Innovation, Sciences et Développement économique Canada
Office de la Propriété Intellectuelle du Canada
Innovation, Science and Economic Development Canada
Canadian Intellectual Property Office
CA 3109605 A1 2020/02/20
(21) 3 109 605
(12) DEMANDE DE BREVET CANADIEN
CANADIAN PATENT APPLICATION
(13) A1

(86) Date de dépôt PCT/PCT Filing Date: 2019/08/12
(87) Date publication PCT/PCT Publication Date: 2020/02/20
(85) Entrée phase nationale/National Entry: 2021/02/12
(86) N° demande PCT/PCT Application No.: US 2019/046124
(87) N° publication PCT/PCT Publication No.: 2020/036852
(30) Priorités/Priorities: 2018/08/13 (CN PCT/CN2018/100206); 2018/11/16 (CN PCT/CN2018/115826)
(51) Cl.Int./Int.Cl. *A61K 31/02* (2006.01), *A61K 31/03* (2006.01), *A61K 31/755* (2006.01), *A61P 35/00* (2006.01), *C12Q 1/68* (2018.01), *G16B 20/10* (2019.01)
(71) Demandeur/Applicant:
BEIJING PERCANS ONCOLOGY CO. LTD., CN
(72) Inventeurs/Inventors:
LI, XIANG, CN;
CHEN, YIYOU, US
(74) Agent: BORDEN LADNER GERVAIS LLP

(54) Titre : BIOMARQUEURS POUR LA CANCEROTHERAPIE
(54) Title: BIOMARKERS FOR CANCER THERAPY

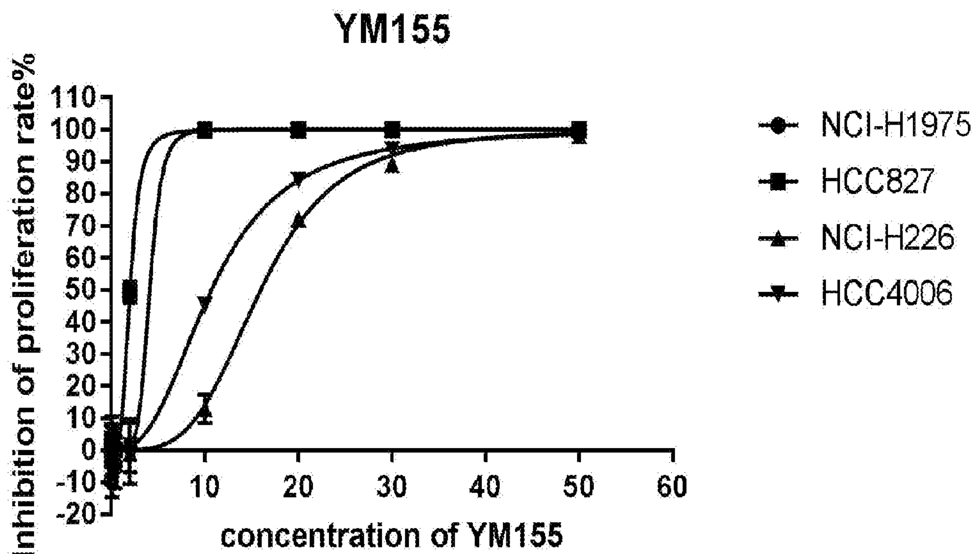



FIG. 2

(57) Abrégé/Abstract:
Provided are methods of using a MYC gene as a biomarker for predicting therapeutic efficacy of survivin inhibitors such as YM155 monobromide in cancer therapy, and related kits, compositions, and methods for diagnosing and treating cancer in a subject in need thereof.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(10) International Publication Number
WO 2020/036852 A1

(43) International Publication Date
20 February 2020 (20.02.2020)

(51) International Patent Classification:
*A61K 31/02* (2006.01) *A61P 35/00* (2006.01)
*A61K 31/03* (2006.01) *C12Q 1/68* (2018.01)
*A61K 31/755* (2006.01) *G16B 20/10* (2019.01)

(21) International Application Number:
PCT/US2019/046124

(22) International Filing Date:
12 August 2019 (12.08.2019)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
PCT/CN2018/100206
13 August 2018 (13.08.2018) CN
PCT/CN2018/115826
16 November 2018 (16.11.2018) CN

(71) Applicant: BEIJING PERCANS ONCOLOGY CO. LTD. [CN/CN]; 5th floor Building 11, Peking University Medical Industry Park, ZGC Life Science Park, Beiqing Road, Beijing, Changping 100000 (CN).

(72) Inventors: LI, Xiang; No.3 Yuanmingyuan West Road, Haidian District, Beijing, Changping 100193 (CN). CHEN, Yiyou; 6802 Graystone Lane, San Jose, California 95120 (US).

(74) Agent: ROGEL, Mark E. et al.; Cooley LLP, 1299 Pennsylvania Avenue, NW, Suite 700, Washington, District of Columbia 20004 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM,

(54) **Title:** BIOMARKERS FOR CANCER THERAPY

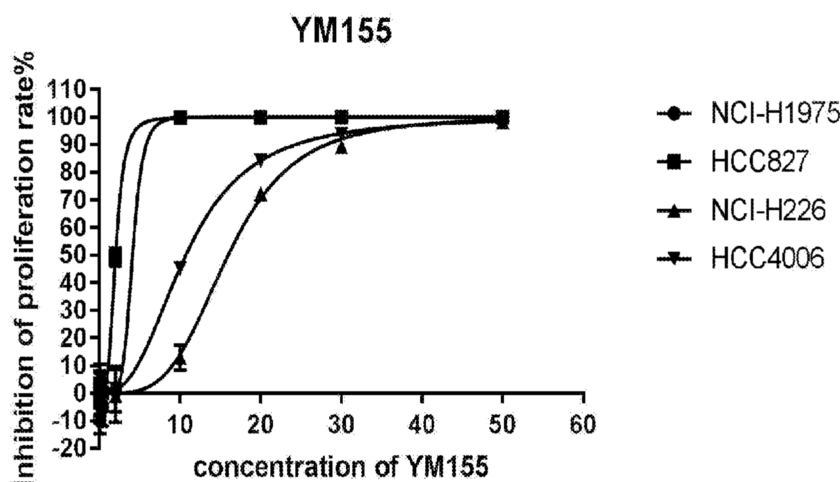



**FIG. 2**

(57) **Abstract:** Provided are methods of using a MYC gene as a biomarker for predicting therapeutic efficacy of survivin inhibitors such as YM155 monobromide in cancer therapy, and related kits, compositions, and methods for diagnosing and treating cancer in a subject in need thereof.

*[Continued on next page]*

WO 2020/036852 A1

TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

**Declarations under Rule 4.17:**

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

**Published:**

— *with international search report (Art. 21(3))*

# Biomarkers for Cancer Therapy

## CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to PCT/CN2018/100206, filed August 13, 2018; and PCT/CN2018/115826, filed November 16, 2018, each of which is incorporated by reference in its entirety.

## BACKGROUND

### *Technical Field*

Embodiments of the present disclosure relate to the use of a MYC gene as a biomarker for predicting therapeutic efficacy of survivin inhibitors such as YM155 monobromide in cancer therapy, and related kits, compositions, and methods for diagnosing and treating cancer in a subject in need thereof.

### *Description of the Related Art*

YM155 monobromide is a small-molecule survivin inhibitor that induces the down-regulation of survivin and exhibits potent antitumor activity (see, e.g., Minematsu et al., Drug Metabolism and Disposition, 37:619-628, 2008). YM-155 exerts anti-tumor effects in various *in vivo* cancer models, including prostate, pancreatic, and lung cancer (see, e.g., Nakahara et al., Cancer Research 67:8014-8021, 2007; and Na et al., PLoS One 7(6), 2012).

However, there is a need in the art to better predict the anti-cancer therapeutic efficacy of YM155 monobromide, and thereby identify patients that will benefit most from treatment with this chemotherapeutic, and others.

## BRIEF SUMMARY

Embodiments of the present disclosure include methods for treating cancer in a subject in need thereof, comprising:

(a) determining MYC gene copy number, or MYC gene chromosomal location site, in a sample of cancer tissue from the subject; and

(b) administering YM155 monobromide [1-(2-Methoxyethyl)-2-methyl-4,9-dioxo-3-(pyrazin-2-ylmethyl)-4,9-dihydro-1H-naphtho[2,3-d] imidazolium bromide], or an analog or derivative thereof, to the subject if MYC gene copy number in the cancer tissue is increased relative to that of a MYC gene copy number reference, or if MYC gene chromosomal location site in the cancer tissue is translocated relative to that of a MYC gene chromosomal location site reference,

thereby treating cancer in the subject in need thereof.

Some embodiments include administering to the subject a chemotherapeutic agent excluding (or other than) YM155 monobromide if MYC gene copy number in the cancer tissue is not

substantially increased relative to that of the MYC gene copy number reference, or if MYC gene chromosomal location site in the cancer tissue is not translocated relative to that of the MYC gene chromosomal location site reference.

Also included are methods for predicting therapeutic response to YM155 monobromide [1-(2-Methoxyethyl)-2-methyl-4,9-dioxo-3-(pyrazin-2-ylmethyl)-4,9-dihydro-1H-naphtho[2,3-d] imidazolium bromide], or an analog or derivative thereof, in a subject with cancer, comprising

(a) determining MYC gene copy number, or MYC gene chromosomal location site, in a sample of cancer tissue from the subject; and

(b) (i) characterizing the subject as responsive to YM155 monobromide therapy if MYC gene copy number in the cancer tissue is increased relative to that of a MYC gene copy number reference, or if the MYC gene chromosomal location site in the cancer tissue is translocated relative to that of a MYC gene chromosomal location site reference; or

(ii) characterizing the subject as non-responsive to YM155 monobromide therapy if MYC gene copy number in the cancer tissue is not substantially increased relative to that of the MYC gene copy number reference, or if the MYC gene chromosomal location site in the cancer tissue is not translocated relative to that of the MYC gene chromosomal location site reference,

thereby predicting therapeutic response to YM155 monobromide in the subject with cancer.

Some embodiments include administering YM155 monobromide to the subject if the subject is characterized as responsive to YM155 monobromide therapy. Some embodiments include administering to the subject a chemotherapeutic agent excluding YM155 monobromide if the subject is characterized as non-responsive to YM155 monobromide therapy.

In some embodiments, the MYC gene copy number in the cancer tissue is increased by about or at least about 1.5, 2, 3, 4, 5, 6, 7, 8, 9, or 10-fold relative to that of the MYC gene copy number reference.

Some embodiments comprise determining MYC gene copy number in the cancer tissue by array comparative genome hybridization (aCGH), single nucleotide polymorphism (SNP) array, copy number variation (CNV) sequencing, or multiplex ligation-dependent probe amplification (MLPA). Some embodiments comprise determining MYC gene chromosomal location site in the cancer tissue by in situ hybridization (ISH), fluorescence in situ hybridization (FISH), next generation sequencing (NGS), or comparative genome hybridization (CGH). Some embodiments comprise obtaining the MYC gene copy number reference from a database, or determining the MYC gene copy number reference from a non-cancerous tissue from a control, optionally by aCGH, SNP array, CNV sequence, or MLPA. Some embodiments comprise obtaining the MYC gene chromosomal location site reference from a database, or determining the MYC gene chromosomal location site reference from a non-cancerous tissue from a control, optionally by ISH, FISH, NGS, or CGH.

Some embodiments comprise obtaining the sample of cancer tissue from the subject. In certain embodiments, the sample of cancer tissue is a surgical sample, a biopsy sample, a pleural

effusion sample, or an ascetic fluid sample obtained from the subject, optionally selected from one or more of lung, blood, breast, gastrointestinal (stomach, colon, rectal), ovarian, pancreatic, liver, bladder, cervical, neuronal, uterine, salivary gland, kidney, prostate, thyroid, or muscle tissue.

In certain embodiments, the subject is a human subject.

In certain embodiments, the cancer is selected from one or more of carcinoma, sarcoma such as rhabdomyosarcoma, for example, alveolar rhabdomyosarcoma (including sarcoma originating in the bones, tendons, cartilage, muscle, fat, fibrous, blood vessels, adipose, and/or connective tissue), neuroblastoma, medulloblastoma, astrocytoma, glioblastoma multiforme, retinoblastoma, myeloma, leukemia, lymphoma (including Hodgkin's lymphoma and Non-Hodgkin's lymphoma), adenosquamous carcinoma, carcinosarcoma, mixed mesodermal tumor, teratocarcinoma, lung cancer (including non-small cell lung cancer, small cell lung cancer, adenocarcinoma, and squamous carcinoma of the lung), breast cancer (including metastatic breast cancer), gastrointestinal cancer, stomach cancer, colorectal cancer, colon cancer, rectal cancer, ovarian cancer, pancreatic cancer, liver cancer, bladder cancer, cervical cancer, glioblastoma, uterine carcinoma, salivary gland carcinoma, kidney or renal cancer (e.g., Wilm's tumor), prostate cancer, thyroid cancer, and head and neck cancer.

In certain embodiments, the MYC gene is selected from MYCC and MYCN. In specific embodiments, the MYC gene is MYCC and the cancer is selected from lung cancers and blood cancers, optionally leukemias and lymphomas. In specific embodiments, the MYC gene is MYCN and the cancer is selected from neuroblastoma, small cell lung cancer, prostate cancer, alveolar rhabdomyosarcoma, medulloblastoma, glioblastoma multiforme, retinoblastoma, and breast cancer.

Also included is the use of a diagnostic kit for determining therapeutic response to YM155 monobromide [1-(2-Methoxyethyl)-2-methyl-4,9-dioxo-3-(pyrazin-2-ylmethyl)-4,9-dihydro-1H-naphtho[2,3-d] imidazolium bromide], or an analog or derivative thereof, therapy in a subject with cancer, comprising means for measuring MYC gene copy number, or MYC gene chromosomal location site, in a sample of tissue from the subject, including cancer tissue and non-cancerous tissue.

In certain embodiments, the means for measuring MYC gene copy number comprise reagents for performing a diagnostic assay selected from one or more of array comparative genome hybridization (aCGH), single nucleotide polymorphism (SNP) array, copy number variation (CNV) sequencing, and multiplex ligation-dependent probe amplification (MLPA) on a human MYC gene.

In certain embodiments, the means for measuring MYC gene chromosomal location site comprise reagents for performing a diagnostic assay selected from one or more of in situ hybridization (ISH), fluorescence in situ hybridization (FISH), next generation sequencing (NGS), and comparative genome hybridization (CGH) on a human MYC gene. Some embodiments comprise a MYC gene copy number reference value obtained from a database, or determined from a non-cancerous tissue from a control. Some embodiments comprise a MYC gene chromosomal location site reference obtained from a database, or determined from a non-cancerous tissue from a control.

Also included are patient care kits, comprising:

(a) means for measuring MYC gene copy number, or MYC gene chromosomal location site, in a sample of tissue from a subject, including cancer tissue and non-cancerous tissue; and

(b) YM155 monobromide [1-(2-Methoxyethyl)-2- methyl-4,9-dioxo-3-(pyrazin-2-ylmethyl)-4,9-dihydro-1H-naphtho[2,3-d] imidazolium bromide], or an analog or derivative thereof.

In certain embodiments, the means for measuring MYC gene copy number comprise reagents for performing a diagnostic assay selected from one or more of array comparative genome hybridization (aCGH), single nucleotide polymorphism (SNP) array, copy number variation (CNV) sequencing, and multiplex ligation-dependent probe amplification (MLPA) on a human MYC gene. In certain embodiments, the means for measuring MYC gene chromosomal location site comprise reagents for performing a diagnostic assay selected from one or more of in situ hybridization (ISH), fluorescence in situ hybridization (FISH), next generation sequencing (NGS), and comparative genome hybridization (CGH) on a human MYC gene.

Some embodiments comprise a MYC gene copy number reference value obtained from a database, or determined from a non-cancerous tissue from a control. Some embodiments comprise a MYC gene chromosomal location site reference obtained from a database, or determined from a non-cancerous tissue from a control.

In some uses or kits, the cancer is selected from one or more of carcinoma, sarcoma such as rhabdomyosarcoma, for example, alveolar rhabdomyosarcoma, (including sarcoma originating in the bones, tendons, cartilage, muscle, fat, fibrous, blood vessels, adipose, and/or connective tissue), neuroblastoma, medulloblastoma, astrocytoma, glioblastoma multiforme, retinoblastoma, myeloma, leukemia, lymphoma (including Hodgkin's lymphoma and Non-Hodgkin's lymphoma), adenosquamous carcinoma, carcinosarcoma, mixed mesodermal tumor, teratocarcinoma), lung cancer (including non-small cell lung cancer, small cell lung cancer, adenocarcinoma, and squamous carcinoma of the lung), breast cancer (including metastatic breast cancer), gastrointestinal cancer, stomach cancer, colorectal cancer, colon cancer, rectal cancer, ovarian cancer, pancreatic cancer, liver cancer, bladder cancer, cervical cancer, glioblastoma, uterine carcinoma, salivary gland carcinoma, kidney or renal cancer (e.g., Wilm's tumor), prostate cancer, thyroid cancer, and head and neck cancer.

In some uses or kits, the MYC gene is selected from MYCC and MYCN. In specific instances, the MYC gene is MYCC and the cancer is selected from lung cancers and blood cancers, optionally leukemias and lymphomas. In particular instances, the MYC gene is MYCN and the cancer is selected from neuroblastoma, small cell lung cancer, prostate cancer, alveolar rhabdomyosarcoma, medulloblastoma, glioblastoma multiforme, retinoblastoma, and breast cancer.

## BRIEF DESCRIPTION OF THE DRAWINGS

**Figure 1** shows the chemical structure of YM155 monobromide (CAS 781661-94-7).

**Figure 2** shows that YM155 inhibits cell proliferation of human lung cancer cell line. NCI-H1975, HCC827, NCI-H226, and HCC4006 cells were cultured in 96-well plates and treated with YM155 at indicated dose (nM). Cell proliferation was detected by EdU proliferation assay. The data presented is mean±SEM.

**Figure 3** shows that YM155 inhibits cell proliferation of human acute myeloid leukemia (AML) cell line. U937 and HL-60 cells were cultured in 96-well plates and treated with YM155 at indicated dose (nM). Cell proliferation was detected by XTT cell viability assay. The data presented is mean±SEM.

**Figure 4** shows that YM155 inhibits cell proliferation of U937 and RAMOS cell line. U937 and RAMOS cells were cultured in 96-well plates and treated with YM155 at indicated dose (nM). Cell proliferation was detected by XTT cell viability assay. The data presented is mean±SEM.

**Figure 5** and **Figure 6** provide genomic information for the human MYC gene (see http://uswest.ensembl.org/Homo_sapiens/Location/View?db=core;g=ENSG00000136997;r=8:127735434-127742951).

**Figure 7** shows that YM155 inhibits cell proliferation of human neuroblastoma cell lines, especially cell lines characterized by increased copy number of N-MYC (see also **Table E3**). Cell proliferation was detected by EdU assay. The data presented is mean±SD.

**Figure 8** shows the location of the MYCN gene on the short (p) arm of chromosome 2 at position 24.3.

**Figures 9A-9B** show the antitumor effect of YM155 in HCC827 xenografts in mice. Figure 9A shows the tumor growth curve, and Figure 9B presents the data as the means ±SD of tumor volume (n=5).** $p<0.01$ compared with the vehicle group.

**Figures 10A-10B** show the antitumor effect of YM155 in HCC4006 xenografts in mice. Figure 10A shows the tumor growth curve, and Figure 10B presents the data as the means ±SD of tumor volume (n=8).

**Figures 11A-11B** show the antitumor effect of YM155 in RAMOS xenografts in mice. Figure 11A shows the tumor growth curve, and Figure 11B presents the data as the means ±SD of tumor volume (n=3). ** $p<0.01$ compared with the vehicle group.

**Figures 12A-12B** show the antitumor effect of YM155 in U937 xenografts in mice. Figure 12A shows the tumor growth curve, and Figure 12B presents the data as the means ±SD of tumor volume (n=3).

**Figures 13A-13B** show the antitumor effect of YM155 in IMR-32 xenografts in mice. Figure 13A shows the tumor growth curve, and Figure 13B presents the data as the means ±SD of tumor volume (n=3).

**Figures 14A-14B** show the antitumor effect of YM155 in SH-SY5Y xenografts in mice. Figure 14A shows the tumor growth curve, and Figure 14B presents the data as the means ±SD of tumor volume (n=2 in vehicle; n=3 in YM155-treated).

## DETAILED DESCRIPTION

Embodiments of the present disclosure relate to the surprising discovery that amplification and/or translocation of the MYC gene in human malignancies associate with increased anti-cancer efficacy of survivin inhibitors, such as YM155 monobromide, and can therefore be used as biomarker(s) to optimize cancer therapy by those agents and others. Without wishing to be bound by any one theory, it is believed that MYC oncogene expression in MYC-amplified or MYC-translocated cancer cells is otherwise pro-apoptotic, except that its pro-apoptotic signaling is negatively-regulated by survivin, a member of the inhibitor of apoptosis (IAP) protein family that inhibits caspases and blocks cell death. Thus, it is expected that survivin inhibitors such as YM155 monobromide can be used to block the apoptosis-inhibiting activity of survivin in the context of MYC amplification and/or translocation, favor the pro-apoptotic signaling of MYC to increase cancer cell death, and thereby provide optimal therapeutic efficacy in this context.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by those of ordinary skill in the art to which the disclosure belongs. Although any methods, materials, compositions, reagents, cells, similar or equivalent similar or equivalent to those described herein can be used in the practice or testing of the subject matter of the present disclosure, preferred methods and materials are described. All publications and references, including but not limited to patents and patent applications, cited in this specification are herein incorporated by reference in their entirety as if each individual publication or reference were specifically and individually indicated to be incorporated by reference herein as being fully set forth. Any patent application to which this application claims priority is also incorporated by reference herein in its entirety in the manner described above for publications and references.

For the purposes of the present disclosure, the following terms are defined below.

The articles “a” and “an” are used herein to refer to one or to more than one (i.e., to at least one) of the grammatical object of the article. By way of example, “an element” means one element or more than one element.

An “antagonist” or “inhibitor” refers to biological structure or chemical agent that interferes with or otherwise reduces the physiological action of another molecule, such as a protein (e.g., survivin). In some instances, the antagonist or inhibitor specifically binds to the other molecule and/or a functional ligand of the other molecule. In some instances, the antagonist or inhibitor down-regulates the expression of the other molecule (e.g., survivin). Included are full and partial antagonists.

An “agonist” or “activator” refers to biological structure or chemical agent that increases or enhances the physiological action of another agent or molecule. In some instances, the agonist specifically binds to the other agent or molecule. Included are full and partial agonists.

By "about" is meant a quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length that varies by as much as 30, 25, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1% to a reference quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length.

Throughout this disclosure, unless the context requires otherwise, the words "comprise," "comprises," and "comprising" will be understood to imply the inclusion of a stated step or element or group of steps or elements but not the exclusion of any other step or element or group of steps or elements.

By "consisting of" is meant including, and limited to, whatever follows the phrase "consisting of." Thus, the phrase "consisting of" indicates that the listed elements are required or mandatory, and that no other elements may be present. By "consisting essentially of" is meant including any elements listed after the phrase, and limited to other elements that do not interfere with or contribute to the activity or action specified in the disclosure for the listed elements. Thus, the phrase "consisting essentially of" indicates that the listed elements are required or mandatory, but that other elements are optional and may or may not be present depending upon whether or not they materially affect the activity or action of the listed elements.

The "half maximal inhibitory concentration" (or "$IC_{50}$") is a measure of the potency of an agent in inhibiting a specific biological or biochemical function. This quantitative measure indicates how much of a particular agent (inhibitor) is needed to inhibit a given biological process (or component of a process, i.e. an enzyme, cell, cell receptor or microorganism) by half. The values are typically expressed as molar concentration. The concentration is commonly used as a measure of antagonist drug potency in pharmacological research. In some instances, $IC_{50}$ represents the concentration of an agent that is required for 50% inhibition in vitro. The $IC_{50}$ of an agent can be determined by constructing a dose-response curve and examining the effect of different concentrations of the agent on the desired activity, for example, inhibition of tumor cell proliferation, tumor-cell killing.

An "increased" or "enhanced" amount is typically a "statistically significant" amount, and may include an increase that is about or at least about 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 7, 8, 9, 10, 15, 20, 30, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, or 1000 fold, or about or at least about 5%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18% , 19%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, 900%, 1000%, relative to that of a reference or control (including all integers and ranges in between). A "decreased" or "reduced" amount is typically a "statistically significant" amount, and may include a decrease that is about or at least about 1.2, 1.4, 1.6, 1.8, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 1000 fold, or about or at least about 5%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18% , 19%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 200%, 300%, 400%,

500%, 600%, 700%, 800%, 900%, 1000%, relative to that of a reference or control (including all integers and ranges in between).

The term “polynucleotide” and “nucleic acid” includes mRNA, RNA, cRNA, cDNA, and DNA including genomic DNA. The term typically refers to polymeric form of nucleotides of at least 10 bases in length, either ribonucleotides or deoxynucleotides or a modified form of either type of nucleotide. The term includes single and double stranded forms of DNA.

A “gene” refers to a hereditary unit consisting of a sequence of DNA that occupies a specific location on a chromosome and codes for a functional molecule or protein. The structure of a gene consists of many elements of which the actual protein coding sequence is often only a small part. These elements include DNA regions that are not transcribed as well as untranslated regions of the RNA. Additionally, genes can have expression-altering regulatory regions that lie many kilobases upstream or downstream of the coding sequence. The information in a gene can also be represented by (or found in) a sequence of RNA or encoded protein.

A “subject” or a “subject in need thereof” includes a mammalian subject such as a human subject.

By “statistically significant” it is meant that the result was unlikely to have occurred by chance. Statistical significance can be determined by any method known in the art. Commonly used measures of significance include the p-value, which is the frequency or probability with which the observed event would occur, if the null hypothesis were true. If the obtained p-value is smaller than the significance level, then the null hypothesis is rejected. In simple cases, the significance level is defined at a p-value of 0.05 or less.

“Substantially” or “essentially” means nearly totally or completely, for instance, 95% or greater of some given quantity.

“Therapeutic response” refers to improvement of symptoms (whether or not sustained) based on the administration of the therapeutic response.

As used herein, the terms “therapeutically effective amount”, “therapeutic dose,” “prophylactically effective amount,” or “diagnostically effective amount” is the amount of an agent needed to elicit the desired biological response following administration.

As used herein, “treatment” of a subject (e.g. a mammal, such as a human) or a cell is any type of intervention used in an attempt to alter the natural course of the subject or cell. Treatment includes, but is not limited to, administration of a pharmaceutical composition, and may be performed either prophylactically or subsequent to the initiation of a pathologic event or contact with an etiologic agent. Also included are “prophylactic” treatments, which can be directed to reducing the rate of progression of the disease or condition being treated, delaying the onset of that disease or condition, or reducing the severity of its onset. “Treatment” or “prophylaxis” does not necessarily indicate complete eradication, cure, or prevention of the disease or condition, or associated symptoms thereof.

The term "wild-type" refers to a gene or gene product (e.g., a polypeptide) that is most frequently observed in a population and is thus arbitrarily designed the "normal" or "wild-type" form of the gene.

Each embodiment in this specification is to be applied to every other embodiment unless expressly stated otherwise.

The present disclosure relates, in part, to the surprising discovery that amplifications and/or translocations of the MYC gene in human malignancies associate with increased anti-cancer efficacy of the chemotherapeutic agent YM155 monobromide, and can thus be used as biomarkers to optimize cancer therapy by that agent and others.

Embodiments of the present disclosure therefore include methods for treating cancer in a subject in need thereof, comprising (a) determining MYC gene copy number, or MYC gene chromosomal location site, in a sample of cancer tissue from the subject; and (b) administering YM155 monobromide [1-(2-Methoxyethyl)-2-methyl-4,9-dioxo-3-(pyrazin-2-ylmethyl)-4,9-dihydro-1H-naphtho[2,3-d] imidazolium bromide] to the subject if MYC gene copy number in the cancer tissue is increased relative to that of a MYC gene copy number reference, or if MYC gene chromosomal location site in the cancer tissue is translocated relative to that of a MYC gene chromosomal location site reference, thereby treating cancer in the subject in need thereof. Certain embodiments include administering to the subject a chemotherapeutic agent excluding (or other than) YM155 monobromide if MYC gene copy number in the cancer tissue is not substantially increased (e.g., the same or less than about 1.1 fold increase) relative to that of the MYC gene copy number reference, or if MYC gene chromosomal location site in the cancer tissue is not translocated relative to that of the MYC gene chromosomal location site reference.

Also included are methods for predicting therapeutic response to YM155 monobromide [1-(2-Methoxyethyl)-2-methyl-4,9-dioxo-3-(pyrazin-2-ylmethyl)-4,9-dihydro-1H-naphtho[2,3-d] imidazolium bromide] in a subject with cancer, comprising (a) determining MYC gene copy number, or MYC gene chromosomal location site, in a sample of cancer tissue from the subject; and (b) (i) characterizing the subject as responsive to YM155 monobromide therapy if MYC gene copy number in the cancer tissue is increased relative to that of a MYC gene copy number reference, or if MYC gene chromosomal location site in the cancer tissue is translocated relative to that of a MYC gene chromosomal location site reference; or (ii) characterizing the subject as non-responsive to YM155 monobromide therapy if MYC gene copy number in the cancer tissue is not substantially increased (e.g., the same or less than about 1.1 fold increase) relative to that of the MYC gene copy number reference, or if MYC gene chromosomal location site in the cancer tissue is not translocated relative to that of the MYC gene chromosomal location site reference, thereby predicting therapeutic response to YM155 monobromide in the subject with cancer. Certain embodiments include administering YM155 monobromide to the subject if the subject is characterized as responsive to YM155 monobromide therapy. Some embodiments include administering to the subject a chemotherapeutic

agent excluding (or other than) YM155 monobromide if the subject is characterized as non-responsive to YM155 monobromide therapy.

The “MYC gene” or “MYC oncogene” refers to a family of proto-oncogenes that encode transcription factors, examples of which include c-Myc (also MYCC) and N-myc (also MYCN).

The MYCC gene encodes a nuclear phosphoprotein that plays a role in cell cycle progression, apoptosis, and cellular transformation. The encoded protein forms a heterodimer with the related transcription factor MAX. This complex binds to the E box DNA consensus sequence and regulates the transcription of specific target genes. There is evidence to show that translation initiates both from an upstream, in-frame non-AUG (CUG) and a downstream AUG start site, resulting in the production of two isoforms with distinct N-termini. In the human genome, the MYCC gene is located on chromosome 8:127, 735, 434-127, 741, 434, forward strand (see, e.g., **Figure 5** and **Figure 6**; and Gene: MYC ENSG00000136997).

The MYCN gene encodes a protein with a basic helix-loop-helix (bHLH) domain. It is located in the cell nucleus and dimerizes with another bHLH protein to bind DNA. MYCN is over-expressed in a number of different types of cancer, including, for example, neuroblastoma, rhabdomyosarcoma, medulloblastoma, astrocytoma, glioblastoma, retinoblastoma, prostate cancer, breast cancer, Wilms’ tumour, and small cell lung cancer (see, for example, Beltran, Mol Cancer Res. 12:815-822, 2014). Indeed, MYCN amplification is an adverse prognostic factor in neuroblastoma. The amplicon (material co-amplified with MYCN) varies among subjects, and in certain instances includes, for example, the DDX1 gene. In some instances, MYCN amplification correlates with a 1p36 deletion and a gain of chromosome 17q. In the human genome, the MYCN gene is located on the the short (p) arm of chromosome 2 at position 24.3 (Cytogenetic Location at 2p24.3; Molecular Location at base pairs 15,940,438 to 15,947,007 on chromosome 2; see also **Figure 8**).

Thus, in certain embodiments, the MYC gene is selected from MYCC and MYCN.

“YM155 monobromide” refers to the small molecule [1-(2-Methoxyethyl)-2-methyl-4,9-dioxo-3-(pyrazin-2-ylmethyl)-4,9-dihydro-1H-naphtho[2,3-d] imidazolium bromide], having the molecular formula $C_{20}H_{19}N_4O_3 \bullet Br$, and the CAS Number 781661-94-7, and includes pharmaceutically-acceptable salts and acids thereof. Also included are biologically-active or equivalent analogs and/or derivatives of YM155 monobromide.

As noted above, in some instances, the MYC gene copy number in the cancer tissue is increased relative to that of the MYC gene copy number reference. In particular embodiments, the MYC gene copy number in the cancer tissue is increased by a statistically significant amount relative to that of the MYC gene copy number reference. In some embodiments, the MYC gene copy number in the cancer tissue is increased by about or at least about 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 7, 8, 9, or 10-fold (or more) relative to that of the MYC gene copy number reference.

The MYC gene copy number in the cancer tissue can be determined by any variety of methods. For example, in some embodiments, the MYC gene copy number is determined by array

comparative genome hybridization (aCGH), single nucleotide polymorphism (SNP) array, copy number variation (CNV) sequencing, or multiplex ligation-dependent probe amplification (MLPA). Certain embodiments thus include the step of determining or detecting copy number of a MYC gene in a sample of cancer tissue from a subject in need thereof. Also included is the step of comparing the copy number of a MYC gene in a sample of cancer tissue relative to that of a MYC gene copy number reference.

The MYC gene chromosomal location site in the cancer tissue can be determined by any variety of methods. For example, in some embodiments, the MYC gene chromosomal location site in the cancer tissue is determined by in situ hybridization (ISH), fluorescence in situ hybridization (FISH), next generation sequencing (NGS), or comparative genome hybridization (CGH). Certain embodiments thus include the step of determining or detecting the MYC gene chromosomal location site in a sample of cancer tissue from a subject in need thereof. Also included is the step of comparing the MYC gene chromosomal location site in the cancer tissue relative to that of a MYC gene chromosomal site reference.

CGH refers to a molecular cytogenetic method for analyzing copy number variations (CNVs) relative to ploidy level in the DNA of a test sample compared to a reference sample, without the need for culturing cells. This technique allows quick and efficient comparisons between two genomic DNA samples arising from two sources, which are most often closely related, because it is suspected that they contain differences in terms of either gains or losses of either whole chromosomes or subchromosomal regions (a portion of a whole chromosome). The technique was originally developed for the evaluation of the differences between the chromosomal complements of solid tumor and normal tissue (see, e.g., Kallioniemi et al., Science. 258 (5083): 818–821, 1992). The use of DNA microarrays in conjunction with CGH techniques has led to the development of a more specific form of array CGH (aCGH), allowing for a locus-by-locus measure of CNV with increased resolution as low as 100 kilobases (see, e.g., Pinkel, Annu Rev Genom Hum Genet. 6:331–354, 2005). CNV is a prevalent form of critical genetic variation that leads to an abnormal number of copies of large genomic regions in a cell, and high-resolution sequence data can be analyzed by next-generation sequencing (NGS) to identify the same (see, e.g., Zhao et al., BMC Bioinformatics. 14 Suppl 11:S1, 2013). MLPA refers to a variation of the multiplex polymerase chain reaction that permits amplification of multiple targets with only a single primer pair (see, e.g., Schouten et al., Nucleic Acids Res. 30 (12): e57, 2002). In situ hybridization (ISH) and fluorescent in situ hybridization (FISH) refer to a type of hybridization that uses a labeled complementary DNA, RNA or modified nucleic acids strand (i.e., probe) to localize a specific DNA or RNA sequence in a portion or section of tissue (in situ) (see, e.g., Parra & Windle, Nature Genetics. 5:17-21, 1993; and Gall & Pardue, PNAS USA. 63: 378–383, 1969). Thus, in some instances, the methods and kits described herein employ any one or more of the foregoing techniques and/or comprise reagents for performing the same.

Examples of a “reference” (e.g., a MYC gene copy number “reference”, a MYC gene chromosomal site “reference”) include a value or amount or location obtained from a database, for example, a value or amount of a “wild-type” MYC gene copy number or a “wild-type” MYC gene chromosomal location site (see, e.g., **Figure 5** and **Figure 6** for a human MYCC gene chromosomal site reference; and **Figure 8** for a human MYCN gene chromosomal site reference). A “reference” also includes a value or amount or location obtained from a non-cancerous tissue from one or more controls, for example, one or more healthy or non-cancerous control subjects (e.g., a population of healthy or non-cancerous control subjects), or one or more corresponding non-cancerous control tissues from the subject being tested. Typically, a “corresponding” non-cancerous control tissue is obtained from the same type of tissue as the cancer tissue being tested. As with the cancer tissue, the MYC gene copy number reference from a non-cancerous control can be determined by any variety of methods, including, for example, by aCGH, SNP array, CNV sequence, and/or MLPA (supra). Similarly, the MYC gene chromosomal location site reference from a non-cancerous control can be determined by any variety of methods, including, for example, ISH, FISH, NGS, and/or CGH (supra).

In some embodiments, the sample of cancer tissue (or non-cancerous control tissue) is a surgical sample, a biopsy sample, a pleural effusion sample, or an ascetic fluid sample from the subject. Particular examples of samples of cancer tissues (or non-cancerous control tissues) include lung, blood, breast, gastrointestinal (stomach, colon, rectal), ovarian, pancreatic, liver, bladder, cervical, neuronal, uterine, salivary gland, kidney, prostate, thyroid, or muscle tissues. Certain embodiments include the step of obtaining the sample of cancer tissue (or non-cancerous control tissue) from the subject, for example, prior to determining MYC gene copy levels or MYC gene chromosomal location site.

In some embodiments, the subject is a human subject.

As noted above, certain embodiments include administering to the subject an anti-cancer agent excluding (or other than) YM155 monobromide if the subject is characterized as non-responsive to YM155 monobromide therapy, for example, if the MYC gene copy number in the cancer tissue is not substantially increased relative to that of the MYC gene copy number reference, or if the MYC gene chromosomal location site in the cancer tissue is not translocated relative to that of the MYC gene chromosomal location site reference. Exemplary anti-cancer agents (other than YM155 monobromide) for administering to a subject characterized as non-responsive to YM155 monobromide therapy include small molecules such as cytotoxic, chemotherapeutic, and anti-angiogenic agents, for instance, those that have been considered useful in the treatment of various cancers. General classes of anti-cancer agents include, without limitation, alkylating agents, anti-metabolites, anthracyclines, anti-tumor antibiotics, platinums, type I topoisomerase inhibitors, type II topoisomerase inhibitors, vinca alkaloids, and taxanes.

Specific examples of anti-cancer agents for administering to a subject characterized as non-responsive to YM155 monobromide therapy include chlorambucil, cyclophosphamide, cilengitide,

lomustine (CCNU), melphalan, procarbazine, thiotepa, carmustine (BCNU), enzastaurin, busulfan, daunorubicin, doxorubicin, gefitinib, erlotinib idarubicin, temozolomide, epirubicin, mitoxantrone, bleomycin, cisplatin, carboplatin, oxaliplatin, camptothecins, irinotecan, topotecan, amsacrine, etoposide, etoposide phosphate, teniposide, temsirolimus, everolimus, vincristine, vinblastine, vinorelbine, vindesine, CT52923, and paclitaxel, and pharmaceutically acceptable salts, acids or derivatives of any of the above. Additional examples of anti-cancer agents include imatinib, crizotinib, dasatinib, sorafenib, pazopanib, sunitinib, vatalanib, geftinib, erlotinib, AEE-788, dichoroacetate, tamoxifen, fasudil, SB-681323, and semaxanib (SU5416) (see Chico et al., Nat Rev Drug Discov. 8:829-909, 2009).

Further examples of anti-cancer agents for administering to a subject characterized as non-responsive to YM155 monobromide therapy include alkylating agents such as thiotepa, cyclophosphamide (CYTOXAN™); alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, trietylenephosphoramide, triethylenethiophosphaoramide and trimethylolomelamine; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, ranimustine; antibiotics such as aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, calicheamicin, carabicin, carminomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin, epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine, 5-FU; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elformithine; elliptinium acetate; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidamine; mitoguazone; mitoxantrone; mopidamol; nitracrine; pentostatin; phenamet; pirarubicin; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK; razoxane; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2"-trichlorotriethylamine; urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; taxoids, *e.g.* paclitaxel (TAXOL®, Bristol-Myers Squibb Oncology, Princeton, N.J.) and

docetaxel (TAXOTERE®, Rhne-Poulenc Rorer, Antony, France); chlorambucil; gemcitabine; 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitomycin C; mitoxantrone; vincristine; vinorelbine; navelbine; novantrone; teniposide; daunomycin; aminopterin; xeloda; ibandronate; CPT-11; topoisomerase inhibitor RFS 2000; difluoromethylomithine (DMFO); retinoic acid derivatives such as Targretin™ (bexarotene), Panretin™ (alitretinoin); ONTAK™ (denileukin diftitox); esperamicins; capecitabine; and pharmaceutically acceptable salts, acids or derivatives of any of the above.

Additional examples of anti-cancer agents for administering to a subject characterized as non-responsive to YM155 monobromide therapy include anti-hormonal agents that act to regulate or inhibit hormone action on tumors such as anti-estrogens including for example tamoxifen, raloxifene, aromatase inhibiting 4(5)-imidazoles, 4-hydroxytamoxifen, trioxifene, keoxifene, LY117018, onapristone, and toremifene (Fareston); and anti-androgens such as flutamide, nilutamide, bicalutamide, leuprolide, and goserelin; and pharmaceutically acceptable salts, acids or derivatives of any of the above.

Further examples of anti-cancer agents for administering to a subject characterized as non-responsive to YM155 monobromide therapy include anti-cancer antibodies such as 3F8, 8H9, abagovomab, adecatumumab, afutuzumab, alemtuzumab, alacizumab (pegol), amatuximab, apolizumab, bavituximab, bectumomab, belimumab, bevacizumab, bivatuzumab (mertansine), brentuximab vedotin, cantuzumab (mertansine), cantuzumab (ravtansine), capromab (pendetide), catumaxomab, cetuximab, citatuzumab (bogatox), cixutumumab, clivatuzumab (tetraxetan), conatumumab, dacetuzumab, dalotuzumab, detumomab, drozitumab, ecromeximab, edrecolomab, elotuzumab, enavatuzumab, ensituximab, epratuzumab, ertumaxomab, etaracizumab, farletuzumab, FBTA05, figitumumab, flanvotumab, galiximab, gemtuzumab, ganitumab, gemtuzumab (ozogamicin), girentuximab, glembatumumab (vedotin), ibritumomab tiuxetan, icrucumab, igovomab, indatuximab ravtansine, intetumumab, inotuzumab ozogamicin, ipilimumab (MDX-101), iratumumab, labetuzumab, lexatumumab, lintuzumab, lorvotuzumab (mertansine), lucatumumab, lumiliximab, mapatumumab, matuzumab, milatuzumab, mitumomab, mogamulizumab, moxetumomab (pasudotox), nacolomab (tafenatox), naptumomab (estafenatox), narnatumab, necitumumab, nimotuzumab, nivolumab, Neuradiab® (with or without radioactive iodine), NR-LU-10, ofatumumab, olaratumab, onartuzumab, oportuzumab (monatox), oregovomab, panitumumab, patritumab, pemtumomab, pertuzumab, pritumumab, racotumomab, radretumab, ramucirumab, rilotumumab, rituximab, robatumumab, samalizumab, sibrotuzumab, siltuximab, tabalumab, taplitumomab (paptox), tenatumomab, teprotumumab, TGN1412, ticilimumab, tremelimumab, tigatuzumab, TNX-650, tositumomab, TRBS07, trastuzumab, tucotuzumab (celmoleukin), ublituximab, urelumab, veltuzumab, volociximab, votumumab, and zalutumumab. Also included are fragments, variants, and derivatives of these antibodies.

The methods described herein can be used in the treatment and/or diagnosis of any variety of cancers or tumors. In some embodiments, the cancer is a primary cancer, i.e., a cancer growing at the anatomical site where tumor progression began and yielded a cancerous mass. In some embodiments, the cancer is a secondary or metastatic cancer, i.e., a cancer which has spread from the primary site or tissue of origin into one or more different sites or tissues. In some instances, the cancer is selected from one or more of carcinoma, sarcoma such as rhabdomyosarcoma, for example, alveolar rhabdomyosarcoma (including sarcoma originating in the bones, tendons, cartilage, muscle, fat, fibrous, blood vessels, adipose, and/or connective tissue ), neuroblastoma, medulloblastoma, astrocytoma, glioblastoma multiforme, retinoblastoma, myeloma, leukemia, lymphoma (including Hodgkin's lymphoma and Non-Hodgkin's lymphoma), adenosquamous carcinoma, carcinosarcoma, mixed mesodermal tumor, teratocarcinoma, lung cancer (including non-small cell lung cancer, small cell lung cancer, adenocarcinoma, and squamous carcinoma of the lung), breast cancer (including metastatic breast cancer), gastrointestinal cancer, stomach cancer, colorectal cancer, colon cancer, rectal cancer, ovarian cancer, pancreatic cancer, liver cancer, bladder cancer, cervical cancer, glioblastoma, uterine carcinoma, salivary gland carcinoma, kidney or renal cancer (e.g., Wilm's tumor), prostate cancer, thyroid cancer, and head and neck cancer.

In specific embodiments, the MYC gene is MYCC and the cancer is selected from lung cancers and blood cancers, optionally leukemias and lymphomas. In specific embodiments, the MYC gene is MYCN and the cancer is selected from neuroblastoma, small cell lung cancer, prostate cancer, alveolar rhabdomyosarcoma, medulloblastoma, glioblastoma multiforme, retinoblastoma, and breast cancer.

In some embodiments, as noted above, the cancer or tumor is a metastatic cancer. Further to the above cancers, exemplary metastatic cancers include, without limitation, bladder cancers which have metastasized to the bone, liver, and/or lungs; breast cancers which have metastasized to the bone, brain, liver, and/or lungs; colorectal cancers which have metastasized to the liver, lungs, and/or peritoneum; kidney cancers which have metastasized to the adrenal glands, bone, brain, liver, and/or lungs; lung cancers which have metastasized to the adrenal glands, bone, brain, liver, and/or other lung sites; melanomas which have metastasized to the bone, brain, liver, lung, and/or skin/muscle; ovarian cancers which have metastasized to the liver, lung, and/or peritoneum; pancreatic cancers which have metastasized to the liver, lung, and/or peritoneum; prostate cancers which have metastasized to the adrenal glands, bone, liver, and/or lungs; stomach cancers which have metastasized to the liver, lung, and/or peritoneum; thyroid cancers which have metastasized to the bone, liver, and/or lungs; and uterine cancers which have metastasized to the bone, liver, lung, peritoneum, and/or vagina; among others.

In certain embodiments, the methods described herein are sufficient to result in tumor regression, as indicated by a statistically significant decrease in the amount of viable tumor, for example, at least a 10%, 20%, 30%, 40%, 50% or greater decrease in tumor mass, or by altered (e.g.,

decreased with statistical significance) scan dimensions. In certain embodiments, the methods described are sufficient to result in stable disease. In certain embodiments, the methods described herein are sufficient to result in clinically relevant reduction in symptoms of a particular disease indication known to the skilled clinician.

The methods for treating cancers can be combined with other therapeutic modalities. For example, a combination therapy described herein can be administered to a subject before, during, or after other therapeutic interventions, including symptomatic care, radiotherapy, surgery, transplantation, hormone therapy, photodynamic therapy, antibiotic therapy, or any combination thereof. Symptomatic care includes administration of corticosteroids, to reduce cerebral edema, headaches, cognitive dysfunction, and emesis, and administration of anti-convulsants, to reduce seizures. Radiotherapy includes whole-brain irradiation, fractionated radiotherapy, and radiosurgery, such as stereotactic radiosurgery, which can be further combined with traditional surgery.

Methods for identifying subjects with one or more of the diseases or conditions described herein are known in the art.

For *in vivo* use, for instance, for the treatment of human disease or testing, the agents described herein are generally incorporated into one or more therapeutic or pharmaceutical compositions prior to administration.

To prepare a therapeutic or pharmaceutical composition, an effective or desired amount of one or more agents is typically mixed with any pharmaceutical carrier(s) or excipient known to those skilled in the art to be suitable for the particular agent and/or mode of administration. A pharmaceutical carrier may be liquid, semi-liquid or solid. Solutions or suspensions used for parenteral, intradermal, subcutaneous or topical application may include, for example, a sterile diluent (such as water), saline solution (*e.g.,* phosphate buffered saline; PBS), fixed oil, polyethylene glycol, glycerin, propylene glycol or other synthetic solvent; antimicrobial agents (such as benzyl alcohol and methyl parabens); antioxidants (such as ascorbic acid and sodium bisulfite) and chelating agents (such as ethylenediaminetetraacetic acid (EDTA)); buffers (such as acetates, citrates and phosphates). If administered intravenously (e.g., by IV infusion), suitable carriers include physiological saline or phosphate buffered saline (PBS), and solutions containing thickening and solubilizing agents, such as glucose, polyethylene glycol, polypropylene glycol and mixtures thereof.

Administration of agents described herein, in pure form or in an appropriate therapeutic or pharmaceutical composition, can be carried out via any of the accepted modes of administration of agents for serving similar utilities. The therapeutic or pharmaceutical compositions can be prepared by combining an agent-containing composition with an appropriate physiologically acceptable carrier, diluent or excipient, and may be formulated into preparations in solid, semi-solid, liquid or gaseous forms, such as tablets, capsules, powders, granules, ointments, solutions, suppositories, injections, inhalants, gels, microspheres, and aerosols. In addition, other pharmaceutically active ingredients

(including other small molecules as described elsewhere herein) and/or suitable excipients such as salts, buffers and stabilizers may, but need not, be present within the composition.

Administration may be achieved by a variety of different routes, including oral, parenteral, nasal, intravenous, intradermal, intramuscular, subcutaneous or topical. Preferred modes of administration depend upon the nature of the condition to be treated or prevented. Particular embodiments include administration by IV infusion.

Carriers can include, for example, pharmaceutically- or physiologically-acceptable carriers, excipients, or stabilizers that are non-toxic to the cell or mammal being exposed thereto at the dosages and concentrations employed. Often the physiologically-acceptable carrier is an aqueous pH buffered solution. Examples of physiologically acceptable carriers include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptide; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as polysorbate 20 (TWEEN™) polyethylene glycol (PEG), and poloxamers (PLURONICS™), and the like.

In some embodiments, one or more agents can be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization (for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacylate)microcapsules, respectively), in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules), or in macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences, 16th edition, Oslo, A., Ed., (1980). The particle(s) or liposomes may further comprise other therapeutic or diagnostic agents.

The precise dosage and duration of treatment is a function of the disease being treated and may be determined empirically using known testing protocols or by testing the compositions in model systems known in the art and extrapolating therefrom. Controlled clinical trials may also be performed. Dosages may also vary with the severity of the condition to be alleviated. A pharmaceutical composition is generally formulated and administered to exert a therapeutically useful effect while minimizing undesirable side effects. The composition may be administered one time, or may be divided into a number of smaller doses to be administered at intervals of time. For any particular subject, specific dosage regimens may be adjusted over time according to the individual need.

Typical routes of administering these and related therapeutic or pharmaceutical compositions thus include, without limitation, oral, topical, transdermal, inhalation, parenteral, sublingual, buccal, rectal, vaginal, and intranasal. The term parenteral as used herein includes subcutaneous injections, intravenous, intramuscular, intrasternal injection or infusion techniques. Therapeutic or

pharmaceutical compositions according to certain embodiments of the present disclosure are formulated so as to allow the active ingredients contained therein to be bioavailable upon administration of the composition to a subject or patient. Compositions that will be administered to a subject or patient may take the form of one or more dosage units, where for example, a tablet may be a single dosage unit, and a container of a herein described agent in aerosol form may hold a plurality of dosage units. Actual methods of preparing such dosage forms are known, or will be apparent, to those skilled in this art; for example, see *Remington: The Science and Practice of Pharmacy*, 20th Edition (Philadelphia College of Pharmacy and Science, 2000). The composition to be administered will typically contain a therapeutically effective amount of an agent described herein, for treatment of a disease or condition of interest.

A therapeutic or pharmaceutical composition may be in the form of a solid or liquid. In one embodiment, the carrier(s) are particulate, so that the compositions are, for example, in tablet or powder form. The carrier(s) may be liquid, with the compositions being, for example, an oral oil, injectable liquid or an aerosol, which is useful in, for example, inhalatory administration. When intended for oral administration, the pharmaceutical composition is preferably in either solid or liquid form, where semi-solid, semi-liquid, suspension and gel forms are included within the forms considered herein as either solid or liquid. Certain embodiments include sterile, injectable solutions.

As a solid composition for oral administration, the pharmaceutical composition may be formulated into a powder, granule, compressed tablet, pill, capsule, chewing gum, wafer or the like. Such a solid composition will typically contain one or more inert diluents or edible carriers. In addition, one or more of the following may be present: binders such as carboxymethylcellulose, ethyl cellulose, microcrystalline cellulose, gum tragacanth or gelatin; excipients such as starch, lactose or dextrins, disintegrating agents such as alginic acid, sodium alginate, Primogel, corn starch and the like; lubricants such as magnesium stearate or Sterotex; glidants such as colloidal silicon dioxide; sweetening agents such as sucrose or saccharin; a flavoring agent such as peppermint, methyl salicylate or orange flavoring; and a coloring agent. When the pharmaceutical composition is in the form of a capsule, for example, a gelatin capsule, it may contain, in addition to materials of the above type, a liquid carrier such as polyethylene glycol or oil.

The therapeutic or pharmaceutical composition may be in the form of a liquid, for example, an elixir, syrup, solution, emulsion or suspension. The liquid may be for oral administration or for delivery by injection, as two examples. When intended for oral administration, preferred composition contain, in addition to the present compounds, one or more of a sweetening agent, preservatives, dye/colorant and flavor enhancer. In a composition intended to be administered by injection, one or more of a surfactant, preservative, wetting agent, dispersing agent, suspending agent, buffer, stabilizer and isotonic agent may be included.

The liquid therapeutic or pharmaceutical compositions, whether they be solutions, suspensions or other like form, may include one or more of the following adjuvants: sterile diluents

such as water for injection, saline solution, preferably physiological saline, Ringer's solution, isotonic sodium chloride, fixed oils such as synthetic mono or diglycerides which may serve as the solvent or suspending medium, polyethylene glycols, glycerin, propylene glycol or other solvents; antibacterial agents such as benzyl alcohol or methyl paraben; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic. Physiological saline is a preferred adjuvant. An injectable pharmaceutical composition is preferably sterile.

A liquid therapeutic or pharmaceutical composition intended for either parenteral or oral administration should contain an amount of an agent such that a suitable dosage will be obtained. Typically, this amount is at least 0.01% of the agent of interest in the composition. When intended for oral administration, this amount may be varied to be between 0.1 and about 70% of the weight of the composition. Certain oral therapeutic or pharmaceutical compositions contain between about 4% and about 75% of the agent of interest. In certain embodiments, therapeutic or pharmaceutical compositions and preparations according to the present invention are prepared so that a parenteral dosage unit contains between 0.01 to 10% by weight of the agent of interest prior to dilution.

The therapeutic or pharmaceutical composition may include various materials, which modify the physical form of a solid or liquid dosage unit. For example, the composition may include materials that form a coating shell around the active ingredients. The materials that form the coating shell are typically inert, and may be selected from, for example, sugar, shellac, and other enteric coating agents. Alternatively, the active ingredients may be encased in a gelatin capsule. The therapeutic or pharmaceutical compositions in solid or liquid form may include a component that binds to agent and thereby assists in the delivery of the compound. Suitable components that may act in this capacity include monoclonal or polyclonal antibodies, one or more proteins or a liposome.

The compositions described herein may be prepared with carriers that protect the agents against rapid elimination from the body, such as time release formulations or coatings. Such carriers include controlled release formulations, such as, but not limited to, implants and microencapsulated delivery systems, and biodegradable, biocompatible polymers, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, polyorthoesters, polylactic acid and others known to those of ordinary skill in the art.

The therapeutic or pharmaceutical compositions may be prepared by methodology well known in the pharmaceutical art. For example, a therapeutic or pharmaceutical composition intended to be administered by injection may comprise one or more of salts, buffers and/or stabilizers, with sterile, distilled water so as to form a solution. A surfactant may be added to facilitate the formation of a homogeneous solution or suspension. Surfactants are compounds that non-covalently interact with

the agent so as to facilitate dissolution or homogeneous suspension of the agent in the aqueous delivery system.

Certain embodiments include the use of a diagnostic kit for determining or predicting a therapeutic response (or responsiveness) to YM155 monobromide [1-(2-Methoxyethyl)-2-methyl-4,9-dioxo-3-(pyrazin-2-ylmethyl)-4,9-dihydro-1H-naphtho[2,3-d] imidazolium bromide] therapy in a subject with cancer, comprising means for measuring MYC gene copy number, or MYC gene chromosomal location site, in a sample of tissue from the subject, including cancer tissue and non-cancerous tissue. Also included are patient care kits, comprising: (a) means for measuring MYC gene copy number, or MYC gene chromosomal location site, in a sample of tissue from a subject, including cancer tissue and non-cancerous tissue; and (b) YM155 monobromide [1-(2-Methoxyethyl)-2-methyl-4,9-dioxo-3-(pyrazin-2-ylmethyl)-4,9-dihydro-1H-naphtho[2,3-d] imidazolium bromide].

In some embodiments, the means for measuring MYC gene copy number comprise reagents for performing a diagnostic assay selected from one or more of array comparative genome hybridization (aCGH), single nucleotide polymorphism (SNP) array, copy number variation (CNV) sequencing, and multiplex ligation-dependent probe amplification (MLPA) on a human MYC gene. In some embodiments, the means for measuring MYC gene chromosomal location site comprise reagents for performing a diagnostic assay selected from one or more of in situ hybridization (ISH), fluorescence in situ hybridization (FISH), next generation sequencing (NGS), and comparative genome hybridization (CGH) on a human MYC gene.

Certain diagnostic or patient care kits include a MYC gene copy number reference value obtained from a database, or determined from a non-cancerous tissue from a control. Some diagnostic or patient care kits include a MYC gene chromosomal location site reference obtained from a database, or determined from a non-cancerous tissue from a control. The kits can also include written instructions, for example, on how to determine MYC gene copy number and/or a MYC gene chromosomal location site in a sample of cancer tissue from a subject, and/or from a non-cancerous control.

In some embodiments, a diagnostic or patient care kit contains separate containers, dividers, or compartments for the composition(s) and informational material(s). For example, the composition(s) or reagents can be contained in a bottle, vial, or syringe, and the informational material(s) can be contained in association with the container. In some embodiments, the separate elements of the kit are contained within a single, undivided container. For example, the composition(s) or reagents are contained in a bottle, vial or syringe that has attached thereto the informational material in the form of a label. In some embodiments, the kit includes a plurality (e.g., a pack) of individual containers, each containing one or more compositions, reagents, and/or unit dosage forms of YM155 monobromide. For example, the kit includes a plurality of syringes, ampules, foil packets, or blister packs, each containing a reagent or a single unit dose of YM155 monobromide.

The containers of the kits can be air tight, waterproof (e.g., impermeable to changes in moisture or evaporation), and/or light-tight.

The patient care kit optionally includes a device suitable for administration of the agent(s), e.g., a syringe, inhalant, dropper (e.g., eye dropper), swab (e.g., a cotton swab or wooden swab), or any such delivery device. In some embodiments, the device is an implantable device that dispenses metered doses of the agent(s). Also included are methods of providing a kit, e.g., by combining the components described herein.

In certain aspects, the diagnostic or therapeutic response tests or methods described herein are performed at a diagnostic laboratory, and the results are then provided to the subject, or to a physician or other healthcare provider that plays a role in the subject's healthcare and cancer treatment. Particular embodiments thus include methods for providing the results of the responsiveness test to the subject in need thereof, or to the physician or other healthcare provider. These results or data can be in the form of a hard-copy or paper-copy, or an electronic form, such as a computer-readable medium.

All publications, patent applications, and issued patents cited in this specification are herein incorporated by reference as if each individual publication, patent application, or issued patent were specifically and individually indicated to be incorporated by reference.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to one of ordinary skill certain changes and modifications may be made thereto without departing from the spirit or scope of the description or appended claims. The following examples are provided by way of illustration only and not by way of limitation. Those of skill in the art will readily recognize a variety of noncritical parameters that could be changed or modified to yield essentially similar results.

## Examples

### Example 1

### Efficacy of YM155 Monobromide in Cancer Cell Lines

Studies were performed to evaluate the efficacy of YM155 monobromide in various cancer cell lines, and also to correlate the efficacy of YM155 monobromide to MYC gene copy number.

Lung Cell Culture. Human lung cancer cell line NCI-H1975, HCC827, NCI-H226 and HCC4006 cells were cultured in RPMI 1640 (Hyclone™, SH30809.01B) supplemented with 10% fetal bovine serum (GEMINI, 900-108). Cultures were incubated at 37°C in 5% $CO_2$. NCI-H1975, HCC827 and NCI-H226 were purchased from Cell Bank, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences. HCC4006 was purchased from CoBioer Biosciences Corporation (Nanjing, China).

Cell Treatment and proliferation assay. NCI-H1975, HCC827, NCI-H226 and HCC4006 cell were seeded in 96-well plates (Corning-Costar, 3603) at 2000 cells/well in 200µl culture medium for 24 hours, respectively. Then cells were treated with YM155 (50, 30, 20, 10, 2, 0.4, 0.08, 0.016 and 0.0032 nM; APExBIO, A4221) or DMSO (0.1%; Amresco, 67-68-5). After 72 hour incubation, cells were labeled with 5-Ethynyl-2'-deoxyuridine (EdU, final concentration at 1uM; Sigma, 900584) for additional 4h under the same culture condition.

After incubation with EdU, cells were fixed with formaldehyde (final concentration at 4%; Thermo, 28908) for 30 min at room temperature. The cells were washed twice with PBS (Hyclone™, SH30256.01) and then permeabilized with 0.5% Triton X-100 (T8787-250ML) in PBS overnight at 4°C. After discarding the supernatant, the cells were incubated with Hoechst 33342 (Invitrogen, H1399) for 1 hour at room temperature and then washed again twice with PBS.

Cells were incubated with staining mix (Beijing Percans Oncology Medical Research Co., Ltd., RUO-00401#150T) for 30 min at room temperature and then washed three times with PBS. The cells were kept in PBS and protected from light throughout experiments.

High content imaging analysis. The treated cells were scanned for image acquisition with CellInsight™ CX5 High-Content Screening (HSC) Platform (Thermo Fisher) that was equipped with filters for Hoechst 33342 (Ex: 386nm) and EdU (Ex: 560nm). The total cell count and EdU-positive cell count were analysis by measuring the signal intensity in the nuclear region.

As shown in **Figure 2**, YM155 inhibits cell proliferation of human lung cancer cell lines. NCI-H1975, HCC827, NCI-H226, and HCC4006 were exposed to different concentrations of YM155 for 72 hours. However, the proliferation inhibitory effects of YM155 were different in these cell lines, with the calculated $IC_{50}$ showed in **Table E1** below. NCI-H1975 and HCC827 with MYC gene amplification (increased MYC gene copy number relative to a reference) were significantly more sensitive to YM155 than NCI-H226 and HCC4006 without MYC gene amplification.

**Table E1. MYCC Gene Copy Number and YM155 $IC_{50}$ in Human Lung Cancer Cell Lines**

| | NCI-H1975 | HCC827 | NCI-H226 | HCC4006 |
|---|---|---|---|---|
| Copy Number | 12.1 | 6.44 | 2.93 | 2.25 |
| Expression | 7.64 | 7.2 | 6.39 | 2.78 |
| YM155 $IC_{50}$ (nm) | **4.122** | **2.015** | 15.99 | 10.73 |

Blood Cell Culture. Human acute myeloid leukemia cells (U937 and HL-60) and Human Burkitt's lymphoma cells (Ramos) were cultured in RPMI 1640 supplemented with 10% fetal bovine serum. Cultures were incubated at 37°C, in 5% $CO_2$. U937 and HL-60 cells were purchased from Cell Bank, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences. Ramos cells were purchased from CoBioer Biosciences Corporation (Nanjing, China). Ramos cells carry the IgH-c-MYC translocation mutation.

Cell Treatment and proliferation assay. U937, HL-60, and Ramos cells were seeded in 96-well plates (Corning-Costar, 3599) at 40000 cells/well in 200µl culture medium treated with YM155 (200, 100, 50, 25, 12.5, 2.5, 1, 0.5nM; APExBIO, A4221) or DMSO (0.1%; Amresco, 67-68-5). After 72 hour incubation, cell proliferation was measured by XTT assay (Cell Proliferation Kit II XTT, Sigma, 11465015001) according to manufacturer's protocols. Briefly, after YM155 treatment, 50 µl of the XTT labeling mixture was added to each well, followed by incubation of the microplate for 4 hours in a humidified atmosphere. The absorbance of wavelength at 490nm ($OD_{490}$) and 650nm ($OD_{650}$) was determined respectively by SpectraMax 190. The $IC_{50}$ was calculated by the value of $OD_{490}$- $OD_{650}$.

As shown in **Figure 3** and **Figure 4**, YM155 inhibits cell proliferation of human acute myeloid leukemia cells and Human Burkitt's lymphoma cells. U937, HL-60, and Ramos were exposed to different concentrations of YM155 for 72 hours. However, the proliferation inhibitory effects of YM155 were different in these cell lines, with the calculated $IC_{50}$ showed in **Table E2** below 5. HL-60 cells with MYC gene amplification (increased MYC gene copy number relative to a reference) and Ramos cells with MYC gene translocation were more sensitive to YM155 than U937 cells without MYC gene amplification or translocation.

**Table E2. MYCC Gene Copy Number and YM155 $IC_{50}$ in Leukemia/Lymphoma Cell Lines**

| | U937 | HL-60 | Ramos |
|---|---|---|---|
| Copy Number | 2.39 | 9.6 | IgH/c-MYC translocation |
| Expression | 8.22 | 9.35 | |
| YM155 $IC_{50}$ (nm) | 116 | **17.07** | **30.83** |

Neuroblastoma Cell Culture. Neuroblastoma cell lines IMR-32, NB-1, KP-N-YN, SK-N-BE, SK-N-SH, and SH-SY5Y cells were cultured in 96-well plates and treated with YM155 at indicated doses (nM). Human neuroblastoma cell line IMR-32 was cultured in MEM (Hyclone™, SH30024.01) supplemented with 10% fetal bovine serum (GEMINI, 900-108); neuroblastoma cell lines NB-1 and KP-N-YN were cultured in RPMI 1640 (HycloneTM, SH30809.01B) supplemented with 10% fetal bovine serum; neuroblastoma cell line SK-N-BE was cultured in F12 (Gibco,11765-054)/MEM(1:1) (HycloneTM, SH30024.01) supplemented with 10% fetal bovine serum; neuroblastoma cell line SH-SY5Y was cultured in in MEM/F12(1:1) supplemented with 10% fetal bovine serum; and neuroblastoma cell line SK-N-SH was cultured in MEM supplemented with 10% fetal bovine serum. Cultures were incubated at 37°C, in 5% CO2.

IMR-32, SH-SY5Y, and SK-N-SH were purchased from Cell Bank, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences. NB-1, KP-N-YN and SK-N-BE cells were purchased from CoBioer Biosciences Corporation (Nanjing, China).

Cell Treatment and proliferation assay. IMR-32, SH-SY5Y, SK-N-SH, NB-1, KP-N-YN, and SK-N-BE cells were seeded in 96-well plates (Corning-Costar, 3603) at 2000 cells/well in 200µl culture medium for 24 hours. Cells were then treated with YM155 (200, 100, 50, 25, 12.5, 6.25, 3.125, 1.5625 and 0.78125 nM; APExBIO, A4221) or DMSO (0.1%; Amresco, 67-68-5). After 72 hour incubation, cells were labeled with 5-Ethynyl-2′-deoxyuridine (EdU, final concentration at 1uM; Sigma, 900584) for additional 4 hours under the same culture condition.

After incubation with EdU, cells were fixed with Formaldehyde (final concentration at 4%; Thermo, 28908) for 30 min at room temperature. The cells were washed twice with PBS (HycloneTM, SH30256.01), then permeabilized with 0.5% Triton X-100 (T8787-250ML) in PBS overnight at 4°C. After discarding the supernatant, the cells were incubated with Hoechst 33342 (Invitrogen, H1399) for 1 hour at room temperature, then washed again twice with PBS. Cells were incubated with staining mix (Beijing Percans Oncology Medical Research Co., Ltd., RUO-00401#150T) for 30 minutes at room temperature, then washed the cells three times with PBS. The cells were kept in PBS and protected from light throughout experiments.

High content imaging analysis. The treated cells were scanned for image acquisition with CellInsight™ CX5 High-Content Screening (HSC) Platform (Thermo Fisher), equipped with filters for Hoechst33342 (Ex: 386nm) and EdU (Ex: 560nm). The total cell count and EdU-positive cell count were analysis by measuring the signal intensity in the nuclear region.

As shown in **Figure 7** and **Table E3** below, YM155 inhibits cell proliferation of neuroblastoma cell lines. It is especially effective at inhibiting cell proliferation of the IMR-32, NB-1, KP-N-YN, and SK-N-BE neuroblastoma cell lines, which are characterized by increased copy number of NMYC.

**Table E3. MYCN Gene Copy Number and YM155 in Neuroblastoma Cell Lines**

| | IMR-32 | NB-1 | KP-N-YN | SK-N-BE | SK-N-SH | SH-SY5Y |
|---|---|---|---|---|---|---|
| Copy Number | 16.4 | 26 | 22.88 | 19.57 | 2.8 | 2.9 |
| Expression | 8.5 | 8.5 | 9.55 | 10.14 | 4.09 | 4.25 |
| YM155 $IC_{50}$ (nm) | **0.62** | **11.71** | **5.89** | **2.46** | 59.06 | >220 |

These data illustrate that MYC gene amplification (i.e., increased MYC gene copy number relative to a reference) and MYC gene translocation can be used to predict responsiveness to YM155 monobromide therapy in cancer, including lung cancers, blood cell cancers, neuroblastomas, and others.

## Example 2

**HCC827/HCC 4006 Xenograft Experiment**

Four to six weeks-old female mice (Balb/C-nu) were purchased from BEIJING HFK BIOSCIENCE Co., Ltd. Cell suspensions (HCC827 (epithelial lung adenocarcinoma): $5x10^6$/0.1ml per mouse; HCC4006: $5x10^6$/0.1ml per mouse) of cell culture medium were inoculated subcutaneously into the right flank of the mice. When tumor size reached around $100mm^3$-$150mm^3$, mice were randomized into treatment and control groups. YM155 (Biochem partner, BCP01864) at 5mg/kg or vehicle (Saline, Shijiazhuang No.4 Pharmaceutical) was administered subcutaneously once daily for five days/week over three weeks. Body weight of mice was assessed twice weekly, and tumor diameter (long diameter and short diameter) was measured using standard calipers. The tumor volume = 1/2* long diameter* (short diameter)$^2$. After two or three weeks observation, mice were sacrificed for analysis. The data are presented in **Figures 9A-9B** and **Figures 10A-10B**.

**Example 3**

**RAMOS/U937 Xenograft Experiments**

Four to six weeks-old female mice (RAMOS: SCID; U937: NOD/SCID) were purchased from BEIJING HFK BIOSCIENCE Co., Ltd. Cell suspensions (RAMOS: $1x10^7$/0.1ml per mouse; U937: $2x10^6$/0.1ml + 0.1ml matrigel per mouse) of cell culture medium were inoculated subcutaneously into the right flank of the mice. When tumor size reached around $100mm^3$-$150mm^3$, mice were randomized into treatment and control groups. YM155 (Biochem partner, BCP01864) at 5mg/kg or vehicle (Saline, Shijiazhuang No.4 Pharmaceutical) was administered subcutaneously once daily for nine days. Body weight of mice was assessed twice weekly, and tumor diameter (long diameter and short diameter) was measured using standard calipers. The tumor volume=1/2* long diameter* (short diameter)$^2$. After two or three weeks observation, mice were sacrificed for analysis. The data are presented in **Figures 11A-11B** and **Figures 12A-12B**.

**Example 6**

**IMR-3/SH-SY5Y Xenograft Experiments**

Four to six weeks-old female mice (IMR-32: NOD/SCID; SH-SY5Y: Balb/C-nu) were purchased from BEIJING HFK BIOSCIENCE Co., Ltd. Cell suspensions (IMR-32: $5x10^6$/0.1ml + 0.1ml matrigel per mouse; SH-SY5Y: $2x10^6$/0.1ml + 0.1ml matrigel per mouse) of cell culture medium were inoculated subcutaneously into the right flank of the mice. When tumor size reached around $100mm^3$-$150mm^3$, mice were randomized into treatment and control groups. YM155 (Biochem partner, BCP01864) at 5mg/kg or vehicle (Saline, Shijiazhuang No.4 Pharmaceutical) was administered subcutaneously once daily for five days/week over 3 weeks. Body weight of mice was assessed twice weekly, and tumor diameter (long diameter and short diameter) was measured using standard calipers. The tumor volume=1/2* long diameter * (short diameter)$^2$. After two or three weeks

observation, mice were sacrificed for analysis. The data are presented in **Figures 13A-13B** and **Figures 14A-14B**.

## CLAIMS

1. A method for treating cancer in a subject in need thereof, comprising:

(a) determining MYC gene copy number, or MYC gene chromosomal location site, in a sample of cancer tissue from the subject; and

(b) administering YM155 monobromide [1-(2-Methoxyethyl)-2-methyl-4,9-dioxo-3-(pyrazin-2-ylmethyl)-4,9-dihydro-1H-naphtho[2,3-d] imidazolium bromide], or an analog or derivative thereof, to the subject if MYC gene copy number in the cancer tissue is increased relative to that of a MYC gene copy number reference, or if MYC gene chromosomal location site in the cancer tissue is translocated relative to that of a MYC gene chromosomal location site reference,

thereby treating cancer in the subject in need thereof.

2. The method of claim 1, comprising administering to the subject a chemotherapeutic agent excluding (or other than) YM155 monobromide if MYC gene copy number in the cancer tissue is not substantially increased relative to that of the MYC gene copy number reference, or if MYC gene chromosomal location site in the cancer tissue is not translocated relative to that of the MYC gene chromosomal location site reference.

3. A method for predicting therapeutic response to YM155 monobromide [1-(2-Methoxyethyl)-2-methyl-4,9-dioxo-3-(pyrazin-2-ylmethyl)-4,9-dihydro-1H-naphtho[2,3-d] imidazolium bromide], or an analog or derivative thereof, in a subject with cancer, comprising

(a) determining MYC gene copy number, or MYC gene chromosomal location site, in a sample of cancer tissue from the subject; and

(b) (i) characterizing the subject as responsive to YM155 monobromide therapy if MYC gene copy number in the cancer tissue is increased relative to that of a MYC gene copy number reference, or if the MYC gene chromosomal location site in the cancer tissue is translocated relative to that of a MYC gene chromosomal location site reference; or

(ii) characterizing the subject as non-responsive to YM155 monobromide therapy if MYC gene copy number in the cancer tissue is not substantially increased relative to that of the MYC gene copy number reference, or if the MYC gene chromosomal location site in the cancer tissue is not translocated relative to that of the MYC gene chromosomal location site reference,

thereby predicting therapeutic response to YM155 monobromide in the subject with cancer.

4. The method of claim 3, comprising administering YM155 monobromide to the subject if the subject is characterized as responsive to YM155 monobromide therapy.

5. The method of claim 3, comprising administering to the subject a chemotherapeutic agent excluding YM155 monobromide if the subject is characterized as non-responsive to YM155 monobromide therapy.

6. The method of any one of claims 1-5, wherein the MYC gene copy number in the cancer tissue is increased by about or at least about 1.5, 2, 3, 4, 5, 6, 7, 8, 9, or 10-fold relative to that of the MYC gene copy number reference.

7. The method of any one of claims 1-6, comprising determining MYC gene copy number in the cancer tissue by array comparative genome hybridization (aCGH), single nucleotide polymorphism (SNP) array, copy number variation (CNV) sequencing, or multiplex ligation-dependent probe amplification (MLPA).

8. The method of any one of claims 1-7, comprising determining MYC gene chromosomal location site in the cancer tissue by in situ hybridization (ISH), fluorescence in situ hybridization (FISH), next generation sequencing (NGS), or comparative genome hybridization (CGH).

9. The method of any one of claims 1-8, comprising obtaining the MYC gene copy number reference from a database, or determining the MYC gene copy number reference from a non-cancerous tissue from a control, optionally by aCGH, SNP array, CNV sequence, or MLPA.

10. The method of any one of claims 1-9, comprising obtaining the MYC gene chromosomal location site reference from a database, or determining the MYC gene chromosomal location site reference from a non-cancerous tissue from a control, optionally by ISH, FISH, NGS, or CGH.

11. The method of any one of claims 1-10, comprising obtaining the sample of cancer tissue from the subject.

12. The method of any one of claims 1-11, wherein the sample of cancer tissue is a surgical sample, a biopsy sample, a pleural effusion sample, or an ascetic fluid sample obtained from the subject, optionally selected from one or more of lung, blood, breast, gastrointestinal (stomach, colon, rectal), ovarian, pancreatic, liver, bladder, cervical, neuronal, uterine, salivary gland, kidney, prostate, thyroid, or muscle tissue.

13. The method of any one of claims 1-12, wherein the subject is a human subject.

14. The method of any one of claims 1-13, wherein the cancer is selected from one or more of carcinoma, sarcoma such as rhabdomyosarcoma for example, alveolar rhabdomyosarcoma, (including sarcoma originating in the bones, tendons, cartilage, muscle, fat, fibrous, blood vessels, adipose, and/or connective tissue), neuroblastoma, medulloblastoma, astrocytoma, glioblastoma multiforme, retinoblastoma, myeloma, leukemia, lymphoma (including Hodgkin's lymphoma and Non-Hodgkin's lymphoma), adenosquamous carcinoma, carcinosarcoma, mixed mesodermal tumor, teratocarcinoma), lung cancer (including non-small cell lung cancer, small cell lung cancer, adenocarcinoma, and squamous carcinoma of the lung), breast cancer (including metastatic breast cancer), gastrointestinal cancer, stomach cancer, colorectal cancer, colon cancer, rectal cancer, ovarian cancer, pancreatic cancer, liver cancer, bladder cancer, cervical cancer, glioblastoma, uterine carcinoma, salivary gland carcinoma, kidney or renal cancer (e.g., Wilm's tumor), prostate cancer, thyroid cancer, and head and neck cancer.

15. The method of any one of claims 1-14, wherein the MYC gene is selected from MYCC and MYCN.

16. The method of claim 15, wherein the MYC gene is MYCC and the cancer is selected from lung cancers and blood cancers, optionally leukemias and lymphomas.

17. The method of claim 15, wherein the MYC gene is MYCN and the cancer is selected from neuroblastoma, small cell lung cancer, prostate cancer, alveolar rhabdomyosarcoma, medulloblastoma, glioblastoma multiforme, retinoblastoma, and breast cancer.

18. Use of a diagnostic kit for determining therapeutic response to YM155 monobromide [1-(2-Methoxyethyl)-2-methyl-4,9-dioxo-3-(pyrazin-2-ylmethyl)-4,9-dihydro-1H-naphtho[2,3-d] imidazolium bromide], or an analog or derivative thereof, therapy in a subject with cancer, comprising means for measuring MYC gene copy number, or MYC gene chromosomal location site, in a sample of tissue from the subject, including cancer tissue and non-cancerous tissue.

19. The use of claim 18, wherein the means for measuring MYC gene copy number comprise reagents for performing a diagnostic assay selected from one or more of array comparative genome hybridization (aCGH), single nucleotide polymorphism (SNP) array, copy number variation (CNV) sequencing, and multiplex ligation-dependent probe amplification (MLPA) on a human MYC gene.

20. The use of claim 18 or 19, wherein the means for measuring MYC gene chromosomal location site comprise reagents for performing a diagnostic assay selected from one or more of in situ hybridization (ISH), fluorescence in situ hybridization (FISH), next generation sequencing (NGS), and comparative genome hybridization (CGH) on a human MYC gene.

21. The use of any one of claims 18-20, comprising a MYC gene copy number reference value obtained from a database, or determined from a non-cancerous tissue from a control.

22. The use of any one of claims 18-20, comprising a MYC gene chromosomal location site reference obtained from a database, or determined from a non-cancerous tissue from a control.

23. The use of any one of claims 18-22, wherein the cancer is selected from one or more of carcinoma, sarcoma such as rhabdomyosarcoma, for example, alveolar rhabdomyosarcoma (including sarcoma originating in the bones, tendons, cartilage, muscle, fat, fibrous, blood vessels, adipose, and/or connective tissue), neuroblastoma, medulloblastoma, astrocytoma, glioblastoma multiforme, retinoblastoma, myeloma, leukemia, lymphoma (including Hodgkin's lymphoma and Non-Hodgkin's lymphoma), adenosquamous carcinoma, carcinosarcoma, mixed mesodermal tumor, teratocarcinoma), lung cancer (including non-small cell lung cancer, small cell lung cancer, adenocarcinoma, and squamous carcinoma of the lung), breast cancer (including metastatic breast cancer), gastrointestinal cancer, stomach cancer, colorectal cancer, colon cancer, rectal cancer, ovarian cancer, pancreatic cancer, liver cancer, bladder cancer, cervical cancer, glioblastoma, uterine carcinoma, salivary gland carcinoma, kidney or renal cancer (e.g., Wilm's tumor), prostate cancer, thyroid cancer, and head and neck cancer

24. The use of any one of claims 18-23, wherein the MYC gene is selected from MYCC and MYCN.

25. The use of claim 24, wherein the MYC gene is MYCC and the cancer is selected from lung cancers and blood cancers, optionally leukemias and lymphomas.

26. The use of claim 24, wherein the MYC gene is MYCN and the cancer is selected from neuroblastoma, small cell lung cancer, prostate cancer, alveolar rhabdomyosarcoma, medulloblastoma, glioblastoma multiforme, retinoblastoma, and breast cancer.

27. A patient care kit, comprising:

(a) means for measuring MYC gene copy number, or MYC gene chromosomal location site, in a sample of tissue from a subject, including cancer tissue and non-cancerous tissue; and

(b) YM155 monobromide [1-(2-Methoxyethyl)-2- methyl-4,9-dioxo-3-(pyrazin-2-ylmethyl)-4,9-dihydro-1H-naphtho[2,3-d] imidazolium bromide], or an analog or derivative thereof.

28. The patient care kit of claim 27, wherein the means for measuring MYC gene copy number comprise reagents for performing a diagnostic assay selected from one or more of array comparative genome hybridization (aCGH), single nucleotide polymorphism (SNP) array, copy number variation (CNV) sequencing, and multiplex ligation-dependent probe amplification (MLPA) on a human MYC gene.

29. The patient care kit of claim 27 or 28, wherein the means for measuring MYC gene chromosomal location site comprise reagents for performing a diagnostic assay selected from one or more of in situ hybridization (ISH), fluorescence in situ hybridization (FISH), next generation sequencing (NGS), and comparative genome hybridization (CGH) on a human MYC gene.

30. The patient care kit of any one of claims 27-29, comprising a MYC gene copy number reference value obtained from a database, or determined from a non-cancerous tissue from a control.

31. The patient care kit of any one of claims 27-30, comprising a MYC gene chromosomal location site reference obtained from a database, or determined from a non-cancerous tissue from a control.

32. The patient care kit of any one of claims 27-31, wherein the MYC gene is selected from MYCC and MYCN.

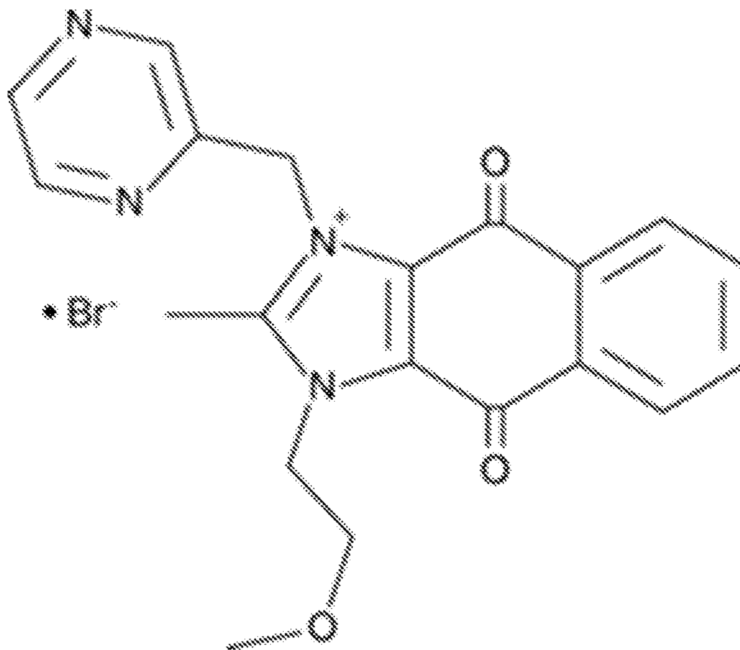



FIG. 1

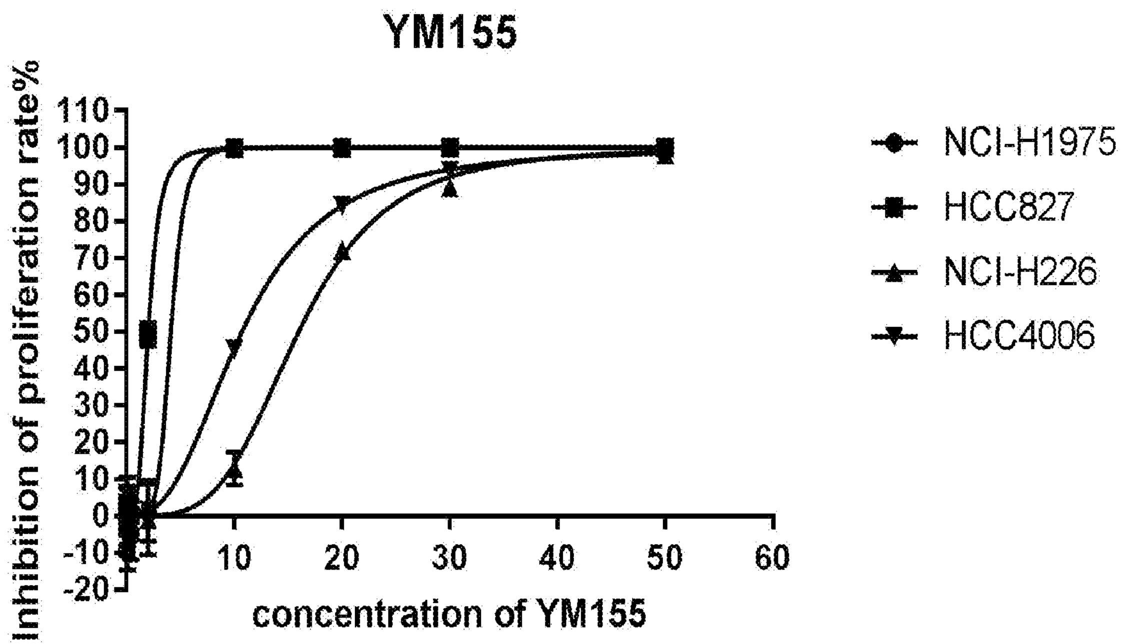



FIG. 2

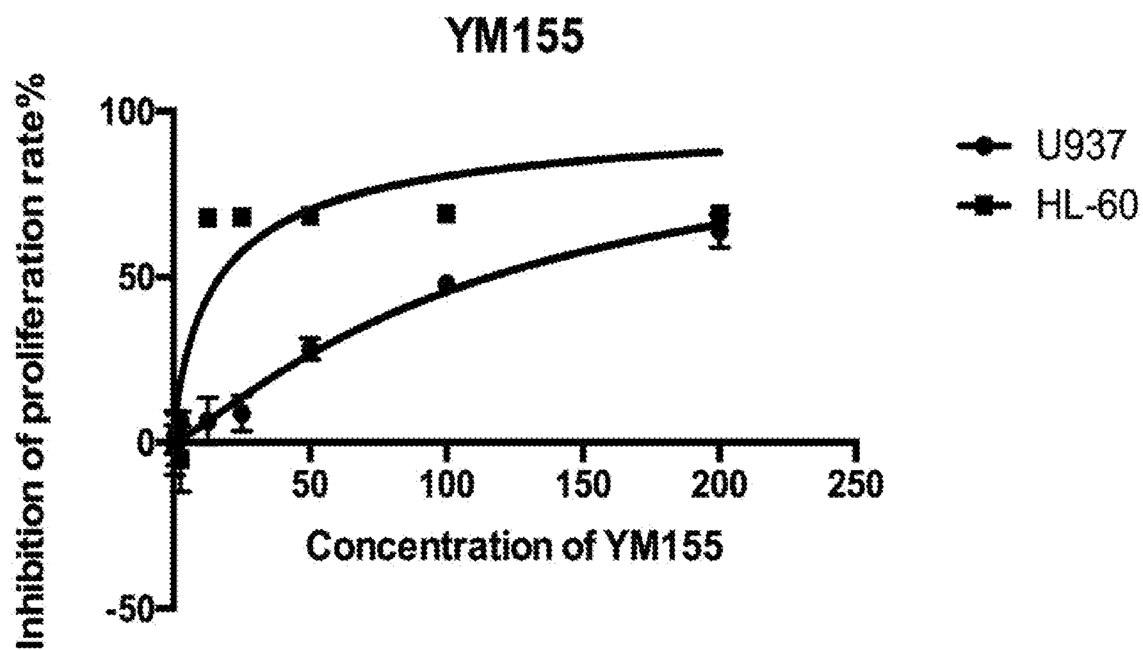



FIG. 3

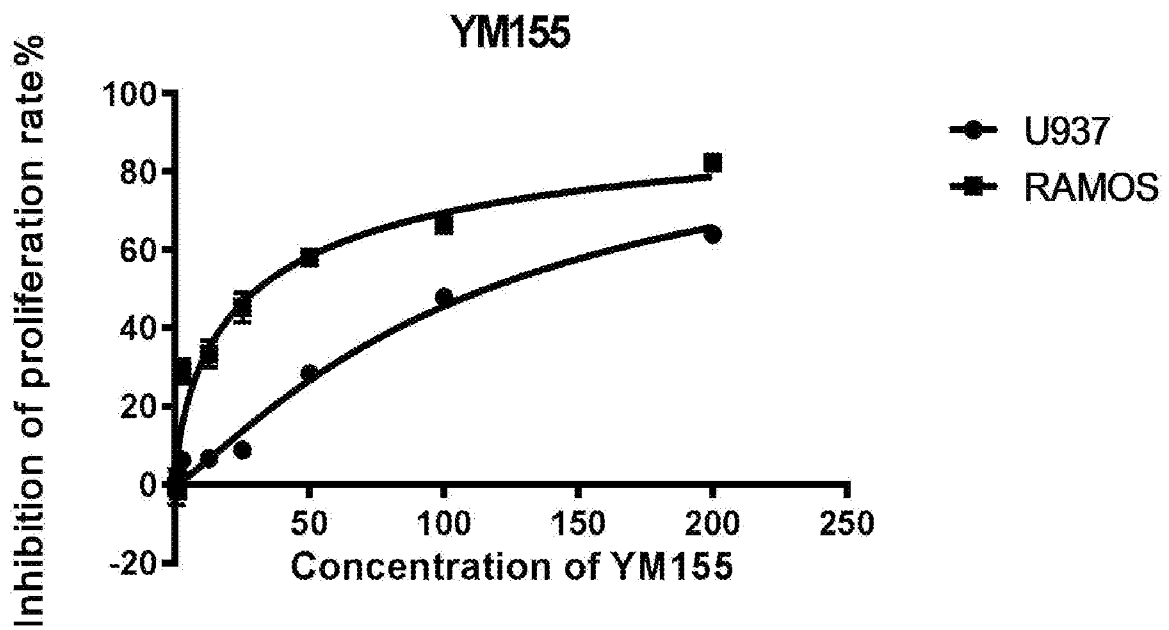
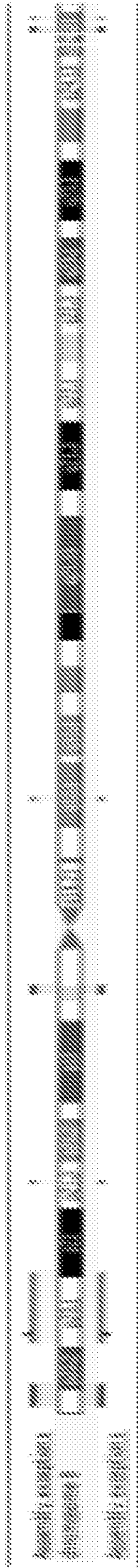



FIG. 4



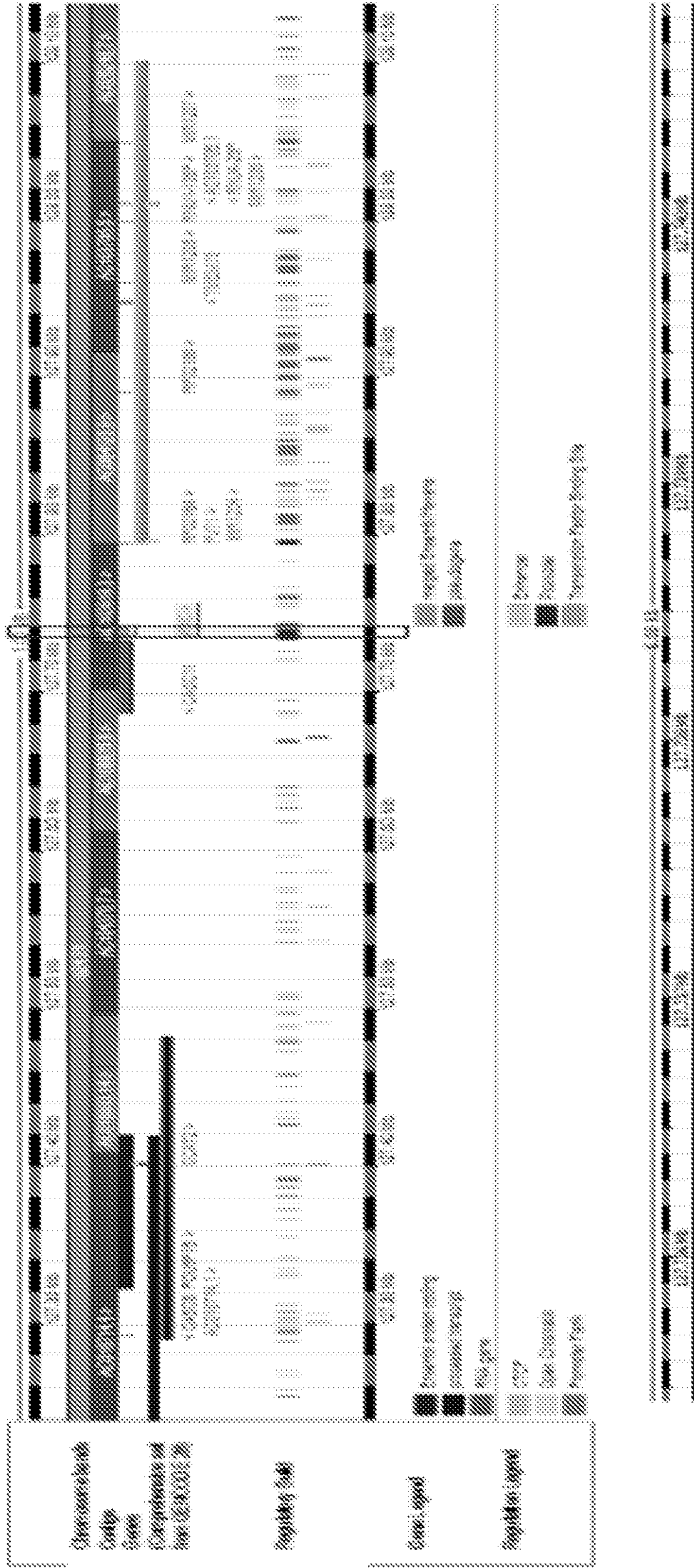




FIG. 5

FIG. 6

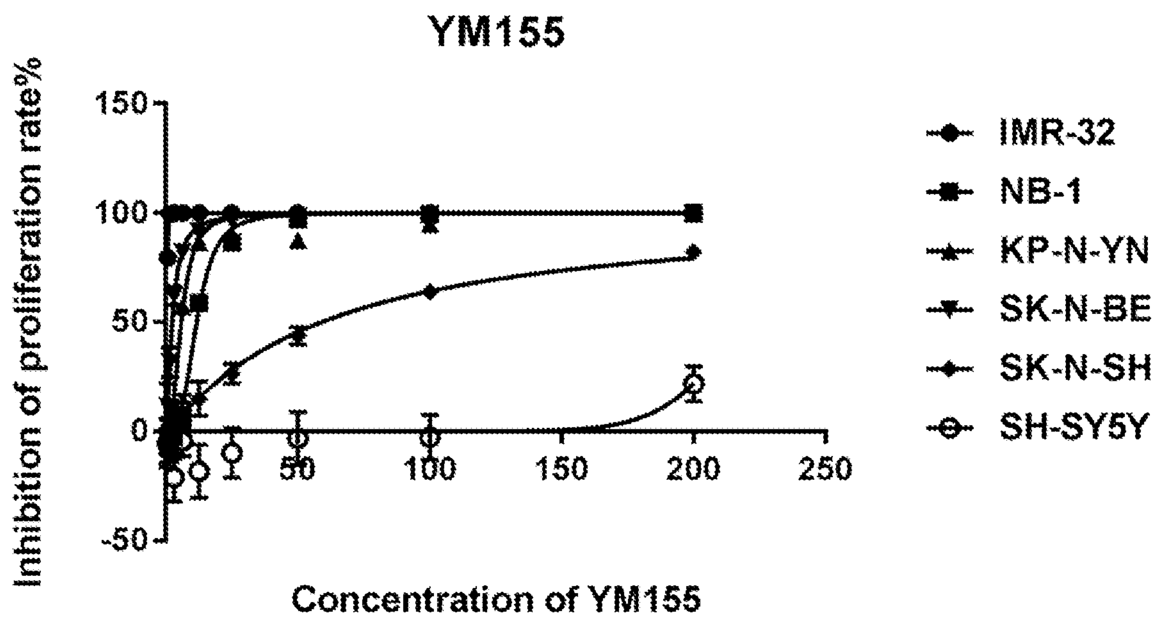



FIG. 7

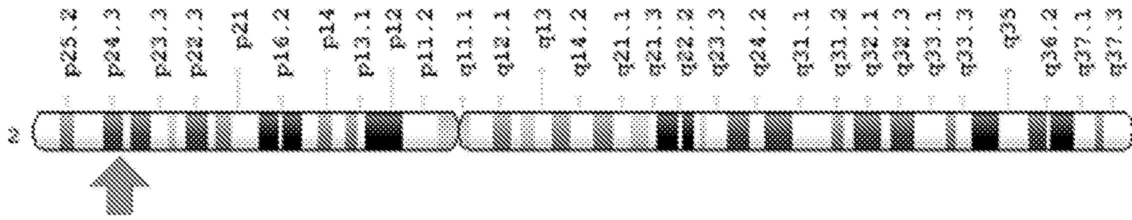
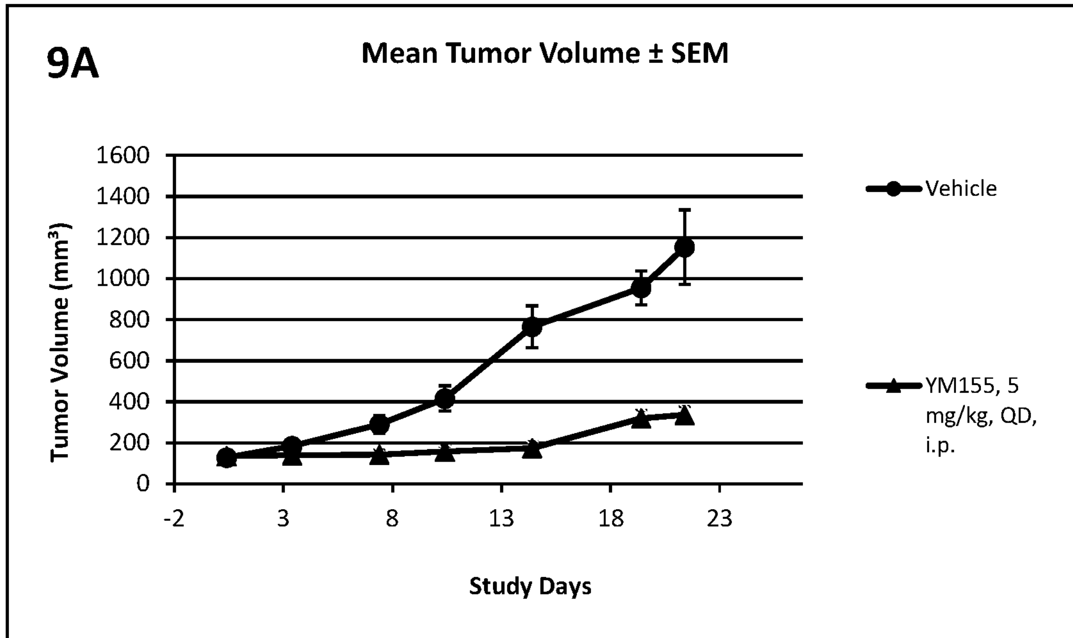
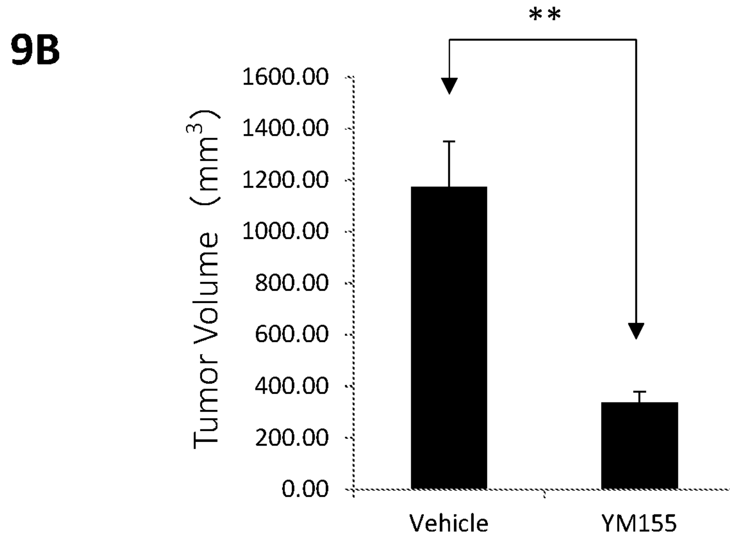
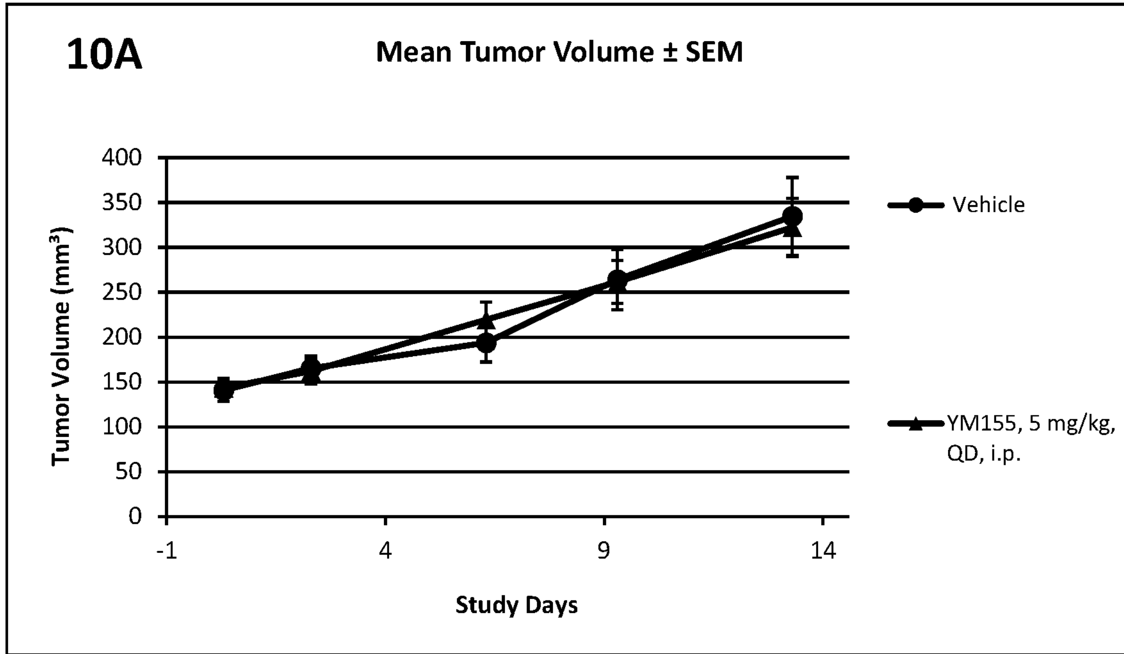


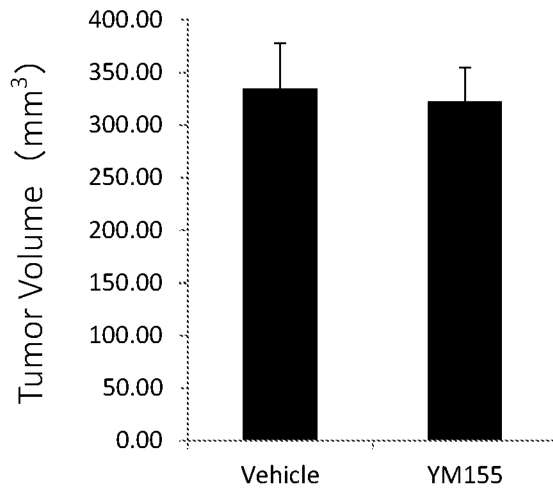

**FIG. 8**



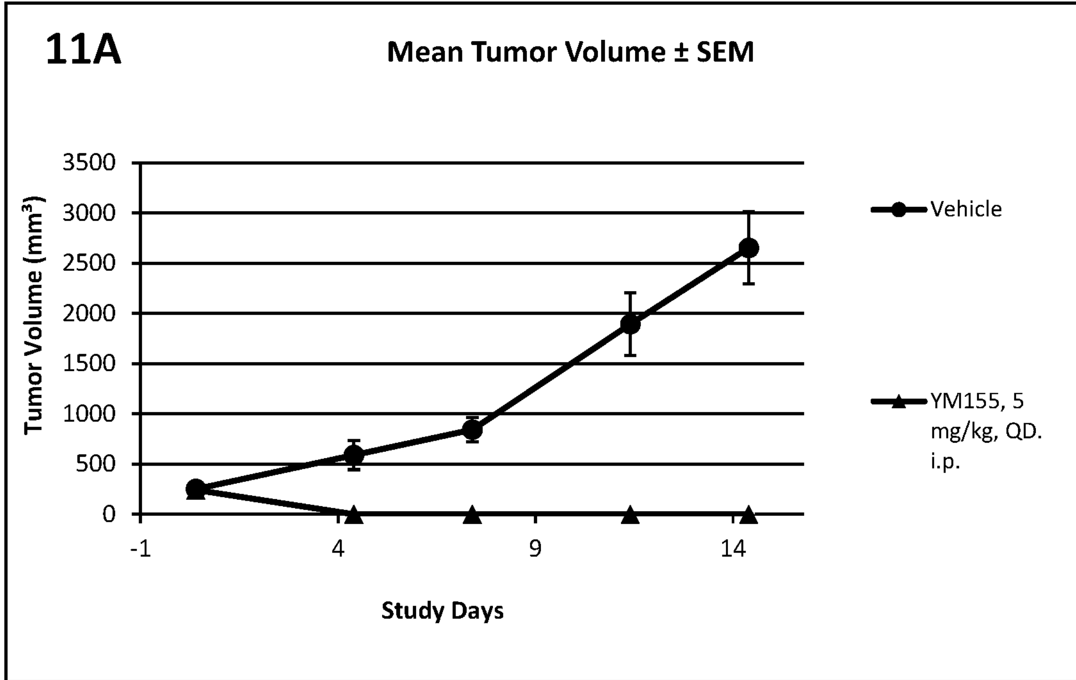



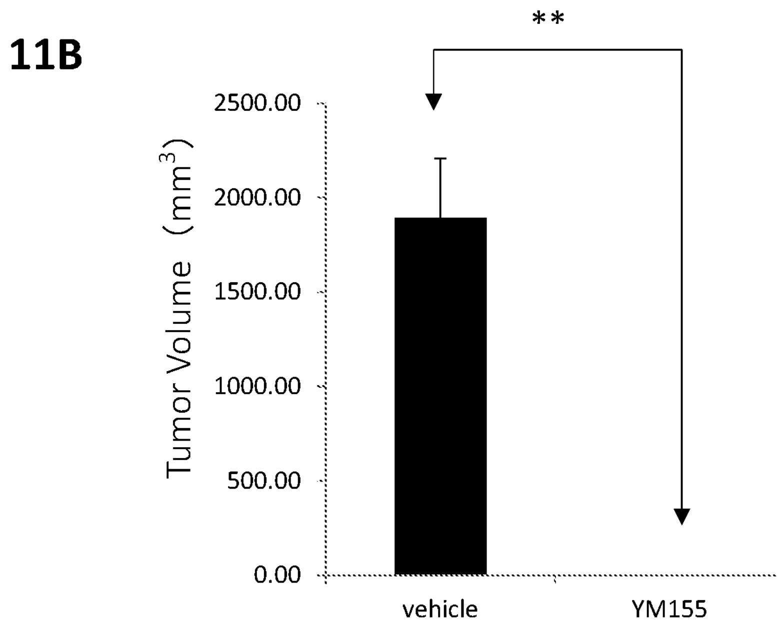

**FIGs. 9A-9B**



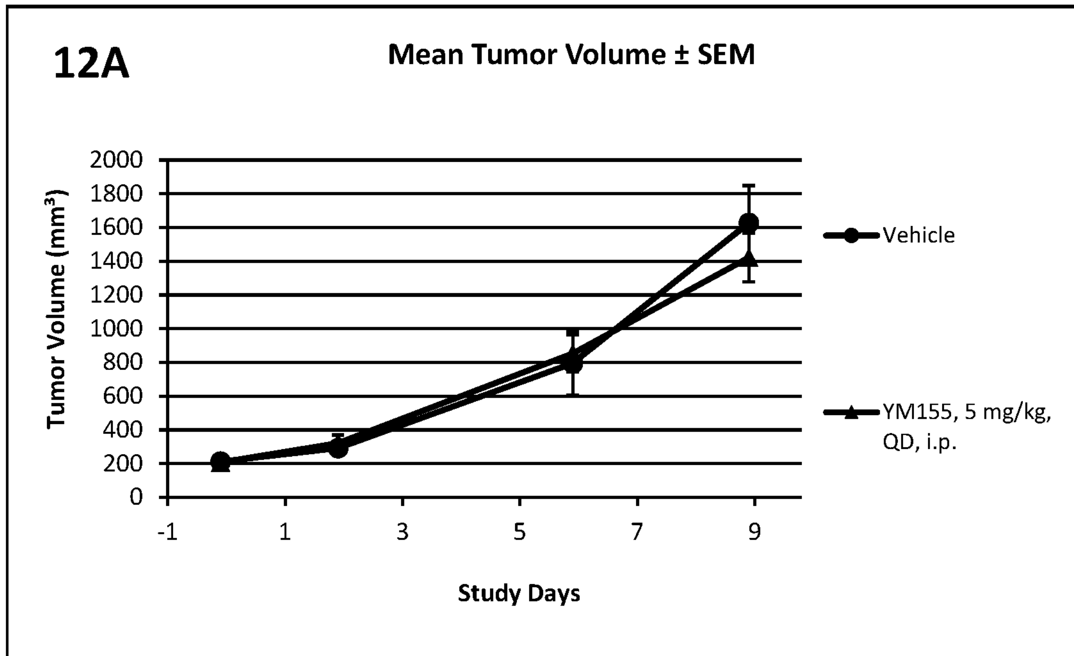



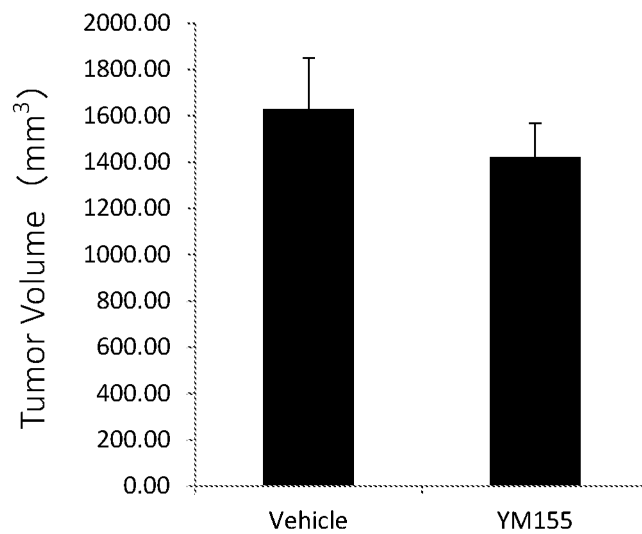

**FIGs. 10A-10B**



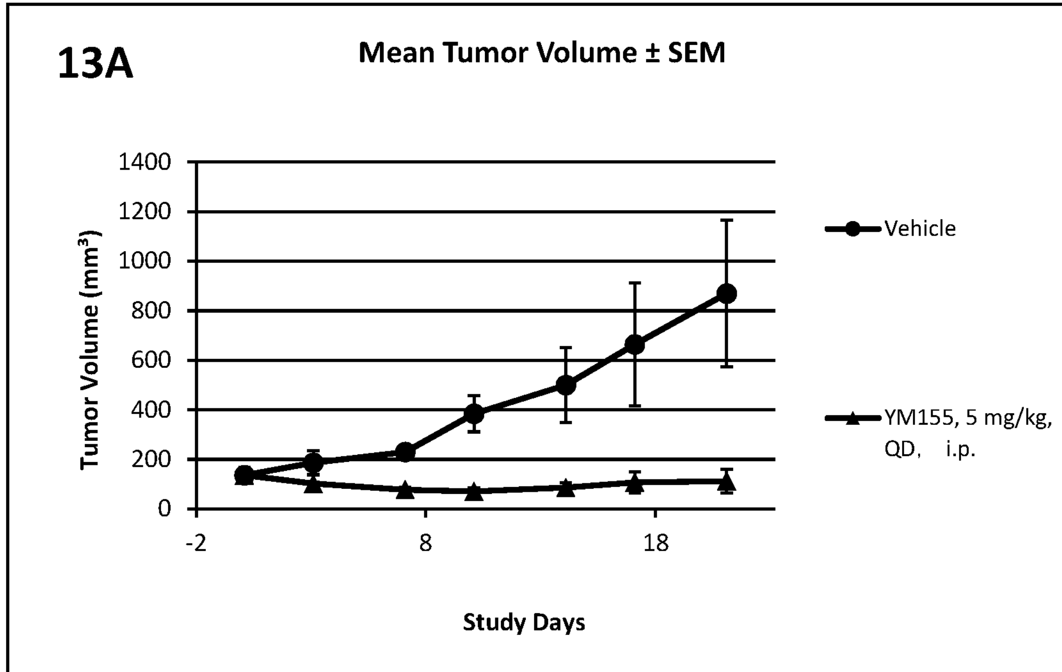



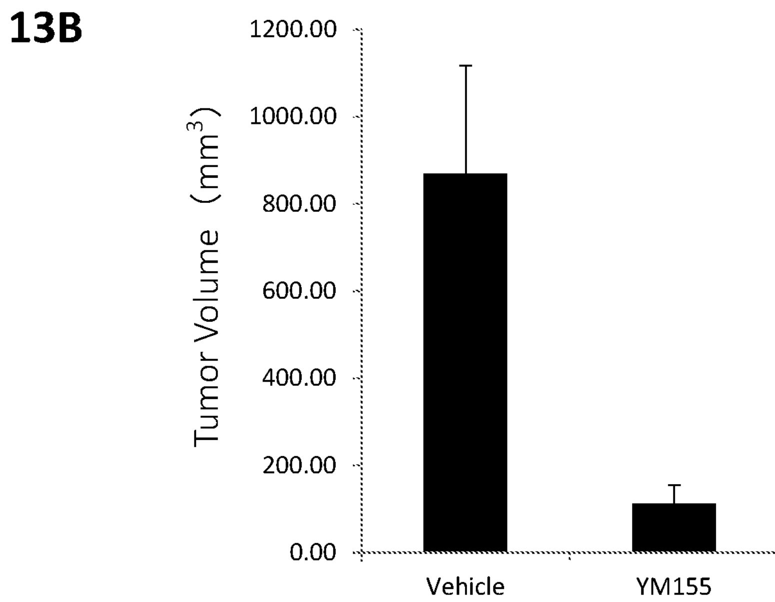

**FIGs. 11A-11B**



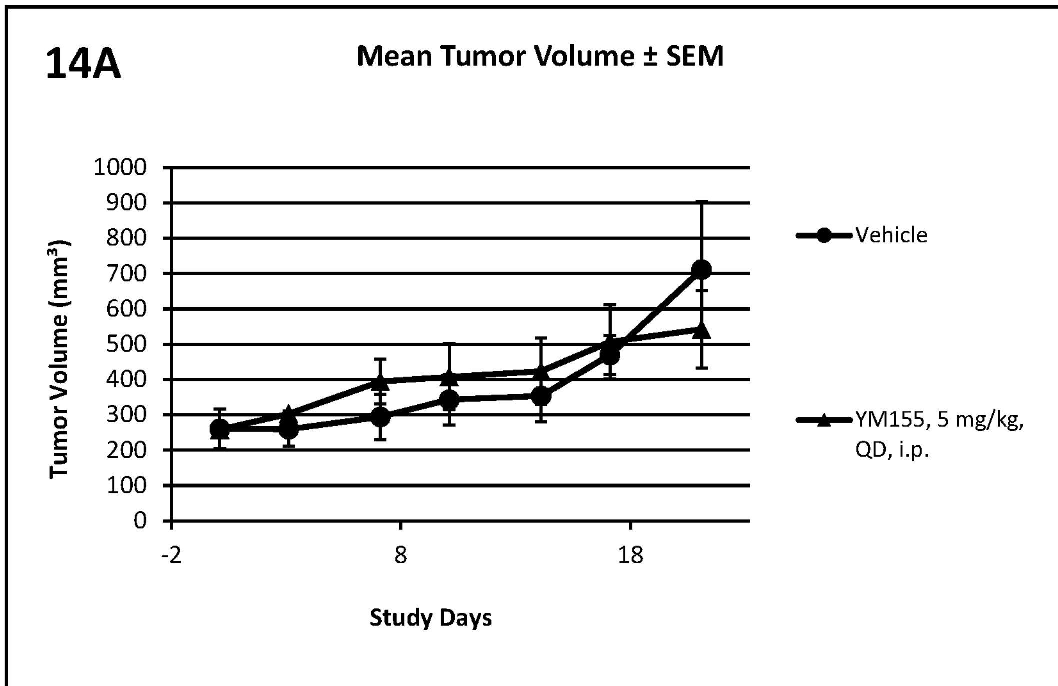



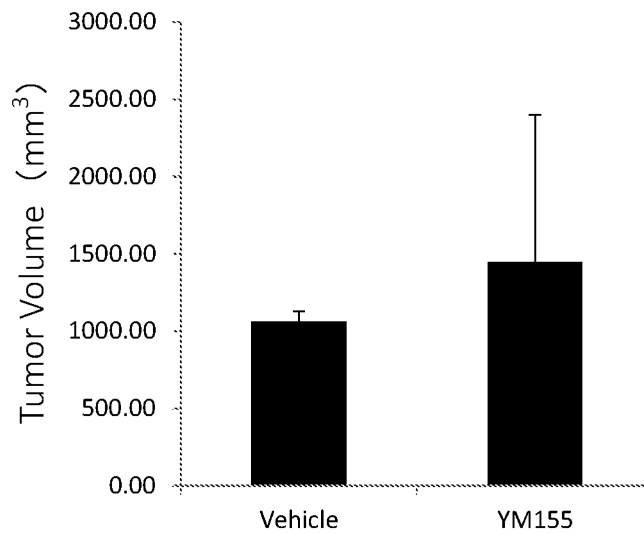

**FIGs. 12A-12B**




**FIGs. 13A-13B**




**FIGs. 14A-14B**

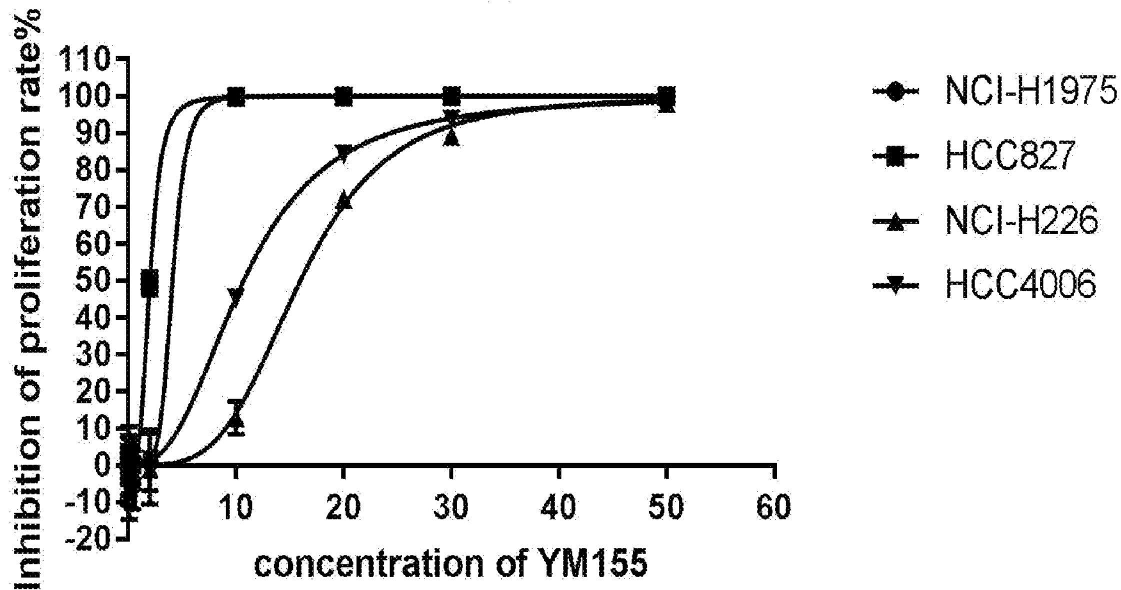



**FIG. 2**