claim 25, wherein the bromodomain inhibitor and the Notch pathway inhibitor are administered concurrently or sequentially.

30

27. The method of claim 25, wherein the Bcl-2 inhibitor and the Notch pathway inhibitor are administered concurrently or sequentially.

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28. The method of claim 25, wherein the bromodomain inhibitor, the Bcl-2 inhibitor, and the Notch pathway inhibitor are administered.

29. The method of claim 25, wherein the bromodomain inhibitor, the Bcl-2 inhibitor and the
5 Notch pathway inhibitor are administered concurrently or sequentially.

30. The method of any one of claims 1-29, wherein the bromodomain inhibitor, the Bcl-2 inhibitor and/or the Notch pathway inhibitor is a small compound or inhibitory nucleic acid molecule.

10

31. The method of any one of claims 1-30, wherein the inhibitory nucleic acid molecule is an siRNA, shRNA, or antisense nucleic acid molecule.

15

32. The method of any one of claims 1- 31, wherein the bromodomain inhibitor is a BET inhibitor.

33. The method of any one of claims 1-32, wherein the bromodomain inhibitor is JQ1 or a derivative thereof.

20

34. The method of any one of claims 1-33, wherein the Bcl-2 inhibitor is G3139, GX15-070, ABT-737 or ABT-199.

35. The method of any one of claims 1-34, wherein the Notch pathway inhibitor is a gamma secretase inhibitor.

25

36. A method comprising:

- (a) measuring nucleus size in a tumor sample from a subject; and
- (b) comparing nucleus size in the tumor sample to a control,

wherein a decreased nucleus size in the tumor sample compared to the control identifies a
30 subject to be treated with a bromodomain inhibitor and/or Bcl-2 inhibitor.

37. The method of claim 36, wherein the nucleus size is nucleus diameter or nucleus volume.

38. A method comprising:

- (a) measuring HPI level in a tumor sample from a subject; and
- (b) comparing the HPI level in the tumor sample to a control,

wherein an increased HPI level in the tumor sample compared to the control identifies a subject
5 to be treated with a bromodomain inhibitor and/or Bcl-2 inhibitor,

wherein the HPI level is HPI-alpha level, HPI-beta level, and/or HPI-gamma level.

39. A method comprising:

- (a) measuring level of a chromatin state biomarker (CSB) in a tumor sample from a
10 subject, the CSB selected from:

- (i) a first CSB group consisting of NPM1, NARG1, RCC1, SSRP1, PRMT3, SAP30, CBX6, CHMP2B, UBE2M, WDR77, HMGB1, CARM1, USP13, HDAC4, COQ3, SET, GATAD2A, PRMT6, HMG20B, DNMT1, ADA, SS18, UBE3A, ZMYND11, and NOC2LL (“Group I CSB”); and

- (ii) a second CSB group consisting of UTX, SIN3A, SAP30L, FLJ20309, RCOR2, ARID5A, UBE2Q2, TRIM24, BAZ2B, SMYD3, EZH2, PHF1, PHF2, BCR, SMARCD3, BMI1, CHD6, FBXL11, SIRT7, ASF1A, RCOR3, CBX4, EPC1, BRD1, and BNF11 (“Group II CSB”); and

- (b) comparing the Group I and/or Group II CSB level with a control,

20 wherein a Group I CSB level that is reduced in the tumor sample compared to a control and/or a Group II CSB level that is elevated in the tumor sample compared to a control identifies a subject to be treated with a bromodomain inhibitor and/or Bcl-2 inhibitor.

40. A method comprising:

- (a) measuring a level of a biomarker in a tumor sample from a subject, the biomarker selected from DTX1, HES4, CD1d, ETS1, ETV6, Runx1, Bcl-2, MYC and CD52; and

- (b) comparing the biomarker level with a control,

wherein a level of DTX1, HES4, or CD1d that is reduced in the tumor sample compared to the control and/or a level of ETS1, ETV6, Runx1, CD52, MYC or Bcl-2 that is elevated in the
30 tumor sample compared to the control identifies a subject to be treated with a bromodomain inhibitor and/or Bcl-2 inhibitor.

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41. The method of any one of claims 38-40, wherein the level is an mRNA level or a protein level.

42. A method comprising:

5 (a) measuring a level of histone modification in a tumor sample from a subject, the histone modification selected from H3K27me3 and H3K9me3; and

(b) comparing the histone modification level with a control, wherein a level of histone modification that is elevated in the tumor sample compared to the control identifies a subject to be treated with a bromodomain inhibitor and/or Bcl-2 inhibitor.

10

43. A method comprising:

(a) measuring a level of H3K27Ac histone modification in a tumor sample from a subject; and

(b) comparing the H3K27Ac histone modification level with a control, 15 wherein a level of H3K27Ac histone modification that is reduced in the tumor sample compared to the control identifies a subject to be treated with a bromodomain inhibitor and/or Bcl-2 inhibitor.

44. A method comprising:

20 (a) measuring a level of H3K4me1 histone modification at a site of elevated H3K27Ac histone modification; and

(b) comparing the H3K4me1 histone modification level with a control, 25 wherein a level of H3K4me1 histone modification at a site of elevated H3K27Ac histone modification that is elevated in the tumor sample compared to the control identifies a subject to be treated with a bromodomain inhibitor and/or Bcl-2 inhibitor.

45. The method of any one of claims 36-44, wherein the control is a nucleus size, an HPI-alpha level, an HPI-beta level, an HPI-gamma level, a CSB level, a histone modification level or a biomarker level selected from DTX1, HES4, CD1d, ETS1, ETV6, Runx1, Bcl-2, MYC and 30 CD52 in a non-tumor sample.

46. The method of any one of claims 36-45, wherein the control is a predetermined threshold.

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47. The method of any one of claims 36-46, further comprising identifying the subject to be treated with a bromodomain inhibitor and/or Bcl-2 inhibitor.

5 48. The method of any one of claims 36-47, further comprising administering to the identified subject a bromodomain inhibitor and/or Bcl-2 inhibitor in an effective amount.

49. The method of claim 48, further comprising administering to the identified subject a Notch pathway inhibitor in an effective amount.

10

50. The method of any one of claims 1-49, wherein the cancer or tumor is a T-ALL.

51. The method of claim 50, wherein the T-ALL is resistant to a Notch pathway inhibitor.

15

52. A method comprising:

administering to a subject having cancer an inhibitor of ARID3B, EZH2, PRMT2, SND1, BRD1, SUV39H1, PRMT5, SS18, BRD4, KDM5D, PRMT7, STAG3L1, CD2BP2, MLL5, SUDS3, CHD1, MINA, CHD8, MORF4L1, or CHRAC1, wherein the cancer is resistant to a Notch pathway inhibitor.

20

53. The method of claim 52, further comprising administering to the subject a Notch pathway inhibitor.

25

54. The method of claim 52 or 53, further comprising administering to the subject a bromodomain inhibitor and/or a Bcl-2 inhibitor in an effective amount to treat the cancer.

55. The method of any one of claims 52-54, wherein the inhibitor is an shRNA, an siRNA, or an antisense nucleic acid molecule.

30

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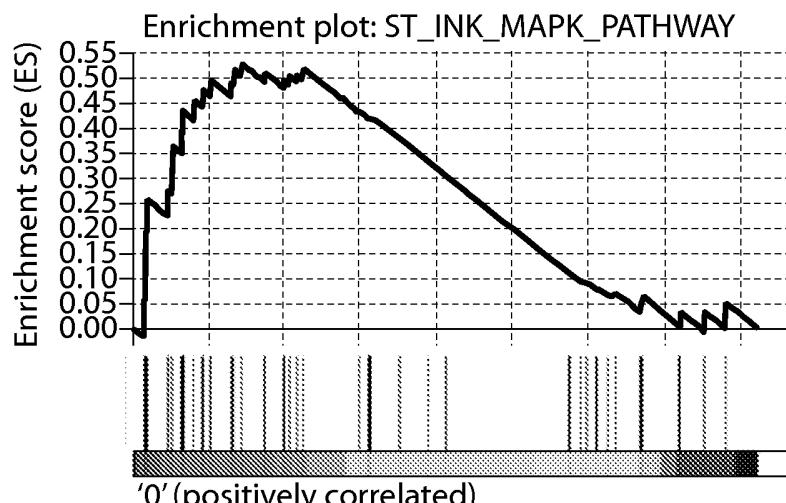
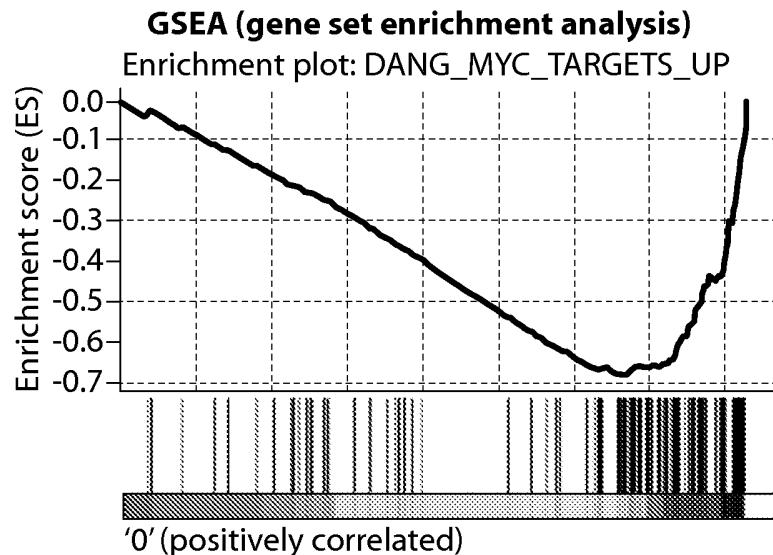
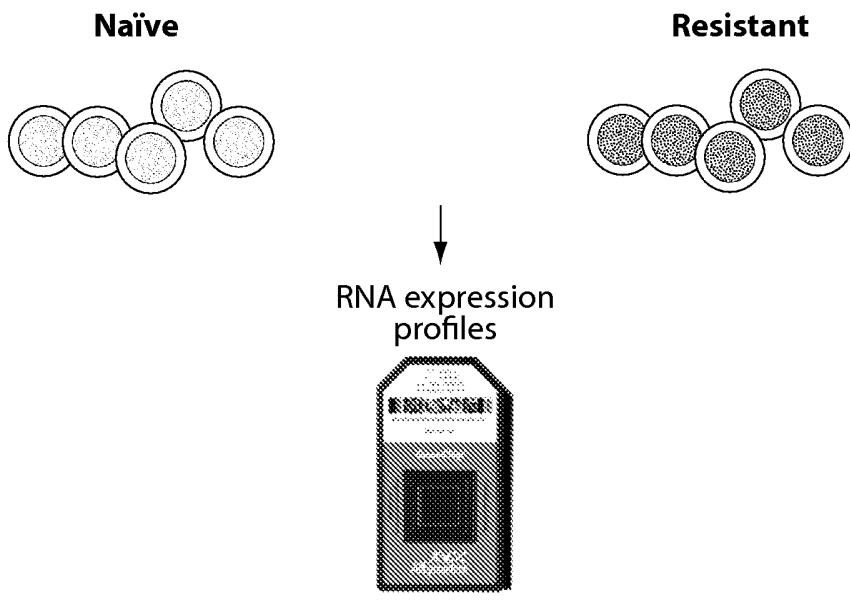


Fig. 1
SUBSTITUTE SHEET (RULE 26)

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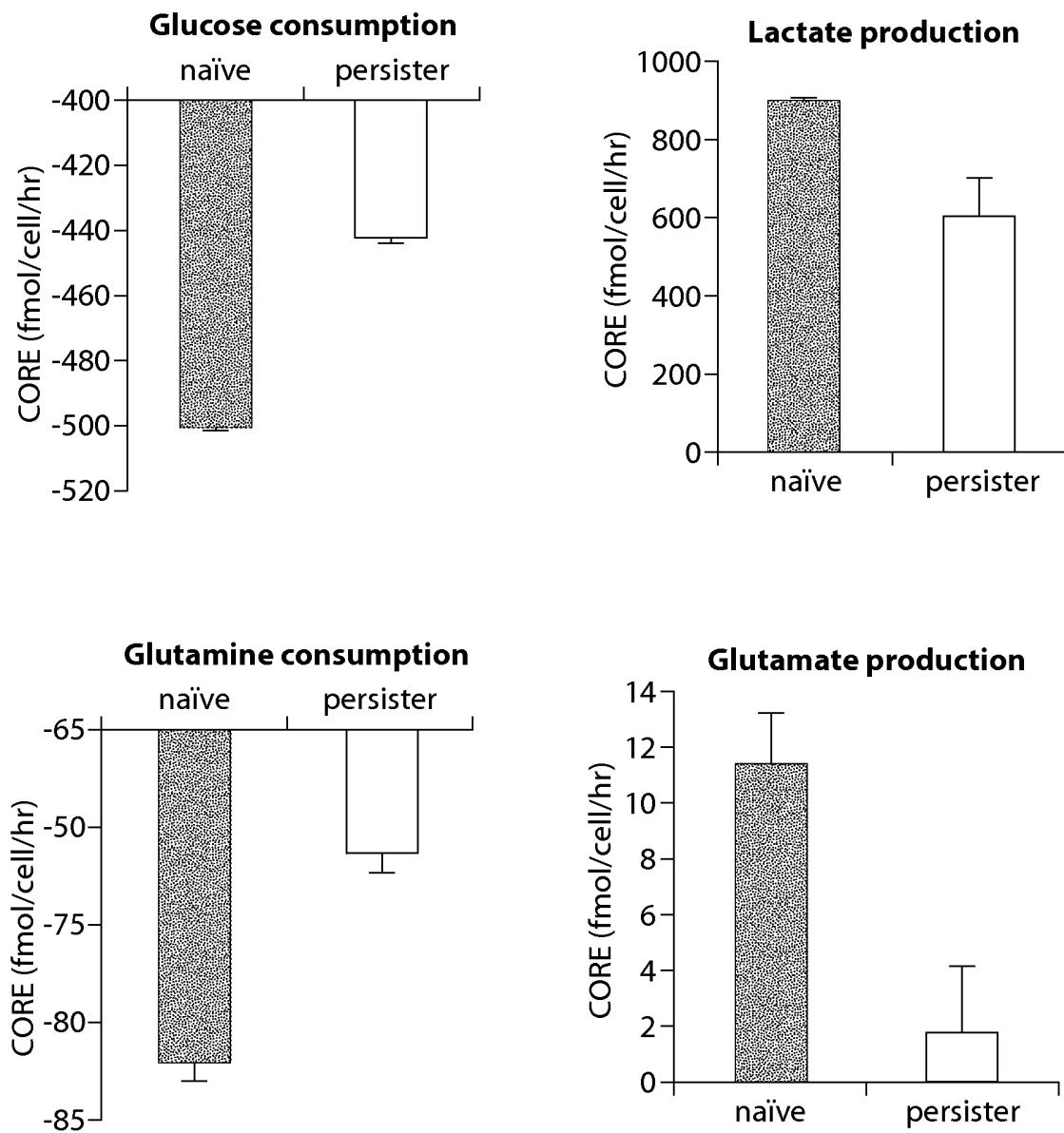
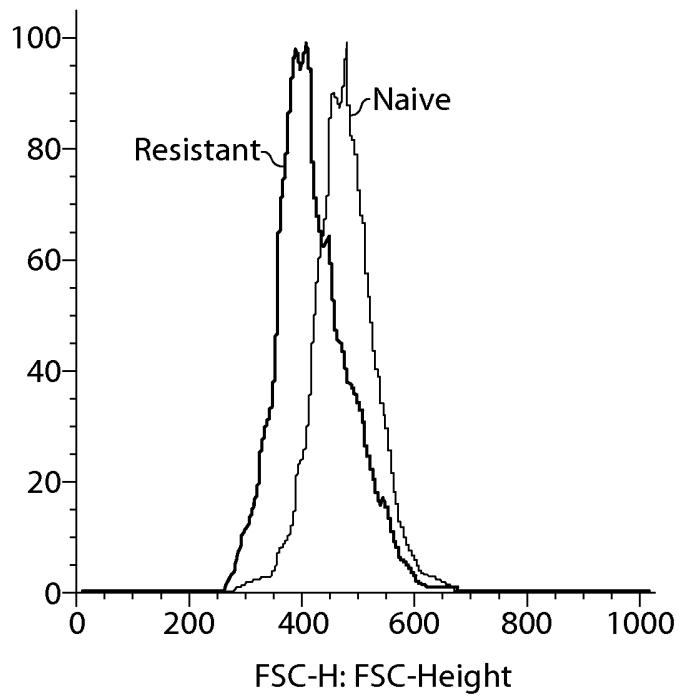
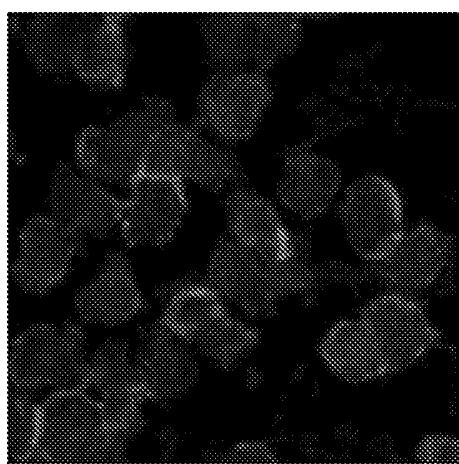


Fig. 2

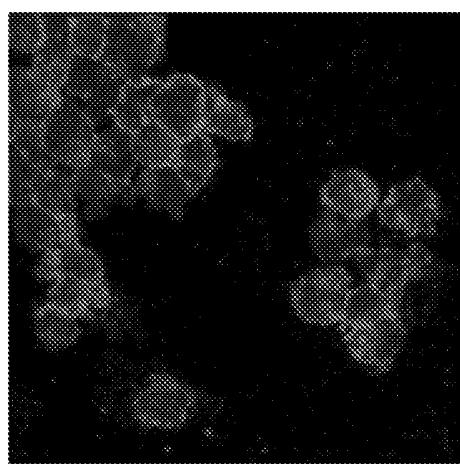
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Forward Scatter**Fig. 3A****DAPI / Actin stain**

Naïve



Resistant

**Fig. 3B**

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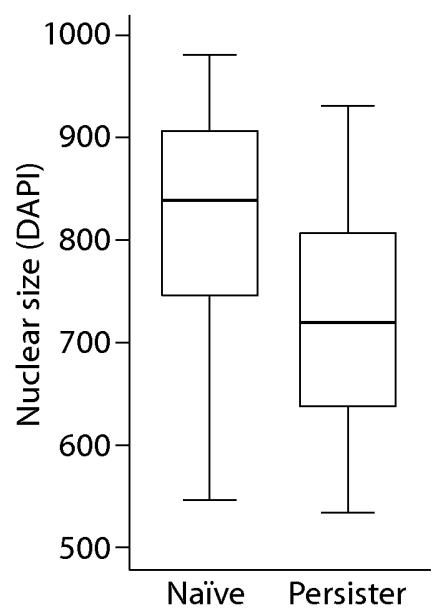


Fig. 3C

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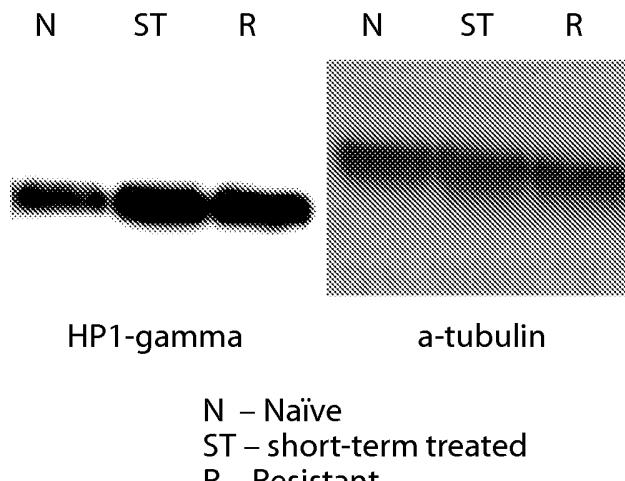


Fig. 4A

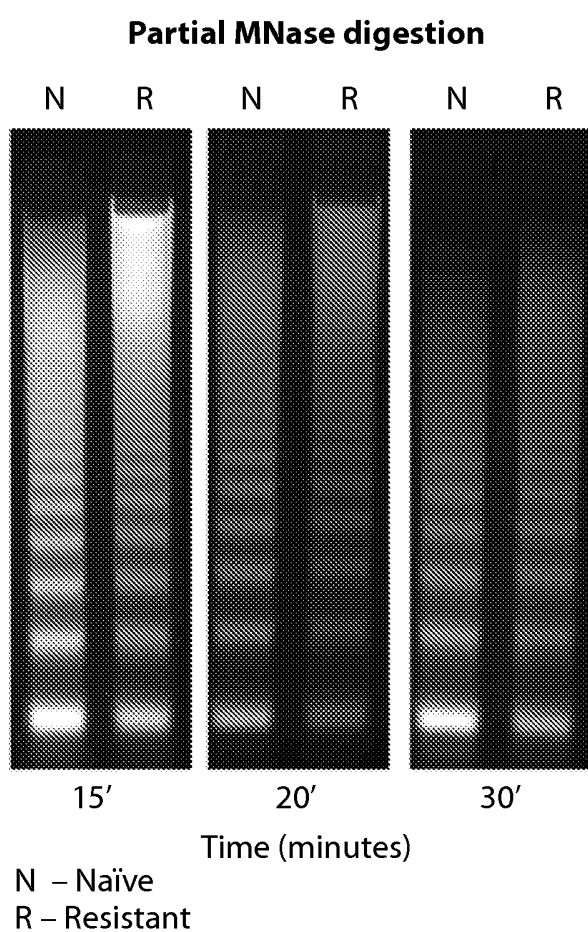
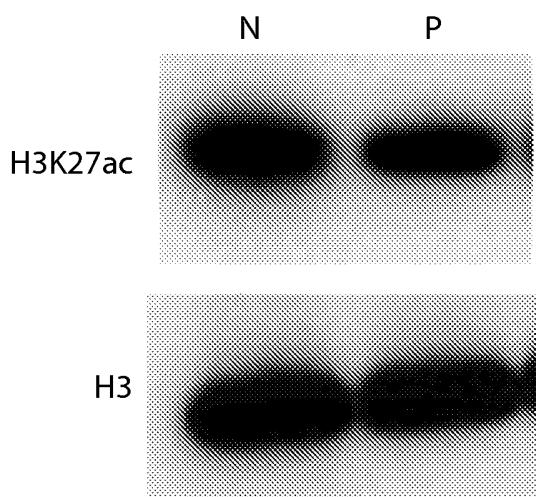
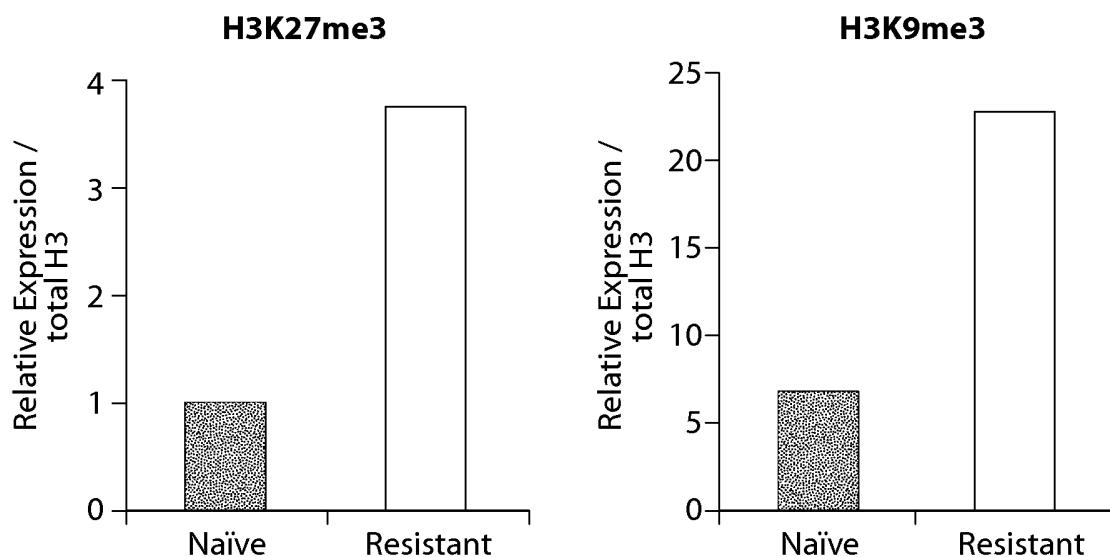


Fig. 4B

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Global levels of repressive chromatin marks**Fig. 5A**

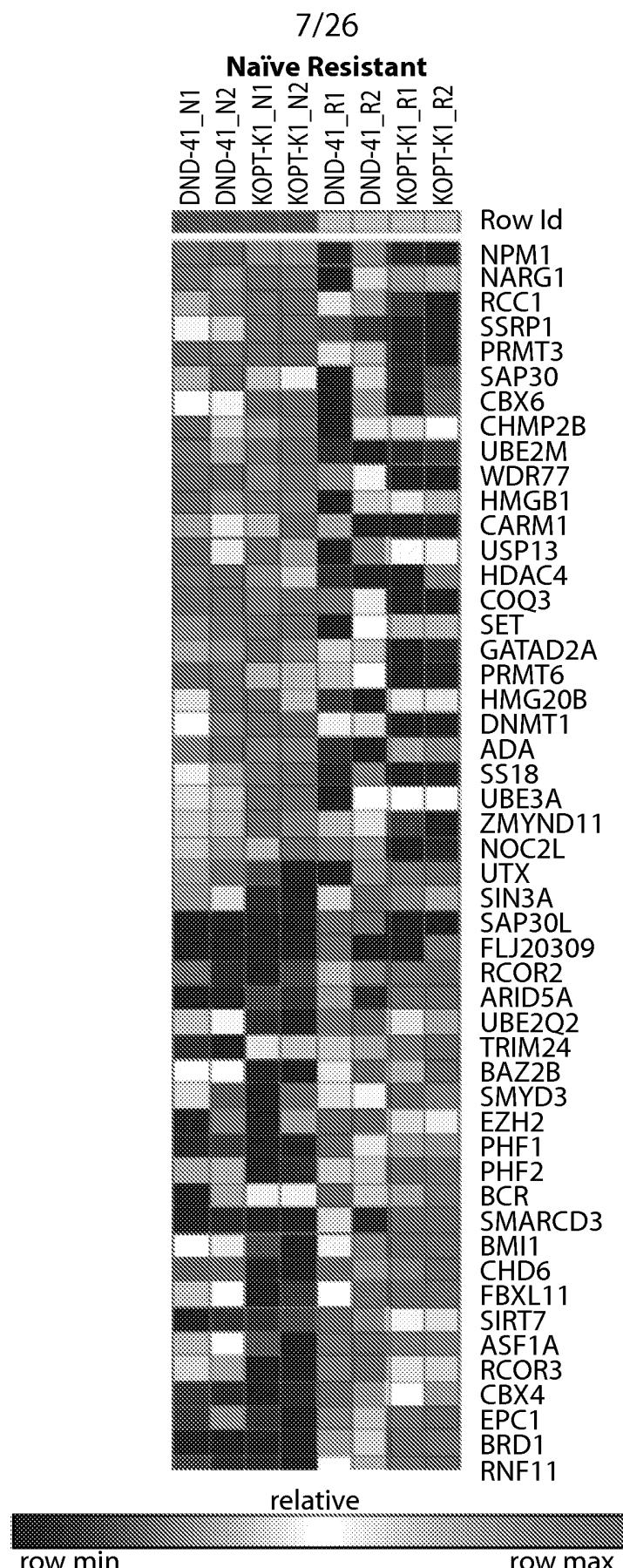


Fig 5R
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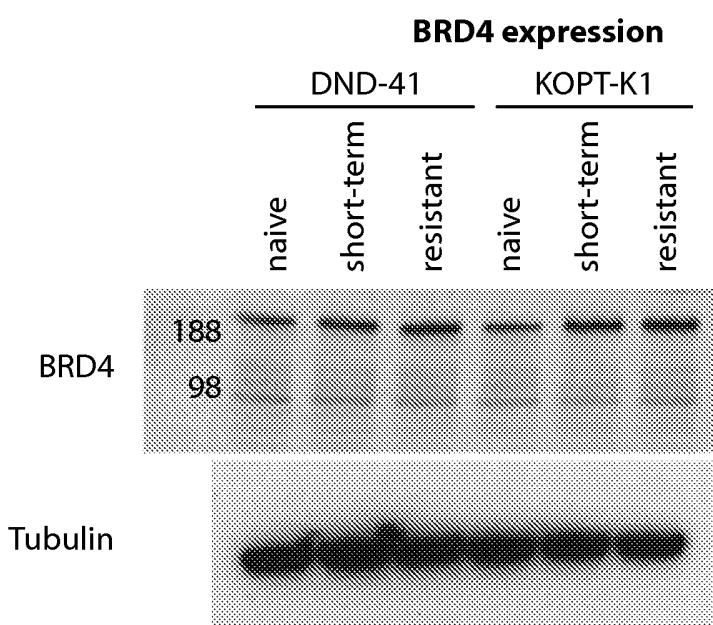


Fig. 6

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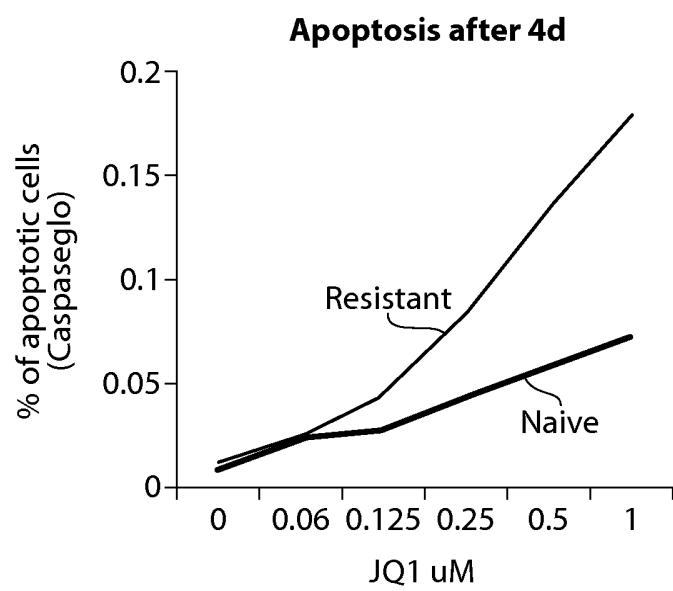
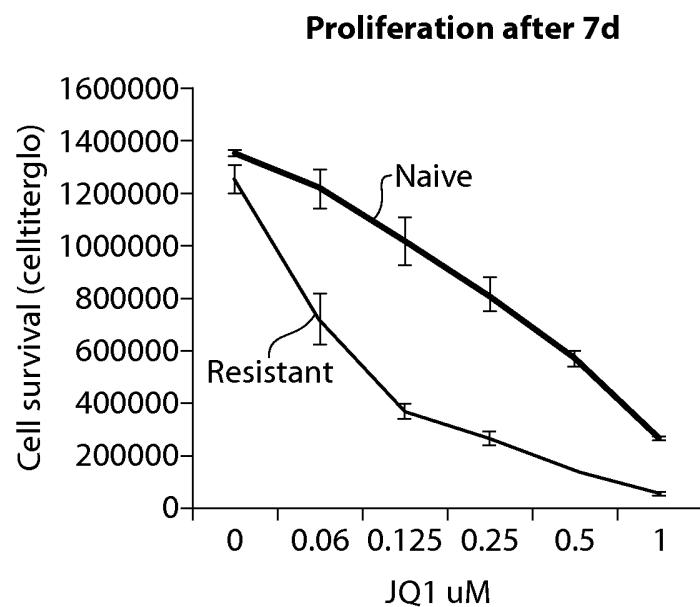
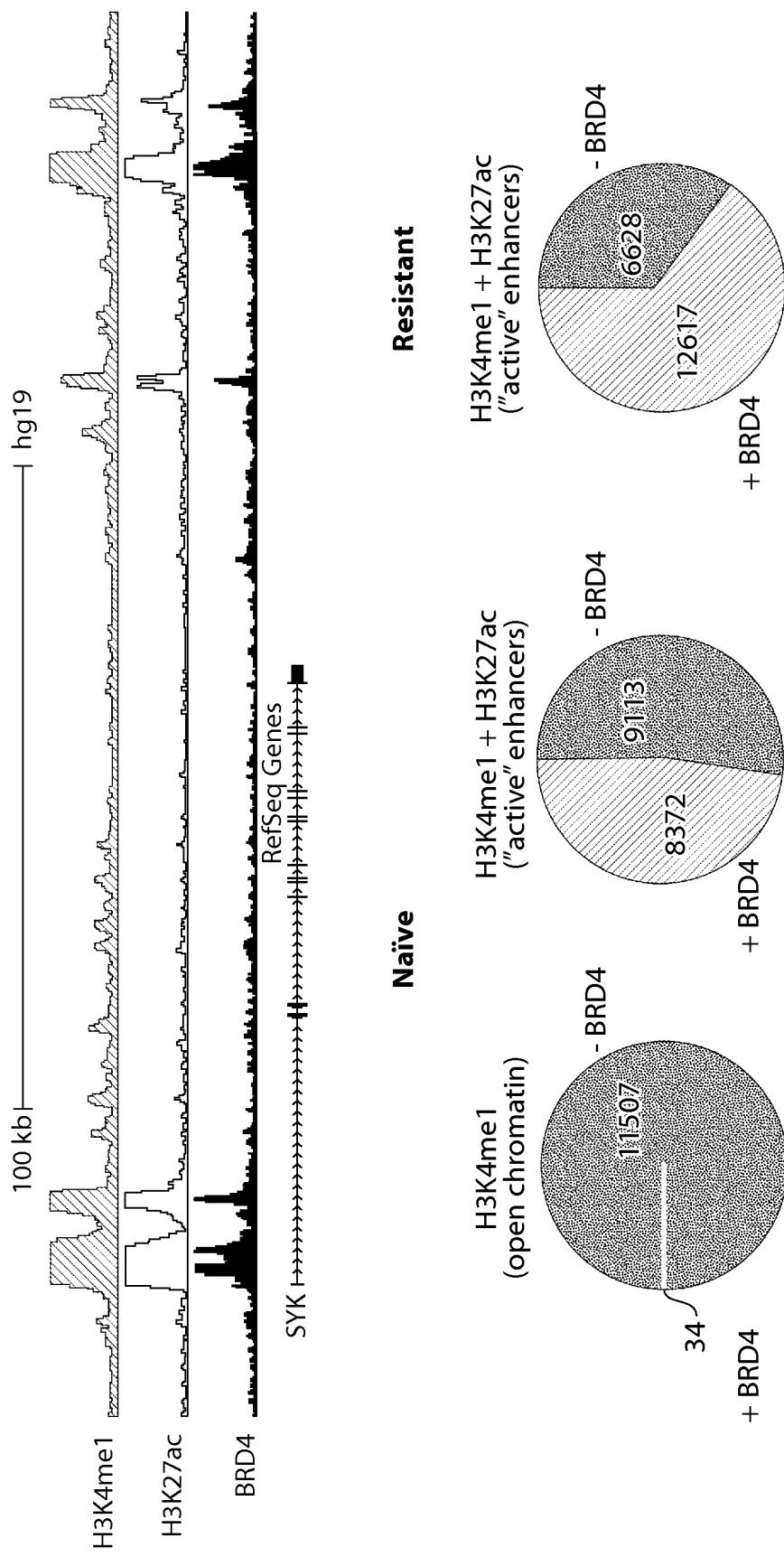


Fig. 7

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**Fig. 8**

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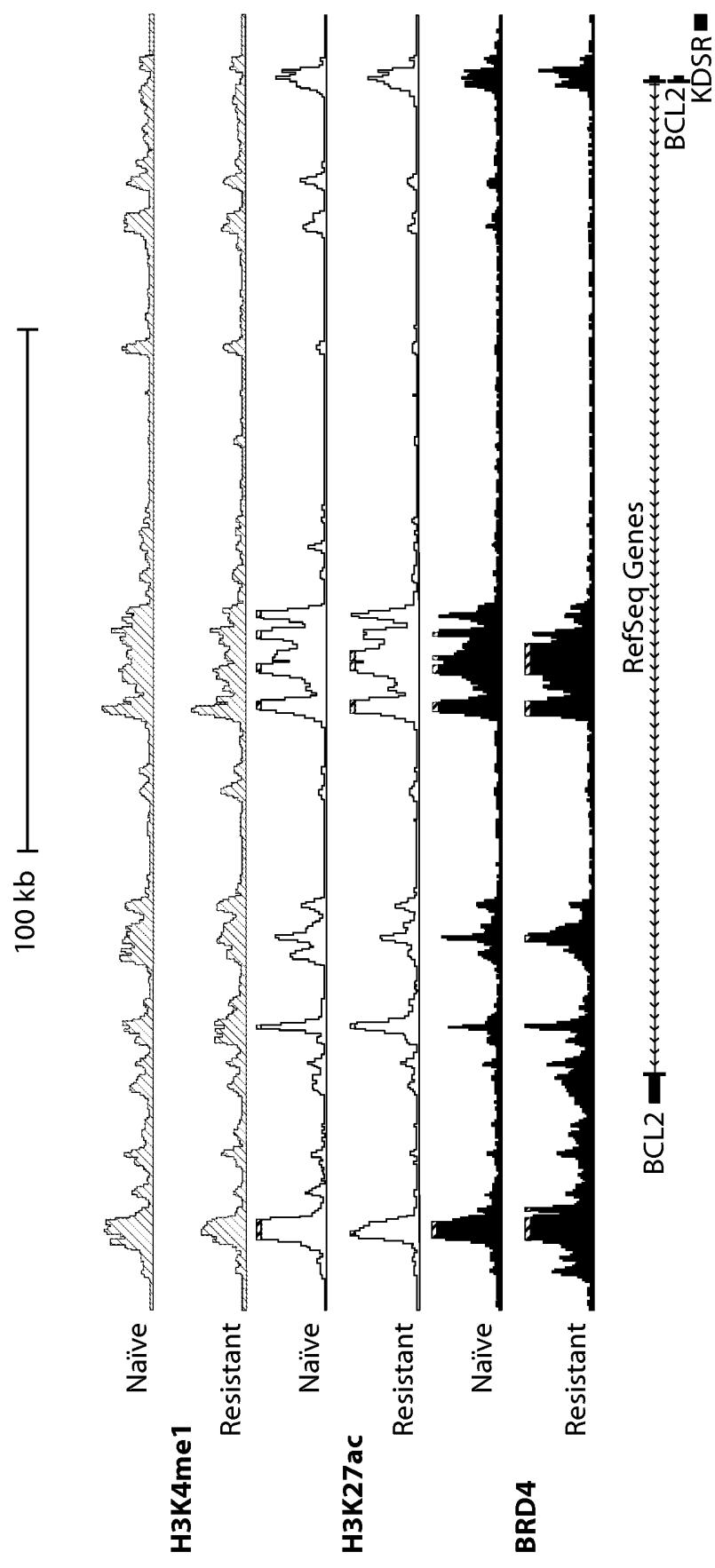


Fig. 9A

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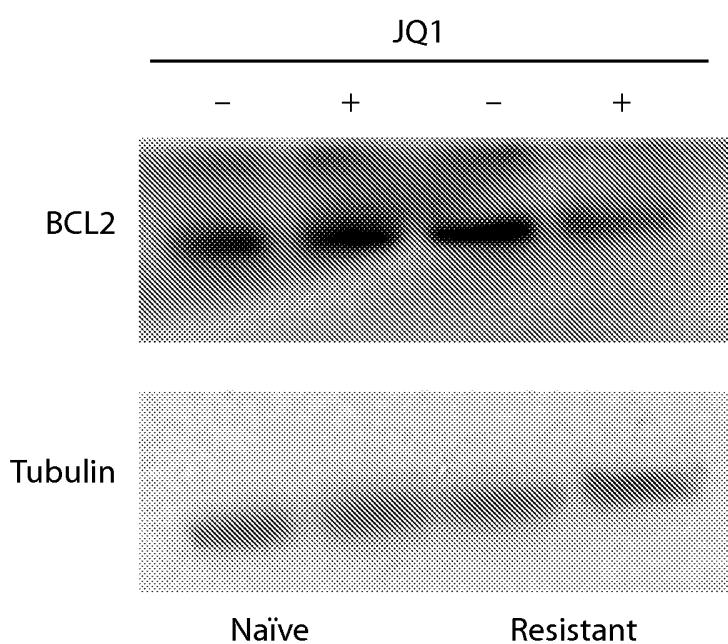


Fig. 9B

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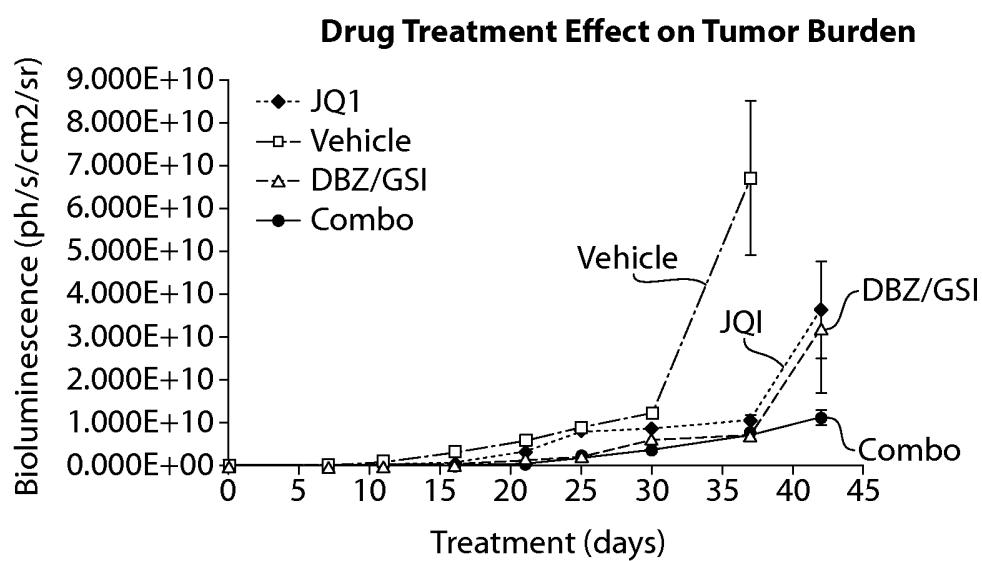
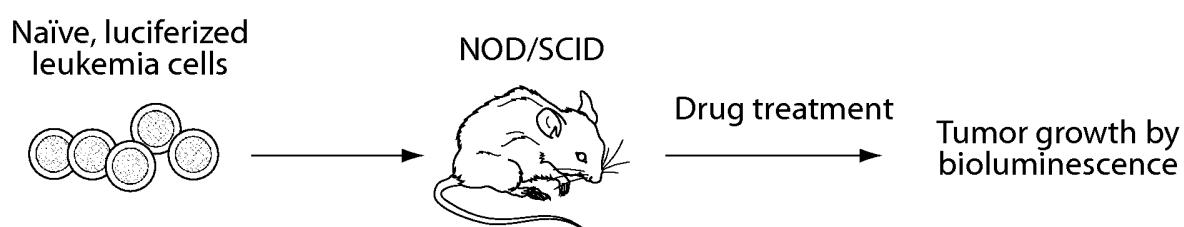


Fig. 10

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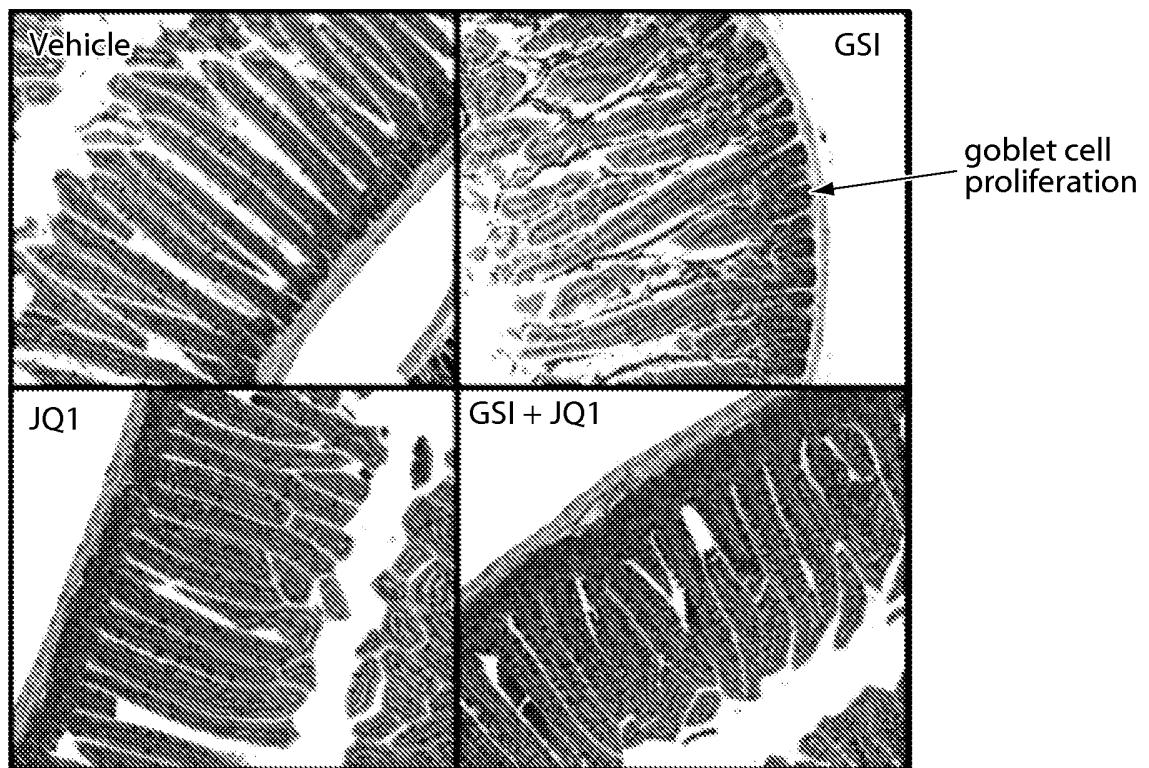


Fig. 11

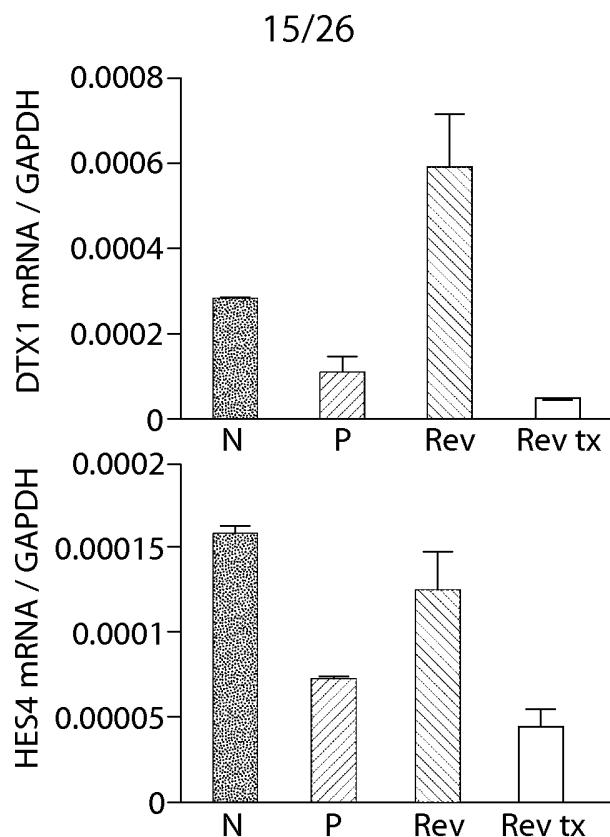


Fig. 12A

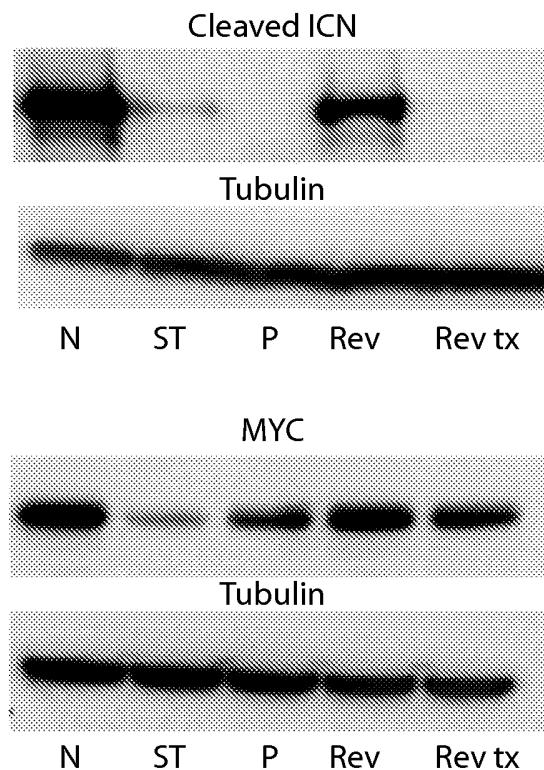


Fig. 12B

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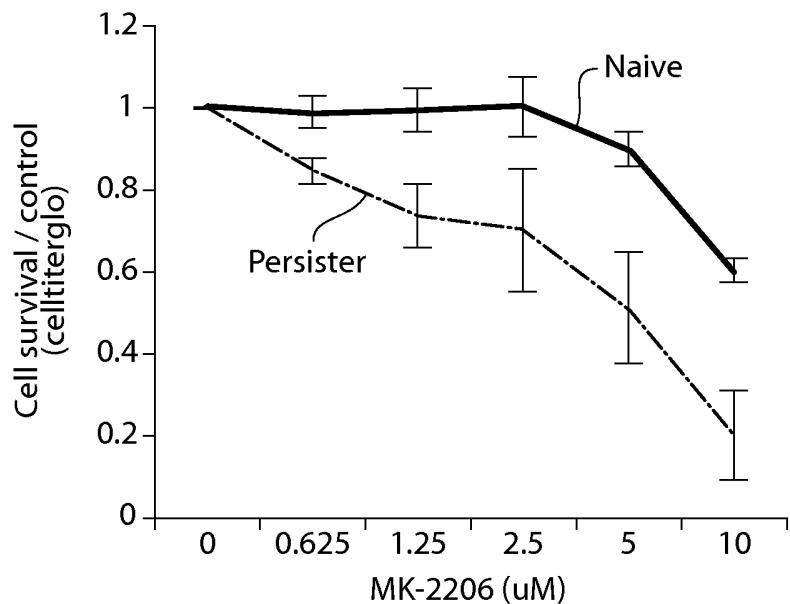


Fig. 13

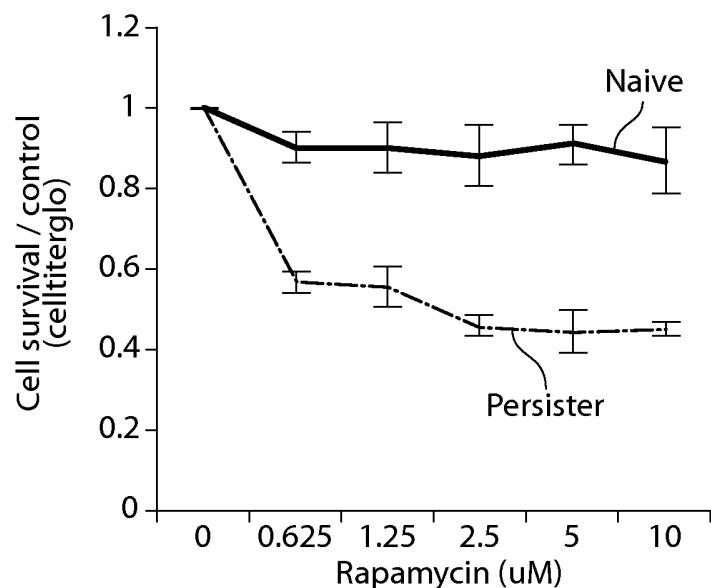
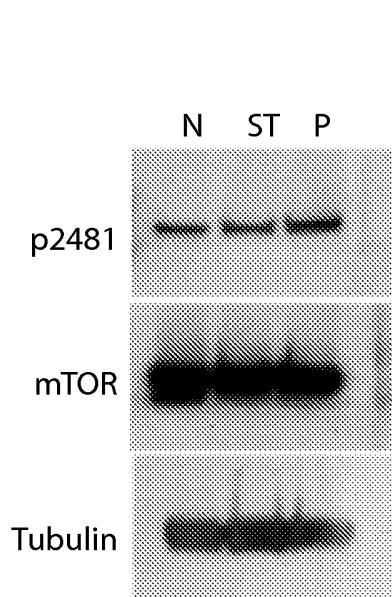


Fig. 14

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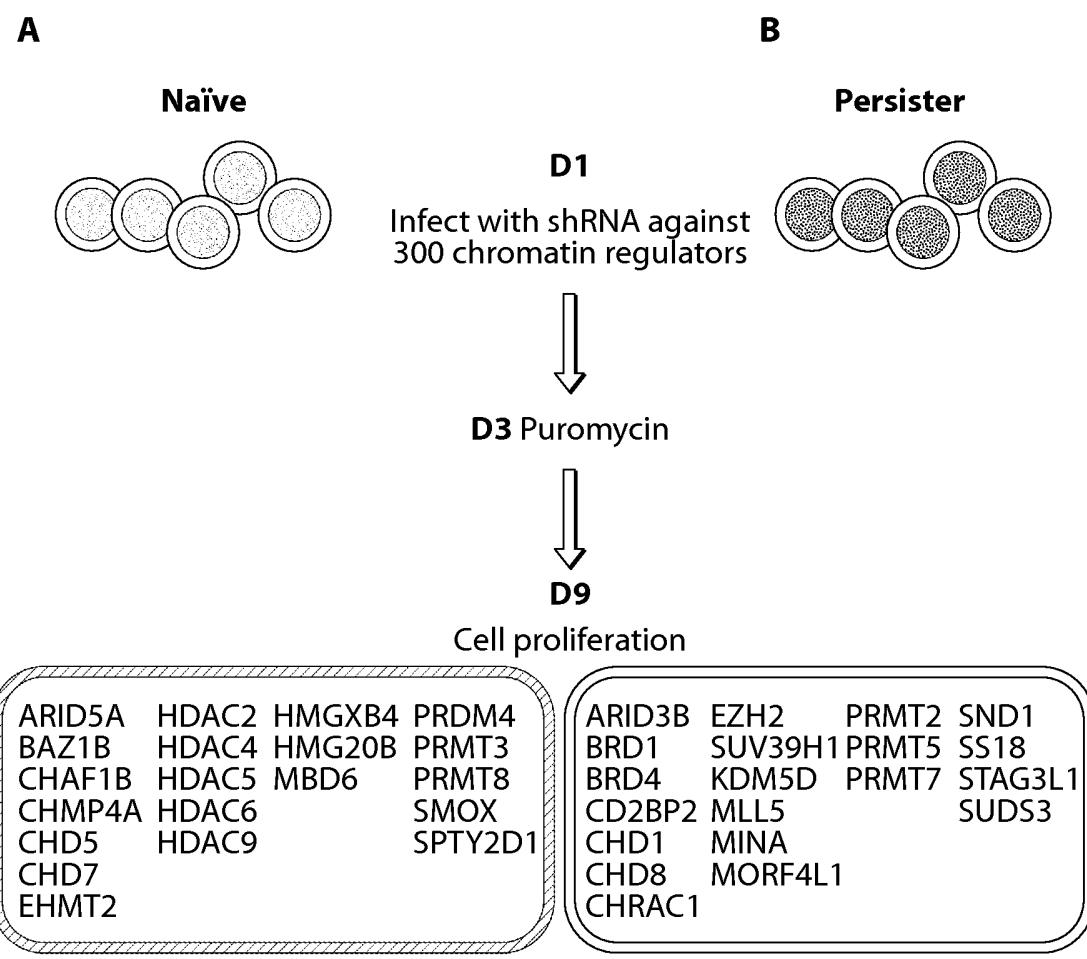


Fig. 15

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Motifs enriched at distal BRD4 sites	Related motif	p- value	% of targets
	ERG/ETS	1E-92	43.2%
	Runx1	1E-48	22.3%

Fig. 16A

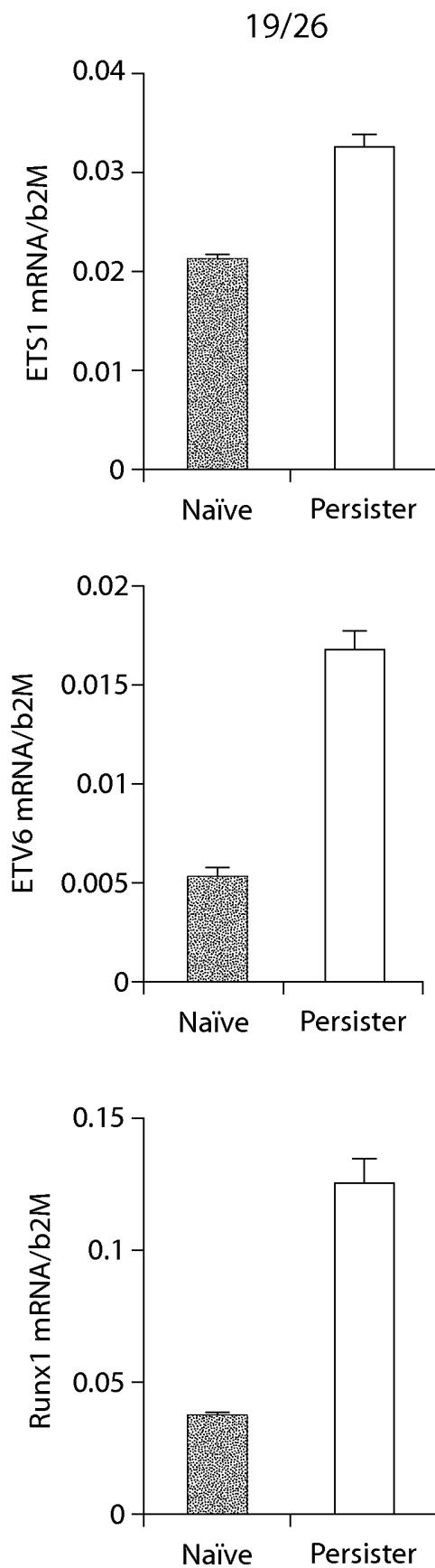


Fig. 16B

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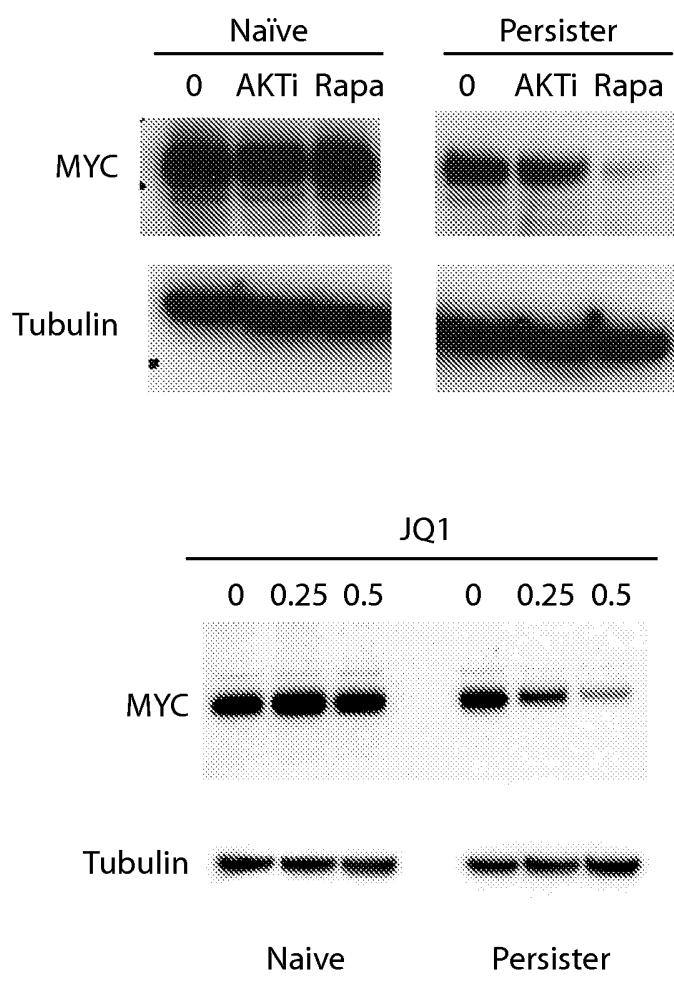


Fig. 17A

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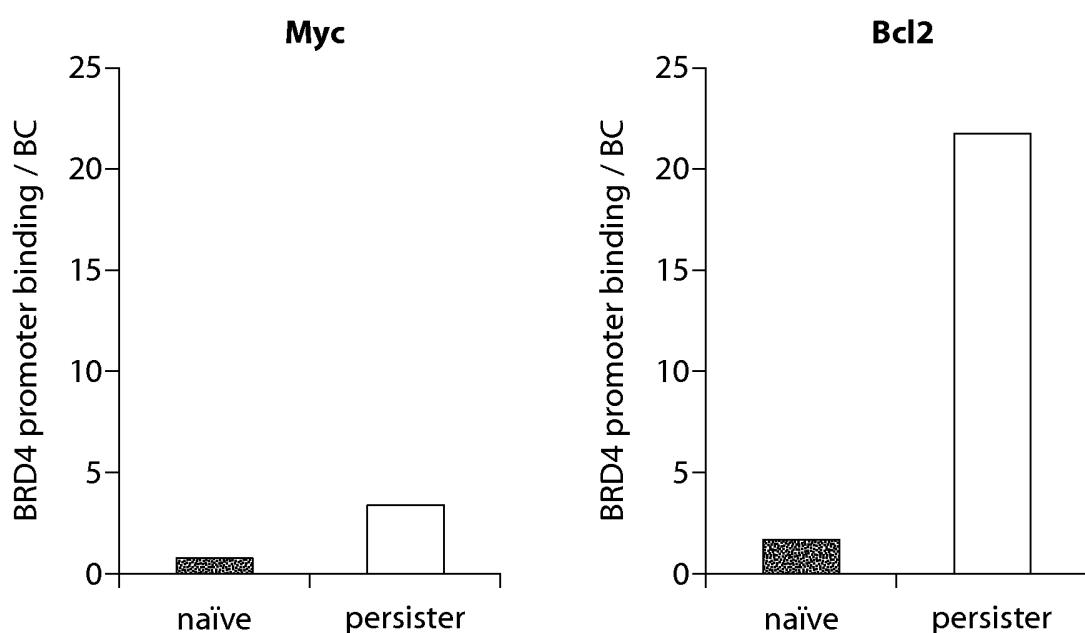


Fig. 17B

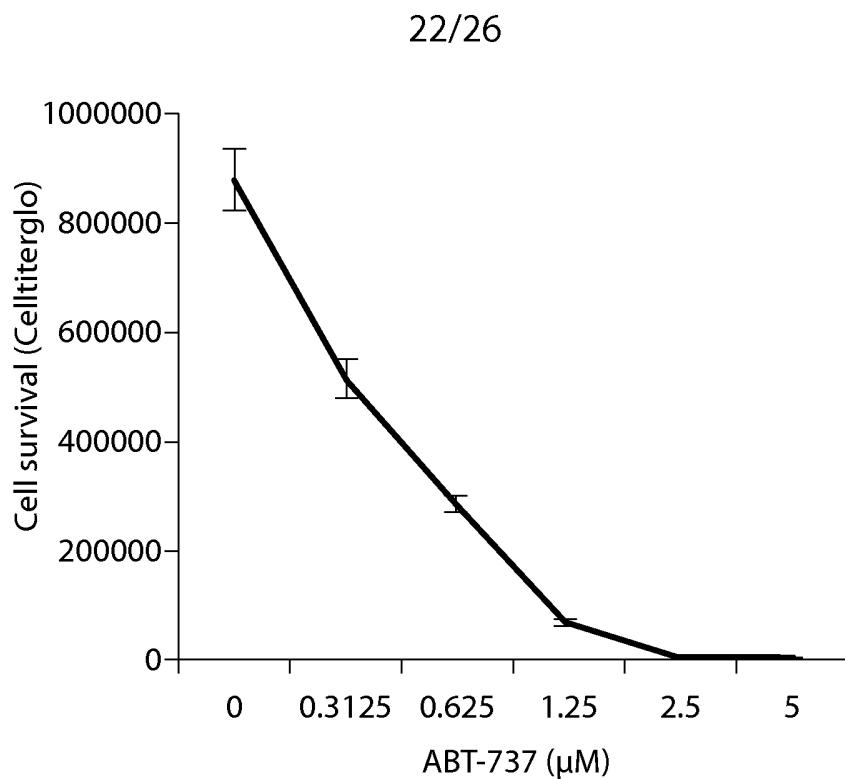


Fig. 18A

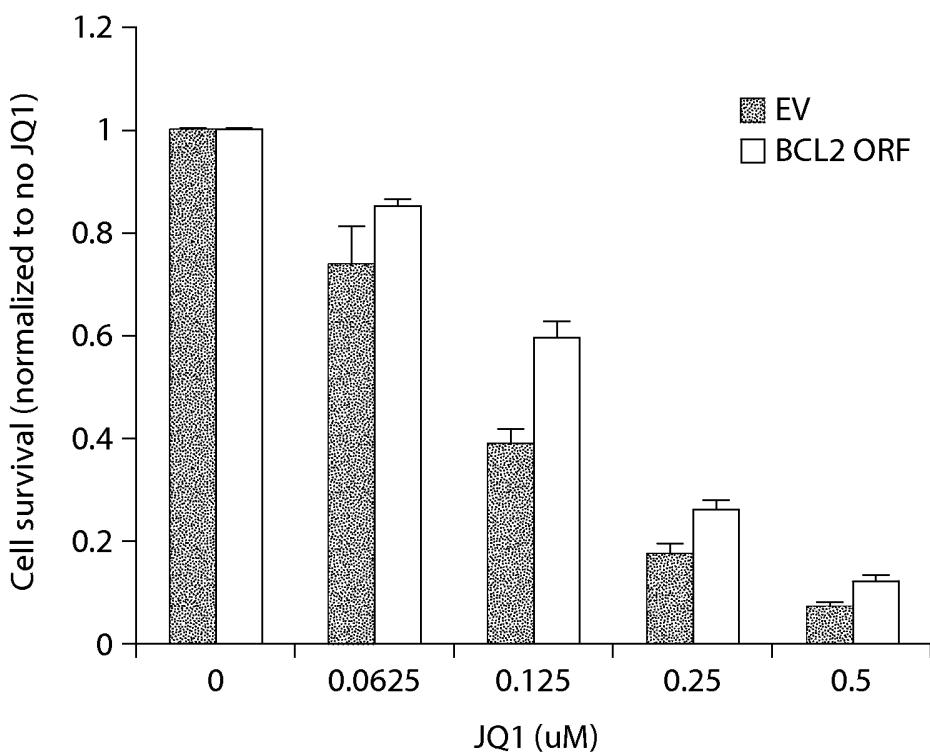


Fig. 18B

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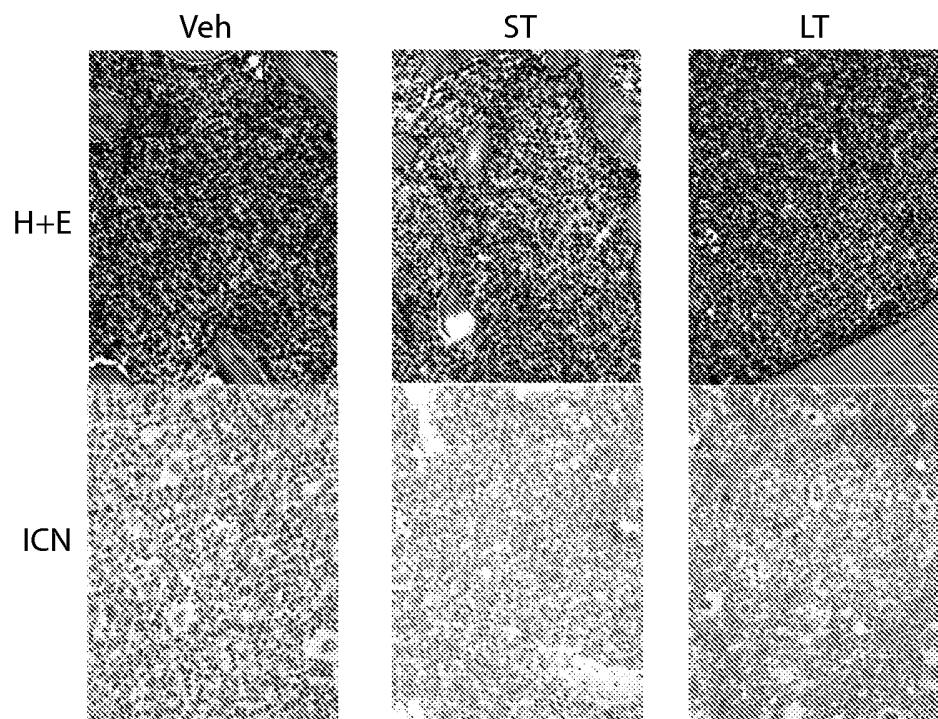


Fig. 19A

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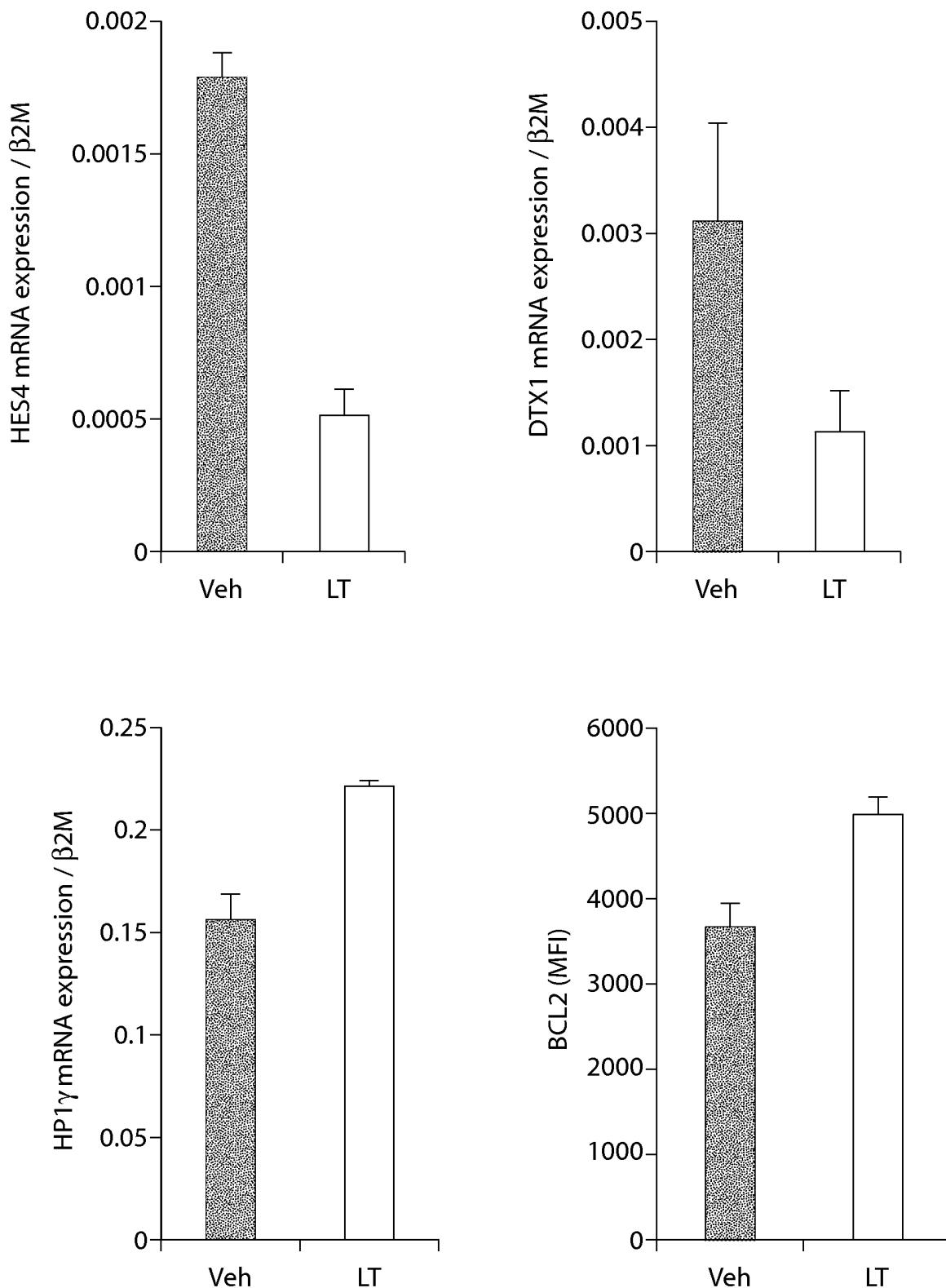


Fig. 19B

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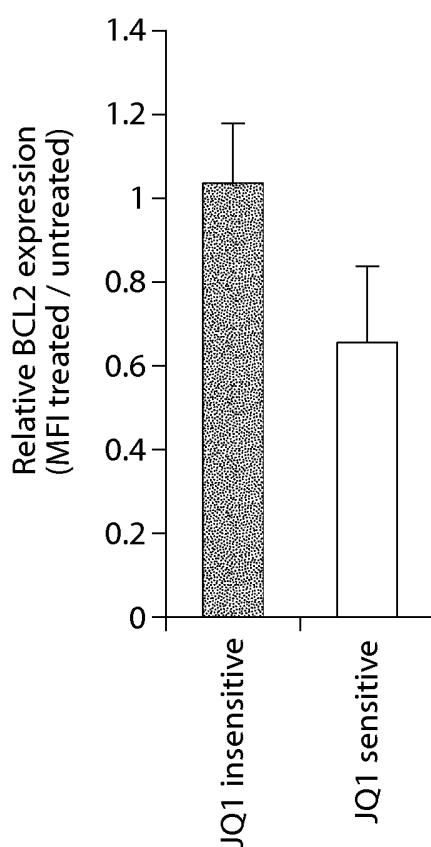
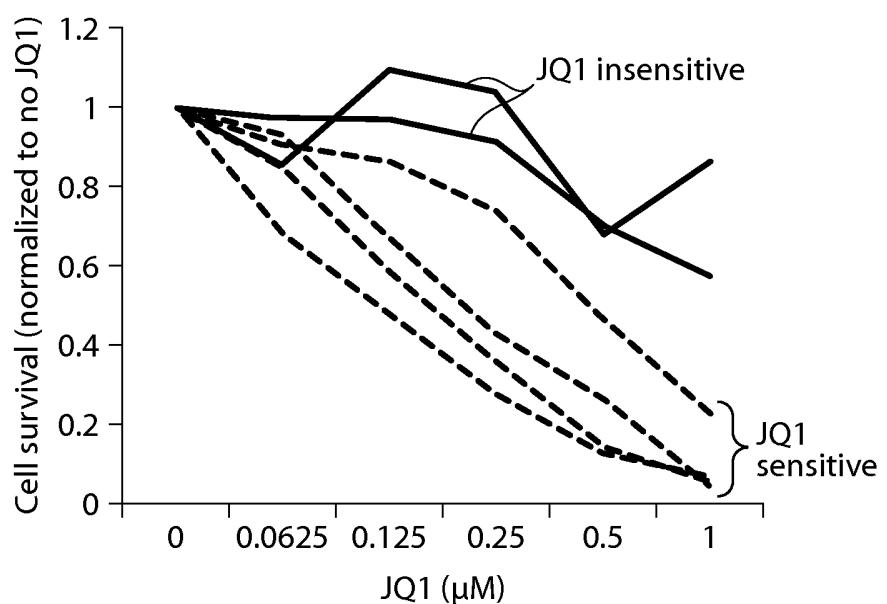


Fig. 20

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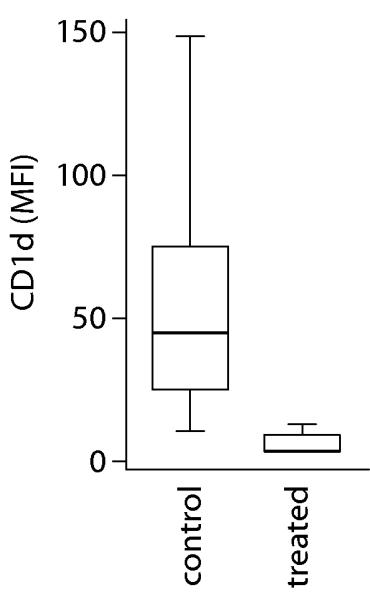
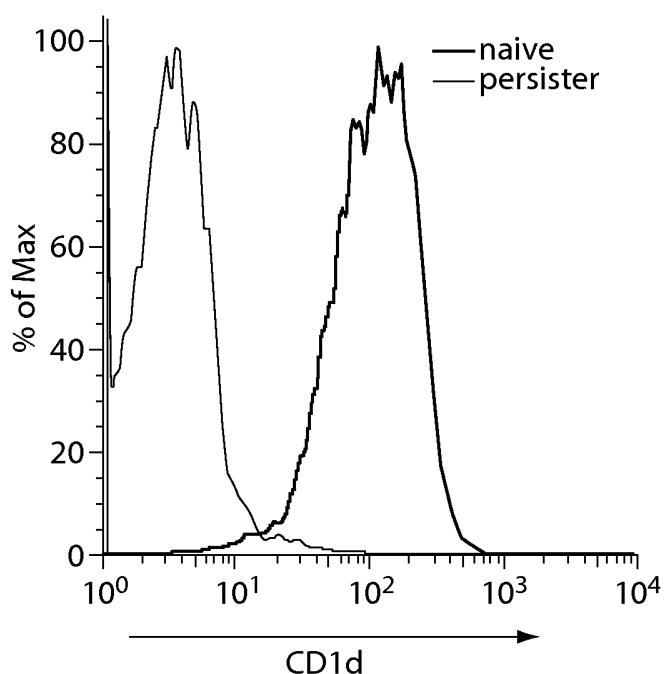


Fig. 21