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(54) Title: METHODS AND MOLECULAR PHARMACODYNAMIC BIOMARKERS FOR MULTIPLE SIGNALING PATHWAYS IN RESPONSE TO CARBOXYAMIDOTRIAZOLE OROTATE

FIG. 2 (a)

	μM CTO					
	2	5	10	2	5	10
EGFRi	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
MEKi	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
HSP90i	-0.2	-0.2	-0.2	-0.4	-0.4	-0.4
P53 stabilisation	0.15	0.15	0.15	[illegible]	[illegible]	[illegible]
WNT / β-catenin i	0	0	0	-0.14	-0.14	-0.14
HDACi	-0.1	-0.12	-0.12	-0.08	-0.16	-0.16
GF	-0.1	-0.1	-0.16	-0.07	-0.01	-0.07
RAS	-0.12	-0.14	-0.14	0	0	0
PI3Ki	0	0	0	0	0	0
PI3K/mTORi	0	0	0	0	0	0
NOTCH / GSi	0	0	0	0	0	0

(57) Abstract: This invention provides methods, pharmacodynamics biomarker signatures for multiple signaling pathways in a cell, sample such as anagen hair, in response to carboxyamidotriazole orotate (CTO) from a subject CTO has demonstrated response in several cancers having different genomic mutations m clinical studies. This invention provides a diagnostic and prognostic assay for monitoring response to CTO ranging from - l00fold to +25 fold differential expression in several transcriptional signatures associated with tumor inhibition including EGFR, MEK, HDAC, RAS, GFS, WNT, HSP90 or non-voltage dependent calcium signaling, while inducing tumor suppressors signatures such as P53 or EGR1 in the anagen hair assay.

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METHODS AND MOLECULAR PHARMACODYNAMIC BIOMARKERS FOR MULTIPLE SIGNALING PATHWAYS IN RESPONSE TO CARBOXYAMIDOTRIAZOLE OROTATE

FIELD OF INVENTION

This invention is related to evaluation of molecular pharmacodynamics markers in response to carboxyamidotriazole orotate (CTO) in ex vivo human anagen hairs obtained from healthy subjects or patients with different diseases. CTO is an orally active agent with antineoplastic activity, that inhibits non-voltage operated Ca2+ channels, blocking both Ca2+ influx into cells and Ca2+ release from intracellular stores and resulting in the disruption of calcium-mediated signal transduction and inhibition of vascular endothelial growth factor (VEGF) signaling, multiple tyrosine kinase signaling, including AKT, MEK-ERK, or Bcr-Abl. More particularly, the present invention relates to the evaluation of molecular pharmacodynamics biomarkers of overall signaling output by transcriptomic assessment of response to CTO given to patients or when added to ex vivo cultured human anagen hairs in vitro.

1. BACKGROUND TO THE INVENTION

The development of new cancer drugs is now based on the identification of genes that are responsible for driving malignancy and the elucidation of the signal transduction pathways that they hijack. Tremendous advances have been made in the area of small molecule kinase inhibitors to develop targeted therapies designed to interfere with critical molecules and signaling pathways driving tumor growth, for example, Imatinib® (BCR-ABL), Gefitnib® (EGFR), Erlotinib® (EGFR) and many other agents under preclinical and clinical development. It is important to focus on the pharmacokinetic and metabolic properties as well as on target potency and molecular selectivity to optimize the effects of targeted therapies. Collin, I and Workman, Cancer Signal Transduction Therapy, 1: 3-23 (2006). In the clinical development of the kinase inhibitors, it is important to develop robust

and informative biomarkers and develop assays for predicting molecular dependence and hence for identifying patients who will benefit from personalized medicine from a particular agent, and the problem of drug resistance developing.

Another challenge facing the development of molecular therapeutics is the likely need to inhibit several oncogenic targets in order to overcome cancers that are driven by several abnormalities, as well as to prevent or neutralize the development of drug resistance. In some cases resistance to a drug may be linked to increased production of molecules (e.g., cytokines, calcium channel signaling, or molecular signaling) in the tumor micro-environment that interferes with the sensitivity and efficacy of the drugs. Therefore, even the most rationally conceived drug molecule may fail because of mutational changes downstream from its intended target or metabolic features of tumors that never allow the drug to reach its target or that trigger feedback mechanism against the drug molecule.

There are several methods currently in use to overcome drug resistance. One is use of cocktails of highly targeted agents that are designed according to the molecular make-up of the specific cancer. Another approach is to use multi-targeted kinase inhibitors (for example Sorafenib®). A further strategy is to use an inhibitor of several kinases that control many oncogenic players and pathways in malignancy, for example inhibitors of histone deacetylases (HDAC) and the HSP90 molecular chaperone. Garon E.B, et al, Mol Cancer Ther., 12: 890-900 (2013); and Witt O at al, Cancer Letters 277: 8-21 (2009).

Screening and structure-based design of targeted drugs against oncogenic markers driving specific cancers are now delivering targeted drugs for preclinical and clinical studies at a rapid rate. However, there is need to study signaling pathways in normal cells to better understand the factors that cause important tumor suppressor proteins to fail as gatekeepers of normal cellular function. There is need to better understand how these tumor suppressor

proteins may be modulated to prevent loss of their normal signaling in response to stress signals.

More particularly, it is important to develop drugs that can aid normal cells to integrate multiple signaling pathways or enhance their role as gate keepers to control growth and proliferation. For example, the P53 transcription factor is a major tumor suppressor protein that serves as a gatekeeper of cellular fate in multicellular organisms. P53 is activated in response to a variety of stress signals and initiates cell cycle arrest, senescence or apoptosis via pathways involving transactivation of P53 target genes. Stambolic V et al., Mol Cell: 317-325 (2001). This universal protection of genetic integrity is however impaired in many human cancers. The new paradigm is to develop agents that target the precise molecular signaling that maintains normal cell cycle, growth and proliferation.

Thus, the rational selection and development of combination treatments is extremely challenging and there is need to develop combinations of targeted drugs and or chemotherapeutic agents based on knowledge of the molecular abnormalities in particular cancers, together with the understanding of the feedback loops that apply upon blockade of a given pathway, as well as enhancing the tumor suppressor signaling pathways of p53 transcription factor and PTEN in normal and cancer cells. .

Carboxyamidotriazole orotate (CTO), an orotate salt of carboxyamidotriazole (CAI) is an inhibitor of receptor-operated calcium channel-mediated calcium influx, and is shown to have anti-proliferative and anti-invasive functions in several human cancer cell lines, including human glioblastoma cells. Ge S et al., Clin Cancer Res 6: 1248-1254 (2000). By interrupting calcium mobilization as a second messenger, CAI can inhibit calcium-sensitive signal transduction pathways, including the release of arachidonic acid and its metabolites; nitric oxide release; the generation of inositol phosphates; and tyrosine phosphorylation Kohn E C et al.,Cancer Res 52:3208-3212 (1992); Kohn E C et al., Proc Natl Acad Sci 92: 1307-

1311 (1995); Felder CF et al., J Pharmacol Exp Therap 257: 967-971 (1990);Hupe DJ et al., J Biol Chem 266: 10136-10142 (1991); Mignen O et al., J Cell Sc 118: 5615-5623 (2005); and Enfissi E et al., Cell Calcium 36: 459-467 (2004). CAI inhibits phosphorylation of cellular proteins STATS and CrkL, and induces apoptosis in imatinib mesylate-resistant chronic myeloid leukemia cells by down-regulating BCR-ABL (Alessandro et al, PLOS 7: 1-13 (2012).

In clinical studies (NCT01107522) CTO given alone was safe and tolerable without determining maximum tolerated dose, in cancer patients with different tumor types and having different genomic mutations, and CTO treatment resulted in cancers responding and demonstrating stable disease or partial response showing tumor shrinkage. Thus enormous efforts are directed to the development of molecular pharmacodynamics biomarkers of signaling outputs of CTO to design combinatorial regimens against molecular targets in different types of cancers. Current methods for assessing pathway activation in tumors involve the measurement of the drug targets, known oncogenes or known tumor suppressors. However, one pathway can be activated at multiple points so it is not feasible to assess pathway activation by evaluating just known cancer associated genes.

It is therefore important to develop the complete molecular signatures of CTO in view of its effect on signaling of multiple kinases, tyrosine kinases and calcium signal transduction pathways. The invention is related to evaluation of the response of molecular pharmacodynamics markers in response to CTO treatment in human cell or tissue samples such as anagen hairs obtained from healthy subjects or from patients, either in vivo or in vitro.

In the in vivo model, anagen hairs are obtained before dosing the patient with CTO, and at different time points after the daily dosing of a therapeutic amount of CTO is given. The patient's clinical status and blood levels of CAI are monitored during this period.

In the in vitro model the anagen hairs are obtained from an untreated subject and the hairs are treated in ex vivo cultures with different doses of CTO which represent the range of doses required for therapeutic efficacy.

In both models, RNA is extracted from the bulbs at the end of anagen hairs, cDNA is then prepared from the RNA and global transcriptional or gene expression levels are determined by microarray analysis or by quantitative PCR (qPCR). Bioinformatic analysis is then conducted to identify CTO induced gene expression changes in anagen hairs. Such a protocol can also be applied to issues other than anagen hair obtained from healthy subjects or patients.

Accordingly, the present invention describes in greater detail, uses of the plucked hair biomarker assay to study effects of CTO on mRNA and protein expression levels in vitro. Plucked scalp hair is an ideal surrogate for measuring direct response to treatment with CTO. Highly vascularized, hair follicle can respond within hours of exposure. Given this vascularization, their epithelial nature and rapid rate of proliferation, the cells in the hair bulb at the base of the plucked hair and the outer root sheath are highly relevant surrogate marker tissue for solid tumors. Highly vascularized, the hair follicle can respond to drug treatment within hours of exposure. Bioinformatic analysis was conducted to identify drug-induced changes in hairs.

3. SUMMARY OF THE INVENTION

The present invention relates to the evaluation of molecular pharmacodynamic biomarkers of multiple signaling pathways in response to CTO given in vivo or in vitro, in ex vivo cultured human anagen hairs. Using a commercially available plucked hair molecular platform assay (Epistem Ltd, Manchester, UK) direct response to treatment with different doses of CTO equivalent to levels of carboxyamidotriazole (CAI) that are therapeutically

achieved in patients, was evaluated to test targeting intracellular signaling pathways in oncology and other therapeutic areas.

Accordingly, the present invention used plucked scalp hair from subjects, extracted the RNA from the bulbs at the end of the anagen hairs, prepared the cDNA from the RNA, determined the gene expression levels by microarray analysis or quantitative PCR (qPCR) and conducted bioinformatics analysis to identify drug induced gene expression changes for CTO and other drugs, for example, Tarceva® (EGFR inhibitor) or BEZ235 (a PI3K inhibitor).

The invention relates to development of molecular pharmacodynamics biomarkers of signaling output by transcriptomic assessment of response to CTO in ex vivo cultured human anagen hairs from human subjects with or without cancer or other diseases. The molecular pharmacodynamics biomarkers of CTO expose include RAS, GFS (PI3K,PI3K/MTOR), MEK, HDAC, NOTCH, WNT-β catenin, HSP90, EGFR, P53, CAIIPA, CAI ex vivo Calcium Signaling Non-voltage dependent, Calcium signaling all genes, Calcium signaling ex vivo, Canonical Calcium Signaling, Canonical Calcium ex vivo, Calcium all genes non-voltage dependent, EGR1, PTEN, TGFβ, CEACAMI, or Dystonin.

In a further aspect the invention provided a method of inhibiting RAS pathway signatures in response to treatment with CTO.

In a further aspect the invention provided a method of inhibiting GFS pathway signatures in response to treatment with CTO.

In a further aspect the invention provided a method of inhibiting MEK pathway signatures in response to treatment with CTO.

In a further aspect the invention provided a method of inhibiting HDAC pathway signatures in response to treatment with CTO.

In a further aspect the invention provided a method of inhibiting NOTCH pathway signatures in response to treatment with CTO.

In a further aspect the invention provided a method of inhibiting WNT-β catenin pathway signatures in response to treatment with CTO.

In a further aspect the invention provided a method of inhibiting HSP90 signatures in response to treatment with CTO.

In a further aspect the invention provided a method of inhibiting EGFR pathway signatures in response to treatment with CTO.

In a further aspect the invention provided a method of inducing P53 pathway signatures in response to treatment with CTO.

In a further aspect the invention provided a method of inhibiting genes associated with non-voltage dependent calcium signaling in response to treatment with CTO. Specifically, the invention provides Signature Scores for the CAI Ingenuity Pathway Analysis, the CAI ex vivo pathway, the calcium signaling pathway and the canonical calcium signaling pathways as pharmacodynamics markers of response to CTO.

In a further aspect the invention provided a method of up regulating EGR1 pathway signatures in response to treatment with CTO.

In a further aspect the invention provided a method of up regulating PTEN pathway signatures in response to treatment with CTO.

In a further aspect the invention provided a method of inducing TGF-β pathway signatures in response to treatment with CTO.

In a further aspect the invention provided a method of down regulating CEACAM1 pathway in response to treatment with CTO.

In a further aspect the invention provided a method of down regulating dystonin pathway in response to treatment with CTO.

The invention also relates to pharmaceutical compositions including CTO and another agent combined to improve sensitivity and efficacy and reduce toxicity while regulating one or more gene signatures including EGFR, MEK, VEGF, HDAC, HSP90, ERK, BCR-ABL, p53, ERG1, CEACAM1, dystonin or genes associates with non-voltage dependent calcium signaling, by monitoring the molecular pharmacodynamics biomarkers of signaling output in response to CTO in ex vivo cultured anagen hairs from a treated mammal.

In an even further aspect the invention provides a method of treating or preventing a condition in a mammal in which the regulation of one or more gene signatures including EGFR, MEK, VEGF, HDAC, HSP90, ERK, BCR-ABL, p53, ERG1, CEACAM1 , dystonin, or genes associates with non-voltage dependent calcium signaling , prevents, inhibits or ameliorates a pathology or a symptomology of the condition, the method including administration of a therapeutically effective amount of CTO as monotherapy or as combinatorial therapy, and monitoring the molecular pharmacodynamics biomarkers of signaling output in response to CTO in ex vivo cultured anagen hairs from a treated mammal.

In another aspect the present invention provides a method of preventing or treating a proliferative condition in a subject, the method including administration of a therapeutically effective amount of CTO alone or in combination with another agent, and monitoring the molecular pharmacodynamics biomarkers of signaling output in response to CTO in ex vivo cultured anagen hairs from a treated mammal. Gene expression patterns can be established in other tissues as well to distinguish between tissues in different disease states or to predict prognosis of a disease such as cancer in response to one or more therapies. Another paradigm is to develop a platform of pharmacodynamics markers to design specific and customized formulation base on the gene expression pattern.

The invention provides a paradigm for rational selection and development of combination treatments based on knowledge of the molecular abnormalities in particular

diseases or cancer, together with the understanding that CTO may be combined with other targeted agents that are constructed to the molecular makeup of the disease or cancer, together with the understanding that of the feedback loops that apply upon blockade of a given pathway blocked by the targeted drug and how these feedback loops may be prevented, to maintain sustained inhibition of the molecular targets that drive the disease as well as in some instances to induce suppressor genes to optimize the treatment outcome.

The invention provides a shift in the method of developing combinatorial drug regimens inhibiting several oncogenic targets with CTO in order to overcome cancers that are driven by several abnormalities, as well as to prevent or neutralize the development of drug resistance by monitoring the pharmacodynamics biomarkers of signaling output in response to CTO and carefully picked combination drugs, by transcriptomic assessment in ex vivo cultured anagen hairs. The current poly-pharmacology approach using several targeted drugs cocktails has not been successful and has posed new problems due to cumulative toxicities of the drugs in the cocktail.

An important embodiment of the invention is the development of the panel of 15-20 gene mRNA expression signatures from hair samples and deploy this on the cDNA samples generated from patients by Affymetrix array data to identify genes differentially expressed in scalp hair samples from untreated and treated patients.

These and other features of the present teachings are set forth herein.

4. BRIEF DESCRIPTION OF FIGURES

FIG. 1a illustrates the different stages of the plucked hair biomarker platform (Epistem, Ltd, Manchester, UK), including: anagen hair collection from the scalp; ex vivo culture of the hairs for 8 and 24 hrs to different doses of CTO and to reference dose of BEZ235 (PI3K inhibitor) and Tarceva® (EGFR inhibitor) for 24 hrs only; RNA isolation and

quality control; Epistem GentRx cDNA amplification; selection of cDNAs passing all quality controls for 5HNVs; sample labeling and microarray hybridization; and bioinformatic analysis using reference databases.

FIG. 1b describes ANOVA analysis of results of transcription response for all contrasts in response to different doses of CTO at 8 hr and 24 hr and for 1 dose of BEZ235 (PI3K inhibitor) and Tarceva® (EGFR inhibitor) for 24 hr only.

FIG. 2a illustrates the results of different doses of CTO on multivariate signatures at 8hr and 24hr periods for 11 different signatures starting with the strongest to lowest inhibition- with EGFRi, MEKi, HSP90i, non-voltage dependent CAI related calcium signaling, HDACi, GF and RAS. WNT/β-catenin was inhibitory at 24hrand no activity was noted for PI3Ki, PI3K/mTOR, NOTCH/GSI. Importantly, P53 was induced and P53 stabilization was noted especially at 24hr.

FIG. 2b lists 13 different signatures studied.

FIG 3a illustrates the Signature Score for the RAS pathway in response to different doses of CTO at 8hr and 24hr compared with BEZ235 and Tarceva® for 24hr.

FIG 3b illustrates the Signature Score for the Growth Factor Signature (GFS) pathway in response to different doses of CTO at 8hr and 24hr compared with BEZ235 and Tarceva® for 24hr.

FIG 3c illustrates the Signature Score for the PI3K pathway in response to different doses of CTO at 8hr and 24hr compared with BEZ235 and Tarceva® for 24hr.

FIG 3d illustrates the Signature Score for the PI3K/mTOR pathway in response to different doses of CTO at 8hr and 24hr compared with BEZ235 and Tarceva® for 24hr.

FIG 3e illustrates the Signature Score for the MEK pathway in response to different doses of CTO at 8hr and 24hr compared with BEZ235 and Tarceva® for 24hr.

FIG 4a illustrates the Signature Score for the HDAC pathway in response to different

doses of CTO at 8hr and 24hr compared with BEZ235 and Tarceva® for 24hr.

FIG 4b illustrates the Signature Score for the NOTCH pathway in response to different doses of CTO at 8hr and 24hr compared with BEZ235 and Tarceva® for 24hr.

FIG 4c illustrates the Signature Score for the WNT β-catenin pathway in response to different doses of CTO at 8hr and 24hr compared with BEZ235 and Tarceva® for 24hr.

FIG 4d illustrates the Signature Score for the HSP90 pathway in response to different doses of CTO at 8hr and 24hr compared with BEZ235 and Tarceva® for 24hr.

FIG 4e illustrates the Signature Score for the EGFR pathway in response to different doses of CTO at 8hr and 24hr compared with BEZ235 and Tarceva® for 24hr.

FIG 4f illustrates the Signature Score for the P53 pathway in response to different doses of CTO at 8hr and 24hr compared with BEZ235 and Tarceva® for 24hr.

FIG 5a illustrates the Signature Score for the CAI IPA pathway in response to different doses of CTO at 8hr and 24hr compared with BEZ235 and Tarceva® for 24hr. Using the Ingenuity Pathways Analysis (IPA) (Ingenuity Systems, Redwood City, CA) for genes influenced by CAI, some suppression of CAI signature in response to CTO treatment was observed, except at 10 μM. When the 29 genes were filtered from IPA for FDA <0.05 and 1.5FC in CTO data set, to select informative genes and determine the direction of change, a 14 gene set resulted.

FIG 5b illustrates the Signature Score for the CAI Ex vivo pathway in response to different doses of CTO at 8hr and 24hr compared with BEZ235 and Tarceva® for 24hr. With the tissue specific direction of change information a strong dose dependent suppression of the IPA CAI list was observed.

FIG 5c illustrates the Signature Score for the Calcium signaling pathway in response to different doses of CTO at 8hr and 24hr compared with BEZ235 and Tarceva® for 24hr. Non-voltage dependent calcium signaling genes were identified from datasets in literature to

inform on regulation.

FIG 5d illustrates the Signature Score for the Calcium signaling for all gene pathways in response to different doses of CTO at 8hr and 24hr compared with BEZ235 and Tarceva® for 24hr. The estimated regulation direction for all genes was identified.

FIG 5e illustrates the Signature Score for the Calcium Signaling pathway in response to different doses of CTO at 8hr and 24hr compared with BEZ235 and Tarceva® for 24hr. ANOVA was used to filter informative genes (FDR <0.05 and 1.5 FC).

Strong suppression of non-voltage dependent calcium genes was noted across CTO treatment in both literature determined regulation and ANOVA determined set.

FIG 6a illustrates the Signature Score for the Canonical Calcium signaling using the KEGG Calcium Signaling and IPA to predict regulation of calcium signaling in response to different doses of CTO at 8hr and 24hr compared with BEZ235 and Tarceva® for 24hr.

FIG 6b illustrates the Signature Score for the Canonical Calcium signaling ex vivo using the KEGG Calcium Signaling and IPA to predict regulation of in response to different doses of CTO at 8hr and 24hr compared with BEZ235 and Tarceva® for 24hr. Suppression noted for 6/78 canonical pathway genes only.

FIG. 7a illustrates the Signature Score for all Signaling genes after merging of the ANOVA filtered gene sets for both the CAI signature (FIG. 5b) and the non-voltage dependent (NVD) gene sets (FIG. 5c).

FIG 7b illustrates results in a panel of 31 CAI/Calcium related genes capable of separating the different CTO doses, both by signature view and by PCA.

FIG.8 illustrates the EGR1 Signaling Pathway.

5. DETAILED DESCRIPTION OF THE INVENTION

This invention is related to carboxyamidotriazole orotate (CTO), an orally active agent with antineoplastic activity, that inhibits non-voltage operated Ca2+ channels, blocking

both Ca2+ influx into cells and Ca2+ release from intracellular stores and resulting in the disruption of calcium-mediated signal transduction and inhibition of vascular endothelial growth factor (VEGF) signaling, multiple tyrosine kinase signaling, including AKT, MEK-ERK, or BCR-ABL. More particularly, the present invention relates to the evaluation of molecular pharmacodynamics biomarkers of signaling output by transcriptomic assessment of response to CTO in vivo and in vitro cultures in ex vivo cultured human anagen hairs. Anagen hairs are obtained at different time points from subjects given varying doses of CTO treatment. This is the approach used in clinical trials of CTO in patients. Alternately, anagen hairs are obtained from untreated subjects and the hairs are treated with varying doses of CTO in vitro-this approach is not subject to physiological conditions of absorption and delivery and was chosen.

The understanding of cancer biology and the human genome has led to the development of new classes of targeted therapies designed to interfere with critical molecules and signaling pathways driving tumor growth and hold promise to improve outcomes. This is particularly important for oncology therapy due to the large number of approved therapies, low response rates and resistance to many current treatments, and clinical importance of optimal and tailored therapy. To improve on the limitations of cytotoxic chemotherapeutic agents, current approaches to drug design in oncology are aimed at modulating specific cell signaling pathways important for tumor growth and survival. In cancer cells, these pathways become deregulated resulting in aberrant signaling, inhibition of apoptosis, increased metastasis and increased cell proliferation.

Though normal cells integrate multiple signaling pathways for controlled growth and proliferation, tumors seem to be heavily reliant on activation of one or two oncogene pathways. Unfortunately the focus on developing new anticancer therapies is not on inducing the multiple signaling pathways that keep controlled growth or proliferation in normal cells

under control. Instead, the components of the aberrant signaling pathways represent attractive selective targets for new anticancer therapies. It seems logical that patients with tumors that are driven by specific oncogenic pathways will respond to therapeutics targeting those pathways. However, one pathway can be activated at multiple points and it is not always feasible to assess pathway activation by evaluating known cancer associated genes. For example, signaling through the phosphatidylinositol 3-kinase (PI3K) pathway is activated by multiple growth factors through receptor tyrosine kinases and has effects on multiple processes, including cell growth and survival, metastatic potential, and drug resistance through gene amplification and/or by pass or new mutations. Many pharmaceutical companies are developing specific inhibitors of one or more signaling pathways and proposing combinations of several specific inhibitors to inhibit multiple oncogene pathways. However,, a major limitation to the overall benefit from targeted therapy is the development of drug resistance. Resistance can occur because of mutations that render the drug target insensitive to the inhibitor or when cancer cells change their dependency on the pathway that is targeted. In the first example, resistance can be overcome by developing new drugs that effectively inhibit resistance-associated mutants, as in the example of dasatinib and nilotinib which are effective on BCR-ABL mutants that confer resistance to imatinib. A second approach is to target multiple signaling pathways simultaneously, and thus prevent the cancer from changing its dependency to another significant pathway, for example combining inhibitors of mitogen-activated protein (MAP)-extracellular signal regulated kinase (ERK) kinase with inhibitors of PI3 kinase. The third approach is to enhance efficacy of the targeted therapy to simultaneously target downstream proteins that protect tumor cells from apoptosis.

However, a new approach to develop molecules, each of which could modulate multiple oncogenic pathways would be a better approach to prevent both drug resistance and serious toxicities. This would be a much harder approach to design the molecule, but this

approach of developing a molecule capable of modeling multiple oncogenic pathways is deemed inefficient and unacceptable strategy. Yet, probably the best way to inhibit several oncogenic targets in order to overcome cancers that are driven by several molecular abnormalities, as well as to prevent drug resistance and toxicity is to develop molecules that modulate multiple oncogenic pathways and /or induce known tumor suppressors.

By using a molecule that modulates the expression of multiple genes, and by integrating the expression data from these multiple genes, a quantitative assessment of the gene expression signatures may be possible. These gene expression signatures for pathway activation and/or inactivation may be used as i) pharmacodynamics biomarkers to monitor the drug induced pathway inhibition in tumors or surrogate markers such as anagen hair, ii) as prediction biomarkers to identify tumors with high level of a particular pathway; and early efficacy biomarkers to get an early readout of efficacy or prevention.

Carboxyamidotriazole orotate (CTO), an orotate salt of carboxyamidotriazole (CAI) is an inhibitor of receptor-operated calcium channel-mediated calcium influx, and is shown to have anti-proliferative and anti-invasive functions in several human cancer cell lines, including human glioblastoma cells (Fiorio Pla et al, 2008; Ge et al, 2000). By interrupting calcium mobilization as a second messenger, CAI can inhibit calcium-sensitive signal transduction pathways, including the release of arachidonic acid and its metabolites; nitric oxide release; the generation of inositol phosphates; and tyrosine phosphorylation (Ge et al, 2000; Kohn et al, 1992). CAI inhibits phosphorylation of cellular proteins STATS and CrkL, and induces apoptosis in imatinib mesylate-resistant chronic myeloid leukemia cells by down-regulating bcr-abl (Alessandro et al, 2008).

Thus enormous efforts are directed to the development of molecular pharmacodynamics biomarkers of signaling outputs of CTO to design combinatorial regimens against molecular targets in different types of cancers. Current methods for

assessing pathway activation in tumors involve the measurement of the drug targets, known oncogenes or known tumor suppressors. However, one pathway can be activated at multiple points so it is not feasible to assess pathway activation by evaluating just known cancer associated genes. The plucked hair biomarker assay is used to study effects of CTO on mRNA and protein expression levels in vitro. Plucked scalp hair is an ideal surrogate for measuring direct response to treatment with CTO. Highly vascularized, hair follicle can respond within hours of exposure. Given this vascularization, their epithelial nature and rapid rate of proliferation, the cells in the hair bulb at the base of the plucked hair and the outer root sheath are highly relevant surrogate marker tissue for solid tumors. Bioinformatic analysis is conducted to identify drug-induced changes in hairs.

The invention relates to development of molecular pharmacodynamics biomarkers of signaling output by transcriptomic assessment of response to CTO in ex vivo cultured human anagen hairs from human subjects with or without cancer or other diseases.

This invention provides methods, pharmacodynamics biomarker signatures for multiple signaling pathways in a cell sample such as anagen hair, in response to carboxyamidotriazole orotate (CTO) from a subject. CTO has demonstrated response in several cancers having different genomic mutations in clinical studies. This invention provides a diagnostic and prognostic assay for monitoring response to CTO ranging from -100 fold to +25 fold differential expression in several transcriptional signatures associated with tumor inhibition including EGFR, MEK, HDAC, RAS, GFS, WNT, HSP90 or non-voltage dependent calcium signaling, while inducing tumor suppressors signatures such as P53 or EGR1 in the anagen hair assay.

A method for quantifying the response to carboxyamidotriazole orotate (CTO) on pharmacodynamics biomarkers of multiple signature pathways , said method comprising:

a) Obtaining a cell sample obtained from a subject and exposing the cell sample to

varying doses of CTO alone, to CTO in combination with another agent or to other agents for different time periods;

b) Isolating the mRNA from the treated cell sample and preparing representative cDNA there from and measuring the transcriptional alteration in expression in the cell sample resulting from CTO exposure;

c) Calculating a signature score for each of the pharmacodynamics biomarkers of multiple signature pathways and quantitating the response to the varying doses of CTO exposure, selecting a list of overlapping genes over expressing at two time periods as listed in Table 1; and

d) Identifying each of the pharmacodynamics biomarkers of multiple signature pathways by at least 3 or more genes in a compiled list and confirming each of the pharmacodynamics biomarkers of multiple signature pathways using reference datasets.

RAS, GROWTH FACTOR, PI3 K Signatures

RAS gene products are involved in kinase signaling pathways that control the transcription of genes, which then regulate cell growth and differentiation. The conversion of RAS from a proto-oncogene usually occurs through a point mutation in the gene, and the altered function can affect the cell in different ways because RAS is involved in many signaling pathways that control cell division and cell death. Mutant ras has been identified in cancers of many origins, including pancreas, colon, lung, thyroid, bladder ovarian, breast, skin, liver kidney and some leukemias. Song, S et al., PLOS ONE 7: 1-11 (2012).

GFS is responsive to phosphatidylinositol 3-kinase (PI3K) pathway perturbation and related to phosphatase and tensin homolog (PTEN) degradation. Loboda A et al. Clin Pharm& Therap 1: 92-96(2009).

Mutations in the 100α subunit of PI3K, called PI3KCA are often responsible for activation of PI3K/AKT and have been reported in several human cancers. Janku F et al., J Clin Oncol 30:777-782 (2012).

EGFR pathway signatures:

In a further aspect the invention provided a method of inhibiting EGFR pathway signatures in response to treatment with CTO. Drugs targeting the EGF receptor (EGFR)-antibodies binding the extracellular domain and small-molecule tyrosine kinase inhibitors have expanded treatment options for several solid tumors. The EFGR gene is frequently up regulated in carcinomas of the breast, kidney, ovary, cervix, and in squamous cell carcinomas. The up regulation is typically due to gene amplification or overexpression. EGFR up regulation in gliomas is most often associated with the rearrangement of the EGFR gene resulting in alterations of its transcript so that such gliomas express both wild type endogenous EGFR as well as episomal mutant form. The EGFR gene is amplified in >50% of glioblastomas.

The EGFR-targeted monoclonal antibodies Cetuximab® and Panitumumab® have been extensively studied in metastatic colorectal cancers. However, the clinical efficacy of EGFR-targeted antibodies is limited by the development of acquired secondary resistance which typically occurs within 3 to 12 months of starting therapy. Multiple mechanisms of secondary resistance to anti-EGFR antibodies have been reported such as expression of EGFR ligands, HER2 amplification, and deregulation of the EGFR recycling process. KRAS mutations arise and are responsible for acquired resistance in half the patients who initially respond to cetuximab or panitumumab. Wang J et al., Mol Cancer Ther 12: 925-936 (2013)

During the past several years four EGFR inhibitors have been approved including cetuximab, panitumumab, gefitnib and erlotinib (Tarceva) and have become standard of care for use in cancer patients. However, the activity reported in unselected patients has been quite

limited and typically develop resistance. The extent of intrinsic and acquired resistance to EGFR inhibitors thus leaves ample room for further anticancer drug development. Gou H-F et al., PLOS ONE 8: 1-6 (2013)

MEK pathway signatures:

In a further aspect the invention provided a method of inhibiting MEK pathway signatures in response to treatment with CTO. The MAPK pathway is commonly activated in human cancers and then activates RAF-MEK-ERK kinase cascade which leads to activation downstream of substrates involved in cell proliferation, survival, transformation, translational control and cytoskeletal rearrangements. Tan N et al., Mol Cancer Ther 12: 853-864 (2013). Small molecule inhibitors targeted this pathway, such as allosteric inhibitors of MEK exhibit anticancer efficacy in vitro and in vivo.

HDAC pathway signatures:

In a further aspect the invention provided a method of inhibiting HDAC pathway signatures in response to treatment with CTO. Histone acetylation is a reversible modification, with deacetylation being catalyzed by histone deacetylases (HDACs). HDACs are represented by 18 genes in humans and are divided into four distinct classes. Several classes of HDAC inhibitors are being evaluated in clinical investigations and indicate that certain HDAC family members are aberrantly expressed in several tumors. Unselective HDAC inhibitors show promising results in leukemias and solid tumors, for example Vorinostat® approved for cutaneous T cell Lymphoma. Witt O et al., Cancer Letter 277: 8-21 (2008). However, some pan-HDAC inhibitors may cause numerous side effects thus requiring selective targeting of HDACs with oncogenic function in cancer cells.

WNT pathway signatures:

In a further aspect the invention provided a method of inhibiting WNT pathway signatures in response to treatment with CTO. The WNT family of signaling molecules

regulates numerous processes in animal development, and WNT malfunction is implicated in various forms of disease including cancer and degenerative diseases. The canonical WNT signaling pathway is regulated at many levels and there is increasing evidence from other systems for crosstalk between WNT signaling and other pathways important in tumorigenesis, converging on B-catenin. B-catenin is a multifunctional protein with distinct molecular roles in cell adhesion at the plasma membrane and in transcription within the nucleus. An increasing number of studies suggest that elevated WNT signaling in glioblastoma (GBM) is initiated by several alternative mechanisms that are involved in different steps of the disease. De Robertis A et al., Mol Cancer Ther 12: 1180-1189 (2013); and Nusse R, Cell Res 15: 28-32 (2005). Therefore, inhibition of WNT signaling may represent a therapeutically relevant approach for GBM treatment.

HSP90 pathway signatures:

In a further aspect the invention provided a method of inhibiting HSP90 pathway signatures in response to treatment with CTO. Heat shock protens serve as molecular chaperones required for stability, post translation modification, and function of multiple client proteins. Expression of HSP is increased at times of physiologic stress, and these effects are believed to support cell survival. HSP90 is over expressed in many tumor types indicating that it may play a role in the survival of cancer cells and thus making it an attractive target for an anticancer agent. Increased HSP90 has been linked to worse prognosis in patients with non-small cell lung cancer. Garon E B et al., Mol Cancer Ther 12: 890-900 (2013).NSCLC arises as a result of several driver mutations, for example EGF receptor mutations are seen in about 10% of NSCLC. HSPs play an important role in neurodegenerative disorders such as Parkinson's disease, Alzheimer's disease or Huntington disease and therefore down regulation of HSP90 has potential beneficial effects in cancer and degenerative diseases.

Non-voltage dependent calcium signaling:

In a further aspect the invention provided a method of inhibiting genes associated with non-voltage dependent calcium signaling in response to treatment with CTO. Kohn E C et al.,Cancer Res 52:3208-3212 (1992); Kohn E C et al., Proc Natl Acad Sci 92: 1307-1311 (1995); Felder CF et al., J Phasrmacol Exp Therap 257: 967-971 (1990Hupe DJ et al., J Biol Chem 266: 10136-10142 (1991); Mignen O et al., J Cell Sc 118: 5615-5623 (2005); and Enfissi E et al., Cell Calcium 36: 459-467 (2004.Calcium signaling non-voltage dependent genes were identified and strong suppression was noted across CTO treatment.

Canonical calcium signaling was analyzed using the KEGG Calcium Signaling Pathway and IPA to predict regulation of canonical calcium signaling. No significant suppression of pathway was noted. Using ANOVA to filter for informative genes (FDR <0.05 & 1.5) it was noted that suppression of canonical calcium genes occurred across CTO treatment, however this only represented 7/68 canonical pathway genes.

TGF-β pathway signatures:

In a further aspect the invention provided a method of inhibiting genes associated with TGF-β signaling in response to treatment with CTO. TGF-β is part of a large family of structurally related cytokines that include bone morphogenic proteins, growth and differentiation factors, activins and inhibins. Nearly every cell type has the ability to secrete TGF-β as well as the ability to respond to TGF-β via the presence of TGF-β receptors on the cell surface. Therefore, gain or loss of function of the TGF-β pathway and its components are to lead to a variety of diseases including cancer. In epithelial cells TGF-β functions as a tumor suppressor, where it inhibits proliferation, induces apoptosis and mediates differentiation. Conversely, in other contexts, TGF-β promotes tumor progression through increasing tumor cell invasion and metastasis. Smith A L et al., Clin Cancer Res 18: 4514-4512 (2012).

CEACAM1 pathway signatures:

In a further aspect the invention provided a method of down regulating CEACAM1 pathway in response to treatment with CTO. CEACAM1 is a member of the carcinoembryionic antigen (CEA) gene family of Ig-like cell-cell adhesion molecules. CEACAM1 is down regulated in epithelial cancers, for example prostate, bladder and colon. Lawson EL et al., PLOS ONE 7: 1-14 (2012).

Dystonin/Bpag1 protein signatures:

In a further aspect the invention provided a method of down regulating dystonin pathway in response to treatment with CTO. Dystonin/Bpag1 proteins are cytoskeletal linkers whose loss of function in mice results in a hereditary sensory neuropathy with a progressive loss of limb coordination. Young K et al., Exp Cell Res 314:2750-2761 (2008).

P53 pathway signatures:

In a further aspect the invention provided a method of inducing P53 pathway signatures in response to treatment with CTO. Normal cells integrate multiple signaling pathways to control growth and proliferation. For example, the p53 transcription factor is a major tumor suppressor protein that serves as a gatekeeper of cellular fate in multicellular organisms. P53 is activated in response to a variety of stress signals and initiates cell cycle arrest, senescence or apoptosis via pathways involving transactivation of p53 target genes. Stambolic V et al., Molecular Cell 8:317-325 (2001). This universal protection of genetic integrity is however impaired in many human cancers.

In a further aspect the invention provided a method of up regulating P53 pathway signatures in response to treatment with CTO. Mutations of PTEN are frequently found in a variety of cancers including brain, breast, endometrial, prostate and kidney tumors. PTEN is a tumor suppressor and is a negative regulator of PI3K/PKB/AKT-dependent cellular survival.

EGR1 pathway signatures:

In a further aspect the invention provided a method of up regulating EGR1 pathway signatures in response to treatment with CTO. The early growth response 1 (EGR1) gene product is a transcription factor with roles in differentiation and growth. The transcription factor EGR1 is a direct regulator of multiple tumor suppressor including TGFβ1, PTEN.and fibronectin. Baron V et al., Cancer gene Therapy 13: 115-124 (2006). In certain human tumor cells and tissues EGR1 exhibits prominent tumor suppressor function and many human tumor cell lines express little or no EGR1 in contrast to their normal counterparts. EGR1 is decreased ot undetectable in non small cell lung cancers, breast tumors and human gliomas. Reexpression of ERG1 in human tumor cell lines inhibits transformation. The mechanism of suppression involves the direct induction of TGF-β1 leading to increased fibronectin, and plasminogen activator inhibitor. Liu, C et al Proc Natl Acad Sci 93: 11831-11836 (1996).

EGR1 is implicated in the regulation of P53 in melanoma cells leading to apoptosis and the proapoptotic suppressor gene PTEN is also directly regulated by EGR1.

The invention also relates to pharmaceutical compositions including CTO and another agent combined to improve sensitivity and efficacy and reduce toxicity while regulating one or more gene signatures including EGFR, MEK, VEGF, HDAC, HSP90, ERK, BCR-ABL, p53, ERG1, CEACAM1 , dystonin, or genes associates with non-voltage dependent calcium signaling, by monitoring the molecular pharmacodynamic biomarkers of signaling output in response to CTO in ex vivo cultured anangen hairs from a treated mammal.

In an even further aspect the invention provides a method of treating or preventing a condition in a mammal in which the regulation of one or more gene signatures including EGFR, MEK, VEGF, HDAC, HSP90, ERK, BCR-ABL, p53, ERG1, CEACAM1 , dystonin, or genes associates with non-voltage dependent calcium signaling , prevents, inhibits or ameliorates a pathology or a symptomology of the condition, the method including administration of a therapeutically effective amount of CTO as monotherapy or as

combinatorial therapy, and monitoring the molecular pharmacodynamics biomarkers of signaling output in response to CTO in ex vivo cultured anangen hairs from a treated mammal.

In another aspect the present invention provides a method of preventing or treating a proliferative condition in a subject, the method including administration of a therapeutically effective amount of CTO alone or in combination with another agent, and monitoring the molecular pharmacodynamics biomarkers of signaling output in response to CTO in ex vivo cultured anagen hairs from a treated mammal. Gene expression patterns can be established in other tissues as well to distinguish between tissues in different disease states or to predict prognosis of a disease such as cancer in response to one or more therapies. Another paradigm is to develop a platform of pharmacodynamics markers to design specific and customized formulation base on the gene expression pattern.

The invention provides a paradigm for rational selection and development of combination treatments based on knowledge of the molecular abnormalities in particular diseases or cancer, together with the understanding that CTO may be combined with other targeted agents that are constructed to the molecular makeup of the disease or cancer, together with the understanding that of the feedback loops that apply upon blockade of a given pathway blocked by the targeted drug and how these feedback loops may be prevented, to maintain sustained inhibition of the molecular targets that drive the disease as well as in some instances to induce suppressor genes to optimize the treatment outcome.

The invention provides a shift in the method of developing combinatorial drug regimens inhibiting several oncogenic targets with CTO in order to overcome cancers that are driven by several abnormalities, as well as to prevent or neutralize the development of drug resistance by monitoring the pharmacodynamics biomarkers of signaling output in response to CTO and carefully picked combination drugs, by transcriptomic assessment in ex vivo

cultured anagen hairs. The current poly-pharmacology approach using several targeted drugs cocktails has not been successful and has posed new problems due to cumulative toxicities of the drugs in the cocktail. The invention provides a rescue solution for targeted and non-targeted drug combinations that fail due to drug resistance mechanisms by combining them with CTO from the start or even after drug resistance is noted, to maintain sincitivity and effectiveness.

An important embodiment of the invention is the development of the panel of up to 15-20 gene mRNA expression signatures from hair samples and deploy this on the cDNA samples generated from patients by Affymetrix array data to identify genes differentially expressed in scalp hair samples from untreated and treated patients.

It is an objective of the present invention to evaluate molecular pharmacodynamic markers in response to carboxyamidotriazole orotate (CTO) in ex vivo human anagen hairs obtained from subjects. CTO is an orally active agent with antineoplastic activity, that inhibits non-voltage operated Ca2+ channels, blocking both Ca2+ influx into cells and Ca2+ release from intracellular stores and resulting in the disruption of calcium-mediated signal transduction and inhibition of vascular endothelial growth factor (VEGF) signaling, multiple tyrosine kinase signaling, including AKT, MEK-ERK, or BCR-ABL. More particularly, the present invention relates to the evaluation of molecular pharmacodynamics biomarkers of signaling output by transcriptomic assessment of response to CTO in ex vivo cultured human anagen hairs.

6. EXAMPLES

Example 1.

The plucked hair biomarker platform developed by Epistem Ltd (Manchester, United Kingdom) was used to assess transcriptomic response to CTO in ex vivo cultured human anagen hairs. Figure 1a describes the overall process steps.

Plucked scalp hair is an ideal surrogate for measuring direct response to CTO treatment. It is a non-invasive and can also be used using samples from patients who have been treated with CTO, in which case the hairs do not need to be treated ex vivo. Hairs growing on the scalp in growth stage (anagen) and which have highly vascularized follicles are suitable.

Donor hairs were plucked from 5 male donor volunteers, immediately transferred to maintenance medium cultures and exposed to varying doses of CTO equivalent to 2 μM, 5 μM, and 10 μM carboxyamidotriazole (CAI). The cultures were either maintained for 8hrs or 24hrs. Controls were used, for example, Tarceva® (EGFR inhibitor) at 1 μM and BEZ235 (PI3K inhibitor) at 1 μM for 24 hrs only. Once in culture hairs were collected at the specified periods for mRNA isolation or protein analysis to ensure quality control. Small amounts of RNA (about up to 500ng) are extracted from the bulbs at the end of the anagen hairs. Representative cDNA was prepared from the RNA and gene expression levels determined by microarray analysis. Biotin labeling, fragmentation and hybridization to 048 Affymetrix U133 plus 2.0 array was done. Bioinformatic analysis was done to identify CTO induced gene expression changes. The whole procedure is given in FIG. 1.

Results obtained show strong transcriptional response for all contrasts. High level of differential expression of transcripts was observed. Tarceva® showed less differential probes than CTO or BEZ235 at this threshold. For CTO differential probes increased in a dose related manner. CTO showed the greatest effect transcriptionally in anagen hair at all doses compared with BEZ235 or Tarceva®. Results are presented in detail in Figure 1b. Biologically relevant alteration of the hair bulb transcriptome ranging from -100fold to +25 fold differential expression was observed at clinically relevant levels of CTO.

Example 2.

The signature scores were assessed as described in detail by Loboda A., et al (Clinical Pharmacology & Therapeutics 86:92-96 (2009) for identification of growth factor gene

signatures, and by Loboda A et al, BMC Medical Genomics 3: 1-11(2010) for identification of signatures of RAS pathway and PI3K. These references are incorporated herein. They describe the Ingenuity Pathway Analysis (Ingenuity Systems, Redwood City, CA); http://www.ingenuity.com software tool to identify signaling pathways that are statistically enriched among growth factor signature genes. The RAS pathway was analyzed using publicly available and literature datasets, e.g., https://array.nci.nih.gov/carray/project/woost-00041.

The results obtained for probes observed at the two time periods were: 1440 at 8 hours and 2961 at 24 hrs. A high degree overlap was observed between the 2 lists (39%, $p<0.0001$). The net result was 558 probes (442 unique annotated genes, ranging -75 to +33 fold at high dose CTO /24hr. Significant results of Signature Scores are presented graphically. The Probe List is presented in Table 1.

Table 1.

Probeset ID	Entrez Gene	Gene Symbol	Gene Title
222450_at	56937	PMEPA1	prostate transmembrane protein, androgen induced 1
233565_s_at	100528031 /// 27111	FKBP1A-SDCBP2 /// SDCBP2	FKBP1A-SDCBP2 readthrough (non-protein coding) /// syndecan binding protein (syntenin)
242832_at	5187	PER1	period homolog 1 (Drosophila)
240463_at	---	---	---
213039_at	23370	ARHGEF18	Rho/Rac guanine nucleotide exchange factor (GEF) 18
235072_s_at	63971	KIF13A	kinesin family member 13A
208926_at	4758	NEU1	sialidase 1 (lysosomal sialidase)
237444_at	---	---	---
239451_at	---	---	---
222288_at	---	---	---
227579_at	2241	FER	fer (fps/fes related) tyrosine kinase
212717_at	9842	PLEKHM1	plexkstrin homology domain containing, family M (with RUN domain) member 1
226853_at	55589	BMP2K	BMP2 inducible kinase
214112_s_at	541578 /// 91966	CXorf40A /// CXorf40B	chromosome X open reading frame 40A /// chromosome X open reading frame 40B

209912_at	7204	TRIO	triple functional domain (PTPRF interacting)
219476_at	79098	C1orf116	chromosome 1 open reading frame 116
238086_at	100129617	LOC100129617	uncharacterized LOC100129617
230721_at	730094	C16orf52	chromosome 16 open reading frame 52
1566079_at	647190	RPS16P5	ribosomal protein S16 pseudogene 5
223839_x_at	6319	SCD	stearoyl-CoA desaturase (delta-9-desaturase)
225671_at	124976	SPNS2	spinster homolog 2 (Drosophila)
212961_x_at	541578	CXorf40B	chromosome X open reading frame 40B
223659_at	84000	TMPRSS13	transmembrane protease, serine 13
229909_at	283358	B4GALNT3	beta-1,4-N-acetyl-galactosaminyl transferase 3
223467_at	51655	RASD1	RAS, dexamethasone-induced 1
235146_at	57458	TMCC3	transmembrane and coiled-coil domain family 3
235548_at	164284	APCDD1L	adenomatosis polyposis coli down-regulated 1-like
206816_s_at	26206	SPAG8	sperm associated antigen 8
242323_at	81579	PLA2G12A	phospholipase A2, group XIIA
224579_at	81539	SLC38A1	solute carrier family 38, member 1
213315_x_at	91966	CXorf40A	chromosome X open reading frame 40A
227314_at	3673	ITGA2	integrin, alpha 2 (CD49B, alpha 2 subunit of VLA-2 receptor)
227093_at	57602	USP36	Ubiquitin specific peptidase 36
200760_s_at	10550	ARL6IP5	ADP-ribosylation-like factor 6 interacting protein 5
201790_s_at	1717	DHCR7	7-dehydrocholesterol reductase
1554980_s_at	467	ATF3	activating transcription factor 3
242235_at	22884	WDR37	WD repeat domain 37
219267_at	51228	GLTP	glycolipid transfer protein
1555786_s_at	645687	LINC00520	long intergenic non-protein coding RNA 520
229734_at	283174	LOC283174	uncharacterized LOC283174
242856_at	---	---	---
201037_at	5214	PFKP	phosphofructokinase, platelet
1562970_at	---	---	---
201465_s_at	3725	JUN	jun proto-oncogene
202067_s_at	3949	LDLR	low density lipoprotein receptor
223639_at	1499	CTNNB1	catenin (cadherin-associated protein), beta 1, 88kDa
201235_s_at	7832	BTG2	BTG family, member 2
225662_at	51776	ZAK	sterile alpha motif and leucine zipper containing kinase AZK
204401_at	3783	KCNN4	potassium intermediate/small conductance calcium-activated channel, subfamily N, member
222906_at	28982	FLVCR1	feline leukemia virus subgroup C cellular receptor 1
238613_at	51776	ZAK	sterile alpha motif and leucine zipper containing kinase AZK
206414_s_at	8853	ASAP2	ArfGAP with SH3 domain, ankyrin repeat and PH domain

			2
210794_s_at	55384	MEG3	maternally expressed 3 (non-protein coding)
226621_at	9180	OSMR	oncostatin M receptor
230682_x_at	8714	ABCC3	ATP-binding cassette, sub-family C (CFTR/MRP), member 3
235668_at	639	PRDM1	PR domain containing 1, with ZNF domain
205483_s_at	9636	ISG15	ISG15 ubiquitin-like modifier
215808_at	5655	KLK10	kallikrein-related peptidase 10
212281_s_at	27346	TMEM97	transmembrane protein 97
212282_at	27346	TMEM97	transmembrane protein 97
226287_at	91057	CCDC34	coiled-coil domain containing 34
213618_at	116984	ARAP2	ArfGAP with RhoGAP domain, ankyrin repeat and PH domain 2
231089_at	100505664	LOC100505664	uncharacterized LOC100505664
227140_at	3624	INHBA	inhibin, beta A
231467_at	---	---	---
202967_at	2941	GSTA4	glutathione S-transferase alpha 4
230323_s_at	120224	TMEM45B	transmembrane protein 45B
224471_s_at	8945	BTRC	beta-transducin repeat containing E3 ubiquitin protein ligase
202708_s_at	8349	HIST2H2BE	histone cluster 2, H2be
242871_at	54852	PAQR5	progestin and adipoQ receptor family member V
205627_at	978	CDA	cytidine deaminase
235542_at	200424	TET3	tet methylcytosine dioxygenase 3
240410_at	---	---	---
236656_s_at	100288911	LOC100288911	uncharacterized LOC100288911
206164_at	9635	CLCA2	chloride channel accessory 2
203159_at	2744	GLS	glutaminase
224991_at	80790	CMIP	c-Maf inducing protein
204258_at	1105	CHD1	chromodomain helicase DNA binding protein 1
228249_at	119710	C11orf74	chromosome 11 open reading frame 74
229013_at	145783	LOC145783	uncharacterized LOC145783
211547_s_at	5048	PAFAH1B1	platelet-activating factor acetylhydrolase 1b, regulatory subunit 1 (45kDa)
226863_at	642273	FAM110C	family with sequence similarity 110, member C
208161_s_at	8714	ABCC3	ATP-binding cassette, sub-family C (CFTR/MRP), member 3
214805_at	1973	EIF4A1	eukaryotic translation initiation factor 4A1
229429_x_at	728855 /// 728875	LOC728855 /// LOC728875	uncharacterized LOC728855 /// uncharacterized LOC728875
202720_at	26136	TES	testis derived transcript (3 LIM domains)
224995_at	56907	SPIRE1	spire homolog 1 (Drosophila)
214771_x_at	23164	MPRIP	myosin phosphatase Rho interacting protein

201939_at	10769	PLK2	polo-like kinase 2
238587_at	84959	UBASH3B	ubiquitin associated and SH3 domain containing B
332113_at	---	---	---
208690_s_at	9124	PDLIM1	PDZ and LIM domain 1
201464_x_at	3725	JUN	jun proto-oncogene
236657_at	100288911	LOC100288911	uncharacterized LOC100288911
215541_s_at	1729	DIAPH1	diaphanous homolog 1 (Drosophila)
238028_at	647024	C6orf132	chromosome 6 open reading frame 132
226893_at	27	ABL2	v-abl Abelson murine leukemia viral oncogene homolog 2
237576_x_at	100506480	LOC100506480	uncharacterized LOC100506480
1552256_a_at	949	SCARB1	scavenger receptor class B, member 1
215255_at	22997	IGSF9B	immunoglobulin superfamily, member 9B
1557258_a_at	8915	BCL10	B-cell CLL/lymphoma 10
240623_at	---	---	---
228754_at	6533	SLC6A6	solute carrier family 6 (neurotransmitter transporter, taurine), member 6
217257_at	6452	SH3BP2	SH3-domain binding protein 2
241036_at	---	---	---
242553_at	8714	ABCC3	ATP-binding cassette, sub-family C (CFTR/MRP), member 3
239358_at	---	---	---
210868_s_at	79071	ELOVL6	ELOVL fatty acid elongase 6
200815_s_at	5048	PAFAH1B1	platelet-activating factor acetylhydrolase 1b, regulatory subunit 1 (45kDa)
208436_s_at	3665	IRF7	interferon regulatory factor 7
208138_at	2520	GAST	gastrin
241780_at	---	---	---
200730_s_at	7803	PTP4A1	protein tyrosine phosphatase type IVA, member 1
219697_at	9956	HS3ST2	heparan sulfate (glucosamine) 3-O-sulfotransferase 2
201693_s_at	1958	EGR1	early growth response 1
218847_at	10644	IGF2BP2	insulin-like growth factor 2 mRNA binding protein 2
230469_at	219790	RTKN2	rhotekin 2
209917_s_at	11257	TP53TG1	TP53 target 1 (non-protein coding)
224329_s_at	84518	CMFN	cornifelin
212253_x_at	100652766 /// 667	DST /// LOC100652766	dystonin /// dystonin-like
238058_at	150381	LOC150381	uncharacterized LOC150381
239334_at	57488	ESYT2	Extended synaptotagmin-like protein 2
222271_at	---	---	---
216718_at	388699	LINC00302	long intergenic non-protein coding RNA 302
219076_s_at	5827	PXMP2	peroxisomal membrane protein 2, 22kDa
204475_at	4312	MMP1	matrix metallopeptidase 1 (interstitial collagenase)
221185_s_at	84223	IQCG	IQ motif containing G

203586_s_at	379	ARL4D	ADP-ribosylation factor-like 4D
217802_s_at	64710	NUCKS1	nuclear casein kinase and cyclin-dependent kinase substrate 1
205767_at	2069	EREG	epiregulin
228360_at	130576	LYPD6B	LY6/PLAUR domain containing 6B
228917_at	---	---	---
228748_at	966	CD59	CD59 molecule, complement regulatory protein
219632_s_at	23729 /// 7442	SHPK /// TRPV1	sedoheptulokinase /// transient receptor potential cation channel, subfamily V, member
238715_at	646014	LOC646014	Uncharacterized LOC646014
218810_at	80149	ZC3H12A	zinc finger CCCH-type containing 12A
225177_at	80223	RABL3IP1	RABL3 family interacting protein 1 (class I)
224454_at	55500	ETNK1	ethanolamine kinase 1
209498_at	634	CEACAM1	carcinoembryonic antigen-related cell adhesion molecule 1 (biliary glycoprotein)
1557257_at	8915	BCL10	B-cell CLL/lymphoma 10
225133_at	51274	KLF3	Kruppel-like factor 3 (basic)
202340_x_at	3164	NR4A1	nuclear receptor subfamily 4, group A, member 1
1556545_at	---	---	---
212474_at	23080	AVL9	AVL9 homolog (S. cerevisiae)
210241_s_at	11257	TP53TG1	TP53 target 1 (non-protein coding)
243543_at	---	---	---
239132_at	4842	NOS1	nitric oxide synthase 1 (neuronal)
222757_s_at	51776	ZAK	sterile alpha motif and leucine zipper containing kinase AZK
201194_at	6415	SEPW1	selenoprotein W, 1
229874_x_at	100506687	LOC100506687	uncharacterized LOC100506687
202557_at	6782	HSPA13	heat shock protein 70kDa family, member 13
239669_at	---	---	---
231907_at	27	ABL2	v-abl Abelson murine leukemia viral oncogene homolog 2
229074_at	30844	EHD4	EH-domain containing 4
205428_s_at	794	CALB2	calbindin 2
205822_s_at	3157	HMGCS1	3-hydroxy-3-methylglutaryl-CoA synthase 1 (soluble)
210869_s_at	4162	MCAM	melanoma cell adhesion molecule
225665_at	51776	ZAK	sterile alpha motif and leucine zipper containing kinase AZK
212781_at	5930	RBBP6	retinoblastoma binding protein 6
232355_at	767579	SNORD114-3	small nucleolar RNA, C/D box 114-3
213288_at	129642	MBOAT2	membrane bound O-acyltransferase domain containing 2
221666_s_at	29108	PYCARD	PYD and CARD domain containing
203072_at	4643	MYO1E	myosin IE
215465_at	26154	ABCA12	ATP-binding cassette, sub-family A (ABC1), member 12

224453_s_at	55500	ETNK1	ethanolamine kinase 1
216935_at	388699	LINC00302	long intergenic non-protein coding RNA 302
209086_x_at	4162	MCAM	melanoma cell adhesion molecule
218833_at	51776	ZAK	sterile alpha motif and leucine zipper containing kinase AZK
209377_s_at	9324	HMGN3	high mobility group nucleosomal binding domain 3
223519_at	51776	ZAK	sterile alpha motif and leucine zipper containing kinase AZK
210138_at	8601	RGS20	regulator of G-protein signaling 20
1558845_at	100506089	LOC100506089	uncharacterized LOC100506089
201819_at	949	SCARB1	scavenger receptor class B, member 1
204310_s_at	4882	NPR2	natriuretic peptide receptor B/guanylate cyclase B (atrionatriuretic peptide receptor B)
239377_at	84285	EIF1AD	eukaryotic translation initiation factor 1A domain containing
224611_s_at	80331	DNAJC5	DnaJ (Hsp40) homolog, subfamily C, member 5
219155_at	26207	PITPNC1	phosphatidylinositol transfer protein, cytoplasmic 1
227163_at	119391	GSTO2	glutathione S-transferase omega 2
209633_at	5523	PPP2R3A	protein phosphatase 2, regulatory subunit B", alpha
219681_s_at	80223	RAB11FIP1	RAB11 family interacting protein 1 (class I)
221860_at	3191	HNRNPL	heterogeneous nuclear ribonucleoprotein L
243296_at	10135	NAMPT	Nicotinamide phosphoribosyltransferase
237133_at	---	---	---
1556000_s_at	55727	BTBD7	BTB (POZ) domain containing 7
204681_s_at	9771	RAPGEF5	Rap guanine nucleotide exchange factor (GEF) 5
215726_s_at	1528	CYB5A	cytochrome b5 type A (microsomal)
210886_x_at	11257	TP53TG1	TP53 target 1 (non-protein coding)
226597_at	92840	REEP6	receptor accessory protein 6
204995_at	8851	CDK5R1	cyclin-dependent kinase 5, regulatory subunit 1 (p35)
236119_s_at	6706	SPRR2G	small proline-rich protein 2G
219228_at	55422	ZNF331	zinc finger protein 331
234971_x_at	113026	PLCD3	phospholipase C, delta 3
201127_s_at	47	ACLY	ATP citrate lyase
226880_at	64710	NUCKS1	Nuclear casein kinase and cyclin-dependent kinase substrate 1
209383_at	1649	DDIT3	DNA-damage-inducible transcript 3
204168_at	4258	MGST2	microsomal glutathione S-transferase 2
239670_at	65268	WNK2	WNK lysine deficient protein kinase 2
208512_s_at	4301	MLLT4	myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila); translocate
213281_at	3725	JUN	Jun proto-oncogene
218310_at	154881 /// 27342	KCTD7 /// RABGEF1	potassium channel

			tetramerisation domain containing 7 /// RAB guanine nucleotide exchan
205151_s_at	9865	TRIL	TLR4 interactor with leucine-rich repeats
218217_at	59342	SCPEP1	serine carboxypeptidase 1
205055_at	3682	ITGAE	integrin, alpha E (antigen CD103, human mucosal lymphocyte antigen 1; alpha polypeptide
215009_s_at	100499177	THAP9-AS1	THAP9 antisense RNA 1 (non-protein coding)
227484_at	57522	SRGAP1	SLIT-ROBO Rho GTPase activating protein 1
239769_at	1009	CDH11	Cadherin 11, type 2, OB-cadherin (osteoblast)
230360_at	342035	GLDN	gliomedin
227112_at	23023	TMCC1	transmembrane and coiled-coil domain family 1
201482_at	5768	QSOX1	quiescin Q6 sulfhydryl oxidase 1
210337_s_at	47	ACLY	ATP citrate lyase
203911_at	5909	RAP1GAP	RAP1 GTPase activating protein
206683_at	7718	ZNF165	zinc finger protein 165
202935_s_at	6662	SOX9	SRY (sex determining region Y)-box 9
218951_s_at	55344	PLCXD1	phosphatidylinositol-specific phospholipase C, X domain containing 1
233488_at	84659	RNASE7	ribonuclease, RNase A family, 7
202562_s_at	11161	C14orf1	chromosome 14 open reading frame 1
208745_at	10632	ATP5L	ATP synthase, H+ transporting, mitochondrial Fo complex, subunit G
236078_at	57707	KIAA1609	KIAA1609
226226_at	120224	TMEM45B	transmembrane protein 45B
213854_at	9145	SYNGR1	synaptogyrin 1
243955_at	---	---	---
222111_at	54639	FAM83B	family with sequence similarity 83, member B
1560296_at	---	---	---
240038_at	---	---	---
211372_s_at	7850	IL1R2	interleukin 1 receptor, type II
202672_s_at	467	ATF3	activating transcription factor 3
218717_s_at	55214	LEPREL1	leprecan-like 1
228366_at	---	---	---
230516_at	115416	MALSU1	Mitochondrial assembly of ribosomal large subunit 1
201920_at	6574	SLC20A1	solute carrier family 20 (phosphate transporter), member 1
209632_at	5523	PPP2R3A	protein phosphatase 2, regulatory subunit B'', alpha
207367_at	479	ATP12A	ATPase, H+/K+ transporting, nongastric, alpha polypeptide
1557256_a_at	---	---	---
200811_at	1153	CIRBP	cold inducible RNA binding protein
205201_at	2737	GLI3	GLI family zinc finger 3
227724_at	728190	LOC728190	uncharacterized LOC728190

205403_at	7850	IL1R2	interleukin 1 receptor, type II
242827_x_at	---	---	---
228084_at	81579	PLA2G12A	phospholipase A2, group XIIA
209365_s_at	1893	ECM1	extracellular matrix protein 1
243279_at	---	---	---
224946_s_at	84317	CCDC115	coiled-coil domain containing 115
218708_at	29107	NXT1	NTF2-like export factor 1
1560531_at	353132	LCE1B	late cornified envelope 1B
207761_s_at	25840	METTL7A	methyltransferase like 7A
206011_at	834	CASP1	caspase 1, apoptosis-related cysteine peptidase
213703_at	150759	LINC00342	long intergenic non-protein coding RNA 342
224595_at	23446	SLC44A1	solute carrier family 44, member 1
224613_s_at	80331	DNAJC5	DnaJ (Hsp40) homolog, subfamily C, member 5
212504_at	22982	DIP2C	DIP2 disco-interacting protein 2 homolog C (Drosophila)
213682_at	10762	NUP50	nucleoporin 50kDa
205247_at	4855	NOTCH4	notch 4
228235_at	84848	MGC16121	uncharacterized protein MGC16121
242873_at	---	---	---
205960_at	5166	PDK4	pyruvate dehydrogenase kinase, isozyme 4
230494_at	6574	SLC20A1	solute carrier family 20 (phosphate transporter), member 1
221360_s_at	81566	CSRNP2	cysteine-serine-rich nuclear protein 2
224480_s_at	84803	AGPAT9	1-acylglycerol-3-phosphate O-acyltransferase 9
210180_s_at	6434	TRA2B	transformer 2 beta homolog (Drosophila)
204621_s_at	4929	NR4A2	nuclear receptor subfamily 4, group A, member 2
217863_at	8554	PIAS1	protein inhibitor of activated STAT, 1
236423_at	---	---	---
223421_at	50626	CYHR1	cysteine/histidine-rich 1
220272_at	54796	BNC2	basonuclin 2
201791_s_at	1717	DHCR7	7-dehydrocholesterol reductase
215574_at	---	---	---
234328_s_at	84648	LCE3D	late cornified envelope 3D
211828_s_at	23043	TNIK	TRAF2 and NCK interacting kinase
58367_s_at	79744	ZNF419	zinc finger protein 419
218950_at	64411	ARAP3	ArfGAP with RhoGAP domain, ankyrin repeat and PH domain 3
1552703_s_at	114769 /// 834	CARD16 /// CASP1	caspase recruitment domain family, member 16 /// caspase 1, apoptosis-related cysteine
219687_at	55733	HHAT	hedgehog acyltransferase
232127_at	1184	CLCN5	chloride channel, voltage-sensitive 5
218377_s_at	10069	RWDD2B	RWD domain containing 2B
210335_at	9182	RASSF9	Ras association (RalGDS/AF-6) domain family (N-terminal)

			member 9
227927_at	---	---	---
227224_at	55103	RALGPS2	Ral GEF with PH domain and SH3 binding motif 2
224778_s_at	57551	TAOK1	TAO kinase 1
229566_at	645638	LOC645638	WDNM1-like pseudogene
202734_at	9322	TRIP10	thyroid hormone receptor interactor 10
201891_at	6455	SH3GL1	SH3-domain GRB2-like 1
237337_at	---	---	---
37152_at	5467	PPARD	peroxisome proliferator-activated receptor delta
209687_at	6387	CXCL12	chemokine (C-X-C motif) ligand 12
203152_at	64976	MRPL40	mitochondrial ribosomal protein L40
201627_s_at	3638	INSIG1	insulin induced gene 1
232593_at	93082	NEURL3	neuralized homolog 3 (Drosophila) pseudogene
224769_at	57551	TAOK1	TAO kinase 1
209792_at	79068	FTO	fat mass and obesity associated
204546_at	9764	KIAA0513	KIAA0513
232224_at	5648	MASP1	mannan-binding lectin serine peptidase 1 (C4/C2 activating component of Ra-reactive fac
239930_at	2590	GALNT2	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 2 (Gal
203178_at	2628	GATM	glycine amidinotransferase (L-arginine:glycine amidinotransferase)
235782_at	---	---	---
218181_s_at	9448	MAP4K4	mitogen-activated protein kinase kinase kinase kinase 4
233520_s_at	202333	CMYA5	cardiomyopathy associated 5
213456_at	25928	SOSTDC1	sclerostin domain containing 1
219528_s_at	64919	BCL11B	B-cell CLL/lymphoma 11B (zinc finger protein)
224945_at	55727	BTBD7	BTB (POZ) domain containing 7
214866_at	5329	PLAUR	plasminogen activator, urokinase receptor
209941_at	8737	RIPK1	receptor (TNFRSF)-interacting serine-threonine kinase 1
226029_at	57216	VANGL2	vang-like 2 (van gogh, Drosophila)
212596_s_at	10042	HMGXB4	HMG box domain containing 4
229873_at	383219	KCTD21	potassium channel tetramerisation domain containing 21
226392_at	5922	RASA2	RAS p21 protein activator 2
226095_at	7326	UBE2G1	ubiquitin-conjugating enzyme E2G 1
214445_at	22936	ELL2	elongation factor, RNA polymerase II, 2
227680_at	284695	ZNF326	zinc finger protein 326
227786_at	90390	MED30	mediator complex subunit 30
222067_x_at	3017	HIST1H2BD	histone cluster 1, H2bd
1569106_s_at	55209	SETD5	SET domain containing 5
231785_at	4909	NTF4	neurotrophin 4

223937_at	27086	FOXP1	forkhead box P1
1558685_a_at	158960	LOC158960	uncharacterized protein BC009467
211965_at	677	ZFP36L1	zinc finger protein 36, C3H type-like 1
39549_at	4862	NPAS2	neuronal PAS domain protein 2
203800_s_at	63931	MRPS14	mitochondrial ribosomal protein S14
1556321_a_at	---	---	---
212321_at	8879	SGPL1	sphingosine-1-phosphate lyase 1
222154_s_at	26010	SPATS2L	spermatogenesis associated, serine-rich 2-like
218774_at	28960	DCPS	decapping enzyme, scavenger
212268_at	1992	SERPINB1	serpin peptidase inhibitor, clade B (ovalbumin), member 1
213134_x_at	10950	BTG3	BTG family, member 3
230669_at	5922	RASA2	RAS p21 protein activator 2
1559901_s_at	388815	LINC00478	long intergenic non-protein coding RNA 478
225298_at	25953	PNKD	paroxysmal nonkinesigenic dyskinesia
242558_at	---	---	---
226043_at	26086	GPSM1	G-protein signaling modulator 1
210236_at	8500	PPFIA1	protein tyrosine phosphatase, receptor type, f polypeptide (PTPRF), interacting protein
214066_x_at	4882	NPR2	natriuretic peptide receptor B/guanylate cyclase B (atrionatriuretic peptide receptor B
240024_at	23541	SEC14L2	SEC14-like 2 (S. cerevisiae)
235462_at	132864	CPEB2	Cytoplasmic polyadenylation element binding protein 2
1554015_a_at	1106	CHD2	chromodomain helicase DNA binding protein 2
235347_at	84859	LRCH3	leucine-rich repeats and calponin homology (CH) domain containing 3
230847_at	56897	WRNIP1	Werner helicase interacting protein 1
201427_s_at	6414	SEPP1	selenoprotein P, plasma, 1
1557905_s_at	960	CD44	CD44 molecule (Indian blood group)
219084_at	64324	NSD1	nuclear receptor binding SET domain protein 1
206176_at	654	BMP6	bone morphogenetic protein 6
219826_at	79744	ZNF419	zinc finger protein 419
212356_at	23351	KHNYN	KH and NYN domain containing
218909_at	26750	RPS6KC1	ribosomal protein S6 kinase, 52kDa, polypeptide 1
230555_s_at	90390	MED30	Mediator complex subunit 30
212687_at	3987	LIMS1	LIM and senescent cell antigen-like domains 1
203098_at	9425	CDYL	chromodomain protein, Y-like
229054_at	677	ZFP36L1	zinc finger protein 36, C3H type-like 1
236039_at	284348	LYPD5	LY6/PLAUR domain containing 5
209661_at	3801	KIFC3	kinesin family member C3
209560_s_at	8788	DLK1	delta-like 1 homolog (Drosophila)

225812_at	619208	C6orf225	chromosome 6 open reading frame 225
227829_at	120071	GYLTL1B	glycosyltransferase-like 1B
238623_at	---	---	---
229415_at	54203	CYCS	cytochrome c, somatic
209222_s_at	9885	OSBPL2	oxysterol binding protein-like 2
1555809_at	83716	CRISPLD2	cysteine-rich secretory protein LCCL domain containing 2
204567_s_at	9619	ABCG1	ATP-binding cassette, sub-family G (WHITE), member 1
232277_at	64078	SLC28A3	solute carrier family 28 (sodium-coupled nucleoside transporter), member 3
237197_at	---	---	---
225209_s_at	118424	UBE2J2	ubiquitin-conjugating enzyme E2, J2
231916_at	4842	NOS1	nitric oxide synthase 1 (neuronal)
212279_at	27346	TMEM97	transmembrane protein 97
204862_s_at	4832	NME3	NME/NM23 nucleoside diphosphate kinase 3
230483_at	---	---	---
212856_at	23151	GRAMD4	GRAM domain containing 4
224650_at	114569	MAL2	mal, T-cell differentiation protein 2 (gene/pseudogene)
202963_at	5993	RFX5	regulatory factor X, 5 (influences HLA class II expression)
225329_at	90550	MCU	mitochondrial calcium uniporter
236274_at	8662	EIF3B	eukaryotic translation initiation factor 3, subunit B
209780_at	57157	PHTF2	putative homeodomain transcription factor 2
218823_s_at	54793	KCTD9	potassium channel tetramerisation domain containing 9
227787_s_at	90390	MED30	mediator complex subunit 30
230296_at	730094	C16orf52	chromosome 16 open reading frame 52
222892_s_at	55287	TMEM40	transmembrane protein 40
210610_at	634	CEACAM1	carcinoembryonic antigen-related cell adhesion molecule 1 (biliary glycoprotein)
230031_at	3309	HSPA5	heat shock 70kDa protein 5 (glucose-regulated protein, 78kDa)
238477_at	10749	KIF1C	kinesin family member 1C
209409_at	2887	GRB10	growth factor receptor-bound protein 10
217995_at	58472	SQRDL	sulfide quinone reductase-like (yeast)
226873_at	54629	FAM63B	family with sequence similarity 63, member B
1553722_s_at	220441	RNF152	ring finger protein 152
204710_s_at	26100	WIPI2	WD repeat domain, phosphoinositide interacting 2
212653_s_at	23301	EHBP1	EH domain binding protein 1
203979_at	1593	CYP27A1	cytochrome P450, family 27, subfamily A, polypeptide 1
244350_at	4651	MYO10	myosin X
223233_s_at	57530	CGN	cingulin
1555967_at	---	---	---

214355_x_at	100128553 /// 100142659 /// 340307 /// 441294 /// 643854	CTAGE15P /// CTAGE4 /// CTAGE6P /// CTAGE8 /// CTAGE9	CTAGE family, member 15, pseudogene /// CTAGE family, member 4 /// CTAGE family, member
214469_at	3012 /// 8335	HIST1H2AB /// HIST1H2AE	histone cluster 1, H2ab /// histone cluster 1, H2ae
212472_at	9645	MICAL2	microtubule associated monoxygenase, calponin and LIM domain containing 2
228115_at	64762	FAM59A	family with sequence similarity 59, member A
228964_at	639	PRDM1	PR domain containing 1, with ZNF domain
230027_s_at	84545	MRPL43	mitochondrial ribosomal protein L43
207318_s_at	8621	CDK13	cyclin-dependent kinase 13
221689_s_at	51227	PIGP	phosphatidylinositol glycan anchor biosynthesis, class P
219270_at	79094	CHAC1	ChaC, cation transport regulator homolog 1 (E. coli)
225299_at	4645	MYO5B	myosin VB
239770_at	83850	ESYT3	extended synaptotagmin-like protein 3
226399_at	79982	DNAJB14	DnaJ (Hsp40) homolog, subfamily B, member 14
226656_at	10491	CRTAP	cartilage associated protein
228852_at	2029	ENSA	endosulfine alpha
206239_s_at	6690	SPINK1	serine peptidase inhibitor, Kazal type 1
210993_s_at	4086	SMAD1	SMAD family member 1
238462_at	84959	UBASH3B	ubiquitin associated and SH3 domain containing B
211962_s_at	677	ZFP36L1	zinc finger protein 36, C3H type-like 1
224666_at	197370	NSMCE1	non-SMC element 1 homolog (S. cerevisiae)
239028_at	130574	LYPD6	LY6/PLAUR domain containing 6
213577_at	6713	SQLE	squalene epoxidase
202011_at	7082	TJP1	tight junction protein 1 (zona occludens 1)
212254_s_at	100652766 /// 667	DST /// LOC100652766	dystonin /// dystonin-like
221701_s_at	64220	STRA6	stimulated by retinoic acid gene 6 homolog (mouse)
239576_at	57509	MTUS1	microtubule associated tumor suppressor 1
234418_x_at	960	CD44	CD44 molecule (Indian blood group)
227985_at	100506098	LOC100506098	uncharacterized LOC100506098
213462_at	4862	NPAS2	neuronal PAS domain protein 2
224975_at	4774	NFIA	nuclear factor I/A
225990_at	91653	BOC	Boc homolog (mouse)
240616_at	---	---	---
219911_s_at	28231	SLCO4A1	solute carrier organic anion transporter family, member 4A1
224970_at	4774	NFIA	nuclear factor I/A
214623_at	26226	FBXW4P1	F-box and WD repeat domain containing 4 pseudogene 1
239478_x_at	55668	C14orf118	chromosome 14 open reading frame 118
226909_at	85460	ZNF518B	zinc finger protein 518B
208670_s_at	23741	EHD1	EP300 interacting inhibitor of differentiation 1

206192_at	1041	CDSN	corneodesmosin
222173_s_at	55357	TBC1D2	TBC1 domain family, member 2
228450_at	144100	PLEKHA7	pleckstrin homology domain containing, family A member 7
1558097_at	253143	PRR14L	proline rich 14-like
219373_at	54344	DPM3	dolichyl-phosphate mannosyltransferase polypeptide 3
230388_s_at	644246	KANSL1-AS1	KANSL1 antisense RNA 1 (non-protein coding)
207098_s_at	55669	MFN1	mitofusin 1
223484_at	84419	C15orf48	chromosome 15 open reading frame 48
244804_at	8878	SQSTM1	sequestosome 1
229679_at	400073	C12orf76	chromosome 12 open reading frame 76
225826_at	326625	MMAB	methylmalonic aciduria (cobalamin deficiency) cblB type
213352_at	23023	TMCC1	transmembrane and coiled-coil domain family 1
211883_x_at	634	CEACAM1	carcinoembryonic antigen-related cell adhesion molecule 1 (biliary glycoprotein)
210387_at	8339 /// 8343 /// 8344 /// 8346 /// 8347	HIST1H2BC /// HIST1H2BE /// HIST1H2BF /// HIST1H2BG /// HIST1H2BI	histone cluster 1, H2bc /// histone cluster 1, H2be /// histone cluster 1, H2bf /// his
210916_s_at	960	CD44	CD44 molecule (Indian blood group)
221432_s_at	81894	SLC25A28	solute carrier family 25 (mitochondrial iron transporter), member 28
218487_at	210	ALAD	aminolevulinate dehydratase
223264_at	59274	MESDC1	mesoderm development candidate 1
206356_s_at	2774	GNAL	guanine nucleotide binding protein (G protein), alpha activating activity polypeptide,
218097_s_at	79004	CUEDC2	CUE domain containing 2
228001_at	757	TMEM50B	transmembrane protein 50B
212441_at	9778	KIAA0232	KIAA0232
201854_s_at	23300	ATMIN	ATM interactor
121_at	7849	PAX8	paired box 8
222143_s_at	64419	MTMR14	myotubularin related protein 14
1558002_at	11171	STRAP	Serine/threonine kinase receptor associated protein
226040_at	---	---	---
226141_at	91050	CCDC149	coiled-coil domain containing 149
1556567_at	4676	NAP1L4	nucleosome assembly protein 1-like 4
226263_at	154007	SNRNP48	small nuclear ribonucleoprotein 48kDa (U11/U12)
212974_at	23353	SUN1	Sad1 and UNC84 domain containing 1
227387_at	54780	NSMCE4A	Non-SMC element 4 homolog A (S. cerevisiae)
232795_at	---	---	---
203936_s_at	4318	MMP9	matrix metallopeptidase 9 (gelatinase B, 92kDa gelatinase, 92kDa type IV collagenase)

225033_at	6482	ST3GAL1	ST3 beta-galactoside alpha-2,3-sialyltransferase 1
209109_s_at	7105	TSPAN6	tetraspanin 6
213351_s_at	23023	TMCC1	transmembrane and coiled-coil domain family 1
203047_at	6793	STK10	serine/threonine kinase 10
220721_at	80110	ZNF614	zinc finger protein 614
1556127_at	23181	DIP2A	DIP2 disco-interacting protein 2 homolog A (Drosophila)
215016_x_at	100652766 /// 667	DST /// LOC100652766	dystonin /// dystonin-like
206576_s_at	634	CEACAM1	carcinoembryonic antigen-related cell adhesion molecule 1 (biliary glycoprotein)
240674_at	3720	JARID2	jumonji, AT rich interactive domain 2
225646_at	1075	CTSC	cathepsin C
1554010_at	3340	NDST1	N-deacetylase/N-sulfotransferase (heparan glucosaminyl) 1
204100_at	7067	THRA	thyroid hormone receptor, alpha
221840_at	5791	PTPRE	protein tyrosine phosphatase, receptor type, E
209978_s_at	25828	TXN2	thioredoxin 2
218530_at	29109	FHOD1	formin homology 2 domain containing 1
235434_at	---	---	---
230063_at	9422	ZNF264	zinc finger protein 264
40420_at	6793	STK10	serine/threonine kinase 10
221027_s_at	81579	PLA2G12A	phospholipase A2, group XIIA
244202_at	---	---	---
212108_at	23197	FAF2	Fas associated factor family member 2
204294_at	275	AMT	aminomethyltransferase
225503_at	207063	DHRSX	dehydrogenase/reductase (SDR family) X-linked
212810_s_at	6509	SLC1A4	solute carrier family 1 (glutamate/neutral amino acid transporter), member 4
214814_at	91746	YTHDC1	YTH domain containing 1
228468_at	84930	MASTL	microtubule associated serine/threonine kinase-like
209108_at	7105	TSPAN6	tetraspanin 6
220444_at	79230	ZNF557	zinc finger protein 557
206172_at	3598	IL13RA2	interleukin 13 receptor, alpha 2
225002_s_at	25870	SUMF2	sulfatase modifying factor 2
212208_at	94239	H2AFV	H2A histone family, member V
228851_s_at	2029	ENSA	endosulfine alpha
209048_s_at	23613	ZMYND8	zinc finger, MYND-type containing 8
211846_s_at	5818	PVRL1	poliovirus receptor-related 1 (herpesvirus entry mediator C)
238909_at	6281	S100A10	S100 calcium binding protein A10
205503_at	5784	PTPN14	protein tyrosine phosphatase, non-receptor type 14
343829_at	673	BRAF	v-raf murine sarcoma viral oncogene homolog B1
244379_at	---	---	---
223351_s_at	55608	ANKRD10	ankyrin repeat domain 10

202633_at	11073	TOPBP1	topoisomerase (DNA) II binding protein 1
214502_at	8970	HIST1H2BJ	histone cluster 1, H2bj
221773_at	2004	ELK3	ELK3, ETS-domain protein (SRF accessory protein 2)
41858_at	27315	PGAP2	post-GPI attachment to proteins 2
212850_s_at	4038	LRP4	low density lipoprotein receptor-related protein 4
223408_s_at	---	---	---
214472_at	3013 /// 8350 /// 8351 /// 8352 /// 8353 /// 8354 /// 8355 /// 8356 /// 8357 /// 8358 /	HIST1H2AD /// HIST1H3A /// HIST1H3B /// HIST1H3C /// HIST1H3D /// HIST1H3E /// HIST1H3F /// HIST1H3G /// HIST1H3H /// HIST1H3I /// HIST1H3J	histone cluster 1, H2ad /// histone cluster 1, H3a /// histone cluster 1, H3b /// histo
225647_s_at	1075	CTSC	cathepsin C
1559977_a_at	284723	SLC25A34	solute carrier family 25, member 34
211347_at	8555	CDC14B	CDC14 cell division cycle 14 homolog B (S. cerevisiae)
1558208_at	---	---	---
227570_at	144110	TMEM86A	transmembrane protein 86A
227492_at	100506658 /// 647859	LOC647859 /// OCLN	occludin pseudogene /// occludin
1558778_s_at	57496	MKL2	MKL/myocardin-like 2
31637_s_at	7067 /// 9572	NR1D1 /// THRA	nuclear receptor subfamily 1, group D, member 1 /// thyroid hormone receptor, alpha
229190_at	100507376	LOC100507376	uncharacterized LOC100507376
236188_s_at	4676	NAP1L4	Nucleosome assembly protein 1-like 4
212503_s_at	22982	DIP2C	DIP2 disco-interacting protein 2 homolog C (Drosophila)
204760_s_at	7067 /// 9572	NR1D1 /// THRA	nuclear receptor subfamily 1, group D, member 1 /// thyroid hormone receptor, alpha
212099_at	388	RHOB	ras homolog family member B
214873_at	91355	LRP5L	low density lipoprotein receptor-related protein 5-like
228181_at	7779	SLC30A1	solute carrier family 30 (zinc transporter), member 1
212763_at	23271	CAMSAP2	calmodulin regulated spectrin-associated protein family, member 2
226285_at	4076	CAPRIN1	cell cycle associated protein 1
213567_at	3840	KPNA4	karyopherin alpha 4 (importin alpha 3)
203927_at	4794	NFKBIE	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, epsilon
208523_x_at	8339 /// 8343 /// 8344 /// 8346 /// 8347	HIST1H2BC /// HIST1H2BE /// HIST1H2BF /// HIST1H2BG /// HIST1H2BI	histone cluster 1, H2bc /// histone cluster 1, H2be /// histone cluster 1, H2bf /// his
219389_at	55061	SUSD4	sushi domain containing 4
202329_at	1445	CSK	c-src tyrosine kinase
238523_at	79786	KLHL36	kelch-like 36 (Drosophila)
1565016_at	3276	PRMT1	protein arginine methyltransferase 1

226409_at	128637	TBC1D20	TBC1 domain family, member 20
229926_at	100500850	MIR3682	microRNA 3682
208527_x_at	8339 /// 8343 /// 8344 /// 8346 /// 8347	HIST1H2BC /// HIST1H2BE /// HIST1H2BF /// HIST1H2BG /// HIST1H2BI	histone cluster 1, H2bc /// histone cluster 1, H2be /// histone cluster 1, H2bf /// his
223598_at	5887	RAD23B	RAD23 homolog B (S. cerevisiae)
243797_at	9262	STK17B	serine/threonine kinase 17b
203317_at	23550	PSD4	pleckstrin and Sec7 domain containing 4
230965_at	9099	USP2	ubiquitin specific peptidase 2
208490_x_at	8339 /// 8343 /// 8344 /// 8346 /// 8347	HIST1H2BC /// HIST1H2BE /// HIST1H2BF /// HIST1H2BG /// HIST1H2BI	histone cluster 1, H2bc /// histone cluster 1, H2be /// histone cluster 1, H2bf /// his
235514_at	151516	ASPRV1	aspartic peptidase, retroviral-like 1
209098_s_at	182	JAG1	jagged 1
1554229_at	153222	CREBRF	CREB3 regulatory factor
209398_at	3006	HIST1H1C	histone cluster 1, H1c
202629_at	10513	APPBP2	amyloid beta precursor protein (cytoplasmic tail) binding protein 2
203428_s_at	25842	ASF1A	ASF1 anti-silencing function 1 homolog A (S. cerevisiae)
238005_s_at	25942	SIN3A	SIN3 transcription regulator homolog A (yeast)
214455_at	8339 /// 8343 /// 8344 /// 8346 /// 8347	HIST1H2BC /// HIST1H2BE /// HIST1H2BF /// HIST1H2BG /// HIST1H2BI	histone cluster 1, H2bc /// histone cluster 1, H2be /// histone cluster 1, H2bf /// his
214073_at	2017	CTTN	cortactin
203140_at	604	BCL6	B-cell CLL/lymphoma 6
232150_at	---	---	---
208546_x_at	8345	HIST1H2BH	histone cluster 1, H2bh
243446_at	84962	AJUBA	ajuba LIM protein
236207_at	6744	SSFA2	sperm specific antigen 2
212016_s_at	5725	PTBP1	polypyrimidine tract binding protein 1
232311_at	567	B2M	Beta-2-microglobulin
219711_at	54807	ZNF586	zinc finger protein 586
208579_x_at	54145 /// 85236	H2BFS /// HIST1H2BK	H2B histone family, member S (pseudogene) /// histone cluster 1, H2bk
239493_at	6129	RPL7	ribosomal protein L7
214074_s_at	2017	CTTN	cortactin
228091_at	55014	STX17	syntaxin 17
234331_s_at	151354	FAM84A	family with sequence similarity 84, member A
212372_at	4628	MYH10	myosin, heavy chain 10, non-muscle

Using the probe list in Table 1, a multivariate analysis of different signatures was conducted using available references to build the following signatures, to analyze the results at different doses of CTO (equivalent to 2 μM, 5 μM, and 10 μM CAI) at 8hrs and 24hrs:

-RAS signature ; Growth factor signature; PI3K/mTOR inhibition; PI3K inhibition; MEK inhibition; HSP90 inhibition; HDAC inhibition; EGFR inhibition; P53 Stabilization; WNT/β-Catenin inhibition; Calcium Signaling; CAI inhibition ; canonical calcium inhibition; and Non-voltage signaling.

Results obtained for the above analyses are summarized in Figure 2b in detail. EGFR, MEK and HDAC pathways were strongly suppressed in response to CTO.

In contrast, the pathway associated with P53 was stabilized in response to CTO.

In addition, genes associated with non-voltage dependent calcium signaling, were strongly suppressed. These results are discussed in detail below.

Example3.

FIG 3a describes results of multivariate signatures for RAS and Growth Factor Signatures. Briefly, modest down regulation in RAS and Growth Factor signatures (GFS) were observed, the inhibition being most obvious at 8hrs. Table 2 gives List of RAS and GFS signatures.

Table 2.

GFS And RAS: Symbol

ABCC5	CYHR1	HIST3H2A	POU2F3	CORO1C	IFRD1	PFKP
ATP6V1B1	DEPTOR	HOXB13	RAMP1	DLEU2	IMPAD1	PNPT1
ATXN3	DNAL4	ING4	SEMA3G	DPH3	KLK6	PSMC4
BCA51	EIF4A2	OVGP1	SEPP1	EIF5	KPNA4	RPS6KA3
BCL2L11	EPHX2	PCMTD1	SIDT2	ENO2	LRP8	S100A2
CALCOCO1	ERBB3	PCMTD2	AREG	HN1	MALL	SERPINB5
CAPN13	HIST1H2AC	PDIA4	BTG3	HSP90AA1	MTHFD1L	SERPINB8

CRBN	HIST1H2BD	PLEKHG4	CEBPG	HSPA4L	PADI1	SLC7A1
						SRXN1
						TIPIN

FIG. 3c describes results on PI3K in response to CTO. Strong suppression of PI3K signature was observed with control BEZ235 treatment but not with CTO.

FIG. 3d describes results on PI3K/mTOR signature. Strong suppression of PI3K/mTOR signature was observed with control BEZ235 treatment but not with CTO.

FIG. 3e describes strong inhibition of MEKi signature in all CTO treatments at 8hrs and 24hrs.

Example4.

FIG. 4a gives results showing weak suppression of HDAC signature in response to CTO treatment. The Signature Score for the HDAC pathway in response to different doses of CTO at 8hr and 24hr is compared with BEZ235 and Tarceva® for 24hr.

FIG. 4b describes no suppression of Notch signature in response to CTO treatment. The Signature Score for the NOTCH pathway in response to different doses of CTO at 8hr and 24hr is compared with BEZ235 and Tarceva® for 24hr.

FIG. 4c describes results showing WNT β-catenin signature shows modes suppression in 24hrs exposure to CTO. The Signature Score for the WNT β-catenin pathway in response to different doses of CTO at 8hr and 24hr is compared with BEZ235 and Tarceva® for 24hr.

FIG. 4d describes strong suppression of HSP90 signature with CTO treatment which is dose dependent. The Signature Score for the HSP90 pathway in response to different doses of CTO at 8hr and 24hr is compared with BEZ235 and Tarceva® for 24hr.

FIG. 4e describes very strong suppression of EGFR signature with CTO treatment and importantly this is stronger than that in Tarceva® at all doses of CTO and time points. The Signature Score for the EGFR pathway in response to different doses of CTO at 8hr and 24hr is compared with BEZ235 and Tarceva® for 24hr

FIG. 4f describes induction of P53 signature with CTO treatment at all doses which is higher at the 24hr time point. The Signature Score for the P53 pathway in response to different doses of CTO at 8hr and 24hr is compared with BEZ235 and Tarceva® for 24hr.

Example5.

FIG 5a illustrates the Signature Score for the CAI IPA pathway in response to different doses of CTO at 8hr and 24hr compared with BEZ235 and Tarceva® for 24hr. Using the Ingenuity Pathways Analysis (IPA) (Ingenuity Systems, Redwood City, CA) for genes influenced by CAI, some suppression of CAI signature in response to CTO treatment was observed, except at 10 μM. When the 29 genes were filtered from IPA for FDA <0.05 and 1.5FC in CTO data set, to select informative genes and determine the direction of change, a 14 gene set resulted.

Table 3 lists the CAI IPA: Symbol

MMP2	ESR1	FOS	PCLG2	MAPK6	MAP15	AKT1	MOS3	HSP90B1
JUN	CCND1	PPAR1	MAPK1	MAPK7	CASP3	AKT2	HSP90AA1	Hsp84-2
CEBPA	MAPK3	PCLG1	MAPK4	MAPK12	HSPA8	AKT3	HSP90AB1	Hsp84-3
								BAG3

FIG 5b illustrates the Signature Score for the CAI Ex vivo pathway in response to different doses of CTO at 8hr and 24hr compared with BEZ235 and Tarceva® for 24hr. With the tissue specific direction of change information a strong dose dependent suppression of the IPA CAI list was observed. Table 4 lists the 14 gene set.

Table 4.

Symbol:

AKT2	CASP3	FOS	HSP90AB1	JUN	CEBPA	MAPK3
BAG3	CCND1	HSP90AA1	HSP90B1	AKT1	MAPK1	MAPK7

FIG 5c illustrates the Signature Score for the Calcium signaling pathway in response to different doses of CTO at 8hr and 24hr compared with BEZ235 and Tarceva® for 24hr.

Non-voltage dependent calcium signaling genes were identified from datasets in literature to inform on regulation. The list is presented in Table 5.

Table 5.

Symbol:

ARG2	CCNA1	CCND2	CCNE2	TNF	CA9
BDNF	CCNA2	CCNE1	CTF1	BRAF	CALR

FIG 5d illustrates the Signature Score for the Calcium signaling for all gene pathway in response to different doses of CTO at 8hr and 24hr compared with BEZ235 and Tarceva® for 24hr. The estimated regulation direction for all genes was identified. The list is provided in Table 6.

Table 6.
Symbol:

ARG2	CCND2	TNF	PPP3R1	CALM3	CAMK2A	CAMK4	ORAI3	TRPC3	TRPC7
BDNF	CCNE1	PPP3CA	PPP3R2	CAMK1	CAMK2B	NOS2	STIM1	TRPC4	BRAF
CCNA1	CCNE2	PPP3CB	CALM1	CAMK1D	CAMK2D	ORAI1	STIM2	TRPC5	CA9
CCNA2	CTF1	PPP3CC	CALM2	CAMK1G	CAMK2G	ORAI2	TRPC1	TRPC6	CALR

FIG 5e illustrates the Signature Score for the Calcium Signaling pathway in response to different doses of CTO at 8hr and 24hr compared with BEZ235 and Tarceva® for 24hr. ANOVA was used to filter informative genes (FDR <0.05 and 1.5 FC).

Strong suppression of non-voltage dependent calcium genes was noted across CTO treatment in both literature determined regulation and ANOVA determined set. Table 7.

Table 7

Symbol:

BDNF	CCNE1	PPP3CC	ORAI3	TNF	CAMK1	ORAI1	BRAF
CCNA1	PPP3CB	CAMK2D	CCND2	CALM3	CAMK2G	STIM2	CALR

FIG 6a illustrates the Signature Score for the Canonical Calcium signaling using the

KEGG Calcium Signaling and IPA to predict regulation of calcium signaling in response to differentdoses of CTO at 8hr and 24hr compared with BEZ235 and Tarceva® for 24hr. Table 8.

Table 8.

Symbol

PPP3CA	CACNA2D4	CHRNB3	HDAC10
PPP3CB	CACNB1	CHRNB4	HDAC11
PPP3CC	CACNB2	CHRND	HDAC2
PPP3R1	CACNB3	CHRNE	HDAC3
PPP3R2	CACNB4	CHRNG	HDAC4
ATP2C1	CACNG1	GRIA1	HDAC5
CACNA1A	CHRFAM7A	GRIA2	HDAC6
CACNA1B	CHRNA1	GRIA3	HDAC7
CACNA1C	CHRNA10	GRIA4	HDAC8
CACNA1D	CHRNA2	GRIK1	HDAC9
CACNA1F	CHRNA3	GRIN1	HTR3A
CACNA1G	CHRNA4	GRIN2A	RYR1
CACNA1H	CHRNA5	GRIN2B	SLC8A1
CACNA1I	CHRNA6	GRIN2C	TNNC1
CACNA1S	CHRNA7	GRIN2D	
CACNA2D1	CHRNA9	GRIN3A	
CACNA2D2	CHRNB1	GRIN3B	
CACNA2D3	CHRNB2	HDAC1	

FIG 6b illustrates the Signature Score for the Canonical Calcium signaling ex vivo using the KEGG Calcium Signaling and IPA to predict regulation of in response to different doses of CTO at 8hr and 24hr compared with BEZ235 and Tarceva® for 24hr. Suppression noted for 6/78 canonical pathway genes only. Table 9

Table 9

Gene Symbol:

HDAC3	HDAC4	PPP3CC	GRIA3	CACNB3	HDAC8	PPP3CC
HDAC4	PPP3CB	ATP2C1	GRIA3	GRIA3	PPP3CC	PPP3CC

Example 7.

FIG. 7a illustrates the Signature Score for all Signaling genes after merging of the ANOVA filtered gene sets for both the CAI signature (FIG. 5b) and the non-voltage dependent (NVD) gene sets (FIG. 5c).

FIG 7b illustrates results in a panel of 31 CAI/Calcium related genes capable of separating the different CTO doses, both by signature view and by PCA.

Table 10 provides the list of 31 genes.

Table 10

Gene Symbol	Source
AKT1	CAI
AKT2	CAI
BAG3	CAI
CASP3	CAI
CCND1	CAI
CEBPA	CAI
FOS	CAI
HSP90AA1	CAI
HSP90AB1	CAI
HSP90B1	CAI
JUN	CAI
MAPK1	CAI
MAPK3	CAI
MAPK7	CAI
BDNF	Non-voltage
BRAF	Non-voltage
CALM3	Non-voltage
CALR	Non-voltage
CAMK1	Non-voltage
CAMK2D	Non-voltage
CAMK2G	Non-voltage
CCNA1	Non-voltage
CCND2	Non-voltage
CCNE1	Non-

	voltage
ORAI1	Non-voltage
ORAI3	Non-voltage
PPP3CB	Non-voltage
PPP3CC	Non-voltage
STIM2	Non-voltage
TNF	Non-voltage
ATP2C1	KEGG
CACNB3	KEGG
GRIA3	KEGG
HDAC3	KEGG
HDAC4	KEGG

Results of bioinformatic analysis of data obtained in response to varying doses of CTO at 8hr and 24hrs indicate that the early growth response 1 gene product (EGR1) was up regulated 6 fold by CTO treatment. In contrast EGR1 was suppressed by BEZ235 and Tarceva®. The up regulation of EGR1 generally regulates multiple tumor suppressor pathways inducing apoptotic downstream events. Liu, C et al Proc Natl Acad Sci 93: 11831-11836 (1996).

Figure 8 illustrates the EGR1 Signaling Pathway

Example 8.

The carcinoembryonic antigen-related cell adhesion molecule (CEACAM1) (also known as CD66a) was down regulated in response to both CTO and Tarceva® treatment.

Dystonin was down regulated by 60 fold in response to CTO treatment.

TGF-β signaling was inhibited in response to treatment with CTO.

The present invention is not to be limited in scope by the embodiment disclosed in the example which is intended as an illustration of one aspect of the invention and any methods which are functionally equivalent are within the scope of the invention. Indeed, various

modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description. Such modifications are intended to fall within the scope of the appended claims.

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, any equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the claims.

What is claimed is:

1. A method for quantifying the response to carboxyamidotriazole orotate (CTO) on pharmacodynamics biomarkers of multiple signature pathways , said method comprising:

   a) Obtaining a cell sample obtained from a subject and exposing the cell sample to varying doses of CTO alone, to CTO in combination with another agent or to other agents for different time periods;

   b) Isolating the mRNA from the treated cell sample and preparing representative cDNA there from and measuring the transcriptional alteration in expression in the cell sample resulting from CTO exposure;

   c) Calculating a signature score for each of the pharmacodynamics biomarkers of multiple signature pathways and quantitating the response to the varying doses of CTO exposure, selecting a list of overlapping genes over expressing at two time periods as listed in Table 1; and

   d) Identifying each of the pharmacodynamics biomarkers of multiple signature pathways by at least 3 or more genes listed in Table 1 and confirming each of the pharmacodynamics biomarkers of multiple signature pathways using reference datasets.

2. The method of claim 1, wherein the pharmacodynamic biomarkers of the signature pathways includes at least 3 or more genes for which pharmacodynamics biomarkers are listed in Table 2.

3. The method of claim 2, wherein the pharmacodynamics biomarker of the signature pathwys is RAS.

4. The method of claim 3, wherein the signaling score of the pharmacodynamic

biomarkers of the signature pathways for RAS is inhibited in response to exposure to CTO.

5. The method of claim 2, wherein the pharmacodynamics biomarker of the signature pathways is GFS.

6. The method of claim 5, wherein the signaling score of the pharmacodynamic biomarkers of the signature pathways for GFS is inhibited in response to exposure to CTO.

7. The method of claim 1, wherein the pharmacodynamics biomarker of the signature pathways is MEKi.

8. The method of claim 5, wherein the signaling score of the pharmacodynamic biomarkers of the signature pathways for MEKi is inhibited in response to exposure to CTO.

9. The method of claim 1, wherein the pharmacodynamics biomarker of the signature pathways is HDAC.

10. The method of claim 9, wherein the signaling score of the pharmacodynamic biomarkers of the signature pathways for HDAC is inhibited in response to exposure to CTO.

11. The method of claim 1, wherein the pharmacodynamics biomarker of the signature pathways is NOTCH.

12. The method of claim 11, wherein the signaling score of the pharmacodynamic biomarkers of the signature pathways for NOTCH is inhibited in response to exposure to CTO.

13. The method of claim 1, wherein the pharmacodynamics biomarker of the signature pathways is WNTβ-catenin.

14. The method of claim 13, wherein the signaling score of the pharmacodynamic

biomarkers of the signature pathways for WNTβ-catenin is inhibited in response to exposure to CTO.

15. The method of claim 1, wherein the pharmacodynamics biomarker of the signature pathways is HSP90.

16. The method of claim 15, wherein the signaling score of the pharmacodynamic biomarkers of the signature pathways for HSP90 is inhibited in response to exposure to CTO.

17. The method of claim 1, wherein the pharmacodynamics biomarker of the signature pathways is EGFR.

18. The method of claim 17, wherein the signaling score of the pharmacodynamic biomarkers of the signature pathways for EGFR is inhibited in response to exposure to CTO.

19. The method of claim 1, wherein the pharmacodynamics biomarker of the signature pathways is P53.

20. The method of claim 19, wherein the signaling score of the pharmacodynamic biomarkers of the signature pathways for P53 is induced in response to exposure to CTO.

21. The method of claim 1, wherein the pharmacodynamic biomarkers of the signature pathways includes at least 3 or more genes for which pharmacodynamics biomarkers are listed in Table 3.

22. The method of claim 21, wherein the pharmacodynamic biomarkers of the signature pathways are Carboxyamidotriazole (CAI) induced signatures identified from IPA.

23. The method of claim 22, wherein the signaling score of the pharmacodynamic biomarkers of the signature pathways induced by CAI is inhibited in response to exposure to CTO.

24. The method of claim 1, wherein the pharmacodynamic biomarkers of the signature pathways includes at least 3 or more genes for which pharmacodynamics biomarkers are listed in Table 4.

25. The method of claim 24, wherein the pharmacodynamics biomarker of the signature pathways are induced by CAI ex vivo.

26. The method of claim 25, wherein the signaling score of the pharmacodynamic biomarkers of the signature pathways for CAI is inhibited in response to exposure to CTO.

27. The method of claim 1, wherein the pharmacodynamic biomarkers of the signature pathways includes at least 3 or more genes for which pharmacodynamics biomarkers are listed in Table 5.

28. The method of claim 27, wherein the pharmacodynamics biomarker of the signature pathways are calcium signaling pathways.

29. The method of claim 28, wherein the signaling score of the pharmacodynamic biomarkers of the signature pathways are calcium signaling pathways is inhibited in response to exposure to CTO.

30. The method of claim 1, wherein the pharmacodynamic biomarkers of the signature pathways includes at least 3 or more genes for which pharmacodynamics biomarkers are listed in Table 6.

31. The method of claim 30, wherein the pharmacodynamics biomarker of the signature pathways are all gene calcium signaling pathways.

32. The method of claim 31, wherein the signaling score of the pharmacodynamic biomarkers of the signature pathways are all gene calcium signaling pathways is inhibited in response to exposure to CTO.

33. The method of claim 1, wherein the pharmacodynamic biomarkers of the signature

pathways includes at least 3 or more genes for which pharmacodynamics biomarkers are listed in Table 7.

34. The method of claim 33, wherein the pharmacodynamics biomarker of the signature pathways are non-voltage dependent calcium signaling pathways.

35. The method of claim 34, wherein the signaling score of the pharmacodynamic biomarkers of the signature pathways are non-voltage dependent calcium signaling pathways is inhibited in response to exposure to CTO.

36. The method of claim 1, wherein the pharmacodynamic biomarkers of the signature pathways includes at least 3 or more genes for which pharmacodynamics biomarkers are listed in Table 8.

37. The method of claim 36, wherein the pharmacodynamics biomarker of the signature pathways are canonical calcium signaling pathways.

38. The method of claim 37, wherein the signaling score of the pharmacodynamic biomarkers of the signature pathways for canonical calcium signaling pathways is inhibited in response to exposure to CTO.

39. The method of claim 1, wherein the pharmacodynamic biomarkers of the signature pathways includes at least 3 or more genes for which pharmacodynamics biomarkers are listed in Table 8.

40. The method of claim 39, wherein the pharmacodynamics biomarker of the signature pathways are canonical calcium signaling pathways.

41. The method of claim 40, wherein the signaling score of the pharmacodynamic biomarkers of the signature pathways for canonical calcium signaling pathways is inhibited in response to exposure to CTO.

42. The method of claim 1, wherein the pharmacodynamic biomarkers of the signature pathways includes at least 3 or more genes for which pharmacodynamics biomarkers

are listed in a reference dataset, wherein the pharmacodynamics biomarker of the signature pathways is EGR1.

43. The method of claim 43, wherein the signaling score of the pharmacodynamic biomarkers of the signature pathways for EGR1 is stimulated in response to exposure to CTO.

44. The method of claim 1, wherein the pharmacodynamic biomarkers of the signature pathways includes at least 3 or more genes for which pharmacodynamics biomarkers are listed in a reference dataset ,wherein the pharmacodynamics biomarker of the signature pathways is CEACAMI.

45. The method of claim 45, wherein the signaling score of the pharmacodynamic biomarkers of the signature pathways for CEACAMI is stimulated in response to exposure to CTO.

46. The method of claim 1, wherein the pharmacodynamic biomarkers of the signature pathways includes at least 3 or more genes for which pharmacodynamics biomarkers are listed in a reference dataset ,wherein the pharmacodynamics biomarker of the signature pathways is TGFβ.

47. The method of claim 47, wherein the signaling score of the pharmacodynamic biomarkers of the signature pathways for TGFβ is stimulated in response to exposure to CTO.

48. The method of claim 1, wherein the pharmacodynamic biomarkers of the signature pathways includes at least 3 or more genes for which pharmacodynamics biomarkers are listed in a reference dataset ,wherein the pharmacodynamics biomarker of the signature pathways is Dystonin.

49. The method of claim 49, wherein the signaling score of the pharmacodynamic biomarkers of the signature pathways for Dystonin is stimulated in response to

exposure to CTO.

50. A method for quantifying the response to carboxyamidotriazole orotate (CTO) on pharmacodynamics biomarkers of non-voltage dependent Calcium signaling pathways, said method comprising:

a) Obtaining a cell sample obtained from a subject treated with varying doses of CTO alone, CTO in combination with another agent or other agents for different time periods;

b) Isolating the mRNA from the treated cell sample and preparing representative cDNA there from and measuring the transcriptional alteration in expression in the cell sample resulting from CTO exposure;

c) Calculating a signature score for each of the pharmacodynamics biomarkers of multiple signature pathways and quantitating the response to different doses CTO exposure in vivo to the varying doses of CTO alone, to CTO in combination with another agent or to other agents, selecting a list of overlapping genes over expressing at different time periods; and

d) Identifying each of the pharmacodynamics biomarkers of multiple signature pathways by at least 3 or more genes listed in Table 10 and quantitating the response in each of the pharmacodynamics biomarkers of multiple signature pathways to different doses CTO exposure in vivo to the varying doses of CTO alone, to CTO in combination with another agent or to other agents.

51. A method for quantifying the response to carboxyamidotriazole orotate (CTO) on pharmacodynamics biomarkers of transcription signatures selected from the group consisting of EGFR, MEK, HDAC, HSP90, WNTβ-catenin, P53, EGR1, PTEN, TGFβ, RAS, GFS, CEACAM1 and Dystonin, said method comprising:

a) Obtaining a cell sample obtained from a subject treated with varying doses of CTO alone, CTO in combination with another agent or other agents for different time periods;

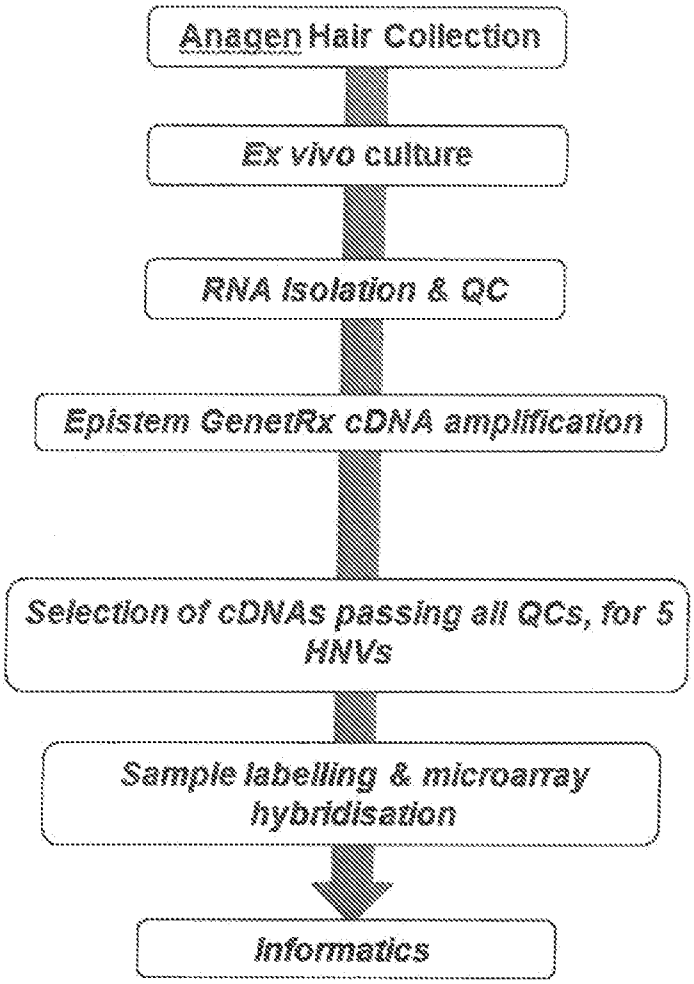
b) Isolating the mRNA from the treated cell sample and preparing representative cDNA there from and measuring the transcriptional alteration in expression in the cell sample resulting from CTO exposure;

c) Calculating a signature score for each of the pharmacodynamics biomarkers of multiple signature pathways and quantitating the response to different doses CTO exposure in vivo to the varying doses of CTO alone, to CTO in combination with another agent or to other agents, selecting a list of overlapping genes over expressing at different time periods; and

d) Identifying each of the pharmacodynamics biomarkers of multiple signature pathways by at least 3 or more genes listed in Table 1or reference datasets and quantitating the response in each of the pharmacodynamics biomarkers of multiple signature pathways to different doses CTO exposure in vivo to the varying doses of CTO alone, to CTO in combination with another agent or to other agents.

FIG. 1

(a)

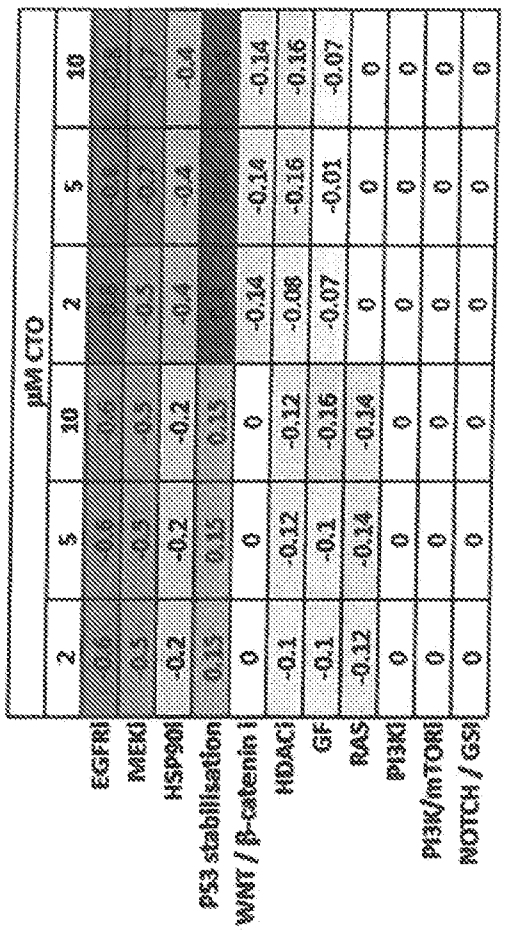


(b)

	CTO						BEZ235	Torceva
	8 Hours			24 Hours				
	2μM	5μM	10μM	2μM	5μM	10μM	1μM	1μM
FDR 0.05 & 1.5 FC	1669	3321	4419	5090	5407	7846	1551	27
Fold Change Range	-17 To 15	-23 To 23	-23 To 29	-43 To 14	-102 To 24	-75 To 32	-8 To 25	-7 To 3

FIG. 2

(a)



(b)

RAS signature:
Growth Factor signature:
PI3K/mTOR inhibition:
PI3K inhibition:
MEK inhibition:
HSP90 inhibition:
HDAC inhibition:
EGFR inhibition:
P53 stabilisation:
WNT/β-Catenin inhibition:
Calcium signalling:
CAI inhibition:
Non-voltage signalling:

FIG.3

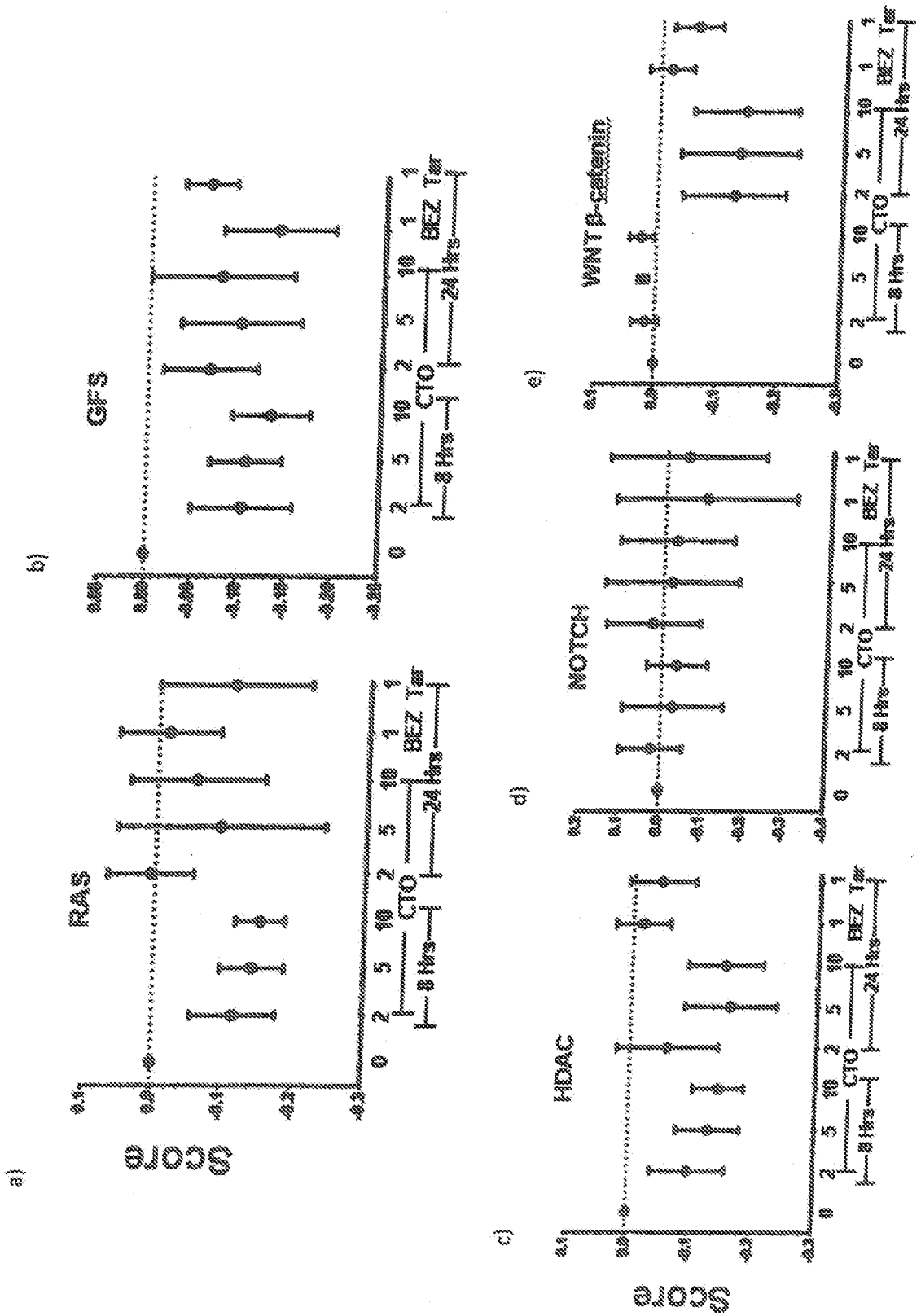

a)
RAS
Score
b)
GFS
c)
HDAC
Score
d)
NOTCH
e)
WNT β-catenin
0 2 5 10 2 5 10 1 1
CTO
BEZ Tar
8 Hrs
24 Hrs

FIG.4

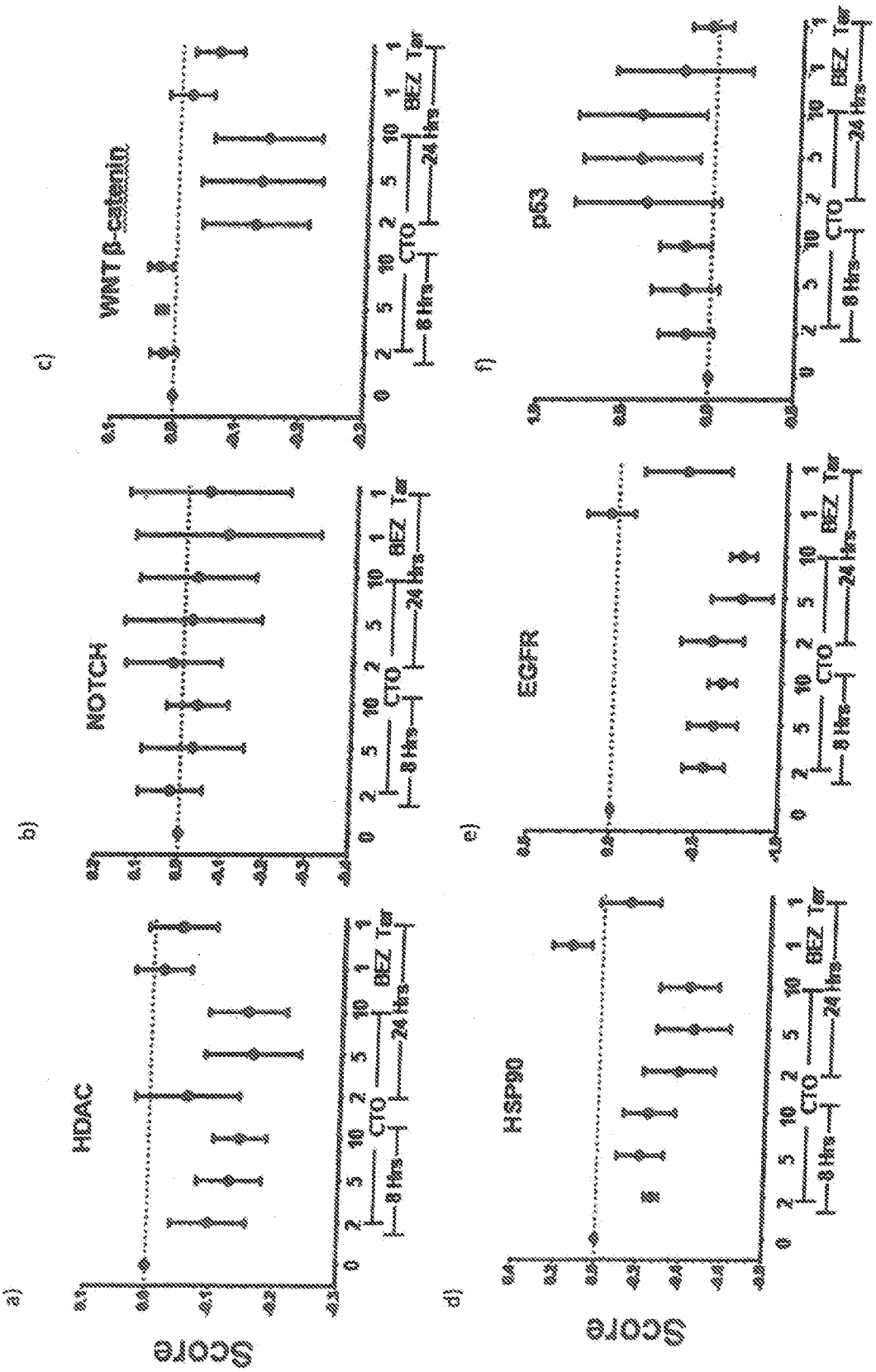

a)
HDAC
Score
b)
NOTCH
c)
WNT β-catenin
d)
HSP90
Score
e)
EGFR
f)
p53
CTO
BEZ
8 Hrs
24 Hrs

FIG.5

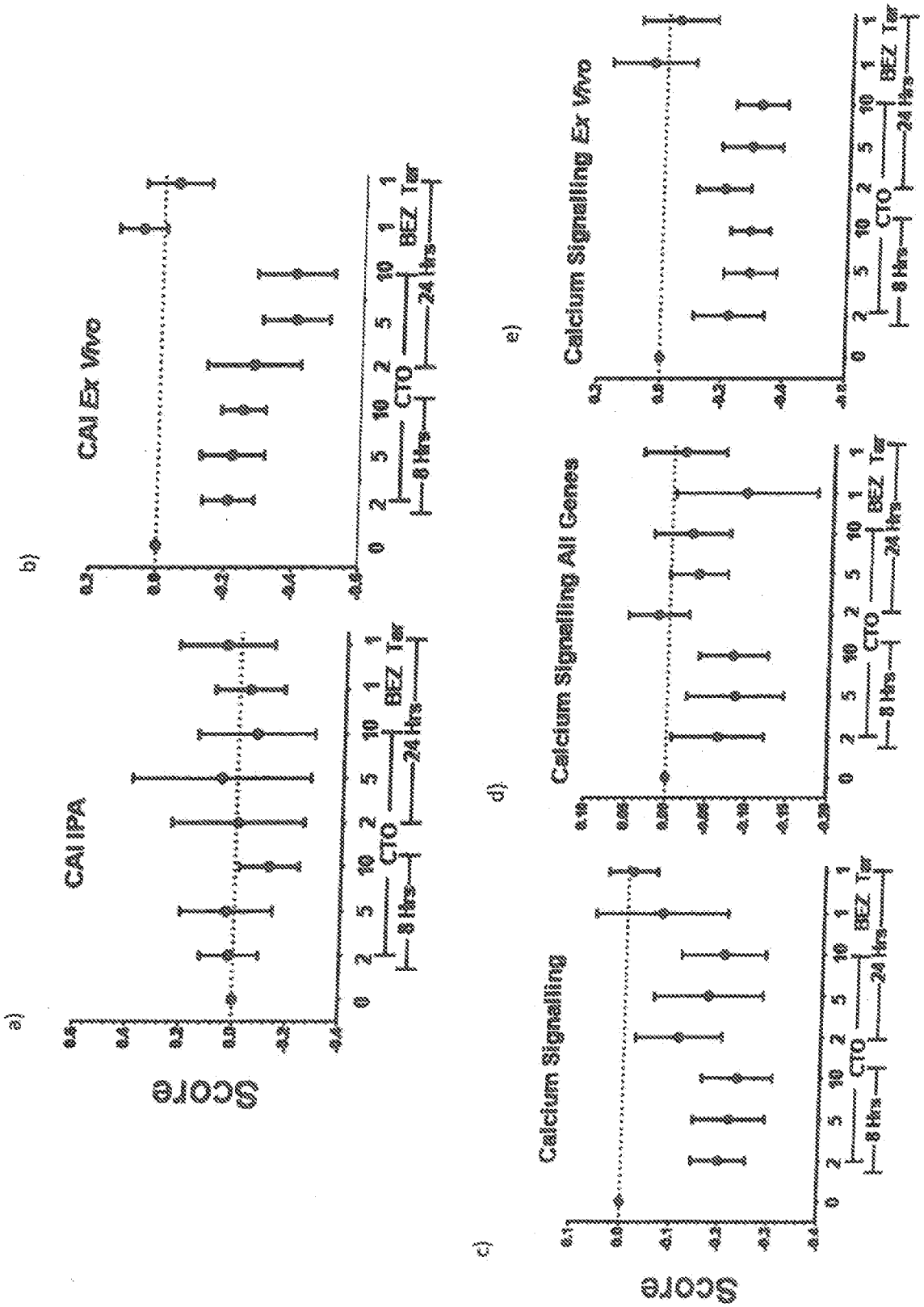

a)
CAI IPA
Score
b)
CAI Ex Vivo
c)
Calcium Signalling
d)
Calcium Signalling All Genes
e)
Calcium Signalling Ex Vivo
0
2
5
10
CTO
8 Hrs
24 Hrs
BEZ Tar

FIG.6

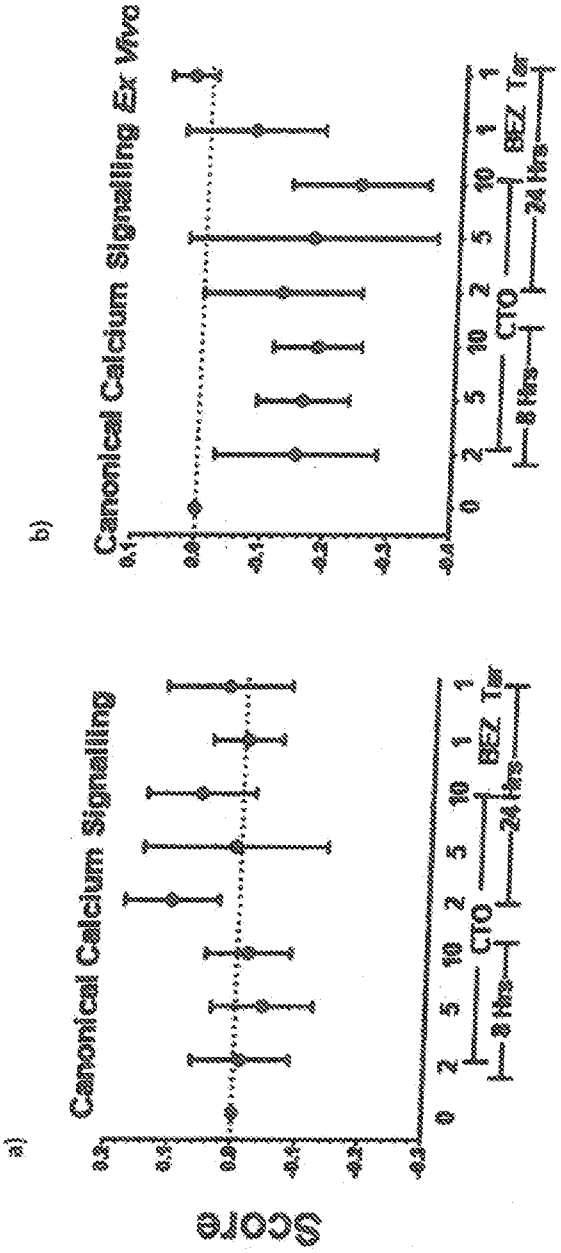

a)
Canonical Calcium Signalling
Score
0.2
0.1
0.0
-0.1
-0.2
-0.3
0
2
5
10
2
3
10
1
1
CTO
BEZ
8 Hrs
24 Hrs
b)
Canonical Calcium Signalling Ex Vivo
0.1
0.0
-0.1
-0.2
-0.3
-0.4
0
2
5
10
2
5
10
1
1
CTO
BEZ
8 Hrs
24 Hrs

FIG.7

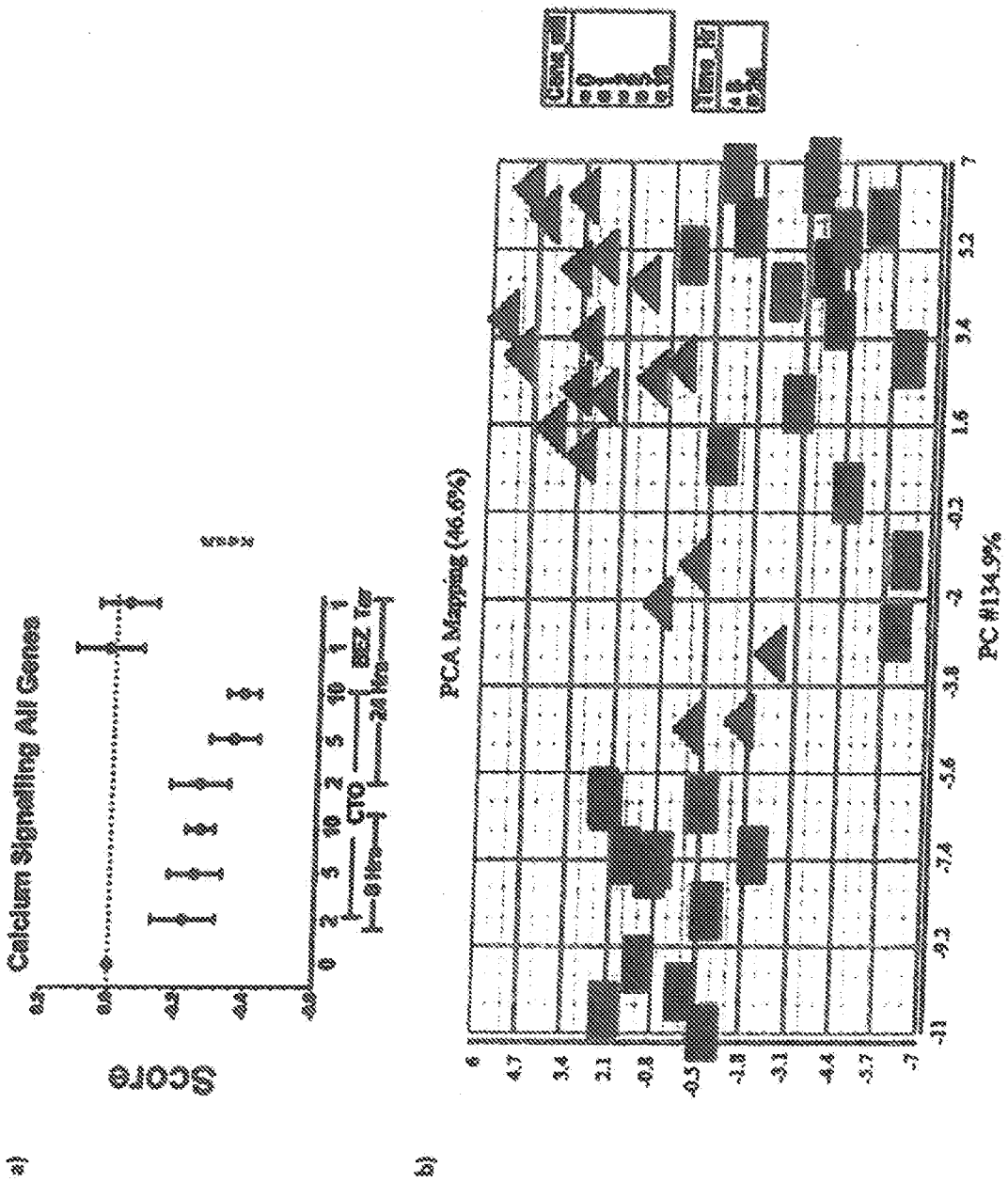
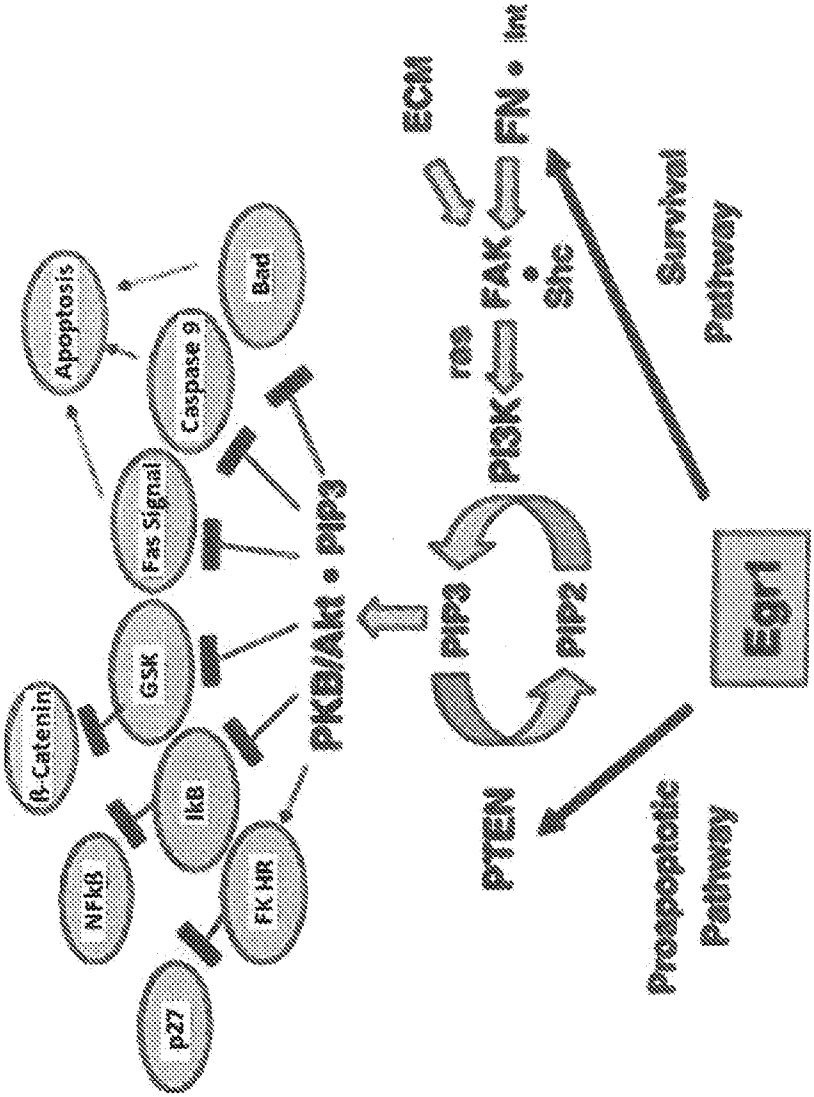

a)
Calcium Signalling All Genes
Score
0.5
0.4
0.3
0.2
0.1
0
2
3
10
2
5
10
1
1
CTO
BEZ 1μg
8 Hrs
24 Hrs
b)
PCA Mapping (46.6%)
PC #134.9%
6
4.7
3.4
2.1
0.8
-0.5
-1.8
-3.1
-4.4
-5.7
-7
-11
-9.2
-7.4
-5.6
-3.8
-2
-0.2
1.6
3.4
5.2
7

FIG.8


Apoptosis
Bad
Caspase 9
Fas Signal
β-Catenin
GSK
NFkB
IkB
p27
FKHR
PKB/Akt • PIP3
PIP3
PIP2
PTEN
ras
PI3K
FAK
Shc
ECM
FN • Int
Egr1
Survival Pathway
Proapoptotic Pathway

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US14/48905

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12Q 1/68 (2014.01)
CPC - C12Q 1/6886, 1/6827, 1/6883
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8): C12Q 1/68 (2014.01)
CPC: C12Q 1/68, 1/6886, 1/6827, 1/6883; USPC: 435/6.14, 6.16, 6.17, 6.18, 6.19, 6.1, 4

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
MicroPatent (US-G, US-A, EP-A, EP-B, WO, JP-bib, DE-C,B, DE-A, DE-T, DE-U, GB-A, FR-A); TotalPatent; ProQuest Dialog; PubMed/PubMed Central; Google; Google Scholar: carboxyamidotriazole, 'carboxyamidotriazole orotate,' 'L651582,' expression, activity, biomarker, 'cell sample,' isolate, extract, 'mRNA,' 'cDNA, 'signature score,' response, reaction, result, 'drug response,' 'overlapping genes'

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CORRADO, C et al. Carboxyamidotriazole-Orotate Inhibits The Growth Of Imatinib-Resistant Chronic Myeloid Leukaemia Cells And Modulates Exosomes-Stimulated Angiogenesis. PLoS ONE. August 2012, Vol. 7, No. 8, e42310; page 2, first column, fourth paragraph to page 3, first column, first paragraph; page 5, figure 2; page 6, first column, first paragraph to page 6, second paragraphs. DOI: 10.1371/journal.pone.0042310.	1-51
A	US 2012/0252689 A1 (WU, Z et al.) October 4, 2012; paragraphs [0046]-[0064], [0087], [0109]	1-51
A	US 2011/0015869 A1 (WATTERS, J et al.) January 20, 2011; paragraphs [0064], [0096], [0129]-[0135], [0157], [0313]; Table 1	1-51
A	US 6239137 B1 (KARMALI, RA et al.) May 29, 2001; abstract	1-51
A	KARMALI, R et al. Carboxyamidotriazole Orotate and Cytotoxic Chemotherapy Have A Synergistic Effect On Tumor Inhibition In Glioblastoma And Colon Xenograft Mouse Models. Cancer Therapy. 20 December 2011, Vol. 8; pages 71-80 [Retrieved on 11 November 2014]; entire document; Retrieved from the Internet: <URL: http://www.cancer-therapy.org/CT/v8/A/HTML/10.Karmali_et_al_71-80.htm>.	1-51
A	KARMALI, R et al. Combinatorial Treatment With Carboxyamidotriazole Orotate And Temozolomide In Sc-Implanted Human LOX IMVI Melanoma Xenografts. Journal of Solid Tumors. 01 October 2012, Vol. 2, No. 5; pages 13-28 [Retrieved on 11 November 2014] ; entire document; Retrieved from the Internet: <URL: http://www.sciedu.ca/journal/index.php/jst/article/viewFile/1529/915>.	1-51

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

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

Date of the actual completion of the international search	Date of mailing of the international search report
21 November 2014 (21.11.2014)	12 DEC 2014
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US14/48905

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	KARMALI, R et al. Treatment With Paclitaxel Orotate And Carboxyamidotriazole Orotate In SC-Implanted OVCAR-5 Human Ovarian Tumor Xenografts. Journal of Cancer Therapy. 4 June 2013, Vol. 4, No. 4; pages 857-871. DOI: 10.4236/jct.2013.44098.	1-51