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(54) Title: PROGNOSIS INDICATORS FOR SOLID HUMAN TUMORS

(57) Abstract: Methods for predicting the clinical outcome of treating a human solid tumor using one or more taxanes are provided. The method includes measuring in the cells of a tumor the expression level of a set of genes whose change is related to chromosomal instability; taking a statistical measure of the expression level of the set of measured genes; and if the statistical measure of the expression level of the set of measured genes is elevated, determining that the prognosis is poor. The sets of genes which are useful in predicting the outcome of treatment of solid tumors using one or more taxanes are indicated. Methods and sets of genes can be used to determine whether a solid tumor is likely to exhibit drug resistance to one or more taxanes in vivo.



WO 2010/144192 A1

PROGNOSIS INDICATORS FOR SOLID HUMAN TUMORS**REFERENCE TO RELATED APPLICATIONS**

[0001] This application claims priority to and the benefit of U.S. Provisional Application No. 61/175,781, filed on May 5, 2009, the entire disclosure of which is
5 incorporated by reference herein.

GOVERNMENT SUPPORT

[0002] The United States government has certain rights to this invention pursuant to Grant Nos. NCI SPORE P50 CA 89393, R21LM008823-01A1 from the National Institute of Health.

10

FIELD

[0003] The present teachings relate generally to the field of cancer diagnostics and treatment, and more specifically to the determination of a likelihood that the outcome of a treatment using microtubule stabilizing drugs and/or carboplatin will be successful.

15

BACKGROUND

[0004] The treatment options for solid human tumors are multifold. Solid tumors can be treated chemotherapeutically, radiologically, surgically, or with a combination of these therapies. Each therapy produces undesirable side effects, which may be extensive enough that some patients cannot complete the course of
20 treatment. The side effects of cancer therapy also have a severe impact on the quality of life of these patients.

[0005] As a result, if the clinician can determine prior to treatment how refractory the tumor will respond to treatment, an appropriate treatment can be selected having the least side effects for the patient. The present teachings provide a method for
25 determining likelihood of clinical outcome based on the malignancy of the tumor.

SUMMARY

[0006] The present teachings relate to methods for predicting the outcome of the treatment of solid human tumors. In various embodiments, the methods generally include measuring in a particular solid tumor cancer type the degree of chromosomal

abnormalities and/or the expression levels of a large number of genes; identifying a subset of the measured genes characteristic of chromosomal instability (CIN); and determining in clinical samples whether the CIN signature accurately predicts the outcome of the treatment of the solid tumor. The methods can include the use of the

5 CIN signature to analyze a tumor of a patient to determine the prognosis of the cancer and whether treatment is likely to be successful. In certain embodiments, the treatment can involve administration of microtubule stabilizing (MTS) agents, such as taxanes, or carboplatin.

[0007] The present teachings also relate to optimizing therapeutic efficacy for

10 treatment of a tumor. In various embodiments, the methods can include measuring in a particular solid tumor cancer type the expression levels of a set of genes characteristic of chromosomal instability (CIN), and determining whether one or more microtubule stabilizing (MTS) agents or carboplatin should be administered to a patient for treatment of a tumor in the patient. For example, the results of the CIN

15 signature analysis may predict that taxane treatment is unlikely to be successful in treating a tumor. Accordingly, the methods may include administering one or more non-taxane drugs, e.g., DNA alkylating agents, to the patient in the treatment of the tumor.

[0008] In some embodiments, the present teachings provide a method of predicting

20 the clinical outcome of treating a patient having a tumor using one or more microtubule stabilizing (MTS) drugs. The method can include the steps of measuring, in a sample comprising a tumor cell from a patient, the mRNA expression levels of at least 10 of the following genes:

1	BRCA1	9	H2AFX	17	RFC3
2	CDC2	10	IMPA2	18	RPA1
3	CDC6	11	MCM3	19	SAE2
4	CHEK1	12	MSH6	20	TOP2A
5	DCLRE1	13	NUP153	21	TOPBP1
6	FDFT1	14	NUP155	22	XPQ1
7	GART	15	NUP205		
8	GPI	16	RCN2		

taking a statistical measure of the mRNA expression level of the measured genes;
and if the statistical measure of the mRNA expression level of the measured genes is
elevated, determining that the patient is a non-responder to MTS drug treatment and
5 that the prognosis is poor.

[0009] Gene expression levels can be measured using any suitable methods,
including, for example, using a microarray or polymerase chain reaction (PCR) (e.g.,
quantitative PCR, such as real-time PCR). In various embodiments, the mRNA
expression levels of more than 10 genes are measured. For example, the mRNA
10 expression levels of 10, 15, 20, or all 22 genes can be measured. In some
embodiments, the method can include the step of administering a suitable MTS drug
to the patient. The method also can include the step of measuring mRNA expression
levels at one or more times after administration of the MTS drug to the patient. The
present teachings can be used with any MTS drug or drugs, such as taxanes (e.g.,
15 paclitaxel or docetaxel) and epothilones.

[0010] In various embodiments, the statistical measure of the mRNA expression
level of the measured genes is a linear combination of the mRNA expression level of
the genes. In some embodiments, the linear combination of the mRNA expression
level of the genes is the mean of logarithms of the mRNA expression levels. In
20 particular embodiments, the linear combination of the mRNA expression level in the
set of genes is a combination of weighted mRNA expression levels.

[0011] In various embodiments, the tumor is a solid tumor, including, by non-
limiting example, breast cancer, ovarian cancer, colorectal cancer, lung cancer,
prostate cancer, medulloblastoma, glioma, and lymphoma.

25 [0012] In some embodiments, the present teachings also provide a method of
selecting a treatment for a patient having a tumor. The method can include the steps
of measuring, in a sample comprising a tumor cell from a patient, the mRNA
expression levels of at least 15 genes of the following set of genes:

1	TPX2	14	MCM7	25	RFC4
2	PRC1	15	RNASEH2A	26	ATAD2
3	FOXMI	16	RAD51API	27	ch-TOG
4	CDC2	17	KIF20A	28	NUP205
5	C20 or P24/TGIF2	18	CDC45L	29	CDC20
6	MCM2	19	MAD2LI	30	CKS2
7	H2AFZ	20	ESPL1	31	RRM2
8	TOP2A	21	CCNB2	32	ELAVL1
9	PCNA	22	FEN1	33	CCNB1
10	UBE2C	23	TTK	34	RRM1
11	MELK	24	CCT5	35	AURKB
12	TRIP13			36	MSH6
13	CNAP1			37	BZH2
				38	CTPS
				39	DKC1
40	OIP5	51	ZWINT	62	SFRS2
41	CDCA8	52	TOPK	63	HDGF
42	PTTG1	53	FLJ10036	64	NXT1
43	CEP55/C10orf3	54	CDCA3	65	NEK2
44	H2AFX	55	ECT2	66	DHCR7
45	CMAS	56	CDC6	67	STK6
46	BRN1	57	UNG	68	NDUFAB1
47	MCM10	58	MTCH2	69	KIAA0286
48	LSM4	59	RAD21	70	KIF4A
49	MTB	60	ACTL6A		
50	ASF1B	61	GPI/MGC13096		

- taking a statistical measure of the mRNA expression level of the measured genes;
and if the statistical measure of the mRNA expression level of the measured genes is
5 elevated, determining that the patient is a responder to carboplatin drug treatment
and/or a non-responder to paclitaxel drug treatment. Gene expression levels can be
measured using any suitable methods, including, for example, using a microarray or
PCR (e.g., quantitative PCR, such as real-time PCR). In various embodiments, the
mRNA expression levels of more than 15 genes are measured. For example, the
10 mRNA expression levels of 20, 25, 30, 35, 40 or 50 more genes can be measured.

[0013] In various embodiments, gene expression levels are measured before onset
of paclitaxel or carboplatin drug treatment. In some embodiments, the method can
include the step of administering carboplatin or recommending administration of
carboplatin to the patient.

- 15 [0014] In various embodiments, the statistical measure of the mRNA expression
level of the measured genes is a linear combination of the mRNA expression level of
the genes. In some embodiments, the linear combination of the mRNA expression
level of the genes is the mean of logarithms of the mRNA expression levels. In

particular embodiments, the linear combination of the mRNA expression level in the set of genes is a combination of weighted mRNA expression levels.

- [0015] In certain embodiments, the method comprises measuring, in a sample comprising a tumor cell from a patient, the mRNA expression level of at least 25 genes in the following set of genes:

1	TPX2	35	AURKB	69	KIAA0286
2	PRCI	36	MSH6	70	KIF4A
3	FOXMI	37	EZH2	71	SNRFB/GC10713
4	CDC2	38	CTPS	72	UCK2
5	C20orf24/TGIF2	39	DKC1	73	PARP1
6	MCM2	40	QIP5	74	RAD54L
7	H2AFZ	41	CDCA8	75	NUSAP1
8	TOP2A	42	PTTG1	76	RFC5
9	PCNA	43	C10 or β	77	TKI
10	UBE2C	44	H2AFX	78	WBP11
11	MELK	45	CMAS	79	SYNCRIP/SNX14
12	TRIP13	46	BRN1	80	BIRC5/AFMID
13	CNAP1	47	MCM10	81	HNRPA8
14	MCM7	48	LSM4	82	TACC3
15	RNASEH2A	49	MTB	83	MKI67
16	RAD51AP1	50	ASF1B	84	CENPF
17	KIF20A	51	ZWINT	85	Spe25
18	CDC45L	52	TOPK	86	C20 or f172
19	MAD2L1	53	FLJ10036	87	PTBP1
20	ESPL1	54	CDCA3	88	DLG7
21	CCNB2	55	ECT2	89	POLR2K
22	FEN1	56	CDC6	90	IARS
23	TTK	57	UNG	91	HPRT1
24	CCT5	58	MTCH2	92	NSDHL
25	RFC4	59	RAD21	93	KNTC2
26	ATAD2	60	ACTL6A	94	RAMP
27	ch-TOG	61	GPI/MGC13096	95	C10 or F7
28	NUP205	62	SFRS2	96	C12 or f14
29	CDC20	63	HDOF	97	SNRPD1
30	CKS2	64	NXT1	98	FLJ20989
31	RRM2	65	NEK2	99	NIF3L1
32	ELAVL1	66	DHCR7	100	DER1
33	CCNB1	67	STK6		
34	RRM1	68	NDUFAB1		

- taking a statistical measure of the mRNA expression level of the measured genes; and if the statistical measure of the mRNA expression level of the measured genes is elevated, determining that the prognosis is poor. The statistical measure can be determined to a 99% confidence level. It should be understood that the other gene sets described herein are equally applicable to the above described method.

- [0016] In certain embodiments, the solid tumor is of a cancer selected from lung cancer, prostate cancer, medulloblastoma, glioma, breast cancer, and lymphoma. In certain embodiments, the solid tumor is of a cancer selected from breast cancer, ovarian cancer, and colorectal cancer.

- [0017] In some embodiments, the statistical measure of the mRNA expression level of the measured genes is a linear combination of the mRNA expression level of the genes in the set of genes. In particular embodiments, the linear combination of the mRNA expression level in the set of genes is a combination of weighted mRNA expression levels. In various embodiments, the linear combination of the mRNA expression level in the set of genes is the mean of the logarithm of each of the mRNA expression levels. In certain embodiments, the statistical measure of the mRNA expression level of the measured genes is elevated relative to the mRNA expression level of the measured genes from a tumor whose prognosis is good.
- 10 [0018] In some embodiments, the present teachings relate to a method for predicting outcome of the treatment of the human solid tumors. In these embodiments, the method generally includes the steps of measuring, in a sample comprising the cells of a tumor from a patient, the mRNA expression level of a set of genes (or subset of a gene set) whose change is related to chromosomal
- 15 instability; taking a statistical measure of the mRNA expression level of the set of measured genes; and if the statistical measure of the mRNA expression level of the set of measured genes is elevated, determining that the prognosis for treatment with a MTS drug is poor.

- [0019] In some embodiments, the present teachings relate to a method for
- 20 predicting the outcome of the treatment of human solid tumors with taxanes such as paclitaxel. In such embodiments, the method can include measuring, in a sample comprising the cells of a tumor from a patient, the mRNA expression level of the following genes (or a subset thereof):

1	BRCA1	9	H2AFX	17	RFC3
2	CDC2	10	IMPA2	18	RPA1
3	CDC6	11	MCM3	19	SAE2
4	CHEK1	12	MSH6	20	TOP2A
5	DCLRE1	13	NUP153	21	TOPBP1
6	FDFT1	14	NUP155	22	XPQ1
7	GART	15	NUP205		
8	GPI	16	RCN2		

taking a statistical measure of the mRNA expression level of the set of measured genes; and if the statistical measure of the mRNA expression level of the set of measured genes is elevated, treating the human solid tumors with agents other than taxanes. In various embodiments, chromosomal instability can be measured by
5 array comparative genomic hybridization (aCGH) and/or counting the number of morphologically visible chromosomal aberrations by the application of chromosome visualization methods such as spectral karyotyping (SKY). Such techniques can be used in conjunction with mRNA expression levels or to correlate and/or corroborate expression levels.

10 [0020] Analytical results and/or measurements described herein, including expression level data, can be displayed, transmitted, or outputted to a user interface device, a computer readable storage medium, or a local or remote computer system. Displaying or outputting a result or measurement means that the results of any of the analyses described herein are communicated to a user using any medium, such as for
15 example, orally, writing, visual display etc., computer readable medium or computer system. It will be clear to one skilled in the art that outputting the result is not limited to outputting to a user or a linked external component(s), such as a computer system or computer memory, but may alternatively or additionally be outputted to internal components, such as any computer readable medium. Computer readable
20 media may include, but are not limited to hard drives, floppy disks, CD-ROMs, DVDs, and DATs. Computer readable media does not include carrier waves or other wave forms for data transmission. It will be clear to one skilled in the art that the taking of a statistical measure as disclosed and claimed herein generally is computer-implemented, but the displaying or outputting step can include
25 communicating to a person orally or in writing.

[0021] Another aspect of the present teachings is a set of genes or data from a set of genes, e.g., expression level data, useful in determining the outcome of treatment of solid tumors. In some embodiments, the set of genes comprises or consists essentially of:

1	TPX2	35	AURKB	69	KIAA0286
2	PRC1	36	MSH6	70	KIF4A
3	FOXMI	37	EZH2	71	SNRFB/MGC10715
4	CDC2	38	CTPS	72	UCK2
5	C20 or f24/TGIF2	39	DKC1	73	PARP1
6	MCM2	40	OIP5	74	RAD54L
7	H2AFZ	41	CDCA8	75	NUSAP1
8	TOP2A	42	PTTG1	76	RFC5
9	PCNA	43	C10orf3/CEP55	77	TK1
10	UBE2C	44	H2AFX	78	WBP11
11	MELK	45	CMAS	79	SYNCRIP/SNX14
12	TRIP13	46	BRN1	80	BIRC5 and AFMID
13	CNAP1	47	MCM10	81	HNRPA8
14	MCM7	48	LSM4	82	TACC3
15	RNASEH2A	49	MTB	83	MKI67
16	RAD51AP1	50	ASF1B	84	CENPF
17	KIF20A	51	ZWINT	85	Sp25
18	CDC45L	52	TOPK	86	C20 or f172
19	MAD2L1	53	FLJ10036	87	FTBP1
20	ESPL1	54	CDCA3	88	DLG7
21	CCNB2	55	ECT2	89	POLR2K
22	FEN1	56	CDC6	90	IARS
23	TTK	57	UNG	91	HPRT1
24	CCT5	58	MTCH2	92	NSDHL
25	RFC4	59	RAD21	93	KNTC2
26	ATAD2	60	ACTL6A	94	RAMP
27	ch-TOG	61	GPI and MGC13096	95	C10 or f7
28	NUP205	62	SFRS2	96	C12 or f14
29	CDC20	63	HDGF	97	SNRPD1
30	CKS2	64	NXT1	98	FLJ20989
31	RRM2	65	NEK2	99	NIF3L1
32	ELAVL1	66	DHCR7	100	DERJ
33	CCNB1	67	STK6		
34	RRM1	68	NDUFAB1		

or

1	TPX2	14	MCM7	25	RFC4
2	PRC1	15	RNASEH2A	26	ATAD2
3	FOXMI	16	RAD51AP1	27	ch-TOG
4	CDC2	17	KIF20A	28	NUP205
5	C20 or f24/TGIF2	18	CDC45L	29	CDC20
6	MCM2	19	MAD2L1	30	CKS2
7	H2AFZ	20	ESPL1	31	RRM2
8	TOP2A	21	CCNB2	32	ELAVL1
9	PCNA	22	FEN1	33	CCNB1
10	UBE2C	23	TTK	34	RRM1
11	MELK	24	CCT5	35	AURKB
12	TRIP13			36	MSH6
13	CNAP1			37	EZH2
				38	CTPS
				39	DKC1
40	OIP5	51	ZWINT	62	SFRS2
41	CDCA8	52	TOPK	63	HDGF
42	PTTG1	53	FLJ10036	64	NXT1
43	CEP55/C10orf3	54	CDCA3	65	NEK2
44	H2AFX	55	ECT2	66	DHCR7
45	CMAS	56	CDC6	67	STK6
46	BRN1	57	UNG	68	NDUFAB1
47	MCM10	58	MTCH2	69	KIAA0286
48	LSM4	59	RAD21	70	KIF4A
49	MTB	60	ACTL6A		
50	ASF1B	61	GPI/MGC13096		

5 or

1	TPX2	10	UBE2C	19	MAD2L1
2	PRC1	11	MELK	20	ESPL1
3	FOXMI	12	TRIP13	21	CCNB2
4	CDC2	13	CNAP1	22	FEN1
5	C20 or f24/TGIF2	14	MCM7	23	TTK
6	MCM2	15	RNASEH2A	24	CCT5
7	H2AFZ	16	RAD51AP1	25	RFC4
8	TOP2A	17	KIF20A		
9	PCNA	18	CDC45L		

or

1	TPX2	24	CCNB1	47	KNITC2
2	FOXMI	25	AURKB	48	MSH2
3	CDC2	26	MSH6	49	NUP155
4	MCM2	27	EZH2	50	POP7
5	H2AFZ	28	OIP5	51	LMNB1
6	TOP2A	29	PTTG1	52	CDKN3
7	PCNA	30	H2AFX	53	LRP8
8	UBE2C	31	ZWINT	54	TYMS
9	MELK	32	CDC6	55	CCNA2
10	TRIP13	33	UNG	56	MTHFD2
11	MCM7	34	RAD21	57	RFC2
12	CDC45L	35	ACTL6A	58	MCM6
13	MAD2L1	36	DHCR7	59	FANCG
14	ESPL1	37	STK6	60	MYBL2
15	CCNB2	38	KIAA0286	61	MCM3
16	FEN1	39	SNRPB/MGC10715	62	NCOA6
17	TTK	40	TK1	63	EIF2C2
18	CCT5	41	HNRPAB	64	TROAP
19	RFC4	42	MCKI67	65	SIL
20	ch-TOG	43	CENPF	66	PRDM1
21	NUP205	44	Spc25	67	POLD2
22	CDC20	45	DLG7	68	EST1B
23	CKS2	46	HPRT1	69	GGH

or

1	BRCA1	9	H2AFX	17	RFC3
2	CDC2	10	IMPA2	18	RPA1
3	CDC6	11	MCM3	19	SAE2
4	CHEK1	12	MSH6	20	TOP2A
5	DCLRE1	13	NUP153	21	TOPBP1
6	FDFT1	14	NUP155	22	XPQ1
7	GART	15	NUP205		
8	GPI	16	RCN2		

- 5 [0022] Data derived from a set of genes can include the expression level measurement of each of the genes in the set or for a subset of genes in a gene set as well as other measurements related to the genes as described herein. The data of the other measurements can be independent of the expression levels. Further, such data can be contained (e.g., stored) on a computer readable medium.
- 10 [0023] The foregoing, and other features and advantages of the present teachings, will be more fully understood from the following figures, description, examples, and claims.

BRIEF DESCRIPTION OF DRAWINGS

[0024] It should be understood that the drawings described below are for illustration purpose only. The drawings are not necessarily to scale, with emphasis generally being placed upon illustrating the principles of the present teachings. The drawings are not intended to limit the scope of the present teachings in any way.

5 [0025] **Figure 1** shows that genes over-expressed in tumors with CIN are repressed by MTS treatment *in vitro* and *in vivo*: (a) The CIN27wp gene expression signature, which correlates with total functional aneuploidy in several cancer types (Carter, et al., (2006) Nat Genet, 38:1043-1048), was analyzed in five gene expression data sets measuring response to MTS treatment. The distribution of
 10 changes induced by MTS was lower in CIN27WP genes (red) than in the set of all measured genes (black) ($P = 3.3e-7$). (b-e) As part of the OV01 clinical trial, expression profiling was performed on ovarian carcinomas before and after 3 cycles of paclitaxel (Px) treatment. (b) For each gene in the CIN70 signature, the median expression amongst patients is compared between the pre-treatment and post-
 15 treatment tumors, with the straight line indicating equivalence. (c) To quantify tumor response, an exponential model was fitted for each patient to the CA125 tumor marker over three cycles of treatment. The resulting coefficient is concordant with the response criteria defined by Rustin et. al. ((2004) J Natl Cancer Inst, 96:487-488) and was used to identify the most paclitaxel-sensitive patients (box).
 20 (d) The median expression of the CIN70 genes is decreased in post-treatment tumors relative to pre-treatment tumors, (e) especially in those patients with the most paclitaxel-sensitive disease (boxed in (c)). Statistics shown are one-sided t-tests. Horizontal bars indicate mean values.

[0026] **Figures 2a-b** show that siRNA silencing of MTS repressed genes impairs
 25 cell viability and promotes cell death: (a) Genes repressed within the two MTS expression signatures that are over-expressed in CIN tumors significantly alter HCT-116 cell viability when targeted by siRNA. The Acumen eX3 cytometer (Acumen TTP Labtech) was used to quantify viable cells 72 hours after siRNA transfection. SDs are displayed for 3 independent experiments. P values shown for Student two
 30 sided T Test in all cases: * $p < 0.05$, ** $p < 0.005$, *** $p < 0.0005$; (b) Genes repressed within the two MTS expression signatures which are over-expressed in CIN tumors

significantly alter cell subG1 fraction in HCT-116 cells when targeted by siRNA. FACS analysis was used to quantify the mean subG1 fraction 72 hours after transfection of siRNA. SDs and p values are displayed for 3 independent experiments.

- 5 [0027] **Figures 2c-d** describe the identification of 22 CIN survival genes and provide a flowchart of analysis resulting in the derivation of the 22 CIN-survival genes: (c) Cells were transfected with siRNA targeting the 50 genes overexpressed in CIN tumors and repressed by MTS agents. Cell viability was quantified by cell titer blue assay 4 days after siRNA transfection in triplicate. Shown are the 22 genes
10 which significantly impair viability in MDA-MB-468, A549 and HCT-116 cell lines; (d) the binomial test with probability correction (BTPC) and rank methods were used to derive two MTS expression signatures. 50 genes were repressed in the MTS signatures and overexpressed in CIN tumors. 22/50 genes impaired cancer cell viability and/or induced apoptosis when silenced by RNA interference in 3 cancer
15 cell lines: HCT-116 (colon), A549 (NSCLC) and MDA-MB-468 (breast).

- [0028] **Figure 3a** describes the quantification of gene repression following paclitaxel treatment of cell lines with increasing chromosomal numerical heterogeneity. Fold change in gene expression post-paclitaxel treatment (24hrs) relative to expression in vehicle control treated cells was determined by qPCR
20 analysis (normalization to 18S and GAPDH) following 24hrs of paclitaxel treatment (50% of the Gi50 concentration) in 3 biological replicate experiments in cell lines with increasing CIN. Colour coding represents the mean fold change in gene expression of 3 biological replicates relative to cells treated with vehicle alone +1SD. MTS repressed genes inducing a significant reduction in cell viability when
25 silenced by siRNA compared to control transfected cells are highlighted in grey. Gene expression following paclitaxel treatment is compared to the HCT-116 cell line displaying the lowest CIN. The significance of the differences in gene expression determined using the unpaired two-sided Student T test: * P value <0.05, ** P value <0.005.

- 30 [0029] **Figure 3b** shows that CIN-survival genes are relatively over-expressed in CIN^{high} COLO205 and SW620 cells compared to near diploid CIN^{low} HCT-116.

Relative quantification of gene expression normalised to 18S and GAPDH in COLO205 and SW620 cells compared to near diploid HCT-116 cells. Graph shows the mean fold change in gene expression compared to HCT-116 cells of 3 biological replicates (+ 1SD).

5 [0030] **Figure 3c** shows that colorectal cancer cell lines with increasing chromosomal numerical heterogeneity uncouple mitotic arrest from cell death. Cell lines were treated with paclitaxel relative to the Gi50, Gi50/2 and Gi50/4 concentrations. Quantification of dying cells (SubG1 fraction) and cells arrested in mitosis (MPM2 positive fraction) were assessed by FACS analysis. The
10 MPM2/SubG1 ratio was calculated by assessing the percentage change in MPM2 and SubG1 cells relative to vehicle control treated cells 24 hours following paclitaxel treatment. The mean ratio of 3 independent experiments is presented (+1SD). Cell lines are represented in ascending order of CIN status (Roschke, et al., (2003) Cancer Res, 63:8634-8647).

15 [0031] **Figure 3d** shows that silencing CIN-survival genes in the SW620 CIN^{high} cell line promotes cell death following 24 hours of paclitaxel Gi50 treatment. Forty-eight hours following transfection of CIN-survival siRNAs, SW620 cells were treated with paclitaxel for 24 hours and cells prepared and analyzed as for **Figure 3c**. The mean MPM2/SubG1 ratio of two independent experiments is presented
20 (+1SD).

[0032] **Figure 3e** shows impaired repression of CIN-survival genes following paclitaxel treatment in an isogenic models of CIN: Quantification of gene repression by qPCR analysis of 21 CIN survival genes following treatment of HCT-116 wild type parental cells and isogenic Mad2+/- cells following 24 hours of paclitaxel
25 treatment (50nM) normalised to 18s. Colour coding represents the mean fold repression + 1SD from 3 biological replicates. P values indicate significantly greater gene repression in the HCT-116 parental cell line (Student one sided T-test).

[0033] **Figure 3f** shows that CIN survival gene repression correlates with cytotoxic response and stable tumor karyotype.

[0034] **Figure 4a** shows that expression of CIN70 genes determines sensitivity to paclitaxel and carboplatin. Figure contrasts basal median gene expression for each CIN70 gene amongst tumors with differing response to paclitaxel and carboplatin. Paclitaxel-resistant tumors have higher median log intensity of the CIN70 signature compared to paclitaxel-sensitive tumors ($p=0.043$). CIN70 gene expression differs significantly between tumors subsequently resistant to paclitaxel and tumors resistant to carboplatin ($p = 0.044$). Statistical tests: Student two-sided T-tests.

[0035] **Figure 4b** shows that residual CIN expression post-treatment is higher in paclitaxel-resistant compared to paclitaxel-sensitive tumors. (Top) Sensitivity defined by the Rustin criteria: The distribution of log2 intensities of CIN70 genes in residual tumors following paclitaxel across all Rustin-resistant (black line) and Rustin-sensitive (red line) patients. (Kolmogorov-Smirnov test, $D = 0.11$, $p = 0.01$). (Bottom) Sensitivity defined by the CA125 coefficient: The same analysis was applied to taxane-resistant (black line: CA125 coefficient >-0.5) and patients most sensitive to paclitaxel (red line: CA125 coefficient <-1 , see Figure 1c) (Kolmogorov-Smirnov test, $D = 0.22$, $p = 8.30e-8$).

[0036] **Figure 4c** shows that expression of CIN and CIN-survival genes is greater in aneuploid, genomically unstable breast cancers. DNA image cytometry was used to classify breast cancers as diploid, genomically stable (dGS), aneuploid, genomically stable (aGS) or aneuploid, genomically unstable (aGU). Box plots summarize the expression of the CIN70, CIN27wp and CIN-survival signatures within each group. P values were calculated using the Student two-tailed T test.

[0037] **Figure S1** shows the results of binomial test with probability correction MTS expression signature demonstrating the most significant repressed and expressed genes following MTS exposure in the 5 datasets. Shown are heatmaps reflecting gene expression changes with log2 fold change values of significantly up (red)- or down (green)-regulated genes in the rows and tumor cell line datasets in the columns, both sorted by hierarchical clustering with euclidean distance (www.bioconductor.org). White cells indicate that this gene was not included on the microarray or removed in the filtering step prior to the meta-analysis.

[0038] **Figure S2** shows the results of binomial test with probability correction MTS expression signature demonstrating the most significant repressed and expressed genes following 5-FU exposure in the 3 datasets. Shown are heatmaps reflecting gene expression changes with log2 fold change values of significantly up (red)- or down (green)-regulated genes in the rows and tumor cell line datasets in the columns, both sorted by hierarchical clustering with euclidean distance (www.bioconductor.org). White cells indicate that this gene was not included on the microarray or removed in the filtering step prior to the meta-analysis.

[0039] **Figure S3a** summarizes repression of genes by MTS using two meta-analysis methods which are over-expressed in the top 15% of genes expressed in CIN tumors with proliferation genes removed (10). List of 50 genes repressed in the MTS expression signatures derived from the two methods that are over-expressed in CIN tumors as part of the CIN signature were chosen for further functional analysis using RNA interference.

[0040] **Figure S3b** describes the validation of MTS expression signature repressed genes in HCT-116 colorectal cancer cell line. Fold change in gene expression ($\pm$ 1SD) determined by TLDA qPCR analysis of genes normalised to 18s RNA expression derived from the two MTS expression signatures following 24hrs of Paclitaxel treatment (50nM) in 3 biological replicate experiments compared to gene expression in vehicle (DMSO) control treated HCT-116 cells. BBC3/Puma, a known taxane induced gene derived from our meta-signature and LAMP2 and WDFY1 (genes whose expression is not influenced by taxane exposure Swanton and Downward unpublished data) were used as controls.

[0041] **Figure S4a** shows that MTS repressed genes promote aneuploidy and cell death when silenced by siRNA. Genes repressed within the two MTS expression signatures that are over-expressed in CIN tumors significantly increase the fraction of aneuploid cells assessed by Acumen and flow cytometry when silenced using RNAi. CIN survival genes are highlighted in grey. **Colour coding:** Lower limit of SD of mean percentage of aneuploid cells from 3 independent experiments is greater than mean aneuploid population in control transfected cells +2 SDs (light grey) or +3 SDs (dark grey) from 3 independent experiments.

[0042] **Figure S4b** shows deconvolution of siRNA smartpools and assessment of cell viability. In particular, experiments in Figure 2b were repeated with the deconvoluted siRNA sequences from the smartpool. The Acumen Explorer laser cytometer was used to quantify viable cells 72 hours after transfection of the smartpool siRNA targeting the gene of interest. Standard deviations are displayed for 2 independent experiments.

[0043] **Figure S4c** assesses target silencing. Fast SYBR green real-time PCR quantification of target silencing (+1SD from 3 independent transfections) was confirmed 72 hours following transfection of HCT-116 cells with siRNAs conferring an effect on cell viability, cell death or aneuploidy compared to gene expression in non-targeting control siRNA transfected cells.

[0044] **Figure S5a** illustrates quantification of gene repression following paclitaxel treatment of MCF-7 and BT549 cell lines. Fold change in gene expression post-paclitaxel treatment (24hrs) relative to expression in vehicle control treated cells was determined by TaqMan qPCR analysis (normalization to GAPDH) following 24hrs of paclitaxel treatment (concentration 50% of the Gi50 dose) in 3 biological replicate experiments in the MCF-7 (CIN^{low}) and the BT549 (CIN^{high}) breast cancer cell lines. Colour coding represents the mean fold change in gene expression of 3 biological replicates relative to cells treated with vehicle alone (+1 standard deviation). CIN survival genes are highlighted in grey. Significance of differential gene repression compared to the MCF-7 cell line following paclitaxel treatment is determined by the 2 tailed Student T test: * P value <0.05, ** P value <0.005.

[0045] **Figure S5b** shows that silencing CIN-survival genes in the SW620 CIN^{high} cell line promotes a synergistic increase in cell death following 24 hours of paclitaxel Gi50 treatment compared to DMSO treated cells. Forty-eight hours following transfection of CIN-survival siRNAs, SW620 cells were treated with paclitaxel for 24 hours and cells prepared and analyzed for SUBG1 content by FACS analysis as for Figure 3c. Results of two biological replicates are shown with standard deviation. Silencing BRCA1, TOP2A, CDC6, H2AFX and NUP205

promotes a significant increase in SUBG1 cells following Paclitaxel exposure compared to DMSO treated cells ($p < 0.05$ Student's T Test).

- [0046] **Figure S5c** shows impaired repression of CIN-survival genes following paclitaxel treatment in an isogenic model of CIN, specifically quantification of gene repression following treatment of HCT-116 wild type parental cells compared to HCT-116 isogenic *hSecurin* (-/-) following 24 hours of paclitaxel treatment (50nM) normalised to 18s. Colour coding represents the mean fold repression + 1SD from 3 biological replicates. P values (* $P < 0.05$) indicate significantly greater gene repression in the HCT-116 parental cell line (one sided Student T-test).
- [0047] **Figure S5d** shows how expression of CIN-survival genes correlates with poorer survival in breast cancer. The mean of the logged expression levels of CIN-survival genes was taken as a single prognostic factor and tested as a predictor of outcome in 4 cancer cohorts. Similar to what was reported by Carter et al. (10), patients were divided into two groups with above or below median expression of the CIN-survival gene signature expression and the prognostic effects examined with Kaplan-Meier survival analysis and Cox proportional hazard model.
- [0048] **Table 1a** provides the datasets used to derive the MTS response signature. Cell lines treated with the MTS and author of the study are shown. The type of array used to derive each gene expression signature (cDNA or Affymetrix) together with the number of genes on the platform, the duration and concentration of drug exposure and the number of biological replicates for each experiment are demonstrated.
- [0049] **Table 1b** shows that genes over-expressed in CIN tumors are significantly repressed following MTS treatment across all datasets. Cell cycle regulated genes were removed from the CIN70 signature and the signature of the top 5% (CIN382WP), 10% (CIN826WP) and 15% (CIN1264WP) of over-expressed genes in CIN tumors. The empirical frequency distribution of CIN27wp, CIN382WP, CIN826WP and CIN1264WP signature genes was compared to the gene expression changes across all datasets following MTS exposure using the one sided bootstrap Kolmogorov-Smirnov test. The test statistic and the p-value for the Kolmogorov-

Smirnov test reveals a significant left shift of the empirical frequency distribution of the CIN genes indicating that genes in the CIN signatures are more likely to be repressed following MTS treatment.

[0050] Table 1c shows that genes over-expressed in CIN tumors are significantly repressed in MTS expression signatures but not in a 5-FU signature. Genes whose expression is influenced by cell cycle phase (20) were removed from the CIN70 signature and the signature of the top 5% (CIN382WP) of over-expressed genes in CIN tumors. Repression of genes in the CIN signature derived from two MTS meta-analysis signature methods (Rank and Binomial Test with Probability Correction, BTPC) with the number of repressed genes after filtering demonstrated for each meta-analysis method are shown. Three published datasets were used to derive a 5-FU signature using the BTPC method. Table demonstrates the level of significance for the representation of MTS or 5-FU-repressed genes in the CIN signature. P values indicate the significance of the association of the CIN signature genes within the MTS or 5-FU expression signatures of repressed genes using the one-sided fisher's exact test with the number of genes overlapping with each CIN dataset shown in parenthesis.

[0051] Table 1d shows that repression of CIN genes occurs preferentially in taxane-sensitive xenografts. Publicly available expression data deriving from paclitaxel-treated human ovarian cancer xenografts in nude mice (19) were analyzed for repression of genes over-expressed in CIN tumors following paclitaxel treatment. The sensitive ovarian cancer cell line (1A9) and its paclitaxel resistant derivative (1A9PTX22) were treated with 60mg/kg of paclitaxel which resulted in tumor responses in 1A9 tumors but not in 1A9PTX22 xenografts. P-values are given for the Kolmogorov-Smirnov test comparing the distribution of genes over-expressed in the CIN signature (with proliferation genes excluded) with the distribution of genes having a negative fold change in ovarian cancer xenografts following paclitaxel treatment of nude mice. CIN genes are significantly repressed in taxane sensitive ovarian cancer xenografts, but not in the paclitaxel-resistant xenograft model.

[0052] Table 2 demonstrates the Gi50 for each cell line and the concentrations of paclitaxel used in each cell line (nM) relative to the cell line Gi50.

[0053] Table 3 summarizes clinical and experimental parameters in 14 diploid genomic stable (dGS), 14 aneuploid genomic stable (aGS), and 16 aneuploid genomic unstable(aGU) breast carcinomas. ER, estrogen receptor; PR, progesterone receptor; pos, positive; neg, negative; ND, not determined.

5

DETAILED DESCRIPTION

[0054] Human solid tumors exhibit differences in outcome even for the same tumor type. Thus, if a clinician can determine which outcome is probable for a specific tumor, the clinician would know if a more or less aggressive treatment regimen can be used. One possibility is to consider the amount of chromosomal aberrations that exists in the specific tumor. It is known for those trained in the art that there is a strong correlation between the total number of chromosomal aberrations in a given tumor and its malignancy. High numbers of chromosomal aberrations are usually associated with a more malignant phenotype.

[0055] With respect to the chromosomal complement of a solid human tumor, the tumor exhibits various aberrations such as multiple trisomies, tetrasomy, and multiple translocations and deletions. These aberrations in chromosomal stability are found in solid tumors of the lung, prostate, breast, brain (both medulloblastoma and glioma), ovaries, colon, and lymph nodes (lymphoma). To quantify the amount of chromosomal aberrations, one may apply any of the following three methods: 1) counting the number of morphologically visible chromosomal aberrations by the application chromosome visualization methods such as spectral karyotyping; 2) quantifying the amount of chromosomal aberrations obtained by array comparative genomic hybridization (aCGH); and 3) quantifying chromosomal aberrations by their effect on the expression level of the genes contained in a given chromosomal region. The latter method, which is an integral part of the present teachings, produces a measure called “functional aneuploidy.”

[0056] The numerical and structural chromosomal aberrations seen in malignancies are a consequence of the aberrant functioning of the cell’s mechanism to maintain genomic integrity. This cellular aberration is called “chromosomal instability” (CIN). Similarly to aneuploidy, its causative mechanism, CIN is also associated with malignancies. High levels of CIN are expected to confer a more

malignant phenotype. Despite the obvious utility of quantifying CIN for clinical diagnostics, its application has been hindered by technical difficulties. The present teachings provide a readily applicable quantification method of CIN in clinical tumor samples.

5 [0057] The expression “level” of a gene can be determined by any method known in the art which measures gene expression products, including, but not limited to, mRNA transcripts and proteins. It should be understood that the amount of gene expression levels need not be determined in absolute terms, but can be determined in relative terms.

10 [0058] A gene expression signature of CIN is derived by the identification of genes with the highest level of correlation between a gene’s expression level and the overall level of chromosomal aberrations across a given set of cancer samples.

[0059] The overall level of chromosomal aberrations in a given clinical sample can be derived by any of the three techniques described herein.

15 [0060] In cancer cells chromosomes can be visualized by spectral karyotyping (SKY) that allows counting the total number of chromosomes and morphological aberrations of chromosomes such as deletions, insertions, translocations, and inversions of various chromosomal regions. In one embodiment the total number of such numerical and morphological aberrations in a cancer cell is used to estimate the
20 overall level of chromosomal aberrations.

[0061] In cancer cells the copy number of each chromosomal region can be measured by array comparative genomic hybridization using microarrays by containing either long cDNA clones targeting the individual chromosomal regions or short DNA probes, such as those used on the so-called single nucleotide
25 polymorphism (SNP) chips. In one embodiment the total number of chromosomal aberrations in a cancer sample is calculated by adding up the deviation of each chromosomal region from the normal chromosomal copy number across the entire genome.

[0062] In cancer cells chromosomal copy number changes have a direct impact on the RNA expression level of the genes contained in a given chromosomal region. Therefore, chromosomal copy number changes can be estimated by calculating the net deviation of the expression level of all genes contained in a given chromosomal region relative to the remainder of the sampled transcriptome.

[0063] First a tumor sample from each of the solid tumors of interest was obtained. A microarray was then used to quantify the expression level of a large number, typically 10,000-20,000 genes in each tumor sample. For a given microarray, each probe or probe set was first mapped to its corresponding transcriptome by sequence mapping and then, through this transcript, the microarray probes were mapped to their respective chromosomal cytobands.

[0064] For each chromosomal cytoband, all of the genes present in the microarray measurement that map to that region are grouped into a set designated B (short for band). In one embodiment, if less than ten genes were mapped to a band, the group was disregarded as statistically unreliable. Although in this embodiment the mapping of genes to the cytobands of the chromosome was used to group the genes, it is contemplated that the grouping of genes into statistically meaningful sets can be accomplished by using windows of equal linear length along the chromosome (5-30 Mb long) or genes can be grouped by neighborhood criteria (20 to 100 genes that are located next to each other on the same chromosome would form a set of genes for further analysis). Also, although ten genes were considered the minimum number of genes necessary to form a group, it is contemplated that other numbers of genes can be used to determine statistical reliability.

[0065] The rest of the genes, i.e., the rest of the transcriptome that is localized somewhere else on the chromosomes and which are measured on the same microarray, are grouped into a set G (short for genome). The sets B and G are disjoint. The distributions of the genes in B and G are then compared using an appropriate statistical metric, such as the t-statistic. In one embodiment, the statistical significance of the group of genes was determined by taking the mean of the log to the base ten of the expression level of each gene in the group B and comparing it with the expression level of the genes from group G. In general, the

statistical metric is formed on a linear combination of the expression level of the genes in the set of genes. The expression levels can be weighted. Other statistical tests, which can be used include: Wilcoxon-Rank test, Signal to Noise ratio, Kolmogorov-Smirnov test and Kruskal-Wallis test

5 [0066] This process is iterated for each gene expression profile in a given cohort such that upon termination, a matrix of t-statistics for each of approximately 350 cytobands per hybridization was obtained. The thus created statistical measures will provide an estimate of the level of aberrant gene expression of a given gene set contained within a given chromosomal region. This is a basis of functional
10 aneuploidy, a measure of the impact of chromosomal aberrations on the transcriptome.

[0067] In addition to the measures outlined above, the overall level of chromosomal aberrations can be characterized by summing up the level of functional aneuploidy across all chromosomal regions. This novel measure is termed
15 total functional aneuploidy.

[0068] For a given set of cancer samples the following measures are obtained: (a) gene expression measurements at the RNA level for typically 10,000-20,000 genes, usually but not exclusively obtained by microarray measurements. In addition to these measurements, the following measures may also be obtained (b) array
20 comparative genomic hybridization across the entire genome and/or (c) a detailed morphological characterization of all chromosomal aberrations.

[0069] For each gene in a given cancer data the gene's expression level across all samples will form a gene expression vector. The total number of chromosomal aberrations in the individual cancer samples as determined by the total number of
25 morphological aberrations, total number of aCGH based chromosomal copy number deviations and total functional aneuploidy will form three additional vectors. Correlation between each gene expression vector and the three vectors characterizing the overall level of chromosomal aberrations is calculated for all genes. The genes with the highest level of correlation to the overall level of
30 chromosomal aberrations will form the CIN gene expression signature.

[0070] A group of expressed genes in a tumor which was difficult to treat showed increased expression relative to tumors which were easier to treat. These genes included:

1	TPX2	35	AURKB	69	KIAA0286
2	PRC1	36	MSH6	70	KIF4A
3	FOXMI	37	EZH2	71	SNRFB/GC10715
4	CDC2	38	CTPS	72	UCK2
5	C20orf24/TGIF2	39	DKC1	73	PARP1
6	MCM2	40	OIP5	74	RAD54L
7	H2AFZ	41	CDCAS	75	NUSAP1
8	TOP2A	42	PTTG1	76	RFC5
9	PCNA	43	C10 or f3	77	TKI
10	UBE2C	44	H2AFX	78	WBP11
11	MELK	45	CMAS	79	SYNCRIP/SNX14
12	TRIP13	46	BRRN1	80	BIRC5/AFMID
13	CNAP1	47	MCM10	81	HNRFB
14	MCM7	48	LSM4	82	TACC3
15	RNASEH2A	49	MTB	83	MKI67
16	RAD51AP1	50	ASF1B	84	CENPF
17	KIF20A	51	ZWINT	85	Spz25
18	CDC45L	52	TOPK	86	C20 or f172
19	MAD2L1	53	FLJ10036	87	PTBP1
20	ESPL1	54	CDCA3	88	DLG7
21	CCNB2	55	ECT2	89	POLR2K
22	FEN1	56	CDC6	90	IARS
23	TTK	57	UNG	91	HPRT1
24	CCT5	58	MTCH2	92	NSDHL
25	RFC4	59	RAD21	93	KNTC2
26	ATAD2	60	ACTL6A	94	RAMP
27	ch-TOG	61	GPI/MGC13096	95	C10 or f7
28	NUP205	62	SFRS2	96	C12 or f14
29	CDC20	63	HDGF	97	SNRPD1
30	CKS2	64	NXT1	98	FLJ20989
31	KRM2	65	NEK2	99	NIF3L1
32	ELAVL1	66	DHCR7	100	DER1
33	CCNB1	67	STK6		
34	RRM1	68	NDUFAB1		

- 5 [0071] Many of these genes are known to be related to chromosomal stability and hence are consistent with chromosomal aberrations as a cause of the malignant phenotype. The application of the method to multiple datasets indicates that the following genes consistently have increased expression in difficult-to-treat tumors:

1	TPX2	25	AURKB	48	MSH2
2	FOXM1	26	MSH6	49	NUP155
3	CDC2	27	EZH2	50	POP7
4	MCM2	28	OIP5	51	LMNB1
5	H2AFX	29	PTTG1	52	CDKN3
6	TOP2A	30	H2AFX	53	LRP8
7	PCNA	31	ZWINT	54	TYMS
8	UBE2C	32	CDC6	55	CCNA2
9	MELK	33	UNG	56	MTHFD2
10	TRIP13	34	RAD21	57	RFC2
11	MCM7	35	ACTL6A	58	MCM6
12	CDC45L	36	DHCR7	59	FANCG
13	MAD2L1	37	STK6	60	MYBL2
14	ESPL1	38	KIAA0286	61	MCM3
15	CCNB2	39	SNRNP and	62	NCOA6
16	FEN1		MGC10715	63	EIF2C2
17	TTK	40	TK1	64	TROAP
18	CCT5	41	HNRPAB	65	SIL
19	RFC4	42	MKI67	66	PRIM1
20	ch-TOG	43	CENPF	67	POLD2
21	NUP205	44	SpC25	68	EST1B
22	CDC20	45	DLG7	69	GGH
23	CKS2	46	HPRT1		
24	CCNB1	47	KNTC2		

[0072] Therefore, by determining that these sets of tumor genes or an appropriate subset thereof have an elevated expression level, the clinician can determine that the tumor is difficult to treat.

- 5 [0073] In certain embodiments, the cell population gene expression approaches described herein can be used to predict in advance of treatment *in vivo* how a tumor may respond to specific cytotoxic drugs, in particular, MTS agents such as taxanes and epothilones, and/or carboplatin. In some embodiments, the methods described herein also can be used after commencement of treatment with taxanes, epothilones, 10 or carboplatin, to monitor the tumor's responsiveness to the drugs. Taxanes include, for example, docetaxel and paclitaxel. Epothilones include, for example, epothilone A, epothilone B, patupilone (EPO906), sagopilone (SH-Y03757A, ZK-EPO), ixabepilone (aza-epothilone B, BMS-247550), epothilone C, KOS-862 (epothilone D), epothilone E, epothilone F, BMS-310705, and KOS-1584 as well as compounds 15 with related microtubule stabilizing activity such as discodermolide, laulimalide, isolaulimalide, taccalonolides and others.

[0074] For example, the application of the present method to multiple datasets indicates that the following genes consistently have increased expression in tumors exhibiting drug resistance to taxanes:

1 BRCA1 9 H2AFX 17 RFC3

2	CDC2	10	IMPA2	18	RPA1
3	CDC6	11	MCM3	19	SAE2
4	CHEK1	12	MSH6	20	TOP2A
5	DCLRE1	13	NUP153	21	TOPBP1
6	FDFT1	14	NUP155	22	XPQ1
7	GART	15	NUP205		
8	GPI	16	RCN2		

The above genes, referred herein as “CIN-survival” genes, were found to be significantly over-expressed in CIN tumors (Carter, et al., (2006) Nat Genet, 38:1043-1048). Data included hereinbelow indicate that cell lines with a high level of CIN (CIN^{high}) fail to repress these CIN-survival genes following paclitaxel exposure as efficiently as cell lines with a low level of CIN (CIN^{low}), despite an efficient mitotic arrest in response to paclitaxel treatment, suggesting an uncoupling of mitotic arrest from cell death in CIN^{high} tumor cells that is associated with an attenuated cytotoxic gene expression program. The data below support the “robust” mitotic arrest observed in chromosomally unstable cell lines following spindle disruption (Tighe, et al., (2001) EMBO Rep, 2:609-614.) and the lack of correlation between cell fate and the duration of mitotic arrest in time-lapse light microscopy studies (Gascoigne, et al., (2008) Cancer Cell, 14:1-12; Orth, et al., (2008) Mol Cancer Ther, 7:3480-3489; Shi, et al., (2008) Cancer Res, 68:3269-3276). These data also illustrate how MTS agents promote either a cytostatic or cytotoxic response that can correlate with the CIN status of the tumor (model in Figure 3f). In particular, CIN signature expression was found to correlate with resistance to paclitaxel and sensitivity to carboplatin in ovarian cancer and the CIN signature is relatively overexpressed in residual, paclitaxel-resistant tumor.

[0075] Thus although cell fate in response to anti-mitotics at the single cell level appears unpredictable and is not genetically predetermined (Gascoigne, et al., (2008) Cancer Cell, 14:1-12), this may not be the case when studying drug response at the population level. Tumor karyotype therefore can be an important determinant of cytotoxic sensitivity in solid human tumors as described herein.

- [0076] Functional annotation of CIN-survival genes within the MTS expression signature can shed light on the mechanisms triggering cell death in response to MTS agents. Transcriptional inhibition during mitosis can alter the balance of short-lived compared to long-lived mRNAs, initiating the degradation of mRNAs encoding proteins which promote cell survival (Blagosklonny (2007) *Cell Cycle*, 6:70-74). In agreement with this hypothesis, it was found that several CIN-survival genes are also repressed following treatment with a transcription inhibitor, Flavopiridol (TOPBP1, CDC2, TOP2A, RFC3 and XPO1) indicating that cell death associated with MTS agents and transcriptional inhibitors may share similar mechanisms.
- 10 [0077] Recently it has been shown that mitotic arrest in response to paclitaxel triggers DNA damage (Dalton, et al., (2007) *Cancer Res*, 67:11487-11492) which can accumulate during a lengthened mitosis contributing to apoptosis due to inefficient DNA repair (Gascoigne, et al., (2008) *Cancer Cell*, 14:1-12). Intriguingly, 9 of the 22 genes in the CIN survival signature have roles in DNA repair. Repression of CIN-survival genes following taxane exposure, such as BRCA1, CHEK1, DCLRE1A, H2AFX, MSH6, RPA1, RFC3, SAE2, and TOPBP1 suggest that failure to initiate efficient mismatch repair or homologous recombination (HR) can contribute to MTS cytotoxicity. It is noteworthy that these genes are overexpressed in CIN tumors and that MMR deficiency is infrequent in CIN colorectal cancers, supporting the potential dependence of CIN on heightened DNA repair activity and a rational basis for their therapeutic resistance to MTS agents. Such a concept is supported by ploidy-specific lethal mutations in genes involved in HR in polyploid yeast models Storchova, et al., (2006) *Nature*, 443:541-547).
- 25 [0078] Using DNA image cytometry combined with gene expression analysis to classify cancers by genomic instability status, the examples below suggest that the CIN signature can be a surrogate marker of CIN *in vivo* and that CIN-survival genes are over-expressed in aneuploid, genomically unstable breast cancer. Furthermore, CIN signature expression appears to be greater in residual paclitaxel resistant compared to sensitive tumors, suggesting that there can be a selection pressure for sustaining CIN in paclitaxel resistant tumors.
- 30

[0079] Intriguingly, aGU tumors are almost exclusively basal-like or Her2-positive breast cancers and analogous to clinical trial data in ovarian cancer described below, might be predicted to be taxane-resistant but platinum-sensitive. Her2 expression confers resistance to taxanes that can be reversed by trastuzumab exposure (Yu, et al., (1998) Mol Cell, 2:581-591; Tan, et al., (2002) Mol Cell, 9:993-1004; Lee, et al., (2002) Cancer Res, 62:5703-5710), and *in vitro* studies of BRCA1 associated breast cancers (frequently associated with basal-like phenotype) have demonstrated sensitivity to platinum drugs and taxane resistance (Bhattacharyya, et al., (2000) J Biol Chem, 275:23899-23903; Quinn, et al., (2003) Cancer Res, 63:6221-6228).
10 This has led to clinical trials assessing the efficacy of a taxane or a platinum agent in patients with Her2 negative, ER negative, PR negative ("triple-negative" breast cancers which histologically often form basal-like tumors) or BRCA carrier metastatic breast cancer.

[0080] Accordingly, not only is unstable aneuploidy associated with poor
15 prognosis in cancer (Carter, et al., (2006) Nat Genet, 38:1043-1048; Kronenwett, et al., (2004) Cancer Res, 64:904-909), CIN also can provide a "catalyst" promoting acquired drug resistance (Duesberg, et al., (2000) Proc Natl Acad Sci U S A, 97:14295-14300; Duesberg, et al., (2001) Proc Natl Acad Sci U S A, 98:11283-11288; Farabegoli, et al., (2001) Cytometry, 46:50-56). For example, it was found
20 that paclitaxel causes repression of CIN-survival genes followed by cell death in diploid cells but not in chromosomally unstable cells. As further demonstrated below using a combination of functional genomic and microarray expression datasets, CIN appears to be functionally associated with altered intrinsic tumor sensitivity to at least two distinct chemotherapy agents (namely, paclitaxel and
25 carboplatin). These results suggest that pre-therapeutic assessment of CIN status can optimize treatment stratification and to clinical trial design using these agents.

[0081] In various embodiments, the expression levels (e.g., mRNA or protein) of a set of genes are utilized to identify patients who potentially will respond favorably to a specific drug treatment and/or patients who potentially will not respond favorably
30 to a specific drug treatment. One or more genes in the set of genes is differentially

expressed (e.g., overexpressed) in difficult to treat cancers. For example, in some embodiments, the set of genes comprises or consists essentially of:

1	TPX2	35	AURKB	69	KIAA0286
2	PRC1	36	MSH6	70	KIF4A
3	FOXM1	37	EZH2	71	SNRFB/MGC10715
4	CDC2	38	CTPS	72	UCK2
5	C20 or f24/TGIF2	39	DKC1	73	PARP1
6	MCM2	40	OIP5	74	RAD54L
7	H2AFZ	41	CDCA8	75	NUSAP1
8	TOP2A	42	PTTG1	76	RFC5
9	PCNA	43	C10orf3/CEP55	77	TK1
10	UBE2C	44	H2AFX	78	WBP11
11	MELK	45	CMAS	79	SYNCRIP/SNX14
12	TRIP13	46	BRN1	80	BIRC5 and AFMID
13	CNAP1	47	MCM10	81	HNRPA8
14	MCM7	48	LSM4	82	TACC3
15	RNASEH2A	49	MTB	83	MKI67
16	RAD51AP1	50	ASF1B	84	CENPF
17	KIF20A	51	ZWINT	85	Sp25
18	CDC45L	52	TOPK	86	C20 or f72
19	MAD2L1	53	FLJ10036	87	PTBP1
20	ESPL1	54	CDCA3	88	DLG7
21	CCNB2	55	ECT2	89	POLR2K
22	FEN1	56	CDC6	90	IARS
23	TTK	57	UNG	91	HPRT1
24	CCT5	58	MTCH2	92	NSDHL
25	RFC4	59	RAD21	93	KNTC2
26	ATAD2	60	ACTL6A	94	RAMP
27	ch-TOG	61	GPI and MGC13096	95	C10 or f7
28	NUP205	62	SFRS2	96	C12 or f14
29	CDC20	63	HDGF	97	SNRPB1
30	CKS2	64	NXT1	98	FLJ20989
31	RRM2	65	NEK2	99	NIF3L1
32	ELAVL1	66	DHCR7	100	DER1
33	CCNB1	67	STK6		
34	RRM1	68	NDUFAB1		

or

1	TPX2	14	MCM7	25	RFC4
2	PRC1	15	RNASEH2A	26	ATAD2
3	FOXM1	16	RAD51AP1	27	ch-TOG
4	CDC2	17	KIF20A	28	NUP205
5	C20 or f24/TGIF2	18	CDC45L	29	CDC20
6	MCM2	19	MAD2L1	30	CKS2
7	H2AFZ	20	ESPL1	31	RRM2
8	TOP2A	21	CCNB2	32	ELAVL1
9	PCNA	22	FEN1	33	CCNB1
10	UBE2C	23	TTK	34	RRM1
11	MELK	24	CCT5	35	AURKB
12	TRIP13			36	MSH6
13	CNAP1			37	EZH2
				38	CTPS
				39	DKC1
40	OIP5	51	ZWINT	62	SFRS2
41	CDCA8	52	TOPK	63	HDGF
42	PTTG1	53	FLJ10036	64	NXT1
43	CEP55/C10orf3	54	CDCA3	65	NEK2
44	H2AFX	55	ECT2	66	DHCR7
45	CMAS	56	CDC6	67	STK6
46	BRN1	57	UNG	68	NDUFAB1
47	MCM10	58	MTCH2	69	KIAA0286
48	LSM4	59	RAD21	70	KIF4A
49	MTB	60	ACTL6A		
50	ASF1B	61	GPI/MGC13096		

or

1	TPX2	10	UBE2C	19	MAD2L1
2	PRC1	11	MELK	20	ESPL1
3	FOXMI	12	TRIP13	21	CCNB2
4	CDC2	13	CNAP1	22	FEN1
5	C20 or C24/TGIF2	14	MCM7	23	TTK
6	MCM2	15	RNASEH2A	24	CCTS
7	H2AFZ	16	RAD51AP1	25	RFC4
8	TOP2A	17	KIF20A		
9	PCNA	18	CDC45L		

or

1	TPX2	24	CCNB1	47	KNTC2
2	FOXMI	25	AURKB	48	MSH2
3	CDC2	26	MSH6	49	NUP155
4	MCM2	27	EZH2	50	POP7
5	H2AFZ	28	OIP5	51	LMNB1
6	TOP2A	29	PTTG1	52	CDKN3
7	PCNA	30	H2AFX	53	LRP8
8	UBE2C	31	ZWINT	54	TYMS
9	MELK	32	CDC6	55	CCNA2
10	TRIP13	33	UNG	56	MTHFD2
11	MCM7	34	RAD21	57	RFC2
12	CDC45L	35	ACTL6A	58	MCM6
13	MAD2L1	36	DHCR7	59	FANCG
14	ESPL1	37	STK6	60	MYBL2
15	CCNB2	38	KIAA0286	61	MCM3
16	FEN1	39	SNRPB/MGC10715	62	NCOA6
17	TTK	40	TK1	63	EIF2C2
18	CCTS	41	HNRPA8	64	TROAP
19	RFC4	42	MKI67	65	SIL
20	ch-TOG	43	CENPF	66	PRIM1
21	NUP205	44	SpC25	67	POLD2
22	CDC20	45	DLG7	68	EST1B
23	CKS2	46	HPRT1	69	GCH

or

1	BRCA1	9	H2AFX	17	RFC3
2	CDC2	10	IMPA2	18	RPA1
3	CDC6	11	MCM3	19	SAE2
4	CHEK1	12	MSH6	20	TOP2A
5	DCLRE1	13	NUP153	21	TOPBP1
6	FDFT1	14	NUP155	22	XPQ1
7	GART	15	NUP205		
8	GPI	16	RCN2		

5

[0082] It will be appreciated that the accuracy and reliability of a prognosis generally will increase as the number of measured genes in the statistical measure increases. However, it also will be appreciated that the expression of less than all (i.e., a subset) of genes in a given gene set can be measured while still obtaining a clinically acceptable prognosis. For example, in some embodiments, the expression levels of between about 10 and about 25 genes (e.g., 10, 12, 14, 16, 18, 20, 22, 24 or

25 genes) in a given gene set are measured and, more preferably, between about 22 and about 25 genes in a given gene set are measured.

[0083] According to various embodiments, a determination is made, for example, a statistical measure, based on the measured gene expressions levels, as to whether a patient is likely to respond to treatment. Information about the likelihood of a favorable (or unfavorable) response to drug treatment can be displayed or outputted to a user interface device, a computer readable storage medium, or a local or remote computer system. Such information can include, for example, the measured levels of one or more genes, the statistical measure of one or more genes, the likely outcome or prognosis, or an equivalent thereof (e.g., a graph, figure, symbol, etc.). Displaying or outputting information means that the information is communicated to a user using any medium, for example, orally, in writing, by visual display computer readable medium, computer system, or other electronic device (e.g., smart phone, personal digital assistant (PDA), laptop, etc.). It will be clear to one skilled in the art that outputting information is not limited to outputting to a user or a linked external component(s), such as a computer system or computer memory, but can alternatively or additionally be outputted to internal components, such as any computer readable medium. Computer readable media can include, but are not limited to hard drives, floppy disks, CD-ROMs, DVDs, and DATs. Computer readable media does not include carrier waves or other wave forms for data transmission. It will be clear to one skilled in the art that the various sample evaluation and diagnosis methods disclosed and claimed herein, can, but need not be, computer-implemented, and that, for example, the displaying or outputting step can be done by, for example, by communicating to a person orally or in writing (e.g., in handwriting).

[0084] According to various embodiments, the measured expression levels of one or more genes, the statistical measure of one or more genes, the likely outcome or prognosis, or an equivalent thereof can be displayed on a screen or a tangible medium or can be transmitted to a person in a medical industry, a medical insurance provider, a health care provider, or to a physician.

[0085] The following examples are provided to illustrate further and to facilitate the understanding of the present teachings and are not in any way intended to limit the invention.

[0086] METHODS

[0087] Datasets: Two MTS expression signatures were derived from publicly available datasets using two meta-analysis methods, namely, MCF-7 and MDA-MB-231 breast cancer cell lines treated with docetaxel (Hernandez-Vargas, et al., (2006).
5 Molecular profiling of docetaxel cytotoxicity in breast cancer cells: uncoupling of aberrant mitosis and apoptosis. Oncogene), ovarian cancer 1A9 xenografts treated with paclitaxel (Bani, et al., (2004). Mol Cancer Ther, 3:111-121), H460 non-small-cell lung carcinoma cell line treated with paclitaxel (GSE 2182) and A549 non-small-cell lung carcinoma cell line treated with epothilone 906 or docetaxel (Chen,
10 et al., (2003) Cancer Res, 63:7891-7899).

[0088] Gene annotation and Preprocessing: The gene-identifier IMAGE ID and GB_ACC number, attached by the manufacturer to each probe on the chips, was translated to Entrez Gene ID using the publicly available software MatchMiner (www.discover.nci.nih.gov/matchminer). In cases where in one dataset there were
15 multiple Entrez Gene IDs matching one identifier, the first matching EntrezGeneID was used based on the 'chain of responsibility' in MatchMiner. This translation was also used across datasets. In cases where there were multiple Entrez Gene IDs matching one identifier across datasets, the first one was selected and applied across datasets.

20 [0089] Meta-analysis Methods:

[0090] A) Binomial test and Binomial Test with Probability Correction (BTPC) Method

[0091] The binomial test with probability correction (BTPC) method was used following the application of Entrez Gene IDs to each of the publicly available
25 datasets. The BTPC method delivers a number of genes which are differentially expressed across experiments in one direction and which show a significant difference in the expression level compared to control samples. In the first step the ordinary binomial test was calculated for each gene by using the dichotomized fold change values ("-1" for negative fold change and "1" one positive fold change) and

a significance threshold of $p \leq 0.05$. Each gene was assessed for differential expression with the moderated t-test, implemented in the limma-package in Bioconductor. The moderated t-test uses a nonparametric empirical Bayes method to shrink the estimated sample variances towards a pooled estimate, resulting in more stable inference when the number of replicates is small. Based on the moderated t-test the p values for each gene were calculated and adjusted for multiple testing by calculating a q value (Storey, et al., (2003) Proc Natl Acad Sci U S A, 100:9440-9445). To account for the quality of the datasets the q values were weighted based on a measure of the spread (the inter quartile range). To filter the genes in a second step, a threshold of the weighted mean q values was used, delivering the signature.

[0092] Following this, a permutation-based false discovery rate (FDR) was calculated to control the genes identified by chance alone. To perform the experiment-wise label permutation simulation, the gene identifiers within one dataset were separated from the values and randomly assigned again. In each permutation a number of genes appear, tagged to be significantly differentially expressed although they are identified by chance alone. Ten permutations were performed and for each permutation the FDR was calculated. The arithmetic mean across all FDRs delivers the overall FDR and therefore the percentage of false positives within the significant genes. Three other meta-analysis methods were calculated and validated by the FDR, but the BTPC method delivered the lowest FDR for up- and down-regulated genes. The standard deviation and the 95% confidence interval for the FDRs were also calculated for each meta-analysis method.

25 [0093] B) Rank method

[0094] The Rank method delivers a number of genes which are regulated with a specific difference to control samples. The genes within each experiment were sorted by the fold change values and a rank was calculated for each gene. Genes were selected based on a rank threshold of 30. Genes were selected for the signature if they appeared in half or more of the datasets and if in all of those datasets genes

were consistently regulated in one direction with no assessment of significance level.

- [0095] CIN Signature: The published CIN signature (CIN70 and CIN27wp) and an extended gene list incorporating the top 5%, 10% and 15% of genes over-expressed in the CIN signature were used in this analysis (Carter, et al., (2006) Nat Genet, 38:1043-1048). Genes were excluded from the final signature (WP) if they were identified as cell cycle regulated transcripts rendering a final gene list: CIN382wp, CIN826wp and CIN1264wp (Whitfield, et al., (2002) Mol Biol Cell, 13:1977-2000).
- 10 [0096] TaqMan low-density arrays (TLDA): HCT116 *hSecurin*^{+/+} and *hSecurin*^{-/-}, A549, HCC-2998, SW620, COLO205, HCT-15, KM12, MCF7, MDA-MB-231, HS578T and BT549 cells were plated at 3×10^6 cells per 10cm plate. Twenty-four hours later, cells were treated for additional 24 hours with 0.5x of the cell specific GI50 of paclitaxel. Total RNA was extracted and cDNA synthesized as described above. The TLDAs (Applied Biosystems) used allowed 8 samples to be simultaneously quantified against 47 pre-loaded specifically designed TaqMan assays with the additional 18S RNA and GAPDH TaqMan assay detecting the endogenous controls. For each port, 100 μ l reaction mixture was added which contained 2 μ l of the 20 μ l RT reaction and 2x TaqMan Universal PCR MasterMix.
- 15 20 The cards were run on the TLDA block of the 7900 HT Real-Time PCR System (Applied Biosystems). Data analysis was performed using the SDS Relative Quantification software (Applied Biosystems).

- [0097] Cell culture and siRNA transfection: HCT-116 wild-type and isogenic *hSecurin*^{-/-} isogenic cells (Jallepalli, et al., (2001). Securin is required for chromosomal stability in human cells. Cell, 105:445-457) were cultured in Dulbecco's Modified Eagle's Medium, supplemented with 10% Fetal Bovine Serum (FBS), 2 mM L-Glutamine, 100 μ g/ml Streptomycin and 100 U/ml Penicillin at 37°C and 10% CO₂. All other cell lines used in this study were from the NCI60 cell panel which are cultured in RPMI medium, supplemented with 10% Fetal Bovine
- 25

Serum (FBS), 2% bicarbonate, 2 mM L-Glutamine, 100 µg/ml Streptomycin and 100 U/ml Penicillin at 37°C and 5% CO₂.

[0098] The siRNA (25nM) / DharmaFect1 (both from Dharmacon) transfection mixture was pipetted before adding 4.5×10^3 of HCT-116 cells per well of a 96 well plate or 3×10^5 of HCT116 cells per well of a 6 well plate in antibiotic-free media. Cells were either processed for analysis or drug-treated 48 hours after transfection.

[0099] Quantification of siRNA cytotoxicity and Acumen Explorer analysis: HCT-116 cells were plated at 6000 cells/well in 96-well plates pre-treated with poly-L lysine. HCT-116 cells underwent reverse transfection with 25nM siRNA and Dharmafect 1 transfection reagent (Dharmacon) according to manufacturer's instructions. Scrambled non-targeting and RISC-free controls were used for each experiment. Forty-eight hours post transfection, cells were prepared for analysis in the Acumen Explorer laser cytometer. Medium was aspirated from the cells and 100µl 80% ethanol in PBS at -20°C was added to each well and cells were incubated at -20°C for 30 minutes. Each well was then washed twice in PBS and 100µl of 0.2mg/ml RNase in PBS was added to each well and incubated for 1 hour at 37°C. The RNase solution was then aspirated and 100µl of 10µM propidium iodide was added to each well. The plate was incubated in the dark for 15 minutes prior to analysis on the Acumen Explorer for quantification of cell number and cell cycle profile. The plates were scanned using a 488nm laser at a sampling resolution of 1µm X direction and 8µm in the Y direction. The whole well was selected for scanning. Cells were identified on the basis of size measurements to exclude debris and large clusters of cells from analysis. This cell population was then sub divided into subG1, G1, S, G2/M, >4n, and 8n sub-populations based on the Total Intensity readout from each cell. The data was exported to give the percentage of cells in each phase of the cell cycle within a well.

[0100] Determination of sub G1 cells and polyploidy by flow cytometry: Cells were fixed with 70% ethanol and incubated with 6µg/ml anti-MPM-2 antibody (Upstate) diluted in PBS/0.2%BSA for 1h. Cells were then washed and incubated with Alexa Fluor® 488 conjugated antibody (Invitrogen) diluted in PBS containing

50 µg/ml RNaseA and 50 µg/ml propidium iodide and analyzed on a LSRII (Becton Dickinson). Cell doublets and debris were excluded from analysis on the basis of their pulse height and area and at least 3×10^4 events were recorded.

[0101] RNA extraction and cDNA synthesis: Total RNA was extracted using the RNeasy system (Qiagen) following the manufacturer's guidelines. The High-capacity cDNA RT kit (Applied Biosystems) was used to synthesize cDNA from 1 µg of total RNA.

[0102] Real-time RT-PCR quantification of mRNA silencing: Total RNA and cDNA were extracted and synthesized as described above. Real-time PCR was performed using the Fast SYBR Green assay (Applied Biosystems) to measure relative transcript levels of target genes, normalised to 18S or RPS18 RNA levels. Target-specific QuantiTect primer assays were purchased from Qiagen.

[0103] Survival analysis: The mean of the logged expression levels of CIN survival or MTS repressed genes as a single prognostic factor was tested as a predictor of long term outcome in 9 published datasets (Bild, et al., (2006) Nature, 439:353-357; Chin, et al., (2006) Cancer Cell, 10:529-541; Ivshina, et al., (2006) Cancer Res, 66:10292-10301; Miller, et al., (2005) Proc Natl Acad Sci U S A, 102:13550-13555; Naderi, et al., (2007) Oncogene, 26:1507-1516; van 't Veer, et al., (2002) Nature, 415:530-536; van de Vijver, et al., (2002) N Engl J Med, 347:1999-2009; Wang, et al., (2005) Lancet, 365:671-679.) Similarly to Carter et al. ((2006) Nat Genet, 38:1043-1048), patients were divided into two groups having above or below median of this average of the CIN survival or MTS expression signature gene expression. For each cohort the prognostic effects of these MTS repressed genes were examined with Kaplan-Meier survival analysis (Kaplan, et al., (1958) J of the American Statistical Association, 53:437-481) and Cox proportional hazard model (Cox, et al., (1984). Analysis of survival data: Chapman and Hall.).

[0104] DNA Image Cytometry: Tissue from 44 surgically removed tumors (Cancer Center Karolinska Institutet, adhering to the guidelines of the local ethical review board) was used for touch preparation slides for quantitative measurement of the nuclear DNA content before it was snap frozen until further processing with

TRIzol reagent (Invitrogen, Carlsbad, CA) for DNA and RNA extraction. The staining procedure on Feulgen-stained touch preparation slides, internal standardization, tumor cell selection and classification of genomic instability status were based on published methods (Kronenwett, et al., (2004) Cancer Res, 64:904-909; Auer, et al., (1984) Cancer Res, 44:394-396). The tumors were classified as belonging to three groups: (i) diploid cases with a distinct peak in the normal 2c region and no cells exceeding 5c, (ii) aneuploid cases with a main peak different from 2c and a *stemline scatter index* (SSI) below or equal 8.8, and (iii) aneuploid samples with a varying numbers of cells (>5%) exceeding 5c (SSI above 8.8). This novel classification system adheres to the parameters established by Kronenwett and colleagues (Kronenwett, et al., (2004) Cancer Res, 64:904-909), who defined the *stemline scatter index* (SSI) as a measurement of clonal heterogeneity in the tumor cell population.

[0105] RESULTS

15 [0106] Genes repressed by MTS are over-expressed in CIN tumors

[0107] A common MTS gene expression signature was derived by meta-analysis of published microarray datasets from four cancer cell lines and one ovarian cancer xenograft model treated with taxanes or epothilone. The datasets used to derive the MTS response signature are shown in Table 1a. Figure S1 shows the most significant repressed and expressed genes following MTS exposure in the 5 datasets. (See Chen, et al., (2003) Cancer Res, 63:7891-7899; Hernandez-Vargas, et al., (2006). Molecular profiling of docetaxel cytotoxicity in breast cancer cells: uncoupling of aberrant mitosis and apoptosis. Oncogene; Bani, et al., (2004) Mol Cancer Ther, 3:111-121) and GSE2182.

25 [0108] Using a list of genes with elevated expression in high CIN tumors (Carter, et al., (2006) Nat Genet, 38:1043-1048), it was tested whether genes repressed by MTS treatment tend to be overexpressed in tumors with high levels of CIN. To avoid non-specific gene expression changes caused by the inhibition of cellular proliferation by MTS treatment, cell cycle regulated genes from the CIN70 signature were excluded, leaving a signature of 27 genes (CIN27wp) (Whitfield, et al., (2002)

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Mol Biol Cell, 13:1977-2000). The empirical frequency distribution of CIN27wp genes was compared to the gene expression changes across all datasets following MTS exposure using the one sided bootstrap Kolmogorov-Smirnov test (Table 1b and Figure 1a). A significant left shift of the empirical frequency distribution of the
5 CIN27wp genes ($p=3.3e-07$) was observed, indicating that genes in the CIN27wp are more likely to be repressed following MTS treatment. Furthermore, it was noted that CIN genes were enriched significantly in the MTS expression signature.

[0109] Comparative data obtained with a different cytotoxic agent, 5-FU, indicate that CIN gene repression is not a general response to cytotoxic agents. Specifically,
10 the enrichment observed with MTS treatment was not evident in the expression signature induced by 5-FU derived by similar methods (Table 1c and Figure S2).

[0110] Furthermore, it was observed that genes over-expressed in CIN tumors are significantly repressed in taxane-sensitive ovarian cancer xenografts, but less repressed in a paclitaxel-resistant xenograft model 24 hours after treatment with
15 60mg/kg of paclitaxel (Table 1d) (Bani, et al., (2004) Mol Cancer Ther, 3:111-121). In addition, ovarian carcinoma data obtained from a CTCR-OV01 stage III/IV ovarian cancer clinical trial show that the majority of the CIN70 and CIN27wp genes were repressed following 3 cycles of paclitaxel chemotherapy (Figure 1b). These results demonstrate that repression of genes associated with CIN occurs
20 following taxane treatment *in vivo*. Consistent with the ovarian xenograft data, significant repression of the median expression values of the CIN70 signature was observed following paclitaxel therapy (Figure 1c-e), with a trend towards greater repression in the most paclitaxel sensitive tumors (Figure 1e). These data suggest that the repression of genes overexpressed in CIN tumors following taxane treatment
25 may be functionally implicated in drug response.

[0111] Validation of MTS repressed genes by real-time PCR

[0112] To test whether the gene expression changes derived from the meta-signature above can be observed following MTS exposure of a cell line not used in the meta-analysis, 50 MTS-repressed genes were identified from the meta-analysis
30 that were also over-expressed in CIN tumors (Figure S3a) (Carter, et al., (2006) Nat

Genet, 38:1043-1048), and among them, 36 of these genes (a number suitable for measurement by qPCR array) were randomly chosen. The near diploid HCT-116 colorectal cancer cell line was exposed to 50nM paclitaxel and the response was measured by qPCR. The 36 genes tested were consistently repressed following
5 paclitaxel exposure, indicating that the meta-analysis methods reliably detected common MTS-induced gene expression changes independent of tumor type (Figure S3b).

[0113] Identification of CIN-survival genes

[0114] In three independent experiments (Figures 2a-b), it was found that the
10 siRNA induced silencing of 22 of the 50 MTS repressed genes significantly impaired cell viability and/or promoted a greater-than-two-fold increase in subG1 DNA content following gene silencing in the HCT-116 cell line. Additionally, silencing all 22 of these genes significantly reduced cell viability in the A549 and MDA-MB-468 cancer cell lines, confirming a role for the expression of these genes
15 in cancer cell survival in multiple tumor types (Figure 2c). Consistent with the contribution of aneuploidy induction following MTS treatment to cell death, 7/22 of these cell viability genes induced aneuploidy following RNAi mediated silencing (Chen, et al., (2002) Cancer Res, 62:1935-1938) (Figure S4a). The viability effects of 7 genes selected for follow-up were validated by siRNA pool deconvolution
20 (Figure S4b), suggesting that these were not off-target effects and confirmed gene silencing by qPCR (Figure S4c).

[0115] In summary, the above results functionally characterized the role of genes overexpressed in CIN tumors that are consistently repressed by MTS agents. The above examples identified 22 “CIN-survival” genes that are overexpressed in CIN
25 tumors, repressed by MTS treatment and impair cancer cell survival when depleted from three cancer cell lines of different tumor origin (flowchart Figure 2d). Conceivably, repression of these genes may contribute to the cytotoxic response following MTS exposure.

[0116] Repression of CIN-survival genes in CIN^{low} but not CIN^{high} cell lines correlates with paclitaxel cytotoxicity
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[0117] As described herein, CIN can be used as a measure of cell-to-cell variability in chromosome number (Geigl, et al., (2008) Trends Genet, 24:64-69). Published SKY data from NCI60 colorectal and breast cancer cell lines were used to define the fraction of normal chromosomes displaying numerical heterogeneity from cell-to-cell as a measure of CIN (Roschke, et al., (2003) Cancer Res, 63:8634-8647).
5 In this study, cell lines are categorized as CIN^{low} if <15% of chromosomes displayed numerical heterogeneity and CIN^{high} if >40% chromosomes exhibited numerical heterogeneity (Roschke, et al., (2003) Cancer Res, 63:8634-8647).

[0118] To investigate whether the CIN status of a cell line correlates with the gene expression response following MTS treatment, six NCI60 colon cancer cell lines
10 with various degrees of CIN were chosen. Each cell line was treated with a paclitaxel concentration equal to half its Gi50 (drug concentration required for 50% growth inhibition, Table 5) for 24 hours and measured gene expression by qPCR. Cell lines with the lowest frequency of CIN elicited repression of genes identified in
15 the MTS signature, including several CIN-survival genes (Figure 3a). In contrast, CIN^{high} cell lines (SW620 and COLO205) elicited significantly less repression of the CIN-survival genes compared to near diploid CIN^{low} HCT-116 cells. In agreement, greater repression of CIN-survival genes was observed in the CIN^{low} MCF-7
20 compared to the CIN^{high} BT549 breast cancer cell lines (Figure S5a). The majority of the CIN-survival genes exhibit increased basal expression in CIN^{high} cells compared to the CIN^{low} HCT-116 cell line (Figure 3b) in agreement with the published CIN signature (Carter, et al., (2006) Nat Genet, 38:1043-1048).

[0119] It was suspected that the relative resistance of CIN^{high} cell lines to paclitaxel-mediated cell death results from the failure of the drug to repress the CIN-
25 survival genes. To address this, tests were performed to determine whether the CIN status of the cell correlates with reduced cell death (assessed by percentage of sub-G1 cells by FACS) in the six colorectal cancer cell lines at different paclitaxel concentrations (Gi50/4, Gi50/2, Gi50 concentration for each cell line; Table 2). It was observed that there is a correlation between increasing CIN status of the six cell
30 lines and an increased proportion of cells arrested in mitosis relative to the percentage of dying cells (MPM2:SubG1 ratio) following 24 hours of paclitaxel

treatment (Gi50/4, Gi50/2, Gi50 for each cell line, correlation coefficient 0.80, 95% confidence interval 0.77-0.86). The SW620 and COLO205 CIN^{high} cell lines that failed to efficiently repress the CIN-survival genes in Figure 3a, displayed the highest MPM2/SubG1 ratios consistent with a cytostatic rather than a cytotoxic response to drug exposure in CIN^{high} cells (Figure 3c).

[0120] In addition, tests were performed to determine whether the silencing of CIN-survival genes increases paclitaxel-mediated cytotoxicity and lowers the MPM2/SubG1 ratio in the CIN^{high} cell line, SW620 (Figure 3d). Following silencing of NUP205, H2AFX, CDC6, RPA1 and TOP2A, a significant decrease in the MPM2/SubG1 ratio (gene silencing was confirmed by qPCR, data not shown) was observed. Silencing of NUP205, H2AFX, CDC6 and RPA1 promoted a significant increase in paclitaxel induced cytotoxicity relative to vehicle treated SW620 cells (Figure S5b), strengthening the model that the repression of CIN-survival genes contributes to cytotoxicity following MTS exposure.

[0121] To substantiate the impaired repression of CIN-survival genes in CIN cells following taxane exposure, the repression of these genes in isogenic models of chromosomal instability was investigated. The results showed significantly less repression of the majority of CIN-survival genes tested in the HCT-116 Mad2^{+/-} and early passage HCT-116 *hSecurin*^{-/-} following 24hrs 50nM paclitaxel exposure compared to parental control cells (Figures 3e and S5c). These data support the impaired repression of CIN survival genes in CIN tumor cells compared to the diploid isogenic pair following MTS exposure.

[0122] In summary, upon taxane treatment, CIN^{high} cancer cell lines and isogenic CIN models do not repress CIN-survival genes as significantly as CIN^{low} cell lines, and basal expression of these genes is also higher in CIN^{high} cell lines. The cytotoxicity of paclitaxel is associated with robust repression of CIN-survival genes that occur in CIN^{low} cells (Figure 3f). These data indicate that CIN may attenuate the cytotoxic response to MTS exposure through impaired CIN-survival gene repression. Consistent with this hypothesis, cytotoxicity in a CIN^{high} cell line can be partially restored by silencing CIN-survival genes.

[0123] CIN ovarian cancers display intrinsic paclitaxel resistance

[0124] The OV01 clinical trial dataset with objective response assessment was used to address whether CIN predicts sensitivity to paclitaxel in contrast to sensitivity to carboplatin in patients with ovarian cancer. Tests were designed to
5 compare the median expression of each CIN70 gene in tumors prior to treatment with paclitaxel or carboplatin monotherapy, and established methods were used to define treatment response according to the fall in serum CA125 level (Rustin, et al., (2004) J Natl Cancer Inst, 96:487-488). The results showed that the majority of the CIN70 genes are overexpressed in paclitaxel resistant and carboplatin sensitive
10 tumors (Figure 4a). It was observed that high median CIN70 signature expression is associated with paclitaxel resistance and low median expression with paclitaxel sensitivity ($p = 0.043$). CIN70 signature expression was significantly greater in tumors subsequently resistant to paclitaxel compared to tumors resistant to carboplatin ($p = 0.044$), supporting the hypothesis that the efficacy of these two
15 cytotoxic agents is differentially altered in CIN tumors. There was no significant relationship between Ki-67 or ABCB1, ABCB4 and ABCB11 expression and response to paclitaxel, supporting the contribution of CIN rather than effects of tumor cell proliferation or drug efflux to paclitaxel resistance (data not shown). There was a strong correlation between the median CIN-survival gene expression
20 and the CIN70 median expression ($R=0.93$, $p<0.0001$) in ovarian cancers derived from this cohort.

[0125] Further tests were performed to investigate whether expression of the CIN signature is increased in residual paclitaxel resistant compared to sensitive tumors. Significant over-expression of CIN70 genes was observed in residual ovarian
25 cancers classified using Rustin criteria as resistant (defined as Ca-125 coefficient >-0.5) compared to sensitive tumors ($p=0.01$, Figure 4b) that was of greater magnitude when taxane-resistant tumors were compared to tumors most sensitive to paclitaxel (defined as Ca-125 coefficient <-1 , $p = 8.30e-8$, Figure 4b). These data suggest that there may be a selection pressure for sustaining CIN in paclitaxel resistant tumors.

30 [0126] These data indicate that CIN, defined by expression of the CIN signature, may predict subsequent resistance to paclitaxel and sensitivity to carboplatin *in vivo*.

This provides clinical evidence implicating CIN in pre-treatment tumors as a marker of taxane resistance *in vivo* and indicates that the efficacy of two common chemotherapy drugs may be differentially influenced by tumor karyotype.

5 **[0127]** Relationship of CIN-survival genes to breast cancer molecular subtype and prognosis

10 **[0128]** Tests were performed to address the relationship between clinical and pathological variables in breast cancer, for which taxanes form a major component of treatment regimens, with expression of the CIN signature and CIN-survival genes in order to better identify patient cohorts that might selectively benefit from taxane
15 schedules. DNA image cytometry methods were used to assess CIN directly in 44 primary breast cancers for which gene expression data were available (GSE11901) (Habermann, et al., (2009) Int J Cancer, 124:1552-1564). This technique is applied to the measurement of the nuclear DNA content in clinical samples, including fine needle aspirate cytology and allows assessment of tumor ploidy status and tumor
20 classification into genomically stable and unstable subtypes (Kronenwett, et al., (2004) Cancer Res, 64:904-909). The 44 breast cancers were classified into three groups; Aneuploid, genomically unstable breast cancers (aGU) which show a larger distribution in their image analysis-quantified DNA content relative to aneuploid, genomically stable (aGS) or diploid, genomically stable (dGS) tumors (clinical data
25 Table 3). The expression levels of the CIN signature and CIN-survival genes in each of these tumors classified for genomic instability status and clinicopathological subtype were assessed by the median centroid method. aGU breast cancers displayed significantly higher expression of the CIN70 ($p=4e-04$), CIN27wp ($p=0.027$) and the CIN-survival gene ($p=0.0024$) signature set than aGS or dGS
30 breast cancer samples (Figure 4c) confirming that expression of the CIN70 and CIN-survival signatures reflect CIN *in vivo*. The aGU tumors that displayed the highest expression of the CIN and CIN-survival gene signatures, incorporated 9/9 basal-like breast cancers and 6 of the 9 Her2-positive cancers in the 44 patient cohort.

30 **[0129]** It has been reported that CIN70 expression signature carries prognostic significance in breast cancer (Carter, et al., (2006) Nat Genet, 38:1043-1048). Consistent with these results, over-expression of CIN-survival genes correlated

significantly with poorer disease-free or disease-specific survival ($P < 0.05$ log-rank test) with an increased hazard ratio for death or relapse in three of four untreated estrogen receptor positive breast cancer cohorts studied (Figure S5b and Sweave analysis).

- 5 [0130] In summary, CIN-survival gene expression is highly correlated both with the CIN signature *in vivo* and CIN determined by DNA image cytometry. The CIN signature (Carter, et al., (2006) Nat Genet, 38:1043-1048) and CIN-survival gene expression carry prognostic power in breast cancer, and the CIN signature carries predictive power and may serve as a surrogate marker for taxane resistance and
- 10 carboplatin sensitivity in ovarian cancer.

[0131] The use of headings and sections in the application is not meant to limit the present teachings; each section can apply to any aspect, embodiment, or feature of the present teachings.

- [0132] Throughout the application, where compositions are described as having, including, or comprising specific components, or where processes are described as
- 15 having, including or comprising specific process steps, it is contemplated that compositions of the present teachings also consist essentially of, or consist of, the recited components, and that the processes of the present teachings also consist essentially of, or consist of, the recited process steps.

- 20 [0133] In the application, where an element or component is said to be included in and/or selected from a list of recited elements or components, it should be understood that the element or component can be any one of the recited elements or components, or can be selected from a group consisting of two or more of the recited elements or components. Further, it should be understood that elements and/or
- 25 features of a composition, an apparatus, or a method described herein can be combined in a variety of ways without departing from the spirit and scope of the present teachings, whether explicit or implicit herein.

[0134] The use of the terms “include,” “includes,” “including,” “have,” “has,” or “having” should be generally understood as open-ended and non-limiting unless specifically stated otherwise.

[0135] The use of the singular herein includes the plural (and vice versa) unless
5 specifically stated otherwise. Moreover, the singular forms “a,” “an,” and “the” include plural forms unless the context clearly dictates otherwise. In addition, where the use of the term “about” is before a quantitative value, the present teachings also include the specific quantitative value itself, unless specifically stated otherwise. As
10 used herein, the term “about” refers to a $\pm 10\%$ variation from the nominal value, unless otherwise indicated or inferred.

[0136] It should be understood that the order of steps or order for performing certain actions is immaterial so long as the present teachings remain operable. Moreover, two or more steps or actions may be conducted simultaneously.

[0137] Where a range or list of values is provided, each intervening value between
15 the upper and lower limits of that range or list of values is individually contemplated and is encompassed within the present teachings as if each value were specifically enumerated herein. In addition, smaller ranges between and including the upper and lower limits of a given range are contemplated and encompassed within the present teachings. The listing of exemplary values or ranges is not a disclaimer of other
20 values or ranges between and including the upper and lower limits of a given range.

[0138] The present teachings encompass embodiments in other specific forms without departing from the spirit or essential characteristics thereof. The foregoing embodiments are therefore to be considered in all respects illustrative rather than limiting on the present teachings described herein. Scope of the present invention is
25 thus indicated by the appended claims rather than by the foregoing description, and all changes that come within the meaning and range of equivalency of the claims are intended to be embraced therein.

[0139] What is claimed is:

CLAIMS

1. A method of predicting the clinical outcome of treating a patient having a tumor using one or more microtubule stabilizing (MTS) drugs, the method comprising:

measuring, in a sample comprising a tumor cell from a patient, the mRNA expression levels of at least 10 of the following genes:

1	BRCA1	9	H2AFX	17	RFC3
2	CDC2	10	IMPA2	18	RPA1
3	CDC6	11	MCM3	19	SAE2
4	CHEK1	12	MSH6	20	TOP2A
5	DCLRE1	13	NUP153	21	TOPBP1
6	FDFT1	14	NUP155	22	XPQ1
7	GART	15	NUP205		
8	GPI	16	RCN2		

taking a statistical measure of the mRNA expression level of the measured genes; and

if the statistical measure of the mRNA expression level of the measured genes is elevated, determining that the patient is a non-responder to MTS drug treatment.

2. The method of claim 1, wherein the mRNA expression levels of 10, 15, 20, or 22 of the following genes is measured.

3. The method of claim 1 or 2, comprising the step of administering a MTS drug to the patient.

4. The method of claim 3, comprising the step of measuring mRNA expression levels at one or more times after administration of the MTS drug to the patient.

5. The method of any one of the preceding claims, wherein the statistical measure of the mRNA expression level of the measured genes is a linear combination of the mRNA expression level of the genes.
6. The method of claim 5, wherein the linear combination of the mRNA expression level of the genes is the mean of logarithms of the mRNA expression levels.
7. The method of any one of the preceding claims, wherein measuring the mRNA expression levels comprises using a microarray or PCR.
8. The method of any one of the preceding claims, wherein the tumor is a solid tumor.
9. The method of claim 8, wherein the solid tumor is of a cancer selected from breast cancer, ovarian cancer, colorectal cancer, lung cancer, prostate cancer, medulloblastoma, glioma, and lymphoma.
10. The method of any one of the preceding claims, wherein the MTS drug is a taxane.
11. The method of claim 10, wherein the taxane is paclitaxel.
12. The method of claim 10, wherein the taxane is docetaxel.
13. The method of any one of claims 1-9, wherein the MTS drug is epothilone.
14. A method of selecting a treatment for a patient having a tumor, the method comprising:
measuring, in a sample comprising a tumor cell from a patient, the mRNA expression levels of at least 15 genes of the following set of genes:

1	TPX2	14	MCM7	25	RFC4
2	PRC1	15	RNASEH2A	26	ATAD2
3	FOXN1	16	RAD51AP1	27	ch-TOG
4	CDC2	17	KIF20A	28	NUP205
5	C20 or F24/TGIF2	18	CDC43L	29	CDC20
6	MCM2	19	MAD2L1	30	CKS2
7	H2AFX	20	ESPL1	31	RRM2
8	TOP2A	21	CCNB2	32	ELAVL1
9	PCNA	22	FEN1	33	CCNB1
10	UBE2C	23	TTK	34	RRM1
11	MELK	24	CCT5	35	AURKB
12	TRIP13			36	MSH6
13	CNAP1			37	EZH2
				38	CTPS
				39	DKC1
40	OIP5	51	ZWINT	62	SFRS2
41	CDCA8	52	TOPK	63	HDGF
42	PTTG1	53	FLJ10036	64	NXT1
43	CEP55/C10orf3	54	CDCA3	65	NEK2
44	H2AFX	55	ECT2	66	DHCR7
45	CMAS	56	CDC6	67	STRK6
46	BRRN1	57	UNG	68	NDUFAB1
47	MCM10	58	MTCH2	69	KJAA0286
48	LSM4	59	RAD21	70	KIF4A
49	MTB	60	ACTL6A		
50	ASF1B	61	GPI/MGC13096		

taking a statistical measure of the mRNA expression level of the measured genes; and

if the statistical measure of the mRNA expression level of the measured genes is elevated, determining that the patient is a responder to carboplatin drug treatment.

15. The method of claim 14, wherein the mRNA expression levels of 20, 25, 30, 35, 40, or 50 or more of the following genes is measured.

16. The method of claim 14, wherein the mRNA expression levels are measured before onset of paclitaxel or carboplatin drug treatment.

17. The method of any one of claims 14-16, wherein the statistical measure of the mRNA expression level of the measured genes is a linear combination of the mRNA expression level of the genes.

18. The method of claim 17, wherein the linear combination of the mRNA expression level of the genes is the mean of logarithms of the mRNA expression levels.
19. The method of any one of claims 14-18, wherein measuring the mRNA expression levels comprises using a microarray or PCR.
20. The method of any one of claims 14-19, wherein the tumor is a solid tumor.
21. The method of claim 20 wherein the solid tumor is of a cancer selected from breast cancer, ovarian cancer, colorectal cancer, lung cancer, prostate cancer, medulloblastoma, glioma, and lymphoma.
22. The method of claim 14, comprising recommending administration of carboplatin to the patient.
23. The method of claim 14, comprising administering carboplatin.

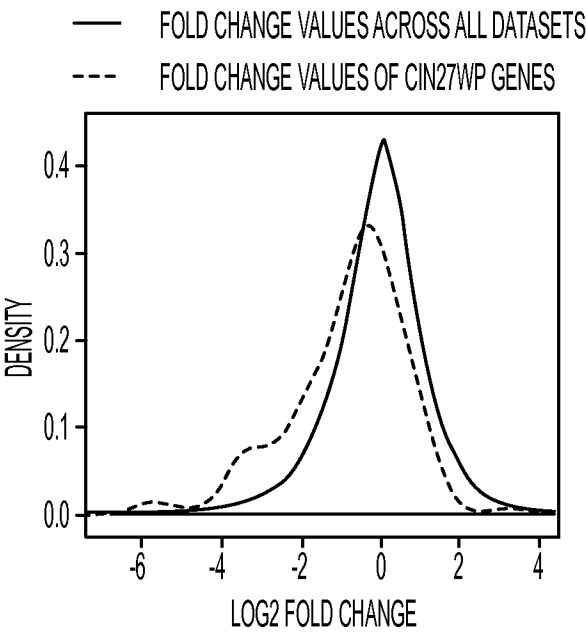


FIG. 1a

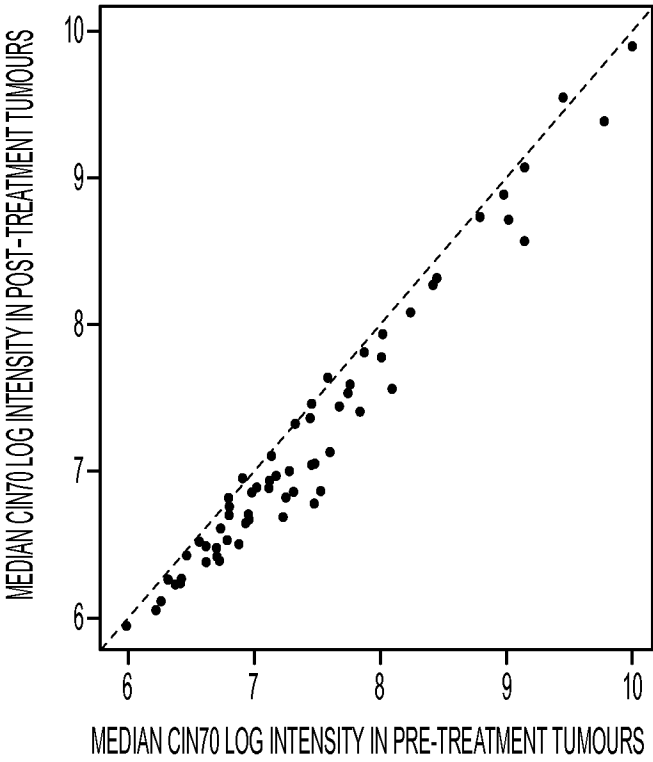
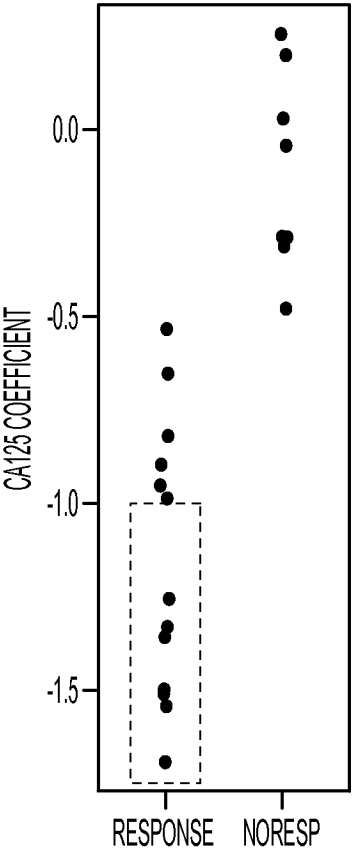


FIG. 1b



RESPONSE BY RUSTIN CRITERIA

FIG. 1c

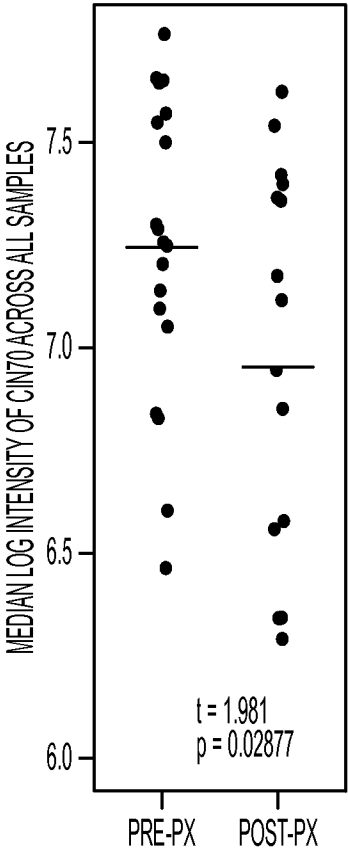


FIG. 1d

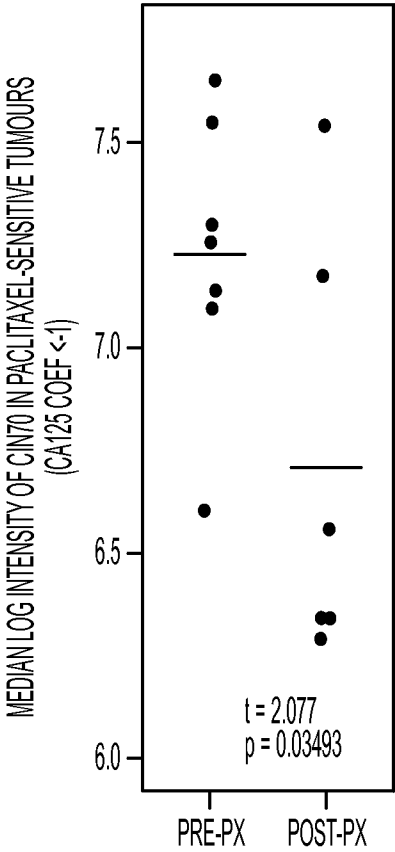


FIG. 1e

3/25

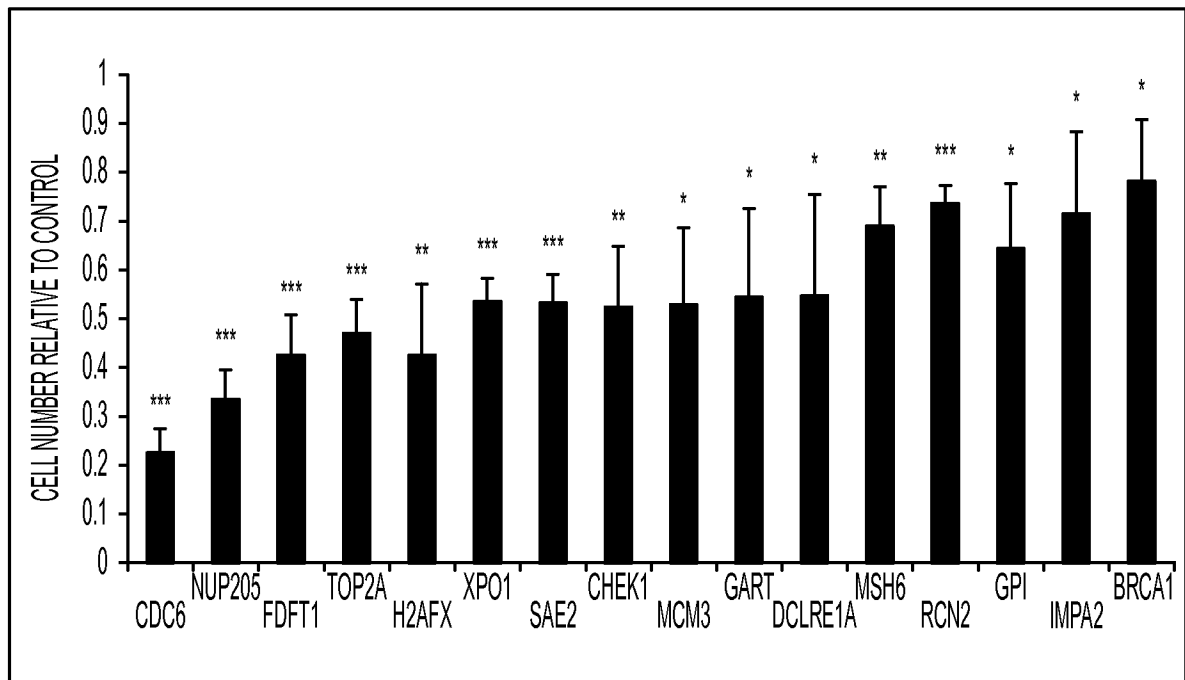


FIG. 2a

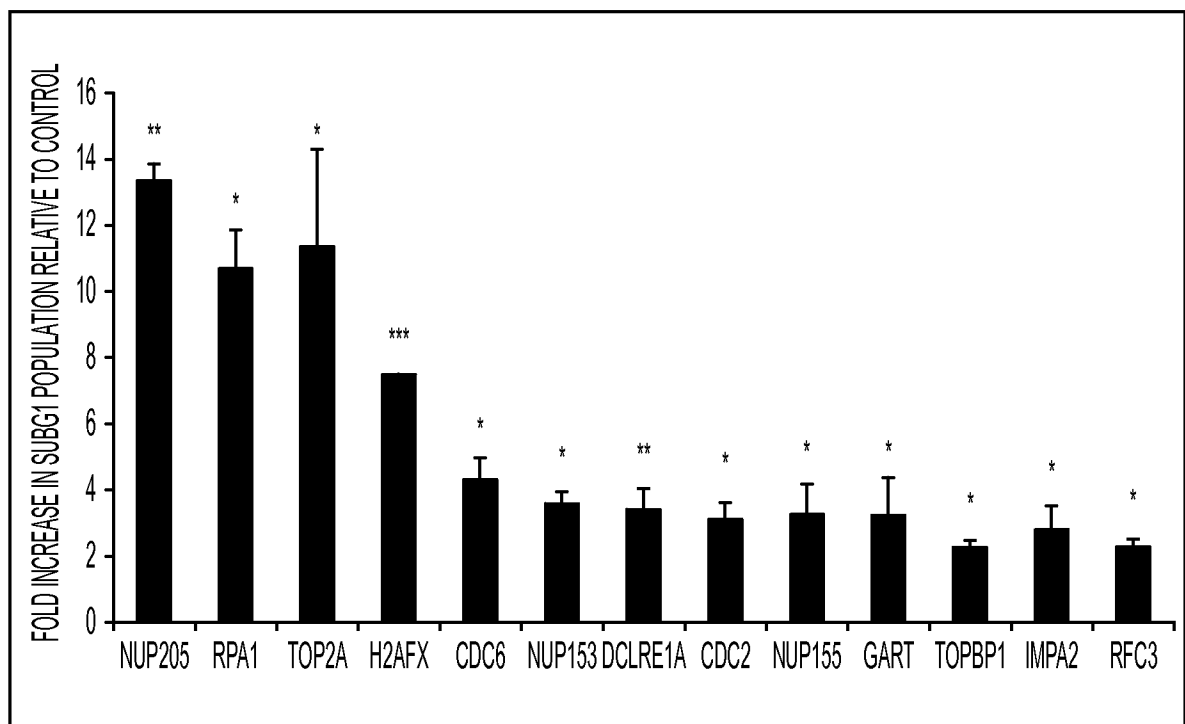


FIG. 2b

4/25

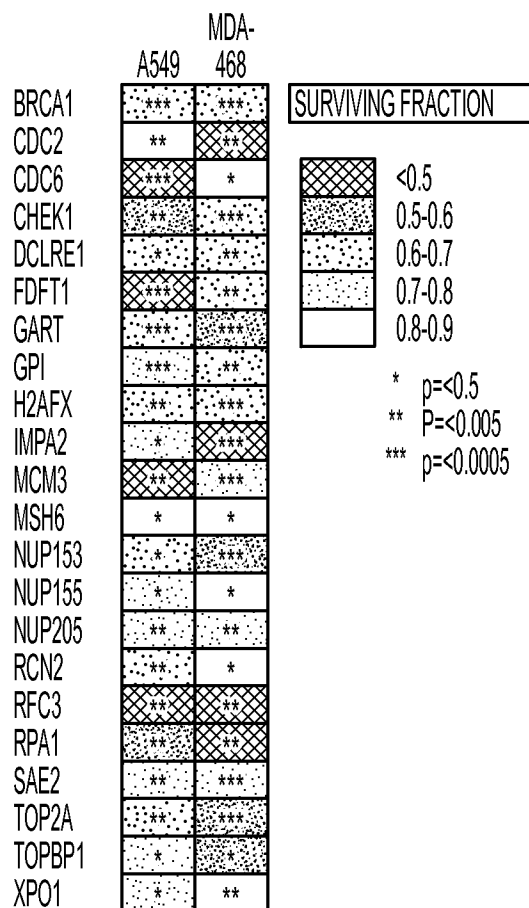


FIG. 2c

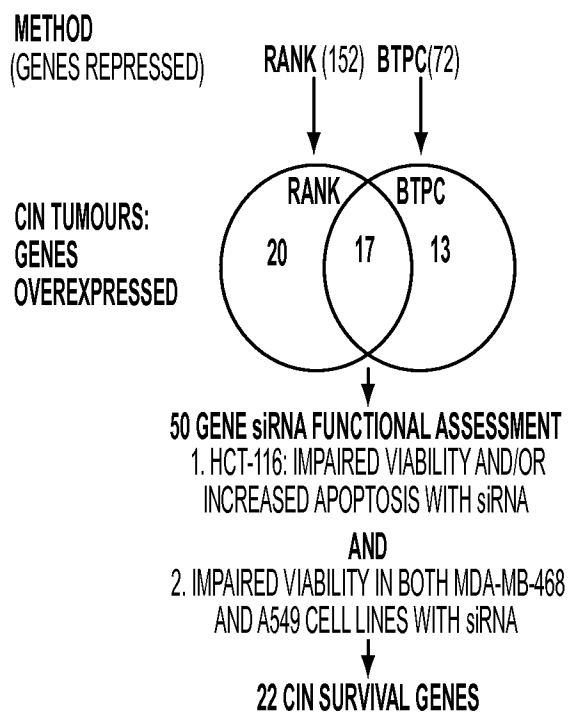
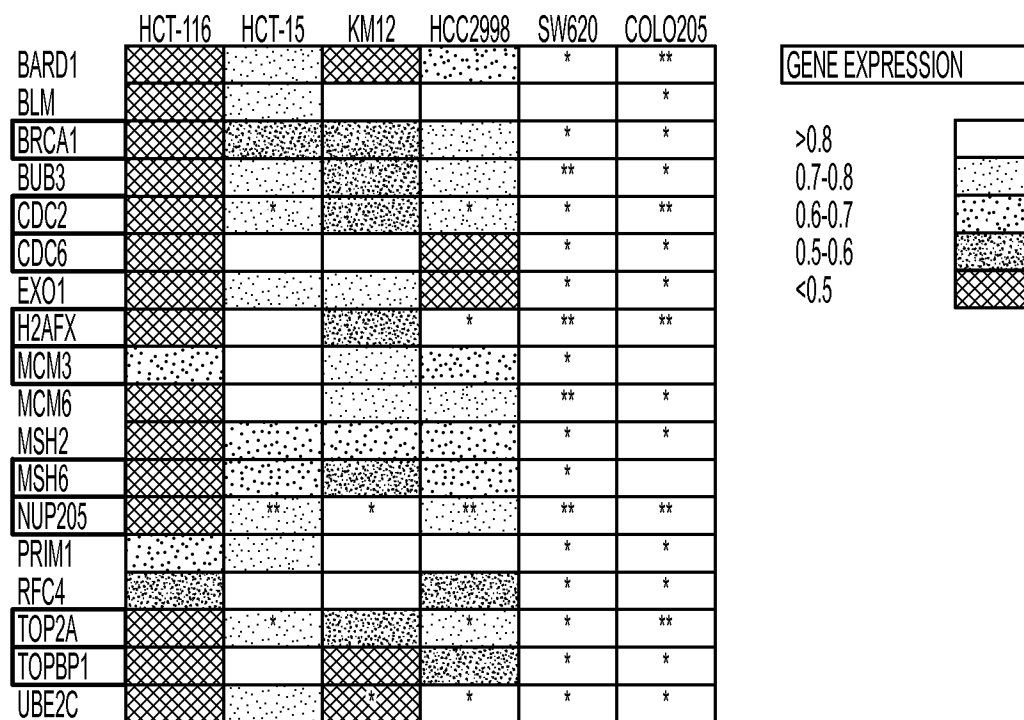


FIG. 2d

5/25



CHROMOSOMAL NUMERICAL HETEROGENEITY

FIG. 3a

6/25

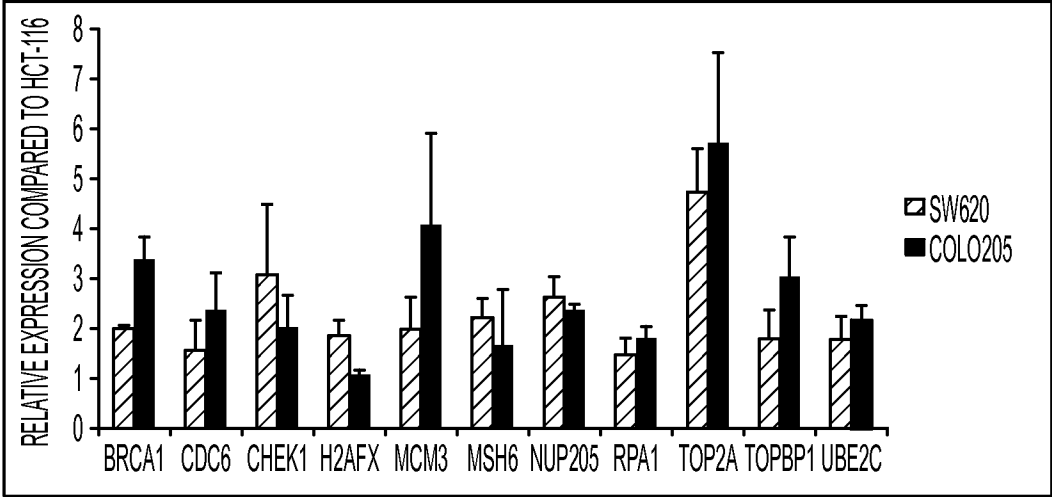


FIG. 3b

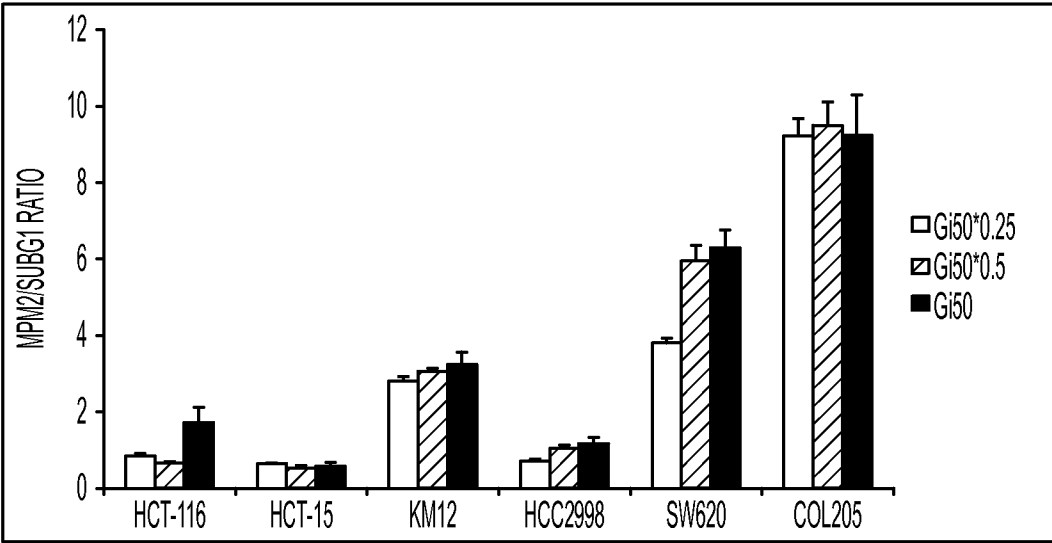


FIG. 3c

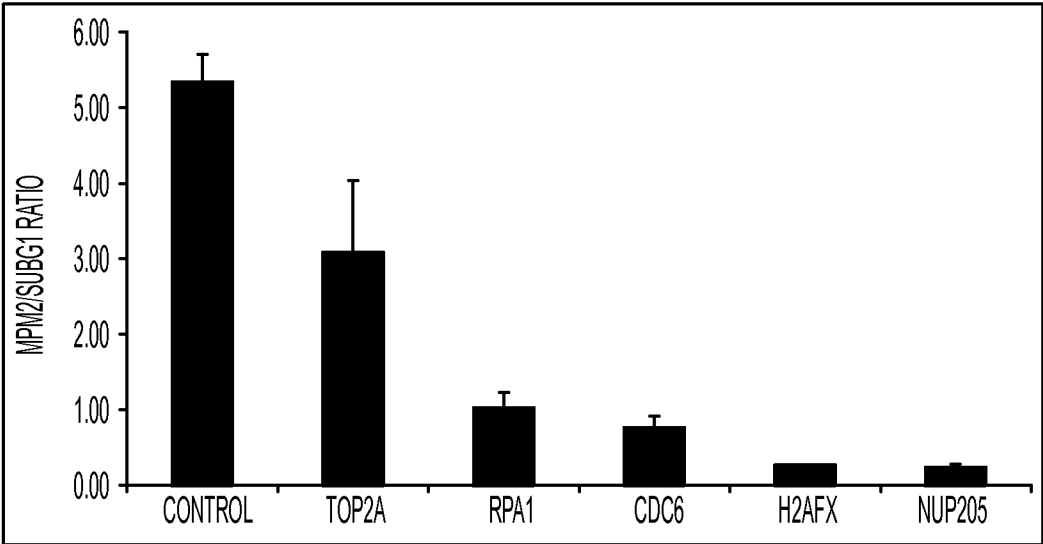


FIG. 3d

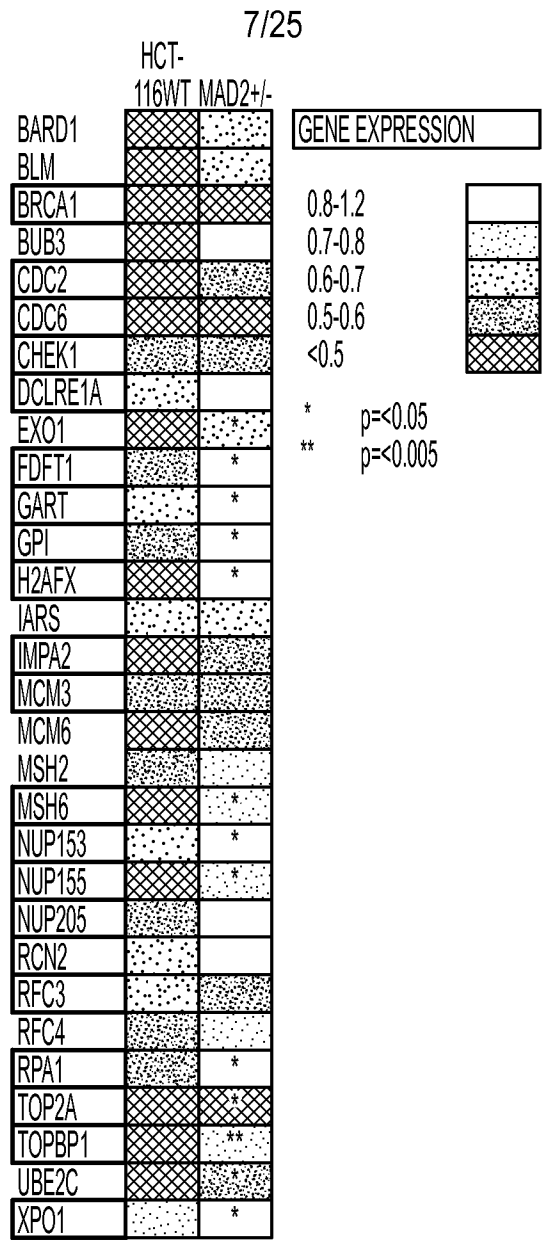


FIG. 3e

	CIN GENE EXPRESSION RELATIVE TO CIN ^{LOW}	MTS-INDUCED CIN GENE EXPRESSION RELATIVE TO NO DRUG	CELL FATE
CIN ^{HIGH}	OVEREXPRESSED	NOT REPRESSED	CYTOSTASIS
CIN ^{LOW}		REPRESSED	CYTOTOXICITY

FIG. 3f

8/25

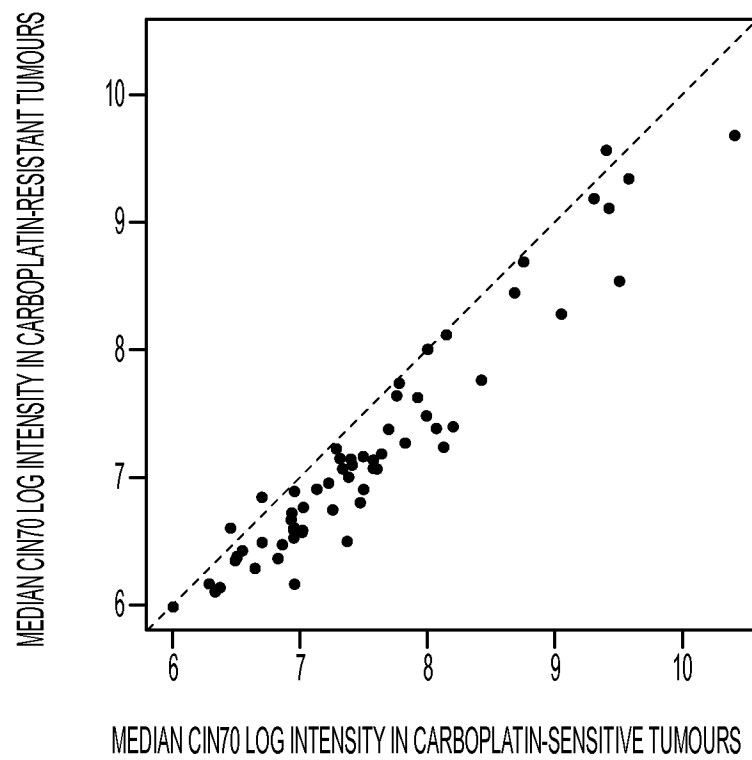
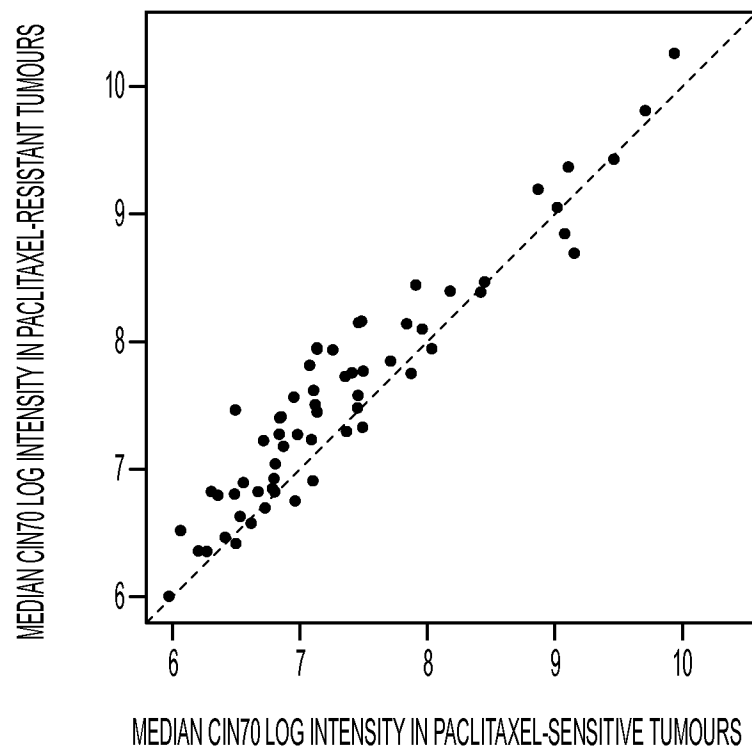


FIG. 4a

9/25

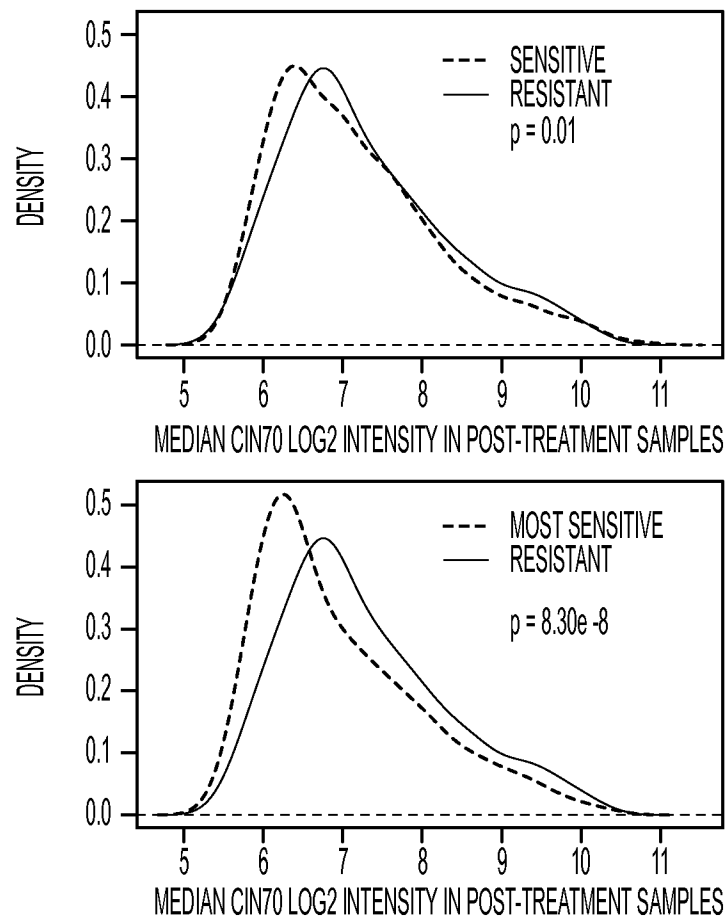


FIG. 4b

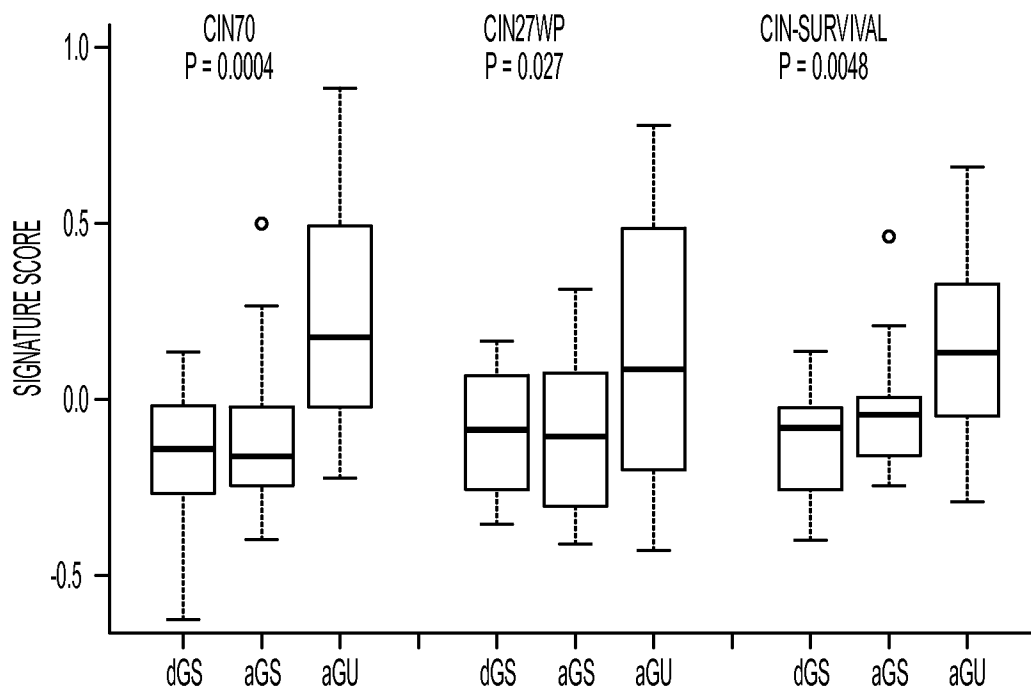
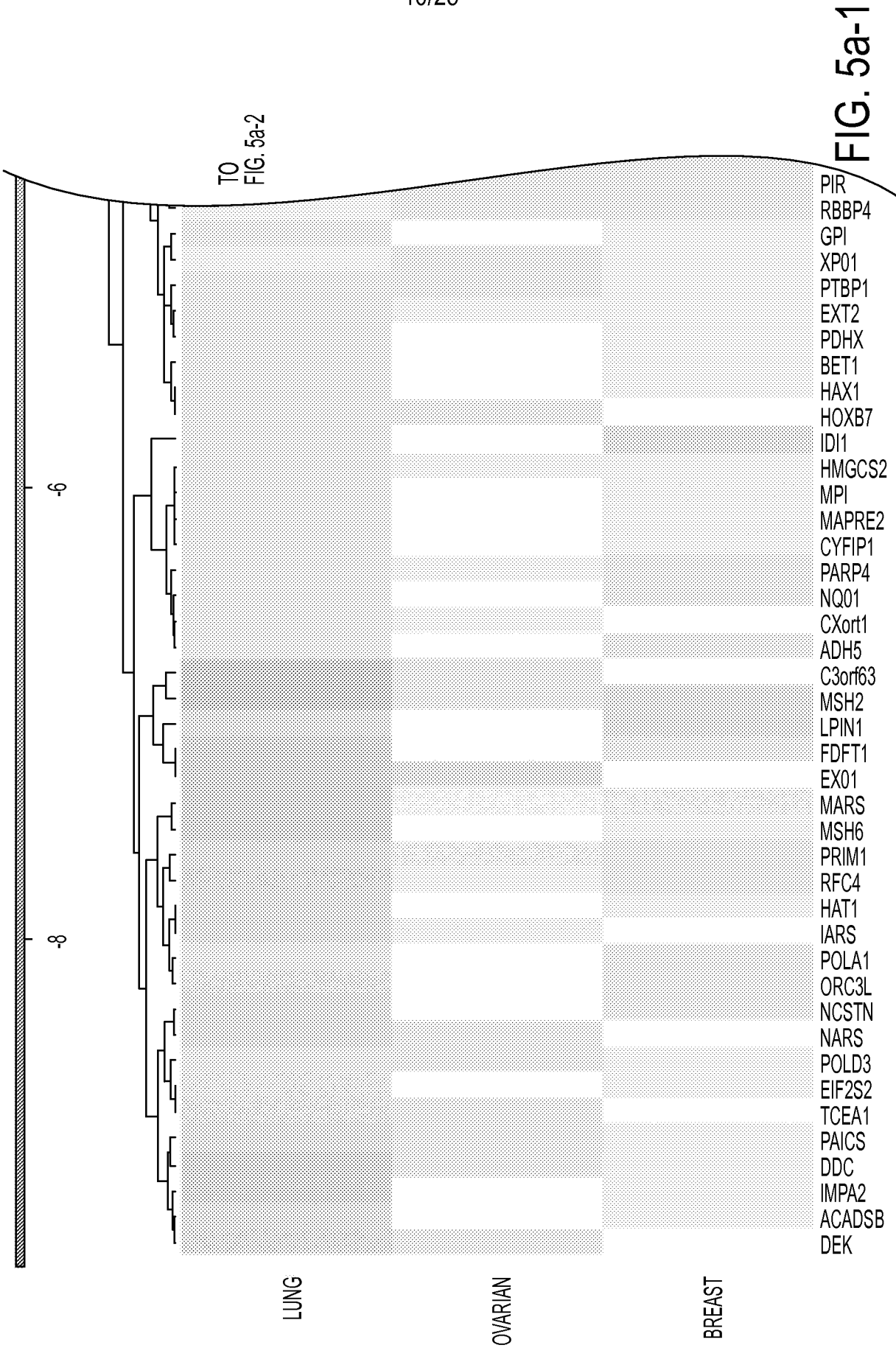
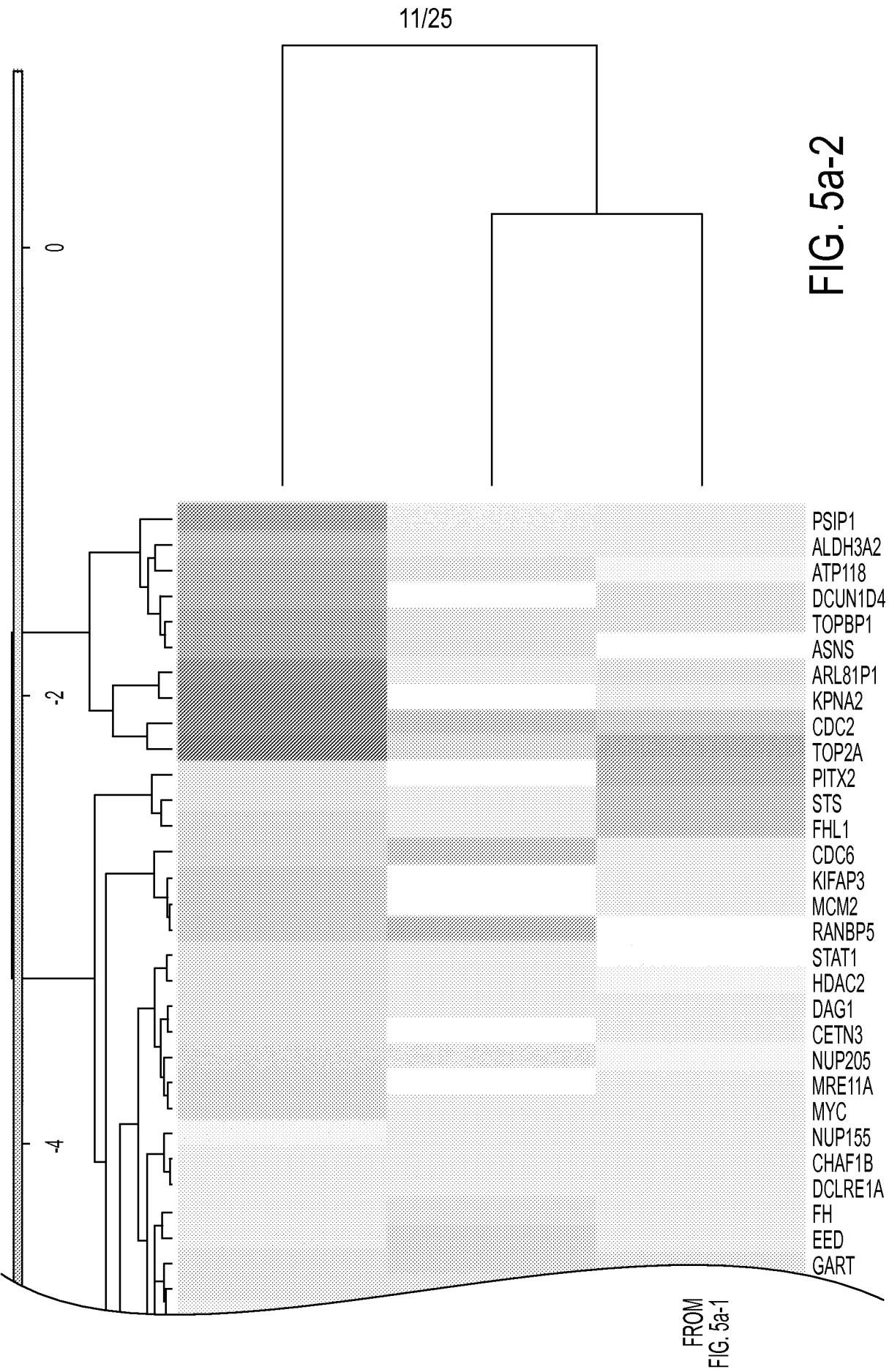


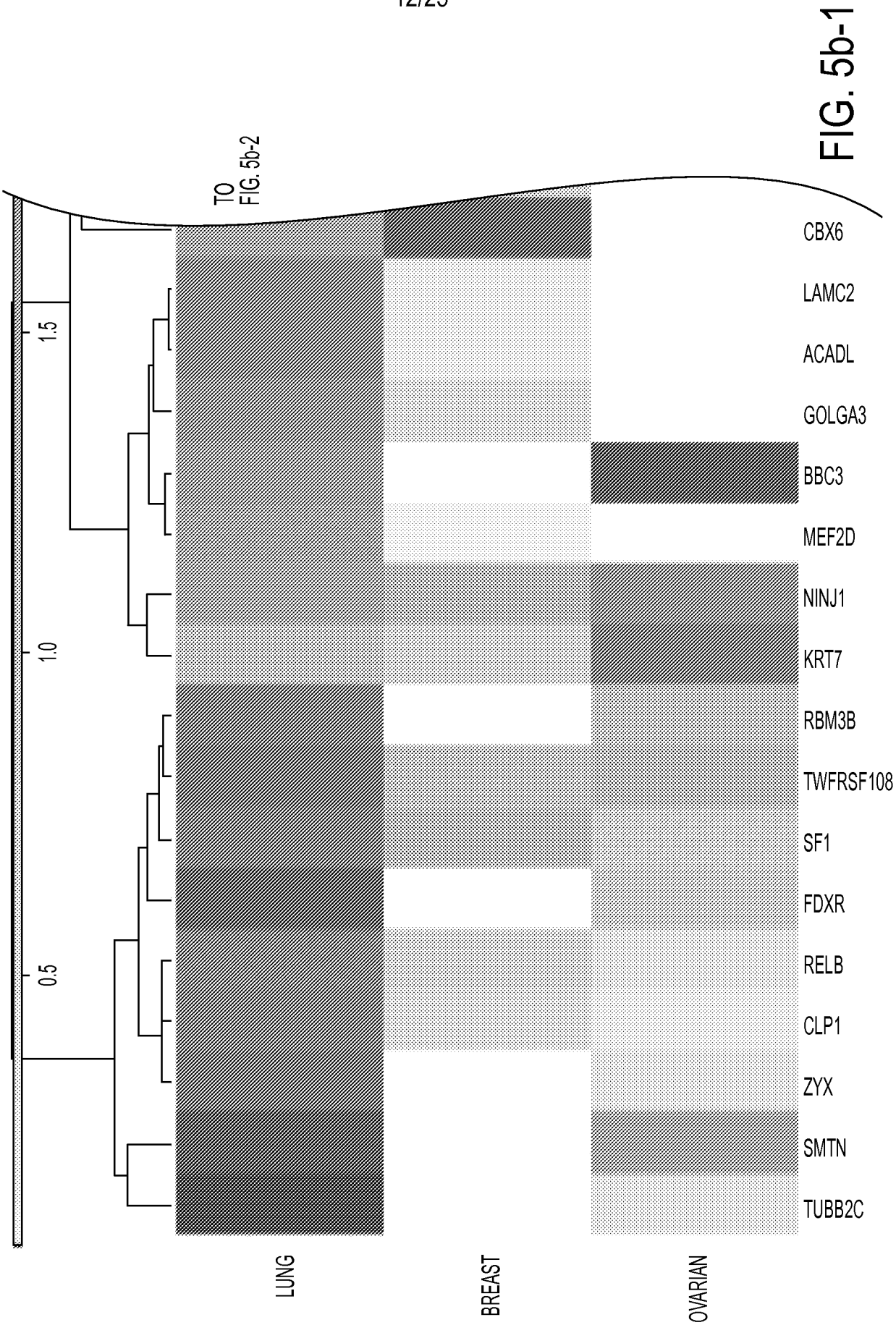
FIG. 4c

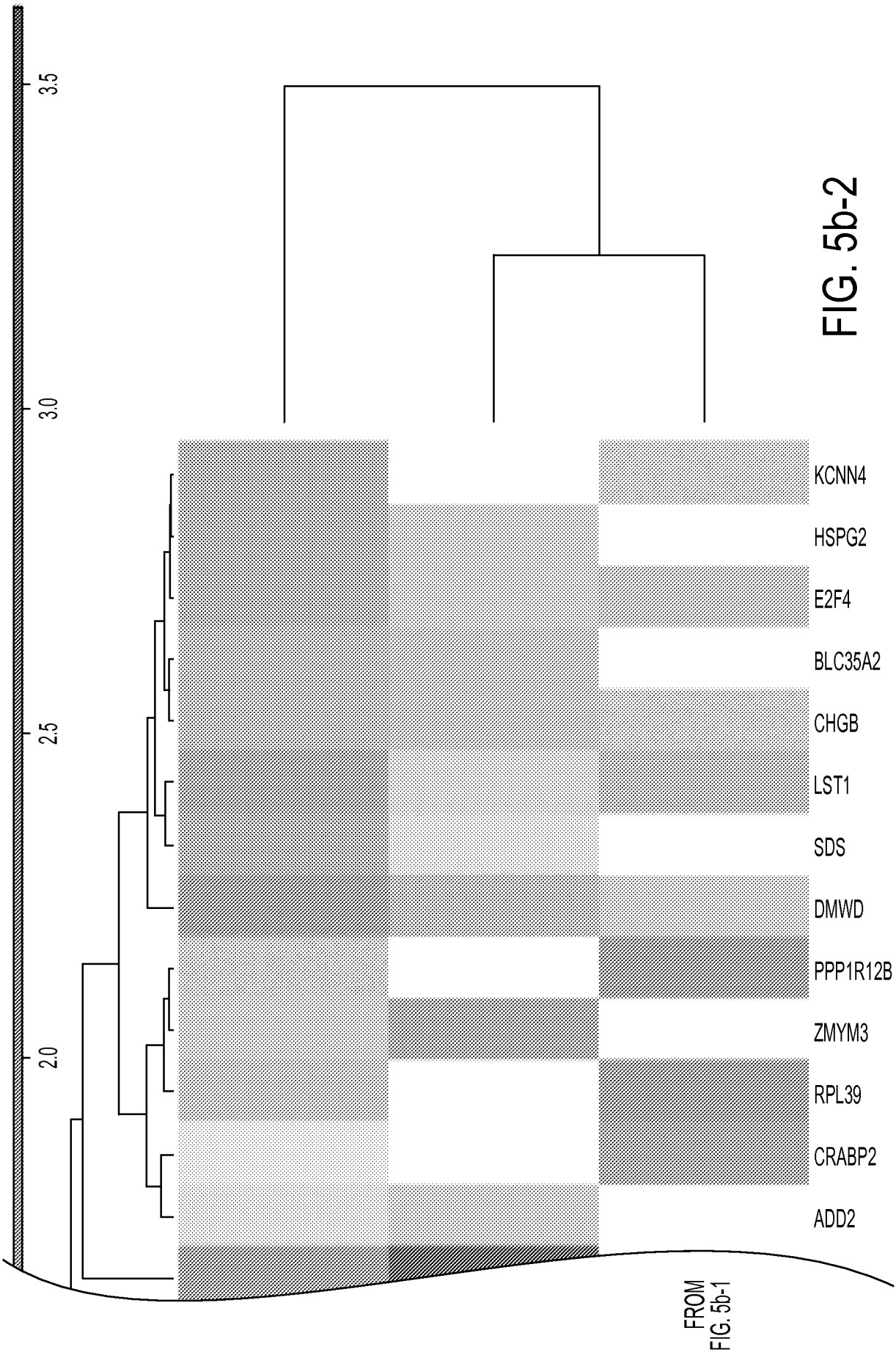
10/25





12/25





TO FIG. 6a-2

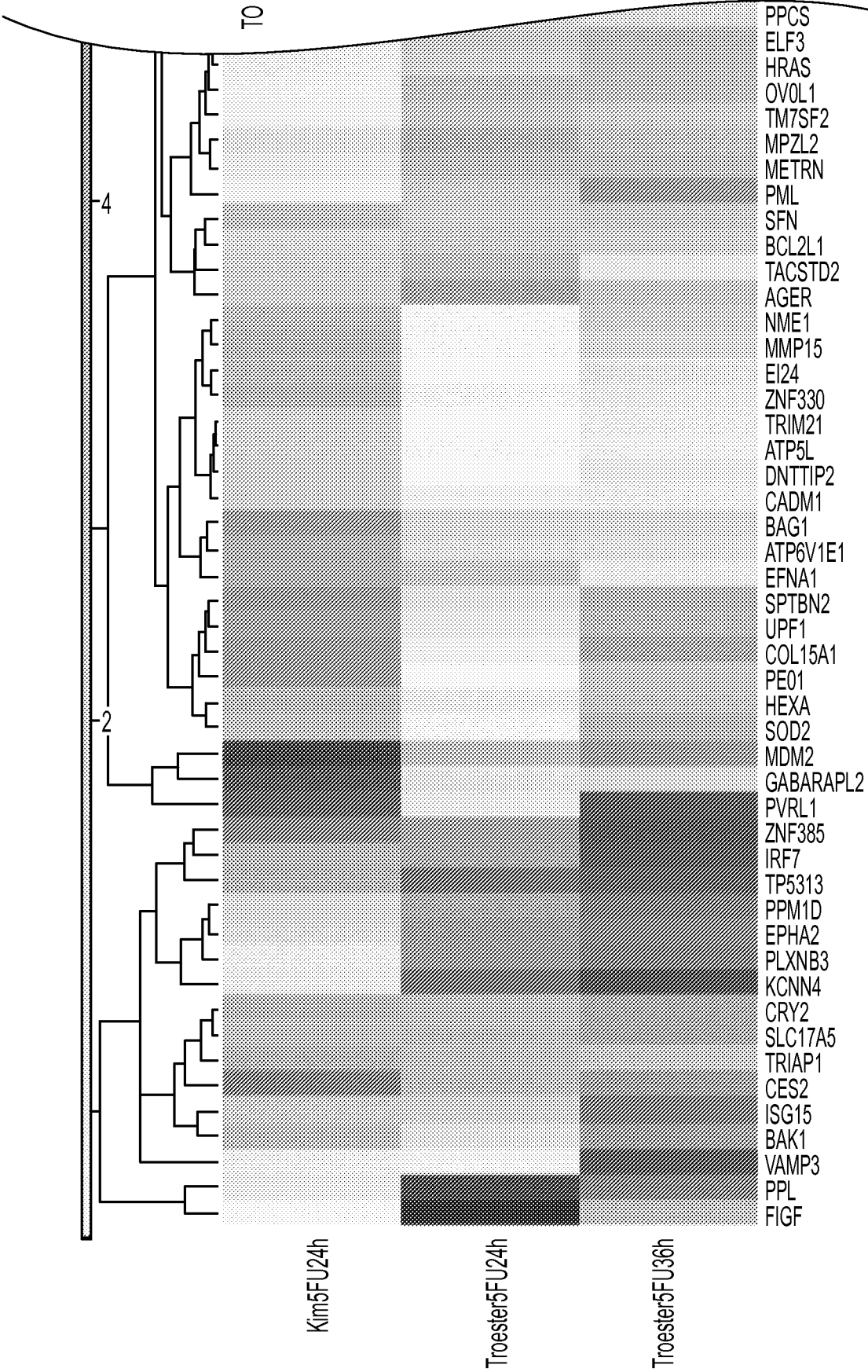
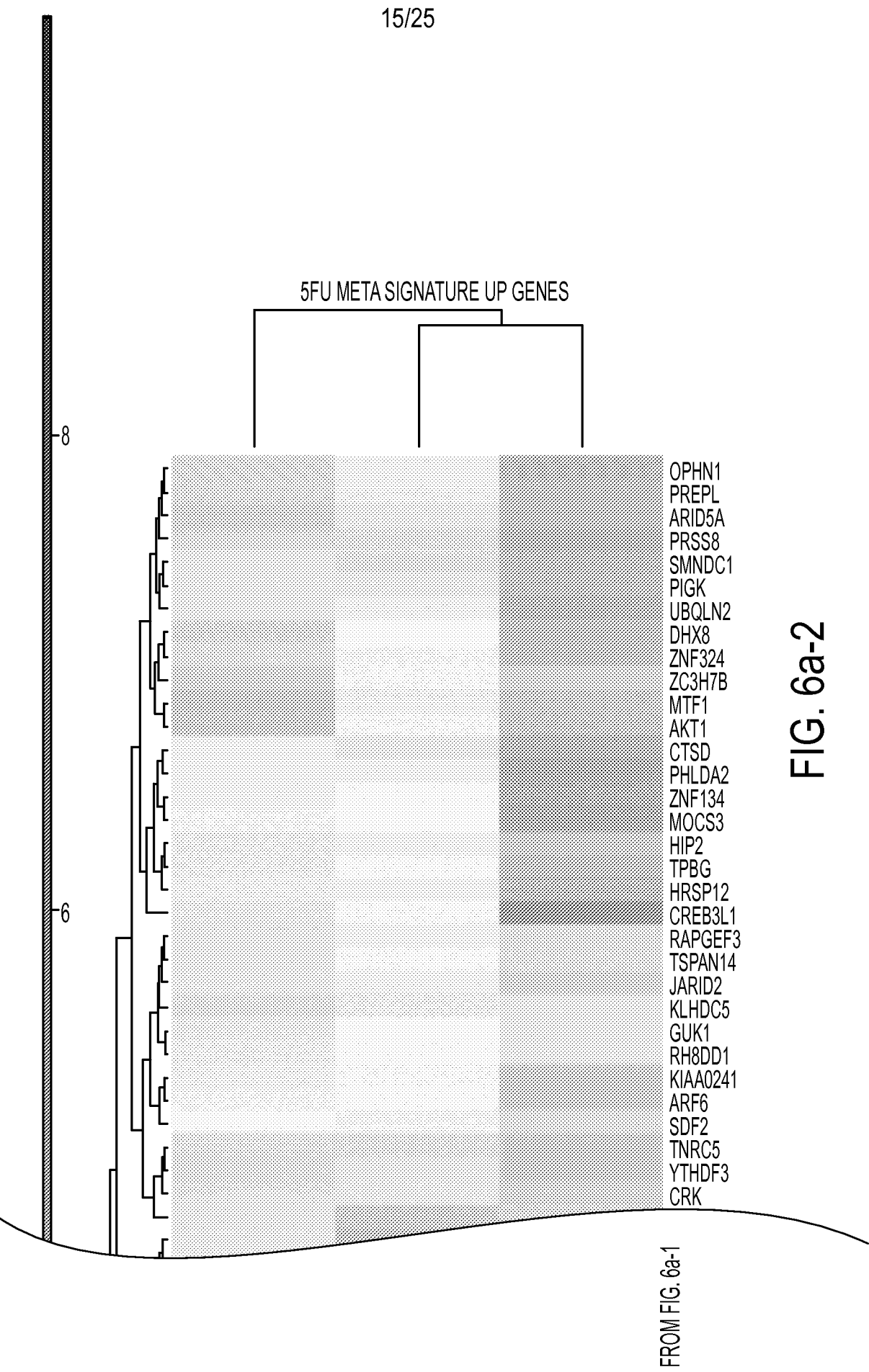
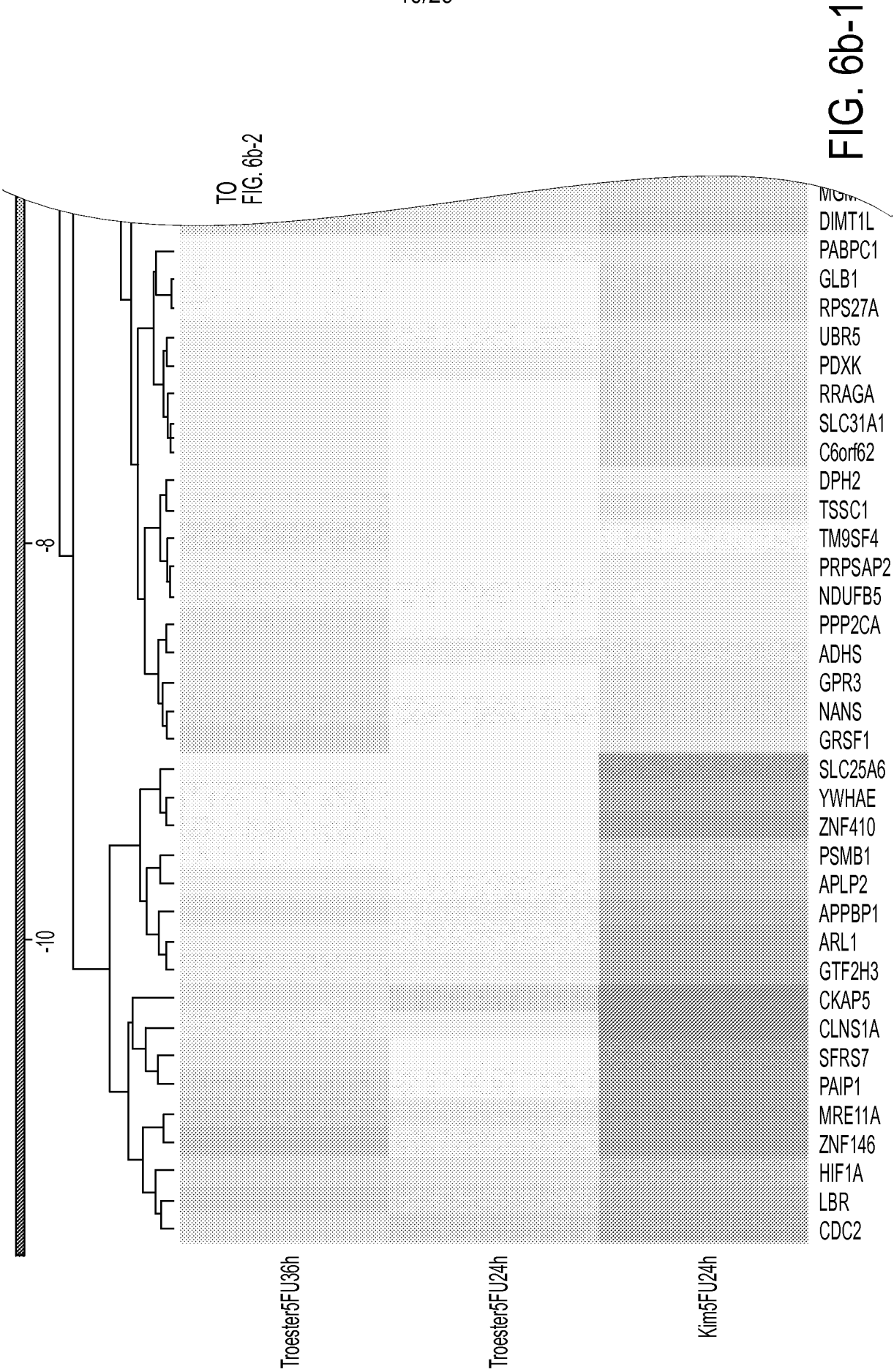


FIG. 6a-1





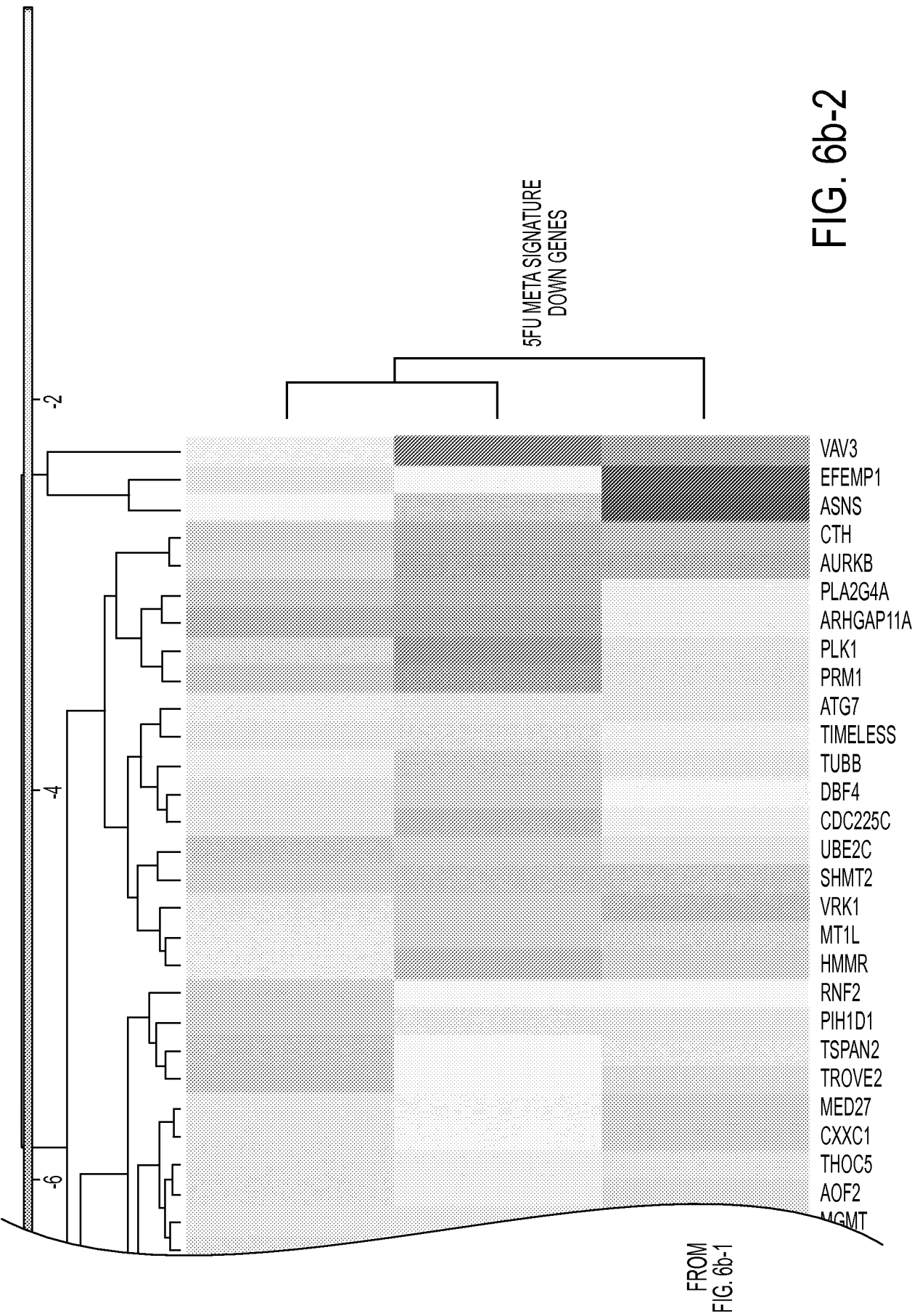


FIG. 6b-2

	RANK 20 BTPC	
ABCE1		
ATP11B		
BARD1		
BLM		
BRCA1		
BUB3		
CDC2		
CDC6		
CHEK1		
DCLRE1A		
DEK		
EED		
EXO1		
FDFT1		
FH		
GART		
GGH		
GPI		
H2AFV		
H2AFX		
HAX1		
HDAC2		
IARS		
IDI1		
IMPA2		
MARS		
MCM3		
MCM6		
MSH2		
MSH6		
MTHFD2		
NUP153		
NUP155		
NUP205		
PDHX		
PIR		
PRIM1		
PTBP1		
RBBP4		
RBL1		
RCN2		
RFC3		
RFC4		
RFC5		
RPA1		
SAE2		
TOP2A		
TOPBP1		
UBE2C		
XPO1		

18/25

FIG. 7a

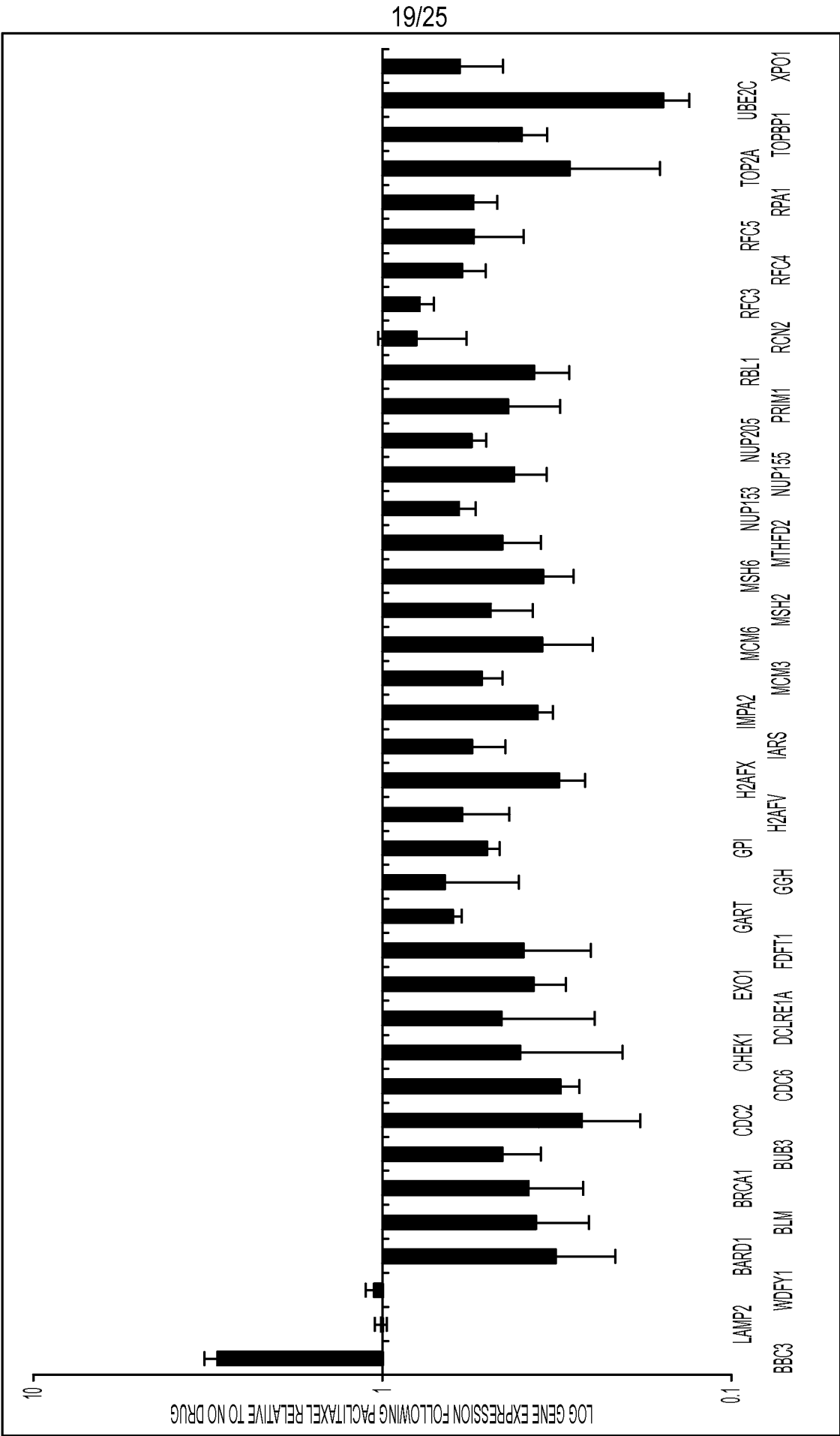


FIG. 7b

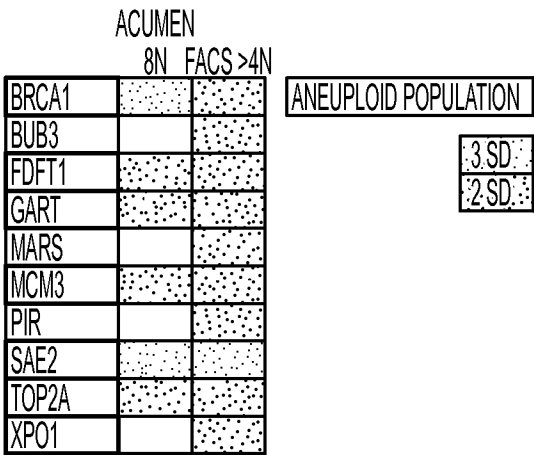


FIG. 8a

21/25

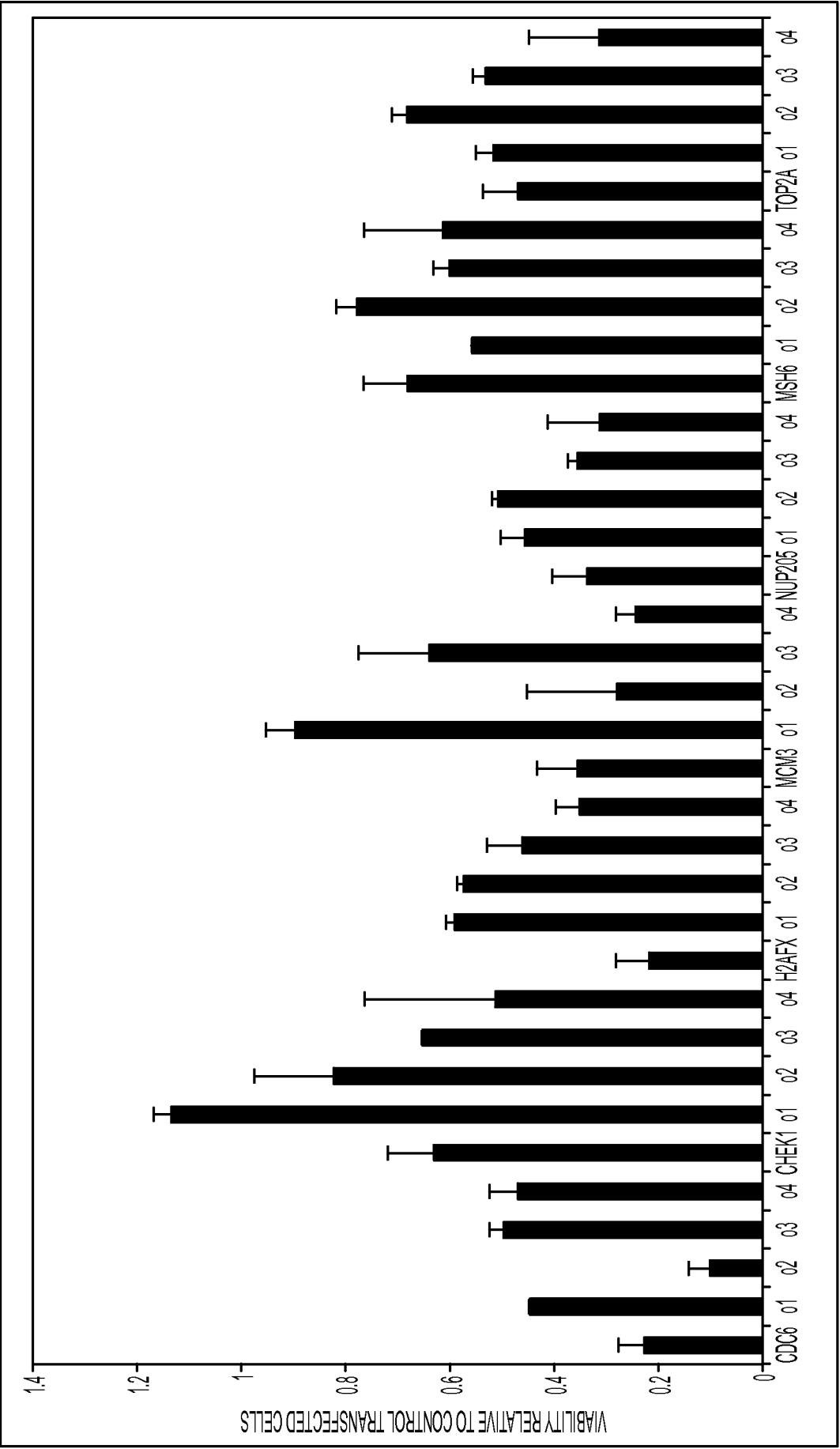


FIG. 8b

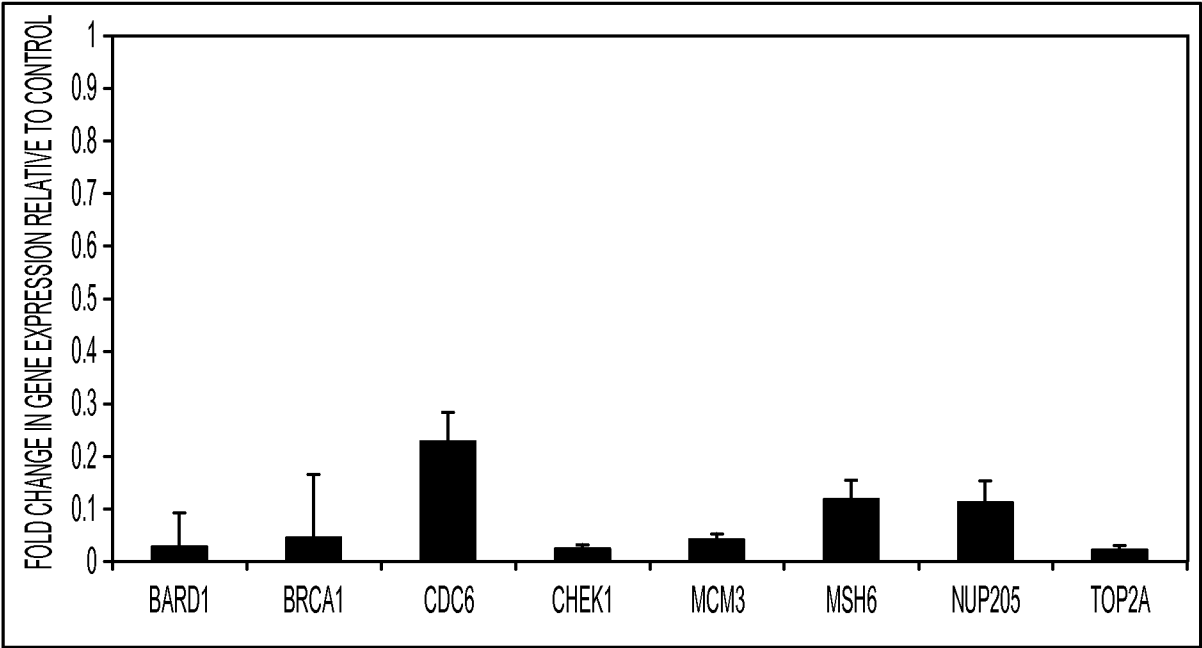


FIG. 8c

23/25

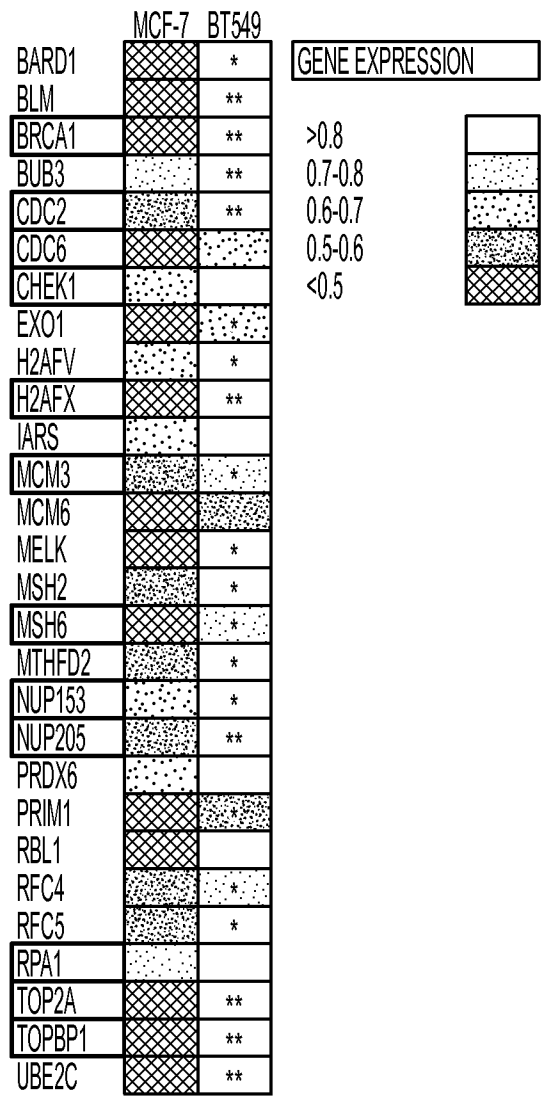


FIG. 9a

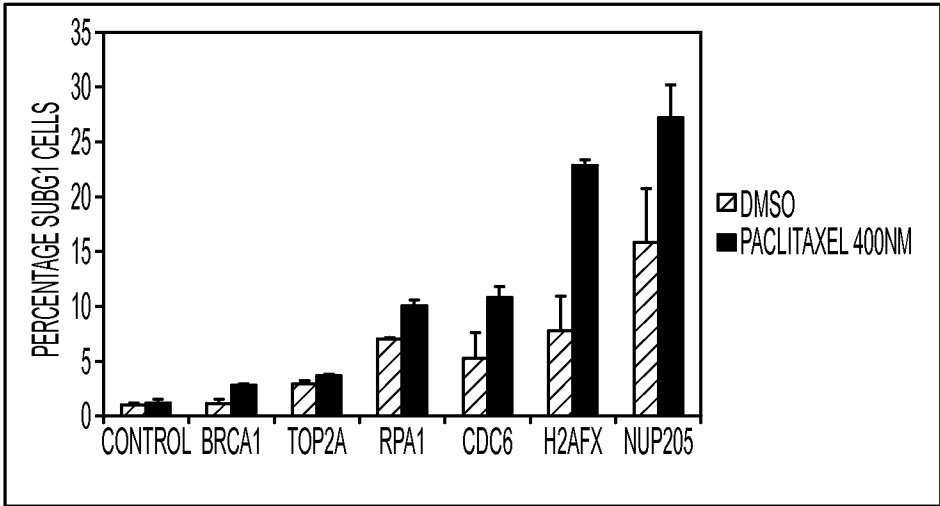


FIG. 9b

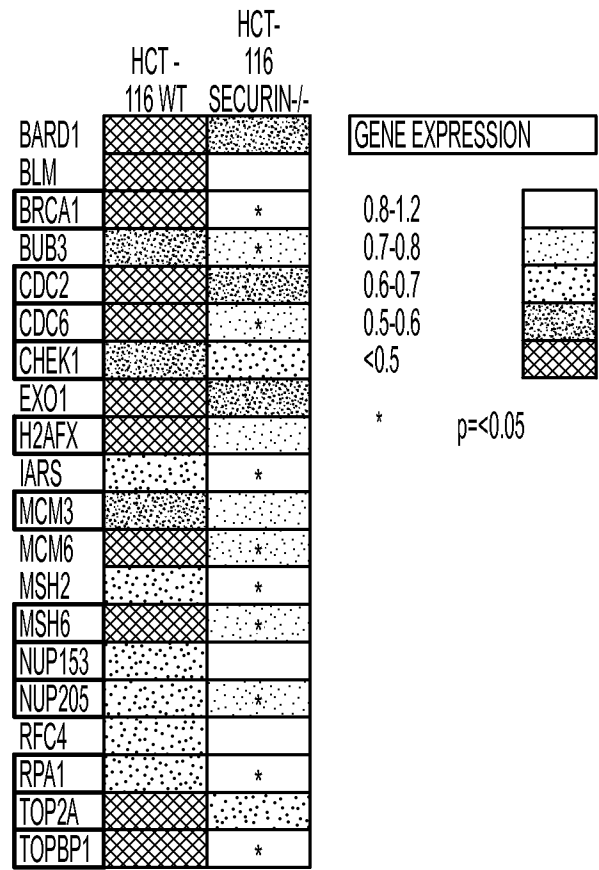


FIG. 9c

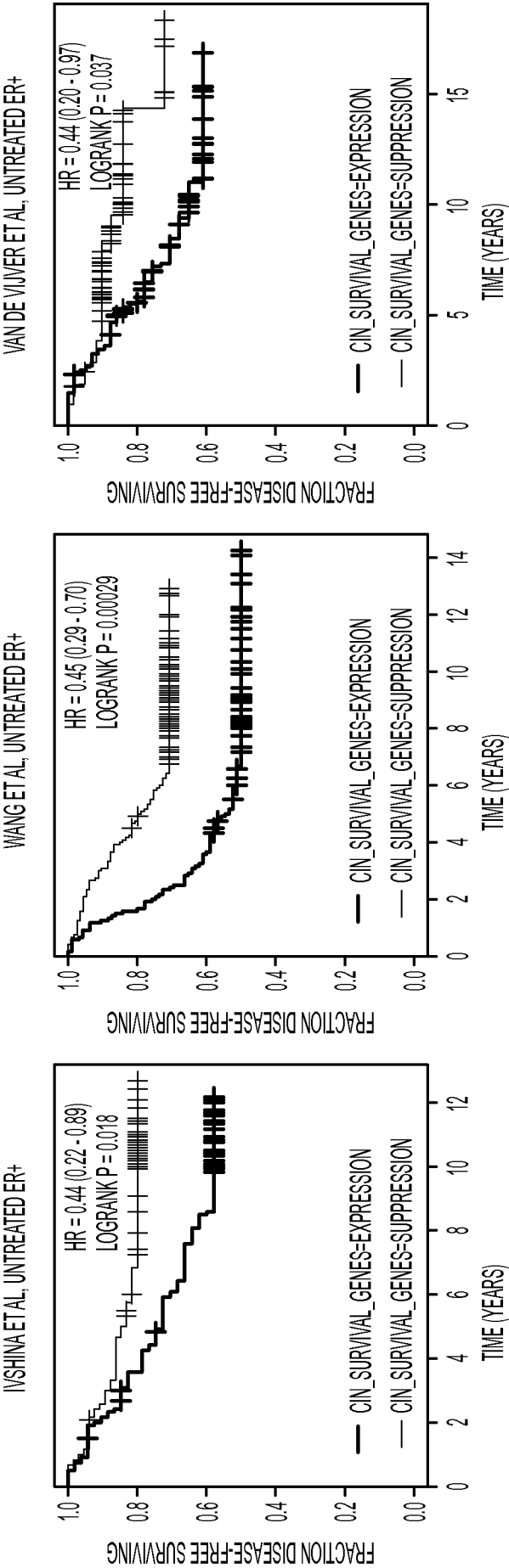


FIG. 9d

INTERNATIONAL SEARCH REPORT

010/033755 US

International application No.

PCT/US 10/33755

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - G01N 33/48 (2010.01)

USPC - 436/94

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)

USPC: 436/94

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

USPC: 436/94 (text search)

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

Electronic data bases searched: PubWEST (PGPB, USPT, EPAB, JPAB); Google Scholar ||| Search terms: cancer, solid tumor (breast, ovarian, lung, colorectal, glioma, prostate) drug resistance, chromosomal instability (CIN tumor), microtubule stabilizing drug, taxane, paclitaxel, docetaxel, gene expression signature, BRCA1, H2AFX, RFC3, CDC2, IMPA2, RPA1

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2007/070621 A2 (CARTER et al.) 21 June 2007 (21.06.2007). Especially abstract, pg 2, pg 5.	1-4
Y	US 2009/0023149 A1 (KNUDSEN). 22 January 2009 (22.01.2009). Especially para [0005], [0021], [0022], [0056].	1-4

☐ 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

30 August 2010 (30.08.2010)

Date of mailing of the international search report

08 SEP 2010

Name and mailing address of the ISA/US

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

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 5-13 and 19-21
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I: claims 1-4, directed to a method of predicting clinical outcome of treating a patient having a tumor using one or more microtubule stabilizing drugs, comprising measuring the mRNA expression of a panel of genes in a tumor sample.

Group II: claims 14-18, 22 and 23, directed to a method of selecting a treatment for a patient having a tumor comprising measuring the mRNA levels of a panel of genes in a tumor cell from the patient, wherein the panel is used to determine if the patient is a responder to carboplatin treatment.

- Please see extra sheet for continuation -

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-4

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

Continuation of Box III: Unity of Invention

The inventions listed as Groups I - II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The special technical feature of the Group I claims is a method of predicting clinical outcome of treating a patient having a tumor using one or more microtubule stabilizing drugs, comprising measuring the mRNA expression of a panel of genes in a tumor sample. The special technical feature of the Group II claims is a method of selecting a treatment for a patient having a tumor comprising measuring the mRNA levels of a panel of genes in a tumor cell from the patient, wherein the panel is used to determine if the patient is a responder to carboplatin treatment.

The common technical elements shared by the above groups do not represent an improvement over the prior art of US 2007/0010469 A1 to Chan et al. (hereinafter "Chan"). In particular, the common technical elements of Groups I and II are: determining responsiveness to treatment with an agent (The polynucleotides described herein, as well as their gene products and corresponding genes and gene products, are of particular interest as genetic or biochemical markers (e.g., in blood or tissues) that will detect the earliest changes along the carcinogenesis pathway and/or to monitor the efficacy of various therapies and preventive interventions; para [0192]) using a set of mRNA markers (polynucleotide libraries of the subject invention generally comprise sequence information of a plurality of polynucleotide sequences; para [0150] - The terms "polynucleotide" and "nucleic acid"...include, but are not limited to, mRNA; para [0050]) wherein elevation of the levels of the marker (where the differentially expressed gene is increased in cancerous cells; para [0172]) by a statistically significant amount (statistical analysis of the fluorescent intensities was applied to each set of duplicate spots to assess the precision and significance of each differential measurement; para [0342]) in tumor cells (differential in the expression level between the tumor and normal samples of each patient; para [0342]) is considered to enable the prediction of potential responsiveness to a therapeutic agent or a target (provide additional evidence of the gene product's use as a therapeutic target, and provide further guidance as to the types of agents that can modulate its activity; para [0362]). The Groups share a further technical element of having some markers in common in the extensive gene panels, but only two (cdc2 and Top2A) are in the minimum claimed gene sets of both Groups (Chan, Table 9 (CDC28, TOP2A); wherein CDC28 was a known alias of cdc2). Therefore, the inventions of Group I and Group II lack unity of invention under PCT Rule 13 because they do not share a same or corresponding special technical feature.