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[Continued on next page]

(54) Title: METHODS AND COMPOSITIONS FOR ASSESSING RESPONSIVENESS OF CANCERS TO BET INHIBITORS

FIG. 16A

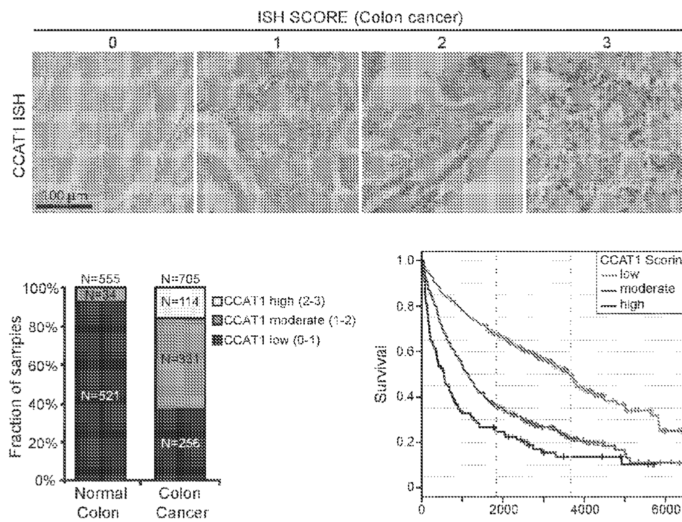

FIG. 16B

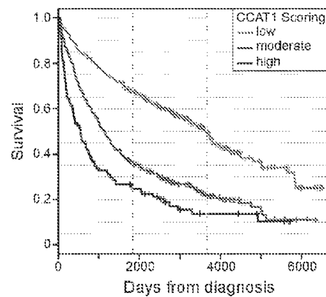

FIG. 16C

(57) Abstract: Methods of treating a subject having cancer with a BET inhibitor, methods of selecting a subject for treatment with a BET inhibitor, methods of predicting responsiveness of a subject having cancer to a BET inhibitor, methods of communicating the likelihood of response of a subject having cancer to a BET inhibitor, and methods of modulating the treatment of a subject undergoing BET inhibitor treatment for cancer are described. The described methods include the steps of performing a nucleic acid-based detection assay to detect the expression level of an eRNA transcribed from a myc-associated enhancer element in a sample containing cancer cells from the subject and determining the expression level of the eRNA in the cells in the sample.

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METHODS AND COMPOSITIONS FOR ASSESSING RESPONSIVENESS OF CANCERS TO BET INHIBITORS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 62/168,691, filed May 29, 2015, which is hereby incorporated by reference in its entirety.

SUBMISSION OF SEQUENCE LISTING ON ASCII TEXT FILE

[0002] The content of the following submission on ASCII text file is incorporated herein by reference in its entirety: a computer readable form (CRF) of the Sequence Listing (file name: 146392032540SEQLIST.txt, date recorded: May 18, 2016, size: 17 KB).

FIELD

[0003] The present disclosure relates to compositions and methods for assessing responsiveness of cancers to BET inhibitors.

BACKGROUND

[0004] Cancer is a leading cause of death and results in over $88 billion dollars in healthcare expenses within the U.S. alone (American Cancer Society. Cancer Facts & Figures 2015. Atlanta, Ga. 2015). Colorectal carcinoma (CRC) is one of the most prevalent and fatal types of cancers accounting for over half a million deaths worldwide annually (Haggar, F.A. & Boushey, R.P. *Clin Colon Rectal Surg* 22, 191-197 (2009)). Genomic analyses of colorectal tumors have uncovered a number of key somatic and germline mutations that drive tumorigenesis at the molecular level and can be linked to well-defined disease stages of tumor progression (Fearon, E.R. *Ann NY Acad Sci* 768, 101-110 (1995); Fearon, E.R. & Vogelstein, B. *Cell* 61, 759-767 (1990); Sillars-Hardebol, A.H., *et al.* T*umor Biol* 31, 89-96 (2010)). Colorectal tumors can be divided into three main subtypes based on these initiating molecular alterations: 1) chromosomal instability (CIN), 2) CpG island methylator phenotype (CIMP) and 3) microsatellite instability (MSI) (Issa, J.P. *Clin Cancer Res* 14, 5939-5940 (2008); Ogino, S. & Goel, A. *J Mol Diagn* 10, 13-27 (2008); Toyota, M., *et al. PNAS* 96, 8681-8686 (1999)). Sixty percent of colon cancers arise from the CIN pathway and are distinguished by aneuploidy and recurrent chromosomal amplifications at distinct genomic

loci. A number of tumor suppressor genes (APC, 5q21; DCC, 18q21) and oncogenes (cMYC, 8q24; MET, 7q; CDK8, CDX2 13q; and PRPF6; 20q) have been shown to reside in these regions of copy number alterations (Adler, A.S., *et al. G&D* 28, 1068-1084 (2014); Salari, K., *et al. PNAS* 109, E3196-3205 (2012); Firestein, R. & Hahn, W.C. *Cancer Res* 69, 7899-7901 (2009); Dulak, A.M., *et al. Cancer Res* 72, 4383-4393 (2012); Fearon, E.R., *et al. Science* 247, 49-56 (1990); Powell, S.M., *et al. Nature* 359, 235-237 (1992)).

[0005] Recent genomic analyses have uncovered epigenetic alterations as major drivers of tumorigenesis (Vogelstein, B., *et al. Science* 339, 1546-1558 (2013); Garraway, L.A. & Lander, E.S. *Cell* 153, 17-37 (2013)). In colon cancer, dysregulation of the epigenome has been recognized to occur at both the histone and DNA methylation level (Lao, V.V. & Grady, W.M. *Nat Rev Gastroentero* 8, 686-700 (2011); Hammoud, S.S., Cairns, B.R. & Jones, D.A. *Curr Opin Cell Biol* 25, 177-183 (2013)). The Wnt effector protein, β-catenin, associates with a number of histone modifying enzymes such as the histone acetyltransferases CBP/p300 and the arginine methyltransferase PRMT2 (Teo, J.L. & Kahn, M. *Adv Drug Deliver Rev* 62, 1149-1155 (2010); Blythe, S.A., Cha, S.W., Tadjuidje, E., Heasman, J. & Klein, P.S. *Dev Cell* 19, 220-231 (2010)). In addition, Wnt pathway genes themselves are repressed via hypermethylation further implying that Wnt signaling may be disrupted through epigenetic targeting at different levels (Ying, Y. & Tao, Q. *Epigenetics* 4, 307-312 (2009); Jiang, X., *et al. Cancer cell* 13, 529-541 (2008); Suzuki, H., *et al. Nat Genetics* 36, 417-422 (2004)). Aberrant DNA hypermethylation has long been recognized as an important etiological cause of tumorigenesis (Baylin, S.B. *Oncology* 2 Suppl 1, S4-11 (2005); Herman, J.G. & Baylin, S.B. *New Engl J Med* 349, 2042-2054 (2003)). In colon tumors, methylation of the MMR gene MLH1 has been found as an alternative pathway of forming MSI-High colon cancer (Issa, J.P. *Ann NY Acad Sci* 910, 140-153; discussion 153-145 (2000)). Widespread CpG island hypermethylation underscores a distinct pathway in colon cancer pathogenesis termed CIMP (Toyota (1999)). Tumors arising through the CIMP pathway comprise 20% of colorectal cancers and are characterized by poor patient outcome. Significant attention has been paid to the role of DNA hypermethylation in epigenetically mediated gene silencing and its significance in colon cancer initiation (Suzuki, H., *et al. Nat Genetics* 31, 141-149 (2002); Baylin, S.B. *Semin Cancer Biol* 12, 331-337 (2002)). However, it is not clear whether these epigenetic targets can be harnessed for therapeutic purposes.

[0006] Based on recent findings in epigenetics research, it is now clear that DNA methylation and histone modification are reversible processes that can be targeted for

therapeutic intervention using small molecule inhibitors of the epigenetic writers (methyltransferases, acetyltransferases, kinases), readers (bromodomain- or chromodomain-containing genes) and erasers (demethylases, deacetylases, phosphatases) (Wee, S., *et al. Ann NY Acad Sci* 1309, 30-36 (2014); Ahuja, N., Easwaran, H. & Baylin, S.B. *J Clin Invest* 124, 56-63 (2014); Azad, N., Zahnow, C.A., Rudin, C.M. & Baylin, S.B. *Clin Oncol* 10, 256-266 (2013); Di Costanzo, A., Del Gaudio, N., Migliaccio, A. & Altucci, L. *Arch Toxicol* 88, 1651-1668 (2014)). For example, the histone acetyl-lysine reader, BRD4, can be targeted for inhibition using drugs that disrupt the bromodomain binding to acetylated histones (Filippakopoulos, P., *et al. Nature* 468, 1067-1073 (2010); Filippakopoulos, P. & Knapp, S. *Drug Discov* 13, 337-356 (2014)). Such drugs are showing promising responses in clinical trials, underscoring the need for additional efforts to identify and characterize epigenetic regulators that may be therapeutically tractable (Filippakopoulos & Knapp (2014)).

[0007] Cancer, including colorectal cancer, is treated using a combination of methods including chemotherapy, radiation therapy, and surgery depending on the cancer type, location, and severity. However, these treatments are expensive and are accompanied by significant adverse effects that negatively impact quality of life. It is presently unclear why some patients respond effectively to certain cancer therapies while others patients fail to respond. For example, the response rates for treating metastatic colorectal cancer with the drug cetuximab ranges between 8.8% when used alone and 22.9% when used in combination with the drug irinotecan (Cunningham *et al.* (2004) *N Engl J Med.* 351:337-45; Saltz *et al.* (2004) *J Clin Oncol.* 22:1201-8).

[0008] Cancer biomarkers can include DNA, mRNA, proteins, metabolites, or processes such as apoptosis or angiogenesis. Cancer biomarkers can be utilized for cancer screening, clinical staging, evaluating treatment responses, and tracking disease progression. However, current biomarkers possess a low diagnostic specificity and sensitivity (Kulasingam *et al* (2008) *Nat. Clin. Pract. Oncol.* 5(10):588-599). The lack of biomarkers for treatment responsiveness contributes to unnecessary, ineffective, and potentially harmful treatments. Identifying biomarkers that predict how a patient will respond to therapy will lead to individualized cancer therapies, thereby decreasing healthcare costs, increasing cancer therapy efficacy, and increasing the quality of life of cancer patients. Thus, an unmet need exists for novel methods to predict responses to specific cancer treatments.

[0009] All references cited herein, including patent applications and publications, are hereby incorporated by reference in their entirety.

SUMMARY

[0010] Accordingly, there is a need for novel methods to treat subjects having cancer with effective treatments and to select cancer patients for effective treatments. The present disclosure provides methods of treating a subject having cancer with a BET inhibitor, methods of selecting a subject for treatment with a BET inhibitor, and methods of modulating the treatment of a subject undergoing BET inhibitor treatment for cancer, where the methods include the step of performing a nucleic acid-based detection assay to detect the expression level of an eRNA transcribed from a myc-associated enhancer element in a sample containing cancer cells from the subject. The teachings herein demonstrate the surprising result that enhancer-derived non-coding RNA can serve as a predictive biomarker and as a pharmacodynamic biomarker of a cancer's responsiveness to BET inhibitor treatment.

[0011] Thus one aspect includes methods of treating a subject having cancer with a BET inhibitor, comprising performing a nucleic acid-based detection assay to detect the expression level of an eRNA transcribed from a myc-associated enhancer element in a sample containing cancer cells from the subject; determining whether the cells in the sample from the subject express the eRNA at a level greater than a reference expression level of the eRNA; and administering an effective amount of the BET inhibitor to the subject if it is determined that the cells in the sample from the subject express the eRNA at a level greater than the reference expression level of the eRNA.

[0012] Another aspect includes methods of selecting a subject for treatment with a BET inhibitor, comprising performing a nucleic acid-based detection assay to detect the expression level of an eRNA transcribed from a myc-associated enhancer element in a sample containing cancer cells from the subject; determining whether the cells in the sample from the subject express the eRNA at a level greater than a reference expression level of the eRNA; and selecting the subject for treatment with a BET inhibitor if the cells in the sample from the subject express the eRNA at a level greater than the reference expression level of the eRNA. In some embodiments, selecting the subject for treatment comprises selecting the subject for inclusion in a clinical trial.

[0013] Another aspect includes methods of modulating the treatment of a subject undergoing BET inhibitor treatment for cancer, comprising performing a nucleic acid-based

detection assay to detect the expression level of an eRNA transcribed from a myc-associated enhancer element in a sample containing cancer cells from the subject; determining the expression level of the eRNA in the cells in the sample; comparing the expression level of the eRNA in the cells in the sample to a reference expression level of the eRNA; and modulating the amount of BET inhibitor administered to the subject based on the difference between the expression level of the eRNA in the cells in the sample and the eRNA reference expression level. In some embodiments, the reference expression level is based on the expression level of the eRNA in the subject at an earlier timepoint during BET inhibitor treatment. In some embodiments, comparing the expression level of the eRNA in the cancer cells to the reference expression level of the eRNA comprises determining that the expression level of the eRNA in the cancer cells is less than the reference expression level of the eRNA and modulating the amount of BET inhibitor administered to the subject comprises maintaining the same level or decreasing the level of BET inhibitor administered to the subject. In some embodiments, comparing the expression level of the eRNA in the cancer cells to the reference expression level of the eRNA comprises determining that the expression level of the eRNA in the cancer cells is the same or more than the reference expression level of the eRNA and modulating the amount of BET inhibitor administered to the subject comprises increasing the level of BET inhibitor administered to the subject.

[0014] Another aspect includes methods of predicting responsiveness of a subject having cancer to a BET inhibitor, comprising performing a nucleic acid-based detection assay to detect the expression level of an eRNA transcribed from a myc-associated enhancer element in a sample containing cancer cells from a subject; determining whether the cells in the sample from the subject express the eRNA at a level greater than a reference expression level of the eRNA; and predicting that the subject will be responsive to a BET inhibitor if it is determined that the cells in the sample from the subject express the eRNA at a level greater than the reference expression level of the eRNA.

[0015] Another aspect includes methods of communicating the likelihood of response of a subject having cancer to a BET inhibitor, comprising: performing a nucleic acid-based detection assay to detect the expression level of an eRNA transcribed from a myc-associated enhancer element in a sample containing cancer cells from a subject; determining whether the cells in the sample from the subject express the eRNA at a level greater than a reference expression level of the eRNA; and communicating to a treatment provider that the cells in the sample from the subject express the eRNA at a level greater than the reference expression

level of the eRNA, wherein the treatment provider administers an effective amount of a BET inhibitor to the subject or selects the subject for treatment with a BET inhibitor based on the communication.

[0016] Another aspect includes methods of treating a subject having cancer with a BET inhibitor, comprising administering to the subject an effective amount of the BET inhibitor wherein cancer cells contained in a sample from the subject were determined to express an eRNA transcribed from a myc-associated enhancer element at a level greater than a reference expression level of the eRNA.

[0017] Another aspect includes methods of treating a subject having cancer with a BET inhibitor, comprising administering to the subject an effective amount of the BET inhibitor wherein the subject was selected for treatment based on a determination that cancer cells contained in a sample from the subject express an eRNA transcribed from a myc-associated enhancer element at a level greater than a reference expression level of the eRNA.

[0018] Another aspect includes methods of treating cancer in a subject comprising administering to the subject an effective amount of a BET inhibitor wherein treatment is based upon the subject having cancer comprising a cancer cell that expresses an eRNA transcribed from a myc-associated enhancer element at a level greater than a reference expression level of the eRNA.

[0019] Another aspect includes methods of treating a cancer cell, wherein the cancer cell expresses an eRNA transcribed from a myc-associated enhancer element at a level greater than a reference expression level of the eRNA, the method comprising providing an effective amount of a BET inhibitor to the cell.

[0020] Another aspect includes method of treating cancer in a subject provided that the subject has been found to have cancer comprising a cancer cell that expresses an eRNA transcribed from a myc-associated enhancer element at a level greater than a reference expression level of the eRNA, the method comprising administering to the subject an effective amount of a BET inhibitor.

[0021] In some embodiments of any of the above aspects, the cancer is selected from the group consisting of colon cancer, lung cancer, pancreatic cancer, bladder cancer, kidney cancer, endometrial cancer, leukemia, prostate cancer, breast cancer, gastric cancer, lung cancer, and ovarian cancer.

[0022] In some embodiments, the cancer is selected from the group consisting of colon cancer, lung cancer, pancreatic cancer, bladder cancer, kidney cancer, and endometrial cancer. In some embodiments the cancer is colon cancer. In some embodiments, the colon cancer is CpG island methylator phenotype (CIMP) (+) colon cancer. In some embodiments of the embodiments of this paragraph, the eRNA comprises a sequence having at least 80% identity to a sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID NO: 3.

[0023] In some embodiments, the cancer is leukemia. In some embodiments where the cancer is leukemia, the eRNA comprises a sequence having at least 80% identity to SEQ ID NO: 4.

[0024] In some embodiments, the cancer is selected from the group consisting of prostate cancer, bladder cancer, kidney cancer, endometrial cancer, breast cancer, gastric cancer, lung cancer, and ovarian cancer. In some embodiments, the cancer is prostate cancer. In some embodiments of the embodiments of this paragraph, the eRNA comprises a sequence having at least 80% identity to SEQ ID NO: 5.

[0025] In some embodiments that may be combined with any of the preceding embodiments, the subject is a human.

[0026] In some embodiments that may be combined with any of the preceding embodiments, the BET inhibitor is selected from the group consisting of JQ1, I-BET 151 (GSK1210151A), I-BET 762 (GSK525762), OTX-015, TEN-010, CPI-203, and CPI-0610.

[0027] In some embodiments that may be combined with any of the preceding embodiments, the nucleic acid-based detection assay detects expression of non-coding, nuclear RNA expressed in the cells in the sample from the subject. In some embodiments that may be combined with any of the preceding embodiments, the nucleic acid-based detection assay is selected from the group consisting of RNAseq, microarray analysis, direct RNA sequencing, in situ hybridization, and quantitative real-time PCR.

[0028] In some embodiments that may be combined with any of the preceding embodiments, the myc-associated enhancer element is a myc-associated super enhancer element.

[0029] In some embodiments that may be combined with any of the preceding embodiments, the reference expression level is based on the expression level of the eRNA in

non-cancer cells. In some embodiments, the non-cancer cells are from the same tissue type as the cancer cells.

[0030] Another aspect includes methods of treating a subject having colon cancer with a BET inhibitor, comprising performing a nucleic acid-based detection assay to detect the expression level of an eRNA comprising a sequence having at least 80% identity to a sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID NO: 3 in a sample containing colon cancer cells from the subject; determining whether the cells in the sample from the subject express the eRNA at a level greater than a reference expression level of the eRNA; and administering an effective amount of the BET inhibitor to the subject if it is determined that the cells in the sample from the subject express the eRNA at a level greater than the reference expression level of the eRNA.

[0031] Another aspect includes kits comprising reagents for detecting the expression level of an eRNA transcribed from a myc-associated enhancer element in a sample. In some embodiments, the kits further comprise instructions for using the reagents. In some embodiments, the kits further comprise a BET inhibitor. In some embodiments, the kits further comprise instructions for performing any of the methods described herein.

[0032] It is to be understood that one, some, or all of the properties of the various embodiments described herein may be combined to form other embodiments of the present disclosure. These and other aspects of the present disclosure will become apparent to one of skill in the art.

BRIEF DESCRIPTION OF THE DRAWINGS

[0033] FIG. 1A shows a schematic diagram of lentiviral expression vectors used to express Cas9 and gRNA. FIG. 1B shows cell viability measured in parental RKO or RKO-Cas9 stably expressing cells 7 days after transduction with gRNAs targeting luciferase or PLK1. FIG. 1C shows a schematic of a CRISPR negative-selection screen that was conducted in RKO-Cas9 cells using an arrayed gRNA library designed and synthesized to target 211 genes involved in epigenetic regulation and cancer (Epi200). Distribution curve shows Z-scores for cell viability for all gRNAs in the Epi200 CRISPR library. Dashed lines (Z-score -1.5, -2.0) indicate cutoffs used for determining whether a gRNA scored as a hit. FIG. 1D shows a scatter gram depicting Z-score values for each gRNA of the 13 genes that scored as hits. Bars represent individual gRNAs. NTC gRNAs and CTNNB1 gRNAs were not

expected to score and are shown for reference. FIG. 1E shows a bar graph demonstrating cell viability effects after BRD4 knockout using 5 independent gRNAs. Error bars indicate standard deviation from three replicates. Accompanying immunoblots display the level of BRD4 depletion 4 days following transduction. FIG. 1F shows validation of gRNA-mediated BRD4 knockout using immunofluorescence. Two independent gRNAs are shown. In merged panels, DNA is blue and BRD4 is red.

[0034] FIGS. 2A-D show bar graphs depicting average cell viability changes conducted on a subset of the top scoring target genes in the primary CRISPR screen. 5 independent gene-specific gRNAs were used per gene. Error bars represent standard deviation from three replicates. Immunoblots beneath bar graphs show the level of protein depletion 4 days following gRNA transduction. Percent protein depletion was quantified using Image J and is displayed under immunoblots. FIG. 2E shows a bar graph demonstrating the range of gRNA mediated protein depletion for 39 gRNAs representing a total of 8 genes.

[0035] FIGS. 3A&B depict BRD4 immunohistochemistry of normal colon (NL), colonic adenoma and colon carcinoma using either a pan-isoform BRD4 (FIG. 3A) or a BRD4-LF specific (FIG. 3A) antibody. FIG. 3B shows photomicrographs illustrating target specificity using wild type (WT) or BRD4 knockout (KO) RKO cells. FIG. 3C depicts immunoblot analysis of isoform specific BRD4 expression in colon cancer cell lines. FIG. 3D shows generation of BRD4 clonal knockout RKO and HCT116 cells. The schematic illustrates the location of gRNAs co-transfected into cells with Cas9. Immunoblot analysis of multiple BRD4 knockout clones. The top BRD4 blots used an antibody recognizing an epitope over the deleted region, and the bottom BRD4 blots used an antibody recognizing a C terminal epitope. FIGS. 3E&F show incucyte analysis used to quantify cell proliferation of RKO (FIG. 3E) or HCT116 (FIG. 3F) BRD4 knockout cells. The blue line represents parental wild-type cells, gray lines represent an individual clone's growth, and the red line represents the average clonal growth. Error bars represent SEM. FIG. 3G shows FACS-based cell cycle analysis of RKO and HCT116 parental and BRD4 knock out cells. FIGS. 3H&I demonstrate that expression of the BRD4 long isoform (LF-wt) rescued cell proliferation defects in HCT116 BRD4 knockout cells. Expression of a non-specific protein (LacZ) or a BRD4 long isoform containing inactivating mutations in both bromodomains (LF-mut) failed to rescue the phenotype. FIG. 3H shows an immunoblot of stably expressing cell lines in FIG. 3I. FIGS. 3J&K show generation of clonal HCT116 cells that lack the C terminal domain of BRD4. FIG. 3J shows a schematic illustrating the location of BRD4 gRNA co-transfected

with Cas9 to knock out the BRD4 long isoform. Immunoblot (below) highlights the 4 resulting BRD4 clones with deleted C termini. FIG. 3K demonstrates incucyte analysis used to quantify cell proliferation of HCT116 cells containing BRD4 C terminal deletions. The blue line represents parental cells, gray lines represent an individual clone's growth, and the red line represents the average clonal growth. Error bars represent SEM.

[0036] FIG. 4A shows an immunoblot (left) demonstrating stable BRD4 knockout cells expressing exogenous LACZ (control), exogenous wild-type BRD4 short isoform (BRD4-wt), or BRD4 short isoform containing bromodomain inactivating mutations. Incucyte analysis (right) was utilized to quantify cell proliferation in the corresponding cell lines. FIG. 4B demonstrates inducible BRD4 knockdown reduces cell proliferation of HCT116 and HT29 cells in vitro. Incucyte anaylsis was utilized to quantify cell proliferation following induction of BRD4 shRNAs with 0.5 μg/ml doxycycline. Error bars represent SEM.

[0037] FIG. 5A shows the generation of stable HT-29 and HCT-116 cell lines containing doxycycline inducible BRD4 shRNAs. Immunoblots show BRD4 protein levels following 4 days of doxycycline treatment in vitro. FIG. 5B depicts IHC staining for BRD4 in representative shBRD4 xenograft tumors after 7 days of sucrose or doxycycline treatment. FIG. 5C shows quantification of BRD4 depletion in xenograft tumors 7 days after doxycycline administration. FIG. 5D shows a line graph depicting tumor volumes from xenografted HCT116 or HT-29 cells measured over time (n = 10 mice/ group). shRNA expression was induced with doxycycline. Error bars represent SEM. ** Indicate statistical significance. FIG. 5E shows quantification of mitotic index using Phospho-histone H3 (Phos H3) IHC following BRD4 knockdown in xenograft tumors. FIG. 5F shows quantification of cMYC by IHC following BRD4 knockdown in xenograft tumors. FIG. 5G shows histological analysis of HT-29 tumors in shNTC or shBRD4 tumors following doxycycline addition. H&E, Alcian Blue, Ki-67, and cMYC staining are shown.

[0038] FIG. 6A shows relative EC50 values in colon cancer cells following JQ1 treatment for 3 days. Blue and red bars highlight sensitive and resistant cell lines used for predictive analysis in FIG. 6B. FIG. 6B shows a schematic illustrating the genomic tools and features utilized for predicting colon cancer sensitivity to BET inhibition. FIG. 6C depicts the relative JQ1 EC50 values in colon cancer cells sorted by CIMP status. Bar represents the mean, error bars represent SEM, and each dot represents a single cell line. FIG. 6D shows a schematic of RNA-seq and ChIP-seq experiments and analysis pipeline. FIG. 6E depicts a

heat map of Brd4 binding at super-enhancers and 10kb flanking regions. Data is displayed for regions with a super-enhancer in at least one cell line. Color scale reflects the density of Brd4 signal. FIG. 6F depicts a heat map and hierarchical clustering of gene expression changes following 500 nM JQ1 treatment (24h). Data are displayed as Log2 (fold change) relative to DMSO control. Genes upregulated or downregulated by greater than 2 fold in at least one cell line are displayed (n=4621). FIG. 6G depicts a heatmap of the top 20 genes differentially regulated by JQ1 in CIMP(+) versus CIMP(-) cell lines. FIG. 6H shows MYC levels examined by immunoblot in colon cancer cell lines treated for 6 or 24 hours with either DMSO or 1 μM JQ1. FIG. 6I shows a comparison of MYC protein depletion in CIMP(+) or CIMP(-) colon cell lines following 1 μM JQ1 treatment for 24 hours. Bars represent average, error bars represent SD, and each dot represents an individual cell line.

[0039] FIG. 7A shows relative EC50 values calculated for colon cancer cell lines treated with JQ1 for 3 days. Bar graph shows the EC50 for each cell line. Blue boxes show the 6 most sensitive (ATRFLOX-SW48) and red boxes show the 6 most resistant (COLO741-LS-180) cell lines that were used for a binomial tail analysis. The features tested are shown on the right. FIG. 7B shows eight gene panel classification for the CIMP-status (defined by Ogino, S., *et al. J Mol Diagn* 9, 305-314 (2007)). Box plot shows the degree of CpG methylation for the CIMP-gene set in each cell line. JQ1 sensitivity is denoted below each cell line and is defined based on an arbitrary cut-off of 0.25 μM EC50. FIGS. 7C&D depict features that were significantly associated with JQ1 sensitivity. Scatter plots show either DNA methylation or expression as a function of the JQ1 sensitivity per given cell line (each dot is one cell line). Rho values (Spearman correlation) and p-values are indicated for each feature.

[0040] FIG. 8A demonstrates sensitivity of colon cancer cell lines to iBET-762. Bar graph depicts relative EC50 values. FIG. 8B shows iBET-762 sensitivity profiles binned according to CIMP status. P-values denote statistical significance (Student's t-test). FIG. 8C shows correlation between iBET-762 and JQ1 EC50 across the entire panel of 20 colon cancer cell lines. Pearson correlation is shown.

[0041] FIG. 9A shows the overlap of genes downregulated by > 2 fold following 500 nM JQ1 treatment (24h) relative to DMSO control in CIMP(+) or CIMP(-) cell lines. Overlap of CIMP(+) common and CIMP(-) common JQ1-downregulated genes. FIG. 9B shows the overlap of Brd4 super-enhancer associated genes in CIMP(+) or CIMP(-) cell lines. Overlap

of CIMP(+) common and CIMP(-) common Brd4 super-enhancer associated genes. FIG. 9C shows GSEA terms enriched in CIMP(+) or CIMP(-) cell lines based on analysis of JQ1-regulated genes. An FDR cutoff of 5% was used. ES (enrichment score), NES (normalized enrichment score).

[0042] FIG. 10A shows quantification of cMYC protein levels at the indicated time points after 1 μM JQ1 treatment in the indicated cell lines. Bar graph shows cMYC levels normalized to DMSO control. CIMP status is defined per Ogino classification. DMSO is shown on the left, JQ1 6 hours in the middle, and JQ1 24 hours on the right for each cell line. FIGS. 10B&C show kinetic analysis of cMYC reduction following 1 μM JQ1 treatment in CIMP(+) (FIG. 10B) or CIMP(-) (FIG. 10C) colon cell lines. Each connecting line and associated dots represent a single cell line. FIG. 10D demonstrates overexpression of V5-tagged cMYC in BRD4 knockout HCT116 cells partially rescues the cell proliferation phenotype. Cell proliferation was measured by Incucyte analysis and quantified by percent confluence. GFP overexpression was used as control (+GFP). cMYC immunoblot (right) confirmed cMYC transgene expression.

[0043] FIG. 11A depicts a Venn diagram showing the overlap of genes downregulated by JQ1 (>2 fold) and associated with a super-enhancer across CIMP(+) cell lines and CIMP(-) cell lines. Super-enhancer-associated, JQ1-regulated genes common to all CIMP(+) cell lines are highlighted. FIG. 11B shows the distribution of Brd4 ChIP-seq signal across enhancers in CIMP(+) cell lines. The y-axis represents input subtracted Brd4 signal. The x-axis represents enhancers ranked by Brd4 signal intensity. Super-enhancers were defined as enhancers that surpassed the inflection point. The CCAT1-associated super-enhancer is highlighted. FIG. 11C depicts genome browser tracks showing input-normalized average BRD4 ChIP-seq signal across the CCAT1 locus for CIMP(+) (blue) and CIMP(-) (red) cell lines. The y-axis represents BRD4/input coverage values averaged for CIMP(+) (n=4) or CIMP(-) (n=2) cell lines. FIG. 11D shows basal RNA levels of CCAT1 in colon cell lines. RNA was measured by RNAseq and presented as Log2 RPKM. CIMP(+) lines are HT-29, HCT 116, COLO 205, and HCT-15, and CIMP(-) lines are COLO 320 and SW 480. FIG. 11E shows relative CCAT1 lncRNA expression in CIMP(+) cells following 500 nM JQ1 treatment. Data is from RNAseq analysis and error bars represent SD. FIG. 11F shows relative cMYC expression in colon cancer cells following 500 nM JQ1 treatment. Data is from RNAseq analysis. CIMP(+) lines are HT-29, HCT 116, COLO 205, and HCT-15, and CIMP(-) lines are COLO 320 and SW 480. Error bars represent SD.

[0044] FIGS. 12A&B depicts genome browser tracks of ChIP-seq for Brd4 (FIG. 12A) and H3K27ac (FIG. 12B) at the MYC and CCAT1 genomic region for CIMP(+) (blue) and CIMP(-) (red) cell lines. The y-axis represents ChIP/input coverage values. FIGS. 12C&D show quantification of input-normalized Brd4 (FIG. 12C) and H3K27ac (FIG. 12D) ChIP-seq signal at the CCAT1 locus. Region was defined by overlap of super-enhancers in CIMP(+) cell lines. The region quantified is highlighted by the dark gray box in FIGS. 12A&B. FIG. 12E shows results from Brd4 ChIP-qPCR at the CCAT1 super-enhancer in CIMP(+) (n=5) and CIMP(-) (n=4) cell lines. Relative Brd4 signal is shown normalized to a negative control locus.

[0045] FIG. 13A depicts genome browser tracks showing H3K27ac signal for colon cancer (HCT-116; blue), prostate cancer (VCaP; red), and leukemia (Jurkat; black) overlapping lncRNA-encoding genes near the Myc locus. The y-axis represents reads per million. FIG. 13B depicts a box and whisker plot of CCAT1, PCAT1, and LOC728724 RNA expression in a panel of cancers. Data was analyzed using RNAseq datasets from The Cancer Genome Atlas (TCGA) and presented as Log10 RPKM. CCAT expression is shown on the left, PCAT1 in the middle, and LOC728724 on the right for each cancer type. FIG. 13C shows correlation analysis between CCAT1 expression and c-MYC downregulation following JQ1 treatment. Cells were treated for 24 hours with either DMSO or 1 μM JQ1 and cMYC expression was quantified by qRT-PCR. Each dot represents a single cell line. FIG. 13D shows correlation analysis between CCAT1 expression and cell line sensitivity to JQ1. Relative EC50 values are calculated based on 3 days of JQ1 treatment. Cell lines with an RPKM ≤ 0.1 were defined as CCAT1 low and cell lines with an RPKM ≥ 1 were defined as CCAT1 high. Each dot represents a single cell line. The bar represents the mean, and error bars represent SEM.

[0046] FIGS. 14A-D shows basal RNA expression of CCAT1 in colon cancer cells (FIG. 14A), CCAT1 in gastric, pancreatic, and lung cancer cells (FIG. 14B), PCAT1 in prostate cancer cells (FIG. 14C), and LOC728724 in blood cancer cells (FIG. 14D). RNA was measured by RNAseq and presented as log2 RPKM. FIGS. 14E-G demonstrate CCAT1(FIG. 14E), PCAT1(FIG. 14F), and LOC728724 (FIG. 14G) expression is dependent on BET activity. Cells were treated for 24 hours with 1 μM JQ1 or DMSO and the indicated lncRNA was quantified by qRT-PCR in the indicated cell lines.

[0047] FIG. 15A shows quantification of cMYC RNA by qRT-PCR in gastric, pancreatic, lung, and colon cancer cells treated for 24 hours with DMSO or 1 μM JQ1. CCAT1 high lines were defined by a CCAT1 RPKM >1 (KATOIII-SNU601 for Gastric; TCCPAN2-CAPAN1 for Pancreatic; NCIH1666-NCIH1944 for Lung; and RKO-SKCO1 for Colon), and CCAT1 low lines were defined by a CCAT1 RPKM <1 (HS746T-HGC27 for Gastric; PANC1-KP4 for Pancreatic; DMS273-NCIH460 for Lung; and SW480-SW620 for Colon). FIGS. 15B-E show correlation analysis between lncRNA expression and cell line sensitivity to JQ1. Relative EC50 values were calculated based on 3 days of JQ1 treatment. Lung (FIG. 15B), pancreatic (FIG. 15C), gastric (FIG. 15D), and colon (FIG. 15E) cell lines with a CCAT1 RPKM ≤ 0.1 were defined as CCAT1 low and cell lines with a CCAT1 RPKM ≥ 1 were defined as CCAT1 high. FIGS. 15F-H show quantification of cMYC RNA by qRT-PCR in prostate cancer cells treated for 24 hours with DMSO or 1 μM JQ1. Correlation analysis between lncRNA expression and cMYC downregulation in prostate cancer cells following JQ1 treatment is shown in FIG. 15F. Prostate cells with a PCAT1 RPKM ≤ 0.1 were defined as PCAT1 low, and cell lines with a PCAT1 RPKM > 0.1 were defined as PCAT1 high (FIG. 15G). FIG. 15H shows a bar graph depicting the level of cMYC reduction per each cell line. FIG. 15I shows quantification of cMYC RNA by qRT-PCR in blood cancer cells treated for 24 hours with DMSO or 1 μM JQ1. Error bars represent SEM. FIG. 15J shows correlation analysis between LOC728724 expression and cMYC downregulation in blood cancer cells following JQ1 treatment. Each dot represents an individual cell line.

[0048] FIG. 16A depicts representative ISH (in situ hybridization) photomicrographs showing CCAT1 expression in colon tumors. ISH scoring system is indicated. FIG. 16B depicts the ISH score breakdown for a panel of normal colon tissues and colon tumors. Each patient sample was scored from triplicate representative tumor cores and the average CCAT1 ISH score was recorded as low (0-1), moderate (1-2), or high (2-3). FIG. 16C shows Kaplan-Meier survival data for patients with colon cancer, separated by CCAT1 ISH score. Patients with high or moderate CCAT1 expression had a worse overall survival compared with low CCAT1 expression.

[0049] FIG. 17A depicts CCAT1 ISH performed on a CCAT1 high (HT-29) or a CCAT1 low (SW620) expressing colon cell line. FIG. 17B demonstrates CCAT1 expression is sensitive to JQ1 treatment. CCAT1 ISH was performed on formalin fixed RKO cell pellets. Cells were pre-treated with either DMSO or 1 μM JQ1. FIG. 17C demonstrates CCAT1

expression in normal human tissues. Box and whisker plots (min to max) highlight CCAT1 expression in normal colon, gastric, lung, and pancreatic human tissue. Results were compiled from TCGA RNAseq data sets. FIG. 17D demonstrates that normal human colon tissue lacks expression of CCAT1, as indicated by CCAT1 ISH.

DETAILED DESCRIPTION

Definitions

[0050] The term “biomarker” or “marker” as used herein refers generally to a molecule, including a gene, non-coding RNA, protein, carbohydrate structure, or glycolipid, the expression of which in or on a mammalian tissue or cell or secreted can be detected by known methods (or methods disclosed herein) and is predictive or can be used to predict (or aid prediction) for a mammalian cell’s or tissue’s sensitivity to, and in some embodiments, to predict (or aid prediction) an individual’s responsiveness to treatment regimes based on BET inhibitors.

[0051] “Percent (%) identity” with respect to the sequence of a reference RNA is defined as the percentage of nucleotides in a candidate sequence that are identical with the nucleotides in the reference RNA sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Percentage identity may also refer to the alignment of a complement of the reference or candidate sequence to the other sequence. Alignment for purposes of determining percent nucleotide sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For purposes herein, however, % nucleotide sequence identity values are generated using the sequence comparison computer program ALIGN-2. The ALIGN-2 sequence comparison computer program was authored by Genentech, Inc., and the source code has been filed with user documentation in the U.S. Copyright Office, Washington D.C., 20559, where it is registered under U.S. Copyright Registration No. TXU510087. The ALIGN-2 program is publicly available from Genentech, Inc., South San Francisco, California, or may be compiled from the source code. The ALIGN-2 program should be compiled for use on a UNIX operating system, including digital

UNIX V4.0D. All sequence comparison parameters are set by the ALIGN-2 program and do not vary.

[0052] As used herein, “treatment” (and grammatical variations thereof such as “treat” or “treating”) refers to clinical intervention in an attempt to alter the natural course of the individual or cell being treated during the course of clinical pathology. Desirable effects of treatment include, but are not limited to, decreasing the rate of disease progression, ameliorating or palliating the disease state, and remission or improved prognosis. For example, an individual is successfully “treated” if one or more symptoms associated with cancer are mitigated or eliminated, including, but not limited to, reducing the proliferation of (or destroying) cancerous cells, decreasing symptoms resulting from the disease, increasing the quality of life of those suffering from the disease, decreasing the dose of other medications required to treat the disease, and/or prolonging survival of individuals.

[0053] As used herein, “delaying progression of a disease” means to defer, hinder, slow, retard, stabilize, and/or postpone development of the disease (such as cancer). This delay can be of varying lengths of time, depending on the history of the disease and/or individual being treated. As is evident to one skilled in the art, a sufficient or significant delay can, in effect, encompass prevention, in that the individual does not develop the disease. For example, a late stage cancer, such as development of metastasis, may be delayed.

[0054] An “individual,” “subject,” or “patient” is a mammal. Mammals include, but are not limited to, domesticated animals (e.g., cows, sheep, cats, dogs, and horses), primates (e.g., humans and non-human primates such as monkeys), rabbits, and rodents (e.g., mice and rats). In some embodiments, the individual, subject, or patient is a human.

[0055] The term “pharmaceutical formulation” refers to a preparation which is in such form as to permit the biological activity of an active ingredient contained therein to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered.

[0056] A “pharmaceutically acceptable carrier” refers to an ingredient in a pharmaceutical formulation, other than an active ingredient, which is nontoxic to a subject., A pharmaceutically acceptable carrier includes, but is not limited to, a buffer, excipient, stabilizer, or preservative.

[0057] An “effective amount” of an agent, e.g., a pharmaceutical formulation, refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic or prophylactic result.

[0058] As is understood in the clinical context, an effective amount of a therapeutic agent (e.g., a BET inhibitor provided herein), drug, compound, or pharmaceutical composition may or may not be achieved in conjunction with another drug, compound, or pharmaceutical composition. Thus, an “effective amount” may be considered in the context of administering one or more therapeutic agents, and a single agent may be considered to be given in an effective amount if, in conjunction with one or more other agents, a desirable result may be or is achieved.

[0059] As used herein, “in conjunction with” refers to administration of one treatment modality in addition to another treatment modality. As such, “in conjunction with” refers to administration of one treatment modality before, during or after administration of the other treatment modality to the individual.

[0060] The term “package insert” is used to refer to instructions customarily included in commercial packages of therapeutic products, that contain information about the indications, usage, dosage, administration, combination therapy, contraindications and/or warnings concerning the use of such therapeutic products.

[0061] The term “sample”, as used herein, refers to a composition that is obtained or derived from a subject having cancer that may contain a cellular and/or other molecular entity that is to be characterized and/or identified, for example based on physical, biochemical, chemical and/or physiological characteristics. For example, the phrase “sample containing cancer cells” and variations thereof refers to any sample obtained from a subject having cancer that may contain the cellular and/or molecular entity that is to be characterized.

[0062] As used herein, a “reference value” or a “reference expression level” can be an absolute value; a relative value; a value that has an upper and/or lower limit; a range of values; an average value; a median value; a mean value; or a value as compared to a particular control or baseline value.

[0063] The term “array” or "microarray", as used herein refers to an ordered arrangement of hybridizable array elements, such as polynucleotide probes (e.g., oligonucleotides) and antibodies, on a substrate. The substrate can be a solid substrate, such as a glass slide, or a

semi-solid substrate, such as nitrocellulose membrane. The nucleotide sequences can be DNA, RNA, or any permutations thereof.

[0064] "Amplification," as used herein, generally refers to the process of producing multiple copies of a desired sequence. "Multiple copies" means at least 2 copies. A "copy" does not necessarily mean perfect sequence complementarity or identity to the template sequence. For example, copies can include nucleotide analogs such as deoxyinosine, intentional sequence alterations (such as sequence alterations introduced through a primer comprising a sequence that is hybridizable, but not complementary, to the template), and/or sequence errors that occur during amplification.

[0065] "Polynucleotide," or "nucleic acid," as used interchangeably herein, refer to polymers of nucleotides of any length, and include DNA and RNA. The nucleotides can be deoxyribonucleotides, ribonucleotides, modified nucleotides or bases, and/or their analogs, or any substrate that can be incorporated into a polymer by DNA or RNA polymerase. A polynucleotide may comprise modified nucleotides, such as methylated nucleotides and their analogs. If present, modification to the nucleotide structure may be imparted before or after assembly of the polymer. The sequence of nucleotides may be interrupted by non-nucleotide components. A polynucleotide may be further modified after polymerization, such as by conjugation with a labeling component. Other types of modifications include, for example, "caps", substitution of one or more of the naturally occurring nucleotides with an analog, internucleotide modifications such as, for example, those with uncharged linkages (e.g., methyl phosphonates, phosphotriesters, phosphoamidates, cabamates, etc.) and with charged linkages (e.g., phosphorothioates, phosphorodithioates, etc.), those containing pendant moieties, such as, for example, proteins (e.g., nucleases, toxins, antibodies, signal peptides, ply-L-lysine, etc.), those with intercalators (e.g., acridine, psoralen, etc.), those containing chelators (e.g., metals, radioactive metals, boron, oxidative metals, etc.), those containing alkylators, those with modified linkages (e.g., alpha anomeric nucleic acids, etc.), as well as unmodified forms of the polynucleotide(s). Further, any of the hydroxyl groups ordinarily present in the sugars may be replaced, for example, by phosphonate groups, phosphate groups, protected by standard protecting groups, or activated to prepare additional linkages to additional nucleotides, or may be conjugated to solid supports. The 5' and 3' terminal OH can be phosphorylated or substituted with amines or organic capping groups moieties of from 1 to 20 carbon atoms. Other hydroxyls may also be derivatized to standard protecting groups. Polynucleotides can also contain analogous forms of ribose or deoxyribose sugars that are

generally known in the art, including, for example, 2'-O-methyl-2'-O- allyl, 2'-fluoro- or 2'-azido-ribose, carbocyclic sugar analogs, α- anomeric sugars, epimeric sugars such as arabinose, xyloses or lyxoses, pyranose sugars, furanose sugars, sedoheptuloses, acyclic analogs and abasic nucleoside analogs such as methyl riboside. One or more phosphodiester linkages may be replaced by alternative linking groups. These alternative linking groups include, but are not limited to, embodiments wherein phosphate is replaced by P(O)S("thioate"), P(S)S ("dithioate"), "(O)NR 2 ("amidate"), P(O)R, P(O)OR', CO or CH 2 ("formacetal"), in which each R or R' is independently H or substituted or unsubstituted alkyl (1-20 C) optionally containing an ether (--O--) linkage, aryl, alkenyl, cycloalkyl, cycloalkenyl or araldyl. Not all linkages in a polynucleotide need be identical. The preceding description applies to all polynucleotides referred to herein, including RNA and DNA.

[0066] "Oligonucleotide," as used herein, generally refers to short, generally single stranded, generally synthetic polynucleotides that are generally, but not necessarily, less than about 200 nucleotides in length. The terms "oligonucleotide" and "polynucleotide" are not mutually exclusive. The description above for polynucleotides is equally and fully applicable to oligonucleotides.

[0067] A "primer" is generally a short single stranded polynucleotide, generally with a free 3'-OH group, that binds to a target potentially present in a sample of interest by hybridizing with a target sequence, and thereafter promotes polymerization of a polynucleotide complementary to the target. A “pair of primers” refer to a 5’ primer and a 3’ primer that can be used to amplify a portion of a specific target gene.

[0068] "Detection" includes any means of detecting, including direct and indirect detection.

[0069] The term "prediction" is used herein to refer to the likelihood that a subject will respond either favorably or unfavorably to a drug, therapeutic agent, or set of drugs or therapeutic agents. In one embodiment, the prediction relates to the extent of those responses. In one embodiment, the prediction relates to whether and/or the probability that a subject will survive or improve following treatment, for example treatment with a particular therapeutic agent, and for a certain period of time without cancer recurrence. The predictive methods of the present disclosure can be used clinically to make treatment decisions by choosing the most appropriate treatment modalities for any particular subject. The predictive methods of the present disclosure can be used to select a subject for treatment, including selecting the

subject for inclusion in a clinical trial. The predictive methods of the present disclosure are valuable tools in predicting if a subject is likely to respond favorably to a treatment regimen, such as a given therapeutic regimen, including for example, administration of a given therapeutic agent, such as a BET inhibitor, or combination, surgical intervention, steroid treatment, etc., or whether long-term survival of the subject, following a therapeutic regimen is likely.

[0070] "Responsiveness of a subject" or "response of a subject" can be assessed using any endpoint indicating a benefit to the subject, including, without limitation, (1) inhibition, to some extent, of cancer progression, including slowing down and complete arrest; (2) reduction in the number of cancer episodes and/or symptoms; (3) reduction in lesional size; (4) inhibition (i.e., reduction, slowing down or complete stopping) of cancer cell infiltration into adjacent peripheral organs and/or tissues; (5) inhibition (i.e. reduction, slowing down or complete stopping) of cancer spread; (6) relief, to some extent, of one or more symptoms associated with the cancer; (7) increase in the length of cancer-free presentation following treatment; and/or (8) decreased mortality at a given point of time following treatment.

[0071] As used herein and in the appended claims, the singular forms "a," "an," and "the" include plural reference unless the context clearly indicates otherwise. For example, reference to an "antibody" is a reference to from one to many antibodies, such as molar amounts, and includes equivalents thereof known to those skilled in the art, and so forth.

[0072] Reference to "about" a value or parameter herein includes (and describes) embodiments that are directed to that value or parameter per se. For example, description referring to "about X" includes description of "X."

[0073] It is understood that aspect and variations of the invention described herein include "consisting" and/or "consisting essentially of" aspects and variations.

[0074] Additional definitions may be found throughout the detailed description.

Methods of the Disclosure

[0075] The present disclosure relates to methods involving biomarkers for use in treating subjects having cancer with a BET inhibitor. "Biomarkers" generally refers to biological molecules, and quantitative and qualitative measurements of the same, that are indicative of a disease state. Predictive biomarkers indicate whether a subject is likely to respond positively to a particular therapy. For example, HER2 profiling is commonly used in breast cancer

patients to determine if those patients are likely to respond to Herceptin® (trastuzumab, Genentech). Pharmacodynamic biomarkers provide a measure of the response to a therapy and so provide an indication of whether a therapy is working. For example, decreasing levels of prostate specific antigen (PSA) generally indicate that anti-cancer therapy for a prostate cancer patient is working. The present disclosure provides methods related to the use of both predictive biomarkers and pharmacodynamic biomarkers.

[0076] Thus, in one aspect, the present disclosure provides methods of treating or delaying progression of cancer in a subject having cancer with a BET inhibitor including the steps of performing a nucleic acid-based detection assay to detect the expression level of an eRNA transcribed from a myc-associated enhancer element in a sample containing cancer cells from the subject; determining whether the cells in the sample from the subject express the eRNA at a level greater than a reference expression level of the eRNA; and administering an effective amount of the BET inhibitor to the subject if it is determined that the cells in the sample from the subject express the eRNA at a level greater than the reference expression level of the eRNA. In certain embodiments, the methods include the steps of performing a nucleic acid-based detection assay to detect the expression level of an eRNA transcribed from a myc-associated enhancer element in a sample containing cancer cells from the subject; determining that the cells in the sample from the subject express the eRNA at a level greater than a reference expression level of the eRNA; and administering an effective amount of the BET inhibitor to the subject having cancer cells that express the eRNA at a level greater than the reference expression level of the eRNA. In one embodiment, the present disclosure provides methods of treating a subject having colon cancer with a BET inhibitor, including the steps of performing a nucleic acid-based detection assay to detect the expression level of an eRNA comprising a sequence having at least 80% identity to SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3 in a sample containing colon cancer cells from the subject; determining whether the cells in the sample from the subject express the eRNA at a level greater than a reference expression level of the eRNA; and administering an effective amount of the BET inhibitor to the subject if it is determined that the cells in the sample from the subject express the eRNA at a level greater than the reference expression level of the eRNA.

[0077] In another aspect, the present disclosure provides methods of selecting a subject for treatment with a BET inhibitor, including the steps of performing a nucleic acid-based detection assay to detect the expression level of an eRNA transcribed from a myc-associated enhancer element in a sample containing cancer cells from the subject; determining whether

the cancer cells from the subject express the eRNA at a level greater than a reference expression level of the eRNA; and selecting the subject for treatment with a BET inhibitor if the cells in the sample from the subject express the eRNA at a level greater than the reference expression level of the eRNA. In certain embodiments, selecting the subject for treatment involves selecting the subject for treatment in a clinical trial. In other embodiments, the method involves selecting a specific BET inhibitor to administer to the subject.

[0078] In another aspect, the present disclosure provides methods of modulating the treatment of a subject undergoing BET inhibitor treatment for cancer, including the steps of performing a nucleic acid-based detection assay to detect the expression level of an eRNA transcribed from a myc-associated enhancer element in a sample containing cancer cells from the subject; determining the expression level of the eRNA in the cancer cells; comparing the expression level of the eRNA in the cancer cells to a reference expression level of the eRNA; and modulating the amount of BET inhibitor administered to the subject based on the difference between the expression level of the eRNA in the cancer cells and the eRNA reference expression level. In another aspect, the present disclosure provides methods of monitoring the treatment of a subject undergoing BET inhibitor treatment, including the steps of performing a nucleic acid-based detection assay to detect the expression level of an eRNA transcribed from a myc-associated enhancer element in a sample containing cancer cells from the subject; determining the expression level of the eRNA in the cancer cells; and comparing the expression level of the eRNA in the cancer cells to a reference expression level of the eRNA. In some embodiments, the steps are repeated at multiple time points while the subject is undergoing BET inhibitor treatment.

[0079] The methods of the present disclosure may be performed by physicians, treatment providers, healthcare workers, hospitals, laboratories, patients, subjects, companies, and other institutions. All of the steps of a method of the present disclosure may be performed by one person, e.g. a physician, or the steps may be performed by multiple people in one institution. The steps may be performed by multiple people or multiple institutions at the direction of one person or one institution. Electronic means may be used to communicate information or data resulting from the step of determining whether cancer cells contained in a sample from a subject express an eRNA transcribed from a myc-associated enhancer element at a level greater than a reference expression level of the eRNA from one person or institution to another person or institution.

[0080] In another aspect, the present disclosure provides methods of predicting responsiveness of a subject having cancer to a BET inhibitor, including the steps of performing a nucleic acid-based detection assay to detect the expression level of an eRNA transcribed from a myc-associated enhancer element in a sample containing cancer cells from a subject; determining whether the cells in the sample from the subject express the eRNA at a level greater than a reference expression level of the eRNA; and predicting that the subject will be responsive to a BET inhibitor if it is determined that the cells in the sample from the subject express the eRNA at a level greater than the reference expression level of the eRNA. In some embodiments, the prediction is communicated to a treatment provider. In another aspect, methods of communicating the likelihood of response of a subject having cancer to a BET inhibitor are provided, which include the steps of performing a nucleic acid-based detection assay to detect the expression level of an eRNA transcribed from a myc-associated enhancer element in a sample containing cancer cells from a subject; determining whether the cells in the sample from the subject express the eRNA at a level greater than a reference expression level of the eRNA; and communicating to a treatment provider that the cells in the sample from the subject express the eRNA at a level greater than the reference expression level of the eRNA, wherein the treatment provider administers an effective amount of a BET inhibitor to the subject or selects the subject for treatment with a BET inhibitor based on the communication. In some embodiments of these aspects of the present disclosure, the steps are performed by a diagnostics company, a diagnostician, a diagnostics professional, or a technician. In other embodiments, the steps are performed by a health care provider. Communication of a prediction or of the likelihood of response of the subject to a treatment provider typically involves communication by electronic means such as, for example, e-mail, text message, entering information into an online database, or updating the subject's electronic medical records. The communication may also occur by in person interaction or telephonic interaction.

[0081] In another aspect, methods of treating or delaying progression of cancer in a subject having cancer with a BET inhibitor including the step of administering to the subject an effective amount of the BET inhibitor where cancer cells contained in a sample from the subject were determined to express an eRNA transcribed from a myc-associated enhancer element at a level greater than a reference expression level of the eRNA are provided. In another aspect, the present disclosure provides methods of treating or delaying progression of cancer in a subject having cancer with a BET inhibitor, including the step of administering to

the subject an effective amount of the BET inhibitor where the subject was selected for treatment based on a determination that cancer cells contained in a sample from the subject express an eRNA transcribed from a myc-associated enhancer element at a level greater than a reference expression level of the eRNA. In another aspect, the present disclosure provides methods of treating or delaying progression of cancer in a subject including the step of administering to the subject an effective amount of a BET inhibitor where treatment is based upon the subject having cancer containing a cancer cell that expresses an eRNA transcribed from a myc-associated enhancer element at a level greater than a reference expression level of the eRNA. In yet another aspect, the present disclosure provides methods of treating a cancer cell, where the cancer cell expresses an eRNA transcribed from a myc-associated enhancer element at a level greater than a reference expression level of the eRNA, the method including a step of providing an effective amount of a BET inhibitor to the cell. In yet another aspect, the present disclosure provides methods of treating cancer or delaying progression of cancer in a subject provided that the subject has been found to have cancer containing a cancer cell that expresses an eRNA transcribed from a myc-associated enhancer element at a level greater than a reference expression level of the eRNA, the method including a step of administering to the subject an effective amount of a BET inhibitor. In some embodiments of these aspects of the present disclosure, the step or steps of the methods are performed by a treatment provider or health care provider such as a physician or nurse.

[0082] In some embodiments of the methods of the present disclosure, the subject is a human.

[0083] The methods of the present disclosure may be used to treat or select subjects having cancer of any type. In some embodiments, the cancer is colon cancer, lung cancer, pancreatic cancer, bladder cancer, kidney cancer, endometrial cancer, leukemia, prostate cancer, breast cancer, gastric cancer, lung cancer, or ovarian cancer. In certain embodiments, the cancer is colon cancer. In other embodiments, the cancer is leukemia, and in some embodiments, the cancer is prostate cancer.

[0084] Colorectal tumors can be divided into three main subtypes based on initiating molecular alterations: 1) chromosomal instability (CIN), 2) CpG island methylator phenotype (CIMP) and 3) microsatellite instability (MSI). In embodiments where the cancer is colon cancer, the colon cancer may be CpG island methylator phenotype (CIMP) (+) colon cancer (Toyota (1999)). Widespread CpG island hypermethylation underscores a distinct pathway

in epigenetically dysregulated CIMP (+) colon cancer pathogenesis. Tumors arising through the CIMP pathway comprise 20% of colorectal cancers and are characterized by poor patient outcome.

[0085] The methods of the present disclosure involve performing a nucleic acid-based detection assay to detect the expression level of an eRNA transcribed from a myc-associated enhancer element in a sample containing cancer cells from the subject. The term “sample”, as used herein, refers to a composition that is obtained or derived from a subject having cancer that may contain a cellular and/or other molecular entity that is to be characterized and/or identified, for example based on physical, biochemical, chemical and/or physiological characteristics. For example, the phrase “sample containing cancer cells” and variations thereof refers to any sample obtained from a subject having cancer that may contain the cellular and/or molecular entity that is to be characterized, such as an eRNA transcribed from a myc-associated enhancer element. The sample, or a “tissue or cell sample,” contains a collection of cells obtained from a tissue of a subject. The source of the tissue or cell sample may be solid tissue as from a fresh, frozen and/or preserved organ or tissue sample or biopsy or aspirate; blood or any blood constituents; bodily fluids such as cerebral spinal fluid, amniotic fluid, peritoneal fluid, or interstitial fluid; cells from any time in gestation or development of the subject. The tissue sample may also be primary or cultured cells or cell lines. Typically, the sample is obtained from a diseased tissue or organ. For example, the sample may be obtained from tissue or an organ where cancer cells are present. In some embodiments, the sample contains both cancer cells and non-cancer cells. In other embodiments, the sample contains only cancer cells. The sample may contain compounds which are not naturally intermixed with the tissue in nature such as preservatives, anticoagulants, buffers, fixatives, nutrients, antibiotics, or the like. For the purposes herein a “section” of a sample is meant a single part or piece of a tissue sample, e.g. a thin slice of tissue or cells cut from a tissue sample. It is understood that multiple sections of tissue samples may be taken and subjected to analysis according to the present disclosure.

[0086] In some embodiments, the cancer cells in the sample are from colon cancer, colorectal cancer, rectal cancer, lung cancer, pancreatic cancer, bladder cancer, kidney cancer, endometrial cancer, leukemia, prostate cancer, breast cancer, gastric cancer, lung cancer, or ovarian cancer. In some embodiments, the cancer cells in the sample are from colon cancer. In some embodiments, the cancer cells in the sample are from CpG island methylator phenotype (CIMP) (+) colon cancer. In some embodiments, the cancer cells in

the sample are from leukemia. In some embodiments, the cancer cells in the sample are from prostate cancer.

BET Inhibitors

[0087] The present disclosure provides methods of treating a subject having cancer with a BET inhibitor, methods of selecting a subject for treatment with a BET inhibitor, and methods of modulating the treatment of a subject undergoing BET inhibitor treatment for cancer. BET inhibitors are typically small molecules that inhibit the activity of the BET family of proteins.

[0088] The BET family of bromodomain-containing proteins comprises 4 proteins (BRD2, BRD3, BRD4 and BRD-t) which contain tandem bromodomains capable of binding to two acetylated lysine residues in close proximity, increasing the specificity of the interaction. BRD2 and BRD3 were reported to associate with histones along actively transcribed genes and may be involved in facilitating transcriptional elongation (Leroy et al, Mol. Cell. 2008 30(1):51 -60), whereas BRD4 appears to be involved in the recruitment of the pTEF-β complex to inducible genes, resulting in phosphorylation of RNA polymerase and increased transcriptional output (Hargreaves et al, Cell, 2009 138(1): 129-145).

[0089] Four main classes of BET inhibitors have been described: (1) thienodiazepines and benzodiazepines; (2) quinolone and quinazoline derivatives; (3) triazolopyridazines; and (4) N-methyl pyridinone and N-methylpyridazinone. Inhibitors from any of these groups may be used in the methods of the present disclosure. Exemplary thienodiazepines and benzodiazepines include, without limitation, the compounds JQ1, I-BET762 (GSK525762), CPI-203, MS417, and OTX-015. Exemplary thienodiazepines and benzodiazepines are disclosed in WO2009084693, WO1998011111, WO2006129623, WO2011143669, WO2011143660, WO2011143651, WO2013030150, WO2011054845, WO2011054844, WO2011161031, WO2012075383, and WO2012151512. Exemplary quinolone and quinazoline derivatives include, without limitation, I-BET151 (GSK1210151A), PFi-1, and RVX-208. Exemplary quinolone and quinazoline derivatives are disclosed in WO2013027168, WO2011054848, WO2011054846, WO2011054843, WO2013024104, WO2008092231, WO2009158404, and WO2010123975. Exemplary triazolopyridazines are disclosed in WO2012174487. Exemplary N-methyl pyridinone and N-methylpyridazinones are disclosed in WO2013097601. Bromodomain ligand dimers of JQ1, which bind to both bromodomains of the BET protein, have also been described as bromodomain inhibitors, as

disclosed in WO2013033268. In addition, bis-aryl compounds, where the aryl rings are separated by two atoms such as ethynyl, ethylene and diazo, and with one aryl ring bearing an amide, sulfonamide or sulfonic acid moiety, have been described as bromodomain inhibitors, as disclosed in WO2012116170. In certain embodiments, the BET inhibitor is JQ1, i-BET 151 (GSK1210151A), i-BET 762 (GSK525762), OTX-015, TEN-010, CPI-203, or CPI-0610.

[0090] Additional BET inhibitors from alternative chemotypes to those described above may also be used in the methods of the present disclosure, which include, without limitation, 3,5-dimethylisoxazoles (Hewings *et al.* (2013) *J Med Chem* 56:3217-27), sulfonamide (Bamborough *et al* (2012) *J Med Chem* 55:587-96), and thiazolidione compounds (Zhao *et al.* (2013) *J Med Chem* 56:3833-51).

Enhancer RNA (eRNA)

[0091] Methods of the present disclosure involve detecting the expression level of an eRNA transcribed from a myc-associated enhancer element in a sample containing cancer cells from a subject. "eRNA," as used herein, refers to any non-coding RNA that is transcribed off of an enhancer regulatory element.

[0092] The majority of non-protein-coding genomic transcripts belong to the group of long non-coding RNAs (IncRNAs), which are greater than 200 nucleotides in length. One subgroup of IncRNAs, termed large intergenic non-coding RNAs (lincRNAs), was described based on distinctive chromatin signature that marks actively transcribed genes. (Khalil AM *et al.*, (2009) *Proc Natl Acad Sci USA* 106: 11667-11672; Guttman M *et al.*, (2009) *Nature* 458:223- 227). lncRNAs are marked by trimethylation of lysine 4 of histone H3 (H3K4me3) at their promoter and trimethylation of lysine 36 of histone H3 (H3K36me3) along the transcribed region. Another subgroup of lncRNAs, termed enhancer RNAs (eRNAs), was recently reported to be transcribed from genomic enhancer regions. (Kim TK *et al.*, (2010) *Nature* 465: 182-187; De Santa F *et al.*, (2010) PLoS Biol 8:elOG0384). Growing evidence suggests that enhancers are actively transcribed by RNA polymerase II to produce enhancer derived long non-coding RNAs (eRNA).

[0093] A "myc-associated enhancer element" is an enhancer that, when bound with certain protein activators, activates the transcription of the c-myc gene. In some embodiments, the myc-associated enhancer element is a myc-associated super enhancer element. A "myc-associated super enhancer element" as used herein refers to a cluster of enhancer elements that may activate transcription of the c-myc gene more strongly than a

myc-associated enhancer element. Typically, super-enhancers differ from enhancers in size, transcription factor binding density and content, ability to activate transcription, and sensitivity to perturbation (Whyte, W.A., *et al. Cell* 153, 307-319 (2013)) Super-enhancers can ramp up the expression of oncogenic factors in a tumor-type specific and lineage dependent manner (Whyte (2013); Dowen, J.M., *et al. Cell* 159, 374-387 (2014); Mansour, M.R., *et al. Science* 346, 1373-1377 (2014)).

[0094] The myc proto-oncogene is a member of the basic helix-loop-helix leucine zipper family of transcription factors (reviewed in (Luscher, B., (2001). Gene 277, 1-14). Believed to bind promoters in as many as 15% of the genes in the human genome (Patel, J.H., Loboda, A.P., Showe, M.K., Showe, L.C. and McMahon, S.B., (2004). *Nat Rev Cancer* 4, 562-568), myc plays a role in a diverse array of processes of cancer development, including angiogenesis, genomic instability, inhibition of differentiation, proliferation, immortalization and metabolism (for review, (Oster, S.K., Ho, C.S., Soucie, E.L. and Perm, L.Z., (2002). *Adv Cancer Res* 84, 81-154)). cMYC upregulation in cancer can occur via numerous mechanisms, including locus amplification, mutation, super-enhancers, and post-translation modifications.

[0095] Colon Cancer Associated Transcript 1 (CCAT1; also known as LOC100507056) is a distinct long noncoding RNA (lncRNA) transcribed off the colon cancer cMYC super-enhancer element. The CCAT1 transcript is located within a demarcated super-enhancer, 500 kb upstream of the cMYC promoter. As described below, the CCAT1 sequence has been annotated in the Ensembl Genome Browser (SEQ ID NO: 1), the NCBI Reference Sequence Database (SEQ ID NO: 2), and by Xiang et al. (SEQ ID NO: 3).

[0096] In some embodiments of the methods of the present disclosure, the eRNA comprises a sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO:3. In such embodiments, the cancer may be colon cancer, lung cancer, pancreatic cancer, bladder cancer, kidney cancer, or endometrial cancer. In certain embodiments the cancer is CpG island methylator phenotype (CIMP) (+) colon cancer.

[0097] SEQ ID NO: 1 is the CCAT sequence as annotated by the Ensembl Genome Browser (record number: ENST00000500112). The sequence for SEQ ID NO: 1 is listed below.

tcatcattaccagctgccgtgttaagcattgcgaaaacgctcacgattcacagaaaaatccatgctgttctttgaaggcattcaagccttaa

tagctagctggatgaatgtttaacttctaggccaggcactactctgtcccaacaataagccctgtacattgggaaaggtgccgagacatg
aactttggtcttctctgcaatccatctggagcattcactgacaacatcgactttgaagttgcactgacctggccagccctgccacttacca
ggttggctctgtatggctaagcgttttctcctaaaatcccttgaaaactgtgagaagaccataagaagatcatatctttaattctatttcacaa
gtcacacaatattccaatcaaatacagatggttgagaaaagtcatccatcttccctccccaccctcccacagcccctcaaccactgccct
gaaacttatatgctgttatccgcagctccatctggagcatcacagctactgtcaaccctgacgctctttctgaaaaaacaccggatggac
atcagaactatttctttaaggatgttactgagccacacaggaaaacttgccttatgattttgaatgcacggatctgatttgactaaacatgat
aactagagaatcacccaatctactcccattttcaactctaaatcatcagagtgtctcaaatccaaagcacacacagaccagcctggccaa
cacggtgaaactccacccctactaaaagtataaaaattatccaggtgtggtggcgggcgcctgtaatccaagctacttgggagtctgga
ggcaggagaatcccttgaacctgggagatggaggttgcagtgagcagagatcacaccaccgcactctagcctgggccacaaatcaa
caacaacaacaacaacaaaaaacaaagcgcacacagagactgaggtcctctttggcattgagaagatggctatgcaagtcccaacta
gcaagtgcaaacttcccagcttcacttctgccagtgtcccttcacccсttctcaaccccactgggaggcaggagggtgcttgacaataa
cagccttggcatcactctgccagggtgtaataggaactgttacaattctgagattctgtgtaagcactggcctttctgcctagaatgccttc
tcctctctttttaactgcatgctcctatttatctttcaaagcccggaaaaaataacactgcacacgggaaatgctcccttcctactgcagtca
tttagatgactctatgccattccattcatttctctttcctaccacagaagtgctttgagattttggagtcagactgcttgaacttgaatcctggc
cctctcatcagagacttgacttattttaggcaagttatataaccaattttacctcagttccttacccataaaatgggtctaatgagagtaccta
ccacacagaattttgatgaaaactgaatgagatgaaggcctttaaggcagtggtccccaaccctggggacacagacaggtaccattttg
tggcctgttaggaactgggccacacagcaggaggtgagcagtgggtgagtgagatcagcgttatttacagctgctccccattgctcac
cttactgcctgagctccacctcctgtcagatcagcagtggcattaaattctcatagcagcacaaaccctgtcatgaactgcacatgcgag
ggatctaggttgtgcgctccttatgagaatctaatgcctaatgacctgtcaccgtctcccatcacccctagatgggagtgtctagttgcag
gaaacaagctcagggcttccactgattctacattatggtgagttgtataattatttcattatataatacaatgtaataataatagaaacacagt
gcacaacaaatgtaatgtgcttgaatcatccccaaaccatcccagtccacggtcttccacattttgtcttttcacaaaattgtcttccacaaa
actggtccctggtgccaaaaaggcttgggaccactgctttaaagcctttgcatagtgcttagaattgaggggaaaaaaaaaacaaaaa
caatgtagctagttgctacaatcactatattggtgagtttc

[0098] SEQ ID NO: 2 is the CCAT1 sequence as annotated by the NCBI Reference Sequence Database (record number: NR_108049.1). The sequence for SEQ ID NO: 2 is listed below.

tttaaatcataccaattgaaccgagccttgtagaaacactatcacctacgcatacctctgcttcttttcattaacctgctatcctctttacaaat
gggattcttcacccactcccttcttctagattagcaatgccctgttaagtaaacgaacacgaaattcaaagggaaacaggagcaatcatc
attaccagctgccgtgttaagcattgcgaaaacgctcacgattcacagaaaaatccatgctgttctttgaaggcattcaagccttaatagc
tagctggatgaatgtttaacttctaggccaggcactactctgtcccaacaataagccctgtacattgggaaaggtgccgagacatgaact
ttggtcttctctgcaatccatctggagcattcactgacaacatcgactttgaagttgcactgacctggccagccctgccacttaccaggttg
gctctgtatggctaagcgttttctcctaaaatcccttgaaaactgtgagaagaccataagaagatcatatctttaattctatttcacaagtcac
acaatattccaatcaaatacagatggttgagaaaagtcatccatcttccctccccaccctcccacagcccctcaaccactgccctgaaac

ttatatgctgttatccgcagctccatctggagcatcacagctactgtcaaccctgacgctctttctgaaaaaacaccggatggacatcaga
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gaaactccacccctactaaaagtataaaaattatccaggtgtggtggcgggcgcctgtaatccaagctacttgggagtctggaggcag
gagaatcccttgaacctgggagatggaggttgcagtgagcagagatcacaccaccgcactctagcctgggccacaaatcaacaaca
acaacaacaacaaaaaacaaagcgcacacagagactgaggtcctctttggcattgagaagatggctatgcaagtcccaactagcaag
tgcaaacttcccagcttcacttctgccagtgtcccttcacccccttctcaaccccactgggaggcaggagggtgcttgacaataacagcct
tggcatcactctgccagggtgtaataggaactgttacaattctgagattctgtgtaagcactggcctttctgcctagaatgccttctcctctc
tttttaactgcatgctcctatttatctttcaaagcccggaaaaaataacactgcacacgggaaatgctcccttcctactgcagtcatttagat
gactctatgccattccattcatttctctttcctaccacagaagtgctttgagattttggagtcagactgcttgaacttgaatcctggccctctca
tcagagacttgacttattttaggcaagttatataaccaattttacctcagttccttacccataaaatgggtctaatgagagtacctaccacac
agaattttgatgaaaactgaatgagatgaaggcctttaaggcagtggtccccaaccct
ggggacacagacaggtaccattttgtggcctgttaggaactgggccacacagcaggaggtgagcagtgggtgagtgagatcagcgt
tatttacagctgctccccattgctcaccttactgcctgagctccacctcctgtcagatcagcagtggcattaaattctcatagcagcacaaa
ccctgtcatgaactgcacatgcgagggatctaggttgtgcgctccttatgagaatctaatgcctaatgacctgtcaccgtctcccatcacc
cctagatgggagtgtctagttgcaggaaacaagctcagggcttccactgattctacattatggtgagttgtataattatttcattatataatac
aatgtaataataatagaaacacagtgcacaacaaatgtaatgtgcttgaatcatccccaaaccatcccagtccacggtcttccacattttgt
cttttcacaaaattgtcttccacaaaactggtccctggtgccaaaaaggcttgggaccactgctttaaagcctttgcatagtgcttagaatt
gaggggggaaaaaaaaaacaaaaacaatgtagctagttgctacaatcactatattggtgagtttcaaaaggaaaagaattctgtcccattt
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aattgtggaccaaaccccaagcaggcagaaagccgtatcttaattattgcaagtatttcaggcaaggtgtggatggccatttgaattcaa
gcagactaggacctgggatgagaaagaaggtgtgtacgtgacttgatctttgaactttagctcaccatctggaagaaggctgagtattct
ctgcactcacatagtagctaatgcctactccccagccacccacaattctttctgtaggaaggctcgctagaatactttgtgatattggatatt
agttccatattctactgtgtatcttagttcaaccaaattgtaatcatctgatatttatttcttttaatataaatataagtatattaagtcttgg

[0099] SEQ ID NO: 3 contains the long isoform of CCAT1 based on 3’ RACE (Xiang et al Cell Research (2014) 24:513–531 PMID: 24662484). The SEQ ID NO: 3 sequence is listed below.

[0100] tttaaatcataccaattgaaccgagccttgtagaaacactatcacctacgcatacctctgcttcttttcattaacctgctatc
ctctttacaaatgggattcttcacccactcccttcttctagattagcaatgccctgttaagtaaacgaacacgaaattcaaagggaaacag
gagcaatcatcattaccagctgccgtgttaagcattgcgaaaacgctcacgattcacagaaaaatccatgctgttctttgaaggcattcaa
gccttaatagctagctggatgaatgtttaacttctaggccaggcactactctgtcccaacaataagccctgtacattgggaaaggtgccg
agacatgaactttggtcttctctgcaatccatctggagcattcactgacaacatcgactttgaagttgcactgacctggccagccctgcca
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ttcacaagtcacacaatattccaatcaaatacagatggttgagaaaagtcatccatcttccctccccaccctcccacagcccctcaacca
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ggccaacacggtgaaactccacccctactaaaagtataaaaattatccaggtgtggtggcgggcgcctgtaatccaagctacttggga
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tgcagtcatttagatgactctatgccattccattcatttctctttcctaccacagaagtgctttgagattttggagtcagactgcttgaacttga
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accattttgtggcctgttaggaactgggccacacagcaggaggtgagcagtgggtgagtgagatcagcgttatttacagctgctcccca
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atgcgagggatctaggttgtgcgctccttatgagaatctaatgcctaatgacctgtcaccgtctcccatcacccctagatgggagtgtcta
gttgcaggaaacaagctcagggcttccactgattctacattatggtgagttgtataattatttcattatataatacaatgtaataataatagaa
acacagtgcacaacaaatgtaatgtgcttgaatcatccccaaaccatcccagtccacggtcttccacattttgtcttttcacaaaattgtctt
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tctcccattcctccccgctcccctctctctttcccaacaggcttggaaagcaggcatcaccatgcctatttaacagttggggtcccttggcc
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ttatgaccaaatggttttacactatcattgaacagtttagtacaaaacatgccaccttttaatctattcattcatttaacaaatactttggagtgtt
taccatgtgccaagtgctgttctaatagacataagctgtgaggttatgcttatctgattctcacagcaacagctttcgagatatgaattggta
tactcatttgacagatgaggaaattgaattcatgtagtgagaaaggaagagctgcaattcagggttactggtttctcctgcactaagcact

gagccacactagaagagaaggcatgaggaagacaaaagtgagaaggtacgagagaagatattaagtagaggtctattagttcctctt
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ccttttaaatgcctgctcattttttggctacctttcaagctctaggaaatactctggaattccaggctgggaggggtttcctttgcagatctgg
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ccttcagctttctgactcacatttttcatgttattaatcagctcttagagtaaggtaaaatctagaacagactttatatacaccctccctcccac
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ataatttttaaagaatatttttcattttttattgaggtataaaatacatgtagtaaggtatgtcaataactcaaatcttatgtgatttttttatgtac
atgtatacctgtgtacacctgtgtaaccactacctaagtcaagatagagaacattttaatcatcttaaaagatttcctgtgtctcttcccacca
atacctgctgatgagcccactctccttacagctatcagcagagttgttttacctgtccttgaacttaatataaatgtaattatactgt

[0101] LOC728724 is also a distinct long noncoding RNA (lncRNA) transcribed off the cMYC super-enhancer element. According to RNA-seq data from The Cancer Genome Atlas, expression of LOC728724 is restricted to leukemia. SEQ ID NO: 4 is the LOC728724 sequence as annotated by the NCBI Reference Sequence Database (record number: NR_033916.1). In some embodiments of the methods of the present disclosure, the eRNA comprises a sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 4. In such embodiments, the cancer is leukemia.

[0102] The SEQ ID NO: 4 sequence is listed below.

tgttggctgaggctccctcttgccggctcctgctcttgaaacacacccaagtgcaatgttcaatcatctacttgtttccaaagttatttgggt
cagcattttcctccacccaccacttaaaactgatttgtttcatacatttgaaatgtatgaaaagccagaaatccaggacccaccctggcctg
ctcccttttctcacctactgtgttaaggataagtcaattgagcttcacttctctaattgaaatcaccgtgctgatggagtctgtctgtggaaat

gaaaggtggctaccataatctgttgggagttgaattaatcctctgggagtgaggctgacctaattactcagaaatactgggcttgctttcat
tctgtggaatacagccatgagaaagaggccagcatgccatttcctaatcttttcttgaattgcctgaaactcacctaacatgaaactgaga
agccttttcatttgaaagtggtatttcttatgtttttatctttttgcttgaaagtattcttaaactaaagaaaggagaaacagaaaaggtgaag
aagccaagaggctggagtttccttttgaatggatattcatccatgaagctgttgattctgtttttctgttcctccagatgtctacacttttacctc
tgcttgcctgatacaaccaccaccagtcacctcacagaataactcactccttaagaatctgatcaggaatcagccttccctgatcacaaa
cactgagtttagaagtccctccacagtgtcccccgtaaccctgtgctacctcactgcctcatagtttagtgccgtttgtgtacatgcctatct
tcgtccatggatccagaacaacttgtaaaaatgactgtacttatcaccttcagtattcaatgactaaactataattttaacatatctggcagaa
tgaatcacttggggatttctgccaacactaccaatatattactcctaatttgtaattattacttagcatggattatagtaccggctgttggaga
gccaatgtggcagaccctaagtggaataaaatgtcagtagcagtctaggagcatatatttattggaatagatcattcattcatttgttcatta
actaatttgttcattaaataaacatttattaagcaaaaaaaaaaaa

[0103] PCAT1 is also a distinct long noncoding RNA (lncRNA) transcribed off the cMYC super-enhancer element. According to RNA-seq data from The Cancer Genome Atlas, PCAT1 shows preferential expression in prostatic carcinoma. SEQ ID NO: 5 is the PCAT1 sequence as annotated by the NCBI Reference Sequence Database (record number: NR_045262.1). In some embodiments of the methods of the present disclosure, the eRNA comprises a sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 5. In such embodiments, the cancer is prostate cancer, bladder cancer, kidney cancer, endometrial cancer, breast cancer, gastric cancer, lung cancer, or ovarian cancer.

[0104] The SEQ ID NO: 5 sequence is listed below.

acacatggatattggatatctgcataggcagcttgctccacgccagtgcctacctgtgcagatgggaaggaaaggaaagtggcaagg
aggcagagaaagcatctgtacccttacaatttggtgagacaagaatgtatgaattcccacaggtcaaattataatgaagaaaggaacct
ctcttgagtacaaagagctacctatggtggtctggagccggaggaccacagcatcaaaggatataagatgcatagccaactgaggaa
cctgagcaattaaagagatccacagttaagtcacacttaactggcacttgtggaagccccgcaaggcctgaaggagagctgacatag
gcaccccggagagccagaatctggatcccatcttaataaggccatgaacaccagtggagaagaggcagaaacaccaatggataag
gaacattcacatctttcttcccatgtgcctctaagtgccagtgcaggccccacaggccaagctacagggagaaaggagatgacgcaaa
ggaacctaactggactttaatcactagaagtgagaagagaaatctattggaacctcccaagataatgccaagggtcaaagggtgcgca
gatacataagaccatggaaataatatcagacaaaaagcagattagagcaattttctttttcgagttcaaaatgggttataaagcagcgga
gacaaaccgcaacatcaccaacgcctttggcccaggaactgctaatgaaggtacagtgcagtcactgttcaggaagttttgcaaagga
gactagagccttgaagatgaggagcatagtgaccagccattggaagtcgacaaagaccaattgagaggaatcattgaagctgatcatc
ttacaactacacgagaagttgtcaaagaacgcaatgttgaccattgtgtggtcttttcgcatttgaagcaaattggaaaggtgaaaaactt
gataagtgggtgccttgtgagctcagcaaaaatccaaaaaaataatcatttttaagtgttgtcttctcttattctacgcaacaacaataacca
ttttgcaatcggattgtgatgtgcaatgaaaagtggatttggggccgggcgcggtggctcacgcctgtaatctcagcactttggaaggc

caaggcgggcagatcacgaggtcaggagatcaagaccgtcctggctaacacggtgaaaccccgtctctactgaaaatacaaaaaatt
agccgggtgtggtggctggcgcctgtagtcccagctacaggctgaggcaggagaatggcatgaacctgggaggcggagcttgcag
tgagccgagaccgtgccactgcactccagcctgggcgacagagcgatactccgtcaaaaaaaaaaaaaaaaaaaaaaaaagacaa
gtggattttatatatggcaaccagcaatgaccagctcagtggctggactgagaagaagctccaaagcacttcccaaagccaaacttgc
accaaaaaaaaggtcagggtcactgtttggtggtctgctgctggtctgatccaccgctgctctctgaatcctggcaaaaccattacatctg
agaagtatgctcaacaaatcaatgagctacgccaaaaactgcagcatctgcagctggcattggtcaacataacgggtccaattcttctcc
acgacaacgctcaactgcaccttgcgcaagcagcgcttcaaaagttgaacaaattgggctacatagtttttcctcatccgccatattcacc
tgacgtcttgccaactaactaccacttcttcaagtatctcaacaactttttgcagggaaaacacttccacaaccagcaggatgcagaaca
cgctttccaagagtttgtcgaatcctgacgcacagatttttatgctacaggaataaactaacttatttctcattggcaaaaatgtgttgattgt
aatggttcctattttgatgaataaatgtgtgtttgagccta

Nucleic-acid based detection assays

[0105] In methods of the present disclosure, nucleic acid-based detection assays are used to detect the expression level of an eRNA transcribed from a myc-associated enhancer element. The nucleic acid-based detection assay may be any assay deemed appropriate by one of skill in the art. For example, the nucleic acid-based detection assay may involve RNAseq, microarray analysis, direct RNA sequencing, in situ hybridization, and quantitative real-time PCR.

[0106] Nucleic acid-based detection assays are well known and include, for example, hybridization assays using complementary DNA probes (such as *in situ* hybridization using labeled riboprobes, Northern blot and related techniques) and various nucleic acid amplification assays (such as RT-PCR using complementary primers specific for eRNA and other amplification-type detection methods, such as, for example, branched DNA, SISBA, TMA and the like).

[0107] In some embodiments, the nucleic acid-based detection assay uses PCR analysis. For example, RT-PCR assays such as quantitative PCR assays are well known in the art. In an illustrative embodiment, the nucleic acid-based detection assay involves producing cDNA from the sample by reverse transcription using at least one primer; amplifying the cDNA so produced using an eRNA polynucleotide as sense and antisense primers to amplify eRNA cDNAs therein; and detecting the presence of the amplified cDNA. In addition, such assays can include one or more steps that allow one to determine the levels of eRNA in a biological sample (e.g. by simultaneously examining the levels of a comparative control mRNA

sequence of a "housekeeping" gene such as an actin family member). Optionally, the sequence of the amplified eRNA cDNA can be determined.

[0108] In some embodiments, the nucleic acid-based detection assay involves in situ hybridization (ISH). In situ hybridization (ISH) is a type of hybridization that uses a labeled complementary DNA or RNA strand as a probe to detect a specific DNA or RNA sequence in a portion or section of tissue (in situ), or, if the tissue is small enough, the entire tissue (whole mount ISH). DNA ISH can be used to determine the structure of chromosomes. RNA ISH is used to measure and localize mRNAs and other RNA transcripts within tissue sections or whole mounts. Sample cells and tissues are usually treated to fix the target transcripts in place and to increase access of the probe. The probe hybridizes to the target sequence at elevated temperature, and then the excess probe is washed away. The probe that was labeled with either radio-, fluorescent-, enzyme-, or antigen-labeled bases is localized and quantitated in the tissue using either autoradiography, fluorescence microscopy, chromogenic detection, or immunohistochemistry, respectively. ISH can also use two or more probes, labeled with radioactivity or the other non-radioactive labels, to simultaneously detect two or more transcripts. In some embodiments, the probe used is a gene-specific probe for detection of human CCAT1 RNA (Affymetrix; VA1-17802) target region 2-2696 in Genbank accessions NR_108049.1.

[0109] Nucleic acid-based detection assays may involve the use of eRNA primers and primer pairs, which allow the specific amplification of the eRNAs of interest or of any specific parts thereof, and probes that selectively or specifically hybridize to the eRNAs of interest or to any part thereof. Probes may be labeled with a detectable marker, such as, for example, a radioisotope, fluorescent compound, bioluminescent compound, a chemiluminescent compound, metal chelator or enzyme. Such probes and primers can be used to detect the presence of eRNA in a sample and as a means for detecting a cell expressing eRNA. As will be understood by the skilled artisan, a great many different primers and probes may be prepared based on the sequences provided in herein and used effectively to amplify, clone and/or determine the presence and/or levels of eRNAs.

[0110] In some embodiments, the nucleic acid-based detection assay involves direct RNA sequencing or RNAseq. Illustrative non-limiting examples of nucleic acid sequencing techniques include, but are not limited to, chain terminator (Sanger) sequencing and dye terminator sequencing. Those of ordinary skill in the art will recognize that because RNA is

less stable in the cell and more prone to nuclease attack RNA is usually reverse transcribed to DNA before sequencing.

[0111] Chain terminator sequencing uses sequence-specific termination of a DNA synthesis reaction using modified nucleotide substrates. Extension is initiated at a specific site on the template DNA by using a short radioactive, or other labeled, oligonucleotide primer complementary to the template at that region. The oligonucleotide primer is extended using a DNA polymerase, standard four deoxynucleotide bases, and a low concentration of one chain terminating nucleotide, most commonly a di-deoxynucleotide. This reaction is repeated in four separate tubes with each of the bases taking turns as the di-deoxynucleotide. Limited incorporation of the chain terminating nucleotide by the DNA polymerase results in a series of related DNA fragments that are terminated only at positions where that particular di- deoxynucleotide is used. For each reaction tube, the fragments are size-separated by electrophoresis in a slab polyacrylamide gel or a capillary tube filled with a viscous polymer. The sequence is determined by reading which lane produces a visualized mark from the labeled primer as one scans from the top of the gel to the bottom.

[0112] Dye terminator sequencing alternatively labels the terminators. Complete sequencing can be performed in a single reaction by labeling each of the di-deoxynucleotide chain- terminators with a separate fluorescent dye, which fluoresces at a different wavelength.

[0113] A variety of nucleic acid sequencing methods are contemplated for use in the methods of the present disclosure including, for example, chain terminator (Sanger) sequencing, dye terminator sequencing, and high-throughput sequencing methods. Many of these sequencing methods are well known in the art. See, e.g., Sanger et al, Proc. Natl. Acad. Sci. USA 74:5463-5467 (1997); Maxam et al, Proc. Natl. Acad. Sci. USA 74:560-564 (1977); Drmanac, *et al, Nat. Biotechnol.* 16:54-58 (1998); Kato, *Int. J. Clin. Exp. Med.* 2: 193-202 (2009); Ronaghi *et al*, *Anal. Biochem.* 242:84-89 (1996); Margulies *et al*, *Nature* 437:376-380 (2005); Ruparel *et al*, *Proc. Natl. Acad. Sci. USA* 102:5932-5937 (2005), and Harris *et al*, *Science* 320: 106-109 (2008); Levene *et al*, *Science* 299:682-686 (2003); Korlach *et al*, *Proc. Natl. Acad. Sci. USA* 105: 1 176-1 181 (2008); Branton *et al*, *Nat. Biotechnol.* 26(10): 1146-53 (2008); Eid *et al*, *Science* 323 : 133-138 (2009); each of which is herein incorporated by reference in its entirety.

[0114] Next-generation sequencing (NGS) methods share the common feature of massively parallel, high-throughput strategies, with the goal of lower costs in comparison to older sequencing methods (see, e.g., Voelkerding *et al.*, *Clinical Chem.*, 55: 641-658, 2009; MacLean *et al.*, *Nature Rev. Microbiol.*, 7: 287-296; each herein incorporated by reference in their entirety). NGS methods can be broadly divided into those that typically use template amplification and those that do not. Amplification-requiring methods include pyrosequencing commercialized by Roche as the 454 technology platforms (e.g., GS 20 and GS FLX), the Solexa platform commercialized by Illumina, and the Supported Oligonucleotide Ligation and Detection (SOLiD) platform commercialized by Applied Biosystems. Non-amplification approaches, also known as single-molecule sequencing, are exemplified by the HeliScope platform commercialized by Helicos Biosciences, and emerging platforms commercialized by VisiGen, Oxford Nanopore Technologies Ltd., Life Technologies/Ion Torrent, and Pacific Biosciences, respectively.

[0115] RNAseq is a technique based on enumeration of RNA transcripts using next-generation sequencing methodologies. RNAseq is a relatively new technology that has been employed for mass sequencing of whole transcriptomes and that offers significant advantages over other methods employed for transcriptome sequencing, such as microarrays, including low levels of background noise, the ability to detect low levels of expression, the ability to detect novel mutations and transcripts, and the ability to use relatively small amounts of RNA (for a review of RNA-seq, see Wang end., *Nat. Rev. Genet.* (2009) 10:57-63).

[0116] In some embodiments, the nucleic acid-based detection assay involves microarray technologies. Using nucleic acid microarrays, RNA in samples containing cancer cells from the subject and samples containing non-cancer cells or other control cells is reverse transcribed and labeled to generate cDNA probes. The probes are then hybridized to an array of nucleic acids immobilized on a solid support. The array is configured such that the sequence and position of each member of the array is known. For example, a selection of genes or non-coding RNAs that have potential to be expressed in certain disease states may be arrayed on a solid support. Hybridization of a labeled probe with a particular array member indicates that the sample from which the probe was derived expresses that gene or non-coding RNA. Differential expression analysis of disease tissue can provide valuable information. Microarray technology utilizes nucleic acid hybridization techniques and computing technology to evaluate the RNA expression profile of thousands of genes or non-coding regions within a single experiment. (see, e.g., WO 01/75166, U.S. Patent 5,700,637,

U.S. Patent 5,445,934, and U.S. Patent 5,807,522; Lockart, Nature Biotechnology, 14:1675-1680 (1996); Cheung, V.G. *et al.*, *Nature Genetics* 21(Suppl):15-19 (1999) for a discussion of array fabrication). DNA microarrays are miniature arrays containing gene fragments that are either synthesized directly onto or spotted onto glass or other substrates. Thousands of genes or genomic regions are usually represented in a single array. A typical microarray experiment involves the following steps: 1) preparation of fluorescently labeled target from RNA isolated from the sample, 2) hybridization of the labeled target to the microarray, 3) washing, staining, and scanning of the array, 4) analysis of the scanned image and 5) generation of expression profiles. Currently, two main types of DNA microarrays are being used: oligonucleotide (usually 25 to 70 mers) arrays and gene expression arrays containing PCR products prepared from cDNAs. In forming an array, oligonucleotides can be either prefabricated and spotted to the surface or directly synthesized on to the surface (in situ).

[0117] The Affymetrix GeneChip® system is a commerically available microarray system which comprises arrays fabricated by direct synthesis of oligonucleotides on a glass surface. Oligonucleotides, usually 25-mers, are directly synthesized onto a glass wafer by a combination of semiconductor-based photolithography and solid phase chemical synthesis technologies. Each array contains up to 400,000 different oligos and each oligo is present in millions of copies. Since oligonucleotide probes are synthesized in known locations on the array, the hybridization patterns and signal intensities can be interpreted in terms of genomic region identity and relative expression levels by the Affymetrix Microarray Suite software. Each genomic region is represented on the array by a series of different oligonucleotide probes. Each probe pair consists of a perfect match oligonucleotide and a mismatch oligonucleotide. The perfect match probe has a sequence exactly complimentary to the particular gene or genomic region and thus measures the expression of the gene or genomic region. The mismatch probe differs from the perfect match probe by a single base substitution at the center base position, disturbing the binding of the target transcript. This helps to determine the background and nonspecific hybridization that contributes to the signal measured for the perfect match oligo. The Microarray Suite software subtracts the hybridization intensities of the mismatch probes from those of the perfect match probes to determine the absolute or specific intensity value for each probe set. Probes are chosen based on current information from Genbank and other nucleotide repositories. The sequences are believed to recognize unique regions of the 3’ end of the gene. A GeneChip Hybridization Oven (“rotisserie” oven) is used to carry out the hybridization of up to 64 arrays at one time.

The fluidics station performs washing and staining of the probe arrays. It is completely automated and contains four modules, with each module holding one probe array. Each module is controlled independently through Microarray Suite software using preprogrammed fluidics protocols. The scanner is a confocal laser fluorescence scanner which measures fluorescence intensity emitted by the labeled cRNA bound to the probe arrays. The computer workstation with Microarray Suite software controls the fluidics station and the scanner. Microarray Suite software can control up to eight fluidics stations using preprogrammed hybridization, wash, and stain protocols for the probe array. The software also acquires and converts hybridization intensity data into a presence/absence call for each gene using appropriate algorithms. Finally, the software detects changes in gene expression between experiments by comparison analysis and formats the output into .txt files, which can be used with other software programs for further data analysis.

Comparing the expression level of eRNA

[0118] Methods of the present disclosure involve determining whether the cells in a sample from a subject express an eRNA at a level greater than a reference expression level of the eRNA or comparing the expression level of the eRNA in the cells in a sample to a reference expression level of the eRNA. In some embodiments, the methods involve determining whether the cancer cells in the sample from the subject express the eRNA at a level greater than a reference expression level of the eRNA or comparing the expression level of the eRNA in the cancer cells in a sample to a reference expression level of the eRNA. In some embodiments, the methods involve determining whether the cancer cells and the non-cancer cells in the sample from the subject express the eRNA at a level greater than a reference expression level of the eRNA or comparing the expression level of the eRNA in the cancer cells and the non-cancer cells in a sample to a reference expression level of the eRNA.

[0119] In some embodiments, the reference expression level is based on the expression level of the eRNA in non-cancer cells. In some embodiments, the non-cancer cells are from the same tissue type as the cancer cells. For example, if the cancer cells are from colon cancer then the non-cancer cells are from healthy colon tissue. In some embodiments, the reference expression level is an average of expression levels of the eRNA from an earlier timepoint or timepoints. In certain embodiments, the reference expression level of the eRNA is measured in a person or persons other than the subject with cancer. In some embodiments, the reference expression level of the eRNA is measured in a person or persons with similar

characteristics to the subject with cancer. In some embodiments, the reference expression level of the eRNA is a combination of multiple expression levels of the eRNA from different sources.

[0120] The reference expression level can be measured in a sample, cell or tissue obtained from a source known, or believed, not to be afflicted with cancer. In some embodiments, the reference expression level is measured in a sample, cell or tissue obtained from a healthy part of the body of the same subject with cancer that is being treated or selected for treatment with methods of the present disclosure. In some embodiments, the reference expression level is measured in a sample, cell or tissue obtained from a healthy part of the body of an individual who is not the subject with cancer that is being treated or selected for treatment with methods of the present disclosure.

[0121] In one aspect, the present disclosure provides methods of modulating the treatment of a subject undergoing BET inhibitor treatment for cancer including the steps of performing a nucleic acid-based detection assay to detect the expression level of an eRNA transcribed from a myc-associated enhancer element in a sample containing cancer cells from the subject; determining the expression level of the eRNA in the cancer cells; comparing the expression level of the eRNA in the cancer cells to a reference expression level of the eRNA; and modulating the amount of BET inhibitor administered to the subject based on the difference between the expression level of the eRNA in the cancer cells and the eRNA reference expression level. In some embodiments, the reference expression level is based on the expression level of the eRNA in the subject at an earlier timepoint during BET inhibitor treatment. The earlier timepoint may be any timepoint during a course of BET inhibitor treatment, including a baseline timepoint such as the timepoint just before or at the same time that the first dose of BET inhibitor is given to the subject. In some embodiments, the method involves determining that the expression level of the eRNA in the cancer cells is less than the reference expression level of the eRNA and modulating the amount of BET inhibitor administered to the subject involves maintaining the same level or decreasing the level of BET inhibitor administered to the subject. In some embodiments, the method involves determining that the expression level of the eRNA in the cancer cells is the same or more than the reference expression level of the eRNA and modulating the amount of BET inhibitor administered to the subject involves increasing the level of BET inhibitor administered to the subject.

[0122] Expression of eRNA is at a level "greater than" the reference expression level if the expression level is at least about 1.5X, 1.75X, 2X, 3X, 4X, 5X, 6X, 7X, 8X, 9X or 10X or more of the reference expression level. Expression of eRNA is at a level "less than" the reference expression level if the expression level is at least about 1.5X, 1.75X, 2X, 3X, 4X, 5X, 6X, 7X, 8X, 9X or 10X less than the reference expression level. The reference expression level may be determined based on any assay known in the art, including but not limited to RNAseq, microarray analysis, direct RNA sequencing, in situ hybridization, and quantitative real-time PCR. Typically, the reference expression level will be determined using the same assay that was used to detect the expression level of the eRNA in the sample containing cancer cells. The reference expression level may be determined qualitatively and/or quantitatively.

Dosage of BET Inhibitor

[0123] Methods of the present disclosure involve administering an effective amount of a BET inhibitor to a subject or modulating the amount of BET inhibitor administered to the subject. An effective amount refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic or prophylactic result. An effective amount of a BET inhibitor may vary according to factors such as the cancer state, age, sex, and weight of the subject, and the ability of the BET inhibitor to elicit a desired response in the subject. An effective amount is also one in which any toxic or detrimental effects of the BET inhibitor are outweighed by the therapeutically beneficial effects.

[0124] A BET inhibitor used in a method of the present disclosure can be administered in an amount of about 0.005 to about 500 milligrams per dose, about 0.05 to about 250 milligrams per dose, or about 0.5 to about 100 milligrams per dose. For example, a BET inhibitor can be administered, per dose, in an amount of about 0.005, 0.05, 0.5, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 350, 400, 450, or 500 milligrams, including all doses between 0.005 and 500 milligrams.

[0125] The dosage of a composition containing a BET inhibitor, or a composition containing the same, can be from about 1 ng/kg to about 200 mg/kg, about 1 μg/kg to about 100 mg/kg, or about 1 mg/kg to about 50 mg/kg. The dosage of a composition can be at any dosage including, but not limited to, about 1 μg/kg. The dosage of a composition may be at any dosage including, but not limited to, about 1 μg/kg, 10 μg/kg, 25 μg/kg, 50 μg/kg, 75 μg/kg, 100 μg/kg, 125 μg/kg, 150 μg/kg, 175 μg/kg, 200 μg/kg, 225 μg/kg, 250 μg/kg, 275

μg/kg, 300 μg/kg, 325 μg/kg, 350 μg/kg, 375 μg/kg, 400 μg/kg, 425 μg/kg, 450 μg/kg, 475 μg/kg, 500 μg/kg, 525 μg/kg, 550 μg/kg, 575 μg/kg, 600 μg/kg, 625 μg/kg, 650 μg/kg, 675 μg/kg, 700 μg/kg, 725 μg/kg, 750 μg/kg, 775 μg/kg, 800 μg/kg, 825 μg/kg, 850 μg/kg, 875 μg/kg, 900 μg/kg, 925 μg/kg, 950 μg/kg, 975 μg/kg, 1 mg/kg, 5 mg/kg, 10 mg/kg, 15 mg/kg, 20 mg/kg, 25 mg/kg, 30 mg/kg, 35 mg/kg, 40 mg/kg, 45 mg/kg, 50 mg/kg, 60 mg/kg, 70 mg/kg, 80 mg/kg, 90 mg/kg, 100 mg/kg, 125 mg/kg, 150 mg/kg, 175 mg/kg, or 200 mg/kg. The above dosages are exemplary of the average case, but there can be individual instances in which higher or lower dosages are merited, and such are within the scope of this disclosure. In practice, the physician determines the actual dosing regimen that is most suitable for an individual subject, which can vary with the age, weight, and response of the particular subject. Such doses may be administered daily or intermittently, e.g., every week or every three weeks (*e.g.,* such that the subject receives from about two to about twenty, or *e.g.,* about six doses of the antibody). An initial higher loading dose, followed by one or more lower doses may be administered. However, other dosage regimens may be useful. The progress of the dosage regime is easily monitored by conventional techniques and assays.

[0126] A therapeutically effective amount of a BET inhibitor required for use in therapy varies with the nature of the condition being treated, the length of time that activity is desired, and the age and the condition of the subject, and ultimately is determined by the attendant physician. Dosage amounts and intervals can be adjusted individually to provide plasma levels of the BET inhibitor that are sufficient to maintain the desired therapeutic effects. The desired dose conveniently can be administered in a single dose, or as multiple doses administered at appropriate intervals, for example as one, two, three, four or more subdoses per day. Multiple doses often are desired, or required. For example, a BET inhibitor can be administered at a frequency of: four doses delivered as one dose per day at four-day intervals (q4d×4); four doses delivered as one dose per day at three-day intervals (q3d×4); one dose delivered per day at five-day intervals (qd×5); one dose per week for three weeks (qwk3); five daily doses, with two days rest, and another five daily doses (5/2/5); or, any dose regimen determined to be appropriate for the circumstance.

Modes of Administration

[0127] Effective amounts of a BET inhibitor can be administered by any suitable route, for example by oral, buccal, inhalation, sublingual, rectal, vaginal, intracisternal or intrathecal through lumbar puncture, transurethral, nasal, percutaneous, i.e., transdermal, or parenteral

(including intravenous, intramuscular, subcutaneous, intracoronary, intradermal, intramammary, intraperitoneal, intraarticular, intrathecal, retrobulbar, intrapulmonary injection and/or surgical implantation at a particular site) administration. Parenteral administration can be accomplished using a needle and syringe or using a high pressure technique. Various dosing schedules including but not limited to single or multiple administrations over various time-points, bolus administration, and pulse infusion are contemplated herein.

[0128] Pharmaceutical compositions containing BET inhibitors can be manufactured, for example, by conventional mixing, dissolving, granulating, dragee-making, emulsifying, encapsulating, entrapping, or lyophilizing processes. Proper formulation is dependent upon the route of administration chosen. When a therapeutically effective amount of a BET inhibitor is administered orally, the composition typically is in the form of a tablet, capsule, powder, solution, or elixir. When administered in tablet form, the composition additionally can contain a solid carrier, such as a gelatin or an adjuvant. When administered in liquid form, a liquid carrier, such as water, petroleum, or oils of animal or plant origin, can be added. The liquid form of the composition can further contain physiological saline solution, dextrose or other saccharide solutions, or glycols.

[0129] When a therapeutically effective amount of a BET inhibitor is administered by intravenous, cutaneous, or subcutaneous injection, the composition is in the form of a pyrogen-free, parenterally acceptable aqueous solution. The preparation of such parenterally acceptable solutions, having due regard to pH, isotonicity, stability, and the like, is within the skill in the art. A preferred composition for intravenous, cutaneous, or subcutaneous injection typically contains an isotonic vehicle.

[0130] BET inhibitors can be readily combined with pharmaceutically acceptable carriers well-known in the art. Such carriers enable the active agents to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a subject to be treated. Pharmaceutical preparations for oral use can be obtained by adding the BET inhibitor to a solid excipient, optionally grinding the resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients include, for example, fillers and cellulose preparations. If desired, disintegrating agents can be added.

[0131] A BET inhibitor can be formulated for parenteral administration by injection, e.g., by bolus injection or continuous infusion. Formulations for injection can be presented in unit dosage form, e.g., in ampules or in multidose containers, with an added preservative. The compositions can take such forms as suspensions, solutions, or emulsions in oily or aqueous vehicles, and can contain formulatory agents such as suspending, stabilizing, and/or dispersing agents.

[0132] Pharmaceutical compositions for parenteral administration include aqueous solutions of the active agent in water-soluble form. Additionally, suspensions of a compound can be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils or synthetic fatty acid esters. Aqueous injection suspensions can contain substances which increase the viscosity of the suspension. Optionally, the suspension also can contain suitable stabilizers or agents that increase the solubility of the compounds and allow for the preparation of highly concentrated solutions. Alternatively, a BET inhibitor can be in powder form for constitution with a suitable vehicle, e.g., sterile pyrogen-free water, before use.

Articles of Manufacture or Kit

[0133] In one aspect of the present disclosure, an article of manufacture or kit including reagents for detecting the expression level of an eRNA transcribed from a myc-associated enhancer element in a sample is provided. In some embodiments, the kit contains reagents for performing RNAseq, microarray analysis, direct RNA sequencing, in situ hybridization, or quantitative real-time PCR. In some embodiments, the kit contains instructions for using the reagents. In some embodiments, the kit contains instructions for performing any of the methods of the present disclosure.

[0134] The article of manufacture or kit typically includes a container and a label or package insert on or associated with the container. Such kits may comprise a carrier means being compartmentalized to receive in close confinement one or more container means such as vials, tubes, and the like. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition which is by itself or combined with another composition effective for performing a nucleic-acid based detection assay. The label or package insert indicates that the composition is used for performing a nucleic-acid based detection assay.

[0135] Each of the container means comprises one of the separate elements to be used in the method. For example, one of the container means may comprise a probe that is or can be detectably labeled. Such probe may be a polynucleotide specific for an eRNA. Where the kit utilizes nucleic acid hybridization to detect the target nucleic acid, the kit may also have containers containing nucleotide(s) for amplification of the target nucleic acid sequence and/or a container comprising a reporter-means, such as a biotin-binding protein, such as avidin or streptavidin, bound to a reporter molecule, such as an enzymatic, florescent, or radioisotope label.

[0136] The kits of the present disclosure will typically comprise the container described above and one or more other containers comprising materials desirable from a commercial and user standpoint, including buffers, diluents, filters, needles, syringes, and package inserts with instructions for use.

[0137] The kits of the present disclosure have a number of embodiments. A typical embodiment is a kit comprising a container, a label on said container, and a composition contained within said container; wherein the composition includes reagents for in situ hybridization, RNAseq, microarray, direct RNA sequencing, or quantitative real-time PCR, the label on said container indicates that the composition can be used to evaluate the presence of eRNA in at least one type of cancer cell, and instructions for using the reagents for evaluating the presence of eRNA in at least one type of cancer cell. The kit can further comprise a set of instructions and materials for preparing a tissue sample and applying reagents to the same section of a tissue sample.

[0138] In some embodiments, the kit includes a BET inhibitor. Other optional components in the kit include one or more buffers (*e.g.*, block buffer, wash buffer, substrate buffer, etc), other reagents such as substrate (*e.g.*, chromogen) which is chemically altered by an enzymatic label, epitope retrieval solution, control samples (positive and/or negative controls), control slide(s) etc.

EXAMPLES

[0139] The following are examples of methods and compositions of the present disclosure. It is understood that various other embodiments may be practiced, given the general description provided above.

Example 1: CCAT1 is a cMYC super-enhancer template RNA that predicts BET sensitivity in colorectal cancer

[0140] An arrayed epigenetic CRISPR library was developed, and a high throughput screen was performed to identify epigenetic modulators in colon cancer (Cong, L., *et al. Science* 339, 819-823 (2013); Jinek, M., *et al. eLife* 2, e00471 (2013); Mali, P., *et al. Science* 339, 823-826 (2013)). A number of essential epigenetic regulators were identified, including BRD4. The results demonstrated that BRD4 inhibition leads to growth arrest and differentiation in the epigenetically dysregulated CIMP(+) class of tumors. CIMP(+) colon cancers were found to be exquisitely dependent on BET activity for cMYC transcription. An integrated transcriptomic and chromatin immunoprecipitation sequencing (ChIP-seq) analysis identified Colon Cancer Associated Transcript 1 (CCAT1; also known as LOC100507056) as a distinct long noncoding RNA (lncRNA) transcribed off the colon cancer cMYC super-enhancer element. Strikingly, CCAT1 expression predicted JQ1 sensitivity and BET-mediated cMYC regulation. These results suggested a novel diagnostic methodology to identify cMYC driven tumors that rely on BET for transcription, which could be translated into a promising strategy for patient selection in clinical trials.

Materials and Methods

Design and construction of gRNA library

[0141] gRNA target sequences were designed using a previously developed algorithm. This algorithm software identifies gRNAs that have the highest probability of disrupting the targeted gene, while minimizing potential off-target effects. Refseq RNAs were grouped by Entrez gene id to identify isoforms for each gene in the target set (Pruitt, K.D., *et al. Nucleic Acids Res* 42, D756-763 (2014)). Gmap was used to align Refseq RNAs to the human genome (hg19) to identify exons (Wu, T.D. & Watanabe, C.K. *Bioinformatics* 21, 1859-1875 (2005); Karolchik, D., *et al. Nucleic Acids Res* (2013)). The gRNA candidate set consisted of 20-mers on either strand flanked at the 3' end by the required PAM (-NGG) entirely contained in an exon in the gene of interest. A 5' G was not required because the 5'-most base would be replaced by G in the final gRNA. The isoform coverage of each candidate gRNA and its proximity to the most distant CDS start in the isoform set was determined. Candidates not in the first 25% of the coding region (CDS) or within 20 bases of the CDS start were eliminated. Potential off-targets for a candidate gRNA were defined as 20-mers flanked by 3' NGG having zero or one mismatch in the 11 3'-most bases, and 4 or fewer mismatches in the 9 5'-

most bases. Each off-target was classified as "exonic," i.e., located anywhere in an exon of another gene, or "genomic." A gRNA candidate was eliminated if it had more than 15 genomic off-target hits, or had any off-target site within 500 kb on the same chromosome. Each potential off-target was scored according to the location and number of mismatches. The more distal the first mismatch from the 3' end (PAM), the greater the penalty; the fewer the mismatches, the greater the penalty. An additional penalty was added for "exonic" off-targets. For each gene of interest, gRNA candidates were ranked by smallest total off-target penalty first (best off-target profile). The top 20 gRNA candidates were displayed, along with detailed off-target information, isoform coverage, and location relative to the CDS start. gRNAs were cloned into the pLKO2 (Sigma; SHC201) lentiviral plasmid as oligonucleotides or by site directed mutagenesis mediated insertion of 19-mer sequences (Genscript). Each gRNA clone was sequenced verified.

Cell culture and generation of stable cell lines expressing Cas9

[0142] All colon, lung, pancreatic, gastric, and blood lines were cultured in RPMI-1640 supplemented with 10% FBS, 2 mmol/L l-glutamine (Invitrogen), and 1% penicillin–streptomycin (Invitrogen). 293T human embryonic kidney cells were grown in Dulbecco's Modified Eagle's Medium (high glucose), 10% FBS, 100 μmol/L nonessential amino acids (Invitrogen), 2 mmol/L l-glutamine (Invitrogen), and 1% penicillin–streptomycin.

[0143] A human codon optimized Cas9 was cloned into pENTR3C dual selection vector (Invitrogen) and Gateway recombined into the lentiviral mammalian expression vector pLenti7.3 (Invitrogen). Lentiviral transduction of RKO cells was conducted at an MOI>1. Stably expressing Cas9 cells were collected 1 week after infection by FACS analysis using the GFP selectable marker.

Lentivirus production and infection

[0144] Lentivirus production and infection was carried out as previously described (Adler, A.S., *et al. Cancer Res* 72, 2129-2139 (2012)). For high throughput production of the gRNA library, lentivirus was generated in 96-well plates. 293T cells were seeded at 55,000 cells/well 18 hours prior to transfection. Cells were subsequently transfected with the pLKO2-gRNA constructs along with pCMV-VSVG and pCMV-8.9 using Lipofectime 2000 (Invitrogen). Six hours after transfection, the media was changed to 200μl of fresh growth media. Viral supernatant was harvested 48 hours after transfection.

CRISPR high throughput arrayed cell viability screen

[0145] RKO-Cas9 cells were seeded (750 cells/well) 18 hours prior to infection in 96 well plates in media containing 8 μg/ml polybrene. Virus particles were added to cells at an average MOI of 3-5 and spin infected at room temperature (1800 rpm, 30 minutes). Stable integration of gRNAs was selected with 2 μg/ml puromycin starting 48 hours after infection. This was accomplished by a media change. To control for lentiviral infection, the primary screen was performed in duplicate for cells receiving puromycin and in singlet for cells not receiving puromycin. Infection and media changes were carried out using robotics (Oasis CB 650; Dynamic Devices). Cell proliferation was measured 7 days after infection using CellTiter-Glo (CTG; Promega). Negative controls (Firefly Luciferase gRNAs) and positive controls (PLK1 gRNAs) were present on each plate in columns 1 and 7.

[0146] Data was normalized on each plate by dividing CTG values of each experimental gRNA by the average of 8 Luciferase gRNA controls that were on the same plate. Z scores were calculated from non-targeting control normalized values. PLK1 and Luciferase gRNA controls were not included for Z score calculations. Hit criteria were established using the following guidelines: 1 gRNA with a Z score < -2 and an additional gRNA with a Z score < -1.5.

Immunoblot Analysis, Immunofluorescence and Antibodies

[0147] The following antibodies were used for immunoblot and immunofluorescence expression analysis: HDAC1 (Cell Signaling; 5356S), CHD1 (Cell Signaling; 4351S), PYGO2 (Epitomics; 3273-1), BRD4 (Epitomics: 5716-1; Bethyl: A301-985A100; Cell Signaling: 12183); BRD3 (Bethyl: A302-368A); BRD2 (A302-583A); KAT8 (Bethyl; A300-992A), AURORA B (BD Biosciences; 611082), KDM3B (Sigma; HPA016610-100ul), B-Catenin (Cell Signaling; 8480S), NSD2 (Bethyl; A303-093A), EZH2 (Cell Signaling; 5246), cMYC (Epitomics 1472-1); ACTIN (MP Biomedicals; 691002); and TUBULIN (Sigma; T6074).

[0148] For immunofluorescence analysis cells were fixed in 2% formaldehyde for 15 minutes, rinsed 2X in PBS, and extracted with PBS containing 0.25% Triton X-100 for 15 minutes. Cells were blocked in either 10% goat or donkey serum and primary antibodies were incubated overnight at 4°C. Cells were washed 3 times in PBS containing 0.1% Triton X-100 and secondary antibodies (Invitrogen) were incubated for 2 hours. DNA was stained with Hoechst 33342 for 15 minutes in PBS. Images were collected on an IN Cell Analyzer

confocal system (GE Healthcare). Images were processed using Image J (NIH) and Photoshop (Adobe).

Generation of BRD4 clonal knockout cells using CRISPR

[0149] BRD4 gRNAs targeting the 5 prime end of BRD4 (target sequences: TTGGTACCGTGGAAACGCC and AAGATCATTAAAACGCCTA) were cloned into pLKO2 (Sigma; SHC201) based on published strategy (Mali (2013)). BRD4 knockout cells were generated by transiently co-transfecting pTKneo-Cas9 and the gRNAs using Lipofectamine LTX (Invitrogen). Untransfected cells were eliminated with a 24 hour Puromycin treatment (2 μg/ml) two days after transfection. Clonal populations were isolated by FACS in 96 well format and screened by immunofluorescence (using Epitomics: 5716-1 antibody) to identify BRD4 knockout cells. Knockout cells were confirmed by immunoblot analysis. Similarly, for BRD4 long isoform specific deletion a target gRNA (target sequence: AAAGAAGGGGCACCCCGGG) was cloned and transfected into cells as above. Clonal knockout cells were screened by immunofluorescence (using Bethyl: A301-985A100 antibody) and confirmed by immunoblot analysis.

Xenograft studies

[0150] Xenograft tumor studies were carried out essentially as described (Adler, A.S., *et al. Cancer Res* 72, 2129-2139 (2012)). Briefly, HT-29 and HCT-116 cells were infected with doxycycline inducible pHUSH-shRNAs targeting BRD4 or NTC control and selected with 2 μg/ml puromycin for stable integration. Target sequences shBRD4-1 (aggaagaggacaagtgcaa) and shBRD4-2 (agaagggagtgaagaggaa) were used. For each cell line, 5 x 106 cells were injected subcutaneously into the backs of female NCr nude mice (Taconic) to initiate tumor growth. Once tumors reached 200 mm^3 in size, hairpin expression was induced with 0.5 mg/ml doxycycline (or sucrose as control). A subset of mice was sacrificed after 7 days of doxycycline treatment to monitor shRNA efficiency. Tumor volume and body weight were measured on remaining mice every 3-4 days until the end of the study.

Calculation of Relative EC50 values

[0151] Cell lines were plated in 96 well format 12 hours prior to drug addition. Compounds were diluted in DMSO with target final concentrations ranging from 20 μM to 3 nM (using 3 fold serial dilutions) and added to cells using the Bravo automated liquid handler (Agilent). Cell number was quantified 3 days after drug addition with CellTiter-Glo. EC50s were calculated using PRISM software.

Identification of genomic features predictive for JQ1 sensitivity

[0152] Using EC50 values as a measure for the response to JQ1 treatment, cell lines were defined as sensitive or resistant based on a 30%- and 70%-quantile cutoff, respectively. To create genomic feature profiles for the classified sensitive (ATRFLOX, HT-29, RKO, CW-2, HCT15, SW48) and resistant (LS180, SW480, LS-174t, GP5d, SW948, Colo-741) cell lines, total copy number (26,347 features), expression (26,225 features) and mutation (314 features) data were retrieved (Klijn, C., *et al. Nat Biotechnol* 33, 306-312 (2015)). The DESeq R package was applied to estimate size factors and obtain dispersion estimates for the associated RNAseq data (Anders, S. & Huber, W. *Genome Biol* 11, R106 (2010)). Gene expression was quantified with variance-stabilized counts. DNA methylation data yielded an additional 35,788 features per cell line. For both expression and methylation data, the classification approach was restricted to the 2000 most variable instances. Furthermore, 14 different measures for CIMP classification including established gene expression signatures (Ogino (2007)) were employed. EMT and MSI status, doubling time, seeding density and alteration status of canonical pathways formed eight additional features.

[0153] The Lasso approach was applied to identify the most reliable features for predicting either the class or the underlying EC50 values of sensitive versus resistant lines (Friedman, J., Hastie, T. & Tibshirani, R. *J Stat Softw* 33, 1-22 (2010)). Using the 'cv.glmnet' function from the R package glmnet, lasso based models were trained and 10-fold cross validations were used to determine the tuning parameter λ yielding minimum cross-validated errors. Features were defined as reliable for predicting JQ1 sensitivity, if the associated β coefficients were not zero.

RNA sequencing and analysis

[0154] Cells were treated for 24 hours with DMSO or 0.5 μM JQ1 in triplicate. Total RNA was isolated with the Qiagen RNAeasy kit and subjected to oligo (dT) capture and enrichment. The resulting mRNA fraction was used to construct complementary DNA libraries. Transcriptome sequencing (RNA-seq) was performed on the Illumina HiSeq Platform using the standard paired-end protocol. In total 28-125 million 75-100 base pair (bp) reads were generated per sample. The RNA-seq reads were then mapped to the human genome (NCBI Build 37) by using GSNAP (Wu and Nacu 2010), allowing a maximum of three mismatches per 75 bp sequencing end, where 65-83% of reads were uniquely mapped to the human reference genome. The differential gene expression was performed with DESeq

both of which are based on a negative binomial distribution model. For analysis of differentially expressed genes, the following cutoffs were used: 2 fold change, median RPKM >0.1, and adjusted p value < 0.01. Clustering was performed with Cluster 3.085 and heatmap representation of differentially expressed genes was generated using Java TreeView (Saldanha, A.J. *Bioinformatics* 20, 3246-3248 (2004)).

Gene set enrichment analysis (GSEA)

[0155] GSEA87 was performed using JQ1/DMSO log2 fold change for each cell line. CIMP(+) classified cell lines (n=4) were compared to CIMP(-) classified cell lines (n=2) using difference of classes as the ranking metric and the Hallmark MSigDB gene set collection. An FDR cutoff of 5% was used.

H3K27ac ChIP-sequencing and analysis

[0156] Cells were fixed with 1% formaldehyde for 5 minutes and quenched with 0.125 M glycine. ChIP was performed using the Diagenode LowCell# ChIP kit with minor modifications. DNA was sonicated to an average length of 100-400 bp on a Covaris E220 platform (duty: 5%, peak incident power: 105 W, cycles per burst: 200, time: 16 min). Sonicated DNA was immunoprecipitated with 2 μg of rabbit anti-H3K27ac antibody (Abcam, ab4729, Lot: GR183919-2) and protein A beads. Complexes were washed and eluted from beads with SDS buffer. ChIP DNA and input DNA was subjected to RNase and proteinase K treatment and crosslinks were reversed by incubation at 65°C. DNA was purified using Qiagen MinElute kit.

[0157] DNA samples were used directly to prepare the indexed Illumina library. ChIP DNA (3 -10ng) or the Input DNA (250-300 ng) was subjected to the standard Illumina library preparation. The process starts from end repair to convert the overhangs into blunt ends. A single “A” nucleotide was then added to the 3’ends of the blunt fragment followed by ligating the standard Illumina adapter to the DNA fragments. The adapter-ligated DNA fragments were then enriched with PCR amplification. Standard Illumina barcode was also added to each library during the library preparation process. The completed libraries were then checked and quantified with Qubit, Bioanalyzer and qPCR. Twenty libraries were then clustered onto the flow cell with 10 libraries in one lane. The libraries were then sequenced with HiSeq2500 using 75 bp, single end read.

[0158] Bioinformatics analysis includes de-multiplexing and filtering followed by alignment to the human genome (hg19) using the BWA algorithm (default settings).

Duplicate reads were removed and uniquely mapped reads were normalized for total read number per sample.

Brd4 ChIP-sequencing and analysis

[0159] Cells were fixed with 1% formaldehyde for 15 min and quenched with 0.125 M glycine. Chromatin was isolated by the addition of lysis buffer, followed by disruption with a Dounce homogenizer. Lysates were sonicated and the DNA sheared to an average length of 300-500 bp. Genomic DNA (Input) was prepared by treating aliquots of chromatin with RNase, proteinase K and heat for de-crosslinking, followed by ethanol precipitation. Pellets were resuspended and the resulting DNA was quantified on a NanoDrop spectrophotometer. Extrapolation to the original chromatin volume allowed quantitation of the total chromatin yield.

[0160] An aliquot of chromatin (30 ug) was precleared with protein A agarose beads (Invitrogen). Genomic DNA regions of interest were isolated using 4 μg of antibody against BRD4 (Bethyl Laboratories, A301-895, Lot. A301-985A100-3). 200 ng of Drosophila S2 chromatin and 0.4 μg of drosophila-specific H2A.v antibody (Active Motif: 39715) were also added to each reaction for normalization purposes. Complexes were washed, eluted from the beads with SDS buffer, and subjected to RNase and proteinase K treatment. Crosslinks were reversed by incubation overnight at 65°C, and ChIP DNA was purified by phenol-chloroform extraction and ethanol precipitation.

[0161] Illumina sequencing libraries were prepared from the ChIP and Input DNAs by the standard consecutive enzymatic steps of end-polishing, dA-addition, and adaptor ligation. After a final PCR amplification step, the resulting DNA libraries were quantified and sequenced on NexSeq 500 (75 nt reads, single end). Reads were aligned to the human genome (hg19) and the Drosophila genome (dm3) using the BWA algorithm (default settings) (Li, H. & Durbin, R. *Bioinformatics* 25, 1754-1760 (2009)). Duplicate reads were removed and only uniquely mapped reads (mapping quality >= 25) were used for further analysis.

[0162] The spike-in normalization was performed by equalizing the Drosophila tag counts across all samples so that the final tag counts were based off of the sample containing the lowest number of Drosophila tags. Then the human tags counts for all samples were proportionally scaled based on the ratios used to adjust the drosophila tag counts. Scaling to the target tag number was performed by randomly removing excess tags.

[0163] Alignments were extended in silico at their 3'-ends to a length of 200 bp, which is the average genomic fragment length in the size-selected library, and assigned to 32-nt bins along the genome. The resulting histograms (genomic "signal maps") were stored in bigWig files. Peak locations for BRD4 were determined using the MACS algorithm (v1.4.2) (Zhang, Y., *et al. Genome Biol* 9, R137 (2008)) with a cutoff of p-value = 1x107. Signal maps and peak locations were used as input data to Active Motif's proprietary analysis program, which creates Excel tables containing detailed information on sample comparison, peak metrics, peak locations and gene annotations.

[0164] MACS peak locations and BAM files were used as input into the ROSE software to identify super-enhancers (Whyte (2013); Loven, J., *et al. Cell* 153, 320-334 (2013)). The default stitching distance of 12.5 kb was used and promoters were not excluded. Super-enhancers were annotated to genes if they fell within 50 kb upstream or downstream of the gene. Heatmap representation of drosophila chromatin normalized BRD4 binding at super-enhancer regions was generated using seqplots (Github). Overlapping super-enhancer regions were grouped into active regions defined by the most upstream start position and the most downstream end position (the union of overlapping intervals). When a super-enhancer was present in only one cell line the active region was defined by that interval. Since super-enhancers have very different sizes, for display purposes an anchored approach was used where super-enhancers were shrunk or expanded to the same length and then signals were displayed with 10 kb flanking regions on both sides.

Calculating input-normalized and averaged ChIP-seq signal

[0165] BRD4 tags for each sample were normalized using drosophila chromatin input method while H3K27ac tags and input tags were normalized to a specific tag number using random downsampling. Coverage was calculated across 10 bp bins using BEDTools (Quinlan, A.R. & Hall, I.M. *Bioinformatics* 26, 841-842 (2010)) and the ratio of ChIP/input across bins was used directly or averaged across CIMP(+) and CIMP(-) lines. Tracks were visualized in the Integrative Genomics Viewer (IGV) (Robinson, J.T., *et al. Nat Biotechnol* 29, 24-26 (2011); Thorvaldsdottir, H., Robinson, J.T. & Mesirov, J.P. *Brief Bioinform* 14, 178-192 (2013)).

Brd4 ChIP-qPCR

[0166] Cells were fixed with 1% formaldehyde for 10 min and quenched with 0.125 M glycine. Crosslinked cells were resuspended and lysed in lysis buffer 1 (50 mM HEPES, pH

7.5, 140 mM NaCl, 1 mM EDTA, 10% glycerol, 0.5% NP-40, 0.25% Triton X-100), followed by lysis buffer 2 (10 mM Tris-HCl, pH 8.0, 200 mM NaCl, 1 mM EDTA, 0.5 mM EGTA). Sonication was performed in sonication buffer (0.1% SDS, 1 mM EDTA, 10 mM Tris-HCl, pH 8.0) on a Covaris E220 platform (duty: 20%, peak incident power: 175 W, cycles per burst: 200, time: 30 min). An aliquot of chromatin was quantified and 60 ug of sonicated DNA was immunoprecipitated with 10 μg of rabbit anti-Brd4 antibody (Bethyl Laboratories, A301-895, Lot. A301-985A100-4) and 100 μl of protein A beads. Complexes were washed and eluted from beads with SDS buffer. ChIP DNA and input DNA were subjected to RNase and proteinase K treatment and crosslinks were reversed by incubation at 65°C. DNA was purified using QIAquick PCR purification kit (Qiagen). qPCR was performed with TaqMan Universal PCR Master Mix (Applied Biosystems) with custom designed primers and probe for CCAT1 (forward: 5'- CAAAGGTCCCAATTTCACACT; reverse: 5'- ACAACTGTGCTCCTGAATGC; probe: 5'- TCCAGTTGGGTTCTCTTTCCTTTGCT) and a negative control locus on chromosome 4 (forward: 5'- GATGGCCCAGTGTAAGCATT; reverse: 5'- TGACTCTGACGATAGCTCTCAAA; probe: 5'- AATGTCCTAGTTTCATAAATTACGGTCACTCTATCTGG).

Gene expression analysis

[0167] For qRT-PCR experiments total RNA was isolated with the RNeasy mini kit (Qiagen). Reverse transcription followed by qPCR was performed with the TaqMan one-step RT-PCR master mix (Applied Biosystems). Samples were normalized to expression of GAPDH mRNA.

Immunohistochemistry

[0168] IHC was performed essentially as described (Adler (2014)). For BRD4 (Epitomics cMYC (Epitomics; 1472-1) and phospho-Histone H3 (Ser10; Upstate Biotechnology), staining was performed on a DAKO autostainer.

Human tissues

[0169] To evaluate CCAT1 expression, a population-based series of patients who had undergone surgical resections for colorectal adenocarcinoma was compiled retrospectively from the pathology archives at St James' University Hospital (Leeds, UK) from 1988 to 2003. Patient demographics and treatment information were obtained from clinical records. Morphology, site, Dukes' stage, grade of differentiation, number of lymph nodes retrieved

and number of positive lymph nodes were compiled from the surgical pathology reports. Survival data were obtained from the Northern and Yorkshire Cancer Registry and Information Service (St James' University Hospital). Inclusion of patients into this cohort was dependent on the availability of archival material and outcome data. TMAs were constructed as above with one core of normal mucosa and three cores of adenocarcinoma per patient. Approval for the use of this tissue was provided by the local research ethics committee.

Non-isotopic in situ hybridization

[0170] Non-isotopic in situ hybridization (ISH) was performed on 4 µm FFPE sections using QuantiGene® ViewRNA ISH Tissue Assay (Affymetrix/Panomics) following the manufacturer's protocol on a Tecan platform equipped to carry out non-isotopic in situ hybridization. Gene-specific probe for detection of human CCAT1 RNA (Affymetrix; VA1-17802) target region 2-2696 in Genbank accessions NR_108049.1 was used on tissue samples. A probe set to Bacillus subtilis dihydropicolinate reductase (dapB) (VF1-11712), target region 1363-2044 in Genbank accession L38424, was used as a negative control. Horseradish peroxidase (HRP) conjugated label probe was used, followed by TSA™ (tyramide signal amplification) to increase sensitivity (Perkin Elmer NEL748001KT). Briefly, TSA Plus DIG stock solution (digoxigenin) was diluted 1:50 in 1x Plus Amplification Diluent and applied to sections and incubated for 10 minutes at room temperature. This was followed by incubation with anti-DIG-AP (Roche 11093274910) diluted 1:500 in TNB blocking buffer with 4% lamb serum (Gibco, 16070-096) for 30 minutes at room temperature. Vulcan Fast Red substrate (Biocare, FR805S) was used for chromogenic detection.

Genomic copy number analysis

[0171] For quantitative copy number analysis, genomic DNA was isolated with the DNeasy blood & tissue kit (Qiagen). Quantitative PCR for cMYC copy number was performed with the TaqMan genotyping master mix using Taqman copy number probes (Applied Biosystems). For colon cell lines and tumors, samples were normalized to copy number at the TERT locus, which is rarely amplified or deleted in colon cancer (Tumorscape).

Accession numbers

[0172] ChIP-seq and RNA-seq data have been deposited in Gene Expression Omnibus with accession number (GSEXXXXX, accession number in progress).

Results

[0173] An arrayed CRISPR loss-of-function screen identified BRD4 as a critical regulator of colon cancer growth.

[0174] To identify epigenetic regulators critical to colon cancer growth, a CRISPR-based loss of function screen was performed in the near diploid RKO colon cancer cell line. Introduction of the Cas9 nuclease and guide RNAs (gRNA) was carried out in a two-step process (FIG. 1A). Lentivirus was utilized to generate RKO cells that stably express Cas9, and cells were subsequently transduced with relevant gRNAs. To examine the specificity and validate this CRISPR system, RKO (wild-type) and RKO-Cas9 cells were infected with gRNAs targeting the firefly luciferase gene and the mitotic kinase PLK1. Overall, five independent gRNAs targeting PLK1 consistently reduced cell viability in the Cas9 expressing cells, but did not hinder the growth of cells lacking Cas9 (FIG. 1B). Three non-targeting luciferase gRNAs did not impact cell proliferation.

[0175] Based on the observed proficiency of gene knockouts, a gRNA library targeting the 5' exons of over 200 genes involved in epigenetic regulation ('Epi200 library') was designed with a median coverage of 5 gRNAs per gene. High throughput cloning and viral production was employed to construct an arrayed lentiviral library containing over 1000 gRNAs. The library was subsequently transduced into RKO-Cas9 cells, infected cells were selected with puromycin 48 hours after infection, and cell viability was measured 7 days after transduction (FIGS. 1C&D). Analysis of the CRISPR screen identified thirteen genes that affected colon cancer proliferation using the criteria that at least two gRNAs score (1st at Z-score<-2.0, 2nd at a Z-score <-1.5) for any given gene (FIG. 1D). Among these genes were known cell cycle regulators such as PLK1, cMYC, AURKB and HDAC137. 7 of the 13 genes scored with all gRNAs meeting the Z-score cut-off, indicating a high level of phenotypic consistency between gene-specific gRNAs (FIG. 1D).

[0176] In order to validate genetic hits from the screen, phenotypic effects were correlated with genotypic activity for each set of gRNAs. Robust gRNA-mediated protein depletion was detected for BRD4, KAT8, CHD1, HDAC1, and AURKB by both immunoblot and immunofluorescence microscopy and positively correlated with the observed cell growth effects (FIGS. 1E& F and FIGS. 2A-D). More than half of all gRNAs tested by immunoblotting (n=39, from 8 genes) were able to reduce the targeted protein by more than 95% (FIG. 2E). As previous CRISPR screens focused on pooled based approaches, these data

illustrated the utility of the CRISPR system in an arrayed based format to provide efficient protein knockout and consistent phenotypic effects (Wang, T., Wei, J.J., Sabatini, D.M. & Lander, E.S. *Science* 343, 80-84 (2014); Shalem, O., *et al. Science* 343, 84-87 (2014); Koike-Yusa, H., Li, Y., Tan, E.P., Velasco-Herrera Mdel, C. & Yusa, K. *Nat Biotechnol* 32, 267-273 (2014)).

[0177] The long isoform of BRD4 is critical for colon cancer cell proliferation.

[0178] Among the novel candidate therapeutic targets that were identified in the CRISPR screen, BRD4 was chosen for further analysis. BRD4 small molecule inhibitors have entered clinical trials in several hematological malignancies (Shi, J. & Vakoc, C.R. *Mol Cell* 54, 728-736 (2014)). As investigations of BRD4 in colon tumors have been limited (Rodriguez, R.M., *et al. J Mol Med (Berl)* 90, 587-595 (2012); Hu, Y., *et al. Int J Mol Sci* 16, 1928-1948 (2015)), BRD4 activity in colon cancer was evaluated. Two alternatively spliced BRD4 transcript variants have been described, a long (BRD4-LF) and short isoform (BRD4-SF) (Floyd, S.R., *et al. Nature* 498, 246-250 (2013); Wang, R., Li, Q., Helfer, C.M., Jiao, J. & You, J. *J Biol Chem* 287, 10738-10752 (2012)). BRD4 expression was characterized by immunohistochemistry in colon tissues (normal, premalignant, and carcinoma) using antibodies that recognize both BRD4 isoforms or specifically the BRD4-LF variant. Overall BRD4 levels remained unchanged at different stages of colonic tumorigenesis, while the BRD4-LF isoform was specifically upregulated during the pre-malignant to malignant transition (adenoma to carcinoma; FIGS. 3A&B). Concordantly, BRD4-LF was consistently expressed in colonic cancer cell lines compared to BRD4-SF (FIG. 3C). While both BRD4 isoforms encode bromodomains, the BRD4-LF has been more strongly implicated in transcriptional regulation due to its C-terminal p-TEFb binding motif (Yang, Z., *et al. Mol Cell* 19, 535-545 (2005); Jang, M.K., *et al. Mol Cell* 19, 523-534 (2005)).

[0179] To further dissect BRD4 function in colon cancer, CRISPR was used to induce N-terminal deletions in BRD4 in RKO and HCT-116 colon cancer cells (FIG. 3D). Clonally derived cell lines nullizygous for BRD4 displayed significant growth retardation in both cancer cell lines (FIG. 3E, F). Consistent with previous findings (Mochizuki, K., *et al. J Biol Chem* 283, 9040-9048 (2008)), BRD4-null cells were marked by cell cycle defects consistent with a G1/S phase delay (FIG. 3G). Since initial findings showed that isoform specific BRD4 expression was dysregulated during colon tumorigenesis (FIGS. 3A&B), isoform specific BRD4 expression was reconstituted by delivery of BRD4-LF or BRD4-SF splice variants to

BRD4-null colon cancer cells. While BRD4-SF was unable to restore growth, expression of BRD4-LF rescued the growth defect of BRD4 knockout cells to wild-type levels (FIGS. 3H& I and FIG. 4A). BRD4 constructs containing bromodomain inactivating mutations failed to rescue the growth defect. To directly test the importance of the pTEFb binding C-terminal motif (CTM) of BRD4-LF, CRISPR was used to ablate the BRD4-LF isoform in colon cancer cells. Analysis of four independent BRD4-LF specific nullizygous HCT-116 cell lines showed reduced cell proliferation (FIG. 3J&K). These data indicate an important role for the BRD4-LF in colon cancer cell proliferation.

[0180] BRD4 loss reduced colon tumor growth and induced cellular differentiation in vivo.

[0181] BRD4, and in particular the BRD4-LF variant, have been implicated in maintaining embryonic pluripotency. Likewise, in cancer, BRD4 inhibition has been shown to induce differentiation effects in hematological malignancies (Alsarraj, J., *et al. Cancer Res* 71, 3121-3131 (2011); Zuber, J., *et al. Nature* 478, 524-528 (2011)). To characterize the effect of acute BRD4 loss on colon tumor growth in vivo, a doxycycline inducible shRNA system was used to deplete BRD4 in implanted tumors (Gray, D.C., *et al. BMC Biotechnol* 7, 61 (2007)). Efficient BRD4 depletion and consequent in vitro growth defects were found with two independent BRD4 shRNAs in HT-29 and HCT-116 cell lines (FIG. 5A, FIG. 4B). Colon cancer cell lines expressing the more potent shBRD4-2 shRNA were then utilized for in vivo tumor xenograft efficacy studies. To measure effects on tumor growth rather than initiation, mice were administered doxycycline after tumors reached 200 mm^3 in size. shBRD4-2 tumors that received doxycycline exhibited efficient BRD4 depletion in vivo and displayed significant tumor regression in both colon tumor xenograft models (FIGS. 5B-D). To characterize the nature of the defect, immunohistochemistry was performed for cell cycle (phospho-Histone H3, cMYC) and apoptosis (cleaved caspase 3) markers on tumor xenografts 7 days after doxycycline-induced BRD4 depletion. Consistent with a cell cycle defect, tumors with reduced BRD4 showed decreased cMYC and phospho-Histone H3 with no significant change in cleaved caspase 3 (FIGS. 5E&F). Histopathological analysis of BRD4-depleted HT-29 xenograft tumors revealed morphological alterations. These included the formation of crypt-like structures with goblet cell differentiation (FIG. 5G) and loss of cancer associated cytological features such as prominent nucleoli and pleomorphic nuclei (FIG. 5G; H&E insets). Immuno-phenotyping analysis showed that these pseudo-crypts mimiced the ordered structure of intestinal epithelium and expressed proliferative markers

such as Ki-67 and cMYC in a graded manner (FIG. 5G, Ki-67 and cMYC, higher expression seen at the pseudo-crypt base). Together, these data indicated that BRD4 is required for tumor growth and maintenance of a de-differentiated state in vivo.

[0182] BET inhibitors preferentially impaired CIMP(+) colon cancer growth.

[0183] To explore the utility of BET inhibition more broadly in colon cancer, the effect of JQ1, a commonly used BET inhibitor, was tested in a panel of 20 colon cancer cell lines with similar proliferation rates (20-40hrs) (Filippakopoulos (2010)). A subset of colon cell lines was distinctly sensitive to JQ1 (FIG. 6A). To investigate the molecular underpinnings of BET inhibitor sensitivity in these cells, genomic features were surveyed that were predictive of JQ1 sensitivity based on the shrinkage and selection method for linear regression, Lasso (FIG. 6B and FIG. 7A) (Ribbing, J., Nyberg, J., Caster, O. & Jonsson, E.N. *J Pharmacokinet Phar* 34, 485-517 (2007)). Using copy number, mutation, DNA methylation, and expression data as well as other classifications such as MSI and CIMP status, 88723 features were generated for each cell line. The Lasso approach selected CIMP status as the most predictive feature to classify lines as sensitive or resistant (FIG. 7B) (Ogino (2007)). The six most sensitive cell lines were all CIMP(+), while the six most resistant cell lines were all CIMP(-) (FIGS. 6A&C).

[0184] Methylation of POU3F2, FAM65B, and GABRB2 as well as expression of ZNF606 were selected as predictive features for the underlying EC50 values. Consistent with the significant enrichment of the CIMP classification in sensitive cell lines, DNA hypermethylation at promoter CpG islands of POU3F2, FAM65B, GABRB2 and ZNF606 was highly correlated with JQ1 sensitivity (FIG. 7C). In the case of ZNF606, a known CpG hypermethylated gene (Lleras, R.A., *et al. Am J Pathol* 178, 1965-1974 (2011)), repressed gene expression correlated strongly with JQ1 activity (FIG. 7D). These findings were confirmed using iBET-762, an additional BET inhibitor with clinical activity[55]. Similar to JQ1, iBET-762 showed greater activity in CIMP(+) cell lines (FIG. 8A&B). JQ1 and iBET-762 EC50’s correlated well to each other, indicating that these effects were on-target (FIG. 8C). These data suggested that CIMP(+) colon cancers are more dependent on BET activity.

[0185] An integrated transcriptomic and genomic approach was used to identify direct BRD4-dependent target genes that account for the increased sensitivity seen in CIMP(+) colon cancer cells. Four CIMP(+) and two CIMP(-) cells were profiled for both JQ1-dependent gene expression changes and BRD4 and histone H3 lysine 27 acetyl (H3K27ac)

genomic enrichment using RNA-seq and ChIP-seq, respectively (FIG. 6D). Previous studies have shown that BRD4 preferentially binds at densely occupied enhancer elements termed super-enhancers (Whyte, W.A., *et al. Cell* 153, 307-319 (2013)), which in some contexts ramps up the expression of oncogenic factors in a tumor-type specific and lineage dependent manner (Whyte, W.A., *et al. Cell* 153, 307-319 (2013); Dowen, J.M., *et al. Cell* 159, 374-387 (2014); Mansour, M.R., *et al. Science* 346, 1373-1377 (2014)). Consistent with these findings, overlapping and cell line specific gene expression changes as well as BRD4 binding at super-enhancers were detected in these six colon cell lines (FIGS. 6E&F, FIG. 9A&B). To identify shared genes and pathways that underlie BET activity in CIMP(+) colon cancer, gene expression changes in response to JQ1 treatment were compared between the CIMP(+) and CIMP(-) cell lines. Gene set enrichment analysis (GSEA) comparing JQ1 transcriptomic changes across all six cell lines revealed significant enrichment of cMYC pathway gene signatures in the CIMP(+) cells (FDR<0.0001) (FIG. 6 and FIG. 9C). A number of important colon cancer associated oncogenic regulators, including EREG and CLDN2, showed CIMP-status specific JQ1 changes in expression (FIG. 6G).

[0186] As cMYC plays a critical role in colon cancer initiation and progression (Fearon, E.R. & Dang, C.V. *Curr Biol* 9, R62-65 (1999); Sansom, O.J., *et al. Nature* 446, 676-679 (2007)), cMYC protein levels were examined by immunoblot analysis after BET inhibition in colon cancer cell lines. cMYC protein was dramatically reduced in CIMP(+) cell lines 24 hours after JQ1 treatment compared to CIMP(-) colon cancers (FIGS. 6H& I). Furthermore, the kinetics of cMYC reduction also occurred earlier in the CIMP(+) cell lines, with the majority of cMYC depleted as early as 6 hours after JQ1 treatment (FIGS. 10A-C). To determine whether cMYC is the main mediator of BET activity in these cells, lentivirus was used to deliver exogenous cMYC in HCT-116 BRD4-null cells. Restoration of cMYC expression in a BRD4-deficient setting led to partial rescue of cell growth (FIG. 10D). These results indicated that CIMP(+) cells are sensitive to loss of cMYC in a BRD4/BET dependent manner.

[0187] CCAT1 is a BET transcriptional target gene and a marker of BET activity near the cMYC locus.

[0188] To identify direct BRD4 targets, the transcriptomic (RNA-seq) and genomic (ChIP-seq) analyses were merged to identify genes that were both downregulated after JQ1 treatment and marked by an adjacent super-enhancer. This integrative approach was

conducted for CIMP(+) and CIMP(-) cell lines independently. Three direct BET targets, PHF15, TRIB3 and CCAT1 (FIG. 11A), were identified by this analysis in the CIMP(+) cells. Of these 3 genes, only TRIB3 and CCAT1 were specific to the CIMP(+) colon cells (FIG. 11A). The CCAT1 transcript was one of the most highly down-regulated genes in CIMP(+) cells and its genomic locus ranked as one of the densest BRD4 enriched enhancers (i.e. 'super-enhancer') in CIMP(+) colon cancer (FIG. 11B). The CCAT1 transcript is located within a demarcated super-enhancer, 500 kb upstream of the cMYC promoter. Both BRD4 binding and H3K27ac levels were enriched at the CCAT1 associated super-enhancer in the CIMP(+) colon cancer cell lines, indicating that its activation may be context specific (FIG. 11C and FIG. 12A-E).

[0189] CCAT1 is a long noncoding RNA (lncRNA) that is expressed in colon, gastric and gallbladder cancer (Ma, M.Z., *et al. Cell Death Disease* 6, e1583 (2015); Mizrahi, I., *et al. J Cancer* 6, 105-110 (2015); Xiang, J.F., *et al. Cell Res* 24, 513-531 (2014)). While CCAT1 has been reported to regulate cMYC expression (Xiang (2014)), its relationship to BET activity has not been previously reported. The basal CCAT1 RNA levels were higher in the CIMP(+) cell lines used in the RNA-seq analysis and correlated with the amount of BRD4 binding and H3K27ac at the CCAT1-associated super-enhancer (FIGS. 11C& D). In the CIMP(+) cell lines, CCAT1 expression was sensitive to JQ1, indicating that it was a direct transcriptional target of BRD4 (FIG. 11E). Consistent with this super-enhancer driving cMYC transcription, JQ1 treatment preferentially reduced cMYC expression in CCAT1 expressing CIMP(+) cells (FIG. 11F). Taken together, these results indicated that CCAT1 is a super-enhancer template RNA and serves as a marker of high BET activity near the cMYC locus.

[0190] CCAT1, PCAT, LOC728724 are BRD4 transcriptional targets and markers for sensitivity to BET inhibitors.

[0191] Growing evidence suggests that enhancers are actively transcribed by RNA polymerase II to produce enhancer derived long non-coding RNAs (eRNA) (Kim, T.K., *et al. Nature* 465, 182-187 (2010); Wang, D., *et al. Nature* 474, 390-394 (2011)). BRD4 has been shown to regulate eRNA expression in murine cells (Kanno, T., *et al. Nat Struct Mol Biol*21, 1047-1057 (2014)). To explore the connection between eRNAs and BET activity at the cMYC locus, the expression of eRNAs by other super-enhancers adjacent to the cMYC locus was examined. H3K27ac was associated with the presence of two distinct eRNAs, PCAT1

(Prostate Cancer Associated Transcript 1) and LOC728724 in prostate and T-ALL, respectively (FIG. 13A) (Mansour, M.R., *et al. Science* 346, 1373-1377 (2014); Asangani, I.A., *et al. Nature* 510, 278-282 (2014)). Both leukemia and prostate cells have been reported to be sensitive to BET inhibition. To determine whether the expression of these eRNAs was restricted to cancer type and super-enhancer activation, the expression of CCAT1, PCAT1 and LOC728724 was examined across different cancer indications using RNA-seq data from The Cancer Genome Atlas (TCGA). These eRNAs showed distinct tumor specificity, with LOC728724 expression restricted to leukemia and PCAT1 showing preferential expression in prostatic carcinoma. While CCAT1 expression has been previously linked to colon cancer, it was also highly expressed in a subset of lung, pancreatic and gastric tumors (FIG. 13B).

[0192] To examine whether CCAT1, PCAT1 and LOC728724 were controlled by BRD4 associated super-enhancers, cell lines were identified for each tumor type that expressed the CCAT1, PCAT1 and LOC728724 eRNAs (FIG. 14A-D) (Klijn, C., *et al. Nature biotechnology* 33, 306-312 (2015)). These cells were treated with JQ1 for 24 hours and the level of each eRNA was assessed by qPCR. BET inhibition led to near complete RNA depletion for each lineage specific eRNA (FIG. 14E-G). These data demonstrated that BET-dependent eRNAs are distinctly expressed in discrete tumor types and serve as pharmacodynamic markers of BET inhibition.

[0193] While the mechanism by which cancers are dependent on BRD4 for cMYC transcription is not well understood, the ability to detect them is of great clinical importance. For example, in hematological malignancies cMYC is an established BRD4 target, while in solid tumor malignancies BET-dependent cMYC transcription is often cell line dependent (Asangani, I.A., *et al. Nature* 510, 278-282 (2014); Lockwood, W.W., Zejnullahu, K., Bradner, J.E. & Varmus, H. *P Natl Acad Sci* 109, 19408-19413 (2012)). The ability of BET-driven eRNA expression to predict which cells utilized BRD4 to modulate cMYC transcription was evaluated. cMYC RNA levels were measured in CCAT1-high and CCAT1-low cell lines following a 24 hour JQ1 treatment. 14 of 16 CCAT1 high cell lines (CCAT1 RPKM threshold>1.0) showed 50% or greater reduction in cMYC levels. Conversely, JQ1 treatment only moderately affected cMYC levels in the 11 cell lines tested that expressed low ($\leq$ 0.5 RPKM) CCAT1 eRNA (FIG. 13C and FIG. 15A). CCAT1 predicted cell line sensitivity to JQ1. Colon, lung, gastric and pancreatic cell lines were treated for 3 days with JQ1 and relative EC50 values were calculated. With the exception of gastric cancer, all other tumor types (pancreatic, lung and colon cells) exhibited a significant

correlation between CCAT1 expression and JQ1 sensitivity (FIG. 13D, FIG. 15B-E). A similar trend was observed in prostate and leukemia, where PCAT1 (FIG. 15F-H) and LOC728724 (FIG. 15I& J) expression were associated with BET-mediated cMYC transcription, respectively. These results indicated that CCAT1 and other eRNAs serve as predictive biomarkers to identify tumors that utilize BET-mediated cMYC transcription for tumor growth.

[0194] Patients with colon tumors expressing high CCAT1 exhibit a poor clinical outcome.

[0195] cMYC upregulation in colon cancer can occur via numerous mechanisms, including locus amplification, mutation, super-enhancers, and post-translation modifications. The ability of CCAT1 expression to identify tumors that rely on a super-enhancer to drive cMYC expression was evaluated. To address this concept a CCAT1 in situ hybridization assay was developed (FIG. 16A and FIG. 17A&B). CCAT1 expression was examined directly in colon tumors (FIG. 16B). CCAT1 expression was scored on a cohort of normal colon tissues (n=555) and colon tumors (n=705), which had associated survival data. Normal colon exhibited weak to no CCAT1 expression which was confirmed using TCGA RNAseq data sets on normal colon tissue (FIG. 16B and FIG. 17C&D). Kaplan-Meier analysis demonstrated that overall patient survival was significantly lower in CCAT1 moderate (ISH score 1-2) or high (ISH score 2-3) compared to CCAT1 low (ISH score 0-1) tumors (FIG. 16C).

Summary

[0196] The importance of epigenetics in neoplasia has been confirmed by the recent discovery that many epigenetic regulators are mutated or dysregulated in human tumors (Watson, I.R., Takahashi, K., Futreal, P.A. & Chin, L. *Nat Rev Genet* 14, 703-718 (2013)) and can furthermore be targeted for therapeutic purposes (Dawson, M.A. & Kouzarides, T. *Cell* 150, 12-27 (2012); Dawson, M.A., Kouzarides, T. & Huntly, B.J. *New Engl J Med* 367, 647-657 (2012)). Herein, the CRISPR technology was used to systematically characterize the epigenetic landscape and identify new therapeutic targets in colon cancer. Unlike other CRISPR based loss of function screens that are based on pooled libraries, the array-based CRISPR library yielded robust and reproducible target depletion and phenotypic response. While advantages exist to both arrayed and pooled screening platforms (Boettcher, M. &

Hoheisel, J.D. *Curr Genomics* 11, 162-167 (2010)), the screen described herein represents an important step in characterizing the CRISPR technology in an arrayed platform.

[0197] The studies described herein identified a number of novel candidate therapeutic targets. BRD4 was pursued, as BRD4 inhibitors have entered clinical trials in several hematological malignancies (Shi, J. & Vakoc, C.R. (2014)). Consistent with the oncogenic effects of BRD4 in other malignancies, genetic or pharmacological inhibition of BRD4 reduced cancer proliferation and abrogated tumor growth in colon cancer xenograft models. BRD4 loss led to tumor differentiation in vivo with crypt-like structures formed in the HT-29 xenografted model. Colon cancer has long been postulated to be a stem-cell driven disease. Consistently, Wnt and Notch pathway inhibitors lead to similar differentiation changes, but are clinically intractable due to accompanying toxicity issues (Bertrand, F.E., Angus, C.W., Partis, W.J. & Sigounas, G. *Cell Cycle* 11, 4344-4351 (2012)). The studies described herein indicated that BRD4 inhibitors offer a clinically tractable path for differentiation therapy in colon cancer.

[0198] Colon tumors characterized by a high degree of CpG island methylation (CIMP) are both biologically and clinically distinct (Lao, V.V. & Grady, W.M. (2011); Suzuki, H., Yamamoto, E., Maruyama, R., Niinuma, T. & Kai, M. *Biochem Bioph Res Co* 455, 35-42 (2014)). CIMP-ness has been correlated with a poor patient outcome and resistance to chemotherapy (Phipps, A.I., *et al. Gastroenterology* 148, 77-87 e72 (2015)). A significant correlation between JQ1 sensitivity and CIMP positivity was observed in colon cancer cells. Furthermore, the effects of JQ1 were linked to its ability to inhibit cMYC expression specifically in CIMP(+) cells. Biologically, CIMP(+) tumors have a low level of CIN and hence disomy at the cMYC locus (Cheng, Y.W., *et al. Clin Cancer Res* 14, 6005-6013 (2008)). The preferential effect of JQ1 on cMYC in this context indicates that cancers with cMYC disomy may be more dependent on super-enhancers and other epigenetic mechanisms to drive cMYC expression.

[0199] The advent of molecular targeted cancer therapy has shed light on the critical role that biomarkers play in identifying responsive patients and fine-tuning drug repurposing efforts. Growing evidence in preclinical models shows that BRD4 activity closely associates with the presence of discrete super-enhancer elements that direct RNA polymerase II mediated transcription. The studies described herein indicated that the pTEFb interaction domain in BRD4-LF is necessary for BRD4 activity in colon cancer. Super-enhancers are

variably formed and require sophisticated methodology such as ChIP-seq to detect. Thus, these elements can be challenging to consistently detect in preserved human tissue and are not amenable as biomarkers in the companion diagnostics setting. The results described herein indicated that distinct enhancer template RNAs such as CCAT1 and PCAT1 can be utilized to uncover super-enhancer activity, an important step in developing biomarkers of BET activity in patients. The results indicated that CCAT1 expression can predict BET inhibitor response in colon, pancreatic and lung cancer. CCAT1 can be readily detected by either RT-PCR or an in situ hybridization assay, making it an ideal biomarker in FFPE tissues. The CCAT1 eRNA itself has been previously shown to regulate cMYC expression albeit at milder levels than what is seen with BET inhibitors (Xiang, J.F. (2014); Younger, S.T. & Rinn, J.L. *Cell Res* 24, 643-644 (2014)). Cumulatively, these data indicated that both BET activity and eRNA expression are necessary to drive cMYC transcription in cancers expressing CCAT1.

[0200] In conclusion, the array-based CRISPR screen described herein identified the BRD4 oncogene as a critical driver of proliferation and the dedifferentiated state in colon cancer. CCAT1, a super-enhancer RNA, predicted growth sensitivity to BET inhibitors and marked cancer cells utilizing BRD4 to drive MYC expression.

What is claimed is:

1. A method of treating a subject having cancer with a BET inhibitor, comprising:
   (a) performing a nucleic acid-based detection assay to detect the expression level of an eRNA transcribed from a myc-associated enhancer element in a sample containing cancer cells from the subject;
   (b) determining whether the cells in the sample from the subject express the eRNA at a level greater than a reference expression level of the eRNA; and
   (c) administering an effective amount of the BET inhibitor to the subject if it is determined that the cells in the sample from the subject express the eRNA at a level greater than the reference expression level of the eRNA.

2. A method of selecting a subject for treatment with a BET inhibitor, comprising:
   (a) performing a nucleic acid-based detection assay to detect the expression level of an eRNA transcribed from a myc-associated enhancer element in a sample containing cancer cells from the subject;
   (b) determining whether the cells in the sample from the subject express the eRNA at a level greater than a reference expression level of the eRNA; and
   (c) selecting the subject for treatment with a BET inhibitor if the cells in the sample from the subject express the eRNA at a level greater than the reference expression level of the eRNA.

3. The method of claim 2, wherein selecting the subject for treatment comprises selecting the subject for inclusion in a clinical trial.

4. A method of modulating the treatment of a subject undergoing BET inhibitor treatment for cancer, comprising:
   (a) performing a nucleic acid-based detection assay to detect the expression level of an eRNA transcribed from a myc-associated enhancer element in a sample containing cancer cells from the subject;

(b) determining the expression level of the eRNA in the cells in the sample;
(c) comparing the expression level of the eRNA in the cells in the sample to a reference expression level of the eRNA; and
(d) modulating the amount of BET inhibitor administered to the subject based on the difference between the expression level of the eRNA in the cells in the sample and the eRNA reference expression level.

5. The method of claim 4, wherein the reference expression level is based on the expression level of the eRNA in the subject at an earlier timepoint during BET inhibitor treatment.

6. The method of claim 5, wherein comparing the expression level of the eRNA in the cancer cells to the reference expression level of the eRNA comprises determining that the expression level of the eRNA in the cancer cells is less than the reference expression level of the eRNA and wherein modulating the amount of BET inhibitor administered to the subject comprises maintaining the same level or decreasing the level of BET inhibitor administered to the subject.

7. The method of claim 5, wherein comparing the expression level of the eRNA in the cancer cells to the reference expression level of the eRNA comprises determining that the expression level of the eRNA in the cancer cells is the same or more than the reference expression level of the eRNA and wherein modulating the amount of BET inhibitor administered to the subject comprises increasing the level of BET inhibitor administered to the subject.

8. A method of predicting responsiveness of a subject having cancer to a BET inhibitor, comprising:
(a) performing a nucleic acid-based detection assay to detect the expression level of an eRNA transcribed from a myc-associated enhancer element in a sample containing cancer cells from a subject;
(b) determining whether the cells in the sample from the subject express the eRNA at a level greater than a reference expression level of the eRNA; and

(c) predicting that the subject will be responsive to a BET inhibitor if it is determined that the cells in the sample from the subject express the eRNA at a level greater than the reference expression level of the eRNA.

9. A method of communicating the likelihood of response of a subject having cancer to a BET inhibitor, comprising:
   (a) performing a nucleic acid-based detection assay to detect the expression level of an eRNA transcribed from a myc-associated enhancer element in a sample containing cancer cells from a subject;
   (b) determining whether the cells in the sample from the subject express the eRNA at a level greater than a reference expression level of the eRNA; and
   (c) communicating to a treatment provider that the cells in the sample from the subject express the eRNA at a level greater than the reference expression level of the eRNA, wherein the treatment provider administers an effective amount of a BET inhibitor to the subject or selects the subject for treatment with a BET inhibitor based on the communication.

10. A method of treating a subject having cancer with a BET inhibitor, comprising administering to the subject an effective amount of the BET inhibitor wherein cancer cells contained in a sample from the subject were determined to express an eRNA transcribed from a myc-associated enhancer element at a level greater than a reference expression level of the eRNA.

11. A method of treating a subject having cancer with a BET inhibitor, comprising administering to the subject an effective amount of the BET inhibitor wherein the subject was selected for treatment based on a determination that cancer cells contained in a sample from the subject express an eRNA transcribed from a myc-associated enhancer element at a level greater than a reference expression level of the eRNA.

12. A method of treating cancer in a subject comprising administering to the subject an effective amount of a BET inhibitor wherein treatment is based upon the subject having cancer comprising a cancer cell that expresses an eRNA transcribed from a

myc-associated enhancer element at a level greater than a reference expression level of the eRNA.

13. A method of treating a cancer cell, wherein the cancer cell expresses an eRNA transcribed from a myc-associated enhancer element at a level greater than a reference expression level of the eRNA, the method comprising providing an effective amount of a BET inhibitor to the cell.

14. A method of treating cancer in a subject provided that the subject has been found to have cancer comprising a cancer cell that expresses an eRNA transcribed from a myc-associated enhancer element at a level greater than a reference expression level of the eRNA, the method comprising administering to the subject an effective amount of a BET inhibitor.

15. The method of any of the above claims, wherein the cancer is selected from the group consisting of colon cancer, lung cancer, pancreatic cancer, bladder cancer, kidney cancer, endometrial cancer, leukemia, prostate cancer, breast cancer, gastric cancer, lung cancer, and ovarian cancer.

16. The method of any of the above claims, wherein the cancer is selected from the group consisting of colon cancer, lung cancer, pancreatic cancer, bladder cancer, kidney cancer, and endometrial cancer.

17. The method of claim 16, wherein the cancer is colon cancer.

18. The method of claim 17, wherein the colon cancer is CpG island methylator phenotype (CIMP) (+) colon cancer.

19. The method of any one of claims 16-18, wherein the eRNA comprises a sequence having at least 80% identity to a sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID NO: 3.

20. The method of claim 15, wherein the cancer is leukemia.

21. The method of claim 20, wherein the eRNA comprises a sequence having at least 80% identity to SEQ ID NO: 4.

22. The method of claim 15, wherein the cancer is selected from the group consisting of prostate cancer, bladder cancer, kidney cancer, endometrial cancer, breast cancer, gastric cancer, lung cancer, and ovarian cancer.

23. The method of claim 22, wherein the cancer is prostate cancer.

24. The method of claim 22 or claim 23, wherein the eRNA comprises a sequence having at least 80% identity to SEQ ID NO: 5.

25. The method of any of the above claims, wherein the subject is a human.

26. The method of any of the above claims, wherein the BET inhibitor is selected from the group consisting of JQ1, I-BET 151 (GSK1210151A), I-BET 762 (GSK525762), OTX-015, TEN-010, CPI-203, and CPI-0610.

27. The method of any of the above claims, wherein the nucleic acid-based detection assay detects expression of non-coding, nuclear RNA expressed in the cells in the sample from the subject.

28. The method of any of the above claims, wherein the nucleic acid-based detection assay is selected from the group consisting of RNAseq, microarray analysis, direct RNA sequencing, in situ hybridization, and quantitative real-time PCR.

29. The method of any of the above claims, wherein the myc-associated enhancer element is a myc-associated super enhancer element.

30. The method of any of the above claims, wherein the reference expression level is based on the expression level of the eRNA in non-cancer cells.

31. The method of claim 30, wherein the non-cancer cells are from the same tissue type as the cancer cells.

32. A method of treating a subject having colon cancer with a BET inhibitor, comprising:

    (a) performing a nucleic acid-based detection assay to detect the expression level of an eRNA comprising a sequence having at least 80% identity to a sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID NO: 3 in a sample containing colon cancer cells from the subject;
    (b) determining whether the cells in the sample from the subject express the eRNA at a level greater than a reference expression level of the eRNA; and
    (c) administering an effective amount of the BET inhibitor to the subject if it is determined that the cells in the sample from the subject express the eRNA at a level greater than the reference expression level of the eRNA.

33. A kit comprising reagents for detecting the expression level of an eRNA transcribed from a myc-associated enhancer element in a sample.

34. The kit of claim 33 further comprising instructions for using the reagents.

35. The kit of claim 33 or claim 34, further comprising a BET inhibitor.

36. The kit of any one of claims 33-35, further comprising instructions for performing any of the methods of claims 1-32.

FIG. 1A

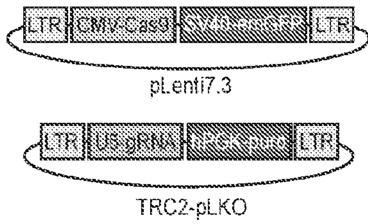


FIG. 1B

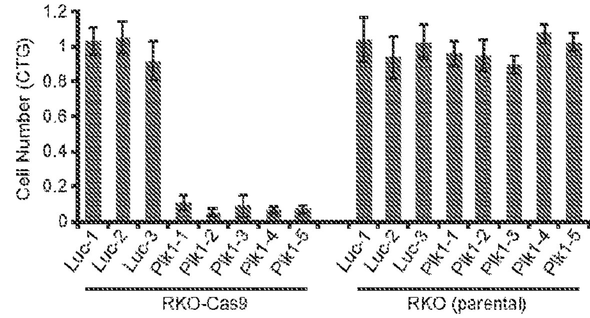


FIG. 1C

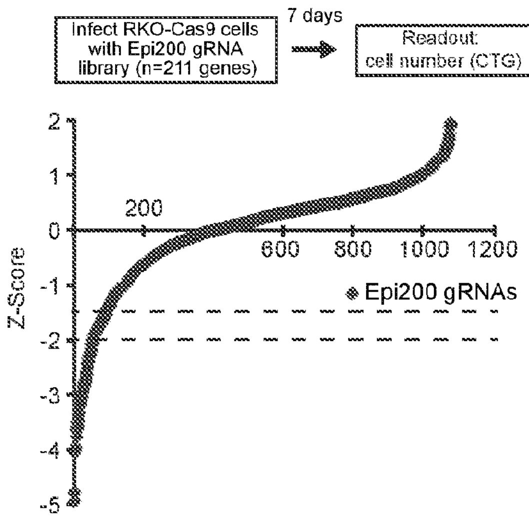


FIG. 1D

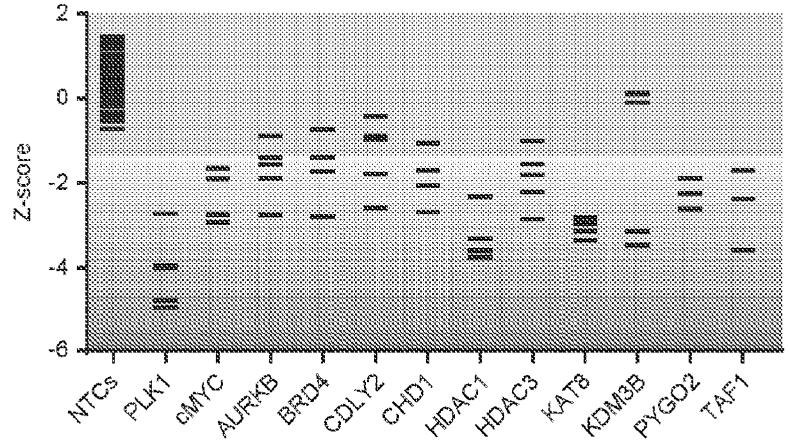


FIG. 1E

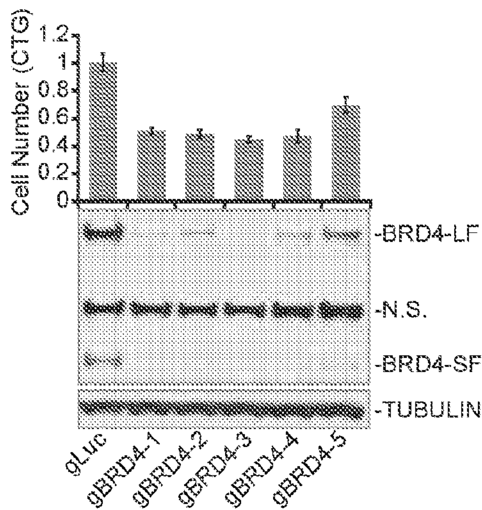


FIG. 1F

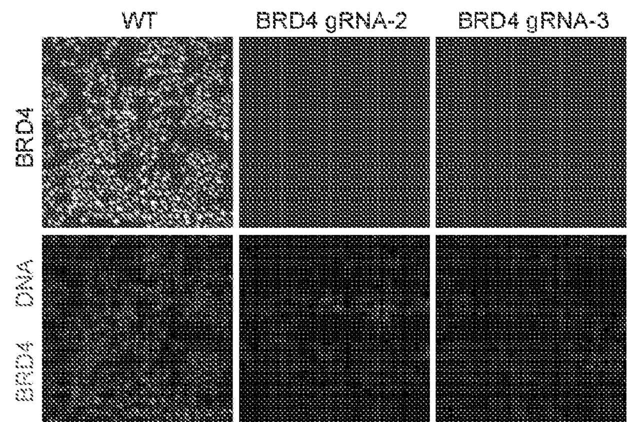


FIG. 2A

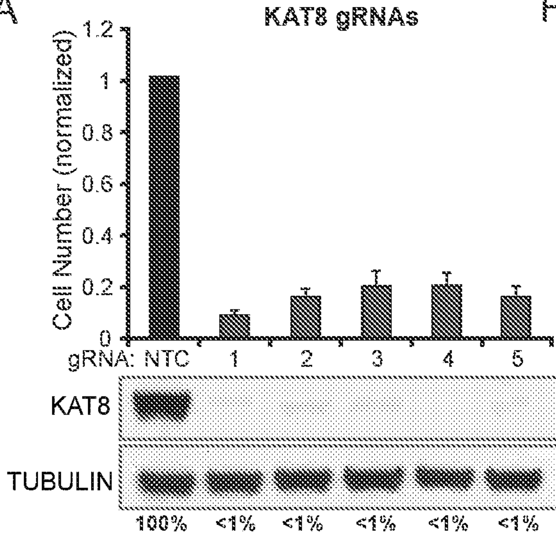


FIG. 2B

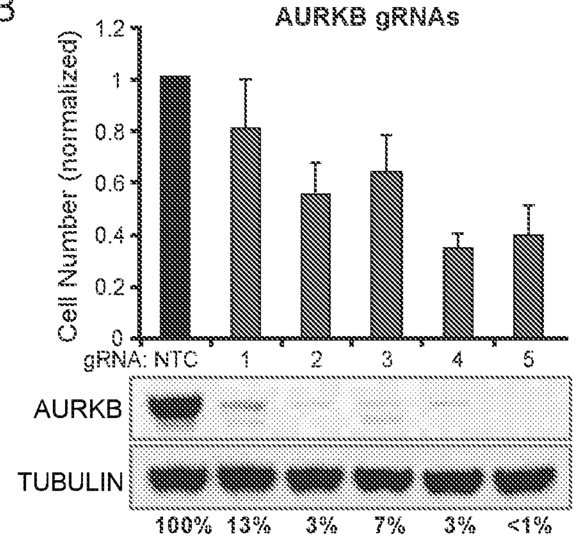


FIG. 2C

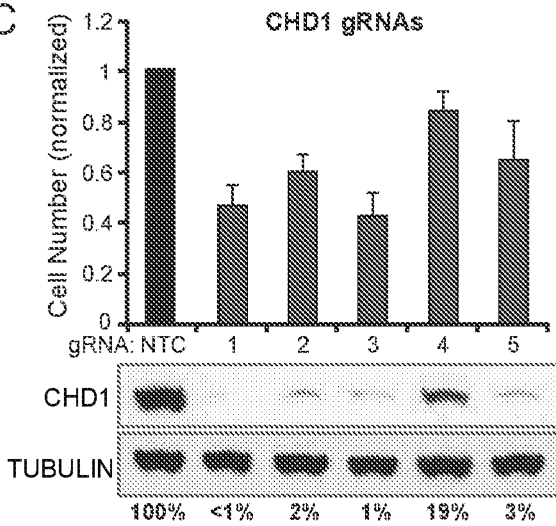


FIG. 2D

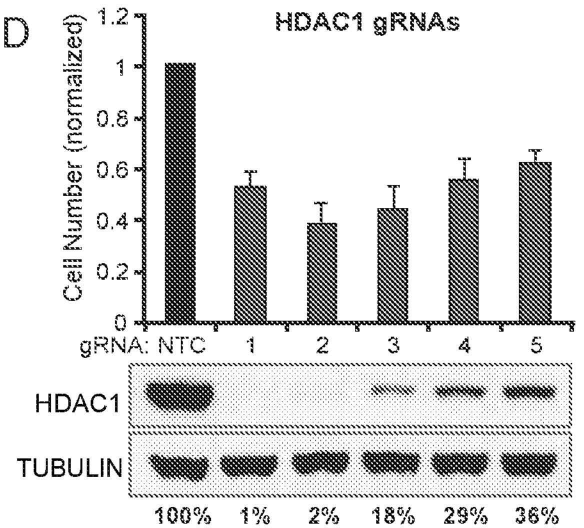


FIG. 2E

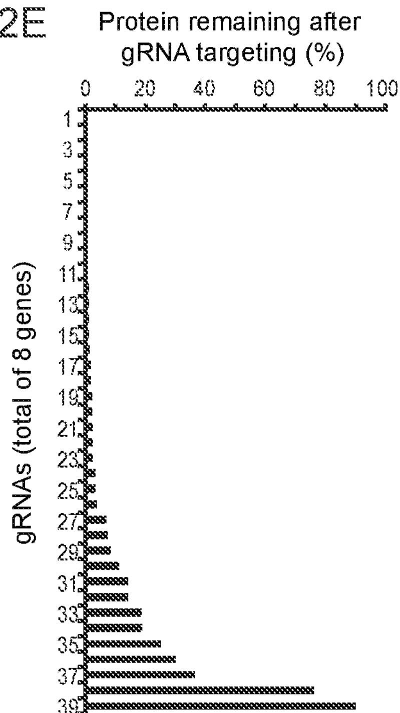


FIG. 3A

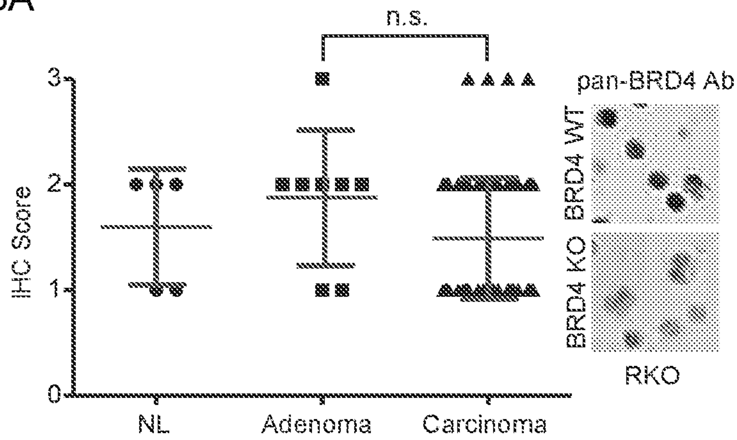


FIG. 3B

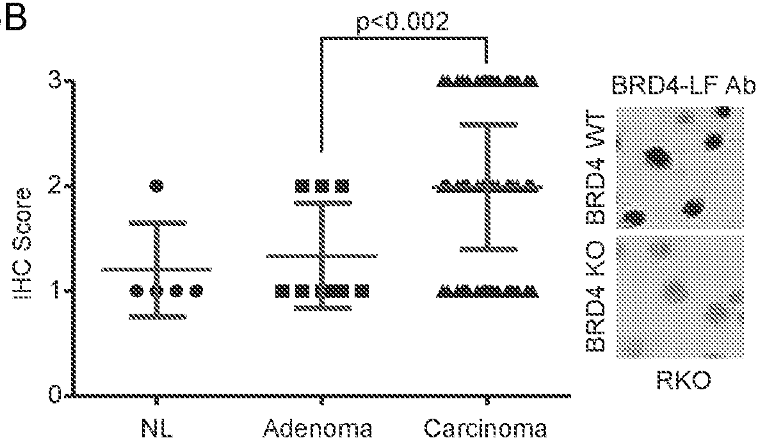


FIG. 3C

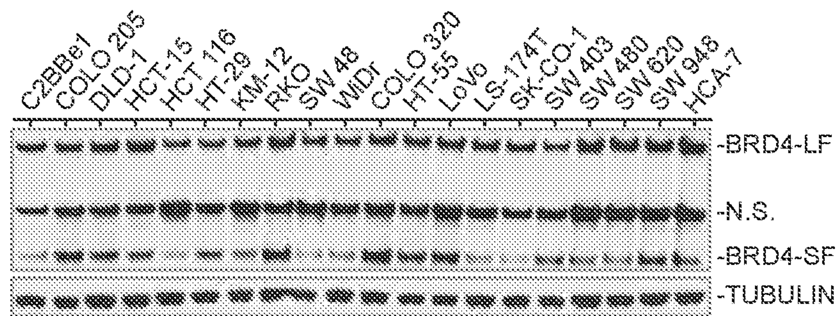


FIG. 3D

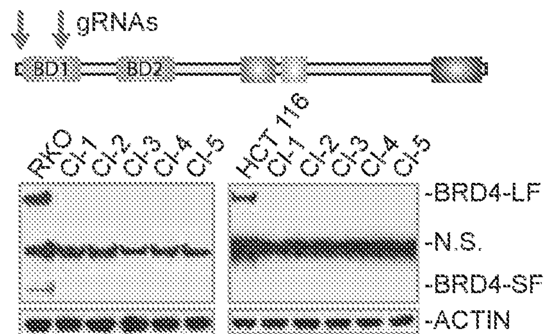


FIG. 3E

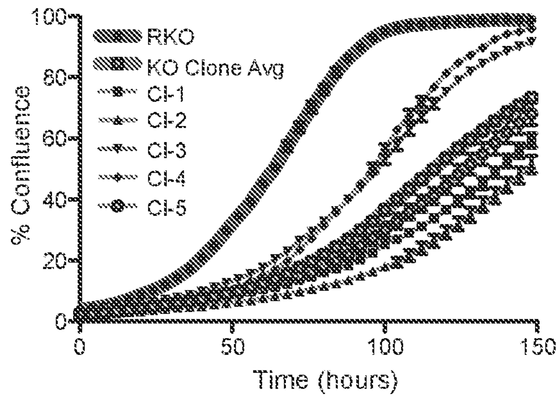


FIG. 3F

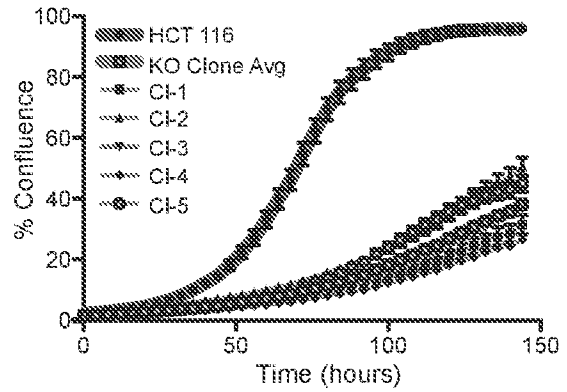


FIG. 3G

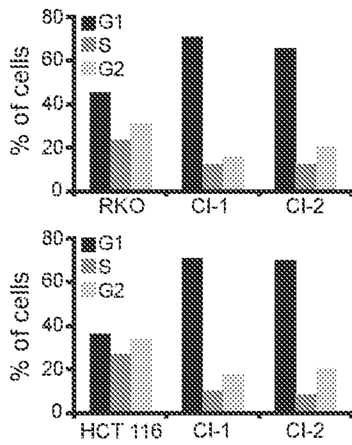


FIG. 3H

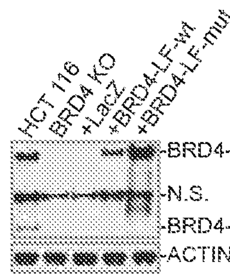


FIG. 3I

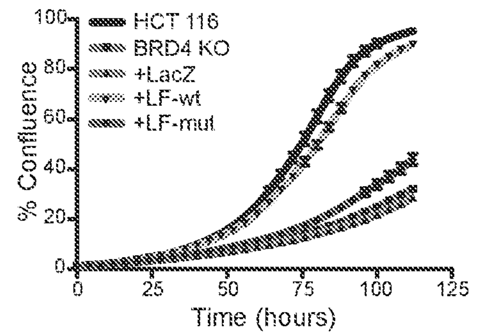


FIG. 3J

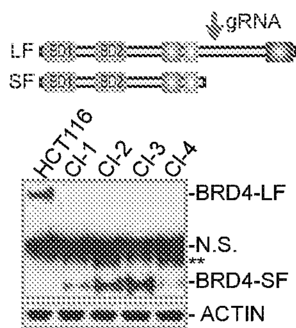


FIG. 3K

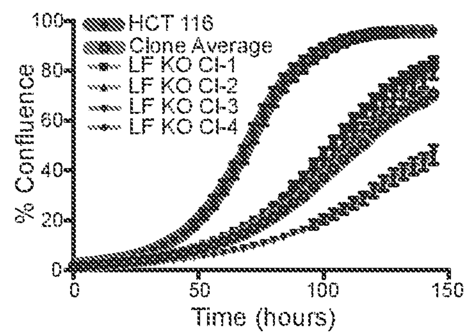
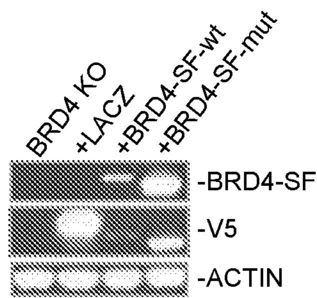


FIG. 4A



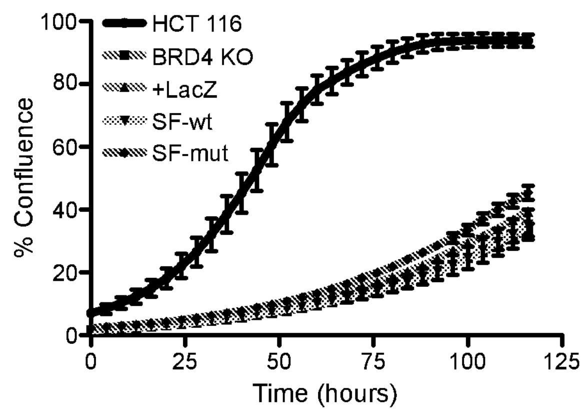
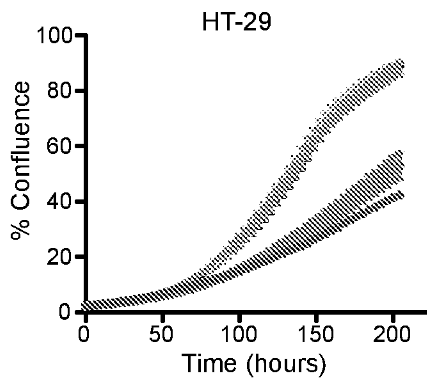


FIG. 4B



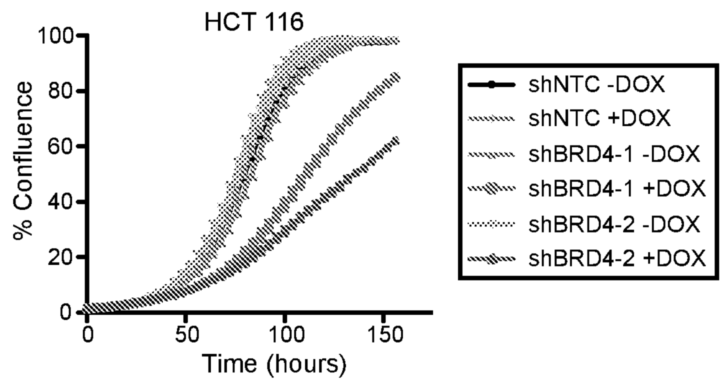


FIG. 5A

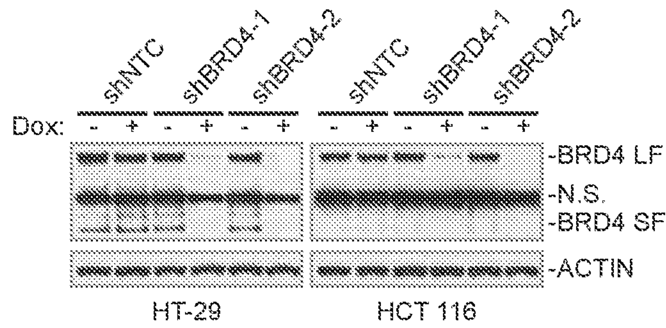


FIG. 5B

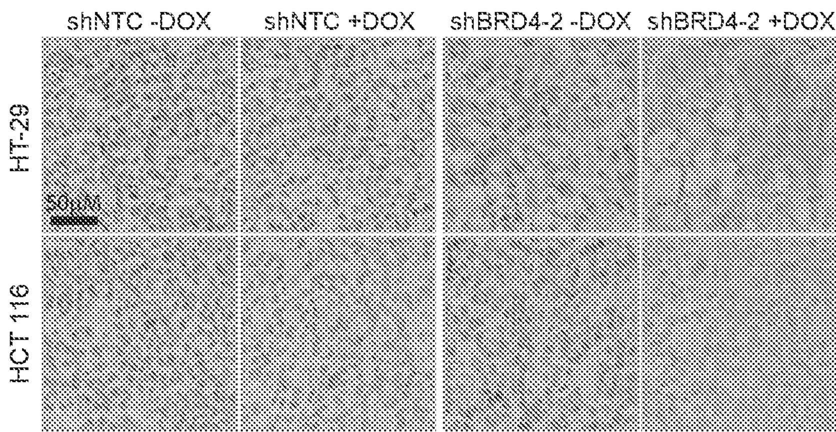


FIG. 5C

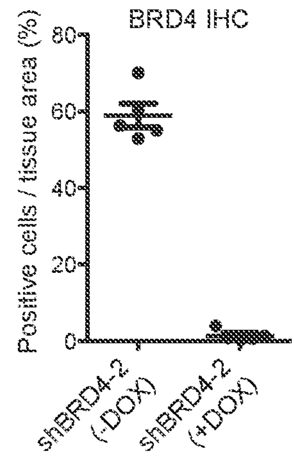


FIG. 5D

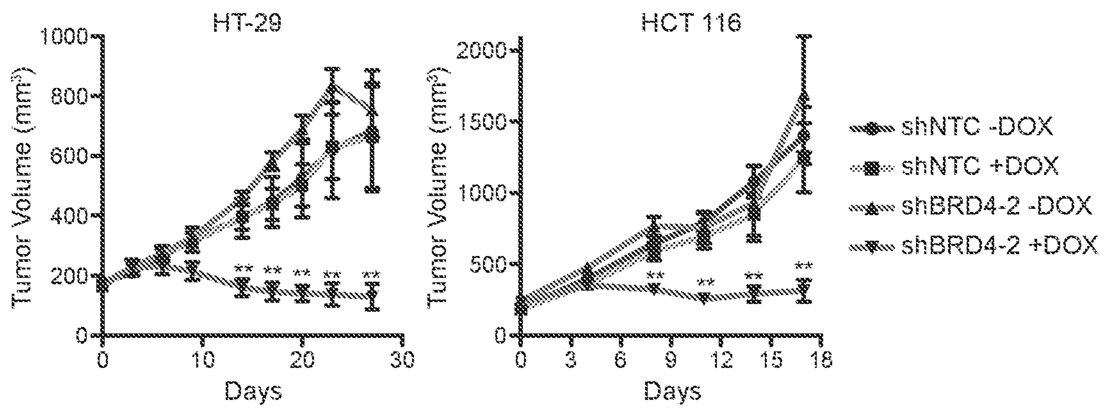


FIG. 5E

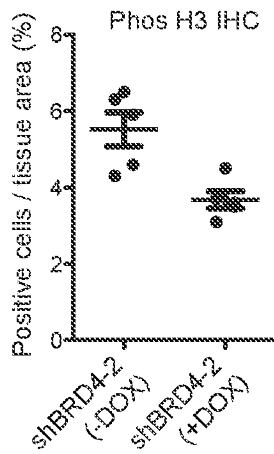


FIG. 5F

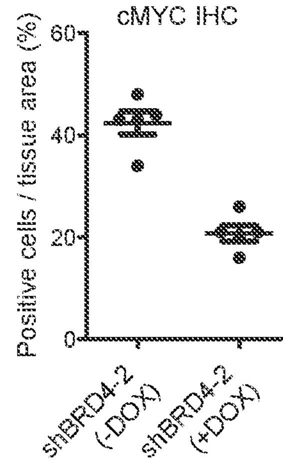


FIG. 5G

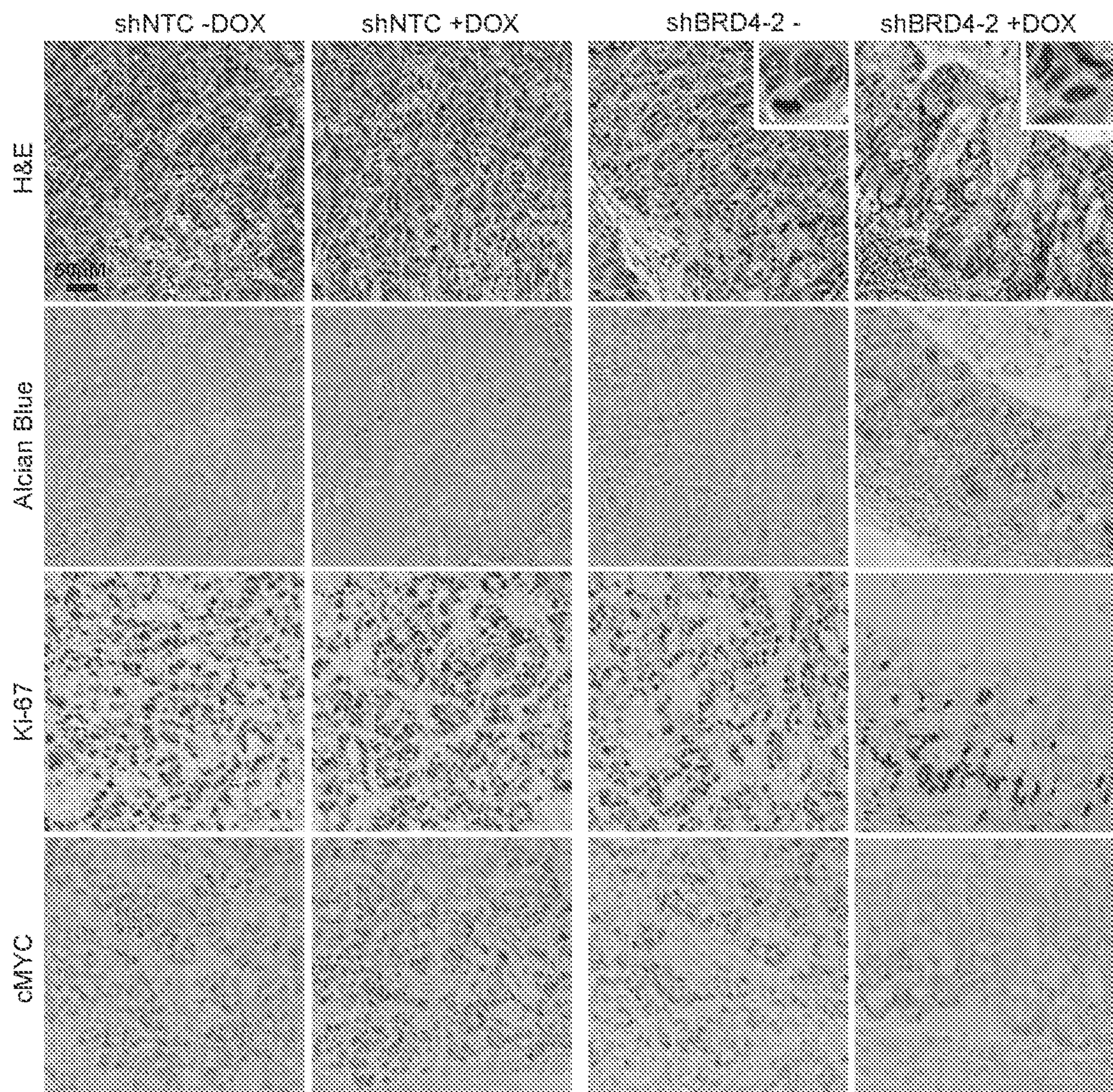


FIG. 6A

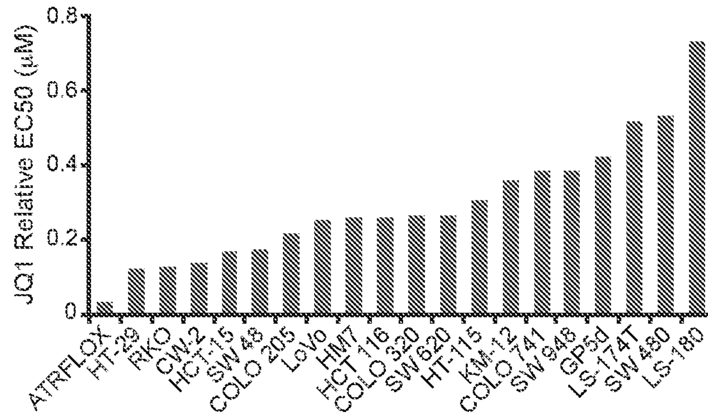


FIG. 6B

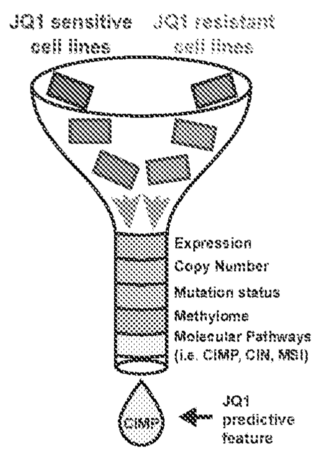


FIG. 6C

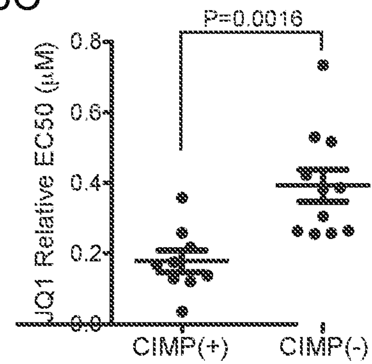


FIG. 6D

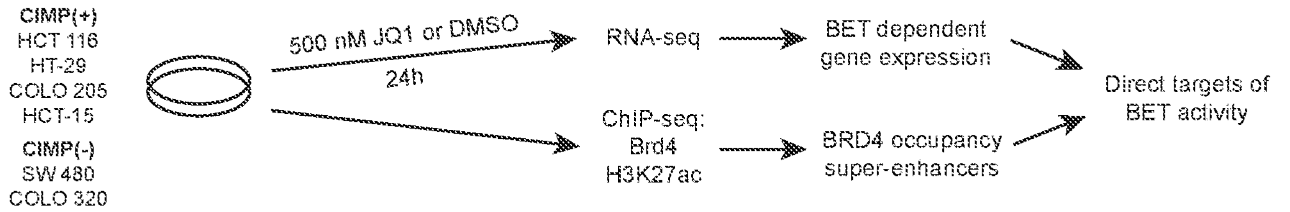


FIG. 6E

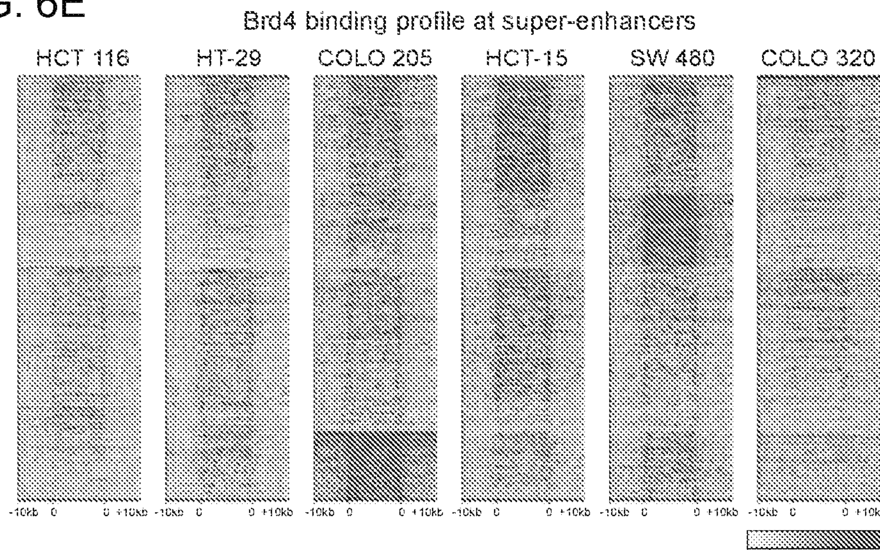


FIG. 6F

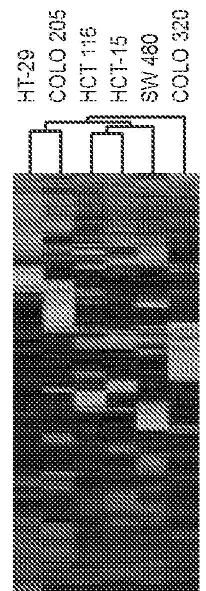


FIG. 6G

Hallmark cMYC Targets v2

Enrichment score (ES)

0.6
0.4
0.2
0.0

Enrichment profile
Hits

CIMP(+)
(positively correlated)

CIMP(-)
(negatively correlated)

CIMP(-)
CIMP(+)

FIG. 6H

HT-29 RKO
6h 24h 6h 24h
JQ1: - + - + - + - +
-cMYC
-ACTIN

COLO 320 SW 480
6h 24h 6h 24h
JQ1: - + - + - + - +
-cMYC
-ACTIN

FIG. 6I

P=0.0007

Relative cMYC protein levels

0.8
0.6
0.4
0.2
0.0

CIMP(+) CIMP(-)

FIG. 7A

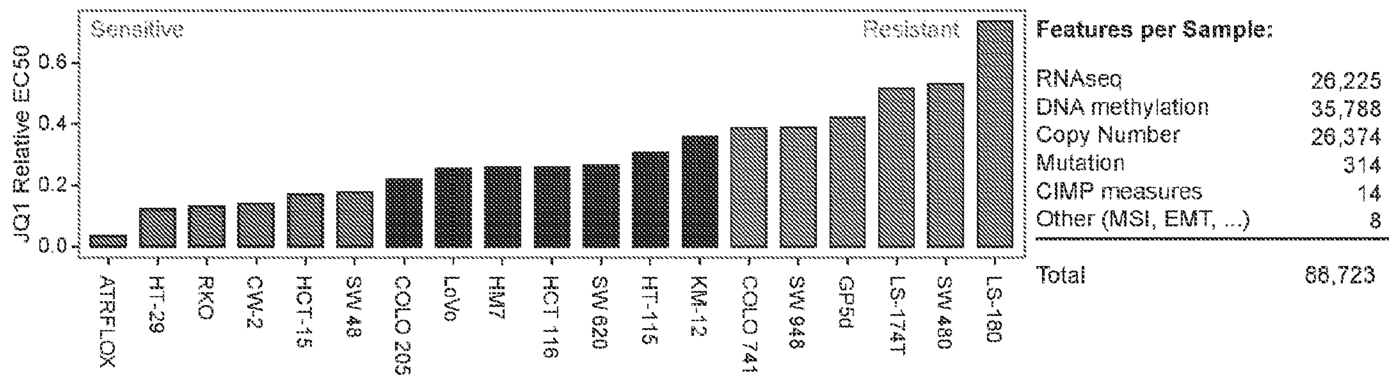


FIG. 7B

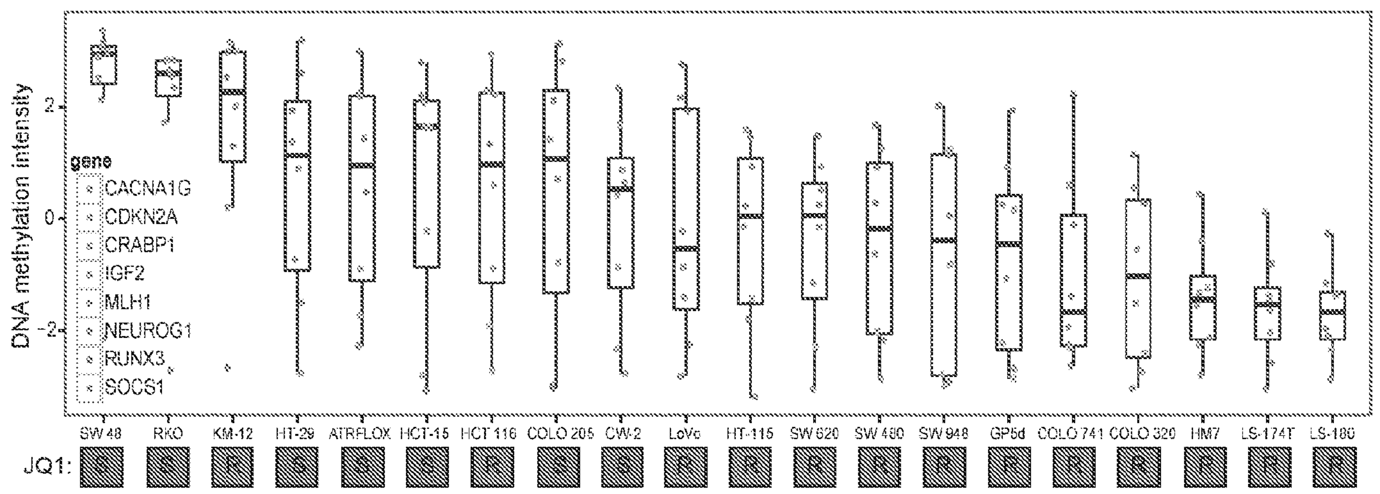


FIG. 7C

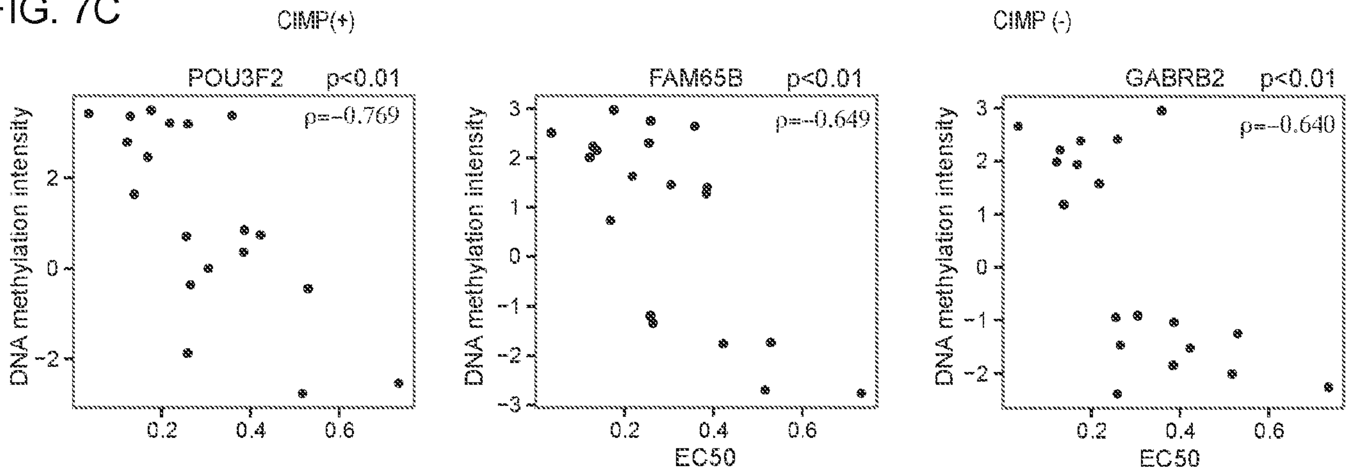


FIG. 7D

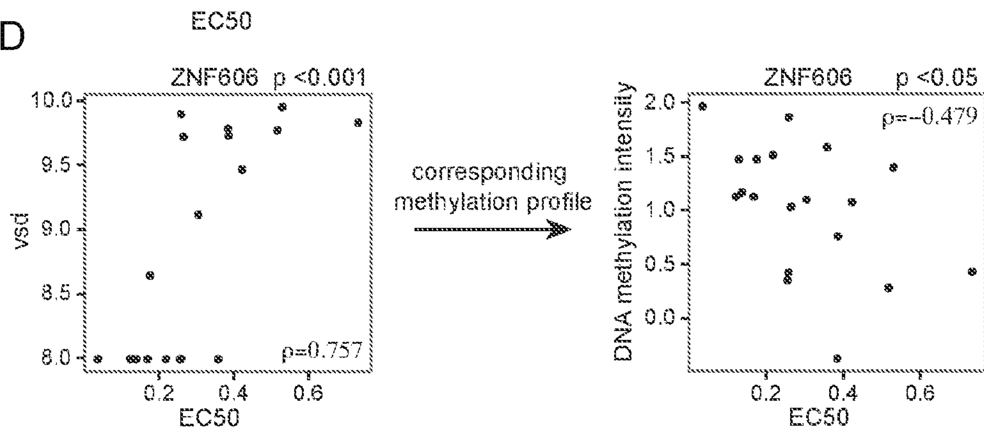


FIG. 8A

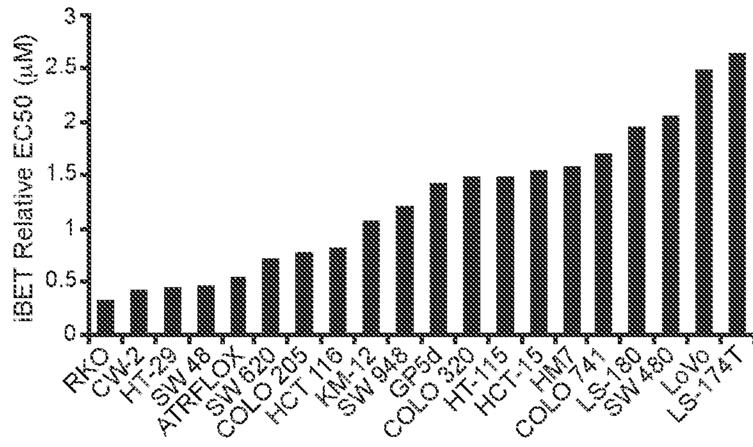


FIG. 8B

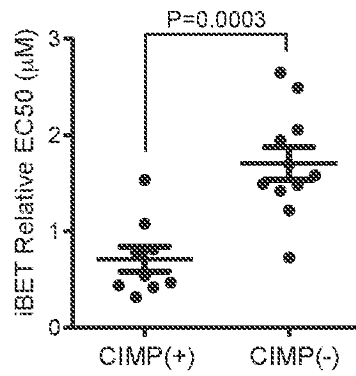


FIG. 8C

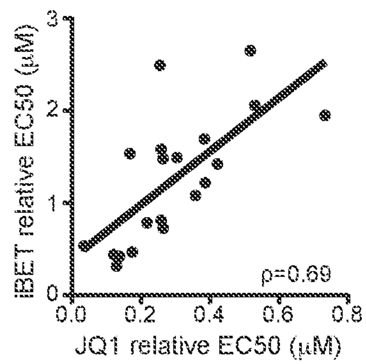
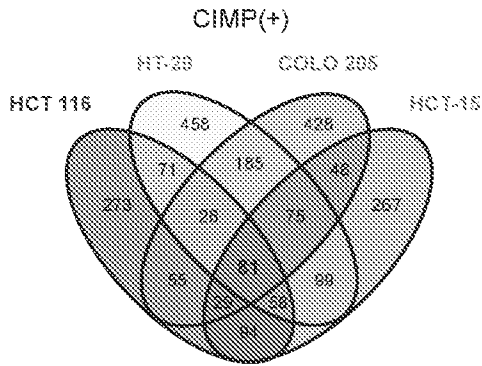


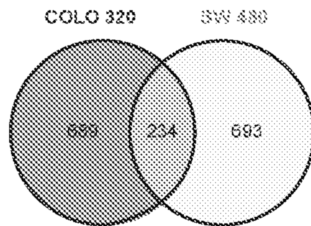
FIG. 9A

RNA-seq:
JQ1 down-regulated genes

CIMP(+)



CIMP(-)



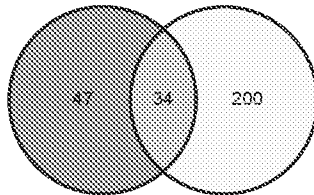
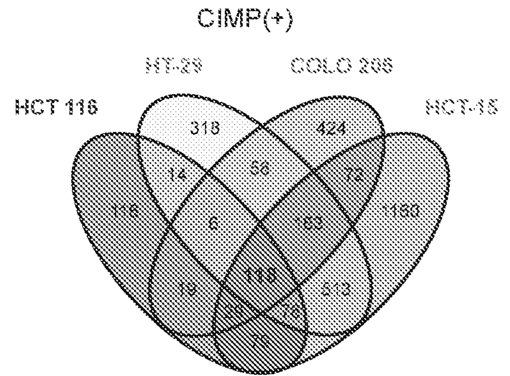


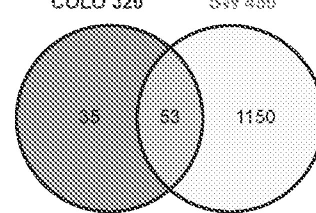
FIG. 9B

ChIP-seq:
Brd4 super-enhancer associated genes

CIMP(+)



CIMP(-)



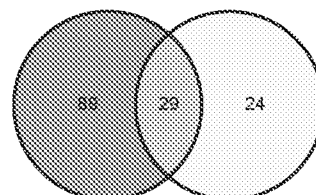


FIG. 9C

GSEA: CIMP(+) enriched terms

NAME	SIZE	ES	NES	FDR q-val
HALLMARK_MYC_TARGETS_V2	58	0.70	2.19	0.0000
HALLMARK_MYC_TARGETS_V1	200	0.57	2.15	0.0000
HALLMARK_MTORC1_SIGNALING	195	0.54	2.02	0.0000
HALLMARK_E2F_TARGETS	200	0.52	1.96	0.0000
HALLMARK_UNFOLDED_PROTEIN_RESPONSE	111	0.52	1.80	0.0009
HALLMARK_APICAL_SURFACE	39	0.56	1.65	0.0041
HALLMARK_G2M_CHECKPOINT	199	0.43	1.63	0.0043
HALLMARK_SPERMATOGENESIS	91	0.45	1.50	0.0173

GSEA: CIMP(-) enriched terms

NAME	SIZE	ES	NES	FDR q-val
HALLMARK_MYOGENESIS	155	-0.50	-1.71	0.0137
HALLMARK_COAGULATION	103	-0.51	-1.65	0.0187
HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION	142	-0.48	-1.62	0.0217

FIG. 10A

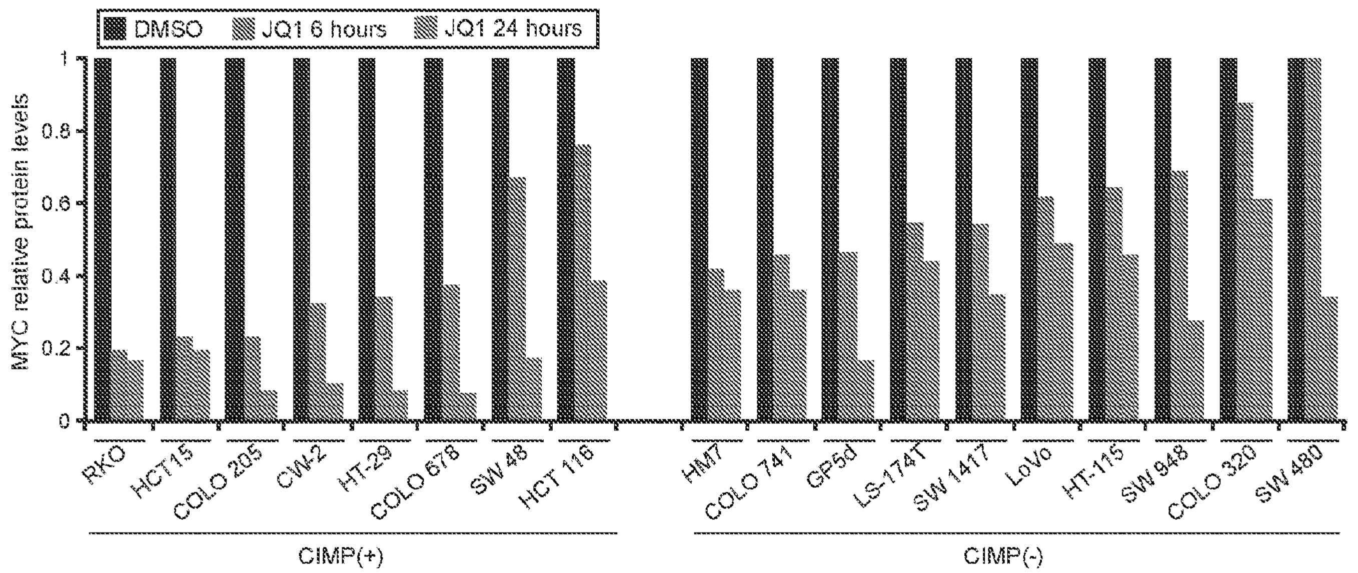


FIG. 10B

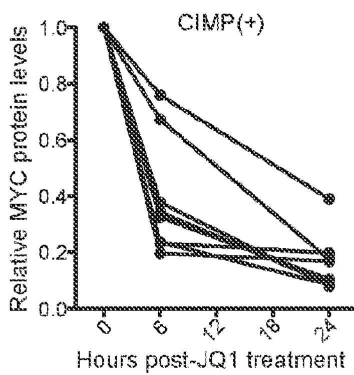


FIG. 10C

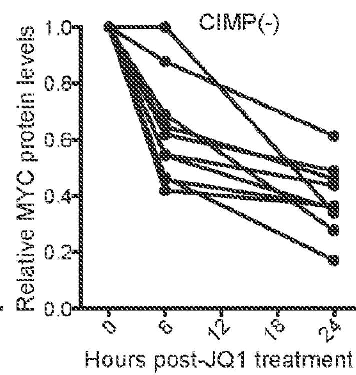


FIG. 10D

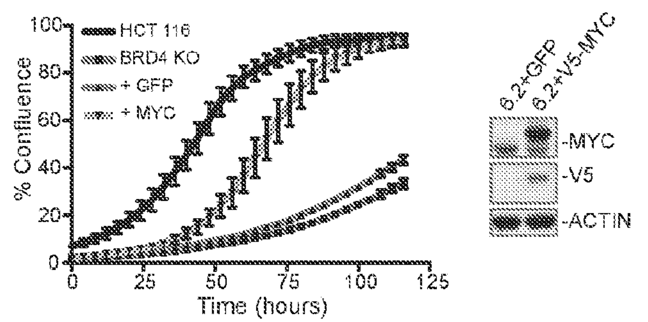
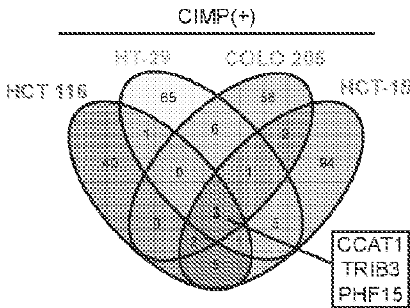


FIG. 11A



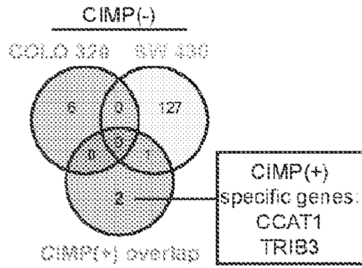


FIG. 11B

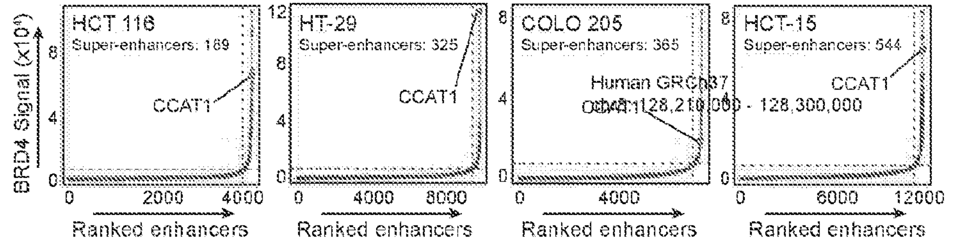


FIG. 11C

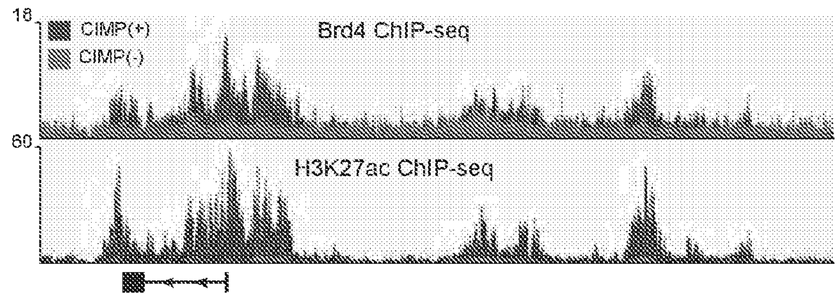


FIG. 11D

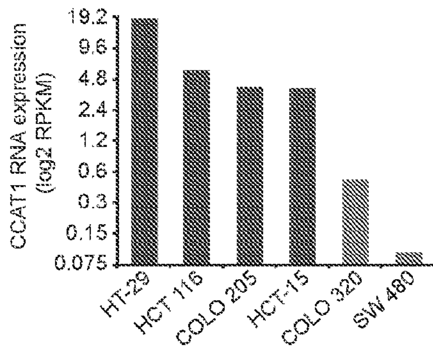


FIG. 11E

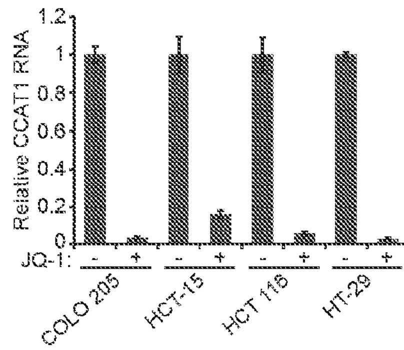


FIG. 11F

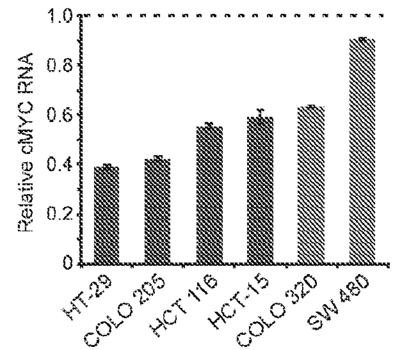


FIG. 12A

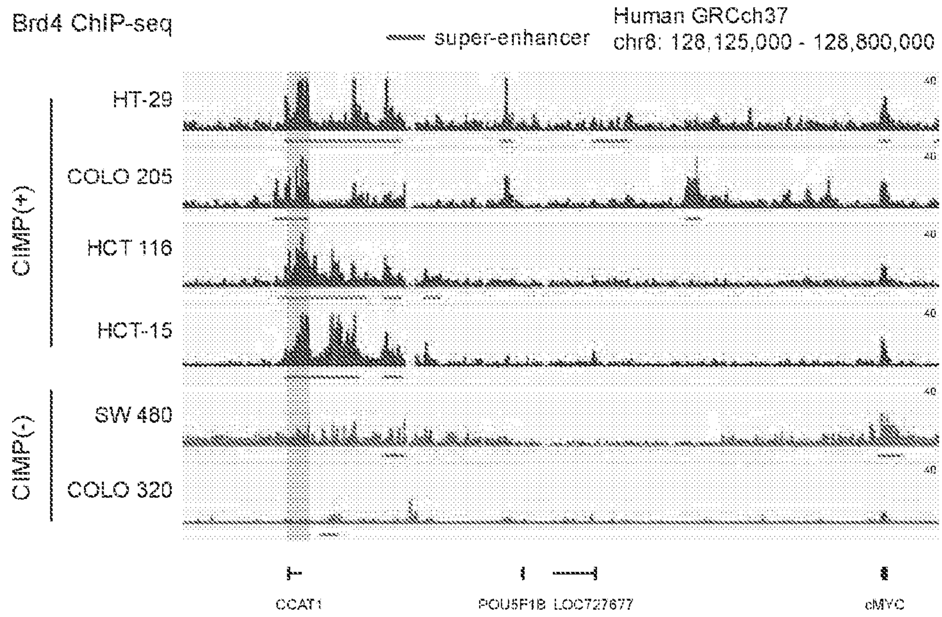


FIG. 12B

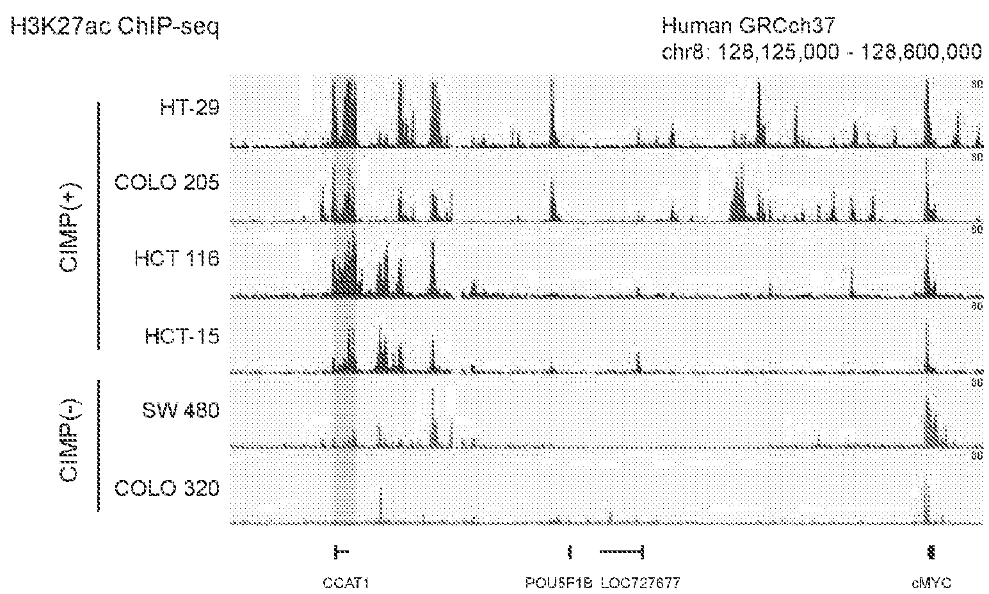


FIG. 12C

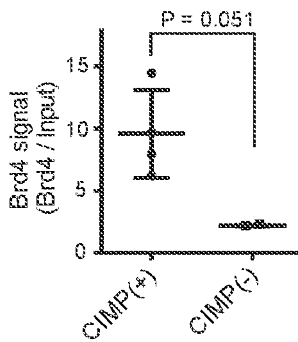


FIG. 12D

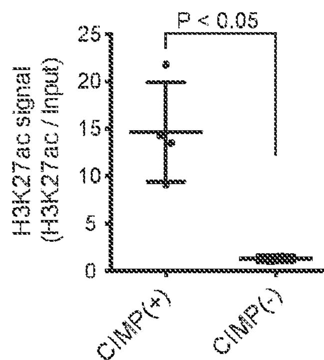


FIG. 12E

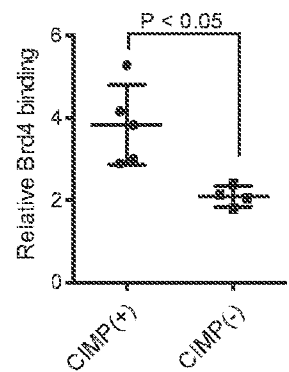


FIG. 13A

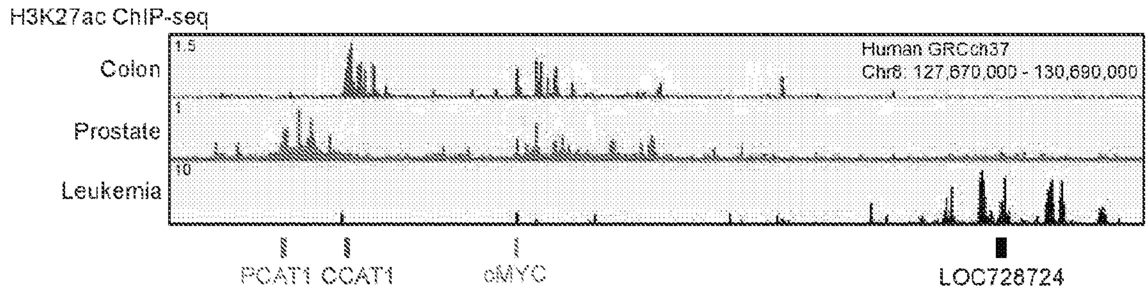


FIG. 13B

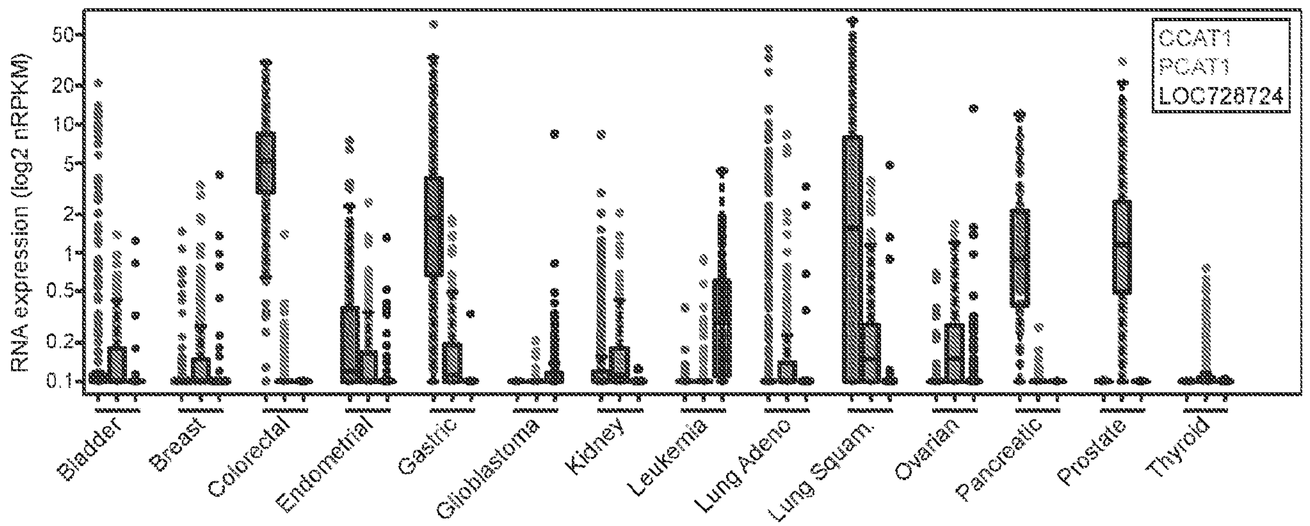


FIG. 13C

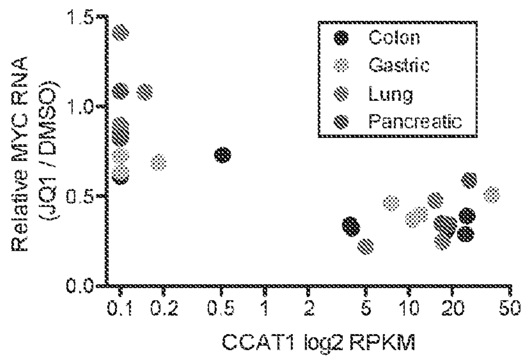


FIG. 13D

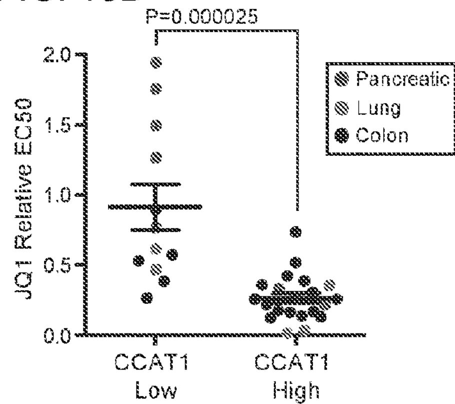


FIG. 14A

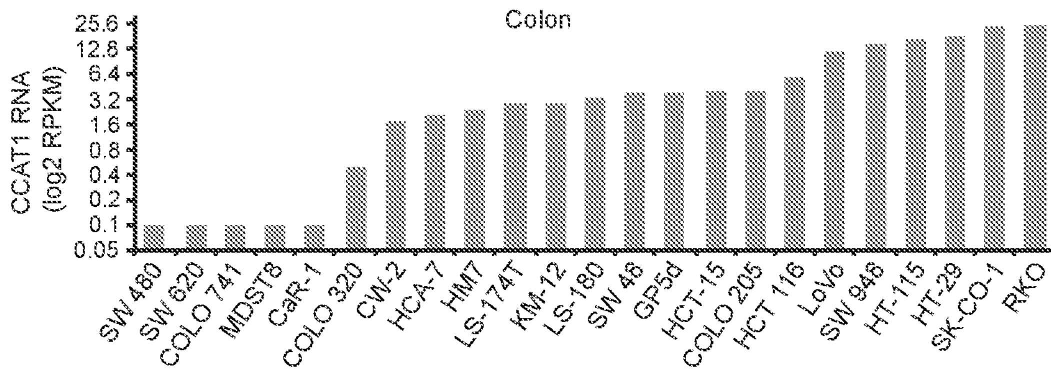


FIG. 14B

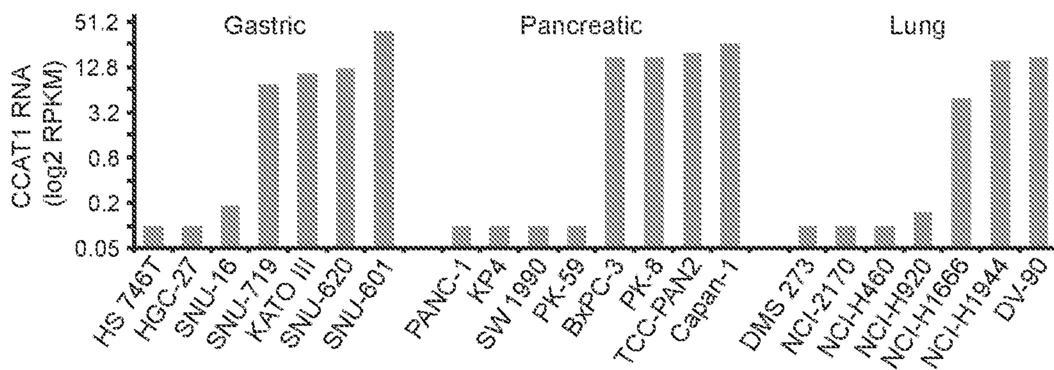


FIG. 14C

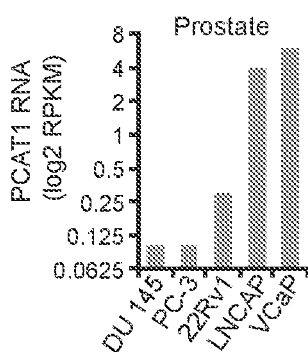


FIG. 14D

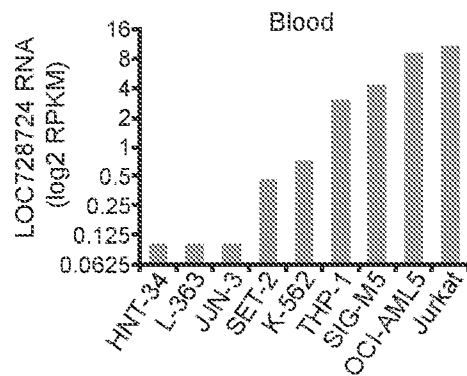


FIG. 14E

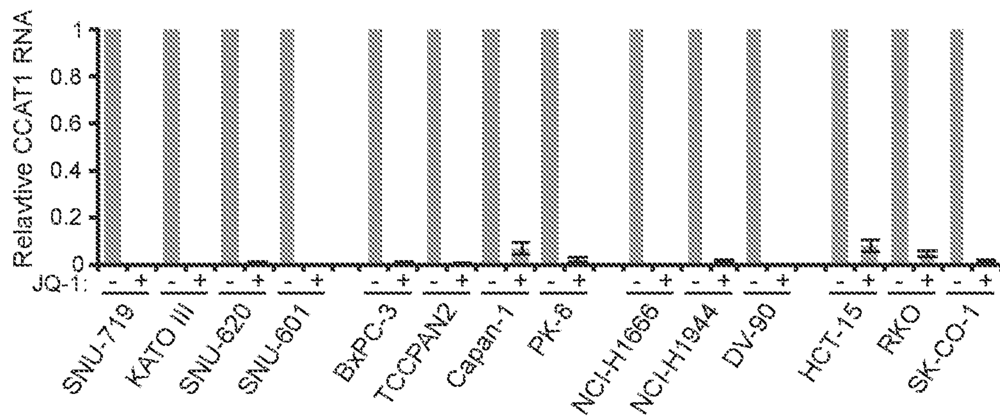


FIG. 14F

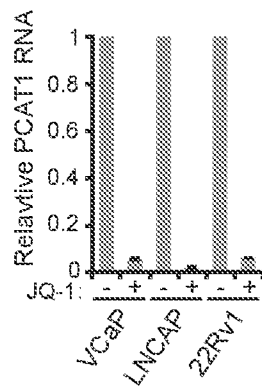


FIG. 14G

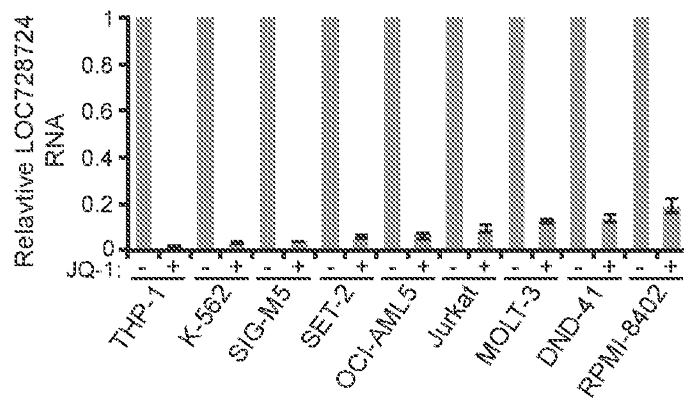


FIG. 15A

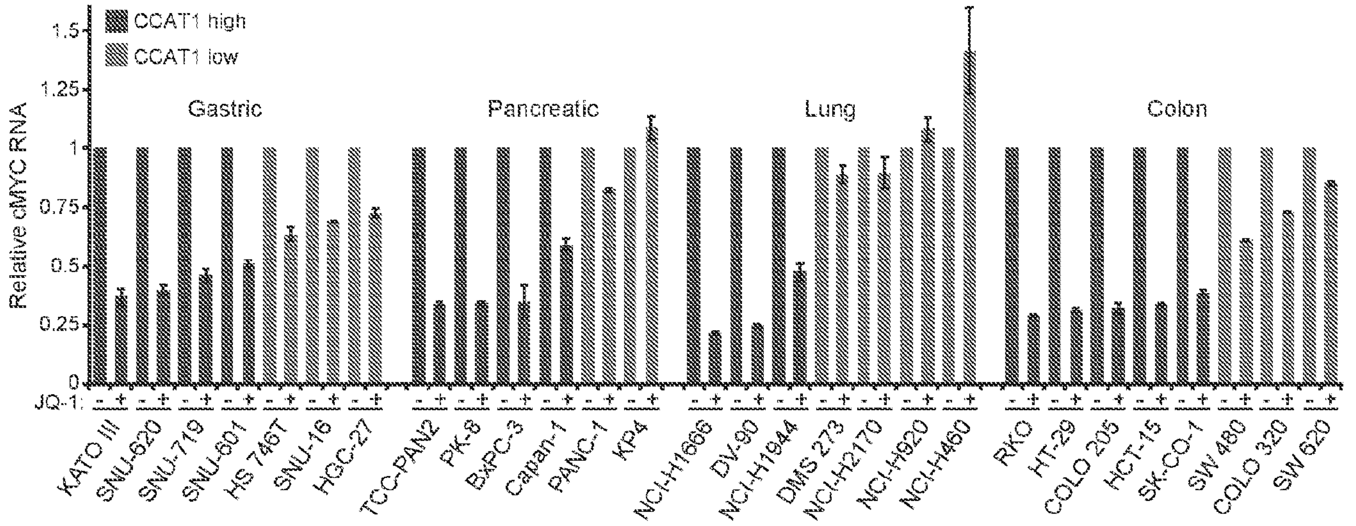


FIG. 15B

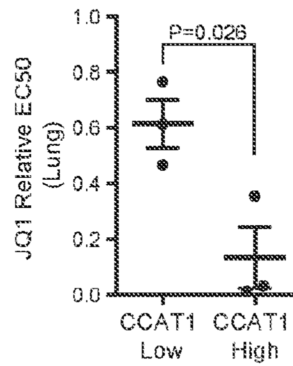


FIG. 15C

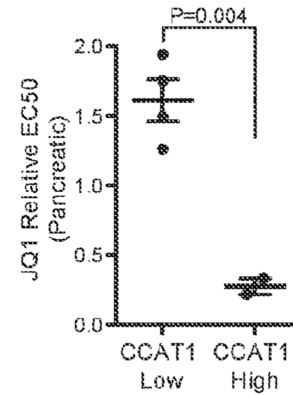


FIG. 15D

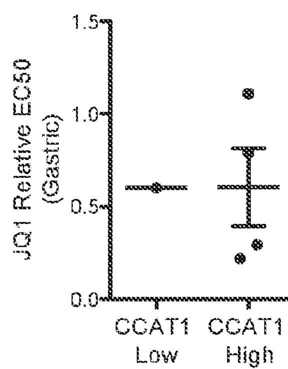


FIG. 15E

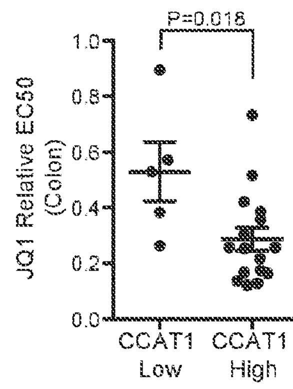


FIG. 15F

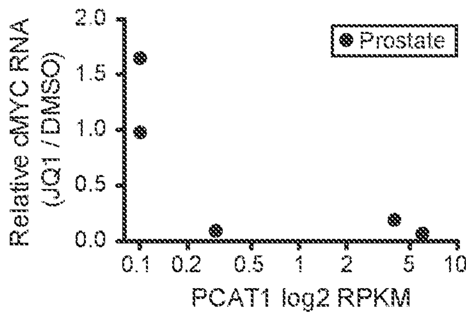


FIG. 15G

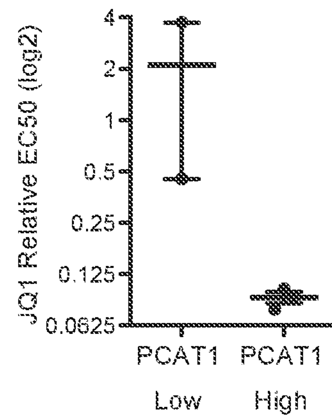


FIG. 15H

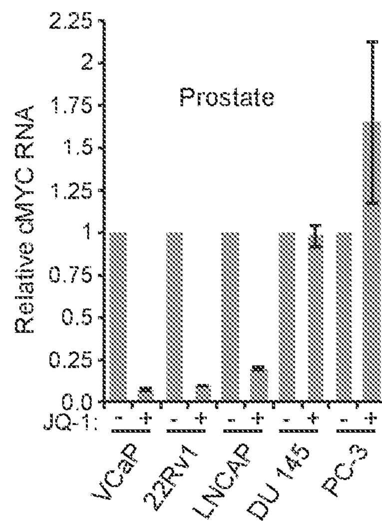


FIG. 15I

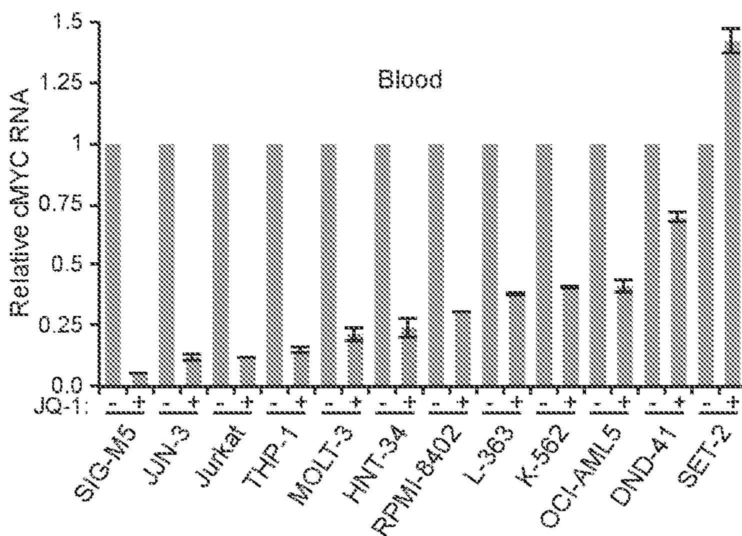


FIG. 15J

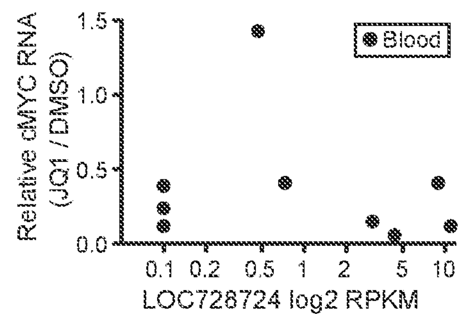
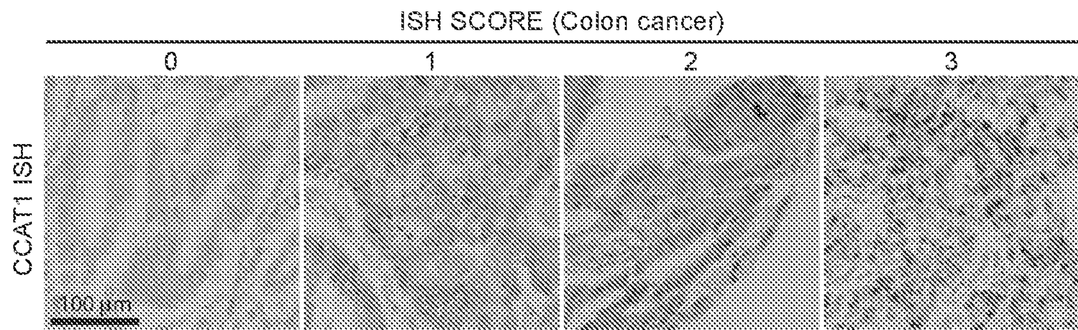


FIG. 16A



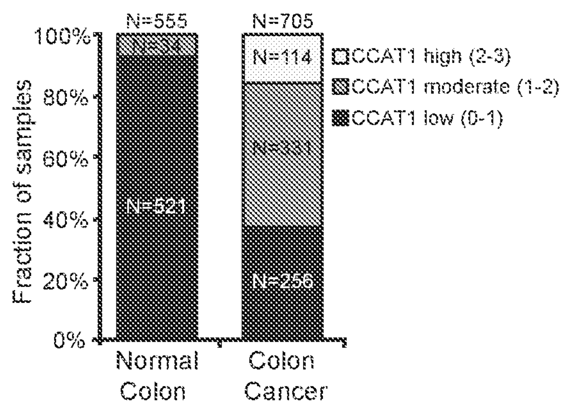


FIG. 16B

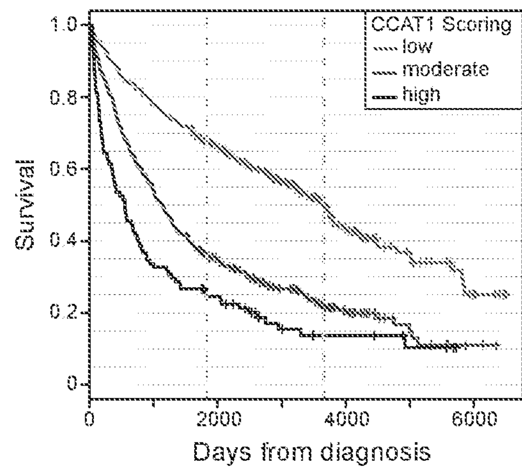


FIG. 16C

FIG. 17A

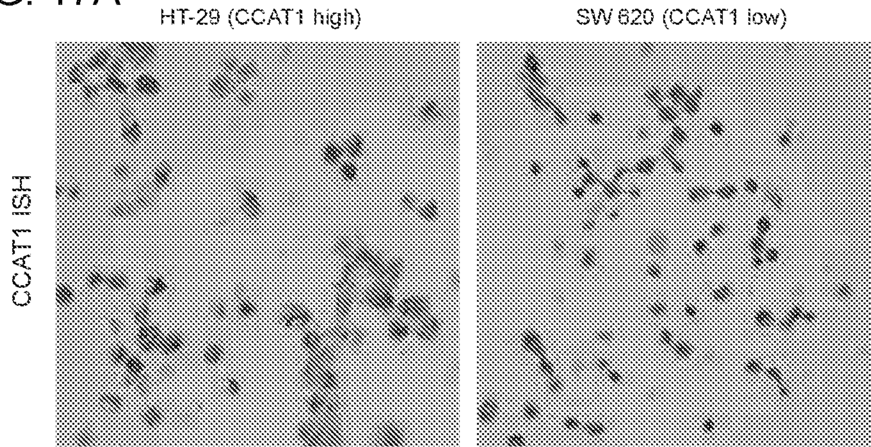


FIG. 17B

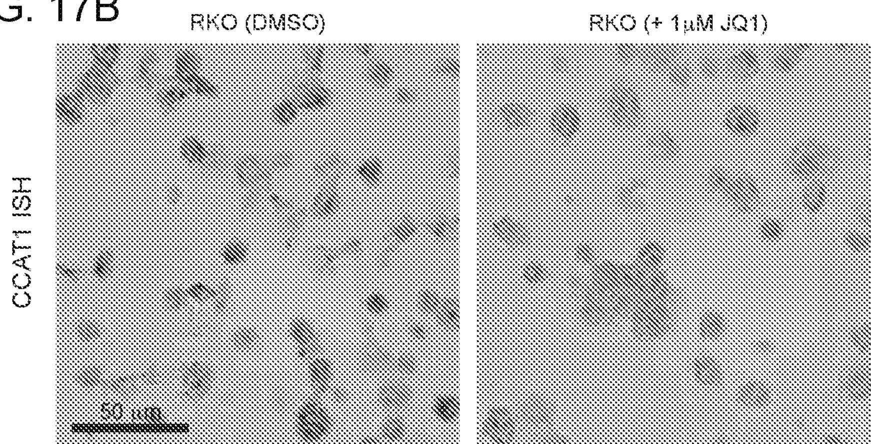


FIG. 17C

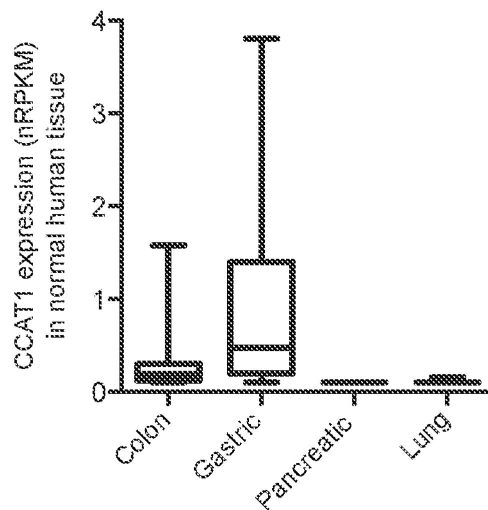
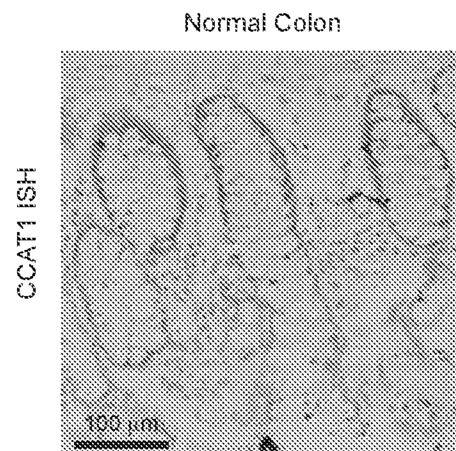


FIG. 17D



INTERNATIONAL SEARCH REPORT

International application No
PCT/US2016/033826

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12Q1/68
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12Q

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

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

EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DEEPANWITA SENGUPTA ET AL: "Disruption of BRD4 at H3K27Ac-enriched enhancer region correlates with decreased c-Myc expression in Merkel cell carcinoma", EPIGENETICS, vol. 10, no. 6, 5 May 2015 (2015-05-05), pages 460-466, XP055297413, US ISSN: 1559-2294, DOI: 10.1080/15592294.2015.1034416 the whole document -----	1-36

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
25 August 2016	14/09/2016

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Botz, Jürgen

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Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE BIOSIS [Online] BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US; November 2013 (2013-11), CHAPUY BJOERN ET AL: "Disruption Of Super Enhancer-Driven Cancer Dependencies In Diffuse Large B-Cell Lymphoma", XP008181342, Database accession no. PREV201400362003 the whole document & BLOOD, vol. 122, no. 21, November 2013 (2013-11), page 3021, 55TH ANNUAL MEETING OF THE AMERICAN-SOCIETY-OF-HEMATOLOGY; NEW ORLEANS, LA, USA; DECEMBER 07 -10, 2013 ISSN: 0006-4971(print) -----	1-36
X	LOVÉN JAKOB ET AL: "Selective Inhibition of Tumor Oncogenes by Disruption of Super-Enhancers", CELL, CELL PRESS, US, vol. 153, no. 2, 11 April 2013 (2013-04-11), pages 320-334, XP028547942, ISSN: 0092-8674, DOI: 10.1016/J.CELL.2013.03.036 the whole document -----	1-36
X	WO 2013/192274 A2 (BROAD INST INC [US]; BRIGHAM & WOMENS HOSPITAL [US]; DANA FARBER CANCE) 27 December 2013 (2013-12-27) the whole document -----	1-36
X	WO 2013/033420 A1 (WHITEHEAD BIOMEDICAL INST [US]; YOUNG RICHARD A [US]; RAHL PETER B [US) 7 March 2013 (2013-03-07) the whole document -----	1-36
X	John A Valenta, et al.: "Combined Therapy With BRD4 Antagonist and JAK Inhibitor Is Synergistically Lethal Against Human Myeloproliferative Neoplasm (MPN) Cells", Blood , 1 November 2013 (2013-11-01), XP008181343, 55th Annual Meeting of the American-Society-of-Hematology, American Society of Hematology, US; New Orleans, LA, USA Retrieved from the Internet: URL:http://www.bloodjournal.org/content/122/21/2842?sso-checked=true [retrieved on 2016-08-25] the whole document -----	1-36

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2016/033826

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

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

1. [X] Claims Nos.: 1, 2, 4, 10-12, 14, 32(all partially)
because they relate to subject matter not required to be searched by this Authority, namely:

see FURTHER INFORMATION sheet PCT/ISA/210

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

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

[ ] The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box II.1

Claims Nos.: 1, 2, 4, 10-12, 14, 32(all partially)

Rule 39 .1 (iv) / 67 .1 (iv) PCT
Claims 1 , 2 , 4 , 10 , 11 , 12 , 14 and 32 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 39.1(iv) / 67.1(iv) PCT. Claims 1 , 2 , 4 , 10 , 11 , 12 , 14 and 32 constitute methods of treatment. This is subject-matter considered by this authority to be covered by the provisions of Rule 39 .1 (iv) / 67 .1 (iv) PCT . The patentability can be dependent on the formulations of the claims. The EPO for example, does not recognize as patentable claims to methods for the treatment of the human or animal body by therapy , but may allow claims to a product, in particular substances or compositions for use in a first or further medical treatment .

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2016/033826

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2013192274 A2	27-12-2013	EP 2861255 A2	22-04-2015
		US 2015174138 A1	25-06-2015
		WO 2013192274 A2	27-12-2013
WO 2013033420 A1	07-03-2013	US 2014371157 A1	18-12-2014
		WO 2013033420 A1	07-03-2013