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(71) Applicant *(for all designated States except US)*: **THE TRANSLATIONAL GENOMICS RESEARCH INSTITUTE** [US/US]; 445 N. 5th Street, Suite 600, Phoenix, AZ 85004 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **BRUCKHEIMER, Elizabeth** [US/US]; 2523 E. Beryl Avenue, Phoenix, AZ 85028 (US). **KIEFER, Jeff** [US/US]; 2523 E. Beryl Avenue, Phoenix, AZ 85028 (US). **MOUSSES, Spyro** [CA/US]; 7328 E. Rustling Pass, Scottsdale, AZ 85255 (US). **SHANMUGAM, Kandavel** [CA/US]; 3881 West Jasper Drive, Chandler, AZ 85226 (US). **YIN, Hongwei (holly)** [CN/US]; 9305 E. Pine Valley Road, Scottsdale, AZ 85260 (US). **WEITMAN, Steve** [US/US]; 9305 E. Pine Valley Road, San Antonio, TX (US).

(74) Agent: **FULLER, Rodney, J.**; Fennemore Craig, P.C., 3003 North Central Avenue, Suite 2600, Phoenix, AZ 85012 (US).

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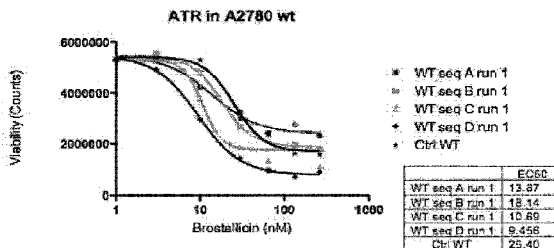
(54) **Title**: METHOD OF ASSESSING SENSITIVITY TO BROSTALLICIN

**FIG. 1A**




| | EC50 |
|---|---|
| WT seq A run 1 | 13.87 |
| WT seq B run 1 | 18.14 |
| WT seq C run 1 | 10.69 |
| WT seq D run 1 | 9.456 |
| Ctrl WT | 25.40 |

(57) **Abstract**: This invention relates to methods assessing resistance or sensitivity of cancer cells and patients to treatment with brostallicin. The method typically comprises: obtaining a cancer cell sample; assessing the expression of at least one sensitizing target in the cancer cell sample; determining if expression of the target in the sample is reduced based on a control; and correlating reduced expression of the target in the cancer cell sample with sensitivity to brostallicin. The invention is also directed to a diagnostic kit for assessing sensitivity of a cancer cells to brostallicin. The kit preferably comprises one or more reagents that detect the level expression of at least one target in the cancer cell sample; and a reporter element that indicates whether the cell sample is sensitive to brostallicin.

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# METHOD OF ASSESSING SENSITIVITY TO BROSTALLICIN

## CROSS REFERENCE TO RELATED APPLICATION

This application claims the benefit of US Provisional Application 61/044,291, filed April 11, 2008 and US Provisional Application 61/080,774, filed July 15, 2008, which each is incorporated in its entirety.

## FIELD OF THE INVENTION

This invention relates to methods and diagnostic kits for assessing whether or not a
10 particular cancer patient will respond to a particular drug, more specifically to the drug brostallicin (PNU-166196).

## BACKGROUND OF THE INVENTION

Despite major advances in the fields of genomics and drug discovery technologies, cancer still kills about 500,000 people per year in the United States. Many new agents with excellent anticancer properties have emerged recently, but most of them fail during the later phases of clinical drug development. Part of the problem is that cancer genomes acquire a variable set of genomic aberrations that are different between cancers. Furthermore, the impact of specific genetic changes on an individual's responsiveness to specific cancer drugs
20 is often unknown. Consequently, current clinical development is largely empirical, lacking molecular intelligence to guide the development towards responsive populations. This lack of insight leads to very large, inefficient clinical trials with unfocused populations that ultimately fail to demonstrate significant therapeutic benefit. Clearly, there is a great need for molecular intelligence to ensure that the development of promising new drugs are focused on cancers that will be particularly vulnerable, and on developing the most rational strategies for drug combinations.

Brostallicin is a synthetic DNA minor groove binder, known to slow the expansion of tumors by killing cancer cells. It also displays synergism in combination with standard cytotoxic agents and targeted therapies in preclinical experimental tumor models. Brostallicin
30 acts by binding covalently to the DNA minor groove, thus interfering with DNA replication. This mechanism of action ultimately leads to tumor cell death. Not all cancer patients respond to a treatment with brostallicin, as some are resistant.

In view of the high morbidity and mortality associated with non-responsive cancer treatments, there is a pronounced need to develop a method and/or diagnostic kit that is able

to determine which patients will respond to a treatment with brostallicin and which will not. The present invention meets these needs, allowing the doctor to selectively pick those cancer patients that will likely respond to treatment with brostallicin from those which should be treated with an alternative therapy drug, thereby saving money and critical treatment time.

## BRIEF SUMMARY OF THE INVENTION

The present invention is based on the discovery that alterations in expression of certain cancer cell targets can be correlated with resistance or sensitivity to brostallicin treatments. The inventors discovered that alterations in genes involved in DNA repair, genes involved in translesion synthesis, and genes involved in histone modification that result in the reduced expression of the target typically increased sensitivity to the drug brostallicin.

It is an object of the invention to provide a method for assessing sensitivity of a cancer cell to treatment with a drug, more specifically treatment with brostallicin. It is also an objective to provide a method of selectively treating cancer patients likely to respond to treatment with brostallicin. It is still further an objective to provide a diagnostic kit suitable for assessing whether treatment of a cancer patient with brostallicin would be effective.

The above and other objectives are met using the methods and kits disclosed herein.

In a preferred embodiment, the invention is directed to a method of assessing sensitivity of a cancer cell to brostallicin. In this embodiment, the method preferably comprises the steps of obtaining a cancer cell sample and assessing the expression of at least one target in the cancer cell sample selected from the group consisting of: ATF1, ATR, BRCA1, BRCA2, CCNG2, EphA5, GNB4, IL13RA2, INPP4B, MCL1, MINPP1, MSH2, NFKBIZ, RAD18, RKHD3, SIAH2, SLC4A10, STK32B, SUV39H2, TRIM7, VHL, and WNK3. Typically, the method further comprises the step of determining if expression of the target in the sample is reduced based on a control; and correlating reduced expression of the target in the cancer cell sample with sensitivity to brostallicin. In certain embodiments, it is preferred that at least two targets have reduced expression.

The cancer cell sample can be of any type of cancer, preferably from bladder cancer, breast cancer, colon cancer, kidney cancer, liver cancer, lung cancer, esophagus cancer, gall bladder cancer, ovarian cancer, pancreas cancer, stomach cancer, cervical cancer, thyroid cancer, prostate cancer, skin cancer, leukemia, B-cell lymphoma, T-cell lymphoma, Hodgkins, lymphoma, non-Hodgkins lymphoma, hairy cell lymphoma, Burkett's lymphoma, fibrosacroma, rhabdomyosarcoma, astrocytoma, neuroblastoma, glioma and schwannomas, melanoma, seminoma, teratocarcinoma, osteosarcoma, xenoderoma pigmentosum,

keratoctanthoma, thyroid follicular cancer, or Kaposi's sarcoma. In a preferred aspect, the cancer sample is breast cancer; ovarian cancer; colon cancer; and pancreatic cancer.

The step of assessing the expression of the target preferably comprises detecting a genomic aberration that reduces expression of the target; measuring mRNA, and/or measuring protein. Detecting a genomic aberration can be accomplished using many techniques; preferably it comprises use of comparative genomic hybridization; array-based comparative genomic hybridization; detecting an inactivating mutation; PCR; and/or DNA sequencing. Measuring mRNA typically involves at least one of the following: RTPCR; microarray analysis; and Northern Blot, whereas measuring protein typically involves at least one of the following: ELISA; mass spectrometry; and Western Blotting.

Reduction in expression of the target is preferably reduced by at least 60%, more preferably at least 70%, and even more preferably at least 75% as compared to a brostallicin resistant control. For example, in one specific embodiment, the expression of the target is less than 20% and more preferably less than 10% as compared to the brostallicin resistant control.

In a preferred embodiment, a control is used to determine if expression of the target is reduced. Preferred controls include: brostallicin resistant cells, brostallicin sensitive cells, lists or databases comprising a log of genomic aberrations that affect expression and/or a log of expression levels of the target in brostallicin resistant cells and/or brostallicin sensitive cells.

In another embodiment, the invention is directed to a method of identifying and treating a cancer patient who may benefit from a treatment with brostallicin drug. The method preferably comprises the steps of obtaining a cancer cell sample from the patient and assessing expression of at least one target in the cancer cell sample. Preferably the method further comprises the step of comparing expression of the target in the cancer cell sample to a control and/or administering an effective amount of brostallicin to the patient when expression of the target is reduced based on a control indicating sensitivity to brostallicin. In a preferred embodiment, the target is ATR, BRCA1, BRCA2, MSH2, RAD18, and VHL

In yet another embodiment, the invention is directed to a diagnostic kit for assessing sensitivity of a cancer cell to brostallicin. The kit preferably comprises one or more reagents that detects the level of expression of at least one target in the cancer cell sample selected from the group consisting of: ATF1, ATR, BRCA1, BRCA2, CCNG2, EphA5, GNB4, IL13RA2, INPP4B, MCL1, MINPP1, MSH2, NFKBIZ, RAD18, RKHD3, SIAH2, SLC4A10, STK32B, SUV39H2, TRIM7, VHL, and WNK3. The kit typically further comprises a reporter element. The reporter element preferably visually indicates whether

expression of the target in the sample is reduced compared to a control indicating sensitivity to brostallicin. The report element, for example, can be a hybridization probe capable of hybridizing to polynucleotides corresponding to target genes and reagents for detecting hybridization. In another embodiment, the reporting element comprises antibodies capable of specifically binding protein products of pre-selected targets and reagents for detecting antibody binding.

Aspects and applications of the invention presented here are described below in the drawings and detailed description of the invention. Unless specifically noted, it is intended that the words and phrases in the specification and the claims be given their plain, ordinary, and accustomed meaning to those of ordinary skill in the applicable arts. The inventors are fully aware that they can be their own lexicographers if desired. The inventors expressly elect, as their own lexicographers, to use only the plain and ordinary meaning of terms in the specification and claims unless they clearly state otherwise and then further, expressly set forth the “special” definition of that term and explain how it differs from the plain and ordinary meaning. Absent such clear statements of intent to apply a “special” definition, it is the inventors’ intent and desire that the simple, plain and ordinary meaning to the terms be applied to the interpretation of the specification and claims.

The inventors are also aware of the normal precepts of English grammar. Thus, if a noun, term, or phrase is intended to be further characterized, specified, or narrowed in some way, then such noun, term, or phrase will expressly include additional adjectives, descriptive terms, or other modifiers in accordance with the normal precepts of English grammar. Absent the use of such adjectives, descriptive terms, or modifiers, it is the intent that such nouns, terms, or phrases be given their plain, and ordinary English meaning to those skilled in the applicable arts as set forth above.

## BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

A more complete understanding of the present invention may be derived by referring to the detailed description when considered in connection with the following illustrative figures.

FIGS. 1A-C show (A) ATR in A2780 wt; (B) ATR in A2780 cis; and (C) a western blot of expression of ATR. ATR is a brostallicin sensitizer. All four siRNA’s appear to knock down the protein relative to the scrambled control in both the parental and cisplatin resistant A2780 cell lines.

FIGS. 2A-C show (A) BRCA1 in A2780 wt; (B) BRCA1 cis; and (C) a western blot of expression of BRCA1, a brostallicin sensitizer.

FIGS. 3A-C show (A) MSH2 in A2780 wt; (B) MSH2 in A2780 cis; and (C) a western blot of expression of MSH2. MSH2 – a Brostallicin sensitizer in ovarian cancer cells. All four siRNA's appear to knock down the protein relative to the scrambled control in both the parental and cisplatin resistant A2780 cell lines. The knockdown seems to be more variable between siRNA's in the A2780 parental line, while the knockdown in the cisplatin resistant line is more uniform among the 4 siRNA's. The antiproliferative studies, MSH2 siRNA B and C demonstrated a greater effect in the cisplatin resistant cell line.

10 FIGS. 4A-B show (A) BRCA2 in A2780 wt and (B) BRCA2 in A2780 cis.

FIGS. 5A-B show (A) VHL in A2780 wt and (B) VHL in A2780 cis.

FIG. 6 shows ATR in MDA-MB-231.

FIG. 7 shows MSH2 in MDA-MB-231.

FIG. 8 shows MCL1 in BT474.

FIG. 9 depicts COMPARE graphs of G150 values from two independent experiments from NCI Development Therapeutics Program. Horizontal bar length (left=resistant and right =sensitive) is proportional to the sensitivity of cell line to brostallicin relative to the mean of all cell lines.

FIG. 10 shows a correlation analysis between two NCI-60 Brostallicin experiments.

20 A quantitative comparison of the two runs was conducted to illustrate concordance to cell line response with a correlation between runs of 0.82.

FIG. 11 shows a list of selected sensitive and resistant cell lines in response to brostallicin. Designation of response categories were chosen by identifying splits within the GI50 response data that resulted in at least one order of magnitude between the two response categories.

FIGS. 12A-B depict Differential Gene Expression Between Sensitive and Resistant Cell Lines**.** (A) Listing of differentially expressed probe sets. A random-variance t-test was used to identity differentially expressed genes between the two classes of cell lines identified in FIG. 11. A stringent significance threshold ($p<0.001$) was used to limit the number of

30 false positive findings. (B) Hierarchical clustering of differentially expressed genes. The statistically significant genes were clustered across all cell lines for visualization of their expression across the full NCI-60 cell line panel. The cell lines coded in green belong to the sensitive class used for differential gene expression analysis and the red coded cell lines

belonging to the resistant class. The black coded cell lines were not used in the original class comparison gene expression analysis.

FIGS. 13A-C is a Gene Set Enrichment Analysis (GSEA) of Brostallicin Response in NCI-60. (A) Top 20 Gene Sets. GSEA was performed on the NCI-60 (U133A/B RMA) gene expression data using the average GI50 response data as a correlation ranking metric. The database of gene sets was composed of a 'high-quality annotated' set of Gene Ontology categories in the biological process, molecular process and cellular component categories. The top twenty gene sets correlated with sensitive cell lines (top) and resistant cell lines (bottom). We observed a number of gene sets associated with DNA repair mechanisms were correlated to brostallicin sensitivity. These sets are highlighted in yellow. (B) Representative Enrichment Plot and Heat-Map. This is an example enrichment plot for the nucleotide-excision repair GO category. A heat map representation of the genes in the category and displaying their respective expression levels is illustrated below the enrichment plot. (C) Leading Edge Analysis. Enrichment gene sets scored in GSEA analysis often contain a sub-set of genes that drive the enrichment toward the correlated phenotype/class. Given the number of sets in DNA repair categories that correlate with brostallicin sensitivity a leading edge analysis was performed to identify those genes (top) that are driving the gene set enrichment. This type of strategy was additionally applied to identify supplemental genes for siRNA library. The interactions between the DNA repair leading edge genes is illustrated in direct interaction network constructed in the MetaCore pathway database tool (GeneGO).

FIGS. 14A-D is a GSEA Analysis of Brostallicin and Additional Minor Groove Binding Agents. Three additional minor groove binding (MGB) agents, with appropriate NCI-60 response vectors, were chosen and submitted to GSEA analysis using the GO gene set library as in FIG. 12. (A) Hierarchical clustering of GO gene set enrichments. The normalized enrichment score (NES) for each MGB agent was used for hierarchical clustering (correlation, average linkage) after the application of variance filter to minimize unchanged GO sets. The red color in the heat map represent those gene sets that are correlated with MGB resistance and blue represent those associated with MGB sensitivity. (B) GO categories enriched for brostallicin sensitivity. This is an enlarged portion of the total dendrogram that highlights those GO categories that are specific for brostallicin sensitivity compared to the other MGB agents. Note the presence of glutathione transferase activity which is in line with the brostallicin mechanism of action. (C) GO categories enriched for brostallicin resistance. Enlarged portion of the total dendrogram corresponding to those GO categories that are preferentially associated with brostallicin resistance. One potential

interesting signaling category, JAK-STAT Cascade is highlighted. (D) GO categories enriched for Yondelis resistance. Enlarged portion of the total dendrogram corresponding to those GO categories that are preferentially associated with Yondelis resistance. Inspection of these GO categories highlight differences in the possible mechanism of action of Yondelis compared to that of other agents such as brostallicin.

FIGS. 15A-D shows the High Throughput RNAi Screen Results. (A) Work flow for the Brostallicin sensitizer identification. (B) Heat map of raw data for all 270 384-well plates in HTS. (C) Plate uniformity measure by CV in the controls wells, using the buffer-only controls. (D) Plate-to-plate variation and drug effects across the whole screen measured by
10 the raw data from in each plate.

FIGS. 16A-D is an Enrichment Analysis of Brostallicin siRNA Confirmation Data. (A-C) Gene ontology analysis. GO analysis was performed to highlight specific molecular concepts, in the form of gene ontology categories, that are enriched in the brostallicin siRNA confirmation list. Briefly, the confirmation list from the HT-RNAi screen was interrogated via GoMiner (discover.nci.nih.gov/gominer/) and results visualized with the VennMaster utility. (A) represents those gene ontology categories enriched ($p<0.05$) in all three gene ontology categories. (B) are those enriched categories in the biological process category and (C) represents those enriched in the cellular component category. (D) Pathway Enrichment Analysis. A complementary enrichment analysis was performed to identify those specific
20 pathways that are enriched in the brostallicin siRNA confirmation list. The analysis was performed in the MetaCore database (GeneGO) using the GeneGO processes data set.

FIGS. 17A-B show potential sensitizers for Brostallicin. siRNA against potential sensitizers (BRCA1 & BRCA2) were reversed transfected into A2780 cisplatin-resistant cell line followed by treatment with 6 different concentrations (specified on X-axis) of Brostallicin. After 96hr of drug treatment, cell viability was measured using CellTiter Glo. IC50 was calculated for each siRNA as well as GFP siRNA control by Prism 5 (GraphPad).

FIG. 18 shows a schematic of the DNA repair associated hits in the brostallicin siRNA screen. Brostallicin sensitivity hits in grey and bolded. That ATR/RAD18 hits are intimately associated with a DNA repair process called translesion synthesis (TLS). TLS is a
30 mechanism during DNA replication in which the standard DNA polymerase is temporarily exchanged for a specialized polymerase that can synthesize DNA across base damage on the template strand. TLS activity has been associated with repairing lesions from monofuntional alkylators (nitrosourea compounds, temozolomide) and bifunctional alkylators (mito C, CDDP). Targeting of CHEK1 is one strategy to inhibit TLS to increase sensitivity to

brostallicin. Inhibiting CHEK1 would reduce stabilization of claspin which will inhibit RAD18 complex binding to chromatin. RAD 18 ubiquitinates PCNA creating a permissive environment for polymerase exchange to allow synthesis through sites of damage.

FIG. 19 shows the translesion synthesis pathway with the brostallicin hits in gray and bold.

## DETAILED DESCRIPTION OF THE INVENTION

In the following description, and for the purposes of explanation, numerous specific details are set forth in order to provide a thorough understanding of the various aspects of the
10 invention. It will be understood, however, by those skilled in the relevant arts, that the present invention may be practiced without these specific details. In other instances, known structures and devices are shown or discussed more generally in order to avoid obscuring the invention. In many cases, a description of the operation is sufficient to enable one to implement the various forms of the invention. It should be noted that there are many different and alternative configurations, devices and technologies to which the disclosed inventions may be applied. The full scope of the inventions is not limited to the examples that are described below.

As described above, in one embodiment, the invention is directed to a method of assessing sensitivity of a cancer cell to brostallicin. The expression of one or more targets in a cancer cell is typically assessed and reduced expression of those targets is correlated with
20 and/or indicative of sensitivity to brostallicin treatments. Positive expression of one or more of the targets may also be correlated with resistance to brostallicin in certain embodiments. Another aspect of the invention comprises assessing the expression of one or more targets in a tumor from a patient, correlating reduced expression of the gene with resistance to brostallicin, and treating the patient with brostallicin if there is reduced expression of the target or targets.

As used herein, a “target” includes any molecular structure produced by a cell and expressed inside the cell, on the cell surface, or secreted by the cell. Targets include proteins, lipids, carbohydrates, nucleic acids including RNA molecules and genomic DNA sequences, genes, subcellular structures, catalytic sites, or any combination of these such as an enzyme,
30 glycoprotein, cell membrane, virus, cell, organ, organelle, or other any other unimolecular or multimolecular structure now known or yet to be disclosed whether alone or in combination. Preferably the target relates to genes involved in DNA repair, genes involved in translesion synthesis, and genes involved in histone modification. Specific, illustrative examples of preferred targets include, but are not limited to one or more of the following: ATF1, ATR,

BRCA1, BRCA2, CCNG2, EphA5, GNB4, IL13RA2, INPP4B, MCL1, MINPP1, MSH2, NFKBIZ, RAD18, RKHD3, SIAH2, SLC4A10, STK32B, SUV39H2, TRIM7, VHL, and WNK3, either alone or in combination.

"Expression" includes all processes through which a target may be produced. Expression thus includes RNA transcription, mRNA splicing, protein translation, protein folding, post-translational modification, membrane transport, associations with other molecules, addition of carbohydrate moieties to proteins, phosphorylation, protein complex formation, lipid synthesis and any other process along a continuum that results in biological material. Expression also encompasses all processes through which the production of material
10 derived from a nucleic acid template may be actively or passively suppressed. Such processes include all aspects of transcriptional and translational regulation. Examples include heterochromatic silencing, transcription factor inhibition, any form of RNAi silencing, microRNA silencing, alternative splicing, protease digestion, post-translational modification, and alternative protein folding.

Expression may be assessed by any number of methods used to detect material derived from a nucleic acid template used currently in the art and yet to be developed. Examples of such methods include any nucleic acid detection method including the following nonlimiting examples, microarray analysis, RNA in situ hybridization, RNAse protection assay, Northern blot, reverse transcriptase PCR, quantitative PCR, quantitative reverse
20 transcriptase PCR, quantitative real-time reverse transcriptase PCR, nucleic acid sequencing, or any other method of detecting a specific nucleic acid, whether or not such method is now known or yet to be disclosed. Other examples include any process of detecting expression that uses an antibody including the following nonlimiting examples, flow cytometry, immunohistochemistry, ELISA, Western blot, and immunoaffinity chromatograpy. Antibodies may be monoclonal, polyclonal, or any antibody fragment including an Fab, $F(ab)_2$, Fv, scFv, phage display antibody, peptibody, multispecific ligand, or any other reagent with specific binding to a target. Such methods also include direct methods used to assess protein expression including the following nonlimiting examples: HPLC, mass spectrometry, protein microarray analysis, PAGE analysis, isoelectric focusing, 2-D gel
30 electrophoresis, and enzymatic assays. Samples from which expression may be detected include single cells, whole organs or any fraction of a whole organ, whether in vitro, ex vivo, in vivo, or post-mortem.

The concept of assessing the expression of a target further encompasses the observation of a condition that indicates reduced or positive expression without direct

measurement of a gene product. In one nonlimiting example, the observation of one or more aberrations of genomic DNA would indicate the lack/reduction of expression of a target encoded by that particular genomic sequence. Such aberrations include point mutations, frameshift mutations, deletions (including interstitial or homozygous deletions,) gene fusions, translocations, inversions, or any chromosomal and/or genomic change that results in reduced expression. This concept also encompasses positive expression that may be observed by genomic amplifications, which may include the presence of multiple copies of one or more genetic loci, whole chromosomes, or any part of a chromosome within a cell. Positive expression of a target typically is indicative of brostallicin resistance, whereas reduced
10 expression of the target is indicate of brostallicin sensitivity.

Other methods used to assess expression include the use of natural or artificial ligands capable of specifically binding a target. Such ligands may that constitutes a target that may be specifically bound by a ligand. Such ligands include antibodies, antibody complexes, conjugates, natural ligands, small molecules, nanoparticles, or any other molecular entity capable of specific binding to a target. Ligands may be associated with a label such as a radioactive isotope or chelate thereof, dye (fluorescent or nonfluorescent,) stain, enzyme, metal, or any other substance capable of aiding a machine or a human eye from differentiating a cell expressing a target from a cell not expressing a target. Additionally, expression may be assessed by monomeric or multimeric ligands associated with substances
20 capable of killing the cell. Such substances include protein or small molecule toxins, cytokines, pro-apoptotic substances, pore forming substances, radioactive isotopes, or any other substance capable of killing a cell.

While a specific target may be identified by a nucleic acid sequence such as a chemical formula or cDNA, mRNA, or protein sequence, the specific target is not limited to the products of that exact formula or sequence. For example, a specific target identified by a nucleic acid sequence encompasses all sequences that, when expression is assessed, yield positive expression when assessed by the same method as the specific target. The following nonlimiting example is included to illustrate this concept: if expression of a specific target in a sample is assessed by immunohistochemistry, and if the sample expresses a sequence
30 different from the sequence used to identify the specific target (e.g. a variation of one or more nucleic acid molecules,) but positive expression is still determined, then the specific target encompasses the sequence expressed by the sample.

Positive expression includes any difference between a cell expressing a specific target and a cell that does not express a specific target. The exact nature of positive expression

varies by the method, but is well known to those practicing a particular method. Positive expression may be assessed by a detector, an instrument containing a detector, or by aided or unaided human eye. Examples include but are not limited to specific staining of cells expressing a target in an IHC slide, binding of RNA from a sample to a microarray and detection by an instrument capable of detecting the binding to said microarray, a high rate of dye incorporation in real-time RTPCR, detection of fluorescence on a cell expressing a target by a flow cytometer, the presence of radiolabeled bands on film in a Northern blot, detection of labeled blocked RNA by RNAse protection assay, cell death measured by apoptotic markers, cell death measured by shrinkage of a tumor, or any other method by which 10 expression may be observed now known or yet to be disclosed.

In some aspects of the invention, reduced expression constitutes a lack of positive expression. In one example, reduced expression corresponds to the lack of a significant difference between a cell in which expression of a particular target is being assessed and a control cell known not to express the particular target. The concept of reduced expression further encompasses insufficient expression to reach or exceed a threshold, cutoff, or level that results in a particular cellular or physiological response. For example, reduced expression may include the expression of a particular target in a test cell that would be positive expression relative to a control cell known not to express the target. However, because the expression of the target in the test cell is insufficient to cause a particular physiological 20 response (e.g. rendering the cell sensitive to a particular drug) the expression in the test cell may still be classified as reduced expression. Similarly, the concept of positive expression also encompasses expression that exceeds a threshold, cutoff or level sufficient to cause a physiological response.

In certain embodiments, it may be useful to compare the expression level of the target to a control. Preferably, the control may be a measure of the expression level of target, in a quantitative form (e.g., a number, ratio, percentage, graph, etc.) or a qualitative form (e.g., band intensity on a gel or blot, etc.). A variety of controls may be used. Levels of the target expression from a brostallicin resistant cell or brostallicin sensitive cell may be used as a control as well. Still other controls may include expression levels present in a database (e.g., 30 a table, electronic database, spreadsheet, etc.) correlated with known expression levels of the target expression for brostallicin resistance cells and/or brostallicin sensitive cells. Preferably reduced expression of the target, as used herein, means expression that is less than the expression of the target in a control known to be resistant to brostallicin and/or expression that is substantially the same or less than a brostallicin sensitive control. Non-limiting

examples of a suitable control, include for instance, the brostallicin resistant and sensitive cell lines listed in FIG. 11. A brostallicin sensitive cell is a cell that is sensitive to treatment with brostallicin such that, for example, it is able to inhibit growth and/or proliferation of the cancer cells.

In still another embodiment, the control is a list and/or database of known conditions that reduce expression of the target. For example, the control may be a list of point mutations, frame shift mutations, deletions, gene fusions, translocations, or inversions that affect the expression of the target. In a preferred embodiment, the control is a mutation known to reduce expression of the target and thereby induce sensitivity to Brostallicin. In this embodiment, detection of the mutation in the cell sample would indicate reduced expression and thus sensitivity to brostallicin.

The invention contemplates assessing the expression of the target in any biological sample from which the expression may be assessed. One skilled in the art would know to select a particular biological sample and how to collect said sample depending upon whether or not expression of germline DNA, tumor DNA, mRNA, or any form of protein is assessed. Examples of sources of samples include but are not limited to biopsy or other *in vivo* or *ex vivo* analysis of prostate, breast, skin, muscle, facia, brain, endometrium, lung, head and neck, pancreas, small intestine, blood, liver, testes, ovaries, colon, skin, stomach, esophagus, spleen, lymph node, bone marrow, kidney, placenta, or fetus. In some aspects of the invention, the sample comprises a fluid sample, such as peripheral blood, lymph fluid, ascites, serous fluid, pleural effusion, sputum, cerebrospinal fluid, amniotic fluid, lacrimal fluid, stool, or urine.

The invention further encompasses kits that facilitate assessing the expression of a target. Such kits will contain one or more reagents that indicate the presence of the target. Contents of such a kit may include one or more of the following alone or in combination: one or more oligonucleotide primers capable of hybridizing to sequences within the target which may be further optimized for use in a PCR based method, an antisense probe to all or part of target sequence, a ligand with specificity to the target mRNA, protein or other measurable gene product, labels, buffers, or any other reagent that may be useful in a method that assesses the expression of a target whether now known or yet to be disclosed. In one embodiment, the method and kit are designed to effectively observe one or more aberrations of genomic DNA that indicate/correlate with the reduction of expression of a target encoded by that particular genomic sequence.

Cancer cells include any cells derived from a tumor, neoplasm, cancer, precancer, cell line, or any other source of cells that have the potential to expand and grow to an unlimited degree. Cancer cells may be derived from naturally occurring sources or may be artificially created. Cancer cells may also be capable of invasion into other tissues and metastasis when placed into an animal host. Cancer cells further encompass any malignant cells that have invaded other tissues and/or metastasized. One or more cancer cells in the context of an organism may also be called a cancer, tumor, neoplasm, growth, malignancy, or any other term used in the art to describe cells in a cancerous state.

Examples of cancers that could serve as sources of cancer cells include solid tumors such as fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon cancer, colorectal cancer, kidney cancer, pancreatic cancer, bone cancer, breast cancer, ovarian cancer, prostate cancer, esophageal cancer, stomach cancer, oral cancer, nasal cancer, throat cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer, uterine cancer, testicular cancer, small cell lung carcinoma, bladder carcinoma, lung cancer, epithelial carcinoma, glioma, glioblastoma multiforme, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, skin cancer, melanoma, neuroblastoma, and retinoblastoma.

Additional cancers that may serve as sources of cancer cells include blood borne cancers such as acute lymphoblastic leukemia ("ALL,"), acute lymphoblastic B-cell leukemia, acute lymphoblastic T-cell leukemia, acute myeloblastic leukemia ("AML"), acute promyelocytic leukemia ("APL"), acute monoblastic leukemia, acute erythroleukemic leukemia, acute megakaryoblastic leukemia, acute myelomonocytic leukemia, acute nonlymphocyctic leukemia, acute undifferentiated leukemia, chronic myelocytic leukemia ("CML"), chronic lymphocytic leukemia ("CLL"), hairy cell leukemia, multiple myeloma, lymphoblastic leukemia, myelogenous leukemia, lymphocytic leukemia, myelocytic leukemia, Hodgkin's disease, non-Hodgkin's Lymphoma, Waldenstrom's macroglobulinemia, Heavy chain disease, and Polycythemia vera.

Expansion of a cancer cell includes any process that results in an increase in the number of individual cells derived from a cancer cell. Expansion of a cancer cell may result from mitotic division, proliferation, or any other form of expansion of a cancer cell, whether in vitro or in vivo. Expansion of a cancer cell further encompasses invasion and metastasis. A cancer cell may be in physical proximity to cancer cells from the same clone or from different clones that may or may not be genetically identical to it. Such aggregations may take the form of a colony, tumor or metastasis, any of which may occur *in vivo* or *in vitro*. Slowing the expansion of the cancer cell may be brought about either by inhibiting cellular processes that promote expansion or by bringing about cellular processes that inhibit expansion.
10 Processes that inhibit expansion include processes that slow mitotic division and processes that promote cell senescence or cell death. Examples of specific processes that inhibit expansion include caspase dependent and independent pathways, autophagy, necrosis, apoptosis, and mitochondrial dependent and independent processes and further include any such processes yet to be disclosed.

In some aspects of the invention, inhibition of the expansion of the cancer cell is achieved through the use of an outside agent applied to the cancer cell for the purpose of slowing the expansion of the cancer cell. Such agents include natural or synthetic ligands, blockers, agonists, antagonists, or activators of receptors, immune cells such as CD8+ T cells, viruses, inhibitors of gene or protein expression such as siRNA or miR's, small molecules,
20 pharmaceutical compositions, or any other composition of matter that when administered to the cancer cell would result in the slowing of the expansion of the cancer cell. The concept of agents that slow the expansion of a cancer cell encompasses restricting access to any natural or artificial agent necessary for cell survival including necessary nutrients, ligands, or cell-cell contacts. Examples of such agents and conditions include treatment with antiangiogenic inhibitors. In some aspects of the invention, the agent that slows the expansion of the cancer cell includes a compound that binds in the minor groove of a DNA molecule. In still further aspects of the invention the agent of choice is brostallicin.

## EXAMPLES

30 Elements and acts in the example are intended to illustrate the invention for the sake of simplicity and have not necessarily been rendered according to any particular sequence or embodiment. The example is further intended to establish possession of the invention by the Inventors.

**Example 1**

A high throughput screen was completed using A2780cis cells against Qiagen's Human Druggable v2.0 siRNA library (the operations manual of which is hereby incorporated by reference in its entirety). The library contains approximately 14,000 individual siRNA sequences covering approximately ~7000 genes. In conjunction with the transfection of the cells with siRNA, the cells were also treated with six different doses of Brostallicin to identify targets that augment the activity the drug. After the initial analysis of results, a total of 400 targets were identified. In addition, 19 targets were selected from the control plates included in the HTS. In the subsequent analysis, four siRNA sequences (A
10 through D) were hit-picked and printed onto assay plates. This was followed by confirmation assays in both A2780 and A2780cis cell lines that were expanded to 8 drug doses of Brostallicin. (Table 1). Two experimental replicates were performed using the same techniques from the HTS.

**Table 1. Drug Concentrations of BROSTALLICIN used in the Confirmation Assays**

| BROSTALLICIN Treatment | Target Growth Inhibition (GI) Value | A2780wt µM | A2780cis µM |
|---|---|---|---|
| Dose 1 | $GI_0$ | 0.0000 (DMSO Vehicle) | 0.0000 (DMSO Vehicle) |
| Dose 2 | $GI_0$ | 0.0000 (DMSO Vehicle) | 0.0000 (DMSO Vehicle) |
| Dose 3 | $GI_{10}$ | 0.003 | 0.003 |
| Dose 4 | $GI_{25}$ | 0.010 | 0.010 |
| Dose 5 | $GI_{30}$ | 0.030 | 0.030 |
| Dose 6 | $GI_{35}$ | 0.065 | 0.065 |
| Dose 7 | $GI_{50}$ | 0.135 | 0.135 |
| Dose 8 | $GI_{70}$ | 0.265 | 0.265 |

Genes with a minimum of two (≥2) sequences that demonstrated Brostallicin sensitization in both experimental replicates were considered a confirmed HTS hit. A total of 148 confirmed hits (siRNA sequences) targeting 59 genes were identified in the A2780cis
20 cell line during the confirmation step (Table 2). These hits were also tested in the A2780wt cells, although fewer of these hits were validated in this cell line (FIGS. 1-5 and Table 2).

**Table 2. List of validated potential sensitizers from HTS in A2780 and A2780cis Cells**

| GENE | **#siRNA_validated**_A2780 cis | **#runs**_confirmed_A2780 WT | **#siRNAs**_confirmed_A2780 WT |
|---|---|---|---|
| ABCC5 | 3 | 0 | 0 |
| ABCF3 | 2 | 2 | 2 |
| ARH | 3 | 2 | 1 |
| ATF1 | 2 | 0 | 0 |
| ATR | 4 | 2 | 2 |
| BRCA1 | 2 | 2 | 1 |
| BRCA2 | 2 | 1 | 3 |
| CCNG2 | 2 | 0 | 0 |
| CTDP1 | 2 | 0 | 0 |
| DKFZp5660084 | 3 | 2 | 1 |
| EHMT1 | 3 | 1 | 2 |
| EPHA5 | 2 | 1 | 2 |
| FOLR1 | 2 | 2 | 1 |
| GNB4 | 3 | 1 | 4 |
| IFITM1 | 2 | 1 | 1 |
| IL13RA2 | 2 | 2 | 1 |
| IL28RA | 2 | 2 | 2* |
| INPP4B | 2 | 0 | 0 |
| LPAL2 | 2 | 2 | 1* |
| MASP2 | 3 | 2 | 1 |
| MCL1 | 3 | 2 | 1 |
| MGC1136 | 2 | 1 | 3 |
| MINPP1 | 2 | 2 | 1* |
| MMP28 | 2 | 2 | 2* |
| MSH2 | 2 | 1 | 2 |
| NFKBIZ | 2 | 1 | 3 |
| NPHP1 | 2 | 1 | 3 |
| NYD-SP25 | 2 | 1 | 3 |
| PPP1R3B | 3 | 1 | 4 |
| PVRL2 | 2 | 2 | 1* |
| RAD18 | 2 | 1 | 1 |
| RAI1 | 2 | 2 | 1 |
| RKHD3 | 3 | 1 | 4 |
| RTN4IP1 | 3 | 2 | 1 |
| SAP18 | 2 | 0 | 0 |
| SET7 | 3 | 2 | 1 |
| SH3GL1 | 2 | 0 | 0 |
| SIAH2 | 2 | 1 | 1 |
| SIPA1 | 2 | 2 | 1 |
| SLC25A2 | 4 | 1 | 4 |
| SLC4A10 | 3 | 1 | 4 |
| SNAI2 | 2 | 2 | 2* |
| STAT2 | 2 | 1 | 1 |
| STK32B | 3 | 2 | 2 |
| SUV39H2 | 4 | 1 | 3 |
| TBL1XR1 | 3 | 1 | 4 |
| TCFL4 | 2 | 1 | 1 |
| TINAG | 3 | 2 | 1 |
| TKTL1 | 2 | 1 | 1 |
| TNFRSF11A | 4 | 1 | 1 |
| TNN | 4 | 2 | 1 |
| TRIM7 | 3 | 1 | 4 |
| TUBA3 | 2 | 2 | 1 |
| VHL | 3 | 1 | 1 |

| GENE | **#siRNA_validated**_A2780 cis | **#runs**_confirmed_A2780 WT | **#siRNAs**_confirmed_A2780 WT |
|---|---|---|---|
| WNK3 | 4 | 2 | 1 |
| YAF2 | 3 | 1 | 1 |
| YEATS4 | 2 | 0 | 0 |
| ZNRF2 | 2 | 2 | 1 |
| ZW10 | 2 | 1 | 1 |

* indicates not the same siRNA

In order to identify any cell line specific Brostallicin potentiators, 18 genes were prioritized from the validated 59-gene list involved in DNA repair and chromatin remodeling pathways (Table 3). These genes would be further validated in two breast cancer cell lines, BT474 and MDA-MB-231. The two breast cancer cell lines were extensively characterized with siRNA transfection and sensitivity to Brostallicin. Four siRNAs per target were hit picked and the study was carried out with 6 different drug doses of Brostallicin for each cell line. Two biological repeats were performed for each cell line for the validation experiment, and only genes with a minimum of two (≥2) sequences that demonstrated brostallicin sensitization in both experimental replicates were considered a validated hit. Out of the 18 genes tested, only 3 genes (ATR, MSH2, and MCL1) are validated in MDA-MB-231 and 2 genes (ATR & MCL1) are validated in BT474 (Table 3 and FIGS. 1-8).

**Table 3. List of validated genes in ovarian and breast cancer cell lines**

| GENE | **#siRNA_validated**_A2780 cis | **#siRNAs**_confirmed A2780 WT | **# siRNA_validated** MDA-MB231 | **#siRNA_validated BT474** |
|---|---|---|---|---|
| ABCC5 | 3 | 0 | | |
| ABCF3 | 2 | 2 | | |
| ATF1 | 2 | 0 | | |
| ATR | 4 | 2 | 4 | 4 |
| BRCA1 | 2 | 1 | 1 | 1 |
| BRCA2 | 2 | 3 | 1 | 1 |
| EHMT1 | 3 | 2 | | |
| FOLR1 | 2 | 1 | | |
| GNB4 | 3 | 4 | | |
| MCL1 | 3 | 1 | 2 | 2 |
| MSH2 | 2 | 2 | 2 | 1 |
| RAD18 | 2 | 1 | | |
| SAP18 | 2 | 0 | | |
| SET7 | 3 | 1 | | |
| SIAH2 | 2 | 1 | | |
| SUV39H2 | 4 | 3 | | |

| GENE | **#siRNA_validated**_A2780 cis | **#siRNAs**_confirmed A2780 WT | **# siRNA_validated** MDA-MB231 | **#siRNA_validated BT474** |
|---|---|---|---|---|
| VHL | 3 | 1 | 1 | 0 |
| ZNRF2 | 2 | 1 | | |

**Example 2**

The Inventors identified three general classes of target molecules involved in the sensitization of cells to brostallicin from the hits identified from the siRNA screen studies: genes involved in DNA repair, genes involved in translesion synthesis, and genes involved in histone modification (see Table 4).

**Table 4. List of Brostallicin sensitizing targets found to be mutated in human cancer**
10 **tissues.**

| Gene | Total Number of Mutations/Fusions found | Total Number of Tissues surveyed |
|---|---|---|
| ATF1 | 104 | 398 |
| ATR | 12 | 422 |
| BRCA1 | 25 | 1832 |
| BRCA2 | 35 | 1872 |
| CCNG2 | 1 | 136 |
| EPHA5 | 5 | 279 |
| GNB4 | 1 | 137 |
| IL13RA2 | 1 | 40 |
| INPP4B | 1 | 137 |
| MINPP1 | 1 | 273 |
| MSH2 | 37 | 1323 |
| NFKBIZ | 1 | 136 |
| RAD18 | 3 | 139 |
| RKHD3 | 1 | 136 |
| SIAH2 | 1 | 136 |
| SLC4A10 | 2 | 136 |
| STK32B | 4 | 286 |
| SUV39H2 | 1 | 136 |
| TRIM7 | 1 | 136 |
| VHL | 1050 | 4525 |
| WNK3 | 5 | 358 |

DNA Repair

DNA repair includes any process by which breaks in genomic DNA may be identified and corrected by a cell, now known or yet to be disclosed. Brostallicin sensitization targets involved in DNA repair include but are not limited to: BRCA1, BRCA2, ATR-A, MSH2, and Rad18. Specifically, a number of genes were found to be involved in translesion synthesis. (See FIG. 18)

Translesion Synthesis

Translesion synthesis (TLS) encompasses cellular processes in which an atypical
10 DNA polymerase capable of synthesizing DNA across base damage on a template strand is used by the cell rather than a standard DNA polymerase that is incapable of traversing a damaged base. TLS activity includes but is not limited to processes that repair lesions caused by monofunctional alkylators such as nitrosourea compounds and temozolomide and bifunctional alkylators such as mito C and CDDP. Brostallicin sensitization targets involved in translesion synthesis include but are not limited to: ATR, CHK1, RAD18, and ZNRF2 (See FIG. 19).

Histone H3 Methylation

Histone H3 methylation includes all cellular processes by which the H3 histone
20 protein is modified at specific amino acid residues, thus modifying its activity in the regulation of chromatin structure. Brostallicin sensitization targets involved in histone H3 methylation include SET7/9, SUV39H2, and EuHMT1.

**Example 3**

The following targets are known to be mutated or otherwise affected by one or more other genomic aberrations such as gene fusions in human cancer tissues:
ATF1, ATR, BRCA1, BRCA2, CCNG2, EphA5, GNB4, IL13RA2, INPP4B, MINPP1, MSH2, NFKBIZ, RAD18, RKHD3, SIAH2, SLC4A10, STK32B, SUV39H2, TRIM7, VHL, and WNK3.

30

**Example 4**

To identify molecular determinants of Brostallicin response that could inform clinical development, we undertook an integrated cellular pharmacogenomics study. We began with the application of advanced knowledge mining tools to explore Brostallicin response data

across the NCI-60 cell line panel and its association to global gene expression profiles. Gene set enrichment analysis (GSEA) revealed unique associations between the Brostallicin sensitivity gene set signature and distinct gene sets representing a number of specific molecular concepts, including significant associations with defined cancer types and aspects of DNA repair. Next, further knowledge mining and integration of orthogonal data sets prioritized a set of approximately 100 candidate genes that were subsequently used to design a focused siRNA library to supplement a larger siRNA library representing 7000 druggable gene targets across the human genome. The combined siRNA library was then used to conduct a high throughput RNA interference (HT-RNAi) phenotype profiling analysis of the
10 A2780 cell line treated with Brostallicin. The results are shown in FIGS. 9-17.

CLAIMS

We claim:

1. A method of assessing sensitivity of a cancer cell to brostallicin, comprising:

(a) obtaining a cancer cell sample;

(b) assessing the expression of at least one target in the cancer cell sample selected from the group consisting of: ATF1, ATR, BRCA1, BRCA2, CCNG2, EphA5, GNB4, IL13RA2, INPP4B, MCL1, MINPP1, MSH2, NFKBIZ, RAD18, RKHD3, SIAH2, SLC4A10, STK32B, SUV39H2, TRIM7, VHL, and WNK3;

(c) determining if expression of the target in the sample is reduced based on a control; and

(d) correlating reduced expression of the target in the cancer cell sample with sensitivity to brostallicin.

2. The method of claim 1, wherein the target is ATR, BRCA1, BRCA2, MSH2, RAD18, and/or VHL.

3. The method of claim 1, wherein the target is BRCA1 and/or BRCA2.

4. The method of claim 1, wherein the cancer cell is selected from the group consisting of: breast cancer; ovarian cancer; colon cancer; and pancreatic cancer.

5. The method of claim 1, wherein at least two targets have reduced expression as compared to a brostallicin resistant control.

6. The method of claim 1, wherein the control is a list or database of genomic aberrations that modify the expression of the target.

7. The method of claim 6, wherein the genomic aberration is a mutation or deletion that reduces expression of the target.

8. The method of claim 1, wherein the step of assessing the expression of the target comprises detecting a genomic aberration that reduces expression of the target.

9. The method of claim 8, wherein detecting a genomic aberration comprises use of at least one of the following: comparative genomic hybridization; array-based comparative genomic hybridization; detecting an inactivating mutation; PCR; and DNA sequencing.

10. The method of claim 8, wherein expression of the target in the cancer cell is reduced by at least 70% based on the control.

11. The method of claim 1, wherein the control is a brostallicin resistant cell; a brostallicin sensitive cell; or a list or database, wherein the list or database comprises a log of genomic aberrations that affect expression or expression levels of the target in brostallicin resistant cells or brostallicin sensitive cells.

12. The method of claim 1, wherein the step of assessing the expression comprises measuring mRNA.

13. The method of claim 12, wherein measuring mRNA comprises at least one of the following: RTPCR; microarray analysis; and Northern Blot.

14. The method of claim 1, wherein the step assessing the expression comprises measuring protein.

15. The method of claim 14, wherein measuring protein comprises at least one of the following: ELISA; mass spectrometry; and Western Blotting.

16. A method of identifying a cancer patient who may benefit from a treatment with brostallicin drug, comprising:

(a) obtaining a cancer cell sample from the patient;

(b) assessing the expression of at least one target in the cancer cell sample selected from the group consisting of: ATF1, ATR, BRCA1, BRCA2, CCNG2, EphA5, GNB4, IL13RA2, INPP4B, MCL1, MINPP1, MSH2, NFKBIZ, RAD18, RKHD3, SIAH2, SLC4A10, STK32B, SUV39H2, TRIM7, VHL, and WNK3; and

(c) comparing expression of the target in the cancer cell sample to a control, wherein reduced expression of the target indicates that the patient may benefit from treatment with brostallicin.

17. A method of treating a cancer patient comprising:

(a) collecting a biological sample from the patient;

(b) assessing the expression of a target selected from the group consisting of: ATF1, ATR, BRCA1, BRCA2, CCNG2, EphA5, GNB4, IL13RA2, INPP4B, MCL1, MINPP1, MSH2, NFKBIZ, RAD18, RKHD3, SIAH2, SLC4A10, STK32B, SUV39H2, TRIM7, VHL, and WNK3 in the biological sample; and

(c) administering an effective amount of brostallicin to the patient when expression of the target is reduced based on a control indicating sensitivity to brostallicin.

18. The method of claim 17, wherein the biological sample comprises a tumor sample.

19. The method of claim 17, wherein the cancer patient presents with a cancer selected from the group consisting of: breast cancer; ovarian cancer; and colon cancer.

20. The method of claim 17, wherein the target is ATR, BRCA1, BRCA2, MSH2, RAD18, and/or VHL.

21. The method of claim 17, wherein at least two targets have reduced expression as compared to a brostallicin resistant control.

22. The method of claim 17, wherein the control is a list or database of genomic aberrations that modify the expression of the target.

23. The method of claim 22, wherein the genomic aberration is a mutation or deletion that reduces expression of the target.

24. The method of claim 17, wherein the step of assessing the expression of the target comprises detecting a genomic aberration that reduces expression of the target and comprising use of at least one of the following: comparative genomic hybridization; array-based comparative genomic hybridization; detecting an inactivating mutation; PCR; and DNA sequencing.

25. The method of claim 17, wherein expression of the target in the cancer cell is reduced by at least 70% based on the control.

26. The method of claim 17, wherein the control is a brostallicin resistant cell; a brostallicin sensitive cell; or a list or database, wherein the list or database comprises a log of genomic aberrations that affect expression or expression levels of the target in brostallicin resistant cells or brostallicin sensitive cells.

27. The method of claim 17, wherein the step of assessing the expression comprises measuring mRNA and/or measuring protein.

28. The method of claim 27, wherein measuring mRNA comprises at least one of the following: RTPCR; microarray analysis; and Northern Blot and measuring protein comprises at least one of the following: ELISA; mass spectrometry; and Western Blotting.

29. A diagnostic kit for assessing sensitivity of a cancer cell to brostallicin, comprising:

(a) one or more reagents that detect the level expression of at least one target in the cancer cell sample selected from the group consisting of: ATF1, ATR, BRCA1, BRCA2,

CCNG2, EphA5, GNB4, IL13RA2, INPP4B, MCL1, MINPP1, MSH2, NFKBIZ, RAD18, RKHD3, SIAH2, SLC4A10, STK32B, SUV39H2, TRIM7, VHL, and WNK3; and

(b) a reporter element, wherein the reporter element indicates whether the cell sample is likely sensitive to brostallicin.

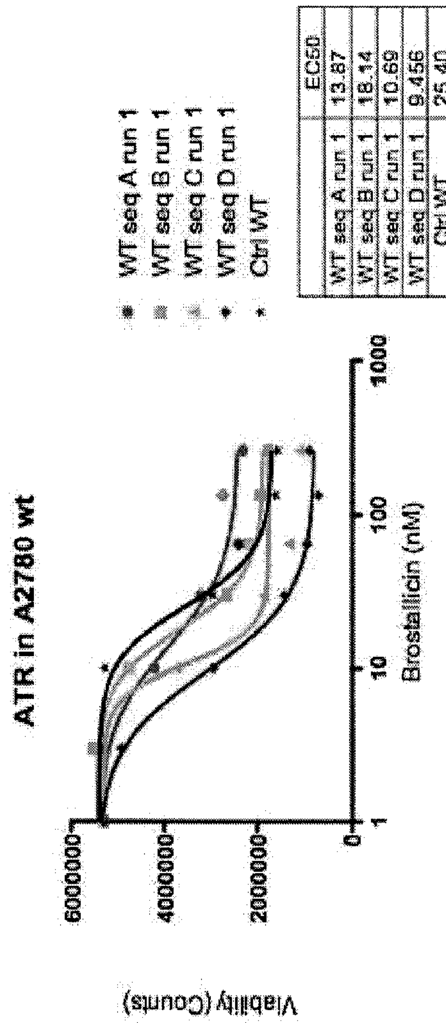
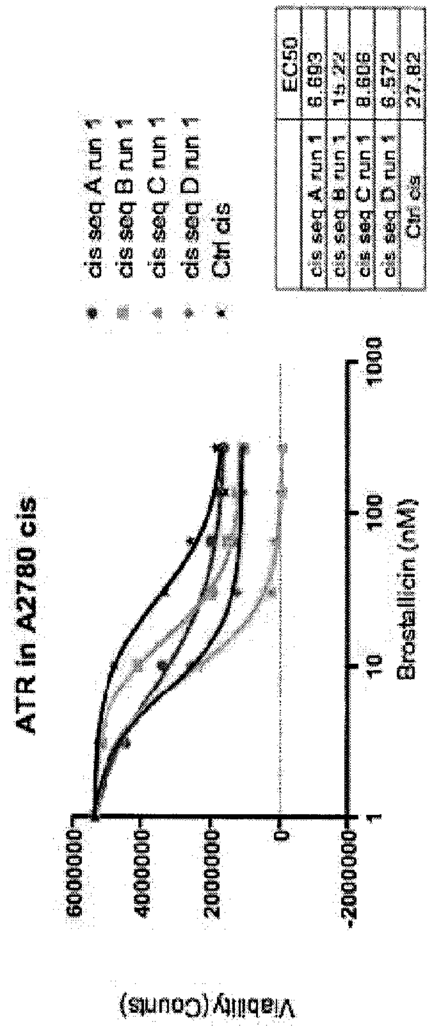



FIG. 1A




| | EC50 |
|---|---|
| cis seq A run 1 | 6.693 |
| cis seq B run 1 | 15.22 |
| cis seq C run 1 | 8.606 |
| cis seq D run 1 | 6.572 |
| Ctrl cis | 27.82 |

FIG. 1B

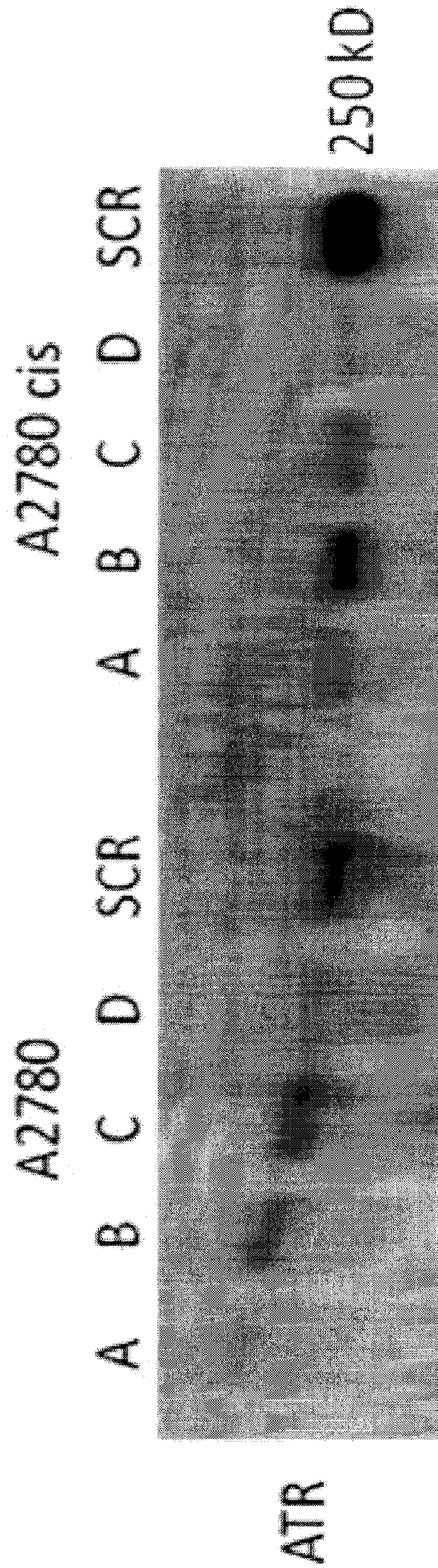
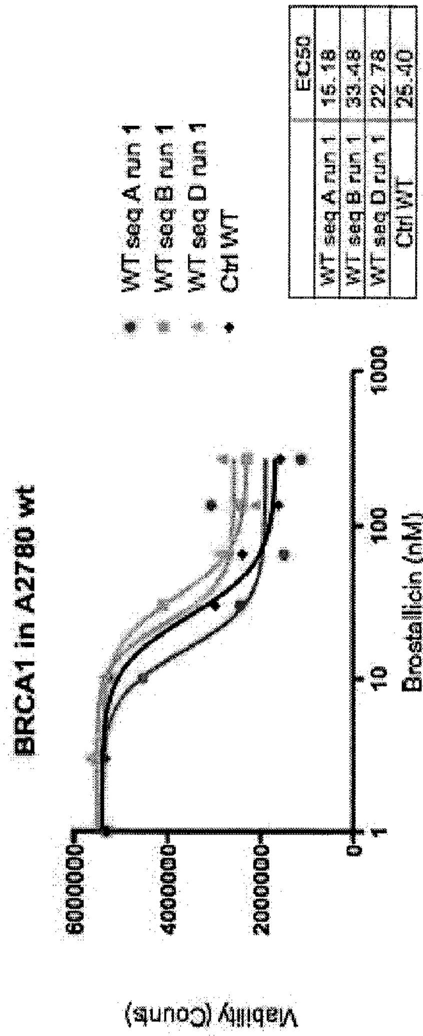



FIG. 1C




| | EC50 |
|---|---|
| WT seq A run 1 | 15.18 |
| WT seq B run 1 | 33.48 |
| WT seq D run 1 | 22.78 |
| Ctrl WT | 25.40 |

**FIG. 2A**

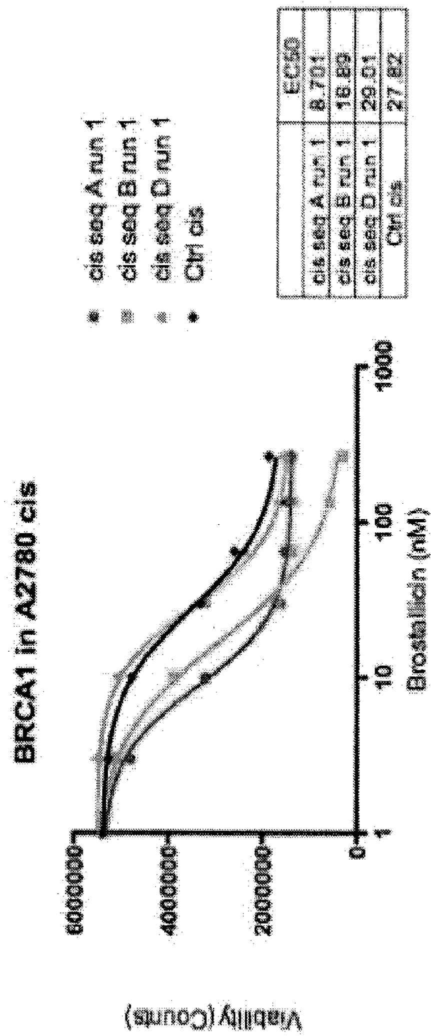



FIG. 2B

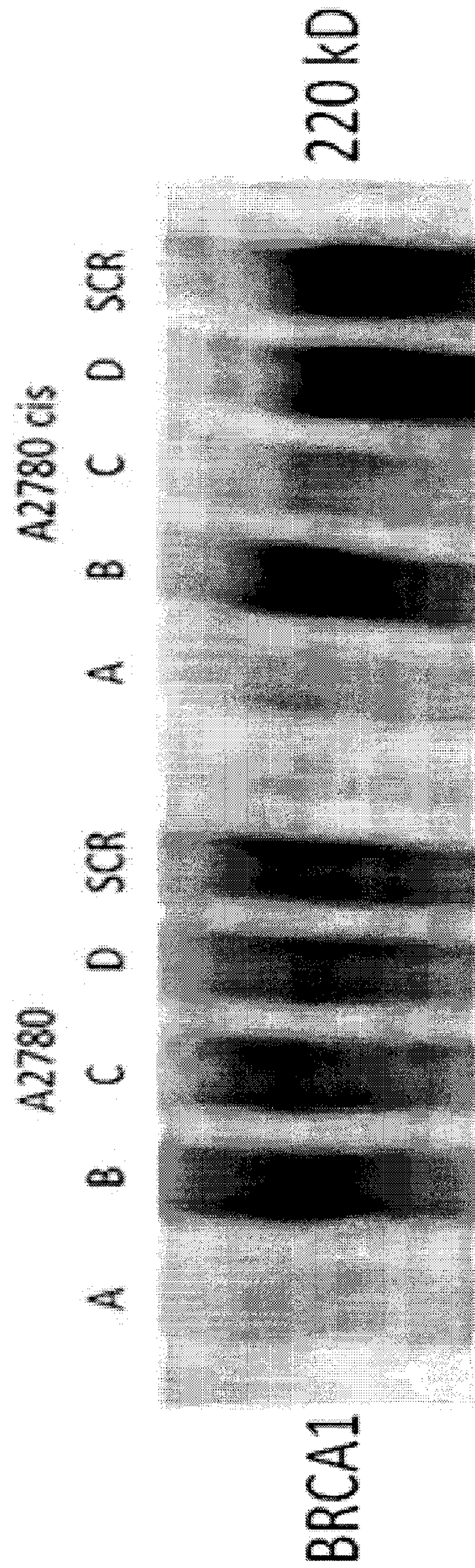
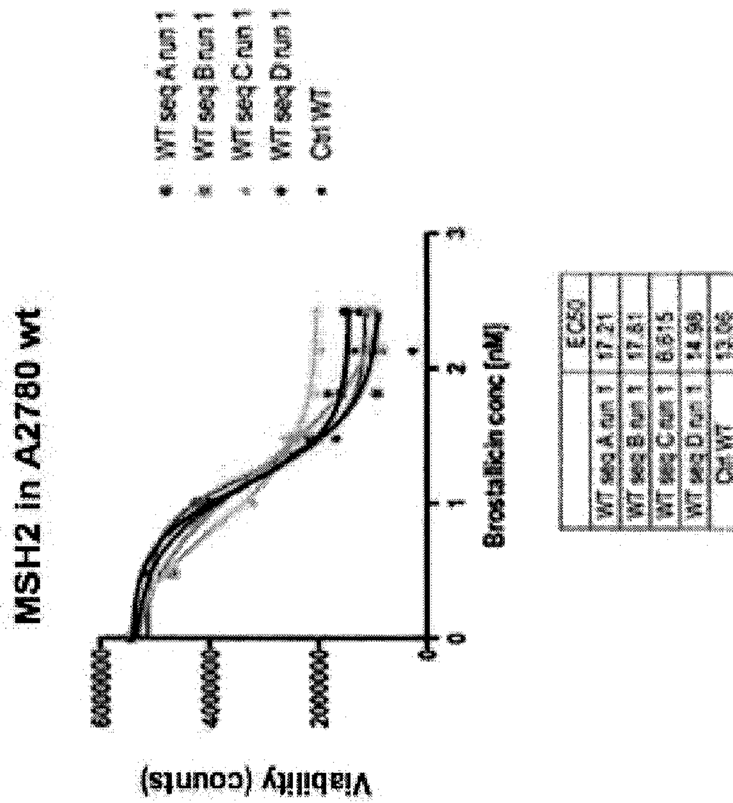
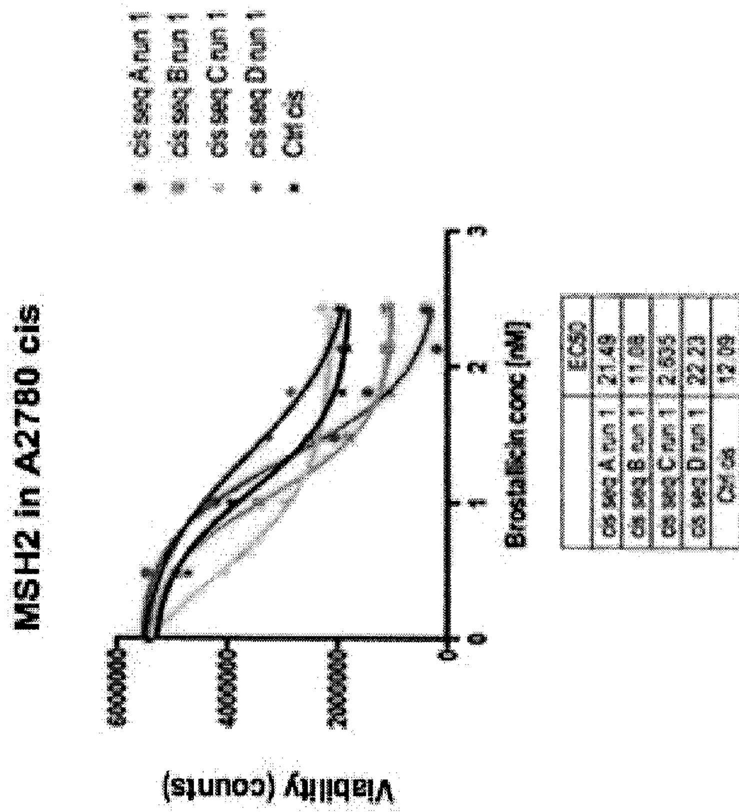



FIG. 2C




| | EC50 |
|---|---|
| WT seq A run 1 | 17.21 |
| WT seq B run 1 | 17.81 |
| WT seq C run 1 | 8.615 |
| WT seq D run 1 | 14.98 |
| Ctrl WT | 13.06 |

FIG. 3A




| | EC50 |
|---|---|
| cis seq A run 1 | 21.49 |
| cis seq B run 1 | 11.08 |
| cis seq C run 1 | 2.635 |
| cis seq D run 1 | 22.23 |
| Ctrl cis | 12.09 |

FIG. 3B

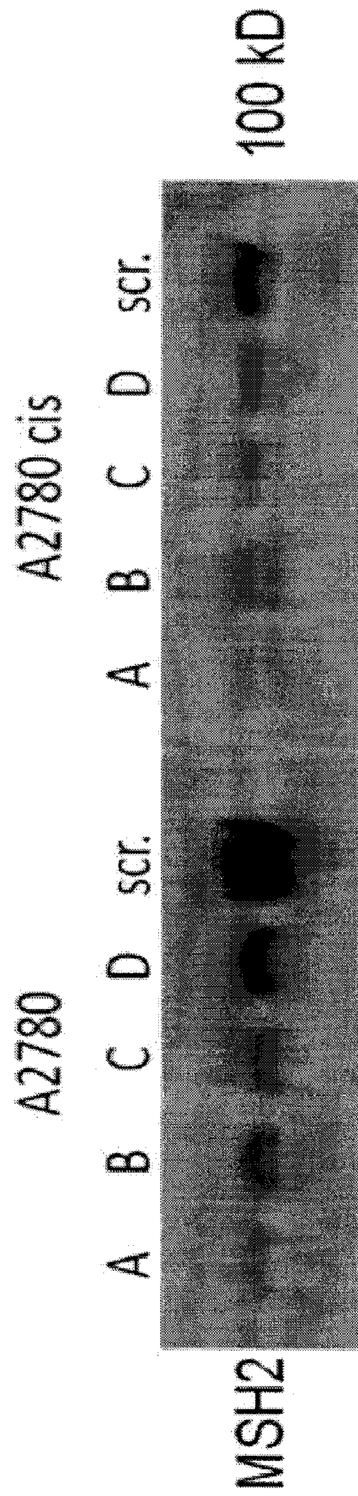



FIG. 3C

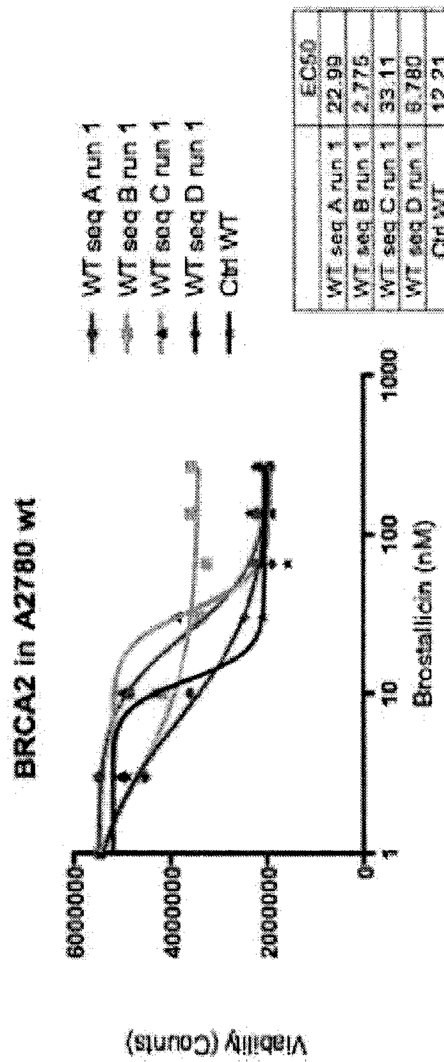
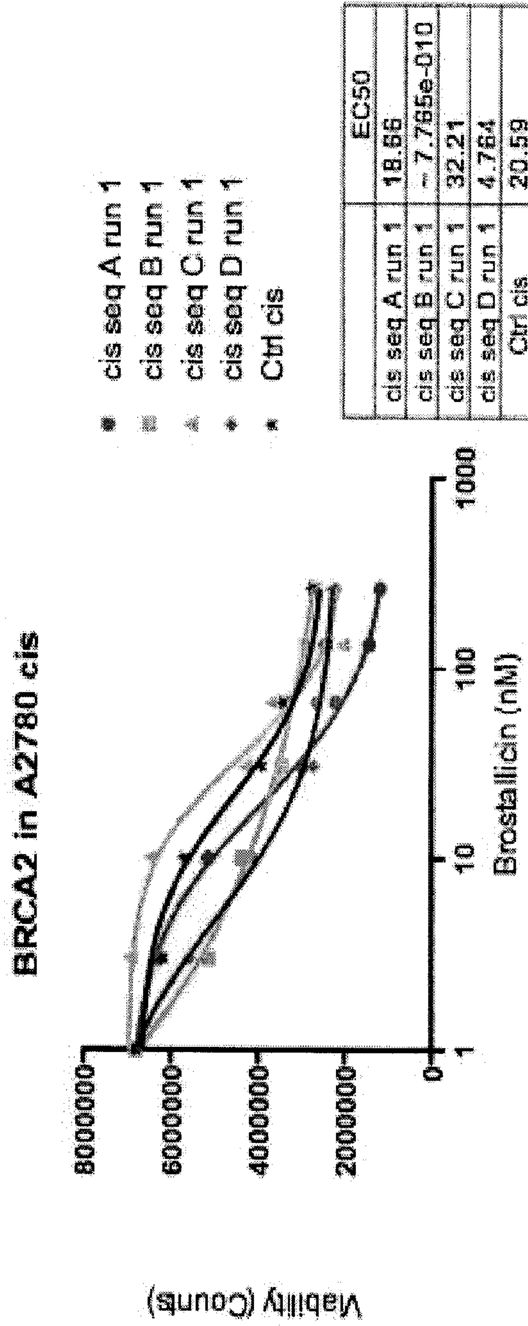
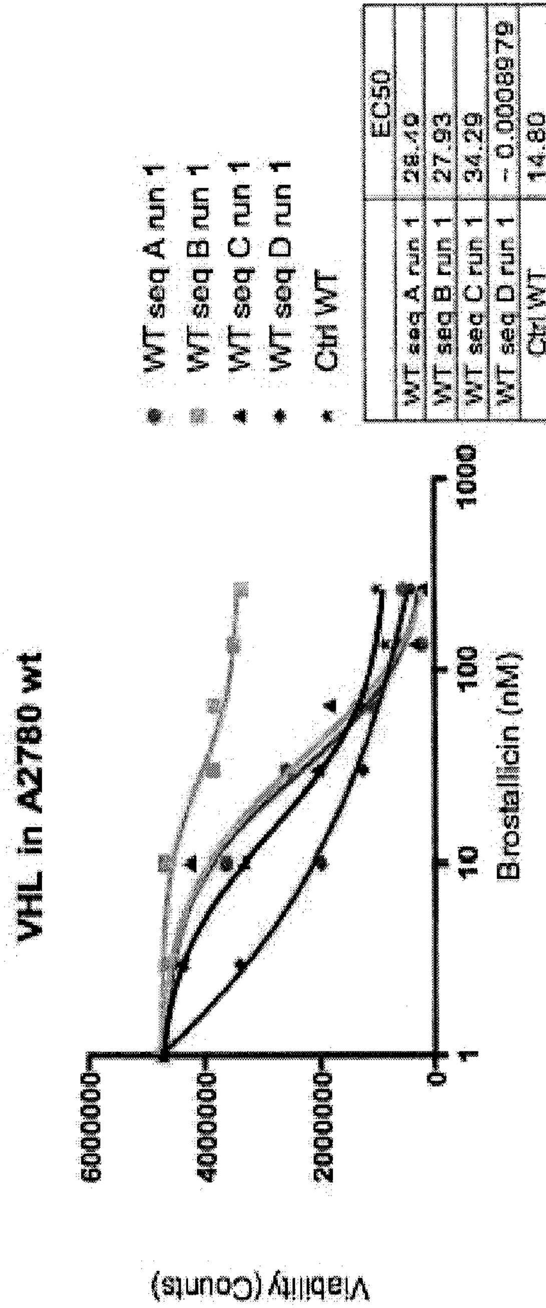



FIG. 4A




| | EC50 |
|---|---|
| cis seq A run 1 | 18.66 |
| cis seq B run 1 | ~ 7.765e-010 |
| cis seq C run 1 | 32.21 |
| cis seq D run 1 | 4.764 |
| Ctrl cis | 20.59 |

FIG. 4B




| | EC50 |
|---|---|
| WT seq A run 1 | 28.49 |
| WT seq B run 1 | 27.93 |
| WT seq C run 1 | 34.29 |
| WT seq D run 1 | ~ 0.0008979 |
| Ctrl WT | 14.80 |

FIG. 5A

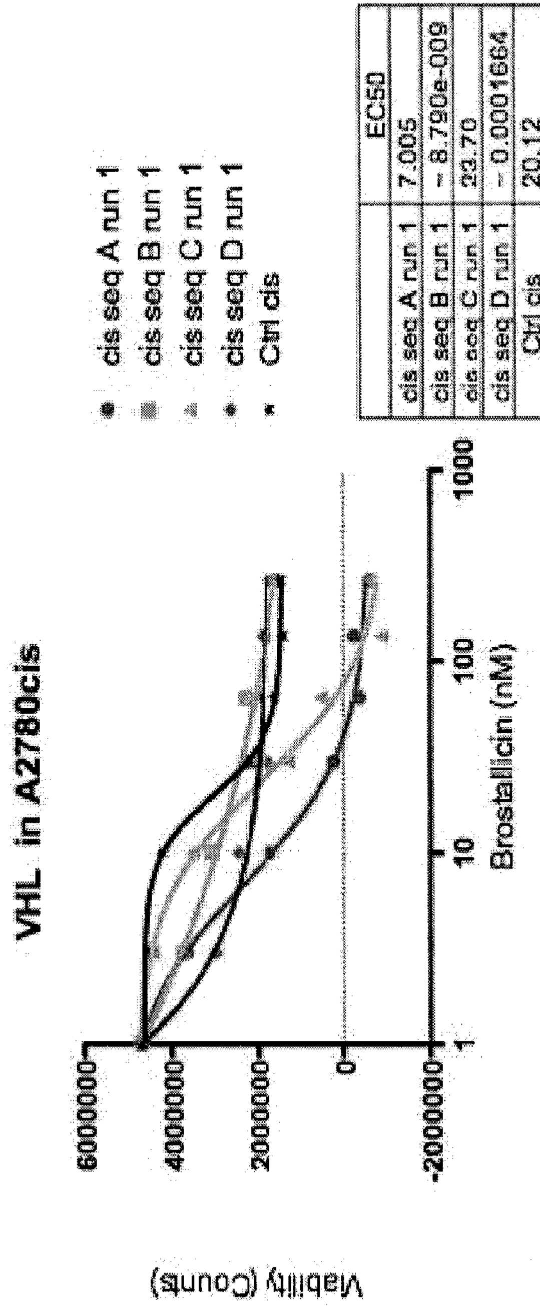



FIG. 5B

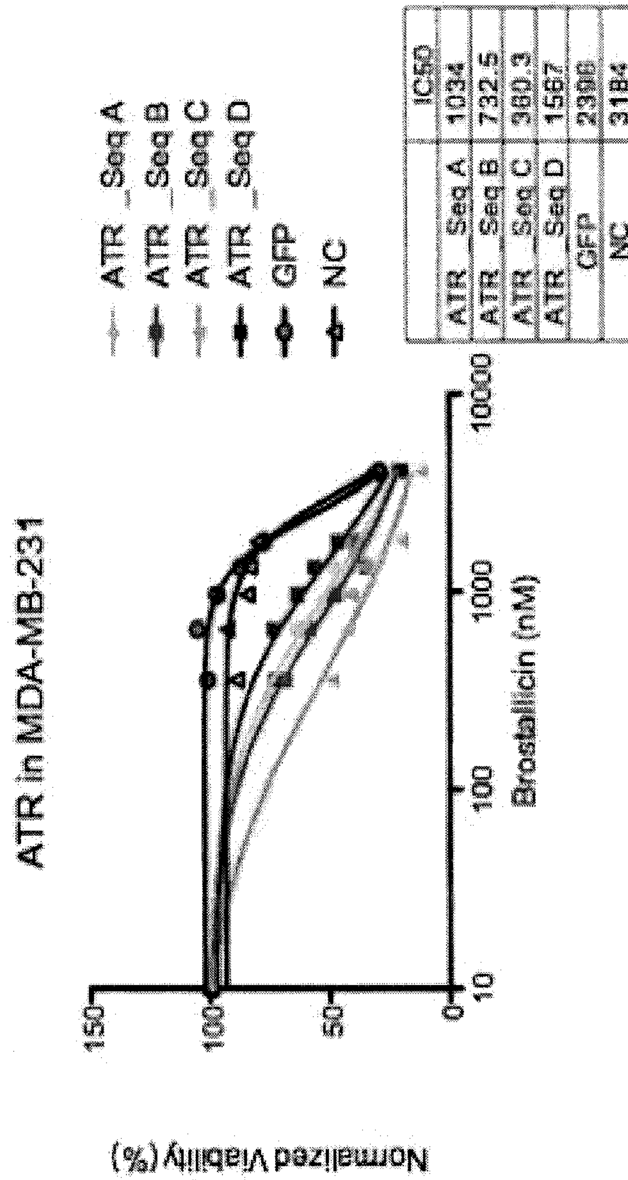
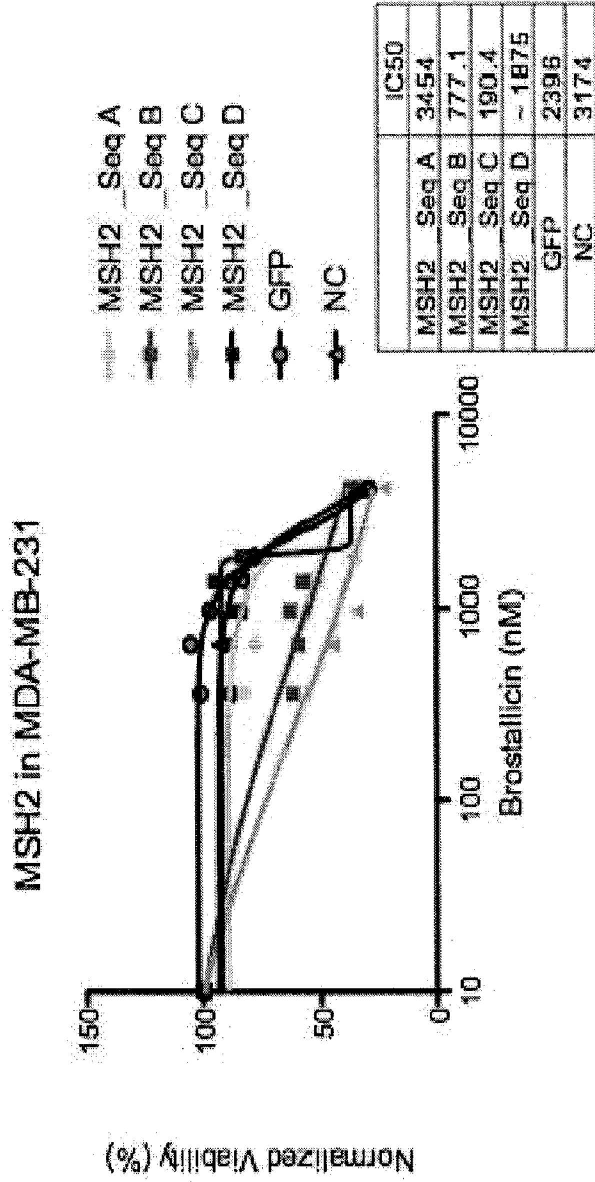
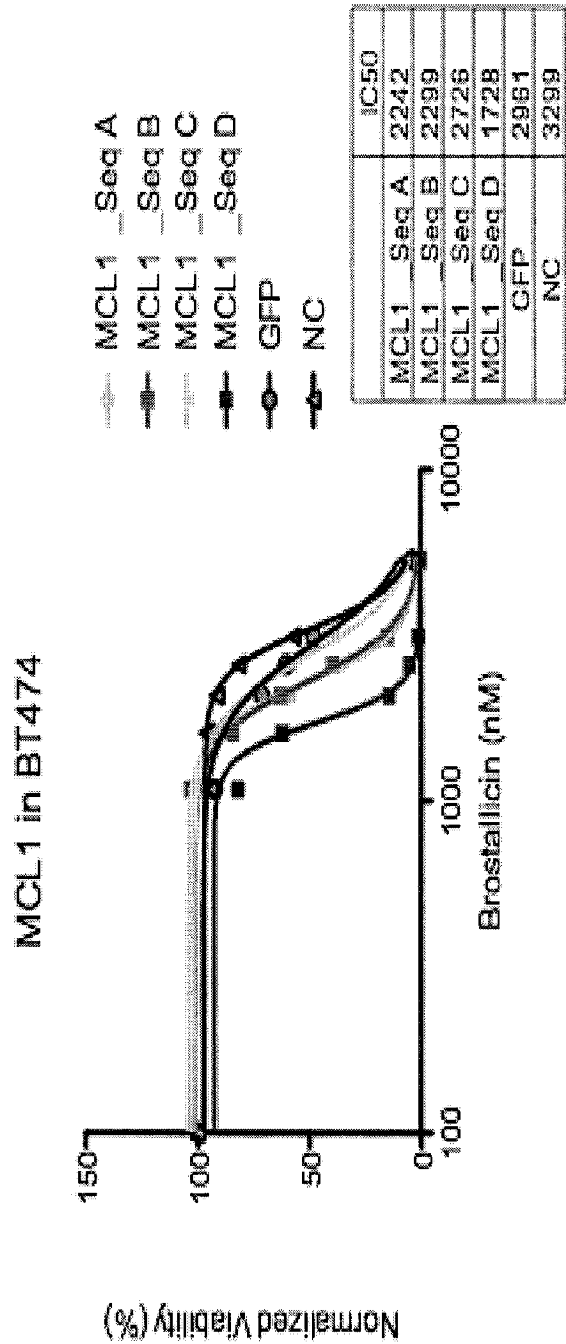



FIG. 6




| | IC50 |
|---|---|
| MSH2 _Seq A | 3454 |
| MSH2 _Seq B | 777.1 |
| MSH2 _Seq C | 190.4 |
| MSH2 _Seq D | ~ 1875 |
| GFP | 2396 |
| NC | 3174 |

FIG. 7




| | IC50 |
|---|---|
| MCL1_Seq A | 2242 |
| MCL1_Seq B | 2299 |
| MCL1_Seq C | 2726 |
| MCL1_Seq D | 1728 |
| GFP | 2961 |
| NC | 3299 |

FIG. 8



SUBSTITUTE SHEET (RULE 26)

| Panel/Cell Line | | $Log_{10}GI50$ |
|---|---|---|
| **Leukemia** | | |
| CCRF-CEM | | -7.59 |
| HL-60(TB) | < | -8.00 |
| K-562 | | -7.59 |
| MOLT-4 | < | -8.00 |
| RPMI-8226 | | -6.49 |
| **Non-Small Cell Lung Cancer** | | |
| A549/ATCC | | -6.83 |
| EKVX | | -5.87 |
| HOP-62 | | -7.32 |
| HOP-92 | | -6.56 |
| NCI-H226 | | -7.90 |
| NCI-H23 | | -7.50 |
| NCI-H322M | | -5.57 |
| NCI-H460 | < | -8.00 |
| NCI-H522 | | -7.71 |
| **Colon Cancer** | | |
| COLO 205 | | -6.86 |
| HCC-2998 | | -6.84 |
| HCT-116 | < | -8.00 |
| HCT-15 | | -6.69 |
| HT29 | | -6.70 |
| KM12 | | -4.91 |
| SW-620 | | -7.29 |
| **CNS Cancer** | | |
| SF-268 | | -7.29 |
| SF-295 | | -6.47 |
| SF-539 | | -5.96 |
| SNB-19 | | -5.93 |
| SNB-75 | | -5.63 |
| U251 | | -6.72 |
| **Melanoma** | | |
| LOX IMVI | | -7.29 |
| MALME-3M | | -7.22 |
| M14 | | -7.32 |
| SK-MEL-2 | | -6.73 |
| SK-MEL-28 | | -7.10 |
| SK-MEL-5 | | -7.31 |
| UACC-257 | | -7.37 |
| UACC-62 | | -7.64 |
| **Ovarian Cancer** | | |
| IGROV1 | | -7.90 |
| OVCAR-3 | | -6.25 |
| OVCAR-4 | | -6.47 |
| OVCAR-5 | | -6.34 |
| OVCAR-8 | | -6.37 |
| SK-OV-3 | | -6.38 |
| **Renal Cancer** | | |
| 786-0 | | -7.59 |
| A498 | | -7.31 |
| ACHN | | -7.75 |
| CAKI-1 | | -7.97 |
| RXF 393 | | -6.77 |
| SN12C | | -7.71 |
| TK-10 | | -5.69 |
| UO-31 | | -6.35 |
| **Prostate Cancer** | | |
| PC-3 | | -6.61 |
| DU-145 | < | -8.00 |
| **Breast Cancer** | | |
| MCF7 | | -7.57 |
| NCI/ADR-RES | | -4.83 |
| MDA-MB-231/ATCC | | -6.24 |
| HS 578T | | -5.54 |
| MDA-MB-435 | | -6.92 |
| BT-549 | | -6.55 |
| T-47D | < | -8.00 |
| MDA-MB-468 | | -7.90 |
| MID | | -6.93 |
| Delta | | 1.07 |
| Range | | 3.17 |

| Panel/Cell Line | | $Log_{10}GI50$ |
|---|---|---|
| **Leukemia** | | |
| CCRF-CEM | | -7.82 |
| HL-60(TB) | | -7.47 |
| K-562 | | -6.98 |
| MOLT-4 | | -7.52 |
| RPMI-8226 | | -6.53 |
| SR | > | -4.00 |
| **Non-Small Cell Lung Cancer** | | |
| A549/ATCC | | -6.82 |
| EKVX | | -5.97 |
| HOP-62 | | -6.53 |
| NCI-H23 | | -7.45 |
| NCI-H322M | | -5.79 |
| NCI-H460 | < | -8.00 |
| NCI-H522 | | -6.86 |
| **Colon Cancer** | | |
| COLO 205 | | -7.53 |
| HCC-2998 | < | -8.00 |
| HCT-116 | | -7.74 |
| HCT-15 | | -6.83 |
| HT29 | | -6.93 |
| KM12 | | -5.26 |
| SW-620 | | -7.45 |
| **CNS Cancer** | | |
| SF-268 | | -7.37 |
| SF-295 | | -7.22 |
| SF-539 | | -6.60 |
| SNB-19 | | -6.32 |
| SNB-75 | | -6.86 |
| U251 | | -7.23 |
| **Melanoma** | | |
| LOX IMVI | | -7.22 |
| MALME-3M | | -7.49 |
| M14 | | -6.87 |
| SK-MEL-2 | | -6.87 |
| SK-MEL-28 | | -6.72 |
| SK-MEL-5 | | -7.57 |
| UACC-257 | | -7.83 |
| UACC-62 | < | -8.00 |
| **Ovarian Cancer** | | |
| IGROV1 | | -6.80 |
| OVCAR-3 | | -6.64 |
| OVCAR-4 | | -6.88 |
| OVCAR-5 | | -6.49 |
| OVCAR-8 | | -6.33 |
| SK-OV-3 | | -6.46 |
| **Renal Cancer** | | |
| 786-0 | | -6.96 |
| A498 | | -7.10 |
| ACHN | < | -8.00 |
| CAKI-1 | | -7.14 |
| SN12C | | -7.88 |
| TK-10 | | -5.93 |
| UO-31 | | -6.14 |
| **Prostate Cancer** | | |
| PC-3 | | -6.86 |
| DU-145 | < | -8.00 |
| **Breast Cancer** | | |
| MCF7 | | -7.94 |
| NCI/ADR-RES | | -5.83 |
| MDA-MB-231/ATCC | | -6.88 |
| HS 578T | | -5.94 |
| MDA-MB-435 | | -6.95 |
| BT-549 | | -7.45 |
| T-47D | < | -8.00 |
| MDA-MB-468 | | -7.73 |
| MID | | -6.99 |
| Delta | | 1.01 |
| Range | | 4.0 |

**FIG. 9**

FIG. 10

FIG. 11



SUBSTITUTE SHEET (RULE 26)

| Gene symbol | Probe set | Fold-change | Geom mean of intensities in RES | Geom mean of intensities in SENS | Permutation p-value | FDR | Parametric p-value |
|---|---|---|---|---|---|---|---|
| FOSL2 | 218880_at | 2.8912722 | 143.5544731 | 49.6509708 | 0.0001 | 0.091739 | 0.0000102 |
| EIF4B | 211937_at | 0.5721367 | 587.3927949 | 1026.665209 | < 1e-07 | 0.17731 | 0.0000686 |
| ULBP2 | 238542_at | 2.1556529 | 120.25891 | 55.7876979 | < 1e-07 | 0.17731 | 0.0000867 |
| ANKRD13C | 223418_x_at | 1.7459075 | 28.2999422 | 16.2093021 | < 1e-07 | 0.17731 | 0.0001058 |
| STIL | 205339_at | 1.5060176 | 102.9902254 | 68.3858062 | 0.0001 | 0.17731 | 0.0001319 |
| FOSL2 | 225262_at | 2.7480515 | 164.3820677 | 59.8176817 | 0.0002 | 0.17731 | 0.0001336 |
|  | 232453_at | 1.8095425 | 31.559447 | 17.4405673 | < 1e-07 | 0.17731 | 0.000138 |
| BIRC2 | 202076_at | 2.1746683 | 271.4505121 | 124.8238673 | 0.0001 | 0.178306 | 0.0001586 |
| ARHGAP21 | 224764_at | 1.6508838 | 80.2463403 | 48.6081087 | 0.0002 | 0.184777 | 0.0001849 |
| PDCD6 | 222380_s_at | 0.6250508 | 51.0750009 | 81.7133555 | 0.0002 | 0.228268 | 0.0002538 |
| TMEPAI | 222450_at | 3.1248931 | 141.8460168 | 45.3922788 | 0.0002 | 0.264587 | 0.0003236 |
| SVIL | 202566_s_at | 1.3715929 | 77.3177244 | 56.3707548 | 0.0008 | 0.286984 | 0.0003829 |
| JUN | 201464_x_at | 3.4283161 | 497.9993762 | 145.2606343 | 0.0007 | 0.289953 | 0.0004191 |
| RUNX2 | 232231_at | 3.4570592 | 111.0099659 | 32.1110982 | 0.0009 | 0.380086 | 0.0006331 |
| TMEPAI | 222449_at | 3.9998423 | 232.6178802 | 58.1567626 | 0.0004 | 0.380086 | 0.0006339 |
| WDFY3 | 212606_at | 1.7324826 | 63.9596813 | 36.9179357 | 0.0009 | 0.421819 | 0.000775 |
| DCBLD1 | 226609_at | 1.8174703 | 57.2094608 | 31.477521 | 0.0003 | 0.421819 | 0.0007973 |
| EIF4B | 211938_at | 0.6469148 | 637.9381321 | 986.1239092 | 0.0007 | 0.422008 | 0.0008499 |
| ITGAV | 202351_at | 2.9759536 | 338.4331714 | 113.7225953 | 0.0008 | 0.422008 | 0.0008915 |
| PPFIBP1 | 203735_x_at | 1.5384496 | 211.2382394 | 137.3059232 | 0.001 | 0.439807 | 0.0009985 |

Sorted by p-value of univariate test, genes are significant at the nominal 0.001 level of the univariate test

**FIG. 12A**

232453_
ULBP2
PPFIBP1
SVIL
ARHGAP2
ANKRD13
TMEPAI
TMEPAI
FOSL2
FOSL2
JUN
RUNX2
WDFY3
BIRC2
ITGAV
DCBLD1
STIL
EIF4B
EIF4B
PDCD6

FIG. 12B

| GENE SETS ENRICHED IN BROSTALLICIN SENSITIVE CELL LINES | ES | NES | NOM p-val | FDR q-val |
|---|---|---|---|---|
| CHROMATIN REMODELING (GO:0006338) | -0.6878 | -1.9394 | 0.0000 | 0.0938 |
| SINGLE-STRANDED DNA BINDING (GO:0003697) | -0.6724 | -1.8470 | 0.0000 | 0.1414 |
| STRUCTURAL CONSTITUENT OF RIBOSOME (GO:0003735) | -0.6028 | -1.6181 | 0.0769 | 0.4949 |
| CYTOSOLIC SMALL RIBOSOMAL SUBUNIT (SENSU EUKARYOTA) (GO:0005843) | -0.6414 | -1.6275 | 0.0385 | 0.5351 |
| CYTOSOLIC LARGE RIBOSOMAL SUBUNIT (SENSU EUKARYOTA) (GO:0005842) | -0.6699 | -1.6535 | 0.0000 | 0.5488 |
| DNA-DIRECTED RNA POLYMERASE ACTIVITY (GO:0003899) | -0.6861 | -1.5099 | 0.0417 | 0.5598 |
| PROTEIN BIOSYNTHESIS (GO:0006412) | -0.4809 | -1.4399 | 0.1176 | 0.5832 |
| NUCLEOLUS (GO:0005730) | -0.4673 | -1.4463 | 0.0816 | 0.5989 |
| TRANSCRIPTION FACTOR TFIID COMPLEX (GO:0005669) | -0.6504 | -1.5106 | 0.0465 | 0.6067 |
| DAMAGED DNA BINDING (GO:0003684) | -0.5305 | -1.4535 | 0.1364 | 0.6094 |
| NUCLEOTIDE-EXCISION REPAIR (GO:0006289) | -0.6047 | -1.6715 | 0.0222 | 0.6156 |
| NUCLEOSOME ASSEMBLY (GO:0006334) | -0.5370 | -1.4851 | 0.0889 | 0.6227 |
| MITOCHONDRIAL INNER MEMBRANE (GO:0005743) | -0.6354 | -1.4601 | 0.0444 | 0.6239 |
| GLUCOSE METABOLISM (GO:0006006) | -0.5778 | -1.5153 | 0.0588 | 0.6407 |
| NUCLEOPLASM (GO:0005654) | -0.4591 | -1.4615 | 0.0600 | 0.6634 |
| DNA RECOMBINATION (GO:0006310) | -0.6070 | -1.5188 | 0.0377 | 0.6982 |
| ATP-DEPENDENT RNA HELICASE ACTIVITY (GO:0004004) | -0.6633 | -1.5208 | 0.0417 | 0.7746 |
| MISMATCH REPAIR (GO:0006298) | -0.5939 | -1.3641 | 0.1636 | 0.8205 |
| RRNA PROCESSING (GO:0006364) | -0.6555 | -1.3506 | 0.1509 | 0.8372 |
| HETEROGENEOUS NUCLEAR RIBONUCLEOPROTEIN COMPLEX (GO:0030530) | -0.6173 | -1.3265 | 0.1429 | 0.8483 |

- 129 / 297 gene sets are upregulated in phenotype **IncreasingBrosResistant_neg**
- 2 gene sets are significantly enriched at FDR < 25%
- 3 gene sets are significantly enriched at nominal pvalue < 1%
- 10 gene sets are significantly enriched at nominal pvalue < 5%

| GENE SETS ENRICHED IN BROSTALLICIN RESISTANT CELL LINES | ES | NES | NOM p-val | FDR q-val |
|---|---|---|---|---|
| HEART DEVELOPMENT (GO:0007507) | 0.6231 | 1.9512 | 0.0000 | 0.1790 |
| EPIDERMIS DEVELOPMENT (GO:0008544) | 0.5711 | 1.8983 | 0.0000 | 0.1373 |
| ACTIN CYTOSKELETON (GO:0015629) | 0.5299 | 1.8499 | 0.0000 | 0.2099 |
| SKELETAL DEVELOPMENT (GO:0001501) | 0.4982 | 1.8332 | 0.0000 | 0.1823 |
| RESPONSE TO UNFOLDED PROTEIN (GO:0006986) | 0.6412 | 1.7995 | 0.0357 | 0.2080 |
| INTERCELLULAR JUNCTION (GO:0005911) | 0.6142 | 1.7127 | 0.0000 | 0.3484 |
| TRANSCRIPTION FACTOR BINDING (GO:0008134) | 0.5064 | 1.7059 | 0.0000 | 0.3261 |
| CYTOKINE ACTIVITY (GO:0005125) | 0.5584 | 1.7031 | 0.0000 | 0.2877 |
| GOLGI APPARATUS (GO:0005794) | 0.5189 | 1.6934 | 0.0000 | 0.2723 |
| TRANSFORMING GROWTH FACTOR BETA RECEPTOR SIGNALING PATHWAY (GO:0007179) | 0.5735 | 1.6488 | 0.0357 | 0.3600 |
| STRUCTURAL CONSTITUENT OF CYTOSKELETON (GO:0005200) | 0.4104 | 1.6470 | 0.0417 | 0.3310 |
| ACTIN BINDING (GO:0003779) | 0.4720 | 1.6447 | 0.0200 | 0.3081 |
| HEPARIN BINDING (GO:0008201) | 0.5668 | 1.6285 | 0.0566 | 0.3315 |
| ENDOPLASMIC RETICULUM LUMEN (GO:0005788) | 0.6857 | 1.6279 | 0.0392 | 0.3086 |
| ORGAN MORPHOGENESIS (GO:0009887) | 0.4398 | 1.6256 | 0.0417 | 0.2929 |
| CELL MOTILITY (GO:0006928) | 0.3945 | 1.6223 | 0.0200 | 0.2825 |
| EXTRACELLULAR MATRIX STRUCTURAL CONSTITUENT (GO:0005201) | 0.5246 | 1.5702 | 0.0204 | 0.3825 |
| INTERMEDIATE FILAMENT (GO:0005882) | 0.5222 | 1.5422 | 0.0200 | 0.4256 |
| RESPONSE TO DRUG (GO:0042493) | 0.5661 | 1.5226 | 0.0909 | 0.4652 |
| ENDOCYTOSIS (GO:0006897) | 0.6051 | 1.5219 | 0.0862 | 0.4435 |

- 168 / 297 gene sets are upregulated in phenotype **IncreasingBrosResistant_pos**
- 5 gene sets are significant at FDR < 25%
- 9 gene sets are significantly enriched at nominal pvalue < 1%
- 20 gene sets are significantly enriched at nominal pvalue < 5%

**FIG. 13A**

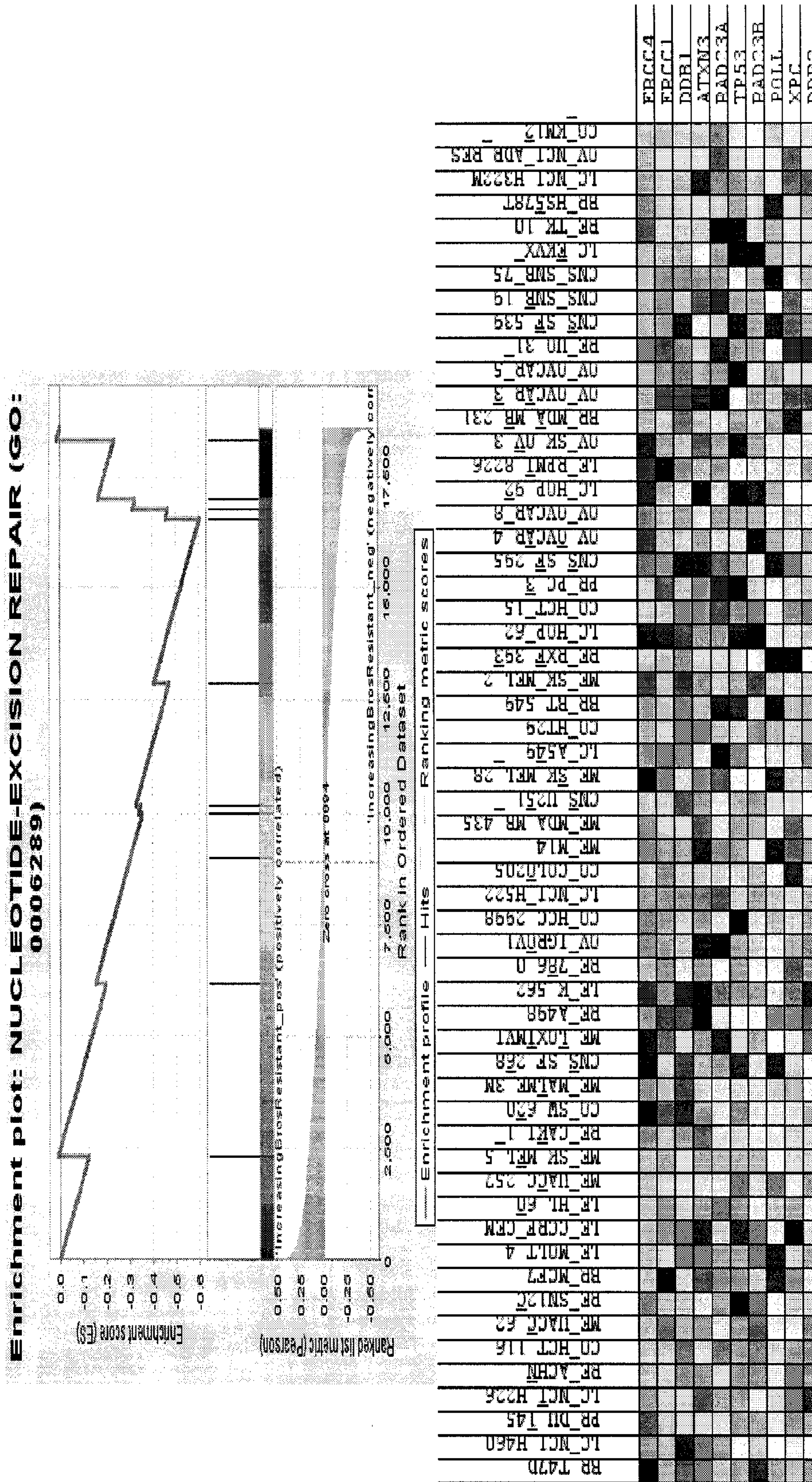
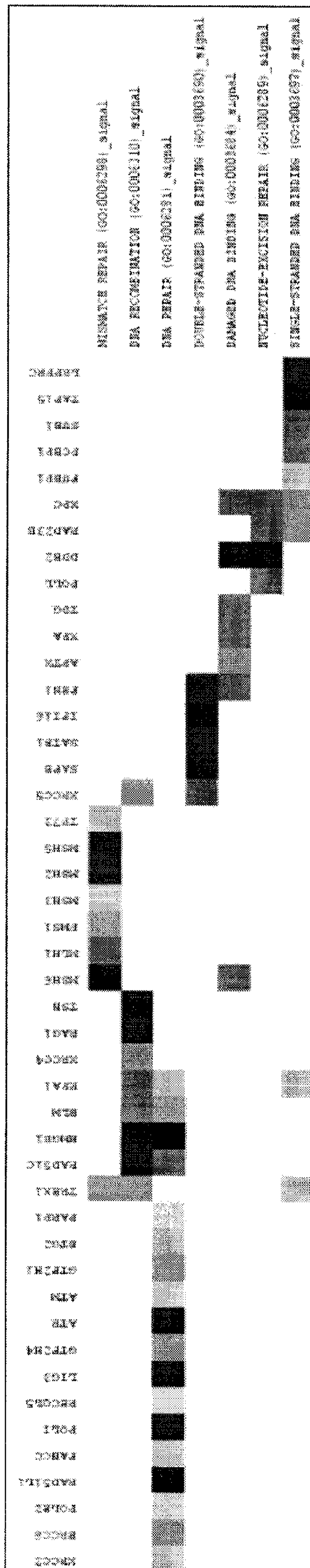



FIG. 13B



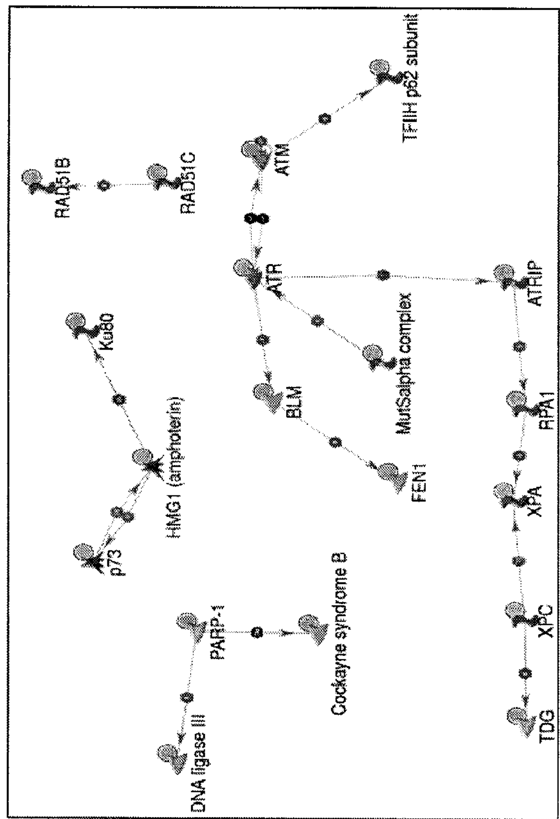



FIG. 13C

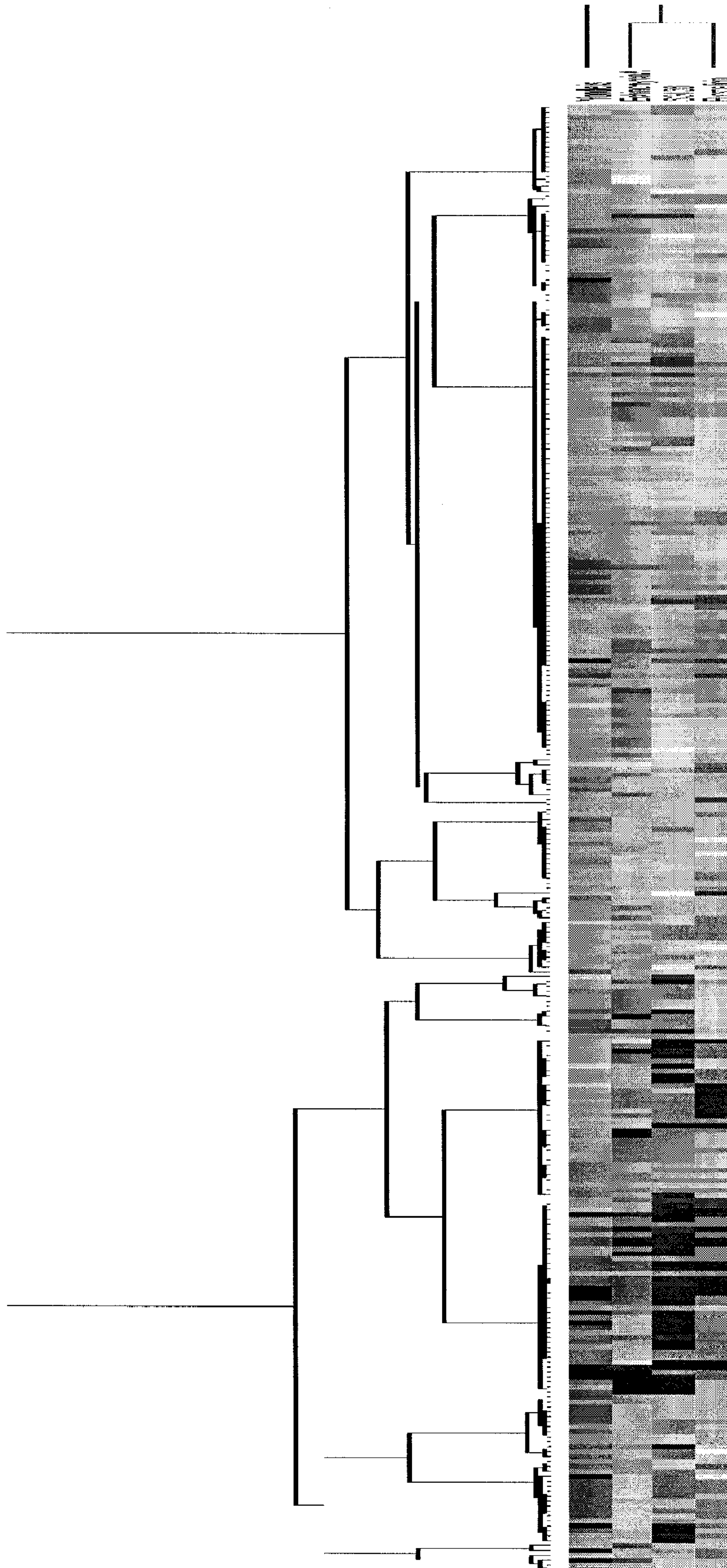


FIG. 14A

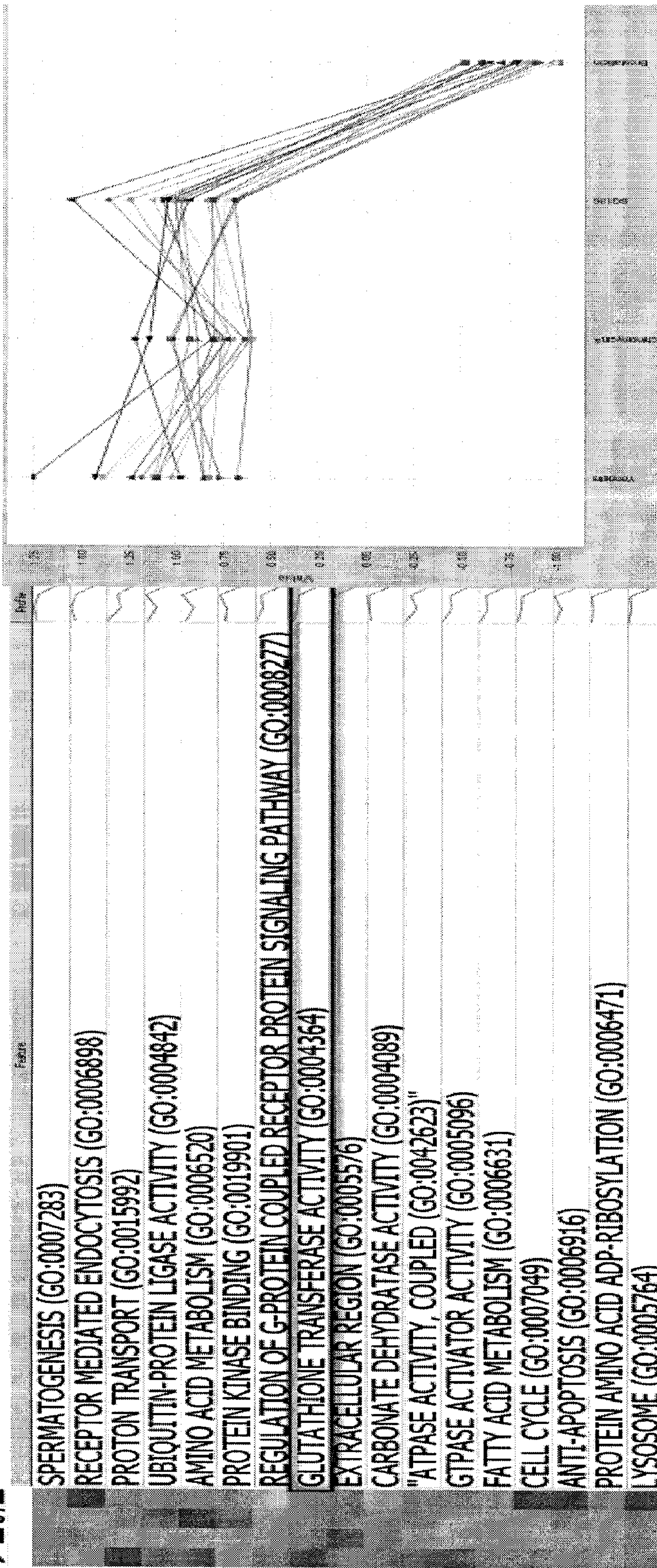
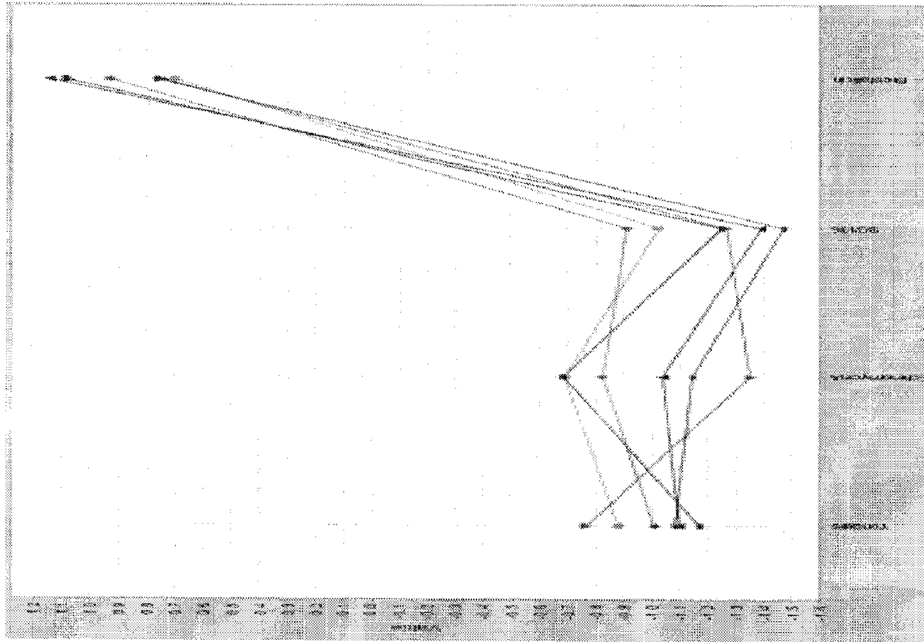


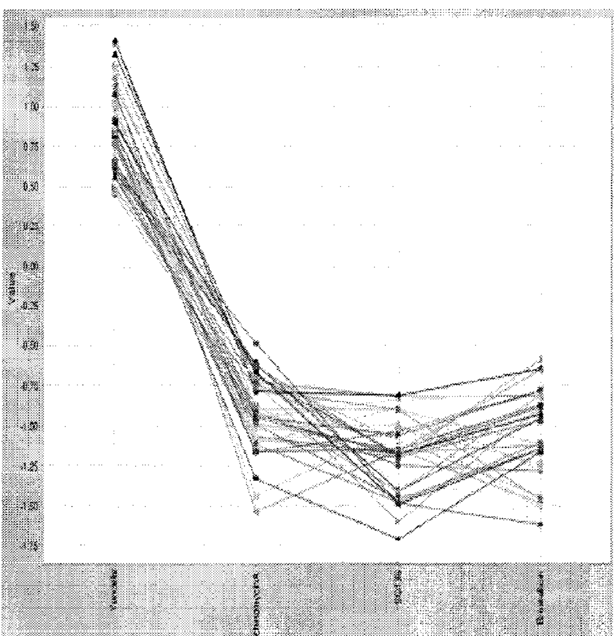

**Fig. 14B**



Yondelis
EchinomycinA
SG136
Brostallicin

| Feature | Profile |
|---|---|
| PROTEIN HETERODIMERIZATION ACTIVITY (GO:0046982) | |
| NLS-BEARING SUBSTRATE IMPORT INTO NUCLEUS (GO:0006607) | |
| AXON GUIDANCE (GO:0007411) | |
| TRANSCRIPTION COREPRESSOR ACTIVITY (GO:0003714) | |
| NEGATIVE REGULATION OF TRANSCRIPTION FROM RNA POLYMERASE II PROMOTER (GO:0000... | |
| JAK-STAT CASCADE (GO:0007259) | |

**FIG. 14C**



Yondelis
EchinomycinA
SG136
Brostallicin

Feature | Profile

STRUCTURAL CONSTITUENT OF RIBOSOME (GO:0003735)
"MITOCHONDRIAL ELECTRON TRANSPORT, NADH TO UBIQUINONE (GO:0006120)"
INTRACELLULAR SIGNALING CASCADE (GO:0007242)
NADH DEHYDROGENASE (UBIQUINONE) ACTIVITY (GO:0008137)
DOUBLE-STRANDED DNA BINDING (GO:0003690)
PROTEIN C-TERMINUS BINDING (GO:0008022)
THYROID HORMONE RECEPTOR BINDING (GO:0046966)
DNA REPAIR (GO:0006281)
CHROMATIN BINDING (GO:0003682)
MITOCHONDRIAL INNER MEMBRANE (GO:0005743)
REGULATION OF TRANSLATIONAL INITIATION (GO:0006446)
GLUCOSE METABOLISM (GO:0006006)
NUCLEOSOME ASSEMBLY (GO:0006334)
CYTOSOLIC SMALL RIBOSOMAL SUBUNIT (SENSU EUKARYOTA) (GO:0005843)
PROTEIN BIOSYNTHESIS (GO:0006412)
ELECTRON CARRIER ACTIVITY (GO:0009055)
"POSITIVE REGULATION OF TRANSCRIPTION, DNA-DEPENDENT (GO:0045893)"
NUCLEOLUS (GO:0005730)
TRANSLATION INITIATION FACTOR ACTIVITY (GO:0003743)
INTRA-GOLGI VESICLE-MEDIATED TRANSPORT (GO:0006891)
MITOCHONDRIAL MATRIX (GO:0005759)
RHO PROTEIN SIGNAL TRANSDUCTION (GO:0007266)
PROTEIN-TYROSINE KINASE ACTIVITY (GO:0004713)
UBIQUITIN-DEPENDENT PROTEIN CATABOLISM (GO:0006511)
RNA POLYMERASE III TRANSCRIPTION FACTOR ACTIVITY (GO:0003709)
PROTEIN FOLDING (GO:0006457)
TRANSCRIPTION FROM RNA POLYMERASE III PROMOTER (GO:0006383)
PEROXISOME (GO:0005777)
ELECTRON TRANSPORT (GO:0006118)
CHROMOSOME ORGANIZATION AND BIOGENESIS (SENSU EUKARYOTA) (GO:0007001)
CYTOCHROME-C OXIDASE ACTIVITY (GO:0004129)
PROTEIN AMINO ACID N-LINKED GLYCOSYLATION (GO:0006487)

FIG. 14D

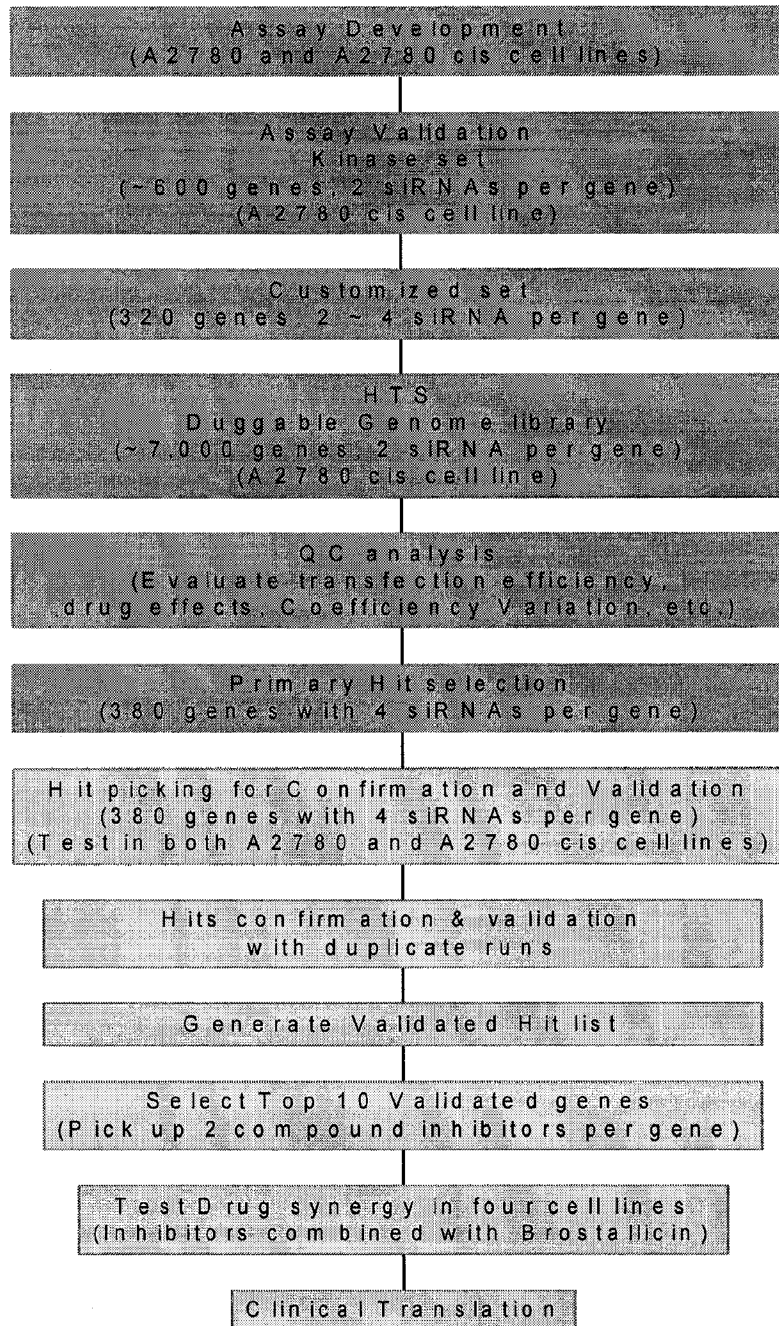



FIG. 15A

FIG. 15B



SUBSTITUTE SHEET (RULE 26)

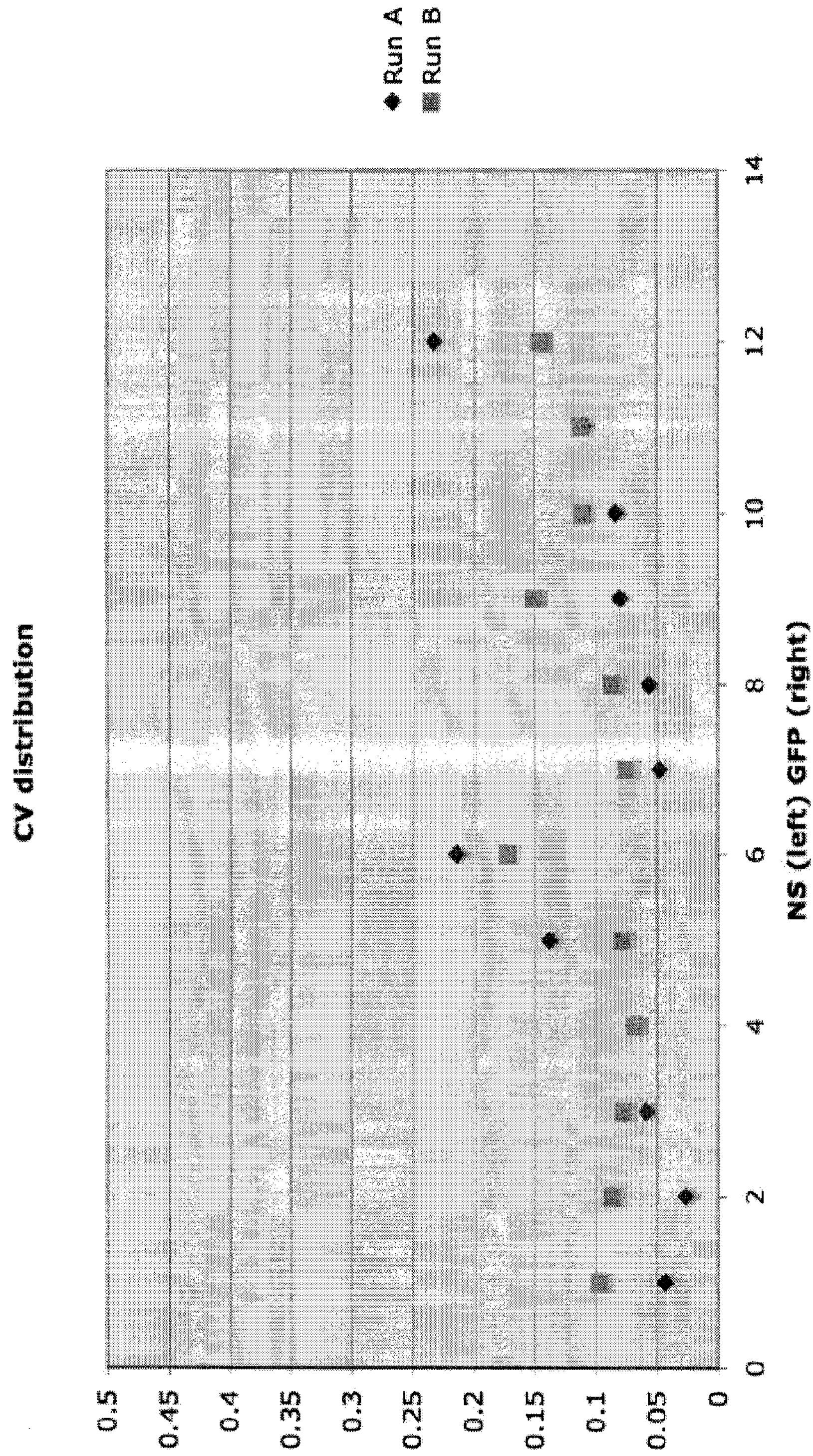



FIG. 15C

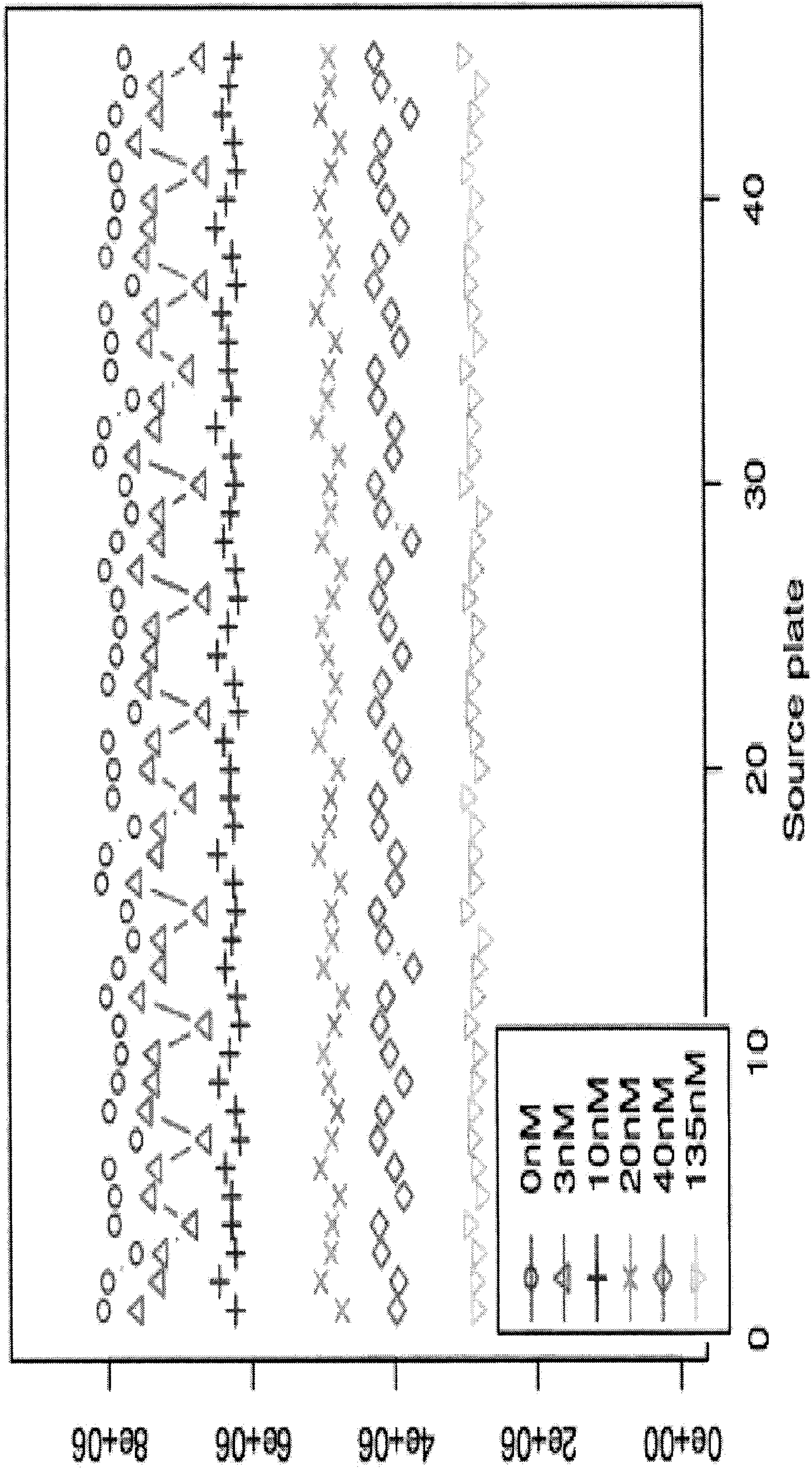



FIG. 15D

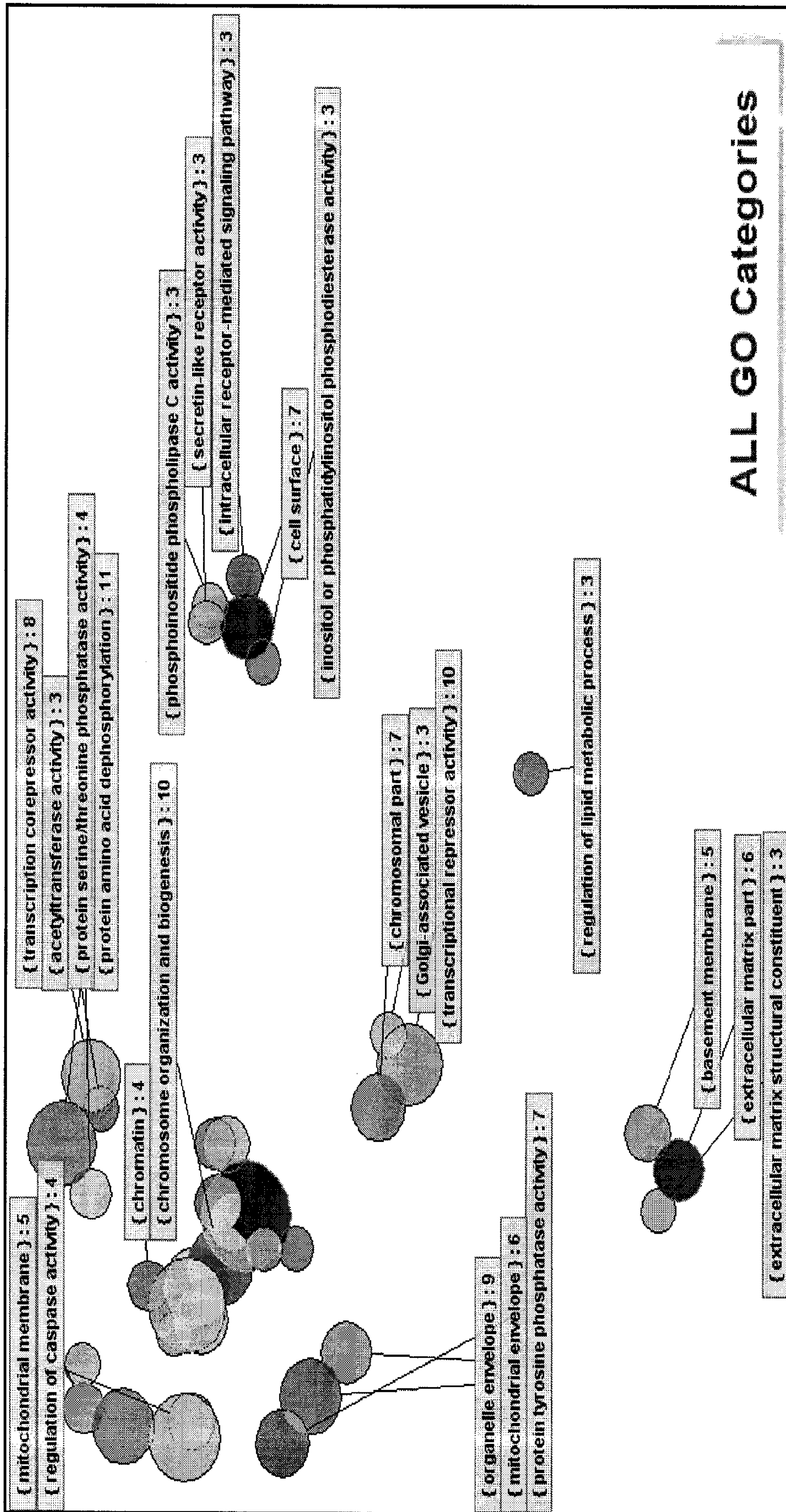



FIG. 16A

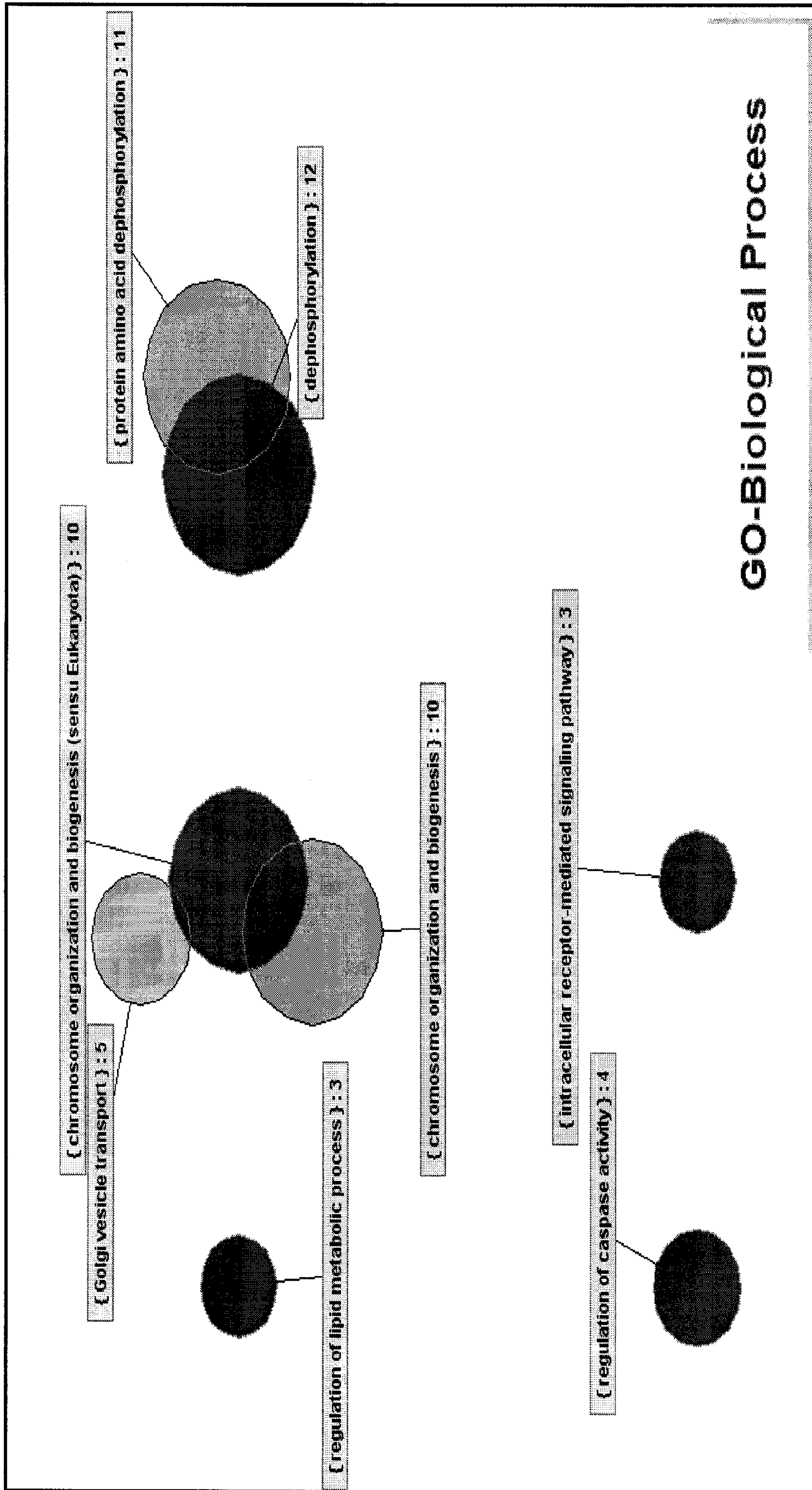



FIG. 16B

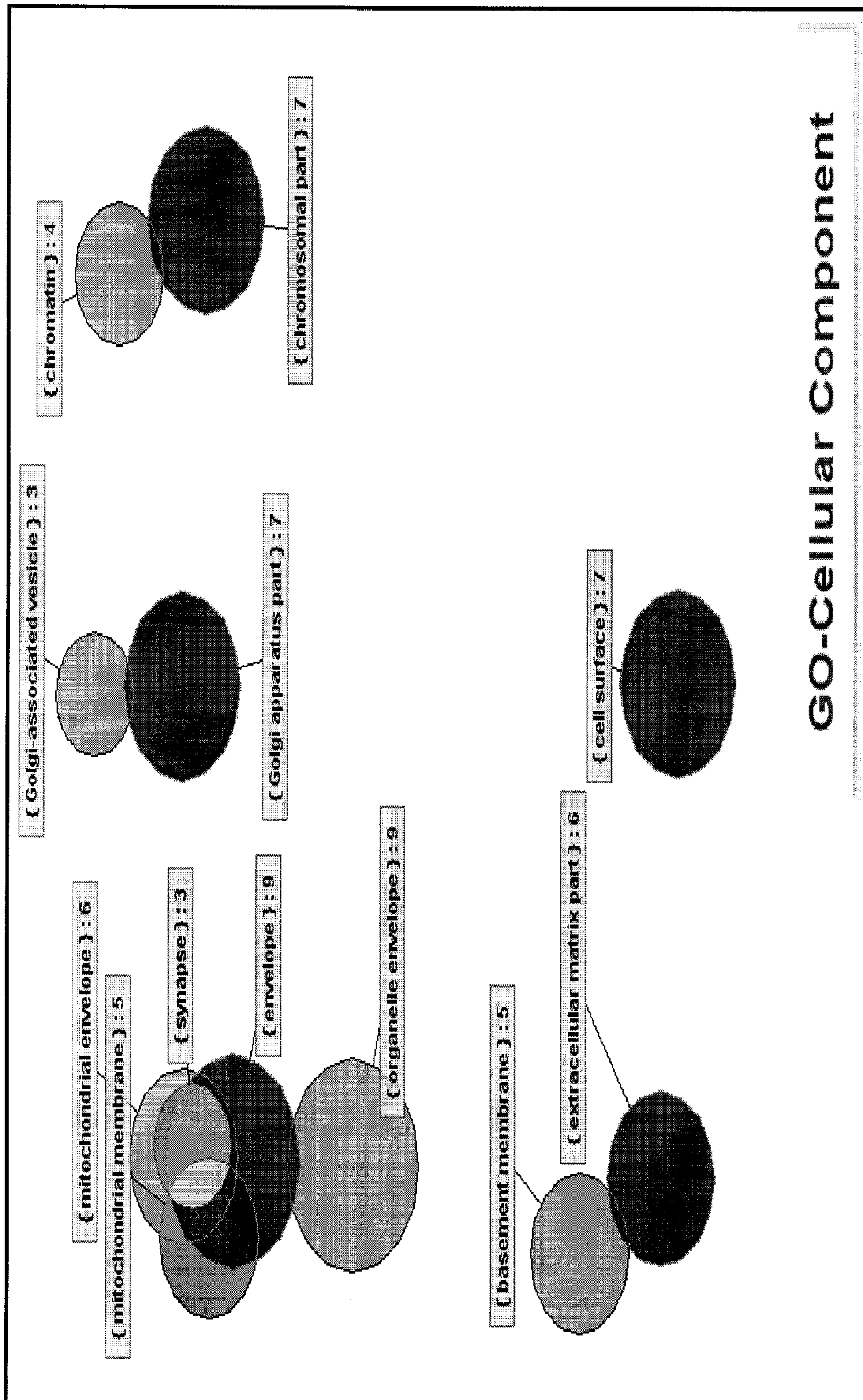



Fig. 16C

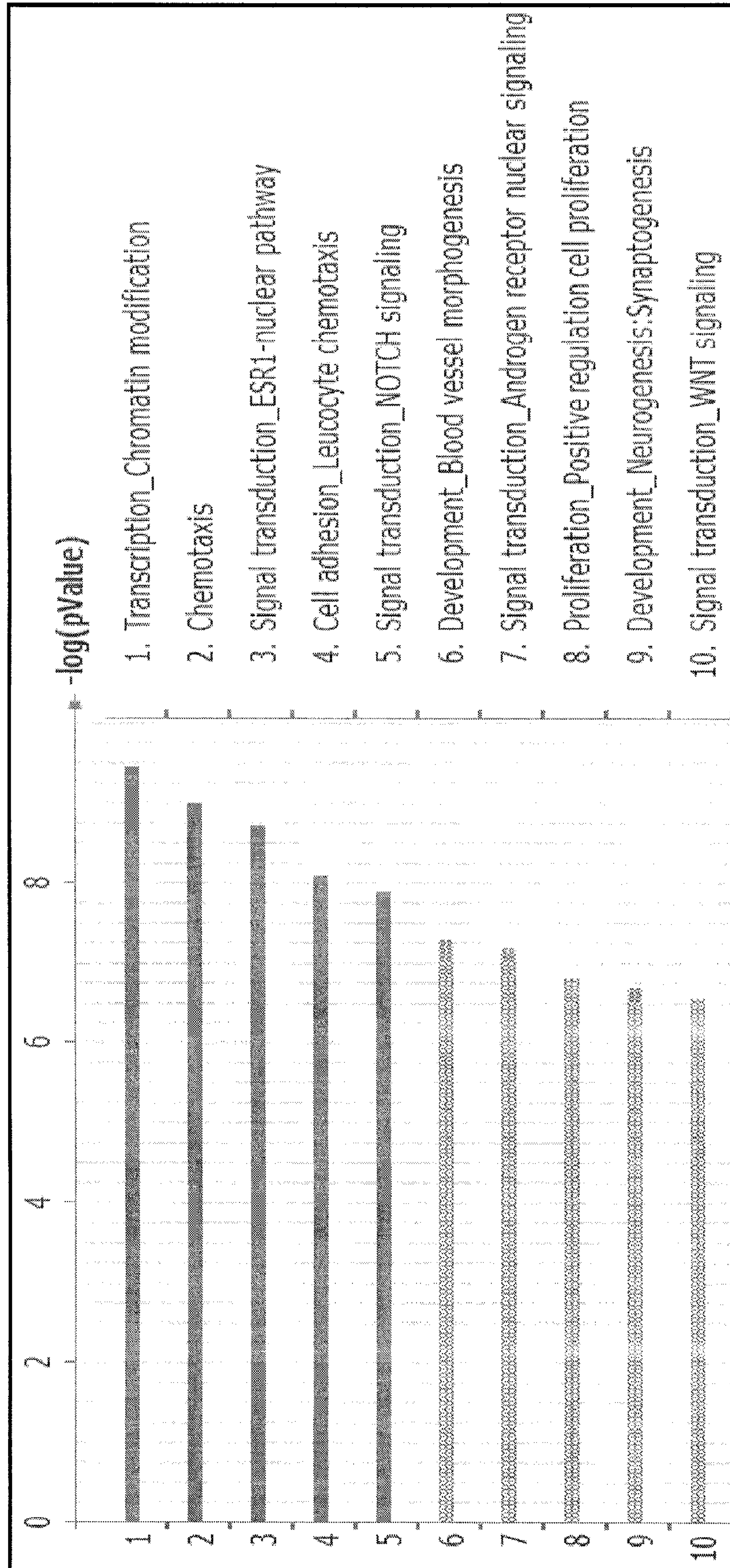
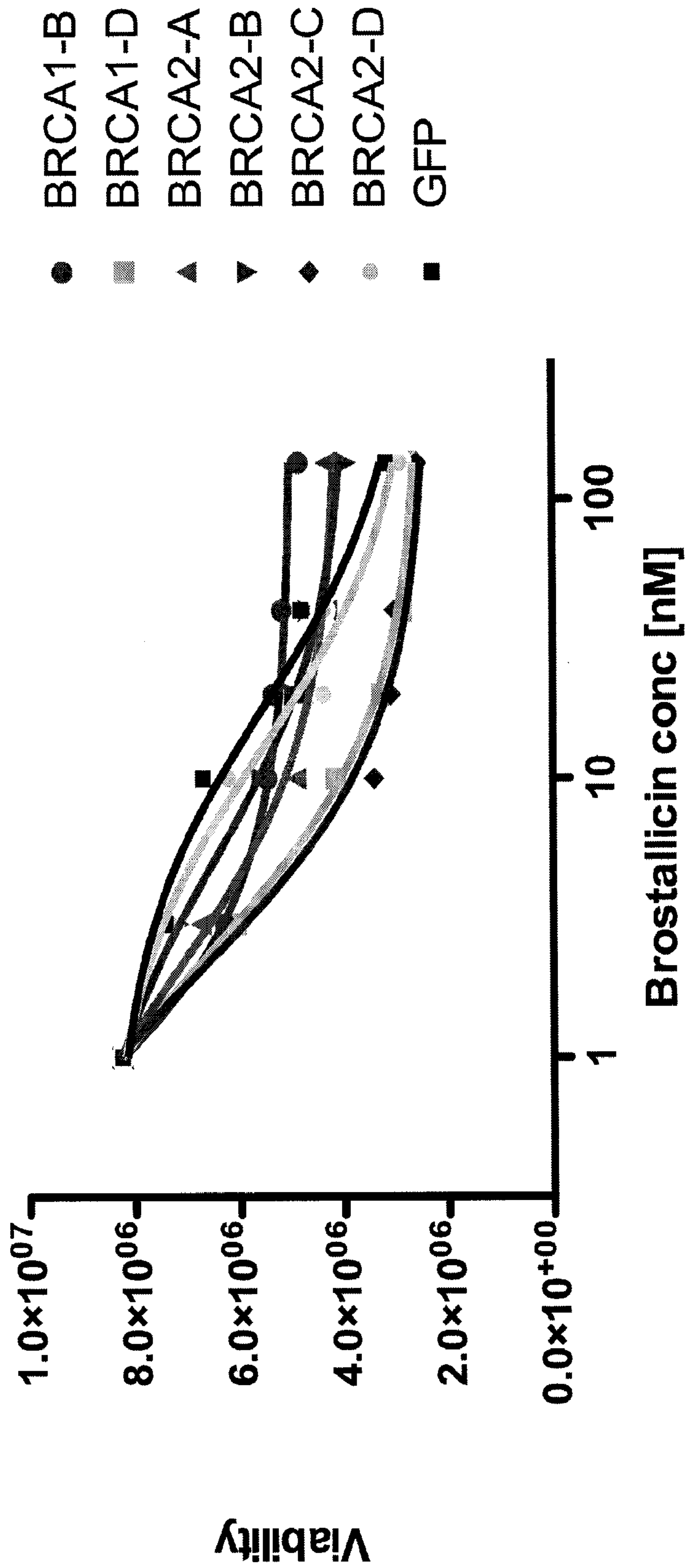
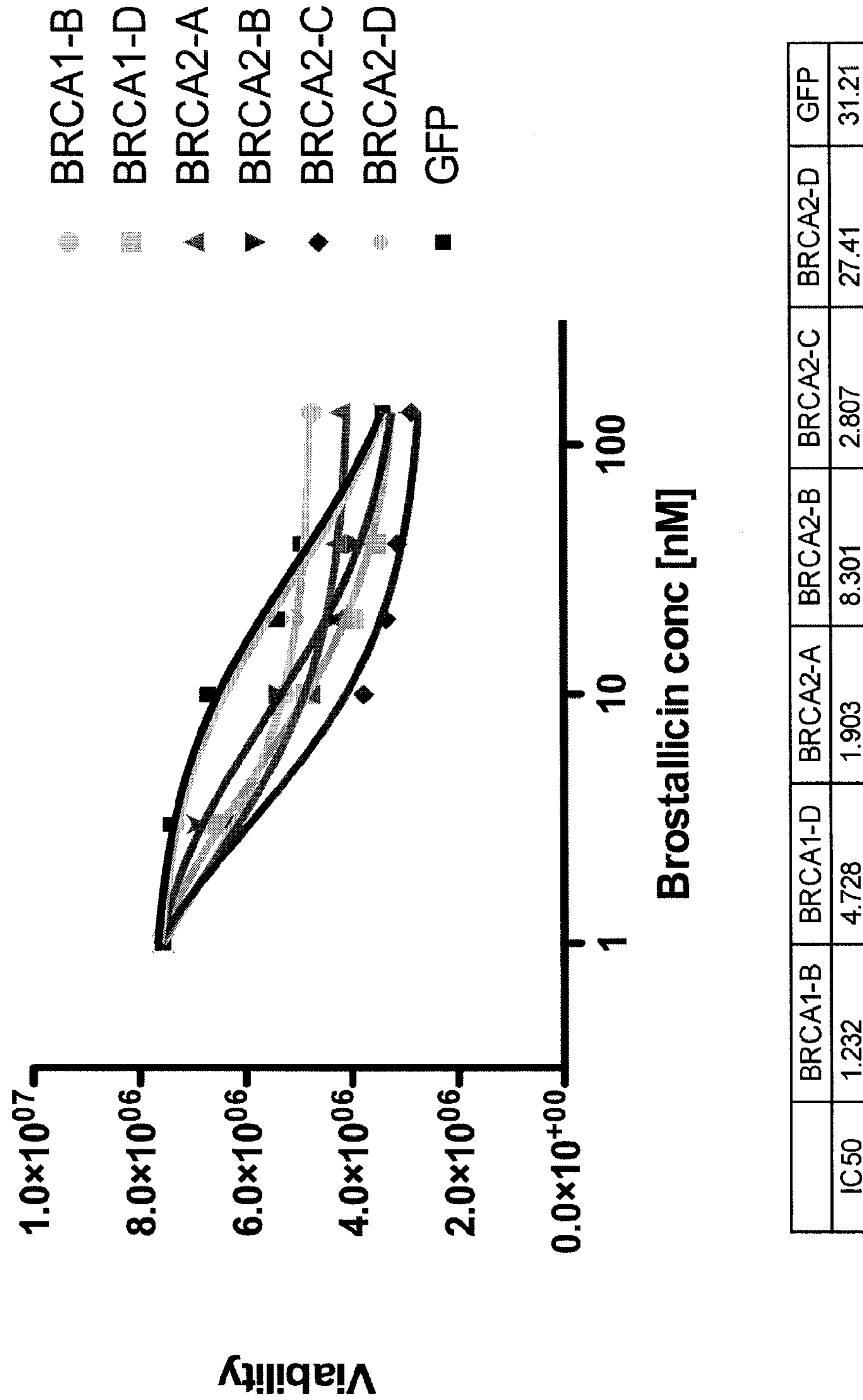



FIG. 16D




| | BRCA1-B | BRCA1-D | BRCA2-A | BRCA2-B | BRCA2-C | BRCA2-D | GFP |
|---|---|---|---|---|---|---|---|
| IC50 | 0.3868 | 2.329 | 1.911 | 5.003 | 1.913 | 12.75 | 18.24 |

FIG. 17A




| | BRCA1-B | BRCA1-D | BRCA2-A | BRCA2-B | BRCA2-C | BRCA2-D | GFP |
|---|---|---|---|---|---|---|---|
| IC50 | 1.232 | 4.728 | 1.903 | 8.301 | 2.807 | 27.41 | 31.21 |

FIG. 17B

# DNA Repair Pathways with Hits Highlighted

Ionizing Radiation

Nucleus

DNA Damage

FANCA FANCC FANCE FANCF FANCG

ATM FANCD2 FANCD2

Intra-S-phase checkpoint response

ATR ATM CTIP Chk2

BRCA2 Rad51 BRCA1 BARD1 BARD1 BRCA1

STAT1 ATF1 E2F Rb Chk1 PLK1 p53 OCT-1 GADD45 SWI/SNF

Expression of IFNγ target genes

Transcriptional Response to DNA Damage

G1/S phase Transition

Checkpoint Regulation

p21CIP1

G2/M Phase

BRCA2 BRCA1 Rad51 BARD1 FANCD2

S phase and G2 arrest

BACH1

BRCA1 BLM MSH2 MLH1 RFC MSH6 NBS1 Rad50 MRE11

DNA Repair

Homologous Recombination

Chromatin Remodelling

ATR PP (1) A, P, PP (10) P, PP (2) CLSPN P, PP (3) Chk1

Translesion Synthesis

UBE2A M, PP (4) RAD18 M, RB (3) PP (4) PCNA M (1) UBE2N ZNRF2 RNF8

FIG. 18

# Translesion Synthesis (TLS)

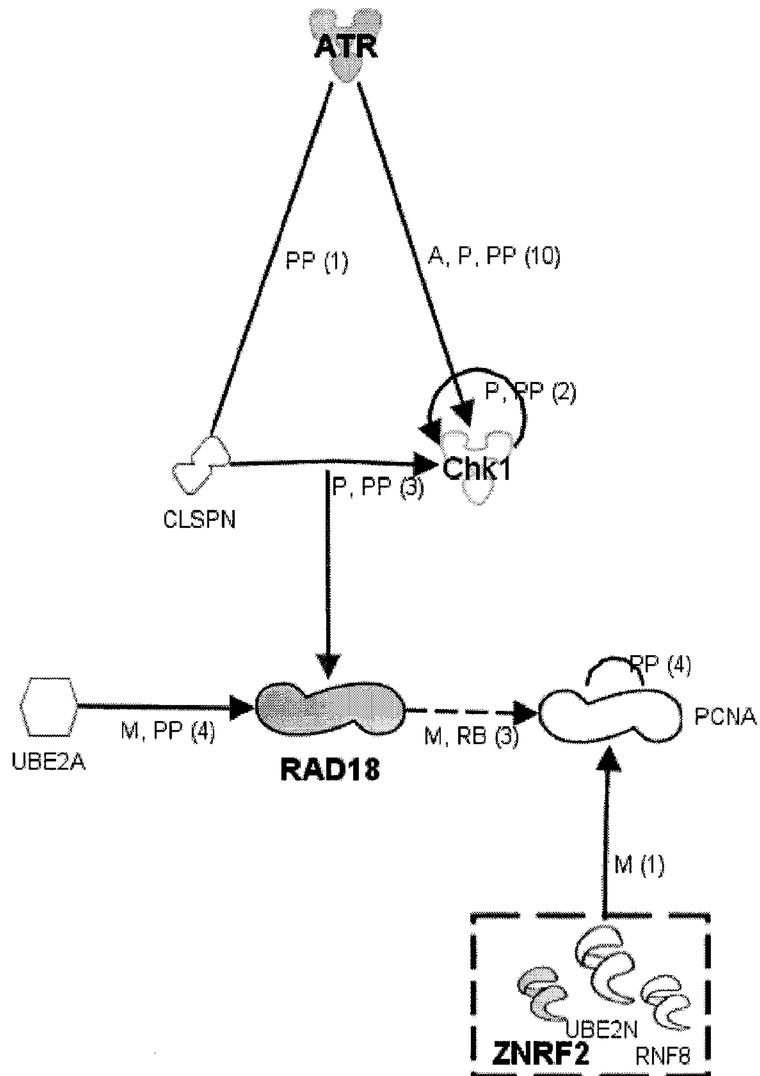



**FIG. 19**

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 09/40404

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12Q 1/68; A61K 31/00 (2009.01)
USPC - 435/6, 424/9.2
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8) - C12Q 1/68; A61K 31/00 (2009.01)
USPC - 435/6, 424/9.2

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
IPC(8) - C12Q 1/68; A61K 31/00 (2009.01) - see keyword below
USPC - 435/6, 424/9.2 - see keyword below

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST(USPT,PGPB,EPAB,JPAB); Medline, Google
Search terms: brostallicin, PNU-166196, BRCA1, sensitivity, expression, control, reduce, correlating, resistant, database, genomic aberrations, mutation, deletion, hybridization, array, rtPCR, sequencing, mRNA, microarray, ELISA, ovarian, colon, pancreatic, cancer

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X<br>---<br>Y | US 2005/0181385 A1 (LINSLEY et al.) 18 August 2005 (18.08.2005), para [0028], [0046], [0047], [0048], [0049], [0050], [0058], [0062], [0068], [0069], [0200], [0203], [0245], [0247], [0279], [0290], [0333], [0349], [0358], [0363], [0369], [0379], [0380], and Table II | 1-5, 11-21, 25-29<br>---<br>6-10, 22-24 |
| A | FEDIER et al. Brostallicin (PNU-166196) - a new DNA minor groove binder that retains sensitivity in DNA mismatch repair-deficient tumour cells. British Journal of Cancer, 2003, Vol. 89, p.1559 - 1565. Abstract | 11, 26 |
| Y | US 2006/0041111 A1 (YAJNIK et al.) 23 February 2006 (23.02.2006), Abstract, para [0004], [0022], [0077], [0170], and [0177] | 6-10, 22-24 |

☐ Further documents are listed in the continuation of Box C. ☐

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

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 20 May 2009 (20.05.2009) | 10 JUN 2009 |


Name and mailing address of the ISA/US
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450
Facsimile No. 571-273-3201

Authorized officer:
Lee W. Young
PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774