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(54) Title: METHODS OF IDENTIFYING ANTINEOPLASTIC AGENTS

(57) Abstract: The expression of particular genes is altered in response to treatment of cancer cells with a DNA damaging agent and a Chk1 inhibitor. Expression of such genes, termed "surrogate marker genes," can be used to identify test compounds as potential antineoplastic agents, to assess the potential *in vivo* efficacy of such agents, and to monitor clinical efficacy of treatment with Chk1 inhibitors.



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METHODS OF IDENTIFYING ANTINEOPLASTIC AGENTS

FIELD OF THE INVENTION

- [01] The invention relates to identification of antineoplastic agents. More particularly, the invention relates to identification of Chk1 inhibitors.

BACKGROUND OF THE INVENTION

- [02] Control of the cell cycle is fundamental to the growth and maintenance of eukaryotic organisms. Eukaryotic cells have evolved control pathways, termed "checkpoints," which ensure that individual steps of the cell cycle are completed before the next step occurs. In response to DNA damage, cell survival is increased both by direct DNA repair mechanisms and by delaying progression through the cell cycle. Depending on the position of the cell within the cycle at the time of irradiation, DNA damage in mammalian cells can prevent passage from G1 into S phase, progression through S phase, or passage from G2 into mitosis. Such checkpoints are thought to prevent deleterious events, such as replication of damaged DNA and the segregation of fragmented chromosomes during mitosis. Hartwell & Kastan, *Science* 266, 1821-28, 1994.
- [03] Mammalian cells treated with agents that inhibit DNA replication or cause DNA damage undergo cell cycle arrest due to the presence of multiple checkpoint response mechanisms. The regulation of cell cycle kinases, and in particular checkpoint kinase, has important implications for the control of proliferative diseases such as cancer. For example, in response to DNA damage in mammalian cells, the tumor suppressor p53 acts in a checkpoint pathway for cell cycle control by inducing the transcription of a cyclin-dependent kinase inhibitor resulting in G1/S arrest.
- [04] Cells deficient in p53 with damaged DNA, however, are unable to arrest in G1/S, a condition that can lead to cancer. It has been estimated that p53 may be non-functional in at least 60% of human cancers. Greenblatt *et al.*, *Canc. Res.* 54, 4855-

78, 1994. Cells lacking both p53 and Chk1 checkpoint controls will re-enter the cell cycle without repairing DNA damage, and ultimately undergo apoptosis.

- [05] Cells in which Chk1 is inhibited become hypersensitive to DNA damaging agents because the cell cycle will not be arrested upon damage to the DNA. Thus, Chk1 inhibitors can be useful as potentiators of DNA damaging agents for treating tumors. For example, the Chk1 kinase inhibitor UCN-01 potentiates camptothecin-induced cytotoxicity. Graves *et al.*, *J. Biol. Chem.* 275, 5600-05, 2000. There is a need in the art for effective model systems in which such agents can be identified and their potential efficacy assessed.

BRIEF DESCRIPTION OF THE FIGURES

- [06] FIG. 1. FACS-based cell cycle analysis following treatment time of microarray experiment (18 hours of CPT treatment and/or 6 hours of treatment with CCR+).
- [07] FIG. 2. Graphs showing differential expression patterns determined using quantitative RT-PCR. FIG. 2A, EGR1, DDIT3. FIG. 2B, CCNE1, CCNE2. FIG. 2C, CCNE2. FIG. 2D, DDIT3, EGR1.
- [08] FIG. 3. Graphs showing dose-dependent effects of Chk1 inhibitors on surrogate marker gene expression. FIG. 3A, cyclin E2. FIG. 3B, EGR1. FIG. 3C, DDIT3.
- [09] FIG. 4. Graphs showing dose-dependent effects of Chk1 inhibitors on surrogate marker gene expression. FIG. 4A, CCNE2. FIG. 4B, EGR1. FIG. 4C, DDIT3.
- [10] FIG. 5. Graph showing cell cycle release fit using CCNE2, EGR1, and DDIT3.

DETAILED DESCRIPTION OF THE INVENTION

- [11] We have found that expression of particular genes is altered in response to treatment of cancer cells with a DNA damaging agent and a Chk1 inhibitor. Changes in expression of these genes, termed "surrogate marker genes," can be used to identify test compounds as potential antineoplastic agents and to assess the potential *in vivo* efficacy of such agents.

- [12] Surrogate marker genes of the invention are differentially expressed in response to the combination of a DNA damaging agent and a test compound that inhibits Chk1. Expression of some surrogate marker genes is increased, whereas expression of others is decreased. Expression of surrogate marker genes of the invention preferably either increases or decreases by at least 1.3-fold in response to the combination of a DNA damaging agent and a test compound. Particularly useful surrogate marker genes are those identified in Tables 2, 3, and 6 for which the “wtd mean ratio” is in bold. Genes whose expression increases in response to a potential antineoplastic agent are those for which the “wtd mean ratio” is greater than 1. Genes whose expression decreases in response to a potential antineoplastic agent are those for which the “wtd mean ratio” is less than one. An especially preferred set of surrogate marker genes includes cyclin E2 (CCNE2), cyclin E1 (CCNE1), DDIT3, and EGR1.
- [13] According to the invention, test compounds can be screened for potential use as antineoplastic agents by assessing their ability to affect expression of at least one surrogate marker gene in a cancer cell treated with a DNA damaging agent. If desired, expression of at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 50, 75, or more surrogate marker genes can be determined.
- [14] Surrogate marker genes can be selected from the group of surrogate marker genes identified in Tables 2, 3, and 6. Methods of the invention involve measuring expression of at least one of the surrogate marker genes identified in Tables 2, 3, and 6 after treatment of a cell with a DNA damaging agent and a test compound. The methods can be carried out either qualitatively or quantitatively and either *in vitro* or *in vivo*.
- [15] Test compounds can be pharmacologic agents already known in the art or can be compounds previously unknown to have any pharmacological activity, including small molecules from compound libraries. Test substances can be naturally occurring or designed in the laboratory. They can be isolated from microorganisms, animals, or plants, or can be produced recombinantly or synthesized by chemical methods known in the art. The function of the test compound can be unknown. Alternatively, the test compound can be a known or suspected Chk1 inhibitor.

- [16] A DNA damaging agent is any chemical compound or treatment method that induces DNA damage when applied to a cell. DNA damaging agents include, but are not limited to, chemosensitizing agents and ionizing irradiation. Any DNA damaging agent can be used in assay methods of the invention.
- [17] Chemosensitizing agents include topoisomerase inhibitors (*e.g.*, camptothecin, daunorubicin or daunomycin, doxorubicin, idarubicin, irinotecan, mitoxantrone, teniposide (VM-26), topotecan), alkylating agents (*e.g.*, mitomycin C, chlorambucil, cyclophosphamide, thiotepa, busulfan), and methylating agents (*e.g.*, methylmethane sulfonate (MMS), temozolomide). Camptothecin is conveniently used.
- [18] Ionizing irradiation that can be used includes, but is not limited to, X-irradiation, gamma-irradiation, UV irradiation, microwaves, electronic emissions, and the like.
- [19] The cancer cell can be a cell of a primary or metastatic tumor, such as a tumor of the colon, breast, lung, prostate, brain, or kidney, or a melanoma, which is isolated from a patient. Alternatively, a cancer cell line, such as DLD-1 (colon), PC-3 (prostate), DU-145 (prostate), H1299 (non-small cell lung), T47D (breast), MDA-MB-435 (breast), or SKOV3 (ovarian), can be used. The cancer cell can be *in vitro* (*i.e.*, in a tissue culture system, including monolayer, aggregate, and explant systems) or *in vivo*, in an experimental animal model, such as a tumor-bearing mouse or rat. In such experimental animal models, the tumor can be naturally occurring or can be implanted, as is known in the art.
- [20] Cancer cells can be contacted with the DNA damaging agent and the test compound simultaneously or sequentially in either order. A chemotherapeutic DNA damaging agent and a test compound can be in separate or in unitary compositions. The amount of DNA damaging agent used should be sufficient to damage the cell's DNA. Those of skill in the art are familiar with numerous DNA damaging agents and can easily identify useful amounts of any particular DNA damaging agent for use either *in vivo* or *in vitro*, as well as routes of administration. Several different concentrations of a test compound can be administered in an assay as part of the evaluation of each test compound.

- [21] Either RNA or protein products can be detected to determine expression of surrogate marker genes, and can be obtained from the contacted cancer cells using well-known isolation methods. If the cancer cells are *in vitro*, the cells can simply be removed from the culture vessel. If the cancer cells are in a tumor in an experimental animal model, a biopsy sample of the tumor can be obtained. Alternatively, surrogate marker gene expression can be measured in blood cells, *e.g.*, peripheral blood mononuclear cells (PBMCs).
- [22] Detecting RNA products of gene expression can be carried out using a variety of methods well known in the art, including, but not limited to, RNA dot blots, slot blots, Northern blots, RT-PCR (including TaqMan RT-PCR), SAGE (serial analysis of gene expression), and electrochemical detection of nucleic acid hybridization (*e.g.*, U.S. Patent 6,361,951). *See also* US 20030096782; Liang & Pardee, *Science* 257, 967, 1992; Higuchi *et al.*, *BioTechnology* 10, 413-17, 1992, and Higuchi *et al.*, *BioTechnology* 11, 1026-30, 1993; Holland *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 88, 7276-80, 1991; Heid *et al.*, *Genome Res.* 6, 986-94, 1996; and Gibson *et al.*, *Genome Res.* 6, 995-1001, 1996. Alternatively, RNA products of expressed surrogate marker genes can be identified by contacting RNA with oligonucleotide probes unique to each of the surrogate marker genes whose expression is to be assayed, either in solution or immobilized on a solid support, such as a membrane or a gene expression "chip." As is well known, an array of oligonucleotide probes can be immobilized on a solid support so that expression of multiple surrogate marker genes can be detected in one assay. Such chips can be purchased commercially (*e.g.*, from Affymetrix or Motorola) or can be custom made.
- [23] Protein products of the disclosed surrogate marker genes can be detected using a variety of techniques known to the art, including immunochemical methods such as radioimmunoassay, Western blotting, and immunohistochemistry. Alternatively, protein synthesis can be determined *in vivo*, in a cell culture, or in an *in vitro* translation system by detecting incorporation of labeled amino acids into protein products.

- [24] As demonstrated in Example 8, EC_{50} values calculated for Chk1 inhibitors correlate well with the inhibitors' IC_{50} . Thus, calculation of an EC_{50} value for a test compound's effect on expression of one or more surrogate marker genes can be used to estimate the potency of the test compound as a Chk1 inhibitor. Such determinations are well suited to be carried out in a medium- to high-throughput format, for example using RT-PCR as is known in the art.
- [25] Determination of expression of one or more surrogate marker genes also can be used to monitor clinical efficacy of treatment with a particular Chk1 inhibitor or a particular dose of a Chk1 inhibitor over time. Biological samples that can be tested to determine gene expression can be, for example, biopsies of the tumor being treated.
- [26] All patents, patent applications, and references cited in this disclosure are expressly incorporated herein by reference. The above disclosure generally describes the present invention. A more complete understanding can be obtained by reference to the following specific examples, which are provided for purposes of illustration only and are not intended to limit the scope of the invention.

EXAMPLE 1

Demonstration of differential gene expression in response to camptothecin

- [27] MDA-MB-435 breast carcinoma cells were pre-incubated with or without 50 nM camptothecin (CPT). Following pre-incubation and treatment, the cells were lysed, total RNA collected, and the poly-A⁺ mRNA fraction purified. The global transcription response to the various culture conditions was assessed by competitive hybridization to a cDNA microarray chip. The "Spot IDs" correspond to publicly available cDNA sequences for the region of the gene represented at that spot on the microarray chip. A subset of data obtained from this experiment is shown in Table 1.
- [28] For hybridizations in this and in the subsequent examples, there are two columns – the weighted mean ratio (wtd mean ratio) and the minimum fold change (min fold change). The minimum fold change is the lower (towards a ratio of one), one-sided 95% confidence limit on the ratio. That is, it represents the minimum change we can

believe with 95% confidence. It can be estimated from the log ratio and standard deviation, assuming that the uncertainty in the ratio is normally distributed. Note that the minimum fold change can never be less than one (hence the minimum function). *See also* Cheng Li and Wing Hung Wong, "Model-based analysis of oligonucleotide arrays: model validation, design issues and standard error application," *Genome Biology* 2001 2(8):research0032.1-0032.11 (published 3 August 2001).

- [29] For data analysis, all of the significance threshold criteria were set for the "min fold change" column, which ensured that the resulting genes would be differentially expressed by greater than the threshold value with 95% confidence (p value < 0.05). The lowest significance threshold used was 1.3-fold. The fold change threshold is an absolute number, and the direction of change (increased or decreased expression) is determined by the looking at the weighted mean ratio. The significance threshold criteria are included in parentheses in each "min fold change" column. The weighted mean ratios representing significant differentials (95% confidence, p value < 0.05) are in bold.
- [30] Table 1 also includes data from an initial Chk1 microarray experiment (Study #1). This experiment was a single replicate, so the algorithm to generate a weighted mean and min fold change could not be used. Because statistical significance could not be assigned by the min fold change calculation, the lowest threshold used as a significance cut-off was 1.5-fold differential expression. The differentials that meet this threshold are in bold.
- [31] CPT treatment resulted in changes in gene expression consistent with its known mechanism of action (MOA). Differential gene expression was observed for genes in the following categories: genes involved in regulating apoptosis, genes involved in the DNA damage repair response, and cell cycle genes.
- [32] There was significant overlap between the observed CPT-dependent differential gene response in Study #1 and the observed response in Study #2. Eighty-two percent of genes with a min fold change $\geq 1.5x$ in Study #2 (55 genes) also were differentially expressed in the Study #1 in response to CPT alone.

Table 1 –Differential Gene Expression in Response to Camptothecin – Mechanism Related Functional Groups**Genes up- or down-regulated in response to camptothecin treatment in BOTH experiments (>1.5x in Study #1, >1.3x in Study #2)**

SpotID	GeneSymbol	GeneName	GeneDesc	Study #1 (+/-) CPT (>1.5x)	Study #2 (+/-) CPT	min fold change (>1.3 x)
APOPTOSIS						
50503	BCL1		B-cell CLL/lymphoma 1	1.5	1.5	1.42
49132	CASP1	caspase 1, ICE	caspase 1, apoptosis-related cysteine protease	2	1.4	1.26
48267	CASP1	caspase 1, ICE	caspase 1, apoptosis-related cysteine protease	1.4	1.7	1.44
50721	CASP4	CASP4	caspase 4, apoptosis-related cysteine protease	1.8	1.4	1.27
49807	CASP4	CASP4	caspase 4, apoptosis-related cysteine protease	1.4	1.4	1.32
49295	FAF1	CGI-03	Fas (TNFRSF6) associated factor 1	0.4	0.5	1.75
48673	LRDD	LRDD	leucine rich repeat and death domain containing pr	3	2	1.45
47924	PIK3CB	p110 (PI3K)	phosphoinositide-3-kinase, catalytic, beta polypep	0.5	0.7	1.37
48988	PIK3CB	p110 (PI3K)	phosphoinositide-3-kinase, catalytic, beta polypep	0.5	0.7	1.36
DNA damage & repair						
50633	ABL2			2.2	1.6	1.46
50132	DDIT3	DNA-damage-inducible transcript 3		5.4	2.6	2.55
49433	DDIT3	DNA-damage-inducible transcript 3		4.3	2.8	2.56
48615	GADD45B	MyD118	growth arrest and DNA-damage-inducible, beta	1.9	1.8	1.49
30128	PCNA		proliferating cell nuclear antigen	1.6	N/A	
49707	PCNA		proliferating cell nuclear antigen	1.2	1.5	1.46

[illegible]

EXAMPLE 2

Effect of compound A (supra-additive, cell cycle release) ("CCR⁺")

- [33] MDA-MB-435 breast carcinoma cells were pre-incubated with or without 50nM CPT and then treated with or without 30nM Compound A. Compound A inhibits Chk1 enzyme activity (ability to phosphorylate cdc25c) in a cell free assay (CFA) with an IC₅₀ of 0.3nM. Compound A inhibits proliferation of MDA-MB-435 cells using a MTS-based proliferation assay with an EC₅₀ of 80nM.
- [34] Following pre-incubation and treatment, the cells were lysed, total RNA collected, and the poly-A⁺ mRNA fraction purified. The global transcription response to the various culture conditions was assessed by competitive hybridization to probes on a cDNA microarray chip.
- [35] In the absence of DNA damaging agents, compound A altered expression of nine genes with a min fold change ≥ 1.3 . Only one gene had a min fold change ≥ 1.5 (1.52x reduction in expression of an unidentified spot). This minimal effect is consistent with the anticipated result, because a Chk1 inhibitor is not expected to have a significant effect in the absence of DNA damage.
- [36] Eighty-nine genes were differentially expressed (≥ 1.3 x min fold change) in response to compound A treatment in the presence of CPT (differentially expressed in comparison to CPT alone) (Table 2).
- [37] As shown in Table 3, CPT + compound A treatment changed the expression of a number of cell cycle-related and DNA damage repair-related genes, compared to CPT treatment alone. In Study #1, cells were treated with compound A for 4 hours (following 18 hours of CPT treatment). In Study #2, cells were treated with Chk1 inhibitors for 6 hours following CPT treatment. The overall response to CPT + compound A was similar between the two studies. Sixty-four percent of genes differentially expressed with > 1.5 min fold change in Study #2 (6hr) also were differentially expressed in Study #1 (4hr). The 6-hour Chk1 inhibitor treatment resulted in an increase in the number of differentially expressed cell cycle-related

genes, compared to the 4 hour Chk1 inhibitor treatment. This difference likely is related directly to the increased treatment time with the Chk1 compound. Of particular note, in both studies (#1 and #2), compound A treatment reversed the CPT-dependent effect on the expression of cyclin E1, E2, CDKN3, and CKS2.

- [38] Table 3 also includes data from an initial Chk1 microarray experiment (Study #1). As noted in Example 1, above, this experiment was a single replicate, so the algorithm to generate a weighted mean and min fold change could not be used. Because statistical significance could not be assigned by the min fold change calculation, the lowest threshold used as a significance cut-off was 1.5-fold differential expression. The differentials that meet this threshold are in bold.

Table 2 - Genes differentially expressed in response to CPT alone and CPT + Compound A

Spot ID	GeneSymbol	GeneName	GeneAssignment		CPT alone		CPT+compound A	
					$\pm$ CPT	$\pm$ CPT	$\pm$ CPT	$\pm$ CPT
					wtd. Means	min fold change (>1.3x)	compound A wtd means	compound A min fold change (>1.3x)
48739					4.54	3.58	1.86	1.67
49056					0.87	1.10	1.59	1.56
50443					0.90	1.08	1.50	1.44
48887					1.09	1.02	1.47	1.38
47778					1.56	1.29	1.45	1.33
50574				semaphorin Y	1.09	1.00	1.41	1.32
47196					1.52	1.36	1.14	1.13
50024					0.67	1.41	1.18	1.10
48604					2.05	1.94	1.14	1.09
50380					1.61	1.50	1.07	1.02
46905					0.72	1.32	1.13	1.00
46767					2.79	1.99	0.72	1.00
48252					0.50	1.79	0.97	1.00
48060					2.03	1.72	1.07	1.00
48899					0.55	1.47	1.31	1.00
49251					0.58	1.41	1.01	1.00

50032						1.54	1.39	1.12	1.00
48812						1.60	1.37	0.92	1.00
50633	ABL2				v-abl Abelson murine leukemia viral oncogene homolog 2 (arg, Abelson-related gene)	1.62	1.46	2.06	1.70
48849	ACVRL1				activin A receptor type II-like 1	0.63	1.47	0.88	1.08
47896	ADAM11	ADAM11			a disintegrin and metalloproteinase domain 11	1.53	1.41	1.02	1.00
47352	ADAM11	ADAM11			a disintegrin and metalloproteinase domain 11	1.45	1.34	1.00	1.00
47913	ADAMTS1	ADAMTS1			a disintegrin-like and metalloprotease (reprolysin type) with thrombospondin type 1 motif, 1	1.49	1.31	1.76	1.24
47060	AF1Q				ALL 1-fused gene from chromosome 1q	1.97	1.90	0.82	1.17
49339	ANXA3	annexin III			annexin A3	1.76	1.54	1.05	1.03
49524	AP1G2				adaptor-related protein complex 1, gamma 2 subunit	1.54	1.31	1.01	1.00
49887	APOBEC1				apolipoprotein B mRNA editing enzyme, catalytic polypeptide 1	2.01	1.64	0.99	1.00
47645	ARF4L				ADP-ribosylation factor 4-like	1.71	1.57	0.97	1.00
47535	ARHGDIA	ARHGDIA			Rho GDP dissociation inhibitor (GDI) alpha	0.74	1.32	1.05	1.01
46843	ARHGDIA	ARHGDIA			Rho GDP dissociation inhibitor (GDI) alpha	0.73	1.31	1.04	1.00
48346	ARHGEF2	ARHGEF2			rho/rac guanine nucleotide exchange factor (GEF) 2	1.57	1.33	1.65	1.52
48277	ARHGEF2	ARHGEF2			rho/rac guanine nucleotide exchange factor (GEF) 2	1.67	1.48	1.62	1.47
48516	ARL6IP				ADP-ribosylation factor-like 6 interacting protein	0.46	2.01	3.14	2.86
46817	ATF3	ATF3			activating transcription factor 3	2.72	2.52	2.46	2.35
50523	ATF4	ATF4			activating transcription factor 4 (tax-responsive enhancer element B67)	1.21	1.16	1.68	1.64
46662	ATF4	ATF4			activating transcription factor 4 (tax-responsive enhancer element B67)	1.30	1.27	1.69	1.56
49244	ATP7A				ATPase, Cu++ transporting, alpha polypeptide (Menkes syndrome)	0.38	2.23	0.96	1.00
49350	BCHE				butyrylcholinesterase	0.68	1.42	0.86	1.12
47979	BDKRB1	BDKRB1			bradykinin receptor B1	1.00	1.00	0.51	1.39
47180	BDKRB2	BDKRB2			bradykinin receptor B2	1.55	1.40	1.09	1.00

47497	BIRC3	cIAP2	baculoviral IAP repeat-containing 3	1.07	1.00	1.56	1.37
49889	BLVRB		biliverdin reductase B (flavin reductase (NADPH))	1.65	1.46	0.90	1.00
46916	BNIP3	BNIP3	BCL2/adenovirus E1B 19kD-interacting protein 3	0.60	1.63	1.00	1.00
47081	BRCA1		breast cancer 1, early onset	1.37	1.30	0.80	1.19
47516	BTG1		B-cell translocation gene 1, anti-proliferative	1.75	1.67	1.73	1.72
49673	BTN3A2	BTN3A2	butyrophilin, subfamily 3, member A2	0.90	1.02	0.68	1.38
48267	CASP1	caspase 1, ICE	caspase 1, apoptosis-related cysteine protease (interleukin 1, beta, convertase)	1.67	1.44	1.10	1.00
49807	CASP4	CASP4	caspase 4, apoptosis-related cysteine protease	1.36	1.32	1.11	1.00
47917	CASP8AP2	CED4, FLASH	CASP8 associated protein 2	1.47	1.24	0.68	1.34
50503	CCND1		B-cell CLL/lymphoma 1	1.46	1.42	0.93	1.00
48015	CCND3	Cyclin D3	cyclin D3	1.14	1.00	0.69	1.36
46608	CCND3	Cyclin D3	cyclin D3	1.27	1.20	0.69	1.35
49850	CCNE1	cyclin E1	cyclin E1	1.78	1.68	0.43	1.98
50521	CCNE1	cyclin E1	cyclin E1	1.61	1.55	0.53	1.37
47926	CCNE2	cyclin E2	cyclin E2	2.41	2.34	0.19	4.86
49967	CCNE2	cyclin E2	cyclin E2	2.33	2.21	0.27	3.26
47899	CCR5	CCR5	chemokine (C-C motif) receptor 5	0.71	1.34	1.17	1.13
47424	CD36L1		CD36 antigen (collagen type I receptor, thrombospondin receptor)-like 1	0.53	1.69	0.94	1.00
50229	CDC2		cell division cycle 2, G1 to S and G2 to M	0.90	1.04	1.53	1.38
50358	CDK7	CDK7	cyclin-dependent kinase 7 (homolog of Xenopus MO15 cdk-activating kinase)	1.02	1.00	1.50	1.47
47382	CDK7	CDK7	cyclin-dependent kinase 7 (homolog of Xenopus MO15 cdk-activating kinase)	1.07	1.03	1.49	1.44
48631	CDKN1A	Waf1, Cip1	cyclin-dependent kinase inhibitor 1A (p21, Cip1)	1.52	1.39	1.38	1.37
49066	CDKN1A	Waf1, Cip1	cyclin-dependent kinase inhibitor 1A (p21, Cip1)	4.65	4.38	1.39	1.18
46842	CDKN3	cyclin-dependent kinase inhibitor 3	cyclin-dependent kinase inhibitor 3 (CDK2-associated dual specificity phosphatase)	0.67	1.35	2.18	1.89

49105	CDKN3	cyclin dependent kinase inhibitor 3	cyclin-dependent kinase inhibitor 3 (CDK2-associated dual specificity phosphatase)	0.68	1.41	1.90	1.70
47175	CED-6	CED-6	CED-6 protein	0.66	1.40	0.94	1.00
48313	CENPA		centromere protein A (17kD)	0.64	1.49	2.14	2.08
49737	CHAF1B		chromatin assembly factor 1, subunit B (p60)	1.57	1.44	0.61	1.46
50590	CKS2		CDC28 protein kinase 2	0.44	2.10	2.94	2.49
47829	CLCN5		chloride channel 5 (nephrolithiasis 2, X-linked, Dent disease)	0.57	1.46	0.88	1.07
49647	COL8A1	COL8A1	collagen, type VIII, alpha 1	0.66	1.34	1.01	1.00
47222	CREB3	CREB-3	cAMP responsive element binding protein 3 (lumen)	1.43	1.39	0.88	1.07
49316	CREB3	CREB-3	cAMP responsive element binding protein 3 (lumen)	1.54	1.43	0.84	1.04
47545	CREBBP	CREB binding protein	CREB binding protein (Rubinstein-Taybi syndrome)	0.86	1.06	1.41	1.33
48924	CSE1L		chromosome segregation 1 (yeast homolog)-like	1.35	1.10	1.43	1.36
48263	CSPG2	CSPG2	chondroitin sulfate proteoglycan 2 (versican)	0.50	1.87	0.91	1.02
50530	CSPG2	CSPG2	chondroitin sulfate proteoglycan 2 (versican)	0.68	1.34	0.97	1.00
49060	CTGF	CTGF	connective tissue growth factor	1.70	1.56	1.12	1.02
47837	CTSG		cathepsin G	2.15	1.65	1.00	1.00
47991	CYC1	cytochrome c-1	cytochrome c-1	0.68	1.42	0.91	1.02
47834	CYP1A1		cytochrome P450, subfamily I (aromatic compound-inducible), polypeptide 1	1.54	1.44	0.87	1.06
50237	CYR61		cysteine-rich, angiogenic inducer, 61	2.23	2.10	1.25	1.18
49433	DDIT3	CHOP	DNA-damage-inducible transcript 3	2.85	2.56	2.74	2.68
50132	DDIT3	CHOP	DNA-damage-inducible transcript 3	2.63	2.55	2.75	2.60
50563	DDX10		DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 10 (RNA helicase)	0.65	1.43	1.18	1.03
48122	DDX15		DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 15	0.65	1.46	0.99	1.00
47559	DGCR6	DGCR6	DiGeorge syndrome critical region gene 6	1.78	1.60	0.75	1.16
50399	DGCR6L		DiGeorge syndrome critical region gene 6 like	1.48	1.32	0.86	1.01

49162	DKFZP761N09121		hypothetical protein DKFZP761N09121	1.47	1.32	1.09	1.05
47451	DOCK1		dedicator of cytokinesis 1	0.69	1.38	0.88	1.03
50383	DTR	HB-EGF, diphtheria toxin receptor	diphtheria toxin receptor (heparin-binding epidermal growth factor-like growth factor)	1.37	1.33	1.38	1.22
46877	DTR	HB-EGF, diphtheria toxin receptor	diphtheria toxin receptor (heparin-binding epidermal growth factor-like growth factor)	1.78	1.51	1.32	1.15
50046	DUSP1	MKP-1, PTPN10	dual specificity phosphatase 1	1.61	1.14	1.68	1.58
49389	DUSP1	MKP-1, PTPN10	dual specificity phosphatase 1	1.59	1.00	1.54	1.35
50362	DUSP10	DUSP10	dual specificity phosphatase 10	1.53	1.50	1.66	1.53
47279	DUSP10	DUSP10	dual specificity phosphatase 10	1.60	1.52	1.58	1.47
46668	DUSP2	PAC-1	dual specificity phosphatase 2	1.03	1.00	1.55	1.34
47277	DUSP6	DUSP6	dual specificity phosphatase 6	0.61	1.48	1.15	1.05
49777	DUSP6	DUSP6	dual specificity phosphatase 6	0.68	1.40	1.05	1.01
49810	DUSP6	DUSP6	dual specificity phosphatase 6	0.65	1.42	1.06	1.00
48657	DYRK4		dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 4	0.72	1.34	1.05	1.01
48032	ECT2	ECT2	epithelial cell transforming sequence 2 oncogene	0.68	1.41	1.43	1.09
47327	EDNRB	EDNRB	endothelin receptor type B	0.64	1.47	0.76	1.10
48859	EGR1		early growth response 1	2.74	2.02	3.74	3.21
48565	EGR2	EGR2	early growth response 2 (Krox-20 (Drosophila) homolog)	1.26	1.09	2.06	1.64
48213	EGR2	EGR2	early growth response 2 (Krox-20 (Drosophila) homolog)	1.18	1.00	1.91	1.30
49474	ELF4	ELF4	E74-like factor 4 (ets domain transcription factor)	1.54	1.37	0.84	1.07
46864	ELF4	ELF4	E74-like factor 4 (ets domain transcription factor)	1.49	1.32	0.88	1.05
48544	ELL2		ELL-RELATED RNA POLYMERASE II, ELONGATION FACTOR	2.12	1.76	1.08	1.04
47274	EMR1		egf-like module containing, mucin-like, hormone receptor-like sequence 1	1.00	1.00	0.52	1.45
47730	ERBB3	ErbB3	v-erb-b2 avian erythroblastic leukemia viral oncogene homolog 3	0.74	1.29	0.69	1.37

49672	ETS2	Ets-2	v-ets avian erythroblastosis virus E26 oncogene homolog 2	0.96	1.00	1.41	1.31
49406	EVA1		epithelial V-like antigen 1	1.81	1.33	1.03	1.00
49637	F2	F2	coagulation factor II (thrombin)	1.00	1.00	0.72	1.33
49295	FAF1	CGI-03	Fas (TNFRSF6) associated factor 1	0.54	1.75	0.85	1.14
49721	FANCG		Fanconi's anemia, complementation group G	1.36	1.28	0.69	1.33
49326	FCGR2A	CD32	Fc fragment of IgG, low affinity IIa, receptor for (CD32)	1.05	1.00	0.70	1.32
48977	FCGR2A	CD32	Fc fragment of IgG, low affinity IIa, receptor for (CD32)	1.04	1.00	0.71	1.30
48489	FKBP1B		FK506-binding protein 1B (12.6 kD)	1.39	1.26	2.09	1.71
50101	FOS	c-fos	v-fos FBJ murine osteosarcoma viral oncogene homolog	1.61	1.13	2.37	1.94
47566	FOS	c-fos	v-fos FBJ murine osteosarcoma viral oncogene homolog	1.64	1.37	1.95	1.78
46829	FOSB	FosB	FBJ murine osteosarcoma viral oncogene homolog B	0.87	1.00	1.68	1.59
48969	FOSL2	FOSL2, Fra2	FOS-like antigen 2	2.28	1.99	1.38	1.17
48270	FOSL2	FOSL2, Fra2	FOS-like antigen 2	2.22	1.98	1.30	1.12
47741	FOXJ1	FOXJ1	forkhead box J1	1.69	1.32	1.00	1.00
47264	GADD45A		growth arrest and DNA-damage-inducible, alpha	1.19	1.09	1.57	1.53
46583	GADD45B	MYD118	growth arrest and DNA-damage-inducible, beta	0.89	1.00	1.69	1.47
48615	GADD45B	MYD118	growth arrest and DNA-damage-inducible, beta	1.76	1.49	1.36	1.01
49728	GALK2		galactokinase 2	0.73	1.32	0.98	1.00
48676	GBE1		glucan (1,4-alpha-), branching enzyme 1 (glyc. branch. enz., Andersen disease, glycogen storage disease type IV)	0.65	1.43	0.93	1.00
50142	GBP1	IFN induced GBP-1	guanylate binding protein 1, interferon-inducible, 67kD	1.49	1.42	0.88	1.10
48066	GBP2	IFN induced GBP-2	guanylate binding protein 2, interferon-inducible	1.53	1.38	0.85	1.12
50373	GBP2	IFN induced GBP-2	guanylate binding protein 2, interferon-inducible	2.11	1.87	0.72	1.06
49092	GCH1	GTP cyclase	GTP cyclohydrolase 1 (dopa-responsive dystonia)	1.92	1.88	0.70	1.15

46977	GCH1	GTP cyclohydrolase I	GTP cyclohydrolase 1 (dopa-responsive dystonia)	1.83	1.52	0.84	1.07
49059	GCH1	GTP cyclohydrolase I	GTP cyclohydrolase 1 (dopa-responsive dystonia)	1.68	1.56	0.73	1.02
46876	GNG11	G protein, gamma 11	guanine nucleotide binding protein 11	1.57	1.51	1.10	1.00
49277	GNG11	G protein, gamma 11	guanine nucleotide binding protein 11	1.38	1.32	1.09	1.00
49692	GRO3	GRO3	GRO3 oncogene	1.00	1.00	1.67	1.34
47411	GRSF1		G-rich RNA sequence binding factor 1	0.71	1.36	1.09	1.03
50070	GS3955		GS3955 protein	0.71	1.33	0.96	1.00
49688	GSK3B	GSK3B	glycogen synthase kinase 3 beta	0.70	1.41	0.97	1.00
49032	GSK3B	GSK3B	glycogen synthase kinase 3 beta	0.73	1.31	1.01	1.00
47831	HEC		highly expressed in cancer, rich in leucine heptad repeats	0.97	1.00	1.45	1.39
48518	HLA-DOA		major histocompatibility complex, class II, DO alpha	1.56	1.31	0.97	1.00
46831	HLA-DPA1	MHCII, DP alpha 1	major histocompatibility complex, class II, DP alpha 1	1.60	1.50	1.09	1.04
50048	HLA-DPA1	MHCII, DP alpha 1	major histocompatibility complex, class II, DP alpha 1	1.53	1.45	1.13	1.03
46863	HLA-DPB1	MHCII, DP beta 1	major histocompatibility complex, class II, DP beta 1	1.50	1.37	1.03	1.00
50381	HLA-DQB1	MHCII, DQ beta 1	major histocompatibility complex, class II, DQ beta 1	1.70	1.61	1.11	1.03
47668	HLA-DRA	MHCII, DR alpha	major histocompatibility complex, class II, DR alpha	1.57	1.45	1.16	1.11
50750	HLA-DRA	MHCII, DR alpha	major histocompatibility complex, class II, DR alpha	1.47	1.40	1.12	1.07
48589	HLA-DRB1	MHCII, DR beta 1	major histocompatibility complex, class II, DR beta 1	1.51	1.35	1.10	1.05
49305	HLA-DRB1	MHCII, DR beta 1	major histocompatibility complex, class II, DR beta 1	1.49	1.38	1.10	1.03
50385	HLA-DRB1	MHCII, DR beta 1	major histocompatibility complex, class II, DR beta 1	1.50	1.34	1.07	1.01
47987	HLA-DRB1	MHCII, DR beta 1	major histocompatibility complex, class II, DR beta 1	1.48	1.35	1.07	1.01
47179	HLA-DRB1	MHCII, DR beta 1	major histocompatibility complex, class II, DR beta 1	1.76	1.70	1.00	1.00
46926	HLA-DRB1	MHCII, DR beta 1	major histocompatibility complex, class II, DR beta 1	1.60	1.38	1.19	1.00

49293	HLA-DRB3	MHCII, DR beta 3	major histocompatibility complex, class II, DR beta 3	1.48	1.40	1.11	1.06
46778	HMG2		high-mobility group (nonhistone chromosomal) protein 2	1.02	1.00	1.48	1.42
49217	HMGIC		high-mobility group (nonhistone chromosomal) protein isoform I-C	0.65	1.37	0.85	1.00
49347	HNRPA3		heterogeneous nuclear ribonucleoprotein A3	0.73	1.33	0.98	1.00
49371	HNRPR		heterogeneous nuclear ribonucleoprotein R	0.71	1.36	1.09	1.06
49100	HSPA4	HSPA4	heat shock 70kD protein 4	0.72	1.36	1.01	1.00
47709	HSPA8	HSPA8	heat shock 70kD protein 8	0.65	1.47	0.89	1.00
48605	HSXIAPAF1	XIAP assoc factor 1	XIAP associated factor-1	1.43	1.31	0.65	1.48
49681	HSXIAPAF1	XIAP assoc factor 1	XIAP associated factor-1	1.44	1.36	0.65	1.46
47284	IDH2		isocitrate dehydrogenase 2 (NADP+), mitochondrial	1.99	1.75	0.76	1.00
46595	IFI35	IFN-induced protein 35	interferon-induced protein 35	1.66	1.45	0.99	1.00
48436	IFI35	IFN-induced protein 35	interferon-induced protein 35	1.60	1.41	0.89	1.00
47659	IL1RAP	IL-1RAP	interleukin 1 receptor accessory protein	0.55	1.69	0.82	1.09
47692	IL1RAP	IL-1RAP	interleukin 1 receptor accessory protein	0.56	1.61	0.82	1.00
50497	IL1RAP	IL-1RAP	interleukin 1 receptor accessory protein	0.58	1.60	0.98	1.00
46856	IL8	interleukin-8	interleukin 8	3.96	3.88	2.95	2.91
49873	INSIG1		insulin induced gene 1	0.70	1.36	1.20	1.17
46618	ISG15	ISG15, IFN stimulated protein	interferon-stimulated protein, 15 kDa	1.58	1.37	0.87	1.00
49322	JUNB	JunB	jun B proto-oncogene	1.60	1.37	1.54	1.46
49319	JUNB	JunB	jun B proto-oncogene	1.78	1.52	1.50	1.14
50205	KIAA0007		KIAA0007 protein	0.70	1.37	1.44	1.37
48862	KIAA0008		KIAA0008 gene product	0.66	1.33	1.83	1.70
47234	KIAA0097		KIAA0097 gene product	0.80	1.13	1.56	1.44

50636	KIAA0101		KIAA0101 gene product	1.83	1.68	0.64	1.26
49727	KIAA0564		KIAA0564 protein	0.65	1.36	1.01	1.00
50765	KIAA0668		KIAA0668 protein	0.62	1.44	1.32	1.18
50568	KNSL5		kinesin-like 5 (mitotic kinesin-like protein 1)	0.76	1.21	1.40	1.39
49240	KPNA3		karyopherin alpha 3 (importin alpha 4)	0.72	1.32	0.84	1.17
50617	KRT20		cytokeratin 20	2.10	1.48	0.98	1.00
48856	KRT2A		keratin 2A (epidermal ichthyosis bullosa of Siemens)	1.77	1.52	1.11	1.00
49362	KRT7		keratin 7	1.80	1.46	1.01	1.00
50021	LAMA4	LAMA4	laminin, alpha 4	0.63	1.52	0.82	1.19
50042	LAMB3	LAMB3	laminin, beta 3 (nicein (125kD), kalinin (140kD), BM600 (125kD))	2.45	2.14	0.95	1.00
48692	LAMC1	LAMC1	laminin, gamma 1 (formerly LAMB2)	0.54	1.63	0.89	1.00
47325	LAMC1	LAMC1	laminin, gamma 1 (formerly LAMB2)	0.66	1.43	0.95	1.00
46939	LDHA		lactate dehydrogenase A	0.66	1.42	0.92	1.00
49163	LGALS3		lectin, galactoside-binding, soluble, 3 (galectin 3)	0.60	1.58	0.97	1.00
49557	LPXN		leupaxin	0.61	1.55	1.03	1.00
48673	LRDD	LRDD	leucine-rich and death domain containing	1.99	1.45	0.61	1.26
49376	MAC30		hypothetical protein	1.11	1.08	0.66	1.47
47158	MAD	MAD	MAX dimerization protein	1.82	1.50	1.54	1.51
47029	MAD	MAD	MAX dimerization protein	1.76	1.58	1.56	1.48
47267	MAD4	MAD4	Mad4 homolog	1.08	1.00	0.55	1.43
47087	MADH3		MAD (mothers against decapentaplegic, Drosophila) homolog 3	1.48	1.42	0.87	1.03
48278	MAP2K3	MKK3	mitogen-activated protein kinase kinase 3	0.83	1.15	1.45	1.42
48028	MAP2K3	MKK3	mitogen-activated protein kinase kinase 3	0.78	1.11	1.37	1.32
49458	MAP4K1	MAP4K1	mitogen-activated protein kinase kinase kinase 1	0.74	1.32	1.04	1.00

49528	MARCO		macrophage receptor with collagenous structure	2.01	1.35	1.02	1.00
47123	MCM2		minichromosome maintenance deficient (S. cerevisiae) 2 (mitotin)	1.46	1.38	0.55	1.52
48470	MCM5		minichromosome maintenance deficient (S. cerevisiae) 5 (cell division cycle 46)	1.17	1.08	0.63	1.37
49996	MHC2TA	MHCII transactivator	MHC class II transactivator	2.05	1.72	0.69	1.15
49452	MHC2TA	MHCII transactivator	MHC class II transactivator	1.95	1.67	0.72	1.09
46610	MIG-6	MIG-6	Gene 33/Mig-6	1.37	1.32	1.24	1.00
49224	MITF		microphthalmia-associated transcription factor	0.61	1.54	1.05	1.00
46825	MKNK2	MKNK2	G protein-coupled receptor kinase 7	1.87	1.46	1.21	1.04
48713	MKNK2	MKNK2	G protein-coupled receptor kinase 7	1.62	1.39	1.25	1.03
47095	MTCP1		mature T-cell proliferation 1	1.48	1.40	0.77	1.27
47772	MTHFD2		methylene tetrahydrofolate dehydrogenase (NAD+ dependent), methenyltetrahydrofolate cyclohydrolase	1.90	1.85	1.25	1.21
47230	MXI1	MXI1	MAX-interacting protein 1	0.71	1.33	0.87	1.08
47732	MYC	c-Myc	v-myc avian myelocytomatosis viral oncogene homolog	0.90	1.07	1.59	1.53
47968	MYLK		myosin, light polypeptide kinase	1.53	1.42	0.88	1.03
48783	MYO5A		myosin VA (heavy polypeptide 12, myoxin)	0.60	1.61	0.87	1.11
47693	NF1	NF1	neurofibromin 1 (neurofibromatosis, von Recklinghausen disease, Watson disease)	0.71	1.31	1.05	1.00
47173	NFKB1	NFkappaB, p105	nuclear factor of kappa light polypeptide gene enhancer in B-cells 1 (p105)	0.85	1.05	1.42	1.37
50676	NFKB2	NFkappaB, p49/p100	nuclear factor of kappa light polypeptide gene enhancer in B-cells 2 (p49/p100)	1.24	1.00	1.34	1.30
48405	NFKBIE	IkB, epsilon	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, epsilon	0.99	1.00	1.55	1.35
49872	NMA		putative transmembrane protein	0.63	1.52	1.00	1.00
46734	NP		nucleoside phosphorylase	1.50	1.43	1.05	1.00
48192	OPA1		optic atrophy 1 (autosomal dominant)	0.71	1.34	0.95	1.00
48976	P2RX5	P2RX5	purinergic receptor P2X, ligand-gated ion channel, 5	1.58	1.36	0.80	1.14

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47935	P4HA1	P4HA1, prolyl-4-hydroxylase alpha1	procollagen-proline, 2-oxoglutarate 4-dioxygenase (proline 4-hydroxylase), alpha polypeptide I	0.68	1.39	1.02	1.00
50722	PCAF	PCAF	p300/CBP-associated factor	0.63	1.52	0.79	1.21
50505	PCAF	PCAF	p300/CBP-associated factor	0.62	1.52	0.86	1.12
48666	PCK2		phosphoenolpyruvate carboxykinase 2 (mitochondrial)	1.63	1.49	1.01	1.00
49707	PCNA		proliferating cell nuclear antigen	1.53	1.46	0.43	1.89
48427	PDE4B	phosphodiesterase 4B	phosphodiesterase 4B, cAMP-specific (dunce (Drosophila)-homolog phosphodiesterase E4)	1.29	1.22	1.43	1.34
50639	PDIR		for protein disulfide isomerase-related	0.76	1.31	0.96	1.00
46602	PEA15	Pea-15, MAT1	phosphoprotein enriched in astrocytes 15	1.36	1.33	1.23	1.15
46919	PFKL		phosphofructokinase, liver	1.39	1.36	0.89	1.00
46901	PIG3		quinone oxidoreductase homolog	1.35	1.30	0.98	1.00
49525	PIGQ		phosphatidylinositol glycan, class Q	1.62	1.36	0.84	1.00
48988	PIK3CB	p110 (PI3K)	phosphoinositide-3-kinase, catalytic, beta polypeptide	0.67	1.36	0.72	1.34
47924	PIK3CB	p110 (PI3K)	phosphoinositide-3-kinase, catalytic, beta polypeptide	0.68	1.37	0.79	1.26
49975	PIM1	pim-1 oncogene	pim-1 oncogene	1.43	1.31	1.01	1.00
48805	PLP1		proteolipid protein 1 (Pelizaeus-Merzbacher disease, spastic paraplegia 2, uncomplicated)	0.65	1.41	0.86	1.06
47776	POLA		polymerase (DNA directed), alpha	0.87	1.10	0.65	1.50
48531	PREP		prolyl endopeptidase	0.63	1.43	0.92	1.04
46646	PRKCE	PKC epsilon	protein kinase C, epsilon	0.47	1.67	0.99	1.00
47998	PSMB9		proteasome (prosome, macropain) subunit, beta type, 9 (large multifunctional protease 2)	1.56	1.43	0.87	1.12
46632	PTGES	PTGES	prostaglandin H synthase	2.12	1.79	1.04	1.00
47618	PTGIS		prostaglandin I2 (prostacyclin) synthase	1.00	1.00	0.54	1.67
48625	PTGS1		prostaglandin-endoperoxide synthase 1 (prostaglandin G/H synthase and cyclooxygenase)	0.59	1.62	1.26	1.22
50353	PTK2	FAK	PTK2 protein tyrosine kinase 2	0.67	1.39	0.85	1.17

49852	PTK2	FAK	PTK2 protein tyrosine kinase 2	0.62	1.53	0.83	1.15
49867	PTN		pleiotrophin (heparin binding growth factor 8, neurite growth-promoting factor 1)	1.48	1.35	0.86	1.06
47981	PTPRG	PTPRG	protein tyrosine phosphatase, receptor type, G	0.34	2.48	0.82	1.01
49533	PTPRG	PTPRG	protein tyrosine phosphatase, receptor type, G	0.36	2.25	0.96	1.00
50348	PTPRG	PTPRG	protein tyrosine phosphatase, receptor type, G	0.43	1.65	0.99	1.00
50632	PTPRJ	PTPRJ	protein tyrosine phosphatase, receptor type, J	0.59	1.45	0.79	1.17
47698	PTPRJ	PTPRJ	protein tyrosine phosphatase, receptor type, J	0.71	1.33	0.89	1.03
47548	PTPRK	PTPRK	protein tyrosine phosphatase, receptor type, K	0.49	1.84	0.99	1.00
48279	PTPRM	PTPRM	protein tyrosine phosphatase, receptor type, M	0.49	1.76	1.11	1.03
48687	PTPRM	PTPRM	protein tyrosine phosphatase, receptor type, M	0.54	1.44	1.13	1.00
50550	PTPRT	PTPRT	protein tyrosine phosphatase, receptor type, T	1.39	1.31	0.88	1.05
50374	RAB7	RAB7	RAB7, member RAS oncogene family	0.68	1.39	1.15	1.06
48258	RAB1F	RAB1F	RAB interacting factor	1.44	1.39	0.84	1.08
47205	RAB1F	RAB1F	RAB interacting factor	1.41	1.38	0.89	1.07
49876	RAD54L		RAD54 (S. cerevisiae)-like	1.41	1.20	0.63	1.43
49565	RASSF1		Ras association (RalGDS/AF-6) domain family 1	1.27	1.12	1.65	1.49
50076	RBBP8		retinoblastoma-binding protein 8	1.48	1.41	0.75	1.26
49120	RELB	l-rel	v-rel avian reticuloendotheliosis viral oncogene homolog B (nuclear factor of kappa light polypeptide gene enhancer in B-cells 3)	1.64	1.20	1.85	1.54
49087	RELB	l-rel	v-rel avian reticuloendotheliosis viral oncogene homolog B (nuclear factor of kappa light polypeptide gene enhancer in B-cells 3)	1.53	1.21	1.72	1.47
46658	RELB	l-rel	v-rel avian reticuloendotheliosis viral oncogene homolog B (nuclear factor of kappa light polypeptide gene enhancer in B-cells 3)	1.36	1.13	1.82	1.33
47933	RIPK2	RIP2, RICK	receptor-interacting serine-threonine kinase 2	1.56	1.50	1.62	1.52
47774	RPA2		replication protein A2 (32kD)	1.58	1.52	0.79	1.22
49934	RPC62		polymerase (RNA) III (DNA directed) (62kD)	1.42	1.33	1.24	1.19
48930	RPS6KA1	RSK1	ribosomal protein S6 kinase, 90kD, polypeptide 1	1.56	1.44	0.44	1.93

50288	RAS2		oncogene TC21	1.43	1.36	1.11	1.06
48190	SCYA20	MIP-3a	small inducible cytokine subfamily A (Cys-Cys), member 20	1.19	1.00	2.11	1.30
49831	SCYC2	SCYC2	small inducible cytokine subfamily C, member 2	1.32	1.31	0.87	1.07
47571	SDC2	heparin sulfate proteoglycan-1	syndecan 2 (heparan sulfate proteoglycan 1, cell surface-associated, fibroglycan)	1.65	1.53	0.80	1.20
48646	SDCBP		syndecan binding protein (syntenin)	0.68	1.38	1.23	1.04
49117	SERPINB1		serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 1	1.66	1.46	1.03	1.00
48415	SERPINB1		serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 1	0.69	1.39	0.99	1.00
49428	SERPINB6		serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 6	1.54	1.38	0.96	1.00
47677	SERPINB6		serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 6	1.42	1.32	0.95	1.00
47710	SERPINB6		serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 6	1.39	1.31	0.96	1.00
48205	SERPINB8		serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 8	1.37	1.31	1.28	1.23
49963	SERPINB8		serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 8	1.43	1.37	1.25	1.23
48281	SERPINE2		serine (or cysteine) proteinase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 2	0.68	1.34	0.85	1.09
50577	SIAT4C		sialyltransferase 4C (beta-galactosidase alpha-2,3-sialyltransferase)	0.62	1.33	1.01	1.00
49880	SLC25A13		solute carrier family 25, member 13 (citrin)	0.59	1.62	0.88	1.02
46903	SLC3A2		solute carrier family 3 (activators of dibasic and neutral amino acid transport), member 2	1.39	1.32	1.87	1.83
50641	SLC5A1		solute carrier family 5 (sodium/glucose cotransporter), member 1	2.01	1.95	0.85	1.13
48182	SNAPC1		small nuclear RNA activating complex, polypeptide 1, 43kD	1.07	1.01	1.47	1.34
49323	SPARC	SPARC	secreted protein, acidic, cysteine-rich (osteonectin)	0.97	1.00	0.70	1.32
48462	SPRR2C		small proline-rich protein 2C	1.01	1.00	1.48	1.41
49446	SRC	Src	v-src avian sarcoma (Schmidt-Ruppin A-2) viral oncogene homolog	1.52	1.35	0.82	1.11
49218	SRD5A2		steroid-5-alpha-reductase, alpha polypeptide 2 (3-oxo-5 alpha-steroid delta 4-dehydrogenase alpha 2)	1.42	1.34	1.08	1.00
49184	SUPV3L1		suppressor of var1 (S. cerevisiae) 3-like 1	1.47	1.43	1.04	1.02

47800	SYTL2			synaptotagmin-like 2		1.63	1.58			0.95	1.00
50707	TCF12	TCF12		transcription factor 12 (HTF4, helix-loop-helix transcription factors 4)		0.72	1.32			0.95	1.00
48433	TEAD1	TEAD1		TEA domain family member 1 (SV40 transcriptional enhancer factor)		0.68	1.43			1.04	1.02
48562	TEAD1	TEAD1		TEA domain family member 1 (SV40 transcriptional enhancer factor)		0.70	1.36			1.02	1.00
50389	TFRC	CD71, transferrin R		transferrin receptor (p90, CD71)		0.71	1.32			0.85	1.10
48513	TGFBR3			transforming growth factor, beta receptor III (betaglycan, 300kD)		0.68	1.32			0.86	1.00
50579	TGIF			TGFB-induced factor (TALE family homeobox)		0.92	1.01			1.45	1.40
48577	THBS3	THBS3		thrombospondin 3		1.61	1.33			0.80	1.04
		TGF beta-inducible early growth response									
46859	TIEG			TGFB inducible early growth response		1.44	1.07			1.62	1.59
48754	TIMP3			tissue inhibitor of metalloproteinase 3 (Sorsby fundus dystrophy, pseudoinflammatory)		0.67	1.31			0.83	1.15
47444	TKT			transketolase (Wernicke-Korsakoff syndrome)		0.65	1.44			0.95	1.00
47918	TNFAIP1	TNFAIP1		tumor necrosis factor, alpha-induced protein 1 (endothelial)		1.61	1.34			1.00	1.00
47642	TNFAIP3	A20, TNFAIP2, Znfinger		tumor necrosis factor, alpha-induced protein 3		1.30	1.26			1.93	1.74
46803	TNFAIP6	TNFAIP6		tumor necrosis factor, alpha-induced protein 6		1.20	1.11			1.38	1.35
50458	TNFRSF21	DR6		tumor necrosis factor receptor superfamily, member 21		0.72	1.33			0.77	1.25
50363	TNFSF12	TWEAK		tumor necrosis factor (ligand) superfamily, member 12		0.70	1.18			3.37	2.73
47542	TRIO	TRIO		triple functional domain (PTPRF interacting)		0.98	1.00			1.35	1.31
49901	TROAP			trophinin associated protein (tastin)		0.84	1.07			1.63	1.52
49779	TXNIP	VDUP1		upregulated by 1,25-dihydroxyvitamin D-3		1.64	1.48			1.59	1.52
47470	TXNIP	VDUP1		upregulated by 1,25-dihydroxyvitamin D-3		1.56	1.41			1.57	1.48
49812	TXNIP	VDUP1		upregulated by 1,25-dihydroxyvitamin D-3		1.57	1.49			1.57	1.47
48761	UBE2C			ubiquitin-conjugating enzyme E2C		0.77	1.26			1.75	1.52
50573	UBE2N			ubiquitin-conjugating enzyme E2N (homologous to yeast UBC13)		0.72	1.32			1.01	1.00

49522	UCP2			uncoupling protein 2 (mitochondrial, proton carrier)	1.42	1.36		0.84	1.03
48807	UNG			uracil-DNA glycosylase	1.41	1.38		0.49	1.96
46709	UROD			uroporphyrinogen decarboxylase	1.47	1.42		0.97	1.00
49536	USP11			ubiquitin specific protease 11	1.82	1.66		0.96	1.00
48262	VAV3	Vav3		vav 3 oncogene	0.66	1.48		0.66	1.46
47298	VAV3	Vav3		vav 3 oncogene	0.70	1.34		0.68	1.35
47787	VGf			VGf nerve growth factor inducible	1.40	1.27		2.27	2.08
50028	XBP1	XBP-1		X-box binding protein 1	1.53	1.50		1.76	1.66
47130	YWHAZ	14-3-3 zeta		tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide	0.71	1.34		1.09	1.03
50730	YWHAZ	14-3-3 zeta		tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide	0.70	1.39		1.11	1.03
50481	YWHAZ	14-3-3 zeta		tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide	0.68	1.31		1.07	1.01
50514	YWHAZ	14-3-3 zeta		tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide	0.69	1.35		1.05	1.00
48979	ZFP36	tristetraprolin		zinc finger protein homologous to Zfp-36 in mouse	1.91	1.71		1.94	1.86
50466	ZFP36	tristetraprolin		zinc finger protein homologous to Zfp-36 in mouse	1.33	1.12		1.88	1.66
49723	ZNF76			zinc finger protein 76 (expressed in testis)	1.65	1.45		1.04	1.00

Table 3 – Genes Differentially Expressed in Response to CPT+ compound A 4-hour (Study #1) and CPT+ compound A 6-hour (Study #2), by mechanism-related functional group

Study #1 (4hr)							Study #2 (6hr)	
Spot ID	GeneSymbol	GeneName	GeneAssignment	CPT+ compound A (≥1.5x)	CPT+ compound A (≥1.5x)	CPT+ compound A min fold change (≥1.3x)		
Cell Cycle								
47516	BTG1		B-cell translocation gene 1, anti-proliferative	1.23	1.73	1.72		
49085	CCNA2	cyclin A2	cyclin A2	1.70	1.33	1.28		
49118	CCNA2	cyclin A2	cyclin A2	1.62	1.33	1.20		
48015	CCND3	Cyclin D3	cyclin D3	0.99	0.69	1.36		
46608	CCND3	Cyclin D3	cyclin D3	0.88	0.69	1.35		
49850	CCNE1	cyclin E1	cyclin E1	0.61	0.43	1.98		
50521	CCNE1	cyclin E1	cyclin E1	0.56	0.53	1.37		
47926	CCNE2	cyclin E2	cyclin E2	0.29	0.19	4.86		
49967	CCNE2	cyclin E2	cyclin E2	0.41	0.27	3.26		
50229	CDC2		cell division cycle 2, G1 to S and G2 to M	1.36	1.53	1.38		
50358	CDK7	CDK7	cyclin-dependent kinase 7 (homolog of Xenopus MO15 cdk-activating kinase)	1.31	1.50	1.47		
47382	CDK7	CDK7	cyclin-dependent kinase 7 (homolog of Xenopus MO15 cdk-activating kinase)	1.26	1.49	1.44		
48631	CDKN1A	Waf1, Cip1 cyclin dependent kinase inhibitor 3	cyclin-dependent kinase inhibitor 1A (p21, Cip1)	1.23	1.38	1.37		
48842	CDKN3		cyclin-dependent kinase inhibitor 3 (CDK2-associated dual specificity phosphatase)	1.61	2.18	1.89		
49105	CDKN3	cyclin dependent kinase inhibitor 3	cyclin-dependent kinase inhibitor 3 (CDK2-associated dual specificity phosphatase)	1.65	1.90	1.70		

		kinase inhibitor 3					
50590	CKS2		CDC28 protein kinase 2	2.51	2.94	2.49	
47123	MCM2		minichromosome maintenance deficient (<i>S. cerevisiae</i>) 2 (mitotin)	0.59	0.55	1.52	
48470	MCM5		minichromosome maintenance deficient (<i>S. cerevisiae</i>) 5 (cell division cycle 46)	0.70	0.63	1.37	
48761	UBE2C		ubiquitin-conjugating enzyme E2C	1.35	1.75	1.52	
DNA damage repair							
49433	DDIT3	CHOP	DNA-damage-inducible transcript 3	2.17	2.74	2.68	
50132	DDIT3	CHOP	DNA-damage-inducible transcript 3	2.13	2.75	2.60	
49721	FANCG		Fanconi's anemia, complementation group G	0.77	0.69	1.33	
47264	GADD45A		growth arrest and DNA-damage-inducible, alpha	1.38	1.57	1.53	
46583	GADD45B	MyD118	growth arrest and DNA-damage-inducible, beta	1.56	1.69	1.47	
49707	PCNA		proliferating cell nuclear antigen	0.52	0.43	1.89	
49876	RAD54L		RAD54 (<i>S. cerevisiae</i>)-like	0.71	0.63	1.43	

EXAMPLE 3

Effect of compound B ("inactive, NEG")

- [39] Compound B does not inhibit Chk1 enzyme activity (ability to phosphorylate cdc25c) at or below 1 μ M (CFA). Compound B is structurally similar to Compound A. Compound B at 30 nM is a control of non-specific, structure-related effects, because Chk1 inhibitory effects would not be anticipated at the 30 nM concentration used in this experiment.
- [40] MDA-MB-435 breast carcinoma cells were pre-incubated with or without 50nM CPT and then treated with or without 30nM compound B. Following pre-incubation and treatment, the cells were lysed, total RNA collected, and the poly-A⁺ mRNA fraction purified. The global transcription response to the various culture conditions was assessed by competitive hybridization to probes on a cDNA microarray chip.
- [41] In the absence of DNA damaging agents, treatment with compound B showed minimal changes, and the genes that were differentially expressed had small changes. Thirty-seven genes were differentially expressed with a min fold change ≥ 1.3 . Twelve genes had ≥ 1.5 x min fold change. Two genes (DFNA5 and "hypothetical") had a min fold change >2 x.
- [42] Treatment with CPT and compound B resulted in differential expression of one gene (Serpina2). Only one gene (SARM) differentially expressed by compound B alone or CPT+ compound B overlaps with differentially expressed genes from compound A or CPT+ compound A groups.

EXAMPLE 4

Effect of Compound C (additive, no cell cycle release) ("CCR")

- [43] Compound C inhibits Chk1 enzyme activity (ability to phosphorylate cdc25c) in a CFA with an IC₅₀ of 0.2nM. Compound C inhibits proliferation of MDA-MB-435 cells, using a MTS-based proliferation assay, with an EC₅₀ of 98nM. MDA-MB-435

breast carcinoma cells were pre-incubated with or without 50 nM CPT and then treated with or without 30 nM Compound C. Following pre-incubation and treatment, the cells were lysed, total RNA collected, and the poly-A⁺ mRNA fraction purified. The global transcription response to the various culture conditions was assessed by competitive hybridization to probes on a cDNA microarray chip. The results are shown in Table 4.

- [44] In the absence of DNA damaging agents, Compound C altered expression of eighty-eight genes with min fold change $\geq 1.3x$. Twenty-two genes had min fold change $> 1.5x$. More genes are differentially expressed in response to Compound C alone than in response to compound A alone, which may indicate off-target effects of Compound C. However, the majority of genes (66) had min fold change < 1.5 , indicating a relatively weak effect.
- [45] Seventy-seven genes had a min fold change $\geq 1.3x$ in response to Compound C treatment in the presence of CPT-dependent DNA damage (Table 4). Compound C (in the presence of CPT-dependent DNA damage) had a large effect on multiple cell cycle- and DNA repair-related genes, although this compound does not show CCR (see further discussion below, comparison w/CPT+ Compound A).

CPT+ compound A vs CPT + compound C – differences that could represent SA/CCR-specific effects

- [46] In direct competitive hybridization, seven genes were differentially expressed, as shown in Table 5. These genes should represent genes that are differentially expressed in only one of the treatment groups. This direct competitive hybridization is the most stringent comparison of these two compounds, since a statistical p-value can be assigned to the differential expression ratio (min fold change is the change with 95% confidence).
- [47] Only one gene (amphiregulin) has a min fold change > 1.5 (1.9x). However, none of the 7 genes in Table 5 are differentially expressed in either the CPT vs. CPT+ compound A comparison or the CPT vs. CPT+ compound C comparison. None of these 7 genes appear to be related to the MOA of Chk1 inhibition. This result

demonstrates that both Compound A and C have similar effects on Chk1 activity and gene expression, although only Compound A caused release from DNA damage-induced arrest at 30 nM.

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Table 4 - Differential gene expression in response to CPT alone and CPT+ compound C

Spot ID	GeneSymbol	GeneName	GeneAssignment	CPT alone		CPT+ Compound C	
				Δ/Δ CPT	wt/means min fold change ($>1.3x$)	Δ/Δ compound C	wt/means min fold change ($>1.3x$)
48739				4.54	3.58	2.10	1.71
50279				1.12	1.08	0.64	1.49
49056				0.87	1.10	1.43	1.39
50024				0.67	1.41	1.24	1.21
46905				0.72	1.32	1.28	1.09
48604				2.05	1.94	1.18	1.09
48060				2.03	1.72	1.19	1.03
46767				2.79	1.99	1.00	1.00
48252				0.50	1.79	0.99	1.00
50380				1.61	1.50	1.08	1.00
48899				0.55	1.47	1.06	1.00
49251				0.58	1.41	0.90	1.00
50032				1.54	1.39	1.08	1.00
48812				1.60	1.37	1.01	1.00
47196				1.52	1.36	1.03	1.00
50633	ABL2		v-abl Abelson murine leukemia viral oncogene homolog 2 (arg, Abelson-related gene)	1.62	1.46	2.00	1.93
46933	ACTB		actin, beta	0.85	1.14	1.47	1.32
48849	ACVRL1		activin A receptor type II-like 1	0.63	1.47	0.97	1.00

47896	ADAM11	ADAM11	a disintegrin and metalloproteinase domain 11	1.53	1.41	0.99	1.00
47352	ADAM11	ADAM11	a disintegrin and metalloproteinase domain 11	1.45	1.34	0.95	1.00
47913	ADAMTS1	ADAMTS1	a disintegrin-like and metalloprotease (reprolysin type) with thrombospondin type 1 motif, 1	1.49	1.31	1.74	1.55
47060	AF1Q		ALL1-fused gene from chromosome 1q	1.97	1.90	0.87	1.07
49339	ANXA3	annexin III	annexin A3	1.76	1.54	1.07	1.06
49524	AP1G2		adaptor-related protein complex 1, gamma 2 subunit	1.54	1.31	0.92	1.00
49887	APOBEC1		apolipoprotein B mRNA editing enzyme, catalytic polypeptide 1	2.01	1.64	1.00	1.00
47645	ARF4L		ADP-ribosylation factor 4-like	1.71	1.57	0.83	1.02
47535	ARHGDIA	ARHGDIA	Rho GDP dissociation inhibitor (GDI) alpha	0.74	1.32	1.13	1.05
46843	ARHGDIA	ARHGDIA	Rho GDP dissociation inhibitor (GDI) alpha	0.73	1.31	1.07	1.00
48346	ARHGEF2	ARHGEF2	rho/rac guanine nucleotide exchange factor (GEF) 2	1.57	1.33	2.18	1.79
48277	ARHGEF2	ARHGEF2	rho/rac guanine nucleotide exchange factor (GEF) 2	1.67	1.48	1.83	1.53
48516	ARL6IP		ADP-ribosylation factor-like 6 interacting protein	0.46	2.01	2.45	2.20
46817	ATF3	ATF3	activating transcription factor 3	2.72	2.52	2.03	1.98
46662	ATF4	ATF4	activating transcription factor 4 (tax-responsive enhancer element B67)	1.30	1.27	1.51	1.46
50523	ATF4	ATF4	activating transcription factor 4 (tax-responsive enhancer element B67)	1.21	1.16	1.57	1.37
49244	ATP7A		ATPase, Cu++ transporting, alpha polypeptide (Menkes syndrome)	0.38	2.23	1.07	1.00
49350	BCHE		butyrylcholinesterase	0.68	1.42	0.96	1.02
47180	BDKRB2	BDKRB2	bradykinin receptor B2	1.55	1.40	0.66	1.00
50448	BIRC3	cIAP2	baculoviral IAP repeat-containing 3	1.43	1.14	1.56	1.47
49889	BLVRB		bliverdin reductase B (flavin reductase (NADPH))	1.65	1.46	1.00	1.00
46916	BNIP3	BNIP3	BCL2/adenovirus E1B 19kD-interacting protein 3	0.60	1.63	1.04	1.00
47081	BRCA1		breast cancer 1, early onset	1.37	1.30	0.87	1.10
47516	BTG1		B-cell translocation gene 1, anti-proliferative	1.75	1.67	1.73	1.68
48267	CASP1	caspase 1, ICE	caspase 1, apoptosis-related cysteine protease (interleukin 1, beta, convertase)	1.67	1.44	1.04	1.00

49807	CASP4	CASP4	caspase 4, apoptosis-related cysteine protease	1.36	1.32	1.12	1.07
50503	CCND1		B-cell CLL/lymphoma 1	1.46	1.42	1.11	1.00
49850	CCNE1	cyclin E1	cyclin E1	1.78	1.68	0.44	1.93
50521	CCNE1	cyclin E1	cyclin E1	1.61	1.55	0.52	1.72
47926	CCNE2	cyclin E2	cyclin E2	2.41	2.34	0.27	2.87
49967	CCNE2	cyclin E2	cyclin E2	2.33	2.21	0.30	2.77
47899	CCR5		chemokine (C-C motif) receptor 5	0.71	1.34	1.22	1.02
47424	CD36L1		CD36 antigen (collagen type I receptor, thrombospondin receptor)-like 1	0.53	1.69	1.01	1.00
50392	CD97	CD97	CD97 antigen	0.96	1.00	1.39	1.33
50358	CDK7	CDK7	cyclin-dependent kinase 7 (homolog of Xenopus MO15 cdk-activating kinase)	1.02	1.00	1.40	1.37
47382	CDK7	CDK7	cyclin-dependent kinase 7 (homolog of Xenopus MO15 cdk-activating kinase)	1.07	1.03	1.36	1.33
49066	CDKN1A	Waf1, Cip1	cyclin-dependent kinase inhibitor 1A (p21, Cip1)	4.65	4.38	1.72	1.54
48631	CDKN1A	Waf1, Cip1	cyclin-dependent kinase inhibitor 1A (p21, Cip1)	1.52	1.39	1.38	1.35
46842	CDKN3	cyclin dependent kinase inhibitor 3	cyclin-dependent kinase inhibitor 3 (CDK2-associated dual specificity phosphatase)	0.67	1.35	1.70	1.66
49105	CDKN3	cyclin dependent kinase inhibitor 3	cyclin-dependent kinase inhibitor 3 (CDK2-associated dual specificity phosphatase)	0.68	1.41	1.51	1.47
47175	CED-6	CED-6	CED-6 protein	0.66	1.40	1.02	1.00
48313	CENPA		centromere protein A (17kD)	0.64	1.49	1.77	1.73
49737	CHAF1B		chromatin assembly factor 1, subunit B (p60)	1.57	1.44	0.54	1.45
50590	CKS2		CDC28 protein kinase 2	0.44	2.10	2.45	2.41
47829	CLCN5		chloride channel 5 (nephrolithiasis 2, X-linked, Dent disease)	0.57	1.46	0.86	1.08
49647	COL8A1	COL8A1	collagen, type VIII, alpha 1	0.66	1.34	1.04	1.00
49316	CREB3	CREB-3	cAMP responsive element binding protein 3 (lumen)	1.54	1.43	1.03	1.00
47222	CREB3	CREB-3	cAMP responsive element binding protein 3 (lumen)	1.43	1.39	0.97	1.00
48953	CSDA	CSDA	cold shock domain protein A	1.03	1.00	1.39	1.34
48263	CSPG2	CSPG2	chondroitin sulfate proteoglycan 2 (versican)	0.50	1.87	0.86	1.10

50530	CSPG2	CSPG2	chondroitin sulfate proteoglycan 2 (versican)	0.68	1.34	0.97	1.00
49060	CTGF	CTGF	connective tissue growth factor	1.70	1.56	0.88	1.08
47837	CTSG	CTSG	cathepsin G	2.15	1.65	0.93	1.00
47991	CYC1	cytochrome c-1	cytochrome c-1	0.68	1.42	1.05	1.00
47834	CYP1A1		cytochrome P450, subfamily 1 (aromatic compound-inducible), polypeptide 1	1.54	1.44	0.99	1.01
50237	CYR61		cysteine-rich, angiogenic inducer, 61	2.23	2.10	0.98	1.00
50132	DDIT3	CHOP	DNA-damage-inducible transcript 3	2.63	2.55	2.26	2.05
49433	DDIT3	CHOP	DNA-damage-inducible transcript 3	2.85	2.56	2.36	1.96
50563	DDX10		DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 10 (RNA helicase)	0.65	1.43	1.22	1.09
48122	DDX15		DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 15	0.65	1.46	1.01	1.00
47559	DGCR6	DGCR6	DiGeorge syndrome critical region gene 6	1.78	1.60	0.88	1.00
50399	DGCR6L		DiGeorge syndrome critical region gene 6 like	1.48	1.32	0.88	1.04
49162	DKFZP761N09121		hypothetical protein DKFZp761N09121	1.47	1.32	1.07	1.02
47451	DOCK1		dedicator of cytokinesis 1	0.69	1.38	0.98	1.00
50383	DTR	HB-EGF, diphtheria toxin receptor	diphtheria toxin receptor (heparin-binding epidermal growth factor-like growth factor)	1.37	1.33	1.21	1.18
46877	DTR	HB-EGF, diphtheria toxin receptor	diphtheria toxin receptor (heparin-binding epidermal growth factor-like growth factor)	1.78	1.51	1.31	1.01
50046	DUSP1	MKP-1, PTPN10	dual specificity phosphatase 1	1.61	1.14	1.68	1.52
49389	DUSP1	MKP-1, PTPN10	dual specificity phosphatase 1	1.59	1.00	1.57	1.39
47279	DUSP10	DUSP10	dual specificity phosphatase 10	1.60	1.52	1.63	1.49
50362	DUSP10	DUSP10	dual specificity phosphatase 10	1.53	1.50	1.62	1.42
47277	DUSP6	DUSP6	dual specificity phosphatase 6	0.61	1.48	1.20	1.11
49810	DUSP6	DUSP6	dual specificity phosphatase 6	0.65	1.42	1.06	1.02
49777	DUSP6	DUSP6	dual specificity phosphatase 6	0.68	1.40	0.98	1.00
48657	DYRK4		dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 4	0.72	1.34	0.97	1.00

48032	ECT2	ECT2	epithelial cell transforming sequence 2 oncogene	0.68	1.41	1.30	1.18
47327	EDNRB	EDNRB	endothelin receptor type B	0.64	1.47	0.74	1.24
48859	EGR1		early growth response 1	2.74	2.02	3.37	3.23
48565	EGR2	EGR2	early growth response 2 (Krox-20 (Drosophila) homolog)	1.26	1.09	2.19	2.12
48213	EGR2	EGR2	early growth response 2 (Krox-20 (Drosophila) homolog)	1.18	1.00	1.89	1.83
49474	ELF4	ELF4	E74-like factor 4 (ets domain transcription factor)	1.54	1.37	0.85	1.13
46864	ELF4	ELF4	E74-like factor 4 (ets domain transcription factor)	1.49	1.32	0.94	1.00
48544	ELL2		ELL-RELATED RNA POLYMERASE II, ELONGATION FACTOR	2.12	1.76	0.97	1.00
47730	ERBB3	ErbB3	v-erb-b2 avian erythroblastic leukemia viral oncogene homolog 3	0.74	1.29	0.74	1.31
49672	ETS2	Ets-2	v-ets avian erythroblastosis virus E26 oncogene homolog 2	0.96	1.00	1.39	1.34
49406	EVA1		epithelial V-like antigen 1	1.81	1.33	1.00	1.00
49295	FAF1	CGI-03	Fas (TNFRSF6) associated factor 1	0.54	1.75	0.97	1.00
49721	FANCG		Fanconi's anemia, complementation group G	1.36	1.28	0.63	1.38
48273	FCGR2A	CD32	Fc fragment of IgG, low affinity IIa, receptor for (CD32)	1.00	1.00	0.69	1.36
50715	FCGR2A	CD32	Fc fragment of IgG, low affinity IIa, receptor for (CD32)	1.05	1.01	0.70	1.35
48489	FKBP1B		FK506-binding protein 1B (12.6 kD)	1.39	1.26	2.12	1.78
50101	FOS	c-fos	v-fos FBJ murine osteosarcoma viral oncogene homolog	1.61	1.13	2.06	1.93
47566	FOS	c-fos	v-fos FBJ murine osteosarcoma viral oncogene homolog	1.64	1.37	1.95	1.90
48969	FOSL2	FOSL2, Fra2	FOS-like antigen 2	2.28	1.99	1.43	1.36
48270	FOSL2	FOSL2, Fra2	FOS-like antigen 2	2.22	1.98	1.40	1.28
47741	FOXJ1	FOXJ1	forkhead box J1	1.69	1.32	1.00	1.00
47264	GADD45A		growth arrest and DNA-damage-inducible, alpha	1.19	1.09	1.36	1.33
46583	GADD45B	MyD118	growth arrest and DNA-damage-inducible, beta	0.89	1.00	1.51	1.41
48615	GADD45B	MyD118	growth arrest and DNA-damage-inducible, beta	1.76	1.49	1.28	1.04
49728	GALK2		galactokinase 2	0.73	1.32	0.94	1.00

48676	GBE1			glucan (1,4-alpha-), branching enzyme 1 (glyc. branch. Enz., Andersen disease, glycogen storage disease type IV)	0.65	1.43	0.94	1.00
50142	GBP1	IFN induced GBP-1		guanylate binding protein 1, interferon-inducible, 67kD	1.49	1.42	1.06	1.00
50373	GBP2	IFN induced GBP-2		guanylate binding protein 2, interferon-inducible	2.11	1.87	0.84	1.13
48066	GBP2	IFN induced GBP-2		guanylate binding protein 2, interferon-inducible	1.53	1.38	0.99	1.00
49092	GCH1	GTP cyclohydrolase I		GTP cyclohydrolase 1 (dopa-responsive dystonia)	1.92	1.88	0.74	1.18
46977	GCH1	GTP cyclohydrolase I		GTP cyclohydrolase 1 (dopa-responsive dystonia)	1.83	1.52	0.80	1.11
49059	GCH1	GTP cyclohydrolase I		GTP cyclohydrolase 1 (dopa-responsive dystonia)	1.68	1.56	0.90	1.00
46876	GNG11	G protein, gamma 11		guanine nucleotide binding protein 11	1.57	1.51	0.98	1.00
49277	GNG11	G protein, gamma 11		guanine nucleotide binding protein 11	1.38	1.32	1.02	1.00
47411	GRSF1			G-rich RNA sequence binding factor 1	0.71	1.36	1.02	1.00
50070	GS3955			GS3955 protein	0.71	1.33	0.97	1.00
49688	GSK3B	GSK3B		glycogen synthase kinase 3 beta	0.70	1.41	1.07	1.00
49032	GSK3B	GSK3B		glycogen synthase kinase 3 beta	0.73	1.31	0.98	1.00
48518	HLA-DOA			major histocompatibility complex, class II, DO alpha	1.56	1.31	1.06	1.00
46831	HLA-DPA1	MHCII, DP alpha 1		major histocompatibility complex, class II, DP alpha 1	1.60	1.50	1.08	1.04
50048	HLA-DPA1	MHCII, DP alpha 1		major histocompatibility complex, class II, DP alpha 1	1.53	1.45	1.08	1.00
46863	HLA-DPB1	MHCII, DP beta 1		major histocompatibility complex, class II, DP beta 1	1.50	1.37	1.14	1.07
50381	HLA-DQB1	MHCII, DQ beta 1		major histocompatibility complex, class II, DQ beta 1	1.70	1.61	1.10	1.00
47668	HLA-DRA	MHCII, DR alpha		major histocompatibility complex, class II, DR alpha	1.57	1.45	1.04	1.01
50750	HLA-DRA	MHCII, DR alpha		major histocompatibility complex, class II, DR alpha	1.47	1.40	1.07	1.00
47179	HLA-DRB1	MHCII, DR beta 1		major histocompatibility complex, class II, DR beta 1	1.76	1.70	1.12	1.08
48589	HLA-DRB1	MHCII, DR beta 1		major histocompatibility complex, class II, DR beta 1	1.51	1.35	1.10	1.06
47887	HLA-DRB1	MHCII, DR beta 1		major histocompatibility complex, class II, DR beta 1	1.48	1.35	1.11	1.05
46926	HLA-DRB1	MHCII, DR beta 1		major histocompatibility complex, class II, DR beta 1	1.60	1.38	1.13	1.04

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49305	HLA-DRB1	MHCII, DR beta 1	major histocompatibility complex, class II, DR beta 1	1.49	1.38	1.07	1.03
50385	HLA-DRB1	MHCII, DR beta 1	major histocompatibility complex, class II, DR beta 1	1.50	1.34	1.09	1.00
49293	HLA-DRB3	MHCII, DR beta 3	major histocompatibility complex, class II, DR beta 3	1.48	1.40	1.11	1.10
49217	HMGIC		high-mobility group (nonhistone chromosomal) protein isoform I-C	0.65	1.37	0.93	1.00
49347	HNRPA3		heterogeneous nuclear ribonucleoprotein A3	0.73	1.33	1.00	1.00
49371	HNRPR		heterogeneous nuclear ribonucleoprotein R	0.71	1.36	1.00	1.00
47414	HSF4		heat shock transcription factor 4	1.23	1.11	0.73	1.31
49100	HSPA4	HSPA4	heat shock 70kD protein 4	0.72	1.36	1.08	1.03
49516	HSPA5	HSPA5	heat shock 70kD protein 5 (glucose-regulated protein, 78kD)	0.92	1.00	1.45	1.42
49328	HSPA5	HSPA5	heat shock 70kD protein 5 (glucose-regulated protein, 78kD)	0.93	1.05	1.38	1.35
47709	HSPA8	HSPA8	heat shock 70kD protein 8	0.65	1.47	0.78	1.13
48605	HSXIAPAF1	1	XIAP associated factor-1	1.43	1.31	0.83	1.15
49681	HSXIAPAF1	1	XIAP associated factor-1	1.44	1.36	0.86	1.01
47284	IDH2		isocitrate dehydrogenase 2 (NADP+), mitochondrial	1.99	1.75	0.77	1.05
46595	IFI35	IFN-induced protein 35	interferon-induced protein 35	1.66	1.45	1.02	1.00
48436	IFI35	IFN-induced protein 35	interferon-induced protein 35	1.60	1.41	0.99	1.00
47659	IL1RAP	IL-1RAP	interleukin 1 receptor accessory protein	0.55	1.69	1.02	1.00
47692	IL1RAP	IL-1RAP	interleukin 1 receptor accessory protein	0.56	1.61	0.94	1.00
50497	IL1RAP	IL-1RAP	interleukin 1 receptor accessory protein	0.58	1.60	0.97	1.00
46856	IL8	interleukin-8	interleukin 8	3.96	3.88	2.48	2.34
49873	INSIG1		insulin induced gene 1	0.70	1.36	1.20	1.15
46618	ISG15	ISG15, IFN stimulated protein	interferon-stimulated protein, 15 kDa	1.58	1.37	0.92	1.00
47989	JUN	c-jun, cJun	v-jun avian sarcoma virus 17 oncogene homolog	1.00	1.00	1.62	1.40
49322	JUNB	JunB	jun B proto-oncogene	1.60	1.37	1.53	1.42
49319	JUNB	JunB	jun B proto-oncogene	1.78	1.52	1.54	1.39

50205	KIAA00007		KIAA00007 protein	0.70	1.37	1.32	1.24
48862	KIAA00008		KIAA00008 gene product	0.66	1.33	1.70	1.61
47234	KIAA00097		KIAA00097 gene product	0.80	1.13	1.60	1.44
50636	KIAA0101		KIAA0101 gene product	1.83	1.68	0.62	1.35
49727	KIAA0564		KIAA0564 protein	0.65	1.36	1.00	1.00
50765	KIAA0668		KIAA0668 protein	0.62	1.44	1.36	1.23
49240	KPNA3		karyopherin alpha 3 (importin alpha 4)	0.72	1.32	0.92	1.03
50617	KRT20		cytokeratin 20	2.10	1.48	1.00	1.00
48856	KRT2A		keratin 2A (epidermal ichthyosis bullosa of Siemens)	1.77	1.52	1.18	1.05
49362	KRT7		keratin 7	1.80	1.46	1.10	1.00
50021	LAMA4	LAMA4	laminin, alpha 4	0.63	1.52	0.94	1.00
50042	LAMB3	LAMB3	laminin, beta 3 (niclin (125kD), kalinin (140kD), BM600 (125kD))	2.45	2.14	1.02	1.00
47325	LAMC1	LAMC1	laminin, gamma 1 (formerly LAMB2)	0.66	1.43	0.92	1.01
48692	LAMC1	LAMC1	laminin, gamma 1 (formerly LAMB2)	0.54	1.63	1.00	1.00
46939	LDHA		lactate dehydrogenase A	0.66	1.42	0.95	1.03
49163	LGALS3		lectin, galactoside-binding, soluble, 3 (galectin 3)	0.60	1.58	1.01	1.00
49557	LPXN		leupaxin	0.61	1.55	1.07	1.03
48673	LRDD	LRDD	leucine-rich and death domain containing	1.99	1.45	0.51	1.58
49376	MAC30		hypothetical protein	1.11	1.08	0.73	1.32
47029	MAD	MAD	MAX dimerization protein	1.76	1.58	1.70	1.66
47158	MAD	MAD	MAX dimerization protein	1.82	1.50	1.50	1.47
47087	MADH3		MAD (mothers against decapentaplegic, Drosophila) homolog 3	1.48	1.42	0.84	1.09
48278	MAP2K3	MKK3	mitogen-activated protein kinase kinase 3	0.83	1.15	1.48	1.42
48028	MAP2K3	MKK3	mitogen-activated protein kinase kinase 3	0.78	1.11	1.41	1.30
49458	MAP4K1	MAP4K1	mitogen-activated protein kinase kinase kinase 1	0.74	1.32	0.99	1.00

49528	MARCO		macrophage receptor with collagenous structure	2.01	1.35	1.00	1.00
47123	MCM2		minichromosome maintenance deficient (S. cerevisiae) 2 (mitotin)	1.46	1.38	0.63	1.49
48470	MCM5		minichromosome maintenance deficient (S. cerevisiae) 5 (cell division cycle 46)	1.17	1.08	0.62	1.52
49996	MHC2TA	MHCII transactivator	MHC class II transactivator	2.05	1.72	0.70	1.33
49452	MHC2TA	MHCII transactivator	MHC class II transactivator	1.95	1.67	0.92	1.00
46610	MIG-6	MIG-6	Gene 33/Mig-6	1.37	1.32	1.13	1.00
49224	MITF		microphthalmia-associated transcription factor	0.61	1.54	1.15	1.04
46825	MKNK2	MNK2	G _i protein-coupled receptor kinase 7	1.87	1.46	1.30	1.12
48713	MKNK2	MNK2	G _i protein-coupled receptor kinase 7	1.62	1.39	1.42	1.12
47095	MTCP1		mature T-cell proliferation 1	1.48	1.40	0.91	1.08
47772	MTHFD2		methylene tetrahydrofolate dehydrogenase (NAD ⁺ dependent), methenyltetrahydrofolate cyclohydrolase	1.90	1.85	1.16	1.10
47230	MXI1	MXI1	MAX-interacting protein 1	0.71	1.33	0.88	1.06
47732	MYC	c-Myc	v-myc avian myelocytomatosis viral oncogene homolog	0.90	1.07	1.45	1.39
47968	MYLK		myosin, light polypeptide kinase	1.53	1.42	0.87	1.05
48783	MYO5A		myosin VA (heavy polypeptide 12, myosin)	0.60	1.61	1.02	1.00
47693	NF1	NF1	neurofibromin 1 (neurofibromatosis, von Recklinghausen disease, Watson disease)	0.71	1.31	1.06	1.02
50542	NFKBIE	I κ B, epsilon	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, epsilon	1.11	1.08	1.53	1.49
48405	NFKBIE	I κ B, epsilon	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, epsilon	0.99	1.00	1.50	1.37
49872	NMA		putative transmembrane protein	0.63	1.52	1.06	1.00
46734	NP		nucleoside phosphorylase	1.50	1.43	1.15	1.08
48941	NR4A1	NAK1	nuclear receptor subfamily 4, group A, member 1	1.32	1.20	1.44	1.36
47897	NR4A2	TINUR	nuclear receptor subfamily 4, group A, member 2	1.37	1.16	1.47	1.43
48192	OPA1		optic atrophy 1 (autosomal dominant)	0.71	1.34	0.99	1.00
48976	P2RX5	P2RX5	purinergic receptor P2X ₅ , ligand-gated ion channel, 5	1.58	1.36	0.91	1.07

47935	P4HA1	P4HA1, prolyl-4-hydroxylase alpha1	procollagen-proline, 2-oxoglutarate 4-dioxygenase (proline 4-hydroxylase), alpha polypeptide I	0.68	1.39	1.03	1.00
50431	PASK		PAS-serine/threonine kinase	1.32	1.19	0.53	1.77
50505	PCAF	PCAF	p300/CBP-associated factor	0.62	1.52	0.84	1.13
50722	PCAF	PCAF	p300/CBP-associated factor	0.63	1.52	0.86	1.03
48666	PCK2		phosphoenolpyruvate carboxykinase 2 (mitochondrial)	1.63	1.49	1.12	1.04
49707	PCNA		proliferating cell nuclear antigen	1.53	1.46	0.48	1.62
48427	PDE4B	phosphodiesterase 4B	phosphodiesterase 4B, cAMP-specific (dunce (Drosophila)-homolog phosphodiesterase E4)	1.29	1.22	1.43	1.39
48556	PDE4B	phosphodiesterase 4B	phosphodiesterase 4B, cAMP-specific (dunce (Drosophila)-homolog phosphodiesterase E4)	1.27	1.23	1.33	1.31
50639	PDIR		for protein disulfide isomerase-related	0.76	1.31	1.02	1.00
46602	PEA15	Pea-15, MAT1	phosphoprotein enriched in astrocytes 15	1.36	1.33	1.21	1.12
46919	PFKL		phosphofructokinase, liver	1.39	1.36	0.91	1.00
46901	PIG3		quinone oxidoreductase homolog	1.35	1.30	1.03	1.00
49525	PIGQ		phosphatidylinositol glycan, class Q	1.62	1.36	0.89	1.00
48988	PIK3CB	p110 (PI3K)	phosphoinositide-3-kinase, catalytic, beta polypeptide	0.67	1.36	0.90	1.09
47924	PIK3CB	p110 (PI3K)	phosphoinositide-3-kinase, catalytic, beta polypeptide	0.68	1.37	0.90	1.08
49975	PIM1	pim-1 oncogene	pim-1 oncogene	1.43	1.31	1.09	1.06
48805	PLP1		proteolipid protein 1 (Pelizaeus-Merzbacher disease, spastic paraplegia 2, uncomplicated)	0.65	1.41	0.87	1.10
47776	POLA		polymerase (DNA directed), alpha	0.87	1.10	0.73	1.32
48531	PREP		prolyl endopeptidase	0.63	1.43	0.94	1.00
49331	PRKAR1A	PKA, regulatory subunit, type I alpha	protein kinase, cAMP-dependent, regulatory, type I, alpha (tissue specific extinguisher 1)	0.95	1.00	1.40	1.37
46646	PRKCE	PKC epsilon	protein kinase C, epsilon	0.47	1.67	0.96	1.00
47998	PSMB9		proteasome (prosome, macropain) subunit, beta type, 9 (large multifunctional protease 2)	1.56	1.43	0.89	1.06
46632	PTGES	PTGES	prostaglandin H synthase	2.12	1.79	1.00	1.00

48625	PTGS1	PTGS1	prostaglandin-endoperoxide synthase 1 (prostaglandin G/H synthase and cyclooxygenase)	0.59	1.62	1.17	1.06
49852	PTK2	FAK	PTK2 protein tyrosine kinase 2	0.62	1.53	0.91	1.05
50353	PTK2	FAK	PTK2 protein tyrosine kinase 2	0.67	1.39	0.95	1.00
49867	PTN		pleiotrophin (heparin binding growth factor 8, neurite growth-promoting factor 1)	1.48	1.35	0.85	1.02
47981	PTPRG	PTPRG	protein tyrosine phosphatase, receptor type, G	0.34	2.48	0.83	1.00
49533	PTPRG	PTPRG	protein tyrosine phosphatase, receptor type, G	0.36	2.25	1.04	1.00
50348	PTPRG	PTPRG	protein tyrosine phosphatase, receptor type, G	0.43	1.65	0.97	1.00
50632	PTPRJ	PTPRJ	protein tyrosine phosphatase, receptor type, J	0.59	1.45	0.88	1.06
47698	PTPRJ	PTPRJ	protein tyrosine phosphatase, receptor type, J	0.71	1.33	0.91	1.00
47548	PTPRK	PTPRK	protein tyrosine phosphatase, receptor type, K	0.49	1.84	1.02	1.00
48687	PTPRM	PTPRM	protein tyrosine phosphatase, receptor type, M	0.54	1.44	0.84	1.04
48279	PTPRM	PTPRM	protein tyrosine phosphatase, receptor type, M	0.49	1.76	1.06	1.00
50550	PTPRT	PTPRT	protein tyrosine phosphatase, receptor type, T	1.39	1.31	0.92	1.02
50374	RAB7	RAB7	RAB7, member RAS oncogene family	0.68	1.39	1.27	1.23
48258	RAB1F	RAB1F	RAB interacting factor	1.44	1.39	0.92	1.07
47205	RAB1F	RAB1F	RAB interacting factor	1.41	1.38	0.92	1.06
49876	RAD54L		RAD54 (S. cerevisiae)-like	1.41	1.20	0.64	1.31
49565	RASSF1		Ras association (RalGDS/AF-6) domain family 1	1.27	1.12	1.53	1.40
50076	RBBP8		retinoblastoma-binding protein 8	1.48	1.41	0.82	1.19
46658	RELB	I-rel	v-rel avian reticuloendotheliosis viral oncogene homolog B (nuclear factor of kappa light polypeptide gene enhancer in B-cells 3)	1.36	1.13	1.94	1.57
49120	RELB	I-rel	v-rel avian reticuloendotheliosis viral oncogene homolog B (nuclear factor of kappa light polypeptide gene enhancer in B-cells 3)	1.64	1.20	1.97	1.55
49087	RELB	I-rel	v-rel avian reticuloendotheliosis viral oncogene homolog B (nuclear factor of kappa light polypeptide gene enhancer in B-cells 3)	1.53	1.21	1.55	1.44
47933	RIPK2	RIP2, RICK	receptor-interacting serine-threonine kinase 2	1.56	1.50	1.38	1.19
47774	RPA2		replication protein A2 (32kD)	1.58	1.52	0.82	1.18
49934	RPC62		polymerase (RNA) III (DNA directed) (62kD)	1.42	1.33	1.24	1.15

48930	RPS6KA1	RSK1	ribosomal protein S6 kinase, 90kD, polypeptide 1	1.56	1.44	0.51	1.73
50288	RRAS2		oncogene TC21	1.43	1.36	1.14	1.10
46978	SCYA28	SCYA28	CC chemokine CCL28	1.07	1.00	0.75	1.30
49831	SCYC2	SCYC2	small inducible cytokine subfamily C, member 2	1.32	1.31	0.86	1.04
47571	SDC2	heparin sulfate proteoglycan-1	syndecan 2 (heparan sulfate proteoglycan 1, cell surface-associated, fibroglycan)	1.65	1.53	0.95	1.00
48646	SDCBP		syndecan binding protein (syntenin)	0.68	1.38	1.16	1.06
48415	SERPINB1		serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 1	0.69	1.39	0.87	1.10
49117	SERPINB1		serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 1	1.66	1.46	0.94	1.00
49428	SERPINB6		serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 6	1.54	1.38	0.99	1.00
47677	SERPINB6		serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 6	1.42	1.32	1.00	1.00
47710	SERPINB6		serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 6	1.39	1.31	1.02	1.00
49963	SERPINB8		serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 8	1.43	1.37	1.25	1.23
48205	SERPINB8		serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 8	1.37	1.31	1.20	1.14
48281	SERPINE2		serine (or cysteine) proteinase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 2	0.68	1.34	0.83	1.00
50577	SIAT4C		sialyltransferase 4C (beta-galactosidase alpha-2,3-sialyltransferase)	0.62	1.33	0.99	1.00
49880	SLC25A13		solute carrier family 25, member 13 (citrin)	0.59	1.62	0.95	1.02
46903	SLC3A2		solute carrier family 3 (activators of dibasic and neutral amino acid transport), member 2	1.39	1.32	1.67	1.59
50641	SLC5A1		solute carrier family 5 (sodium/glucose cotransporter), member 1	2.01	1.95	0.98	1.00
47960	SLC7A5		solute carrier family 7 (cationic amino acid transporter, y+ system), member 5	1.21	1.09	1.74	1.67
49446	SRC	Src	v-src avian sarcoma (Schmidt-Ruppin A-2) viral oncogene homolog	1.52	1.35	0.71	1.14
49218	SRD5A2		steroid-5-alpha-reductase, alpha polypeptide 2 (3-oxo-5 alpha-steroid delta 4-dehydrogenase alpha 2)	1.42	1.34	1.15	1.05
49184	SUPV3L1		suppressor of var1 (S. cerevisiae) 3-like 1	1.47	1.43	1.06	1.04
47800	SYTL2		synaptotagmin-like 2	1.63	1.58	0.81	1.11
47227	TANK	I-TRAF, TANK	TRAF family member-associated NFKB activator	0.93	1.00	1.39	1.31

50238	TARS		threonyl-tRNA synthetase	0.98	1.00	1.36	1.33
50707	TCF12	TCF12	transcription factor 12 (HTF4, helix-loop-helix transcription factors 4)	0.72	1.32	0.92	1.00
48562	TEAD1	TEAD1	TEA domain family member 1 (SV40 transcriptional enhancer factor)	0.70	1.36	1.12	1.03
48433	TEAD1	TEAD1	TEA domain family member 1 (SV40 transcriptional enhancer factor)	0.68	1.43	1.07	1.00
50389	TFRC	CD71, transferrin R	transferrin receptor (p90, CD71)	0.71	1.32	0.84	1.14
48513	TGFBR3		transforming growth factor, beta receptor III (betaglycan, 300kD)	0.68	1.32	0.79	1.14
50579	TGIF		TGFB-induced factor (TALE family homeobox)	0.92	1.01	1.44	1.36
47987	THBS1	THBS1	thrombospondin 1	0.80	1.19	0.64	1.33
48577	THBS3	THBS3	thrombospondin 3	1.61	1.33	0.79	1.11
48754	TIMP3		tissue inhibitor of metalloproteinase 3 (Sorsby fundus dystrophy, pseudoinflammatory)	0.67	1.31	0.94	1.01
47444	TKT		transketolase (Wernicke-Korsakoff syndrome)	0.65	1.44	1.04	1.02
47918	TNFAIP1	TNFAIP1	tumor necrosis factor, alpha-induced protein 1 (endothelial)	1.61	1.34	1.03	1.00
48770	TNFAIP3	A20, TNFAIP2, Zn finger	tumor necrosis factor, alpha-induced protein 3	1.31	1.00	1.70	1.64
47642	TNFAIP3	A20, TNFAIP2, Zn finger	tumor necrosis factor, alpha-induced protein 3	1.30	1.26	1.68	1.34
50458	TNFRSF21	DR6	tumor necrosis factor receptor superfamily, member 21	0.72	1.33	0.81	1.01
50363	TNFSF12	TWEAK	tumor necrosis factor (ligand) superfamily, member 12	0.70	1.18	2.67	2.12
47470	TXNIP	VDUP1	upregulated by 1,25-dihydroxyvitamin D-3	1.56	1.41	1.50	1.46
49812	TXNIP	VDUP1	upregulated by 1,25-dihydroxyvitamin D-3	1.57	1.49	1.46	1.42
49779	TXNIP	VDUP1	upregulated by 1,25-dihydroxyvitamin D-3	1.64	1.48	1.45	1.42
50573	UBE2N		ubiquitin-conjugating enzyme E2N (homologous to yeast UBC13)	0.72	1.32	0.99	1.00
49522	UCP2		uncoupling protein 2 (mitochondrial, proton carrier)	1.42	1.36	0.94	1.01
48807	UNG		uracil-DNA glycosylase	1.41	1.38	0.60	1.44
49258	UP	uridine phosphorylase	uridine phosphorylase	0.99	1.00	1.43	1.35
49129	UP	uridine phosphorylase	uridine phosphorylase	0.98	1.00	1.45	1.32

[illegible]

Table 5 – Differential Gene Expression in Direct Competitive Hybridization CPT+ Compound A vs. CPT+ Compound C

Spot ID	GeneSymbol	GeneName	GeneAssignment	direct competitive hybridization		individual hybridizations				(compared to CPT alone)							
				CPT + compound A	vs CPT + compound C	CPT +/- compound A		CPT +/- compound A	CPT +/- compound C	CPT +/- compound A	CPT +/- compound C						
										vs CPT + compound C							
										wtd means		min fold change (>1.3x)		wtd means		min fold change (>1.3x)	
46982	AREG		amphiregulin (schwannoma-derived growth factor)	2.06	1.92	1.00	1.00	1.00	1.00	1.00	1.00						
46569				0.55	1.43	1.28	1.00	0.74	1.00	1.00							
46895	C1orf29		hypothetical protein, expressed in osteoblast	1.69	1.42	0.87	1.00	1.00	1.00	1.00							
46987	TNNT1		troponin T1, skeletal, slow	0.74	1.32	1.18	1.05	0.96	1.01	1.01							
50373	GBP2	IFN induced GBP-2	guanylate binding protein 2, interferon-inducible	1.42	1.32	0.72	1.06	0.84	1.13	1.13							
46811	SELL	L-selectin	selectin L (lymphocyte adhesion molecule 1)	1.44	1.30	1.11	1.00	1.10	1.00	1.00							
47562	KIAA1919		KIAA1919 protein	0.76	1.30	1.18	1.04	0.94	1.04	1.04							

EXAMPLE 5

- [48] A manual comparison was done of expression responses to CPT+ compound A to CPT+ compound C. The results are shown in Table 6. This comparison serves as an internal control of the direct competitive hybridization, discussed above. However, this type of comparison is not as stringent as the direct comparison, since no p-value can be assigned to any observed differences.
- [49] Twenty-four most highly regulated genes in CPT+ compound A (min fold change $\geq 1.67x$) also are similarly regulated by CPT+ compound C. Sixty percent of all CPT+ compound A reg

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- [50] ulated genes (54 genes) also are differentially expressed in response to CPT+ compound C. The majority of the CPT+ compound A regulated genes that are not significantly regulated by CPT+ compound C show a similar expression trend in CPT+ compound C, although the degree of differential expression does not meet the cut-off criteria of ≥ 1.3 .

Cell cycle and other MOA-related genes

- [51] Most MOA-related genes differentially expressed in response to CPT+ compound A were similarly affected by CPT+ compound C (*e.g.*, cycE1, cycE2, cdkn3, NFkB components, cks2, cdk7, p21). There are a few MOA-related genes differentially expressed only by CPT+ compound A. However, every gene but UBE2C (1.52x) and FosB (1.59x) has min fold change $< 1.5x$, so these differential responses are weak. Although a few genes do miss the threshold cut off in the CPT+ compound C group, expression of these genes shows a similar trend of min fold change to that seen in CPT+ compound A.

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Table 6 - Comparison of Differential Gene Expression in CPT vs. CPT+ compound A hybridization and CPT vs. CPT+ compound C hybridization

Spot ID	GeneSymbol	GeneName	GeneAssignment	CPT +/- compound A wid means	CPT +/- compound A min fold change (≥1.3x)	CPT +/- compound C wid means	CPT +/- compound C min fold change (≥1.3x)
48739				1.86	1.67	2.10	1.71
50279				0.72	1.22	0.64	1.49
49056				1.59	1.56	1.43	1.39
48887				1.47	1.38	1.30	1.10
50574			semaphorin Y	1.41	1.32	1.22	1.04
47778				1.45	1.33	1.70	1.03
50443				1.50	1.44	1.27	1.01
46569				1.28	1.00	0.74	1.00
50633	ABL2		v-abl Abelson murine leukemia viral oncogene homolog 2 (arg, Abelson-related gene)	2.06	1.70	2.00	1.93
46933	ACTB		actin, beta	1.11	1.06	1.47	1.32
47913	ADAMTS1	ADAMTS1	a disintegrin-like and metalloprotease (reprolysin type) with thrombospondin type 1 motif, 1	1.76	1.24	1.74	1.55
46982	AREG	amphiregulin	amphiregulin (schwannoma-derived growth factor)	1.00	1.00	1.00	1.00
48346	ARHGEF2	ARHGEF2	rho/rac guanine nucleotide exchange factor (GEF) 2	1.65	1.52	2.18	1.79
48277	ARHGEF2	ARHGEF2	rho/rac guanine nucleotide exchange factor (GEF) 2	1.62	1.47	1.83	1.53
48516	ARL6IP		ADP-ribosylation factor-like 6 interacting protein	3.14	2.86	2.45	2.20
46817	ATF3	ATF3	activating transcription factor 3	2.46	2.35	2.03	1.98
46662	ATF4	ATF4	activating transcription factor 4 (tax-responsive enhancer element B67)	1.69	1.56	1.51	1.46
50523	ATF4	ATF4	activating transcription factor 4 (tax-responsive enhancer element B67)	1.68	1.64	1.57	1.37
47979	BDKRB1	BDKRB1	bradykinin receptor B1	0.51	1.39	1.00	1.00
50448	BIRC3	ciAP2	baculoviral IAP repeat-containing 3	1.50	1.24	1.56	1.47

47497	BIRC3	clAP2	baculoviral IAP repeat-containing 3	1.56	1.37	1.45	1.16
47516	BTG1		B-cell translocation gene 1, anti-proliferative	1.73	1.72	1.73	1.68
49673	BTN3A2	BTN3A2	butyrophilin, subfamily 3, member A2	0.68	1.38	0.73	1.18
46895	C1orf29		hypothetical protein, expressed in osteoblast	0.87	1.00	1.00	1.00
47917	CASP8AP2	CED4, FLASH	CASP8 associated protein 2	0.68	1.34	0.74	1.20
48015	CCND3	Cyclin D3	cyclin D3	0.69	1.36	0.80	1.15
46608	CCND3	Cyclin D3	cyclin D3	0.69	1.35	0.80	1.08
49850	CCNE1	cyclin E1	cyclin E1	0.43	1.98	0.44	1.93
50521	CCNE1	cyclin E1	cyclin E1	0.53	1.37	0.52	1.72
47926	CCNE2	cyclin E2	cyclin E2	0.19	4.86	0.27	2.87
49967	CCNE2	cyclin E2	cyclin E2	0.27	3.26	0.30	2.77
50392	CD97	CD97	CD97 antigen	1.35	1.28	1.39	1.33
50229	CDC2		cell division cycle 2, G1 to S and G2 to M	1.53	1.38	1.34	1.22
50358	CDK7	CDK7	cyclin-dependent kinase 7 (homolog of Xenopus MO15 cdk-activating kinase)	1.50	1.47	1.40	1.37
47382	CDK7	CDK7	cyclin-dependent kinase 7 (homolog of Xenopus MO15 cdk-activating kinase)	1.49	1.44	1.36	1.33
49066	CDKN1A	Waf1, Cip1	cyclin-dependent kinase inhibitor 1A (p21, Cip1)	1.39	1.18	1.72	1.54
48631	CDKN1A	Waf1, Cip1	cyclin-dependent kinase inhibitor 1A (p21, Cip1)	1.38	1.37	1.38	1.35
46842	CDKN3	cyclin dependent kinase inhibitor 3	cyclin-dependent kinase inhibitor 3 (CDK2-associated dual specificity phosphatase)	2.18	1.89	1.70	1.66
49105	CDKN3	cyclin dependent kinase inhibitor 3	cyclin-dependent kinase inhibitor 3 (CDK2-associated dual specificity phosphatase)	1.90	1.70	1.51	1.47
48313	CENPA		centromere protein A (17kD)	2.14	2.08	1.77	1.73
49737	CHAF1B		chromatin assembly factor 1, subunit B (p60)	0.61	1.46	0.54	1.45
50590	CKS2		CDC28 protein kinase 2	2.94	2.49	2.45	2.41
47545	CREBBP	CREB binding protein	CREB binding protein (Rubinstein-Taybi syndrome)	1.41	1.33	1.43	1.21
48953	CSDA	CSDA	cold shock domain protein A	1.25	1.14	1.39	1.34
48924	CSE1L		chromosome segregation 1 (yeast homolog)-like	1.43	1.36	1.38	1.29
50132	DDIT3	CHOP	DNA-damage-inducible transcript 3	2.75	2.60	2.26	2.05

49433	DDIT3	CHOP	DNA-damage-inducible transcript 3	2.74	2.68	2.36	1.96
50046	DUSP1	MKP-1, PTPN10	dual specificity phosphatase 1	1.68	1.58	1.68	1.52
49389	DUSP1	MKP-1, PTPN10	dual specificity phosphatase 1	1.54	1.35	1.57	1.39
47279	DUSP10	DUSP10	dual specificity phosphatase 10	1.58	1.47	1.63	1.49
50362	DUSP10	DUSP10	dual specificity phosphatase 10	1.66	1.53	1.62	1.42
46668	DUSP2	PAC-1	dual specificity phosphatase 2	1.55	1.34	1.45	1.27
48859	EGR1		early growth response 1	3.74	3.21	3.37	3.23
48565	EGR2	EGR2	early growth response 2 (Krox-20 (Drosophila) homolog)	2.06	1.64	2.19	2.12
48213	EGR2	EGR2	early growth response 2 (Krox-20 (Drosophila) homolog)	1.91	1.30	1.89	1.83
47274	EMR1		egf-like module containing, mucin-like, hormone receptor-like sequence 1	0.52	1.45	1.00	1.00
47730	ERBB3	ErbB3	v-erb-b2 avian erythroblastic leukemia viral oncogene homolog 3	0.69	1.37	0.74	1.31
49672	ETS2	Ets-2	v-ets avian erythroblastosis virus E26 oncogene homolog 2	1.41	1.31	1.39	1.34
49637	F2	F2	coagulation factor II (thrombin)	0.72	1.33	0.77	1.24
49721	FANCG		Fanconi's anemia, complementation group G	0.69	1.33	0.63	1.38
48273	FCGR2A	CD32	Fc fragment of IgG, low affinity IIa, receptor for (CD32)	0.71	1.19	0.69	1.36
50715	FCGR2A	CD32	Fc fragment of IgG, low affinity IIa, receptor for (CD32)	0.71	1.23	0.70	1.35
48977	FCGR2A	CD32	Fc fragment of IgG, low affinity IIa, receptor for (CD32)	0.71	1.30	0.72	1.28
49326	FCGR2A	CD32	Fc fragment of IgG, low affinity IIa, receptor for (CD32)	0.70	1.32	0.72	1.14
48489	FKBP1B		FK506-binding protein 1B (12.6 kD)	2.09	1.71	2.12	1.78
50101	FOS	c-fos	v-fos FBJ murine osteosarcoma viral oncogene homolog	2.37	1.94	2.06	1.93
47566	FOS	c-fos	v-fos FBJ murine osteosarcoma viral oncogene homolog	1.95	1.78	1.95	1.90
46829	FOSB	FosB	FBJ murine osteosarcoma viral oncogene homolog B	1.68	1.59	1.26	1.21
48969	FOSL2	FOSL2, Fra2	FOS-like antigen 2	1.38	1.17	1.43	1.36
47264	GADD45A		growth arrest and DNA-damage-inducible, alpha	1.57	1.53	1.36	1.33
46583	GADD45B	MyD118	growth arrest and DNA-damage-inducible, beta	1.69	1.47	1.51	1.41
50373	GBP2	IFN induced GBP-2	guanylate binding protein 2, interferon-inducible	0.72	1.06	0.84	1.13

49692	GRO3	GRO3	GRO3 oncogene	1.67	1.34	1.00	1.00
47831	HEC		highly expressed in cancer, rich in leucine heptad repeats	1.45	1.39	1.24	1.18
46778	HMG2		high-mobility group (nonhistone chromosomal) protein 2	1.48	1.42	1.35	1.26
47414	HSF4		heat shock transcription factor 4	0.93	1.00	0.73	1.31
49516	HSPA5	HSPA5	heat shock 70kD protein 5 (glucose-regulated protein, 78kD)	1.27	1.23	1.45	1.42
49328	HSPA5	HSPA5	heat shock 70kD protein 5 (glucose-regulated protein, 78kD)	1.32	1.29	1.38	1.35
48605	HSXIAPAF1	HSXIAPAF1	XIAP associated factor-1	0.65	1.48	0.83	1.15
49681	HSXIAPAF1	HSXIAPAF1	XIAP associated factor-1	0.65	1.46	0.86	1.01
46856	IL8	interleukin-8	interleukin 8	2.95	2.91	2.48	2.34
47989	JUN	c-jun, cJun	v-jun avian sarcoma virus 17 oncogene homolog	1.24	1.20	1.62	1.40
49322	JUNB	JunB	jun B proto-oncogene	1.54	1.46	1.53	1.42
49319	JUNB	JunB	jun B proto-oncogene	1.50	1.14	1.54	1.39
50205	KIAA0007		KIAA0007 protein	1.44	1.37	1.32	1.24
48862	KIAA0008		KIAA0008 gene product	1.83	1.70	1.70	1.61
47234	KIAA0097		KIAA0097 gene product	1.56	1.44	1.60	1.44
50636	KIAA0101		KIAA0101 gene product	0.64	1.26	0.62	1.35
47562	KIAA1919		KIAA1919 protein	1.18	1.04	0.94	1.04
50568	KNSL5		kinesin-like 5 (mitotic kinesin-like protein 1)	1.40	1.39	1.22	1.15
48673	LRDD	LRDD	leucine-rich and death domain containing	0.61	1.26	0.51	1.58
49376	MAC30		hypothetical protein	0.66	1.47	0.73	1.32
47029	MAD	MAD	MAX dimerization protein	1.56	1.48	1.70	1.66
47158	MAD	MAD	MAX dimerization protein	1.54	1.51	1.50	1.47
47267	MAD4	MAD4	Mad4 homolog	0.55	1.43	1.00	1.00
48278	MAP2K3	MKK3	mitogen-activated protein kinase kinase 3	1.45	1.42	1.48	1.42
48028	MAP2K3	MKK3	mitogen-activated protein kinase kinase 3	1.37	1.32	1.41	1.30
47123	MCM2		minichromosome maintenance deficient (S. cerevisiae) 2 (mitotin)	0.55	1.52	0.63	1.49

48470	MCM5			minichromosome maintenance deficient (S. cerevisiae) 5 (cell division cycle 46)	0.63	1.37	0.62	1.52
49996	MHC2TA	MHCII transactivator		MHC class II transactivator	0.69	1.15	0.70	1.33
47732	MYC	c-Myc		v-myc avian myelocytomatosis viral oncogene homolog	1.59	1.53	1.45	1.39
47173	NFKB1	NFkappaB, p105		nuclear factor of kappa light polypeptide gene enhancer in B-cells 1 (p105)	1.42	1.37	1.32	1.27
50676	NFKB2	NFkappaB, p49/p100		nuclear factor of kappa light polypeptide gene enhancer in B-cells 2 (p49/p100)	1.34	1.30	1.32	1.23
50542	NFKBIE	IkB, epsilon		nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, epsilon	1.54	1.28	1.53	1.49
48405	NFKBIE	IkB, epsilon		nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, epsilon	1.55	1.35	1.50	1.37
48941	NR4A1	NAK1		nuclear receptor subfamily 4, group A, member 1	1.47	1.21	1.44	1.36
47897	NR4A2	TINUR		nuclear receptor subfamily 4, group A, member 2	1.21	1.05	1.47	1.43
50431	PASK			PAS-serine/threonine kinase	0.60	1.19	0.53	1.77
49707	PCNA			proliferating cell nuclear antigen	0.43	1.89	0.48	1.62
48427	PDE4B	phosphodiesterase 4B		phosphodiesterase 4B; cAMP-specific (dunce (Drosophila))-homolog phosphodiesterase E4)	1.43	1.34	1.43	1.39
48556	PDE4B	phosphodiesterase 4B		phosphodiesterase 4B; cAMP-specific (dunce (Drosophila))-homolog phosphodiesterase E4)	1.33	1.20	1.33	1.31
48988	PIK3CB	p110 (PI3K)		phosphoinositide-3-kinase, catalytic, beta polypeptide	0.72	1.34	0.90	1.09
47776	POLA			polymerase (DNA directed), alpha	0.65	1.50	0.73	1.32
49331	PRKAR1A	PKA, regulatory subunit, type I alpha		protein kinase, cAMP-dependent, regulatory, type I, alpha (tissue specific extinguisher 1)	1.30	1.27	1.40	1.37
47618	PTGIS	PTGIS		prostaglandin I2 (prostacyclin) synthase	0.54	1.67	1.00	1.00
49876	RAD54L			RAD54 (S. cerevisiae)-like	0.63	1.43	0.64	1.31
49565	RASSF1			Ras association (RalGDS/AF-6) domain family 1	1.65	1.49	1.53	1.40
46658	RELB	I-rel		v-rel avian reticuloendotheliosis viral oncogene homolog B (nuclear factor of kappa light polypeptide gene enhancer in B-cells 3)	1.82	1.33	1.94	1.57
49120	RELB	I-rel		kappa light polypeptide gene enhancer in B-cells 3)	1.85	1.54	1.97	1.55
49087	RELB	I-rel		v-rel avian reticuloendotheliosis viral oncogene homolog B (nuclear factor of kappa light polypeptide gene enhancer in B-cells 3)	1.72	1.47	1.55	1.44
47933	RIPK2	RIP2, RICK		receptor-interacting serine-threonine kinase 2	1.62	1.52	1.38	1.19
48930	RPS6KA1	RSK1		ribosomal protein S6 kinase, 90kD, polypeptide 1	0.44	1.93	0.51	1.73

48190	SCYA20	MIP-3a	small inducible cytokine subfamily A (Cys-Cys), member 20	2.11	1.30	1.00	1.00
46978	SCYA28	SCYA28	CC chemokine CCL28	0.77	1.10	0.75	1.30
46811	SELL	L-selectin	selectin L (lymphocyte adhesion molecule 1)	1.11	1.00	1.10	1.00
46903	SLC3A2		solute carrier family 3 (activators of dibasic and neutral amino acid transport), member 2	1.87	1.83	1.67	1.59
47960	SLC7A5		solute carrier family 7 (cationic amino acid transporter, y+ system), member 5	1.42	1.28	1.74	1.67
48182	SNAPC1		small nuclear RNA activating complex, polypeptide 1, 43kD	1.47	1.34	1.30	1.19
49323	SPARC	SPARC	secreted protein, acidic, cysteine-rich (osteonectin)	0.70	1.32	0.88	1.06
48462	SPRR2C		small proline-rich protein 2C	1.48	1.41	1.28	1.14
47227	TANK	I-TRAF, TANK	TRAF family member-associated NFKB activator	1.34	1.12	1.39	1.31
50238	TARS		threonyl-tRNA synthetase	1.32	1.17	1.36	1.33
50579	TGIF		TGFB-induced factor (TALE family homeobox)	1.45	1.40	1.44	1.36
47987	THBS1	THBS1	thrombospondin 1	0.85	1.00	0.64	1.33
46859	TIEG	TGF beta-inducible early growth response A20, TNFAIP2, Zn finger	TGFB inducible early growth response	1.62	1.59	1.50	1.28
48770	TNFAIP3	TNFAIP3	tumor necrosis factor, alpha-induced protein 3	1.62	1.22	1.70	1.64
47642	TNFAIP3	A20, TNFAIP2, Zn finger	tumor necrosis factor, alpha-induced protein 3	1.93	1.74	1.68	1.34
46803	TNFAIP6	TNFAIP6	tumor necrosis factor, alpha-induced protein 6	1.38	1.35	1.23	1.14
50363	TNFSF12	TWEAK	tumor necrosis factor (ligand) superfamily, member 12	3.37	2.73	2.67	2.12
46987	TNNT1		troponin T1, skeletal, slow	1.18	1.05	0.96	1.01
47542	TRIO	TRIO	triple functional domain (PTPRF interacting)	1.35	1.31	1.20	1.16
49901	TROAP		trophin associated protein (tastin)	1.63	1.52	1.30	1.22
47470	TXNIP	VDUP1	upregulated by 1,25-dihydroxyvitamin D-3	1.57	1.48	1.50	1.46
49812	TXNIP	VDUP1	upregulated by 1,25-dihydroxyvitamin D-3	1.57	1.47	1.46	1.42
49779	TXNIP	VDUP1	upregulated by 1,25-dihydroxyvitamin D-3	1.59	1.52	1.45	1.42
48761	UBE2C		ubiquitin-conjugating enzyme E2C	1.75	1.52	1.49	1.28
48807	UNG		uracil-DNA glycosylase	0.49	1.96	0.60	1.44

49258	UP	uridine phosphorylase	uridine phosphorylase	1.29	1.16	1.43	1.35
49129	UP	uridine phosphorylase	uridine phosphorylase	1.31	1.21	1.45	1.32
47298	VAV3	Vav3	vav 3 oncogene	0.68	1.35	0.70	1.20
48262	VAV3	Vav3	vav 3 oncogene	0.66	1.46	0.80	1.08
47787	VGF		VGF nerve growth factor inducible	2.27	2.08	2.30	2.02
50028	XBP1	XBP-1	X-box binding protein 1	1.76	1.66	1.80	1.77
48979	ZFP36	tristetraprolin	zinc finger protein homologous to Zfp-36 in mouse	1.94	1.86	1.73	1.63
50466	ZFP36	tristetraprolin	zinc finger protein homologous to Zfp-36 in mouse	1.88	1.66	1.56	1.42

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EXAMPLE 6

Cell cycle release assay

- [52] To confirm the ability of the CCR⁺ compound to release cells from CPT-induced cell cycle arrest, cells were treated with 50 nM CPT for 18 hours, followed by 6 hours of CCR⁺. As shown in Figure 1 and Table 7, CPT induced S phase arrest with 81% of cells accumulating in S phase. Following 6 hours of incubation with the CCR⁺ compound, cells were released from CPT-induced S-phase arrest and progressed into G2/M. The ability of CCR⁺ to release cells from CPT-induced arrest after 6 hours suggested that transcriptional changes in response to 6 hours of CCR⁺ treatment would be relevant to the compound's MOA, and differentially expressed genes could be considered as reasonable biomarkers of Chk1 inhibitor activity.

Table 7

<i>6hr post CCR⁺ treatment</i>	<i>% G0/G1</i>	<i>% S</i>	<i>% G2/M</i>	<i>% G0/G1</i>	<i>% S</i>	<i>% G2/M</i>
<i>No treatment</i>	43.41	39.3	17.29	43.05	39.8	17.15
	42.69	40.3	17.01			
<i>50nM CPT</i>	5.58	78.65	15.77	4.895	81.245	13.86
	4.21	83.84	11.95			
<i>30nM CCR⁺</i>	45.06	38.47	16.47	44.29	38.995	16.715
	43.52	39.52	16.96			
<i>50nM CPT+30nM CCR⁺</i>	3.98	14.61	81.41	3.915	13.545	82.545
	3.85	12.48	83.68			

EXAMPLE 7

Validation of Chk1 inhibition surrogate marker genes by quantitative RT-PCR

- [53] Validation of several surrogate markers of Chk1 inhibition, including cyclin E1 (CCNE1), cyclin E2 (CCNE2), DDIT3, and EGR1, was carried out using quantitative RT-PCR (qRT-PCR). qRT-PCR using the same mRNA used for the transcriptional profiling confirmed the transcriptional changes of the surrogate marker genes. RNA was prepared using the RNeasy kit (Qiagen Corp., Valencia, CA) according to manufacturer's instructions, then two-step qRT-PCR was carried out. cDNA was generated by combining 4 µl of eluted RNA with the reaction buffer containing MMLV RT 10x buffer (Ambion, Inc., Austin, TX), 10 pmoles oligo dT (Ambion), 10 pmoles random decamers

- (Ambion), 125 μ M each dNTP (Roche Applied Science, Indianapolis, IN), 20 units RNase inhibitor (Ambion), and 50 units of MMLV reverse transcriptase (Ambion). The reaction was incubated for 1 hour at 42°C, and 2 μ l of the cDNA was then used for the PCR reaction.
- [54] Cyclin E1, cyclin E2, DDIT3, EGR1, and GAPDH PCR primers and probes were from Assays-on-Demand Gene Expression Kits (Applied Biosystems, Foster City, CA). The PCR reaction was done according to the Assays-on-Demand protocol for a 50 μ l reaction, with 22.5 μ l from the cDNA preparation used for each PCR reaction. The reaction parameters were as follows: 10 minute hold at 95°C, 45 cycles of 15 seconds at 95°C, 1 minute at 60°C.
- [55] As shown in Figures 2A and 2B, the differential expression pattern for these markers confirmed the array results.
- [56] To further confirm the reproducibility of the array results, three of these markers (CCNE2, DDIT3, and EGR1) were selected for further validation. A new experiment was performed in a 96-well plate format, under the same treatment conditions as that used for the transcriptional profiling. RNA was harvested following incubation with CPT, the Chk1 inhibitor compounds, and the inactive control compound. qRT-PCR was then performed as described above.
- [57] As shown in Figures 2C and 2D, the observed expression changes in response to CPT+compounds compared to CPT alone for the three markers were similar to changes observed in both the initial array results and the confirmatory qRT-PCR (Figures 2A and 2B).

EXAMPLE 8

Chk1 inhibitors have dose dependent effect on surrogate marker gene expression

- [58] The qRT-PCR data from the 96-well plate experiment (Figures 2C and 2D) suggested that the active Chk1 inhibitors (CCR⁺ and CCR⁻, *i.e.*, compounds A and C) altered the

CPT-dependent changes in surrogate marker mRNA expression to different degrees. For example, CPT+CCR⁺ appeared to induce CCNE2 to a greater extent than CPT+CCR⁻ (compared to CPT alone).

- [59] A dose response study was performed to investigate whether these markers could be used to rank compounds according to their potency against Chk1. For the initial dose response experiment (with CCR⁺, CCR⁻, and inactive compounds), cells were seeded at 5×10^4 cells/well in 96-well flat bottom plates in OptiMEM I + 4% FBS. Cells were pre-incubated with 50 nM CPT for 18 hours, and then incubated with 1, 3, 10, 30, 100, and 300 nM of the CCR⁺, CCR⁻, or inactive compound for 6 hours.
- [60] As shown in Figure 3, while the inactive compound (in the presence of CPT) had no effect on gene expression, the three markers showed a dose-dependent response to the active compounds (CCR⁺ and CCR⁻) in the presence of DNA damage. Further, the CCR⁺ compound had a larger effect on gene expression than the CCR⁻ compound, consistent with the observation that CCR⁺ was more effective in the functional cell cycle release assay.
- [61] To further investigate the potential to use these surrogate markers to rank the potency of Chk1 inhibitor compounds, cells were treated with an additional 15 compounds. MDA-MB-435 cells were seeded at 5×10^4 cells/well in 96-well flat bottom plates in OptiMEM I + 4% FBS. Cells were pre-incubated with 50 nM CPT for 18 hours and then incubated with 5, 15, 50, 150, 450, and 1000 nM of the experimental compounds for 6 hours. A broader range of concentrations was used in this experiment compared to the initial study to allow detection of a dose-response across the range of IC₅₀ values of this set of compounds. Twelve active Chk1 inhibitors and three inactive compounds were selected for this expanded validation study.
- [62] The results for a subset of compounds are shown in Figure 4. Similar to the results with the initial compounds in Figure 3, these additional compounds also showed a range of efficacies in their ability to alter gene expression. Based on the dose-response analyses, EC₅₀ values were generated for the initial 3 compounds (CCR⁺, CCR⁻, inactive/NEG) as well as for the additional 15 compounds (Table 8). The results of this expanded study

indicate that these markers are dose responsive for multiple compounds. Further, the ranking of the compounds based on the gene expression EC_{50} generally reflects the *in vitro* enzyme IC_{50} . Therefore, it is possible to use gene expression as measured by RT-PCR to determine rank order of potency of inhibitors against an enzyme *in situ*, in this case Chk1.

[63] Table 8

Compound	Chk1 IC50 (nM)	CCNE2 EC50 (nM)	EGR1 EC50 (nM)	DDIT3 EC50 (nM)
143124.1 (CCR+)	0.3	13.15	19.01	6.61
147161.1(active2)	0.2	14.84	64.19	No fit
158114.1(active3)	0.5	35.40	237.74	60.03
147168.1	1	54.10	289.90	48.80
162899.1	0.3	20.39	51.19	50.69
161461.2	1.5	106.24	676.49	85.80
143813.1(active1)	0.8	33.74	162.56	28.58
151022.1	1	60.21	468.93	42.17
151021.1	2	349.43	367.46	263.16
154248.1	4	400.38	583.28	197.29
163662.1	0.8	62.23	164.94	126.11
147166.1	4	316.14	427.16	231.48
157600.2(act/inact4)	0.5	94.76	657.27	182.84
157265.1 (CCR-)	0.2	40.10	83.91	26.84
155143.1 (NEG)	1700	>1000	>1000	>1000
156740.1(inact6)	56	>1000	>1000	>1000
157449.1	0.4	213.86	581.42	234.33
165993.1(inact5)	>10000	401.55	619.62	93.30

EXAMPLE 9

Surrogate marker expression correlates with compounds' effects on in vitro cell cycle assay.

- [64] The direct *in vitro* measure of functional Chk1 inhibition is the ability of an inhibitor to release MDA-MB-435 cells from CPT-induced S phase arrest. To confirm that the selected Chk1 inhibitor compounds were acting through the correct mechanism and were able to release cells from CPT-induced cell cycle arrest, cell cycle analyses were done using all 18 compounds included in the surrogate marker study. The EC₅₀ generated for each compound is included in Table 9.
- [65] A correlation analysis was carried out between the EC₅₀s generated from the qRT-PCR dose response experiments (Table 8) and from the cell cycle release assay (Table 9) to

determine how well the selected markers of Chk1 inhibition correlated with a direct functional *in vitro* cell cycle release assay. A plot of predicted EC₅₀ (based on RT-PCR results) and experimental EC₅₀ (detected by FACS) are shown in Figure 5.

- [66] Spearman correlation analysis demonstrated that the EC₅₀s of the surrogate markers correlated well with the cell cycle release EC₅₀s, indicating that the selected surrogate markers are predictive of the ability of the Chk1 inhibitor compounds to release cells from CPT-induced cell cycle arrest with p values of <0.02.
- [67] While the use of a direct functional assay is sometimes preferable to indirect methods for the evaluation of novel, targeted small molecule inhibitors, it is not always feasible to use such an assay, particularly if medium or high throughput is desirable. In the case of Chk1 inhibitors, the cell cycle release assay, while a direct measure of Chk1 inhibition, is very labor intensive, particularly if dose response analyses are done. As a result, identification of biomarkers whose changes in expression correlate well with the functional effect of these inhibitors has clear utility. For example, RT-PCR can be used to assay many compounds rapidly in 96-well or other multiplexed format, as demonstrated here.
- [68] As experimental Chk1 inhibitors advance into *in vivo* models and through to clinical trials, there will be an ongoing need for markers that are significantly correlated with Chk1 inhibition and more importantly, the functional effect of this inhibition on release of DNA-damage cells from cell cycle arrest. The biomarkers identified here appear to be effective in ranking the potency of the compounds in inhibiting Chk1 and in their ability to release cells from DNA damage-induced cell cycle arrest. In the clinical setting, these markers could be used to monitor drug exposure and efficacy, where direct measurement of efficacy is often not possible or practical.

[69] Table 9

Compound	Chk1 IC50 (nM)	CCR EC50 (nM)
143124.1 (CCR+)	0.3	8
147161.1	0.2	13
158114.1	0.5	30
147168.1	1	33
162899.1	0.3	39
161461.2	1.5	(141) (47)
143813.1	0.8	50-150
151022.1	1	125
151021.1	2	150
154248.1	4	210
163662.1	0.8	360
147166.1	4	400
157600.2	0.5	700
157265.1 (CCR-)	0.2	-
155143.1 (NEG)	1700	-
156740.1	56	-
157449.1	0.4	-
165993.1	>10000	N/A

WHAT IS CLAIMED IS:

1. A method of identifying an antineoplastic agent, comprising the steps of:
contacting cancer cells with a DNA damaging agent and a test compound;
and
determining expression of at least one surrogate marker gene selected from the group consisting of surrogate marker genes identified in Tables 2, 3, and 6, wherein a greater than 1.3-fold change in expression of the at least one surrogate marker gene identifies the test compound as a potential antineoplastic agent.
2. The method of claim 1 wherein expression of at least two surrogate marker genes is determined.
3. The method of claim 1 wherein expression of at least three surrogate marker genes is determined.
4. The method of claim 1 wherein expression of at least four surrogate marker genes is determined.
5. The method of claim 1 wherein expression is determined by quantitative measurements of mRNA or protein levels.
6. The method of claim 1 wherein the at least one surrogate marker gene is selected from the group consisting of cyclin E2 (CCNE2), cyclin E1 (CCNE1), DDIT3, and EGR1.
7. The method of claim 4 wherein the at least four surrogate marker genes are CCNE2, CCNE1, DDIT3, and EGR1.
8. The method of claim 1 wherein the DNA damaging agent is a chemosensitizing agent.

9. The method of claim 8 wherein the chemosensitizing agent is a topoisomerase inhibitor.

10. The method of claim 9 wherein the topoisomerase inhibitor is camptothecin.

11. The method of claim 1 wherein the cancer cells are *in vitro*.

12. The method of claim 11 wherein expression is determined in a high throughput format.

13. The method of claim 1 wherein the cancer cells are *in vivo*.

14. A method for assessing *in vivo* efficacy of a Chk1 inhibitor for cancer treatment, comprising the steps of:

administering to an experimental animal model bearing a tumor a DNA damaging agent and a Chk1 inhibitor; and

determining expression of at least one surrogate marker gene selected from the group consisting of surrogate marker genes identified in Tables 2, 3, and 6, wherein a greater than 1.3-fold change in expression of the at least one surrogate marker gene identifies the Chk1 inhibitor as potentially efficacious for the treatment of cancer.

15. The method of claim 14 wherein expression of at least two surrogate marker genes is determined.

16. The method of claim 14 wherein expression of at least three surrogate marker genes is determined.

17. The method of claim 14 wherein expression of at least four surrogate marker genes is determined.

18. The method of claim 14 wherein expression is determined by quantitative measurements of mRNA or protein levels.

19. The method of claim 14 wherein the at least one surrogate marker gene is selected from the group consisting of CCNE2, CCNE1, DDIT3, and EGR1.

20. The method of claim 17 wherein the at least four surrogate marker genes are CCNE2, CCNE1, DDIT3, and EGR1.

21. The method of claim 14 wherein the DNA damaging agent is a chemosensitizing agent.

22. The method of claim 21 wherein the chemosensitizing agent is a topoisomerase inhibitor.

23. The method of claim 22 wherein the topoisomerase inhibitor is camptothecin.

24. A method of estimating potency of a test compound to inhibit Chk1, comprising the steps of:

incubating cancer cells *in vitro* for a predetermined period of time with a DNA damaging agent;

incubating the cancer cells with increasing concentrations of a test compound;

determining expression of at least one surrogate marker gene selected from the group consisting of surrogate marker genes identified in Tables 2, 3, and 6 in the presence of each concentration of the test compound, wherein at least one concentration of the test compound results in a greater than 1.3-fold change in expression of the at least one surrogate marker gene; and

using changes in expression of the at least one surrogate marker gene at different concentrations of the test compound to calculate the EC₅₀ value of the test compound for altering expression of the at least one surrogate marker gene; and

using the calculated EC_{50} value to estimate an IC_{50} value for the test compound for inhibition of Chk1.

25. The method of claim 24 wherein expression of at least two surrogate marker genes is determined.

26. The method of claim 24 wherein expression of at least three surrogate marker genes is determined.

27. The method of claim 24 wherein expression of at least four surrogate marker genes is determined.

28. The method of claim 24 wherein expression is determined by quantitative measurements of mRNA or protein levels.

29. The method of claim 24 wherein the at least one surrogate marker gene is selected from the group consisting of cyclin E2 (CCNE2), cyclin E1 (CCNE1), DDIT3, and EGR1.

30. The method of claim 29 wherein the at least four surrogate marker genes are CCNE2, CCNE1, DDIT3, and EGR1.

31. The method of claim 24 wherein the DNA damaging agent is a chemosensitizing agent.

32. The method of claim 31 wherein the chemosensitizing agent is a topoisomerase inhibitor.

33. The method of claim 32 wherein the topoisomerase inhibitor is camptothecin.

34. The method of claim 24 wherein the test compound is known to inhibit Chk1.

35. The method of claim 24 which is carried out in a high throughput format.

36. A method to aid in monitoring clinical efficacy of a Chk1 inhibitor, comprising the steps of:

determining expression of at least one surrogate marker gene selected from the group consisting of surrogate marker genes identified in Tables 2, 3, and 6 in a biological sample obtained from a patient undergoing treatment with a DNA damaging agent and a specified dose of a Chk1 inhibitor, wherein a greater than 1.3-fold change in expression of the at least one surrogate marker gene indicates clinical efficacy of the specified dose of the Chk1 inhibitor.

37. The method of claim 36 wherein expression of at least two surrogate marker genes is determined.

38. The method of claim 36 wherein expression of at least three surrogate marker genes is determined.

39. The method of claim 36 wherein expression of at least four surrogate marker genes is determined.

40. The method of claim 36 wherein expression is determined by quantitative measurements of mRNA or protein levels.

41. The method of claim 36 wherein the at least one surrogate marker gene is selected from the group consisting of cyclin E2 (CCNE2), cyclin E1 (CCNE1), DDIT3, and EGR1.

42. The method of claim 41 wherein the at least four surrogate marker genes are CCNE2, CCNE1, DDIT3, and EGR1.

43. The method of claim 36 wherein the DNA damaging agent is a chemosensitizing agent.

44. The method of claim 43 wherein the chemosensitizing agent is a topoisomerase inhibitor.

45. The method of claim 44 wherein the topoisomerase inhibitor is camptothecin.

FIG. 1

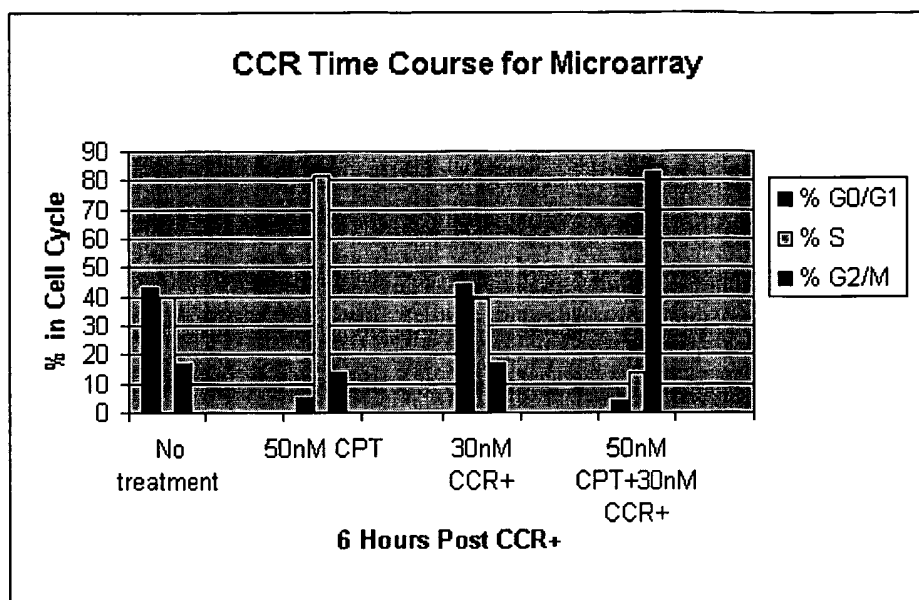


FIGURE 2A

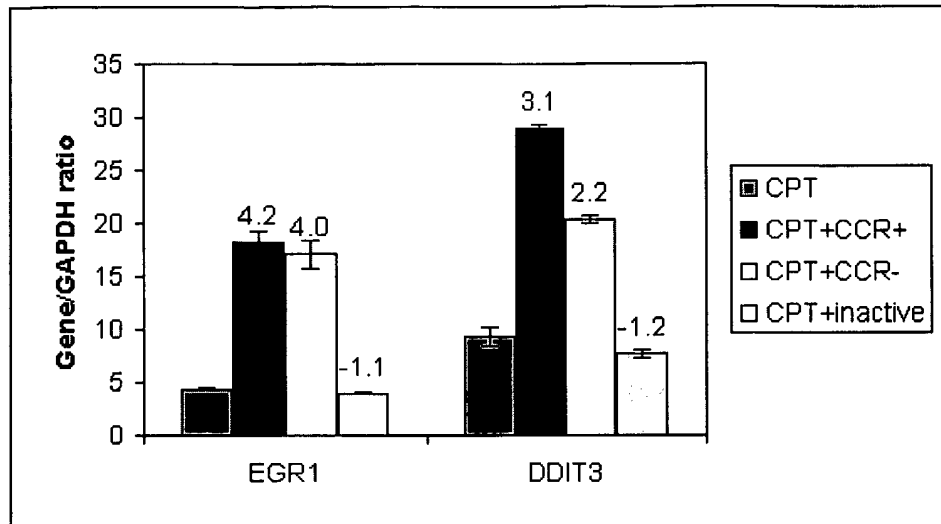


FIGURE 2B

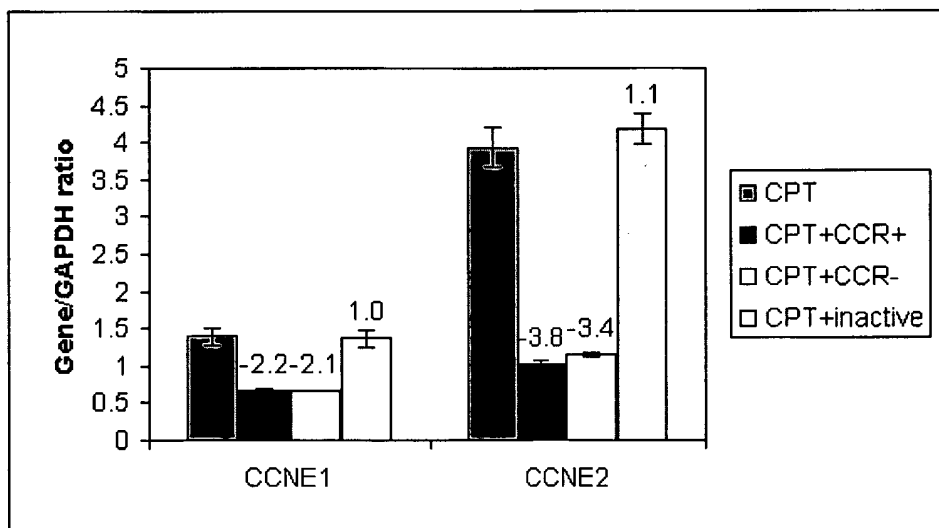


FIGURE 2C

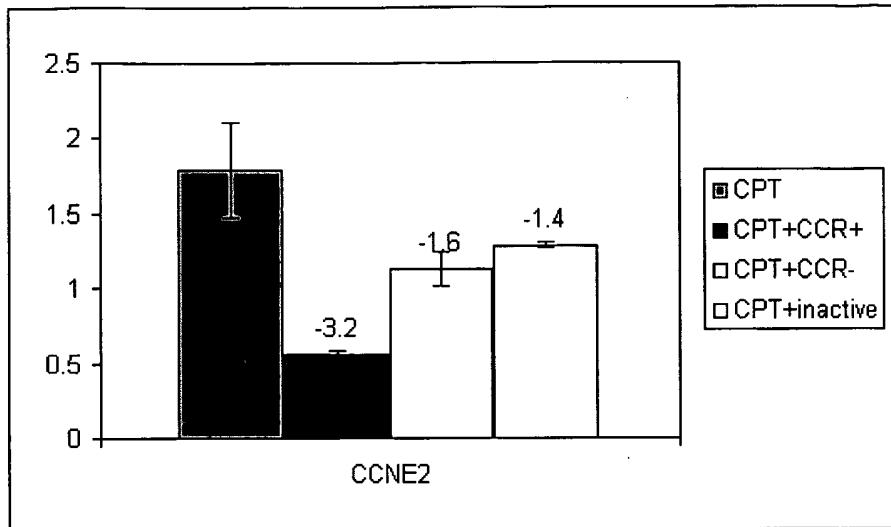


FIGURE 2D

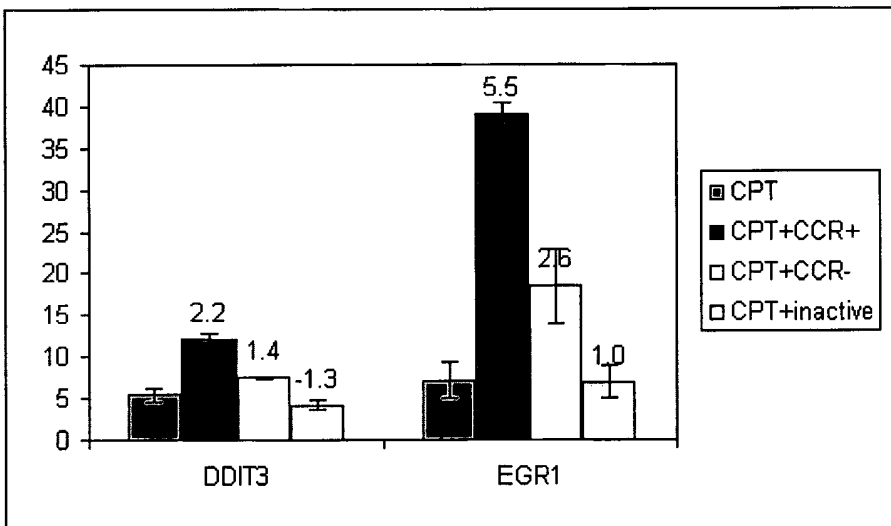


FIGURE 3A

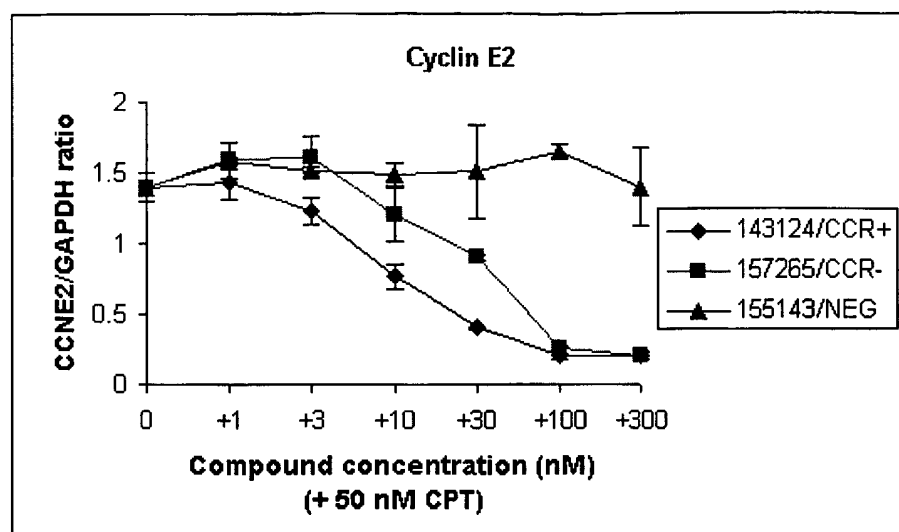


FIGURE 3B

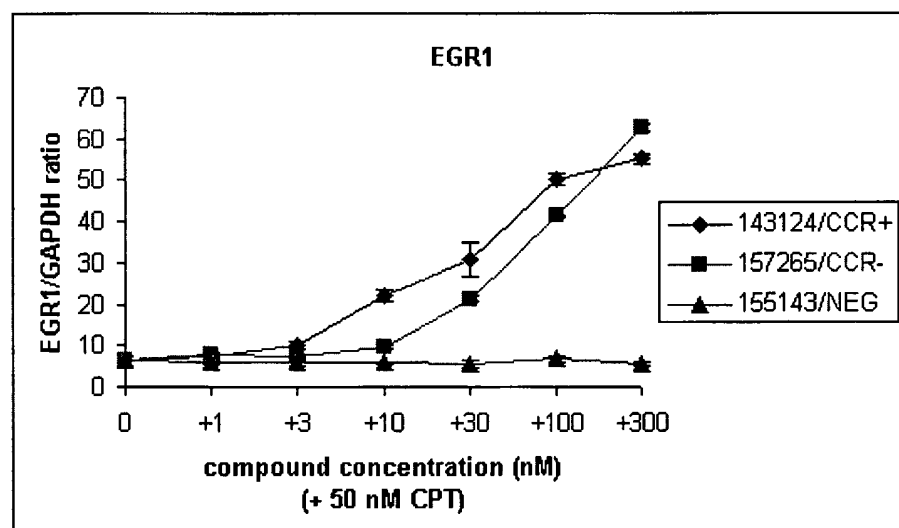


FIGURE 3C

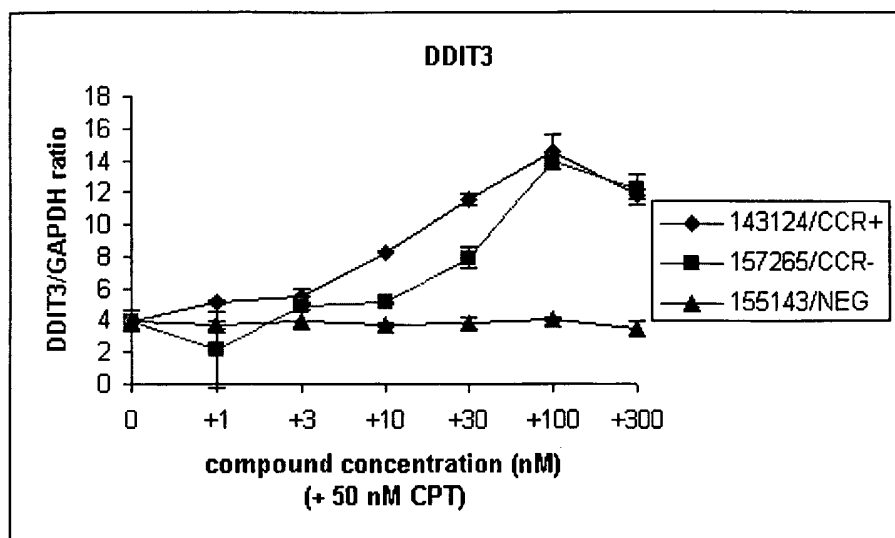


Figure 4A

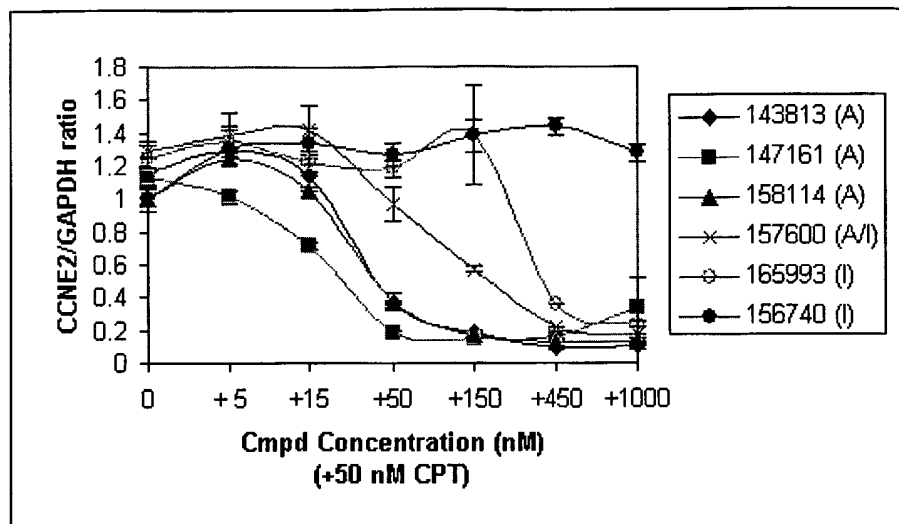


FIGURE 4B

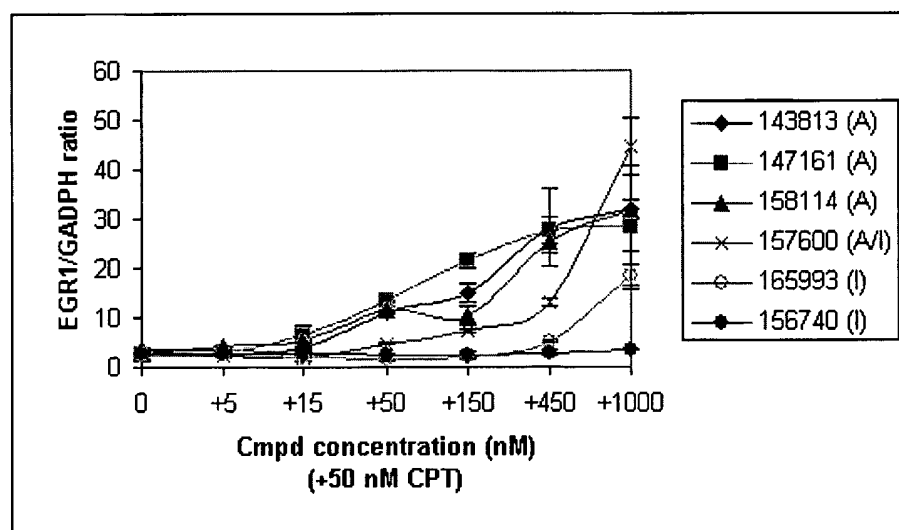


FIGURE 4C

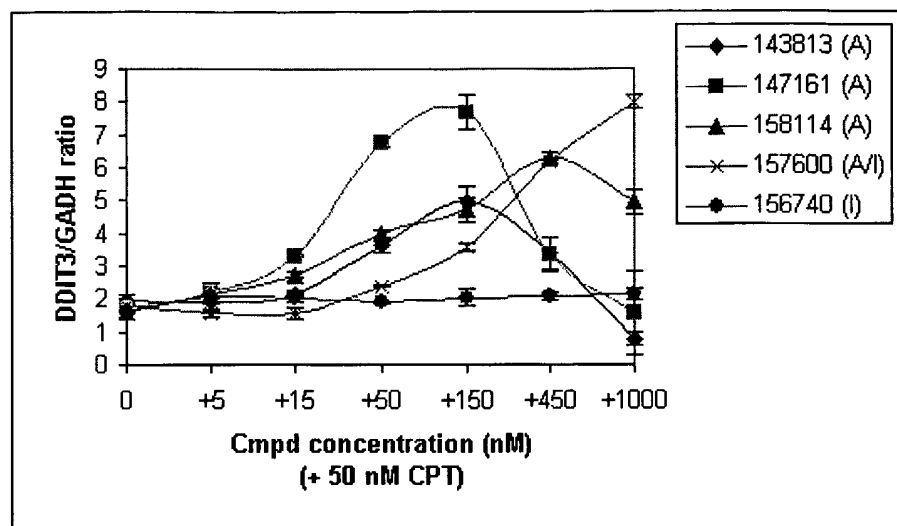


Figure 5

