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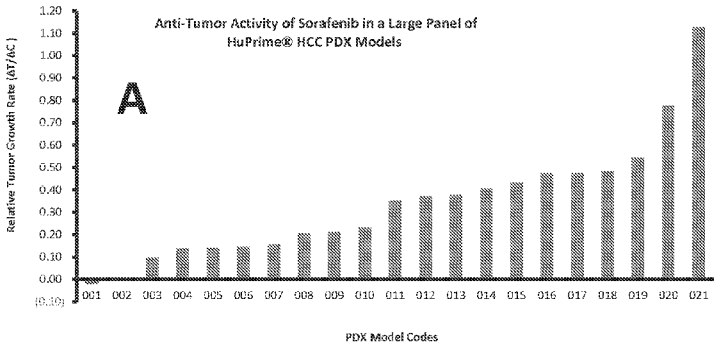
(54) Title: GENE EXPRESSION SIGNATURES PREDICTIVE OF SUBJECT RESPONSE TO A MULTI-KINASE INHIBITOR AND METHODS OF USING THE SAME

FIG. 1A



(57) Abstract: Gene expression signature predictive of cancer patient response to multi-kinase inhibitor is disclosed. Also disclosed are methods predicting the efficacy of the multi-kinase inhibitor for treating cancer in a patient. Also disclosed are methods for distinguishing responders from non-responders to a multi-kinase inhibitor in treating cancer. Also disclosed are methods for treating a cancer patient with a multi-kinase inhibitor.

GENE EXPRESSION SIGNATURES PREDICTIVE OF SUBJECT RESPONSE TO A MULTI-KINASE INHIBITOR AND METHODS OF USING THE SAME

CROSS-REFERENCE TO RELATED APPLICATIONS

[001] The present application claims priority to, and the benefit of International Patent Application Serial No. PCT/CN2013/082487, filed August 28, 2013, which is herein incorporated by reference in its entirety for all purposes.

FIELD OF THE INVENTION

[002] The present invention relates to treatment of subjects as well as identification and selection of subjects for treatment with a multi-kinase inhibitor, e.g., Sorafenib, Sunitinib, Axitinib, Vandetanib, Pazopanib, Cabozantinib, etc. or a salt, solvate, or physiologically functional derivative thereof, or a mixture thereof. Provided are methods, reagents, and tools for the prediction, diagnosis, prognosis, and therapy of a disease such as cancer.

BACKGROUND OF THE INVENTION

[003] Hepatocellular carcinoma (HCC) is an aggressive tumor and the fifth deadliest cancers worldwide. It is particularly prevalent in East Asia and in man (Parkin DM, Bray F, Ferlay J, Pisani P.Global cancer statistics, 2002. CA Cancer J Clin 2005;55:74-108. El-Serag HB, 7. Rudolp.h KL. Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. Gastroenterology 2007;132:2557-76). Its incidence is increasing steadily in both the United States and China (1). So far, there have been few effective treatment options. The common standard chemotherapy (e.g. doxorubicin) has little clinical benefit.

[004] The only approved target therapy available is Sorafenib that recently became available (1). Sorafenib is a multi-kinase inhibitor targeting vascular endothelial growth factor (VEGF)-mediated angiogenesis and blocking the RAF/extracellular signal regulated kinase (ERK) kinase (MEK)/ERK cascade. It is the first drug found to prolong survival of patients with advanced hepatocellular carcinoma (HCC), and has been marketed for treatment of advanced renal cell carcinoma and unresectable HCC. However, increase survival for HCC patients is by modest 3 months. Like many new generation target therapies, Sorafenib also has unfavorable side effects. Many HCC patients do not respond well to Sorafenib both in term of safety and lack of efficacy. There is a clear need for predictive markers of Sorafenib to help selecting the patient population that may benefit the most.

[005] It was reported that the baseline level of phosphorylated ERK (pERK) might be a relevant marker from the initial single arm phase II study of Sorafenib, and a few reports have been published on relationship between the serum markers (e.g. soluble c-KIT, HGF, AFP, VEGF, etc) and the outcome of Sorafenib treatment, but these results are either very preliminary or not consistent across studies. Thus, the utility of these factors as potential predictive markers need to be further evaluated, and new strategies like genomic-based molecular signature analysis could be useful to identify new predictive markers, and help us better understand the mechanism of action of Sorafenib in HCC.

SUMMARY OF THE INVENTION

[006] The present invention provides a panel of gene markers that can be used to predict a subject's responsiveness/resistance to a drug. In some embodiments, the gene markers include SEC14L2, H6PD, TMEM140, SLC2A5, ACTA1, IRF8, STAT2, UGT2A1, functional variants, fragments, or orthologs thereof.

[007] The present invention also provides a collection of activity profile of a panel of gene markers comprising at least two or more gene markers selected from the group consisting of SEC14L2, H6PD, TMEM140, SLC2A5, ACTA1, IRF8, STAT2, UGT2A1, functional variants, fragments, or orthologs thereof. In some embodiments, the activity profile is collected from more than one subject that is responding to a drug. In some embodiments, the activity profile is collected from more than one subject that is resistant to a drug. In some embodiments, the drug is a multi-kinase inhibitor. In some embodiments, the multi-kinase inhibitor targets to vascular endothelial growth factor (VEGF)-mediated angiogenesis and blocks the RAF/extracellular signal regulated kinase (ERK) kinase (MEK)/ERK cascade. In some embodiments, the multi-kinase inhibitor comprises Sorafenib, or a salt, solvate, or physiologically functional derivative thereof. In some embodiments, the collection of activity profile is collected from the subjects, parts of the subjects, or cells derived from the subjects before, during and/or after being treated with the drug. In some embodiments, the panel of gene markers includes at least two, three, or four of gene markers of the present invention.

[008] The present invention also provides methods for determining a subject's responsiveness or resistance to a drug. In some embodiments, the drug is a multi-kinase inhibitor. In some embodiments, the multi-kinase inhibitor targets to vascular endothelial growth factor (VEGF)-mediated angiogenesis and blocks the RAF/extracellular signal regulated kinase (ERK) kinase (MEK)/ERK cascade. In some embodiments, the multi-kinase inhibitor comprises Sorafenib, or a salt, solvate, or physiologically functional

derivative thereof. In some embodiments, the methods comprise measuring activity profile of a panel of gene markers. In some embodiments, the methods further comprise comparing the activity profile of the panel to a predetermined activity profile. In some embodiments, the subject's responsiveness is determined based on the comparison between the activity profile of the panel of gene markers and the predetermined activity profile. In some embodiments, the subject is determined to be responsive to the drug if the activity profile of the panel is within the predetermined activity profile, or determined to be resistant to the drug if the activity profile of the panel is not within the predetermined activity profile. In some embodiments, activity profile derived from subjects that are not responsive to the drug can also be used. For example, the subject is determined to be not responsive to the drug if the activity profile of the panel is within the activity profile derived from subjects that are not responsive to the drug.

[009] In some embodiments, the activity profile of a panel of gene markers includes one or more parameters describing gene expression level, RNA activity level, and/or protein activity level. In some embodiments, the activity profile is reflected by a quantitative signature value or a set of signature values that are calculated on the basis of gene expression level, RNA activity level, and/or protein activity level.

[0010] In some embodiments, the panel comprises at least two, three, or four of gene markers. In some embodiments, the gene markers include at least one or more markers selected from the group consisting of SEC14L2, H6PD, TMEM140, SLC2A5, ACTA1, IRF8, STAT2, and UGT2A1, or any combination thereof.

[0011] In some embodiments, the activity profile is gene expression level, wherein a similar or lower expression of one or more gene markers in comparison to the predetermined activity profile before the treatment of the multi-kinase inhibitor indicates the responsiveness of the subject, and a higher expression of one or more gene markers in comparison to the predetermined activity profile before the treatment of the multi-kinase inhibitor indicates the resistance of the subject.

[0012] Alternatively, a normalization or stabilization in the activity level of a biomarker of the present invention toward a predetermined standard level after the treatment indicates responsiveness of subject. In some embodiments, a normalization or stabilization of SEC14L2, H6PD, TMEM140, SLC2A5, ACTA1, IRF8, STAT2, and/or UGT2A1 toward a predetermined standard level indicates the responsiveness of the subject. In some embodiments, an absence of normalization and stabilization of SEC14L2, H6PD, TMEM140, SLC2A5, ACTA1, IRF8, STAT2, and/or UGT2A1 toward a predetermined standard level

indicates the resistance of the subject to the treatment. As used herein, when the level of a biomarker goes toward the predetermined standard level, it is called normalization. As used herein, when the level of a biomarker reduces its speed of going away from the predetermined standard level, it is called stabilization.

[0013] In some embodiments, the multi-kinase inhibitor is Sorafenib, Axitinib, Vandetanib, Pazopanib, Cabozantinib, or any combination thereof.

[0014] In some embodiments, the subject is a human with cancer. In some embodiments, the cancer is hepatocellular carcinoma.

[0015] The present invention also provides methods for administering a drug to a subject. In some embodiments, the drug is a multi-kinase inhibitor. In some embodiments, the methods comprise testing the subject for activity profile of a panel of gene markers. In some embodiments, the panel of gene markers includes at least one or more markers selected from the group consisting of SEC14L2, H6PD, TMEM140, SLC2A5, ACTA1, IRF8, STAT2, and UGT2A1. In some embodiments, the methods further comprise comparing the activity profile of the panel to a predetermined activity profile. In some embodiments, the comparison is conducted before, during, or after administration of the drug. In some embodiments, the drug comprises at least one multi-kinase inhibitor. In some embodiments, the multi-kinase inhibitor targets to vascular endothelial growth factor (VEGF)-mediated angiogenesis and blocks the RAF/extracellular signal regulated kinase (ERK) kinase (MEK)/ERK cascade. In some embodiments, the multi-kinase inhibitor is administered to the subject if the activity profile of the panel is within the predetermined activity profile derived from a population of subjects that are responding to the drug.

[0016] The present invention also provides an array comprising probes for detection of at least two or more gene markers. In some embodiments, the gene markers are selected from the group consisting of SEC14L2, H6PD, TMEM140, SLC2A5, ACTA1, IRF8, STAT2, and UGT2A1. In some embodiments, the array is a microarray.

BRIEF DESCRIPTION OF THE DRAWINGS

[0017] FIG. 1 shows evaluation of efficacy of Sorafenib in a large panel of HuPrime HCC PDXs. FIG. 1A: $\Delta T/\Delta C$ was calculated, where ΔT and ΔC were the mean tumor volume changes of the treated and control groups, respectively on a given day, as indicated by arrows in FIGs B-E. *:$p<0.05$; **:$p<0.01$; ***:$p<0.001$. FIGs 1B to 1E show representative HCC-HuPrime® respond to Sorafenib, model codes 001, 006, 020, and 021, respectively.

[0018] FIG. 2 shows baseline pERK level detected by IHC is not associated with the model response to Sorafenib.

[0019] FIG. 3 shows Expression of signature genes is predictive of Sorafenib treatment effect to HCC in PDX models. The average mRNA expression intensity, in log2-scale, of the marker genes, is designated as the signature score.

[0020] FIG. 4 shows RAF/extracellular signal regulated kinase (ERK) kinase (MEK)/ERK cascade.

DETAILED DESCRIPTION OF THE INVENTION

[0021] As used herein, the following terms shall have the following meanings:

[0022] The verb “comprise” as is used in this description and in the claims and its conjugations are used in its non-limiting sense to mean that items following the word are included, but items not specifically mentioned are not excluded.

[0023] The term "a" or "an" refers to one or more of that entity; for example, “a gene” refers to one or more genes or at least one gene. As such, the terms "a" (or "an"), "one or more" and "at least one" are used interchangeably herein. In addition, reference to “an element” by the indefinite article "a" or "an" does not exclude the possibility that more than one of the elements are present, unless the context clearly requires that there is one and only one of the elements.

[0024] The invention provides isolated, chimeric, recombinant or synthetic polynucleotide sequences. As used herein, the terms “polynucleotide”, “polynucleotide sequence”, “nucleic acid sequence”, “nucleic acid fragment”, and “isolated nucleic acid fragment” are used interchangeably herein and encompass DNA, RNA, cDNA, whether single stranded or double stranded, as well as chemical modifications thereof. These terms encompass nucleotide sequences and the like. A polynucleotide may be a polymer of RNA or DNA that is single- or double-stranded, that optionally contains synthetic, non-natural or altered nucleotide bases. A polynucleotide in the form of a polymer of DNA may be comprised of one or more segments of cDNA, genomic DNA, synthetic DNA, or mixtures thereof. Nucleotides (usually found in their 5′-monophosphate form) are referred to by a single letter designation as follows: “A” for adenylate or deoxyadenylate (for RNA or DNA, respectively), “C” for cytidylate or deoxycytidylate, “G” for guanylate or deoxyguanylate, “U” for uridylate, “T” for deoxythymidylate, “R” for purines (A or G), “Y” for pyrimidines (C or T), “K” for G or T, “H” for A or C or T, “I” for inosine, and “N” for any nucleotide. In some

embodiments, the isolated, chimeric, recombinant or synthetic polynucleotide sequences are derived from gene markers of the present invention.

[0025] Single letter amino acid abbreviations used herein have their standard meaning in the art, and all peptide sequences described herein are written according to convention, with the N-terminal end to the left and the C-terminal end to the right.

[0026] The invention provides expression gene signatures that can be used to predict patient response to a drug. As used herein, the term “gene” refers to any segment of DNA associated with a biological function. Thus, genes include, but are not limited to, coding sequences and/or the regulatory sequences required for their expression. Genes can also include nonexpressed DNA segments that, for example, form recognition sequences for other proteins. Genes can be obtained from a variety of sources, including cloning from a source of interest or synthesizing from known or predicted sequence information, and may include sequences designed to have desired parameters.

[0027] The invention provides homologous and orthologous polynucleotides and polypeptides. As used herein, the term "homologous" or "homologue" or "ortholog" is known in the art and refers to related sequences that share a common ancestor or family member and are determined based on the degree of sequence identity. The terms “homology”, “homologous”, “substantially similar” and “corresponding substantially” are used interchangeably herein. They refer to nucleic acid fragments wherein changes in one or more nucleotide bases do not affect the ability of the nucleic acid fragment to mediate gene expression or produce a certain phenotype. These terms also refer to modifications of the nucleic acid fragments of the instant invention such as deletion or insertion of one or more nucleotides that do not substantially alter the functional properties of the resulting nucleic acid fragment relative to the initial, unmodified fragment. It is therefore understood, as those skilled in the art will appreciate, that the invention encompasses more than the specific exemplary sequences. These terms describe the relationship between a gene found in one species, subspecies, variety, cultivar or strain and the corresponding or equivalent gene in another species, subspecies, variety, cultivar or strain. For purposes of this invention homologous sequences are compared. "Homologous sequences" or "homologues" or "orthologs" are thought, believed, or known to be functionally related. A functional relationship may be indicated in any one of a number of ways, including, but not limited to: (a) degree of sequence identity and/or (b) the same or similar biological function. Preferably, both (a) and (b) are indicated. The degree of sequence identity may vary, but in some embodiments, is at least 50% (when using standard sequence alignment programs known in the art), at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at

least 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least 98.5%, or at least about 99%, or at least 99.5%, or at least 99.8%, or at least 99.9%. Homology can be determined using software programs readily available in the art, such as those discussed in Current Protocols in Molecular Biology (F.M. Ausubel et al., eds., 1987) Supplement 30, section 7.718, Table 7.71. Some alignment programs are MacVector (Oxford Molecular Ltd, Oxford, U.K.), ALIGN Plus (Scientific and Educational Software, Pennsylvania) and AlignX (Vector NTI, Invitrogen, Carlsbad, CA). Another alignment program is Sequencher (Gene Codes, Ann Arbor, Michigan), using default parameters.

[0028] The invention provides probes and primers that are derived from the nucleic acid sequences of the signature genes. The term "probe" as used herein refers to an oligonucleotide which is capable of specific annealing to the amplification target. The term "primer" as used herein refers to an oligonucleotide which is capable of annealing to the amplification target allowing a DNA polymerase to attach, thereby serving as a point of initiation of DNA synthesis when placed under conditions in which synthesis of primer extension product is induced, i.e., in the presence of nucleotides and an agent for polymerization such as DNA polymerase and at a suitable temperature and pH. The (amplification) primer is preferably single stranded for maximum efficiency in amplification. Preferably, the primer is an oligodeoxyribonucleotide. The primer must be sufficiently long to prime the synthesis of extension products in the presence of the agent for polymerization. The exact lengths of the primers will depend on many factors, including temperature and composition (A/T vs. G/C content) of primer. A pair of bi-directional primers consists of one forward and one reverse primer as commonly used in the art of DNA amplification such as in PCR amplification. In some embodiments, the primers or probes hybridize with any of SEQ ID NOs. 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, and 29. In some embodiments, the primers or probes hybridize with any of SEQ ID NOs. 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, and 29 under stringent hybridization conditions. As used herein, stringent hybridization conditions can be 6×SSC (0.9 M NaCl, 0.09 M sodium citrate, pH 7.4) at 65° C.

[0029] The terms "array" or "matrix" refer to an arrangement of addressable locations or "addresses" on a device. The locations can be arranged in two-dimensional arrays, three-dimensional arrays, or other matrix formats. The number of locations may range from several to at least hundreds of thousands. Most importantly, each location represents a totally independent reaction site. A "nucleic acid array" refers to an array containing nucleic acid probes, such as oligonucleotides or larger portions of genes. The nucleic acid on the array is preferably single-stranded. Arrays wherein the probes are oligonucleotides are

referred to as “oligonucleotide arrays” or “oligonucleotide chips.” A “microarray,” also referred to herein as a “biochip” or “biological chip,” is an array of regions having a density of discrete regions of at least about 100/cm^2, and preferably at least about 1000/cm^2. The regions in a microarray have typical dimensions, for example, diameters, in the range of between about 10-250 μm, and are separated from other regions in the array by about the same distance. None limiting examples of compositions and methods for making and using arrays are described in U.S. Patent Nos. 5202231, 5695940, 5525464, 5445934, 5744305, 5677195, 5800992, 5871928, 5795716, 5700637, 6054270, 5807522, and 6110426, each of which is incorporated by reference herein in its entirety for all purposes.

[0030] The term “sample” or “biological sample” as used herein, refers to a sample obtained from an organism or from components (e.g., cells) of an organism. The sample may be of any biological tissue or fluid. The sample may be a sample which is derived from a patient. Such samples include, but are not limited to, sputum, blood, blood cells (e.g., white blood cells), tissue or biopsy samples (e.g., tumor biopsy), urine, peritoneal fluid, and pleural fluid, patient derived xenografts (PDXs), or cells therefrom. Biological samples may also include sections of tissues such as frozen sections taken for histological purposes.

[0031] The term “marker” encompasses a broad range of intra- and extra-cellular events as well as whole-organism physiological changes. A marker may be represent essentially any aspect of cell function, for example, but not limited to, levels or rate of production of signaling molecules, transcription factors, metabolites, gene transcripts as well as post-translational modifications of proteins. Marker may include partial and/or whole genome analysis of transcript levels, rates, and/or stability, and partial and/or whole proteome analysis of protein levels, activity and/or modifications. A signature may also refer to a gene or gene product which is up- or down-regulated in a compound-treated subject having the disease compared to an untreated diseased cell. That is, the gene or gene product is sufficiently specific to the treated cell that it may be used, optionally with other genes or gene products, to identify, predict, or detect efficacy of a small molecule. Thus, in some embodiments, a signature is a gene or gene product that is characteristic of efficacy of a compound in a diseased cell or the response of that diseased cell to treatment by the compound.

[0032] The term “baseline level” refers to a standard control for “normal” levels (i.e., patients without disease, patients responding to a drug, or patients not responding to a drug, etc.), but can also be comparative, e.g., where low baseline levels is compared to the levels of other subjects having the disease.

[0033] The term "multi-kinase inhibitor" refers to a composition that can reduce or block the action of more than one protein kinases. The inhibitor can reduce or block the action of a serine kinase, a tyrosine kinase, a threonine kinase and/or other types of kinases. The inhibitor can target to vascular endothelial growth factor (VEGF)-mediated angiogenesis and block the RAF/extracellular signal regulated kinase (ERK) kinase (MEK)/ERK cascade. Examples of multi-kinase inhibitors include, but are not limited to composition comprising one or more drugs such as Sorafenib (e.g., Nexavar®), vemurafenib (e.g., Zelboraf®), sunitinib (e.g., Sutent®), axitinib (e.g., Inlyta®), vandetanib (e.g., Caprelsa®), cabozantinib (e.g., Cometriq®), ponatinib (e.g., Iclusig®), ruxolitinib (e.g., Jakafi®), regorafenib (e.g., Stivarga®), crizotinib (e.g., Xalkori®), a salt, a solvate, or a physiologically functional derivative thereof, or a mixture thereof.

[0034] The term "Sorafenib" refers to salt of 4-{4-[({[4-Chloro-3-(trifluoromethyl)phenyl]amino}carbonyl)amino]phenoxy}-N-methylpyridine-2-carboxamide. The synthesis and use of 4-{4-[({[4-Chloro-3-trifluoromethyl)phenyl]amino}carbonyl)amino]phenoxy}-N-methylpyridine-2-carboxamide and many other ureas, as well as pharmaceutically acceptable salts thereof such as salts, formulations, physiologically functional derivatives, such as those described in a number of applications including, but not limited to, international applications WO 00/42012, WO 00/41698, WO 02/062763, WO 03/354950, WO 02/085859, WO 03/047579, WO 04/15653, WO 07/053573, WO 08/008733, WO 09/106825, WO 09/054004, WO 09/111061, WO/2013/000909, US Patent Nos. 7235576, 7351834, 7897623, 8445687, and U.S. Patent Application Publication No. 2013/0012550, each of which is incorporated herein by reference in its entirety.

[0035] The term "Sunitinib" (as known as SU11248, or Sutent) refers to N-(2-diethylaminoethyl)-5-[(Z)-(5-fluoro-2-oxo-1H-indol-3-ylidene)methyl]-2,4-dimethyl-1H-pyrrole-3-carboxamide, as well as pharmaceutically acceptable salts thereof such as salts, formulations, physiologically functional derivatives, such as those described in a number of applications including, but not limited to, international applications WO/2011/004200A1, WO/2010/011834A2, WO/2011/128699A2, WO/2011/104555A2, WO/2009/067686A2, WO/2009/067674A2, WO/2010/039798A2, WO/2011/100325A2, WO/2012/088522A1, WO/2009/124037A1, WO/2010/049449A2, WO/2009/157011A1, US Patent Nos. 6573293, 7125905, 7211600, and U.S. Patent Application Publication Nos. US20110263671, US20100256392, US20110034703, US20130190512, US20090247767, US20100160646, US20090062368, US20110275690, US20110092717, US20110112164, each of which is incorporated herein by reference in its entirety.

[0036] The term “Axitinib” (as known as AG013736 or Inlyta) refers to N-Methyl-2-[[3-[(E)-2-pyridin-2-ylethenyl]-1H-indazol-6-yl]sulfanyl]benzamide, as well as pharmaceutically acceptable salts thereof such as salts, formulations, physiologically functional derivatives, such as those described in a number of applications including, but not limited to, international applications WO/2013/046133A1 and WO/2011/038467A1, U.S. Patent Nos. 6534524, 7141581, and U.S. Patent Application Publication Nos. US20090062347 and US20120244116, each of which is incorporated herein by reference in its entirety.

[0037] The term “Vandetanib” (as known as INN or Caprelsa) refers to N-(4-bromo-2-fluorophenyl)-6-methoxy-7-[(1-methylpiperidin-4-yl)methoxy]quinazolin-4-amine, as well as pharmaceutically acceptable salts thereof such as salts, formulations, physiologically functional derivatives, such as those described in a number of applications including, but not limited to, U.S. Patent Nos. 7173038, 8067427, and RE42353, each of which is incorporated herein by reference in its entirety.

[0038] The term “Pazopanib” (as known as Votrient) refers to 5-[[4-[(2,3-Dimethyl-2H-indazol-6-yl)methylamino]-2-pyrimidinyl]amino]-2-methylbenzolsulfonamide, as well as pharmaceutically acceptable salts thereof such as salts, formulations, physiologically functional derivatives, such as those described in a number of applications including, but not limited to, international applications WO/2010/036796A1, WO/2012/073254A1, WO/2011/050159A1, WO/2011/009016A1, WO/2011/085007A1, WO/2012/103060A1, WO/2011/039648A1, WO/2011/140343A1, WO/2013/043529A1, and WO/2011/146458A1; U.S. Patent Nos. 7105530, 7262203, and 8114885; and U.S. Patent Nos. US20110301113, US20120197019, US20120165354, US20110281901, US20130012531, US20120028918, and US20120232102, each of which is incorporated herein by reference in its entirety.

[0039] The term “Cabozantinib” (as known as Cometriq or XL184) refers to N-(4-((6,7-Dimethoxyquinolin-4-yl)oxy)phenyl)-N-(4-fluorophenyl)cyclopropane-1,1-dicarboxamide, as well as pharmaceutically acceptable salts thereof such as salts, formulations, physiologically functional derivatives, such as those described in a number of applications including, but not limited to, U.S. Patent No. 7579473, which is incorporated herein by reference in its entirety.

[0040] The terms "treating" and "treatment" as used herein refer to an approach for obtaining beneficial or desired results including clinical results. For purposes of this invention, beneficial or desired clinical results include, but are not limited to, one or more of the following: decreasing the severity and/or frequency one or more symptoms resulting from the disease, diminishing the extent of the disease, stabilizing the disease (*e.g.*, preventing or delaying the worsening of the disease), delay or slowing the progression of the disease,

ameliorating the disease state, decreasing the dose of one or more other medications required to treat the disease, and/or increasing the quality of life. "Treating" a patient with a formulation described herein includes management of an individual to inhibit or cause regression of a disease or condition.

[0041] The term "effective amount" refers to the amount of one or more compounds that renders a desired treatment outcome. An effective amount may be comprised within one or more doses, i.e., a single dose or multiple doses may be required to achieve the desired treatment endpoint.

[0042] A “therapeutically effective amount” refers to an amount of one or more compounds sufficient to produce a desired therapeutic outcome (e.g., reduction of severity of a disease or condition). In one embodiment, the therapeutically effective amount refers to a therapeutically effective plasma concentration of a multi-kinase inhibitor. A “prophylactically effective amount” refers to an amount of a pharmaceutical formulation including one or more compounds sufficient to prevent or reduce severity of a future disease or condition when administered to an individual who is susceptible and/or who may develop a disease or condition.

[0043] By "pharmaceutically acceptable" is meant a material that is not biologically or otherwise undesirable, i.e., the material may be incorporated into a pharmaceutical composition administered to a patient without causing any significant undesirable biological effects or interacting in a deleterious manner with any of the other components of the composition in which it is contained. When the term "pharmaceutically acceptable" is used to refer to a pharmaceutical carrier or excipient, it is implied that the carrier or excipient has met the required standards of toxicological and manufacturing testing or that it is included on the Inactive Ingredient Guide prepared by the U.S. Food and Drug administration.

[0044] The term "disorder" or "disease" used interchangeably herein, refers to any alteration in the state of the body or one of its organs and/or tissues, interrupting or disturbing the performance of organ function and/or tissue function (e.g., causes organ dysfunction) and/or causing a symptom such as discomfort, dysfunction, distress, or even death to a subject afflicted with the disease.

[0045] The term “subject”, "individual" or “patient” refers to an animal, for example, a mammal and includes, but is not limited to, human, bovine, horse, feline, canine, rodent, or primate. In some embodiments, the individual is a human.

[0046] The term "derivative" as used herein includes derivatives, analogs, prodrugs, and unnatural precursors.

[0047] The term “pharmaceutically acceptable salt" refers to a salt which retains the biological effectiveness of the compound and which is not biologically or otherwise undesirable.

[0048] Additional terms shall be defined, as required, in the detailed description that follows.

Cancer

[0049] The present invention provides a method for determining a subject’s responsiveness or resistance to one or more drug, such as a multi-kinase inhibitor. In some embodiments, the drug is used to treat cancer.

[0050] In some embodiments, the cancer is selected from the group consisting of cancers of the tongue, mouth, pharynx, and oral cavity, esophageal cancer, stomach cancer, gastrointestinal stromal tumor, cancer of the small intestine, anal cancer, cancer of the anal canal, anorectal cancer, liver cancer, intrahepatic bile duct cancer, gallbladder cancer, biliary cancer, cancer of other digestive organs, cancer of the larynx, bone and joint cancer, uterine cancer, cervical cancer, uterine corpus cancer, cancer of the vulva, vaginal cancer, testicular cancer, penile cancer, urinary bladder cancer, kidney cancer, renal cancer, cancer of the ureter and other urinary organs, ocular cancer, brain and nervous system cancer, CNS cancers, and thyroid cancer, comprising administering to a subject in need thereof a therapeutically effective amount of β-lapachone, or a pharmaceutically acceptable salt thereof, in combination with a pharmaceutically acceptable carrier, wherein said β-lapachone, or a pharmaceutically acceptable salt thereof, treats said cancer selected from the group consisting of cancers of the tongue, mouth, pharynx, and oral cavity, esophageal cancer, stomach cancer, gastrointestinal stromal tumor, cancer of the small intestine, anal cancer, cancer of the anal canal, anorectal cancer, liver cancer, intrahepatic bile duct cancer, gallbladder cancer, biliary cancer, cancer of other digestive organs, cancer of the larynx, bone and joint cancer, uterine cancer, cervical cancer, uterine corpus cancer, cancer of the vulva, vaginal cancer, testicular cancer, penile cancer, urinary bladder cancer, kidney cancer, renal cancer, cancer of the ureter and other urinary organs, ocular cancer, brain and nervous system cancer, CNS cancers, and thyroid cancer.

[0051] In some embodiments, the cancer is a liver cancer. In some embodiments, the cancer is hepatocellular carcinoma (HCC), mesenchymal tissue, sarcoma, hepatoblastoma, cholangiocarcinoma, angiosarcoma, hemangiosarcoma, lymphoma, or mixture thereof.

Gene Markers

[0052] The present invention provides a panel of gene markers. In some embodiments, one or more members of the panel of gene markers can be used to determine a subject's responsiveness or resistance to a drug, such as a multi-kinase inhibitor.

[0053] In some embodiments, In some embodiments, the multi-kinase inhibitor targets to vascular endothelial growth factor (VEGF)-mediated angiogenesis and reduces the activity of or blocks the RAF/extracellular signal regulated kinase (ERK) kinase (MEK)/ERK cascade (see FIG. 4), including, but not limited to, Sorafenib (e.g., Nexavar®), vemurafenib (e.g., Zelboraf®), sunitinib (e.g., Sutent®), axitinib (e.g., Inlyta®), vandetanib (e.g., Caprelsa®), cabozantinib (e.g., Cometriq®), ponatinib (e.g., Iclusig®), ruxolitinib (e.g., Jakafi®), regorafenib (e.g., Stivarga®), crizotinib (e.g., Xalkori®), a salt, a solvate, or a physiologically functional derivative thereof, or a mixture thereof.

[0054] In some embodiments, the panel of gene markers include at least one, two, three, four, five, six, sever, or eight of the following genes:

[0055] SEC14L2: a.k.a. SEC14-Like2, Squalene Transfer Protein, TAP, Supernatant Protein Factor, C22orf6, SPF, KIAA1186, KIAA1658, Tocopherol-Associated Protein, Alpha-Tocopherol-Associated Protein, or HTAP, e.g., having the nucleotide sequence of GenBank ID AL096881, and polypeptide sequence of UniProtKB:O76054, or functional variants, fragments, or orthologs thereof. In some embodiments, the SEC14L2 marker comprises transcript variant 1 (SEQ ID NO: 1) or SEC14L2 isoform 1 (SEQ ID NO: 2), functional variants, fragments, or orthologs thereof. In some embodiments, the SEC14L2 marker comprises transcript variant 2 (SEQ ID NO: 3) or SEC14L2 isoform 2 (SEQ ID NO: 4), functional variants, fragments, or orthologs thereof. In some embodiments, the SEC14L2 marker comprises transcript variant 3 (SEQ ID NO: 5) or SEC14L2 isoform 3 (SEQ ID NO: 6), functional variants, fragments, or orthologs thereof. In some embodiments, an activity of SEC14L2 within a predetermined activity level indicates that the subject is responsive to the treatment of a multi-kinase inhibitor. In some embodiments, an activity of SEC14L2 in a subject similar to, or lower than a predetermined activity level indicates that the subject is responsive to the treatment of a multi-kinase inhibitor. In some embodiments, an activity of SEC14L2 in a subject higher than a predetermined activity level indicates that the subject is

not responsive to the treatment of a multi-kinase inhibitor. As used herein, the term "similar" refers to that there is no significant statistical difference between the activity of a gene marker in a subject and a predetermined activity level. As used herein, the term "lower" or "higher" refers to that there is a statistical difference between the activity of a gene marker in a subject and a predetermined activity level to determine there the activity of a gene marker in a subject is less or more when compared to a predetermined activity level.

[0056] H6PD: a.k.a. hexose-6-phosphate dehydrogenase (glucose 1-dehydrogenase), GDH/6PGL Endoplasmic Bifunctional Protein, GDH, Glucose 1- Dehydrogenase, Glucose Dehyrogenase, Glucose Dehydrogenase, G6PDH, Salivary Dehydrogenase, 6-Phosphogluconolactonase, CORTRD1, G6PD, H Form, EC 1.1.1.49, EC 2.7.4.3, or EC 3.1.1.31, e.g., having the nucleotide sequence of GenBank ID CAA10071.1, and the polypeptide sequence of UniProtKB: O95479, or functional variants, fragments, or orthologs thereof. In some embodiments, the H6PD marker comprises transcript variant 1 (SEQ ID NO: 7) or H6PD isoform 1 (SEQ ID NO: 8), functional variants, fragments, or orthologs thereof. In some embodiments, the H6PD marker comprises transcript variant 2 (SEQ ID NO: 9) or H6PD isoform 2 (SEQ ID NO: 10), functional variants, fragments, or orthologs thereof. In some embodiments, an activity of H6PD within a predetermined activity level indicates that the subject is responsive to the treatment of a multi-kinase inhibitor. In some embodiments, an activity of H6PD in a subject similar to, or lower than a predetermined activity level indicates that the subject is responsive to the treatment of a multi-kinase inhibitor. In some embodiments, an activity of H6PD in a subject higher than a predetermined activity level indicates that the subject is not responsive to the treatment of a multi-kinase inhibitor.

[0057] TMEM140: a.k.a. transmembrane protein 140, e.g., having the nucleotide sequence of GenBank ID NM_018295.3, and the polypeptide sequence of UniProtKB: Q9NV12, or functional variants, fragments, or orthologs thereof. In some embodiments, the TMEM140 marker comprises TMEM140 transcript SEQ ID NO: 11 or TMEM140 polypeptide SEQ ID NO: 12, functional variants, fragments, or orthologs thereof. In some embodiments, an activity of TMEM140 within a predetermined activity level indicates that the subject is responsive to the treatment of a multi-kinase inhibitor. In some embodiments, an activity of TMEM140 in a subject similar to, or lower than a predetermined activity level indicates that the subject is responsive to the treatment of a multi-kinase inhibitor. In some embodiments, an activity of TMEM140 in a subject higher than a predetermined activity level indicates that the subject is not responsive to the treatment of a multi-kinase inhibitor.

[0058] SLC2A5: a.k.a., solute carrier family 2 (facilitated glucose/fructose transporter), member 5, Glucose Transporter-Like Protein 5, GLUT51, Solute Carrier Family 2, Facilitated Glucose Transporter Member 5, Glucose Transporter Type 5, Small Intestine, Fructose Transporter, GLUT-5, e.g., having the nucleotide sequence of GenBank ID NM_001135585 or NM_003039, and the polypeptide sequence of UniProtKB: P22732, or functional variants, fragments, or orthologs thereof. In some embodiments, the SLC2A5 marker comprises transcript variant 1 (SEQ ID NO: 13) or SLC2A5 isoform 1 (SEQ ID NO: 14), functional variants, fragments, or orthologs thereof. In some embodiments, the SLC2A5 marker comprises transcript variant 2 (SEQ ID NO: 15) or SLC2A5 isoform 2 (SEQ ID NO: 16), functional variants, fragments, or orthologs thereof. In some embodiments, an activity of SLC2A5 within a predetermined activity level indicates that the subject is responsive to the treatment of a multi-kinase inhibitor. In some embodiments, an activity of SLC2A5 in a subject similar to, or lower than a predetermined activity level indicates that the subject is responsive to the treatment of a multi-kinase inhibitor. In some embodiments, an activity of SLC2A5 in a subject higher than a predetermined activity level indicates that the subject is not responsive to the treatment of a multi-kinase inhibitor.

[0059] ACTA1, a.k.a., actin, alpha 1, skeletal muscle, CFTDM, ACTA, MPFD, NEM3, NEM2, ASMA, Actin, Alpha Skeletal Muscle, CFTD1, Nemaline Myopathy Type 3, NEM1, Alpha-Actin-1, or CFTD, e.g., having the nucleotide sequence of GenBank ID NM_001100.3, and the polypeptide sequence of UniProtKB: P68133, or functional variants, fragments, or orthologs thereof. In some embodiments, the ACTA1 marker comprises ACTA1 transcript SEQ ID NO: 17 or ACTA1 polypeptide SEQ ID NO: 18, functional variants, fragments, or orthologs thereof. In some embodiments, an activity of ACTA1 within a predetermined activity level indicates that the subject is responsive to the treatment of a multi-kinase inhibitor. In some embodiments, an activity of ACTA1 in a subject similar to, or lower than a predetermined activity level indicates that the subject is responsive to the treatment of a multi-kinase inhibitor. In some embodiments, an activity of ACTA1 in a subject higher than a predetermined activity level indicates that the subject is not responsive to the treatment of a multi-kinase inhibitor.

[0060] IRF8: a.k.a., interferon regulatory factor 8, Interferon Consensus Sequence Binding Protein 1, ICSBP, H-ICSBP, ICSBP1, Interferon Consensus Sequence-Binding Protein, or IRF-8, e.g., having the nucleotide sequence of GenBank ID NM_002163.2, NM_001252275.1 or NM_006798.3, and the polypeptide sequence of UniProtKB: Q02556, or functional variants, fragments, or orthologs thereof. In some embodiments, the IRF8 marker comprises IRF8 transcript SEQ ID NO: 19 or IRF8 polypeptide SEQ ID NO: 20,

functional variants, fragments, or orthologs thereof. In some embodiments, an activity of IRF-8 within a predetermined activity level indicates that the subject is responsive to the treatment of a multi-kinase inhibitor. In some embodiments, an activity of IRF8 in a subject similar to, or lower than a predetermined activity level indicates that the subject is responsive to the treatment of a multi-kinase inhibitor. In some embodiments, an activity of IRF8 in a subject higher than a predetermined activity level indicates that the subject is not responsive to the treatment of a multi-kinase inhibitor.

[0061] STAT2: a.k.a., signal transducer and activator of transcription 2, P113, STAT113, Interferon Alpha Induced Transcriptional Activator, Signal Transducer And Activator Of Transcription 2, Signal Transducer And Activator Of Transcription 2, ISGF-32, e.g., having the nucleotide sequence of GenBank ID NM_005419.3 or NM_198332.1, and the polypeptide sequence of UniProtKB: P52630, or functional variants, fragments, or orthologs thereof. In some embodiments, the STAT2 marker comprises transcript variant 1 (SEQ ID NO: 21) or STAT2 isoform 1 (SEQ ID NO: 22), functional variants, fragments, or orthologs thereof. In some embodiments, the STAT2 marker comprises transcript variant 2 (SEQ ID NO: 23) or STAT2 isoform 2 (SEQ ID NO: 24), functional variants, fragments, or orthologs thereof. In some embodiments, an activity of STAT2 within a predetermined activity level indicates that the subject is responsive to the treatment of a multi-kinase inhibitor. In some embodiments, an activity of STAT2 in a subject similar to, or lower than a predetermined activity level indicates that the subject is responsive to the treatment of a multi-kinase inhibitor. In some embodiments, an activity of STAT2 in a subject higher than a predetermined activity level indicates that the subject is not responsive to the treatment of a multi-kinase inhibitor.

[0062] UGT2A1: a.k.a., UGT2A2, UDP glucuronosyltransferase 2 family, polypeptide A1, complex locus; UDP Glucuronosyltransferase 2 Family, Polypeptide A1; UDP Glycosyltransferase 2 Family, Polypeptide A1; UDP-Glucuronosyltransferase 2A1; UDPGT 2A1; UGT2A2; or EC 2.4.1.17, e.g., having the nucleotide sequence of GenBank ID NM_001252274.1 NM_001252275.1 or NM_006798.3, and the polypeptide sequence of UniProtKB: Q9Y4X1, or functional variants, fragments, or orthologs thereof. In some embodiments, the UGT2A1 marker comprises transcript variant 1 (SEQ ID NO: 25) or UGT2A1 isoform 1 (SEQ ID NO: 26), functional variants, fragments, or orthologs thereof. In some embodiments, the UGT2A1 marker comprises transcript variant 2 (SEQ ID NO: 27) or UGT2A1 isoform 2 (SEQ ID NO: 28), functional variants, fragments, or orthologs thereof. In some embodiments, the UGT2A1 marker comprises transcript variant 2 (SEQ ID NO: 29) or UGT2A1 isoform 2 (SEQ ID NO: 30), functional variants, fragments, or orthologs thereof. In

some embodiments, an activity of UGT2A1 within a predetermined activity level indicates that the subject is responsive to the treatment of a multi-kinase inhibitor. In some embodiments, an activity of UGT2A1 in a subject similar to, or lower than a predetermined activity level indicates that the subject is responsive to the treatment of a multi-kinase inhibitor. In some embodiments, an activity of UGT2A1 in a subject higher than a predetermined activity level indicates that the subject is not responsive to the treatment of a multi-kinase inhibitor.

[0063] The present invention also provides activity profiles of a panel of gene markers comprising at least one, two, three, four, five, six, seven, eight or more gene markers. In some embodiments, the gene markers are selected from the group consisting of SEC14L2, H6PD, TMEM140, SLC2A5, ACTA1, IRF8, STAT2, and UGT2A1, or functional variants, fragments, or orthologs thereof.

[0064] In some embodiments, while more than one gene markers of the present application are used, a determination of responsiveness can be made based on activities of one or more gene markers. Under such situations, a conclusion can be made that the subject is most likely responsive or most likely not responsive to the treatment. In some embodiments, while more than one gene markers of the present application are used, the activity of each gene marker carries the same “weight” or determination factor with respect to determining whether the subject is responsive to the treatment of a multi-kinase inhibitor. In some embodiments, while more than one gene markers of the present application are used, the activity of each gene marker carries different “weight” or determination factor with respect to determining whether the subject is non-responsive to the treatment of a multi-kinase inhibitor. In some embodiments, the weight can be determined by a predictive power of the marker, which can be quantified by the coefficient of determination (r2) and the associated p-value. In some embodiments, higher values of r2 and/or lower values of p-value indicate better predictive power.

[0065] In some embodiments, while more than one gene markers of the present application are used and some gene markers indicate the subject is responsive to the treatment of a multi-kinase inhibitor, while some gene markers indicate that the subject may not be responsive to the treatment of a multi-kinase inhibitor, or the result is inconclusive based on the activity of all gene markers used, a conclusion can be made based on overall activities of the gene markers used or a particular subset of gene markers used, e.g., that the subject is most likely responsive or most likely not responsive to the treatment based on the numbers of gene marker indicating the responsiveness. For example, when a total of *n* gene markers are tested (*n*=2, 3, 4, or more etc.), if *m* gene markers indicate that the subject

is responsive to the treatment, while *m* is not smaller than *n-m*, then a conclusion can be made that the subject is likely responsive to the treatment. If *m* is smaller than *n-m*, then a conclusion can be made that the subject is likely not responsive to the treatment.

[0066] As used herein, the term "activity profile" refers to a set of data representing distinctive features or characteristics of one or more gene markers. Such features or characteristics include, but are not limited to, transcript abundance, transcript stability, transcription rate, translation rate, post-translation modification, protein abundance, protein stability, and/or protein enzymatic activity, etc. In some embodiments, the activity profile comprises data related to gene expression level of each gene marker.

[0067] In some embodiments, a collection of activity profiles of a panel of gene markers is provided. In some embodiments, the collection comprises activity profiles is obtained from a specific population of subjects. In some embodiments, the specific population of subjects consists of subjects that are responsive to a multi-kinase inhibitor. In some embodiments, the specific population of subjects consists of subjects that are not responsive to a multi-kinase inhibitor.

[0068] In some embodiments, the collection comprises activity profiles that are statistically homogeneous in one or more aspects, e.g., statistically homogeneous in one or more quantitative or semi-quantitative parameters describing the features and characteristics of the activity profiles. In some embodiments, the quantitative parameters include, but are not limited to, transcript abundance, transcript stability, transcription rate, translation rate, post-translation modification, protein abundance, protein stability, and/or protein enzymatic activity, etc. Whether a group of activity profiles are statistically homogeneous or not in one or more aspects can be determined by any suitable statistic test and/or algorithm known to one skilled in the art.

[0069] In some embodiments, one or more of the gene markers increase its activity in response to the multi-kinase inhibitor. In some embodiments, one or more of the gene markers decrease its activity in response to the multi-kinase inhibitor. In some embodiments, one or more of the gene markers remains its activity in response to the multi-kinase inhibitor. As used herein, the term "gene activity" refers to gene expression level, RNA activity level, or protein activity level. As used herein, the term "RNA activity level refers to mRNA abundance, synthesis rate, and/or stability, etc. As used herein, the term "protein activity level" refers to protein abundance, synthesis rate, stability, enzymatic activity, phosphorylation rate, etc.

[0070] In some embodiments, the collection of activity profiles of one or more gene markers of the present invention is obtained from one or more tests. The test can be performed by the subject himself/herself, by a doctor, by a nurse, by a test lab, by a healthcare provider, or any other parties capable of doing the test. The test results containing the collection of activity profiles can be then analyzed by the same party or by a second party, such as the subject himself/herself, a doctor, a nurse, a test lab, a healthcare provider, a physician, a clinical trial personnel, a hospital, a lab, a research institute, or any other parties capable of analyzing the test to determine if the subject is responsive to the drug.

[0071] Despite how the activity of the gene markers of the present invention changes after the treatment of the multi-kinase inhibitor, for all gene markers described herein, their expression level is lower before the treatment when compared to the average expression level of randomly selected patients, or patients not responding to the treatment. Therefore, the expression level of patients responding to the treatment can be used as a reference. When the expression level of one or more of the presently described gene markers is within the expression level of patients responding to the treatment, it indicates the responsiveness of the subject. In other words, before the multi-kinase inhibitor treatment, the expression of these gene markers of a given subject in comparison to a predetermined expression level of a population of subjects responding to the treatment can be used to predict the probability of response to multi-kinase inhibitor treatment. Alternatively, the expression level of patients not responding to the treatment can also be used. For example, before the multi-kinase inhibitor treatment, when the expression level of one or more of the presently described gene markers of a given subject is within the expression level of patients not responding to the treatment, it indicates the non-responsiveness of the subject.

Methods

[0072] Also provided are methods of using the panel of gene markers of the present invention.

[0073] In some embodiments, methods for determining a subject's responsiveness or resistance to a drug are provided. In some embodiments, the multi-kinase inhibitor targets to vascular endothelial growth factor (VEGF)-mediated angiogenesis and reduces the activity of or blocks the RAF/extracellular signal regulated kinase (ERK) kinase (MEK)/ERK cascade (see Figure 4).

[0074] In some embodiments, the drug comprises one or more multi-kinase inhibitors, such as an inhibitor that can target to vascular endothelial growth factor (VEGF)-mediated angiogenesis and block the RAF/extracellular signal regulated kinase (ERK) kinase (MEK)/ERK cascade, including but not limited to, Sorafenib (e.g., Nexavar®), vemurafenib (e.g., Zelboraf®), sunitinib (e.g., Sutent®), axitinib (e.g., Inlyta®), vandetanib (e.g., Caprelsa®), cabozantinib (e.g., Cometriq®), ponatinib (e.g., Iclusig®), ruxolitinib (e.g., Jakafi®), regorafenib (e.g., Stivarga®), crizotinib (e.g., Xalkori®), a salt, a solvate, or a physiologically functional derivative thereof, or a mixture thereof. In some embodiments, the drug is Sorafenib, Axitinib, Vandetanib, Pazopanib, Cabozantinib, a salt, a solvate, or a physiologically functional derivative thereof, or a mixture thereof.

[0075] In some embodiments, the drug is used to treat a disease associated with cancer. In some embodiments, the cancer is a liver cancer or a kidney cancer. In some embodiments, the liver cancer is hepatocellular carcinoma.

[0076] In some embodiments, the methods comprise measuring activity profile of a panel of gene markers in a sample collected from the subject comprising at least one or more markers selected from the group of SEC14L2, H6PD, TMEM140, SLC2A5, ACTA1, IRF8, STAT2, and UGT2A1.

[0077] In some embodiments, the methods further comprise comparing the activity profile of the panel to a predetermined activity profile derived from a population of subjects responding to a multi-kinase inhibitor, wherein the subject is determined to be responsive to the multi-kinase inhibitor if the activity profile of the panel is within the predetermined activity profile.

[0078] In some embodiments, the methods further comprise comparing the activity profile of the panel to activity profile derived from a population of subjects not responding to a multi-kinase inhibitor, wherein the subject is determined to be not responsive to the multi-kinase inhibitor if the activity profile of the panel is within the activity profile.

[0079] As used herein, the term “predetermined level”, “predetermined activity profile” or a “reference activity profile” refers to a standardized data or data set representing the average, representative features or characteristics of one or more gene markers in the population of subjects responsive to a multi-kinase inhibitor treatment. Such features or characteristics include, but are not limited to, transcript abundance, transcript stability, transcription rate, translation rate, post-translation modification, protein abundance, protein stability, and/or protein enzymatic activity, etc. In some embodiments, the specific

population of subjects are consisting of about 5, about 10, about 20, about 50, about 100, about 200, about 300, about 400, about 500, about 1000, about 5000, about 10K, or more individual subjects. The predetermined activity profile can be a standardized data or data set collected before, during, or after the specific population of subjects has been all exposed to a multi-kinase inhibitor. In some embodiments, the predetermined activity profile is a standardized data or data set collected before the specific population of subjects has been all exposed to a multi-kinase inhibitor. (or from sample from cell, tumor cells,

[0080] In some embodiments, the predetermined activity profile is a predetermined bar or threshold level. A higher than predetermined bar or threshold activity of a given gene marker of the present invention in a subject indicates that the subject is not responsive to the treatment, while a similar or lower activity than predetermined bar or threshold activity of a given gene marker of the present invention in a subject indicates that the subject is responsive to the treatment.

[0081] In some embodiments, the predetermined activity profile is a predetermined range. An activity of a given gene marker of the present invention in a subject higher or outside the range indicates that the subject is not responsive to the treatment, while an activity of a given gene marker of the present invention in a subject within or lower than the range indicates that the subject is responsive to the treatment.

[0082] It is understood that instead of obtaining the predetermined activity profile from a group of subjects known to be responsive to the treatment, a “negative predetermined activity profile” can be obtained from subjects known to be not responsive to the treatment. In some embodiments, an activity of a given gene marker of the present invention in a subject similar to, or higher than a negative predetermined activity level indicates that the subject is not responsive to the treatment of a multi-kinase inhibitor. In some embodiments, an activity of a given gene marker of the present invention in a subject lower than a negative predetermined activity level indicates that the subject is responsive to the treatment of a multi-kinase inhibitor.

[0083] As used herein, a subject is “responsive” to a multi-kinase inhibitor when the vascular endothelial growth factor (VEGF)-mediated angiogenesis in the subject is reduced or blocked by the multi-kinase inhibitor, and the activity of the RAF/extracellular signal regulated kinase (ERK) kinase (MEK)/ERK cascade in the subject is reduced or blocked, which can be tested by any suitable methods known to one skilled in the art. In some embodiments, the responsiveness can be reflected by antitumor activity of a multi-kinase inhibitor. In some embodiments, the antitumor activity of a multi-kinase inhibitor can be

measured by %ΔT/ΔC, wherein ΔT = tumor volume change in the treatment group and ΔC = tumor volume change in control group. In some embodiments, the antitumor activity of a multi-kinase inhibitor can be measured in any suitable sample collected from a subject, part of a subject, or PDX derived from a subject. In some embodiments, a subject is considered responsive to a multi-kinase inhibitor when a predicted %ΔT/ΔC is less than about 90%, less than about 85%, less than about 80%, less than about 75%, less than about 70%, less than about 65%, less than about 60%, less than about 55%, less than about 50%, less than about 45%, less than about 40%, less than about 35%, less than about 30%, less than about 25%, less than about 20%, less than about 15%, less than about 10%, less than about 5%, or even less depending on the types of the multi-kinase inhibitor and the types of the tumor. In some embodiments, the subject is predicted to be responsive to a multi-kinase inhibitor when a predicted %ΔT/ΔC corresponding to the activity profiles of one or more gene markers is less than about 40%.

[0084] As used herein, the sentence "the activity profile of the panel is within the predetermined activity profile" refers to that the activity profile been analyzed is similar to the predetermined activity profile, for example, the parameters describing the activity profile are close to the parameters describing the predetermined activity profile, or within the variation range of a predetermined activity profile, e.g., the parameters are within the variation range based on a confidence interval of 90% constructed from the parameters describing the predetermined activity profile.

[0085] The activity profile of the panel can be described by any suitable parameters. In some embodiments, the activity profile of the panel is described by signature values associated with one or more gene markers expressed in the subject to be evaluated. Accordingly, the predetermined activity profile is described by the signature value associated with the gene markers expressed in the reference group.

[0086] In some embodiments, when the activity profile of the panel of gene markers in a sample collected from a subject been analyzed is within a predetermined activity profile based on a population of subjects responsive to a multi-kinase inhibitor treatment, the subject been analyzed is determined to be responsive to the multi-kinase inhibitor.

[0087] Alternatively, when the activity profile of the panel of gene markers in a sample collected from a subject been analyzed is within a standardized data or data set representing the average, representative features or characteristics of one or more gene markers in the population of subjects not responsive to a multi-kinase inhibitor treatment, the subject been analyzed is determined to be not responsive to the multi-kinase inhibitor.

[0088] Methods for administering a multi-kinase inhibitor to a subject are provided. In some embodiments, the methods comprise testing the subject for activity profile of a panel of gene markers comprising at least one or more gene markers. In some embodiments, the gene markers are selected from the group consisting of SEC14L2, H6PD, TMEM140, SLC2A5, ACTA1, IRF8, STAT2, and UGT2A1.

[0089] In some embodiments, the methods further comprises comparing the activity profile of the panel to a predetermined activity, wherein the multi-kinase inhibitor is administered to the subject if the activity profile of the panel is within the predetermined activity profile. In some embodiments, the multi-kinase inhibitor targets to vascular endothelial growth factor (VEGF)-mediated angiogenesis and reduces the activity of or blocks the RAF/extracellular signal regulated kinase (ERK) kinase (MEK)/ERK cascade (see FIG. 4).

[0090] The activity profile of a panel of gene markers can be determined by any suitable methods known to one skilled in the art. In some embodiments, a biological sample is taken from a subject and analyzed. The gene activity can be gene copy number, gene amplification number, or promoter activity, etc. RNA activity can be mRNA abundance, synthesis rate, and/or stability, etc. Protein activity can be protein abundance, synthesis rate, stability, enzymatic activity, phosphorylation rate, modifications, binding activity, etc. In some embodiments, the biological sample is then typically assayed from the presence of one or more gene expression products such as RNA, mRNA, cDNA, cRNA, protein, etc.

[0091] In some embodiments, mRNA from a biological sample is directly used in determining the levels of expression of one or more genes by hybridization. In some particular embodiments, RNA is obtained from a biological sample. The RNA is then transformed into cDNA (complementary DNA) copy using methods known in the art. In some particular embodiments, the cDNA is labeled with a fluorescent label or other detectable label. The cDNA is then hybridized to a substrate containing a plurality of probes of interest. A probe of interest typically hybridizes under stringent hybridization conditions to at least one DNA sequence of a gene signature. In certain embodiments, the plurality of probes are capable of hybridizing to the sequences derived from the gene markers selected from SEC14L2, H6PD, TMEM140, SLC2A5, ACTA1, IRF8, STAT2, and UGT2A1 under the hybridization conditions. In some embodiments, the conditions comprise using 6×SSC (0.9 M NaCl, 0.09 M sodium citrate, pH 7.4) at 65° C. The probes may comprise nucleic acids. The term “nucleic acid” encompasses known nucleotide analogs or modified backbone residues or linkages, which are synthetic, naturally occurring, and non-naturally occurring, which have similar binding properties as the reference nucleic acid, and which are

metabolized in a manner similar to the reference nucleotides. Examples of such analogs include, without limitation, phosphorothioates, phosphoramidates, methyl phosphonates, chiral-methyl phosphonates, peptide-nucleic acids (PNAs).

[0092] In certain cases, the probes will be from about 15 to about 50 base pairs or more in length. The amount of cDNA hybridization can be measured by assaying for the presence of the detectable label, such as a fluorophore. The quantification of the hybridization signal can be used to generate a score for a particular sequence or set of sequences in the gene signature for a particular patient or plurality of patients.

[0093] Included within the scope of the invention are DNA arrays or microarrays containing a plurality of sequences that hybridize under stringent hybridization conditions to one or more of the gene sequences of the markers. An example of a substrate containing one or more probes of interest is a plurality of DNA probes that are affixed to a substrate. In certain embodiments, the substrate may comprise one or more materials such as gel, nitrocellulose, nylon, quartz, glass, metal, silica based materials, silica, resins, polymers, etc., or combinations thereof. Typically, the DNA probes comprise about 10-50 bp of contiguous DNA. In certain embodiments, the DNA probes are from about 20 to about 50 bp of contiguous DNA. In certain embodiments, the present invention relates to kits which comprising a microarray directions for its use. The kit may comprise a container which comprises one or more microarrays and directions for their use.

[0094] The biological sample may also be analyzed for gene expression of one or more gene markers using methods that can detect nucleic acids including, but not limited to, PCR (polymerase chain reaction); RT-PCT (reverse transcriptase-polymerase chain reaction); quantitative or semi-quantitative PCR, etc.

[0095] In certain embodiments, the levels of gene expression are measured by detecting the protein expression products of the genes or DNA sequences. The levels of protein products may be measured using methods known in the art including the use of antibodies which specifically bind to a particular protein. These antibodies, including polyclonal or monoclonal antibodies, may be produced using methods that are known in the art. These antibodies may also be coupled to a solid substrate to form an antibody chip or antibody microarray. Antibody or protein microarrays may be made using methods that are known in the art.

[0096] Once the levels of gene expression have been measured then a signature value/score is calculated. Examples of how to calculate a signature value/score are

described herein. In some embodiments, the average mRNA expression intensity, in log2-scale, of the marker gene is designated as the signature score. The signature value/score is then compared to the signature value associated with the predetermined activity profile to predict the subject's response to multi-kinase inhibitor treatment. In some embodiments, the predicted subject's response is measured by a predicted %ΔT/ΔC, wherein ΔT = tumor volume change in the treatment group and ΔC = tumor volume change in control group.

[0097] The signature value can be calculated by any suitable method. In some embodiments, the signature value is calculated by a pre-determined algorism. In some embodiments, a value is assigned to each gene marker based on its expression level. Non-limiting examples of methods for calculating signature value are described in Chang et al. (SIGNATURE: A workbench for gene expression signature analysis, BMC Bioinformatics 2011, 12:443), Kawaguchi et al. (Gene expression signature-based prognostic risk score in patients with glioblastoma, Cancer Sci. 2013 Jun 7. [Epub ahead of print]), Cuzick et al. (Prognostic value of an RNA expression signature derived from cell cycle proliferation genes in patients with prostate cancer: a retrospective study, Lancet Oncol. 2011 Mar;12(3):245-55. doi: 10.1016/S1470-2045(10)70295-3.), Sánchez-Navarro (An 8-gene qRT-PCR-based gene expression score that has prognostic value in early breast cancer., BMC Cancer. 2010 Jun 28;10:336. doi: 10.1186/1471-2407-10-336.), Shi et al. (A Network-Based Gene Expression Signature Informs Prognosis and Treatment for Colorectal Cancer Patients, PLoS ONE, 2012, 7(7):e412), Matsui et al. (Developing and Validating Continuous Genomic Signatures in Randomized Clinical Trials for Predictive Medicine, Clinical Cancer Research, 2012, 2012; doi: 10.1158/1078-0432.CCR-12-1206), Lyng et al. (Gene Expression Signatures That Predict Outcome of Tamoxifen-Treated Estrogen Receptor-Positive, High-Risk, Primary Breast Cancer Patients: A DBCG Study, PLoS ONE, 2013, 8(1):e54078), and Zhao et al. (Combining Gene Signatures Improves Prediction of Breast Cancer Survival, PLoS ONE, 2011, 6(3):e17845), each of which is incorporated herein by reference in its entirety.

[0098] Although there is no absolute threshold expression level required in order to determine if a subject is responding to a treatment, one skilled in the art would be able to determine a suitable standard threshold based on the types of the multi-kinase inhibitor and the diseased to be treated. For example, in some embodiments, to be convenient, when the predicted %ΔT/ΔC associated with the signature value of gene markers is less than about 40% (or other preferred value), the subject can be determined to be responding to a given multi-kinase inhibitor.

[0099] In some embodiments, the methods of the present invention can be applied on a dosage basis. For example, for each pre-determined dosage of the same multi-kinase inhibitor, a set of gene markers can be identified, and these gene markers can be used to determine if a specific subject is responding to a specific multi-kinase at the pre-determined dosage. None limiting examples of dosage to be administered include, about 0.1 μg/kg, about 1 μg/kg, about 10 μg/kg, about 100 μg/kg, about 1 mg/kg, about 10 mg/kg, about 50 mg/kg, about 100 mg/kg, or more.

[00100] In some embodiments, the methods of the present invention can be applied on an administration method basis. For example, for each pre-determined drug administration method of the same multi-kinase inhibitor, a set of gene markers can be identified, and these gene markers can be used to determine if a specific subject is responding to the multi-kinase inhibitor by using the pre-determined drug administration method. None limiting examples of a route for administration include, mucosal, enteral, parental, transdermal/transmucosal, and inhalation. In one embodiment, the mucosal route is via the nasal, oropharyngeal, ocular, or genitourinary mucosa. In another embodiment, the enteral route is oral, rectal or sublingual. Still in another embodiment, the parenteral route is any one of intraarterial, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, and submucosal injection or infusion. Still in another embodiment, the transdermal/transmucosal route is topical. Still in another embodiment, the inhalation route is intranasal, oropharyngeal, intratracheal, intrapulmonary or transpulmonary.

[00101] In some embodiments, the methods of the present invention can be applied on a drug combination basis. For example, for each pre-determined drug combination of a multi-kinase inhibitor and a non-multi-kinase inhibitor, or a combination of two or more multi-kinase inhibitors, a set of gene markers can be identified, and these gene markers can be used to determine if a specific subject is responding to the drug combination comprising a multi-kinase inhibitor.

[00102] In some embodiments, the methods of the present invention can be applied on a formulation basis. For example, for each pre-determined drug formulation of a given multi-kinase inhibitor, a set of gene markers can be identified, and these gene markers can be used to determine if a specific subject is responding to the multi-kinase inhibitor by using the pre-determined drug formulation.

[00103] The gene markers and associated methods of the present invention can be used for all suitable purposes. In some embodiments, they are used in prospective clinical trial. In some embodiments, they are used in clinical treatment/prevention practice.

[00104] Also provided are methods to discover predictive gene markers. In some embodiments, the methods comprise a "phase II clinical trial-like" study. In some embodiments, the methods comprise applying one or more drugs to cells derived from a subject and measuring the expressing profile of one or more genes in the cells. In some embodiments, the study comprises using PDX models. In some embodiments, the study comprises using bioinformatic and statistic analysis. In some embodiments, the bioinformatic and statistic analysis are used to identify a panel of specific gene markers which have a high correlation with drug response or drug resistance. The potential applications include, but are not limited to: a) discovery of biomarkers for early stage clinical drug candidate; b) indication selection and expansion; c) life cycle management for marketed drugs; d) novel indication discovery for "me-too" drugs.

[00105] In some embodiments, the efficacy of Sorafenib, Sunitinib, Axitinib, Vandetanib, Pazopanib, Cabozantinib or other multi-kinase inhibitors is measured on a panel of PDX models. In some embodiments, each panel has multiple mice, at least 3, 4, 5, 6, 7, 8, 9, 10 or more. In some embodiments, a control group and a treatment group is included. The control group receives vehicle only. The treatment group receives Sorafenib, Sunitinib, Axitinib, Vandetanib, Pazopanib, Cabozantinib or other multi-kinase inhibitors.

[00106] In some embodiments, for each PDX panel, drug efficacy is quantified by $\%\Delta T/\Delta C$, wherein ΔT is the tumor volume change in the treatment group and ΔC is the tumor volume change in control group. In some embodiments, mRNA expression levels of 1, 2, 3, 4, 5, 6, 7, 8 or more tested marker genes are profiled by microarray, RNAseq, and/or RT-PCR. In some embodiments, the protein levels of the markers are profiled by immunoassay. In some embodiments, a signature score is calculated based on the expressions or the protein levels of the marker genes, and its predictive power is quantified by the coefficient of determination (r2) and the associated p-value. In some embodiments, a higher values of r2 and/or lower values of p-value indicate better predictive power. In some embodiments, a p-value at least smaller than 0.05 is used for considering the signature have any predictive power.

[00107] This invention is further illustrated by the following examples which should not be construed as limiting. The contents of all references, patents and published patent applications cited throughout this application, as well as the Figures and the Sequence Listing, are incorporated herein by reference.

EXAMPLES

Example 1. Significant numbers of HCC HuPrime® models are sensitive or partially sensitive to Sorafenib.

[00108] Experimental models capturing human HCC oncogenic mechanisms are critical to evaluate HCC treatments and discover biomarkers predictive of patient response. Patient derived xenograft (PDX) models mirror patients' histopathological and genetic profiles (2-7). We have recently established a large collection of HCC-PDX models (named as HCC-HuPrime®, or patient avatars) by engrafting the treatment naïve patient tumor tissue fragments into immunocompromised mice. We tested Sorafenib on a randomized cohort of the HCC-HuPrime in clinical trial-like study. The study led to identification of "responders and non-responders". We next expression-profiled these models using microarray GeneChip technology. By applying statistic analysis, we have identified a specific molecular signature that are associated with the response, or called HuSignature®. The gene expression signature, consist of only a few genes, can be predictive of the response of HCC patients to Sorafenib and also amendable for developing the companion diagnostics to used for patient stratification in the clinic. This signature can be used as guide to treat the HCC patient likely to be responders, while avoid treating those unlikely responders, thus maximizing treatment benefit while minimizing an individualized.

[00109] We have established a large of panel of HCC PDX models by transplanting surgically removed tumor tissues from treatment naïve Asian HCC patients via subcutaneous engraftment in Balb/c nude mice. The take-rate for HCC engraftment is at ~20%, moderate as compared to high take-rate for colorectal (CRC) (8), and similar to those for NSCLC (9, 10). We were interested in identifying models that would respond or not respond to Sorafenib. To this end, we tested a cohort of randomly selected 21 HCC HuPrime by treating them with Sorafenib. The results demonstrated that majority of HCC models treated by daily oral administration of Sorafenib at the doses of 50 mg/kg showed varying degree of responses to the treatment as measured by $\%^{\Delta T}/_{\Delta C}$ (FIG. 1) (Table 1). 13 out of 21 PDXs (62%) achieved effective tumor growth inhibition with $\%^{\Delta T}/_{\Delta}$ <40% ($p<0.05$). On the other hand, there are also models that are quite or partially resistant to Sorafenib, non- or poor responders, including models LI0334 and LI0050 as shown in FIG. 1.

Table 1.

	Model#	Sorafenib	ΔT/ΔC(day14-day15)	P value
001	LIM348	50mg/kg p.o.qd×14	(0.02)	0.001
002	LIM1025	50mg/kg p.o. qd×14	0.01	<0.001
003	LIM752	50mg/kg p.o. qd×14	0.10	<0.001
004	LIM1081	50mg/kg p.o. qd×13	0.14	0.015
005	LIM1098	50mg/kg qdx14 p.o.	0.14	0.008
006	LIM612	50mg/kg p.o.Qd×22	0.15	0.001
007	LIM1035	50mg/kg p.o. qd×14	0.16	0.005
008	LIM1005	50mg/kg p.o. qd×14	0.21	0.015
009	LIM574	50mg/kg p.o. qd×14	0.21	0.002
010	LIM941	50mg/kg p.o.5days on/2 days off	0.23	<0.001
011	LIM1074	50mg/kg p.o. qd×13	0.35	0.032
012	LIM801	50mg/kg p.o.5days on/2 days off	0.37	0.001
013	LIM1054	50mg/kg p.o. qd×14	0.38	0.045
014	LIM1097	50mg/kg p.o. qd×14	0.41	0.046
015	LIM1057	p.o. 50mg/kg, qd×14	0.43	0.003
016	LIM1068	50mg/kg p.o. qd×13	0.47	0.024
017	LIF1069	50mg/kg p.o. qd×14	0.47	0.024
018	LIM1078	50mg/kg p.o. qd×13	0.48	0.055
019	LIM1088	50mg/kg p.o. qd×13	0.54	0.182
020	LIMsh050	50mg/kg p.o. qd×14	0.78	0.134
021	LIM334	50mg/kg p.o.5days on/2 days off	1.13	0.659

Example 2. HCC-PDX can be classified into three major categories per global gene expression profiling

[00110] HCC is a disease of diverse types. Global gene expression profiling of patient tumor samples have revealed that HCC can be classified into three major subtypes. Recently, transcriptome analysis has classified HCC into three major categories with distinct clinical parameters as well as cellular differentiation[1]. They are S1 (stem-like group with activation of the WNT pathway and TGF-β), S2 (activation of MYC and AKT), and S3 (hepatocyte differentiation). HCC-PDXs) are believed to be predictive experimental models by maintaining the original patients' histopathological and genetic profiles[2,3]. This present study attempted to test this hypothesis by demonstrating whether HCC-avatar have similar genomic profiles as the patient tumors in classification and biological properties.

[00111] First, all described HCC-PDXs were used in avatar trial were profiled for global gene expression as previously described (9, 10). We assessed the resemblance of our "HCC-Avatars" to patient tumors using the same algorithm that was used to classify clinical patient samples[1]. Twenty-two HCC-PDX models can be classified into 3 groups, among which S1 and S2 are more closely related (see, Table 2). Using the 572 genes generated from the algorithm, the same classifications were obtained by hierarchical clustering and principal component analysis (PCA), except for two outliers. The results demonstrate that our cohort of HCC-PDXs can be divided into the same three subclasses as those from patient samples[1].

[00112] Using miRNA expression-criterion developed by Luk et al., our collection of HCC PDXs were classified into two classes, 14q32.2-hi and 14q32.2-lo. When compared to the S1, S2, S3 subclasses determined by mRNA profiling described above, S1 belongs to 14q32.2-lo, and S2/3 belong to 14q32.2-hi (except LI1025, LI1078) (see, Table 2).

Table 2. Classification of HCC HuPrime® by mRNA and miRNA profiling

PDX model	by mRNA	by 14q32.2 miRNA cluster
LI0612	class 1	low expression
LI0941	class 1	low expression
LI0348	class 1	NA
LI1646	class 1	low expression
LI1074	class 1	Low expression
LI1639	class 1	low expression
LI0801	class 1	low expression
LI1055	class 1	Low expression

LI1098	class 1	low expression
LI1057	class 1	low expression
LI1058	class 1	low expression
LI1054	class 1	low expression
LI0050	class 2	high expression
LI0752	class 2	high expression
LI1025	class 2	
LI0574	class 2	
LI1035	low expression	high expression
LI1068	NA	high expression
LI1069	class 3	high expression
LI1078	class 3	low expression
LI1081	class 3	NA
LI1088	class 3	high expression

[00113] Serum AFP and tumor AFP-mRNA levels were found to be strongly and positively correlated, and also associated with S2/3[1] and 14q32.2-hi[4], consistent with previous reports[1]. Six stem-like markers were found not to be associated with S1[1], nor with 14q32.2-hi[4], thus different from the report by Luk et al[4]. Activation of c-MET, as defined by response to c-MET inhibitor, seems only observed in S1, consistent with one of previous observations[5], but not the other[4]. When all these models were treated with Sorafenib, a multi-kinase inhibitor, there seemed to be no correlation in the tumor responses between the subclasses (data not shown). We are currently investigating the relevant drug response to the classification in order to investigate the utility of this classification. Nevertheless, our data suggested that HCC-HuPrime® are good representative of patient tumors, and thus are thus likely predictive experimental models.

Example 3. Identification of HCC gene expression signature predictive of response to Sorafenib.

[00114] A statistical method based on linear regression was used to identify predictive biomarker (i.e., gene signature) using global gene expression levels and the measured Sorafenib treatment effect on HCC-PDX as by %ΔT/ΔC as described above. By pre-set statistic criteria including p-value<0.0001, range of gene expression across tested HCC-PDX > 4 folds, no outlier in the linear regression analysis, we can derive a signature consisting of 8 genes that demonstrated good predictivity (e.g., FIG. 1, and Table 3). It is worth noting that a signature with more genes will create better relevance and smaller p-value, while have less practical values in the clinical application. The stringency of the preset criteria will determine the number of signature genes.

[00115] Although these signature genes are identified purely by statistical analysis and no biological knowledge was used to do any filtration before and after the analysis, these signature genes may be used to predict a subject's response to a multi-kinase inhibitor. Several genes are related to glucose and fructose metabolism. Two genes are in the Interferon (IFN) mediated pathways (note: a Phase I clinical trial is ongoing for using Peginterferon Alfa-2b with Sorafenib in patients with unresectable or metastatic clear cell renal carcinoma, see ClinicalTrials.gov Identifier No.: NCT00589550, which is incorporated herein by reference in its entirety).

Table 3. Exemplary Signature genes

Gene ID	Gene name	Gene annotation	Reference
SEC14L2	SEC14-like 2 (S. cerevisiae), a.k.a., SEC14 (S. Cerevisiae)-Like, SEC14-Like Protein, C22orf6, Tocopherol-Associated Protein, TAP, TAP1, hTAP. Alpha-Tocopherol-Associated Protein, Squalene Transfer Protein, SPF, KIAA1186, or KIAA1658	This gene encodes a cytosolic protein which belongs to a family of lipid-binding proteins including Sec14p, alpha-tocopherol transfer protein, and cellular retinol-binding protein. The encoded protein stimulates squalene monooxygenase which is a downstream enzyme in the cholesterol biosynthetic pathway. Alternatively spliced transcript variants encoding different isoforms have been identified for this gene. (provided by RefSeq)	http://www.genecards.org/cgi-bin/carddisp.pl?gene=SEC14L2 SEQ ID NO: 1 SEC14L2 transcript variant 1 SEQ ID NO: 2 SEC14L2 isoform 1 SEQ ID NO: 3 SEC14L2 transcript variant 2 SEQ ID NO: 4 SEC14L2 isoform 2 SEQ ID NO: 5 SEC14L2 transcript variant 3 SEQ ID NO: 6 SEC14L2 isoform 3
H6PD	hexose-6-phosphate dehydrogenase (glucose 1-dehydrogenase), a.k.a., GDH, Glucose Dehyrogenase, CORTRD1, G6PDH, 6-phophogluconolactonase, G6PD, H form, GDH/6PGL Endoplasmic, Bifuntional Protein, Glucose-1-Dehydrogenase, Glocose Dehydrogenase, Glucose-6-Phosphate, Dehydrogenase, Salivary, EC 1.1.1.49, EC 2.7.4.3, or EC 3.1.1.31	Oxidizes glucose-6-phosphate and glucose, as well as other hexose-6-phosphates	http://www.genecards.org/cgi-bin/carddisp.pl?gene=H6PD SEQ ID NO: 7 H6PD transcript variant 1 SEQ ID NO: 8 H6PD isoform 1 SEQ ID NO: 9 H6PD transcript variant 2 SEQ ID NO: 10 H6PD isoform 2

Gene ID	Gene name	Gene annotation	Reference
TMEM140	transmembrane protein 140	NA	http://www.genecards.org/cgi-bin/carddisp.pl?gene=TMEM140 SEQ ID NO: 11 TMEM140 transcript SEQ ID NO: 12 TMEM140 protein
SLC2A5	solute carrier family 2 (facilitated glucose/fructose transporter), member 5, a.k.a., GLUT5, Glucose Transporter Type 5, Small intestine, GLUT-5, Glucose Transporter-Like Protein 5, or Fructose Transporter	Cytochalasin B-sensitive carrier. Seems to function primarily as a fructose transporter	http://www.genecards.org/cgi-bin/carddisp.pl?gene=SLC2A5 SEQ ID NO: 13 SLC2A5 transcript variant 1 SEQ ID NO: 14 SLC2A5 isoform 1 SEQ ID NO: 15 SLC2A5 transcript variant 2 SEQ ID NO: 16 SLC2A5 isoform 2
ACTA1	actin, alpha 1, skeletal muscle, a.k.a., ACTA, Nemaline Myopathy Type 3, ASMA, CFTD1, NEM3, CFTD, CFTDM, MPFD, NEM1, NEM2, Actin, Alpha Skeletal Muscle, or Alpha-actin-1	Actins are highly conserved proteins that are involved in various types of cell motility and are ubiquitously expressed in all eukaryotic cells	http://www.genecards.org/cgi-bin/carddisp.pl?gene=ACTA1 SEQ ID NO: 17 ACTA1 transcript SEQ ID NO: 18 ACTA1 protein
IRF8	interferon regulatory factor 8, a.k.a., ICSBP1, Interferon Consensus Sequence Binding Protein 1, Interferon Consensus Binding Protein, H-ICSBP, UCSBP, or IRF-8,	Specifically binds to the upstream regulatory region of type I IFN and IFN-inducible MHC class I genes (the interferon consensus sequence (ICS)). Plays a negative regulatory role in cells of the immune system	http://www.genecards.org/cgi-bin/carddisp.pl?gene=IRF8 SEQ ID NO: 19

Gene ID	Gene name	Gene annotation	Reference
			IRF8 transcript SEQ ID NO: 20 IRF8 protein
STAT2	signal transducer and activator of transcription 2, 113kDa, a.k.a., ISGF-3, P113, STAT113, Interferon alpha Induced, Transcriptional Activator, Signal Transducer And Activator of Transcription 2, p113	Signal transducer and activator of transcription that mediates signaling by type I IFNs (IFN-alpha and IFN-beta). Following type I IFN binding to cell surface receptors, Jak kinases (TYK2 and JAK1) are activated, leading to tyrosine phosphorylation of STAT1 and STAT2. The phosphorylated STATs dimerize, associate with ISGF3G/IRF-9 to form a complex termed ISGF3 transcription factor, that enters the nucleus. ISGF3 binds to the IFN stimulated response element (ISRE) to activate the transcription of interferon stimulated genes, which drive the cell in an antiviral state	http://www.genecards.org/cgi-bin/carddisp.pl?gene=STAT2 SEQ ID NO: 21 STAT2 transcript variant 1 SEQ ID NO: 22 STAT2 isoform 1 SEQ ID NO: 23 STAT2 transcript variant 2 SEQ ID NO: 24 STAT2 isoform 2
UGT2A1 / UGT2A2	UDP glucuronosyltransferase 2 family, polypeptide A1, complex locus, a.k.a., UDP-Glucuronosyltransferase 2A1, UDPGT 2A, UDP Glycosyltransferase 2 Family, Polypeptide A1, UGT2A2, or EC 2.4.1.17	UDP-glucuronosyltransferases catalyze phase II biotransformation reactions in which lipophilic substrates are conjugated with glucuronic acid to increase water solubility and enhance excretion. They are of major importance in the conjugation and subsequent elimination of potentially toxic xenobiotics and endogenous compounds. Active on odorants and seems to be involved in olfaction; it could help clear lipophilic odorant molecules from the sensory epithelium	http://www.genecards.org/cgi-bin/carddisp.pl?gene=UGT2A1 SEQ ID NO: 25 UGT2A1 transcript variant 1 SEQ ID NO: 26 UGT2A1 isoform 1 SEQ ID NO: 27 UGT2A1 transcript variant 2 SEQ ID NO: 28 UGT2A1 isoform 2

Gene ID	Gene name	Gene annotation	Reference
			SEQ ID NO: 29 UGT2A1 transcript variant 3 SEQ ID NO: 30 UGT2A1 isoform 3

Materials and Methods

[00116] Patient tumor samples and engraftment in immunocompromised mice. Freshly and surgically removed tumor tissues were obtained from the patients diagnosed as HCC through collaboration with the Beijing Keluoen Translational Medicine Institute and Hebei Medical University Fourth Hospital with approval by the Institutional Review Boards of Hebei Medical University Fourth Hospital and the informed consents from patients. The engraftments of patient tumor fragments into immunocompromised mice subcutaneously have been broadly described by others. Briefly, the tumors were sliced into 3x3x3 mm3 fragments and inoculated subcutaneously on the flank of mice (Balb/c nude, 6-8 wks old, female, Beijing HFK Bioscience Co. Ltd., Beijing, China). The tumor growth was monitored twice weekly by using a caliper. The established tumor models from these patient samples, called passage 0 or P0, were serially re-engrafted to maintain tumors, these subsequent passages were called P1, 2, 3… (< 10). When tumors sizes reach 500-700 mm3 (1/2 length x width^2), they were harvested for the next round of engraftment to passage the tumors and to conduct studies of pharmacology, histopathology, immunohistology, cellular and molecular analysis. All procedures were performed under sterile conditions at Crown Bioscience SPF facility. All studies involving experimental animals were carried out in strict accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Committee on the Ethics of Animal Experiments of Crown Bioscience, Inc. (Crown Bioscience IACUC Committee).

[00117] Evaluation of antitumor activity. When tumor volume reaches 100-150mm3, the mice were randomly grouped into two groups of 5 mice with similar average tumor volume. The control group was treated immediately after grouping with vehicle control (PBS, weekly IP injection for two weeks); the treatment groups were treated with one of the followings: cetuximab (weekly IP injection for two weeks, 1 mg/mouse, Merck KGaA), erlotinib (daily oral, 50 mg/kg, Nanjing Angel Pharmaceutical Co.), crizotinib (daily oral, 50 mg/kg, Selleckchem.com). The tumor growth was monitored twice weekly, and %ΔT/ΔC value were calculated for assessing tumor response to the treatment (ΔT = tumor volume change in the treatment group and ΔC = tumor volume change in control group).

[00118] IHC analysis of HuPrime® tumors. Standard immuno-histochemistry was used to analyze tumor tissues from the HuPrime models. Briefly, the tissues were fixed in 10% neutral buffered formalin and embedded in paraffin per standard histological procedures. After

deparaffinization and rehydratation, 3-μm thick tissue sections were pretreated at 95°C in 0.01M sodium citrate, pH6.0 solution for 30 min, followed by staining with rabbit anti-human monoclonal pERK or pEGFR antibody (Cell Signaling, Boston, USA). Positive staining was detected using Ultra Vision LP large Volume Detection System HRP Polymer (Ready-To- Use) Kit (Lab Vision, Fremont, CA). DAB was used as the chromogenic substrate, and sections were counterstained with Gill's Hematoxylin (Fisher Scientific, Fair Lawn, USA). The test specimens were then scored independently by three investigators in a blinded fashion per following criteria: 0, no staining; 1+, minimal staining; 2+, moderate staining; 3+, strong staining. Areas of most intensity were identified by scanning tumor sections at low power (×100), and then images were photographed at high magnification (×400) using Olympus BX51 microscopy system with DP71 digital camera (Olympus, Melville, NY).

[00119] Expression profiling of HCC-PDX and gene copy number analysis. Fresh HCC HuPrime™ tumor tissues were collected from the tumor-bearing mice, snap-frozen, and stored at -80°C before being used for genetic and genomic analysis. For gene profiling analysis, the total RNA was isolated from the frozen tissues using Trizol (Invitrogen, Carlsbad, CA) per the manufacturer's instructions, and purified using RNeasy mini columns (Qiagen). RNA quality was assessed on a Bioanalyzer (Agilent). Only RNA samples with high quality (RIN>8) were used for expression profiling assays on Affymetrix HG-U219 array plates following standard protocol (GeneChip® 3'IVT Expression Kit, User Manual, Affymetrix, P/N 702646 Rev. 8). Raw CEL data sets of all samples were normalized by RMA algorithm. Probe set intensity was expressed as Log (2) transformed values. For SNP/CNV assay using Affymetrix® SNP6.0 chips, genomic DNA was isolated and purified using Genomic DNA Tissue and Blood Isolation Kit (Qiagen) following manufacturer's instruction. DNA processing and chip hybridization were performed following standard Affymetrix protocol (Affymetrix® Genome-Wide Human SNP Nsp/Sty 6.0 User Guide, P/N 702504, Rev. 4). Raw CEL data was QC-ed and filtered to remove low call-rate samples, and gene copy number analysis were performed by PICNIC and/or PennCNV methods. For some of the samples, the relative gene copy numbers were determined by qPCR. Briefly, the same genomic DNAs were subjected to amplification using MET specific primers (**SEQ ID NO: 31,** MET-F: GCTGGTGGTCCTACCATACATG; **SEQ ID NO: 32,** MET-R: CTGGCTTACAGCTAGTTTGCCA) by SYBR Green based quantitative PCR. The mammalian LINE-1 retrotransposon gene was used as a reference. The q-PCR data was analyzed on the chromo4 system using Opticon Monitor 3 software to generate the raw data. The raw data was then processed using the delta CT relative quantification method. ΔCT= (CT

value of target gene)—(CT value of reference gene). Delta CT values were then converted into intensity value (POWER(ΔCT,-2)). All data was normalized to that of a sample with known MET copy number to obtain relative MET copy number.

Discussion

[00120] Hepatocellular carcinoma (HCC) is a heterogeneous. The only approved target therapy is Sorafenib, either as monotherapy or in combination with chemotherapy. However, the treatment so far has shown limited overall benefit. However, as a multi-kinase inhibitor, this is largely due to high toxicity and also low overall efficacy, since there is lack of patient stratification that can identify the likely responders who can actually benefit from the treatment and the likely non-responders who can avoid the unnecessary toxicity. Therefore, developing such an individualized treatment plan would improve the overall treatment benefits. Unfortunately, so far clinical practice of Sorafenib has yet to reveal effective biomarkers to support such individualized treatments.

[00121] HCC-PDXs capture the original patient disease physiology as well as the underlying genetic diversity for each patient (2). These experimental models can be used as "patient surrogates or xenopatients (12)" to test drug candidates prior to the inception of clinical development. A large collection of established HCC-PDXs, or a library of HCC-PDX, can represent heterogeneity and diversity of the diseases. With their availability, they can be used to run a randomized phase II clinical trial-like study on any given drug. Such trials not only reveal the overall benefit of treatment by demonstrating % of responders, but also to potentially reveal valuable biomarkers (or molecular signatures) to be associated with these responders, if the models have been comprehensively profiled, as the ones described in this report. These biomarkers, once validated in the clinic, can potentially be used for patient stratification.

[00122] This report described a clinical trial-like study under well controlled experimental parameters and using a panel of randomized HuPrime® HCC models of full genetic annotations. The statistic analysis of our trial data indeed revealed a specific gene expression signature, HCC-Sorafenib-HuSignature™). This signature can be used to identify patients with higher probability to respond, or not respond to, Sorafenib, in a prospective clinical trial and also in clinical treatment practice.

[00123] In general, our HuTrial/HuSignature™ platform exemplified in this report is designed to discover predictive signatures by conducting a "phase II clinical trial-like" study by employing large collection of genome-defined HuPrime® PDX models and by using bioinformatic and statistic analysis. The basis of process is that the outcome can enable bioinformatician and biostatistician to identify specific genetic signature which has a high correlation with drug response or drug resistance. The resulting signature ("training set") can be further confirmed by either running more studies with additional HuPrime® models, or in a prospective clinical trial study ("test set"). The potential applications of this platform include: a) discovery of biomarkers for early stage clinical drug candidate; b) indication selection and expansion; c) life cycle management for marketed drugs; d) novel indication discovery for "me-too" drugs

[00124] The most important cost driver for drug development is the high failure rate in late stage clinical development (13). The need to reduce drug attrition is especially acute in the field of oncology, where drugs often fail not because of toxicity but rather lack of efficacy. The successful development of drugs like trastuzumab, imatinib, and gefitinib has demonstrated the critical need to identify biomarkers in order to select patients which are most likely to benefit from the drug treatment. Our HuTrial/HuSignature™ platform could be a very powerful tool to be used to minimize the attrition during drug clinical development.

[00125] Patient derived xenografts (PDXs) are considered experimental models mimicking patient tumors, or "patient avatars". We performed a clinical-like trial (phase II-like) using a cohort of 21 hepatocellular carcinoma (HCC) patient derived xenografts (PDXs), also called patient avatar trial, for testing their response to multi-kinase inhibitor Sorafenib. 13 out of 21 PDXs (62%) achieved effective tumor growth inhibition with $\Delta T/\Delta C < 40\%$ ($p<0.05$) by the treatment of Sorafenib at the doses of 50 mg/kg. Gene expression profiles of these PDXs were analyzed to reveal the mRNA expression signatures that may be predictive of the response to Sorafenib. This signature can be potentially used to develop companion diagnostics to stratify patent treatment, or individualized treatment.

Example 4. Identifying and using gene markers

[00126] The efficacy of Sorafenib, Sunitinib, Axitinib, Vandetanib, Pazopanib, and Cabozantinib is measured on a panel of HCC PDXs each of which has multiple mice, at least 3, in both the control group that receives vehicle. The treatment group receives Sorafenib, Sunitinib, Axitinib, Vandetanib, Pazopanib, or Cabozantinib.

[00127] For each PDX, drug efficacy is quantified by %ΔT/ΔC wherein ΔT is the tumor volume change in the treatment group and ΔC is the tumor volume change in control group. The mRNA expression levels or the protein levels of 2, 3, 4, 5, 6, 7, 8 or more marker genes are profiled by microarray, RNAseq, and/or RT-PCR. In some embodiments, the markers include SEC14L2, H6PD, TMEM140, SLC2A5, ACTA1, IRF8, STAT2, and/or UGT2A1. In some embodiments, other markers can be included.

[00128] A signature score is calculated based on the expressions or the protein levels of the marker genes, and its predictive power is quantified by the coefficient of determination (r2) and the associated p-value. Higher values of r2 and/or lower values of p-value indicate better predictive power. A p-value at least smaller than 0.05 is needed for considering the signature have any predictive power. In some embodiments, one or more particular marker is especially useful for predicting the responsiveness of a subject to a particular multi-kinase inhibitor.

[00129] The preceding merely illustrates the principles of the invention. It will be appreciated that those skilled in the art will be able to devise various arrangements which, although not explicitly described or shown herein, embody the principles of the invention and are included within its spirit and scope. Furthermore, all examples and conditional language recited herein are principally intended to aid the reader in understanding the principles of the invention and the concepts contributed by the inventors to furthering the art, and are to be construed as being without limitation to such specifically recited examples and conditions.

[00130] Moreover, all statements herein reciting principles, aspects, and embodiments of the invention as well as specific examples thereof, are intended to encompass both structural and functional equivalents thereof. Additionally, it is intended that such equivalents include both currently known equivalents and equivalents developed in the future, i.e., any elements developed that perform the same function, regardless of structure. The scope of the present invention, therefore, is not intended to be limited to the exemplary embodiments shown and described herein. Rather, the scope and spirit of the present invention is embodied by the appended claims.

[00131] Unless defined otherwise, all technical and scientific terms herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials, similar or equivalent to those described herein, can be used in the practice or testing of the present invention, the preferred methods and

materials are described herein. All publications, patents, and patent publications cited are incorporated by reference herein in their entirety for all purposes.

[00132] The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention.

[00133] While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth and as follows in the scope of the appended claims.

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Each of references mentioned above is herein incorporated by reference in its entirety for all purposes.

Claims

1. A method for determining a subject's responsiveness to a multi-kinase inhibitor comprising

measuring activity profile of a panel of gene markers comprising at least one or more markers selected from the group consisting of SEC14L2, H6PD, TMEM140, SLC2A5, ACTA1, IRF8, STAT2, and UGT2A1,

comparing the activity profile of the panel to a predetermined activity profile,

wherein the subject's responsiveness is determined based on the comparison between the activity profile of the panel of gene markers and the predetermined activity profile, and

wherein the multi-kinase inhibitor targets to vascular endothelial growth factor (VEGF)-mediated angiogenesis and blocks the RAF/extracellular signal regulated kinase (ERK) kinase (MEK)/ERK cascade.

2. The method of claim 1, wherein the activity profile of a panel of gene markers includes gene expression level, RNA activity level, or protein activity level.

3. The method of claim 1, wherein the panel comprises at least two, three, or four of gene markers.

4. The method of claim 1, wherein the predetermined activity profile is gene expression level.

5. The method of claim 1, wherein the multi-kinase inhibitor is Sorafenib, Sunitinib, Axitinib, Vandetanib, Pazopanib, Cabozantinib, or mixture thereof.

6. The method of claim 1, wherein the subject is a human with hepatocellular carcinoma.

7. A method for administering a multi-kinase inhibitor to a subject comprising

testing the subject for activity profile of a panel of gene markers comprising at least one or more markers selected from the group consisting of SEC14L2, H6PD, TMEM140, SLC2A5, ACTA1, IRF8, STAT2, and UGT2A1,

comparing the activity profile of the panel to a predetermined activity profile,

wherein the multi-kinase inhibitor is administered to the subject if the activity profile of the panel is within the predetermined activity profile and wherein the multi-kinase inhibitor targets to vascular endothelial growth factor (VEGF)-mediated angiogenesis and blocks the RAF/extracellular signal regulated kinase (ERK) kinase (MEK)/ERK cascade.

8. A collection of activity profile of a panel of gene markers comprising at least two or more gene markers selected from the group consisting of SEC14L2, H6PD, TMEM140, SLC2A5, ACTA1, IRF8, STAT2, and UGT2A1.

9. An array comprising probes for detection of at least two or more gene markers selected from the group consisting of SEC14L2, H6PD, TMEM140, SLC2A5, ACTA1, IRF8, STAT2, and UGT2A1.

10. The method of claim 1, wherein the subject is determined to be responsive to the multi-kinase inhibitor treatment when the activity profile of a panel of gene markers is within the predetermined activity profile.

11. The method of claim 1, wherein the subject is determined to be not responsive to the multi-kinase inhibitor treatment when the activity profile of a panel of gene markers is not within the predetermined activity profile.

12. The method of claim 1, wherein the subject is determined to be responsive to the multi-kinase inhibitor treatment when the activity profile of a panel of gene markers is similar to, or lower than the predetermined activity profile.

13. The method of claim 1, wherein the subject is determined to be not responsive to the multi-kinase inhibitor treatment when the activity profile of a panel of gene markers is higher than the predetermined activity profile.

14. A method for determining a subject's responsiveness to a multi-kinase inhibitor comprising

measuring activity profile of a panel of gene markers comprising at least one or more markers selected from the group consisting of SEC14L2, H6PD, TMEM140,

SLC2A5, ACTA1, IRF8, STAT2, and UGT2A1,

comparing the activity profile of the panel to a negative predetermined activity profile,

wherein the subject's responsiveness is determined based on the comparison between the activity profile of the panel of gene markers and the negative predetermined activity profile, and

wherein the multi-kinase inhibitor targets to vascular endothelial growth factor (VEGF)-mediated angiogenesis and blocks the RAF/extracellular signal regulated kinase (ERK) kinase (MEK)/ERK cascade.

15. The method of claim 14, wherein the subject is determined to be responsive to the multi-kinase inhibitor treatment when the activity profile of a panel of gene markers is not within the negative predetermined activity profile.

16. The method of claim 14, wherein the subject is determined to be not responsive to the multi-kinase inhibitor treatment when the activity profile of a panel of gene markers is within the negative predetermined activity profile.

17. The method of claim 14, wherein the subject is determined to be responsive to the multi-kinase inhibitor treatment when the activity profile of a panel of gene markers lower than the negative predetermined activity profile.

18. The method of claim 14, wherein the subject is determined to be not responsive to the multi-kinase inhibitor treatment when the activity profile of a panel of gene markers is similar to , or higher than the negative predetermined activity profile.

19. A method for administering a multi-kinase inhibitor to a subject comprising

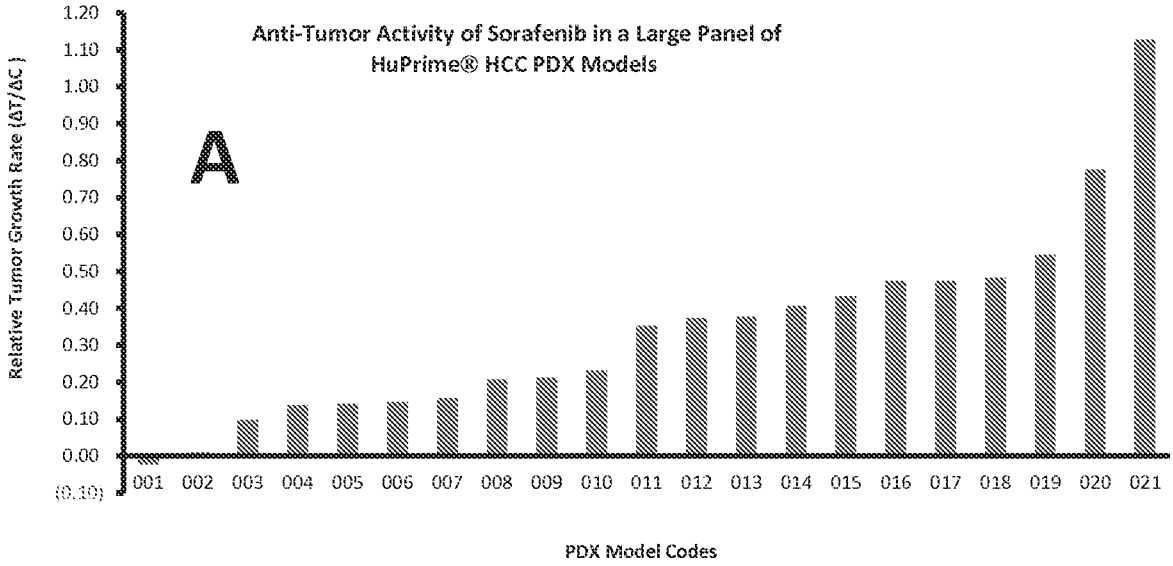
testing the subject for activity profile of a panel of gene markers comprising at least one or more markers selected from the group consisting of SEC14L2, H6PD, TMEM140, SLC2A5, ACTA1, IRF8, STAT2, and UGT2A1,

comparing the activity profile of the panel to a negative predetermined activity profile,

wherein the multi-kinase inhibitor is administered to the subject if the activity profile of

the panel is not within the negative predetermined activity profile and wherein the multi-kinase inhibitor targets to vascular endothelial growth factor (VEGF)-mediated angiogenesis and blocks the RAF/extracellular signal regulated kinase (ERK) kinase (MEK)/ERK cascade.

FIG. 1A


Anti-Tumor Activity of Sorafenib in a Large Panel of HuPrime® HCC PDX Models
A
Relative Tumor Growth Rate (ΔT/ΔC)
1.20
1.10
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
(0.10)
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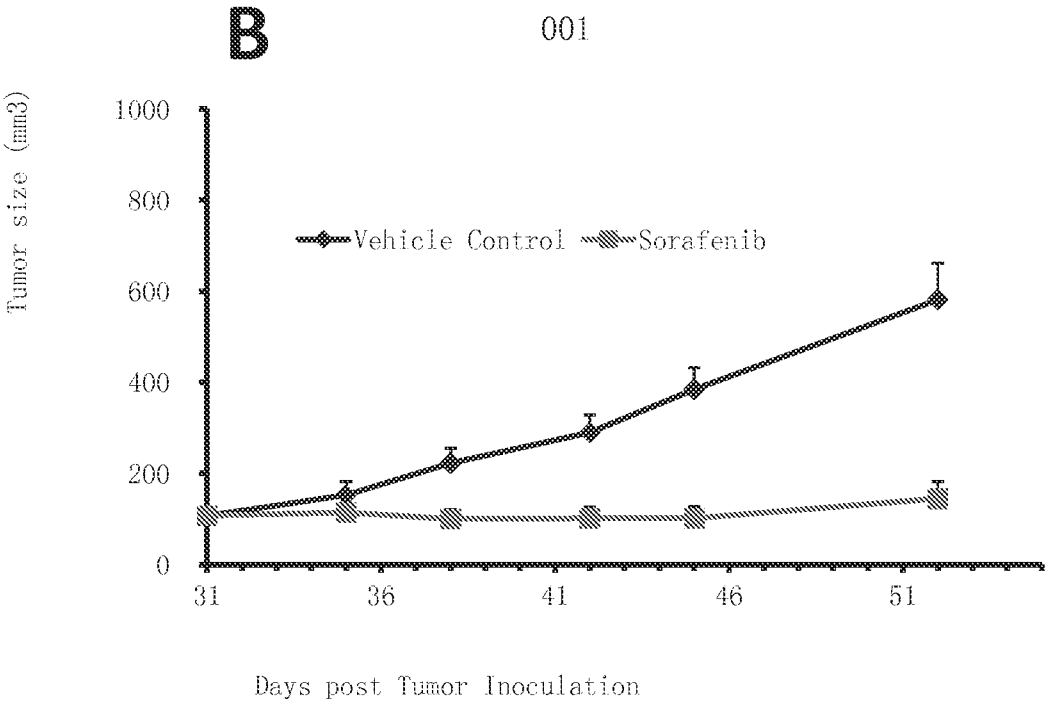
PDX Model Codes

FIG. 1B


B
001
Tumor size (mm3)
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800
600
400
200
0
Vehicle Control
Sorafenib
31
36
41
46
51
Days post Tumor Inoculation

FIG. 1C

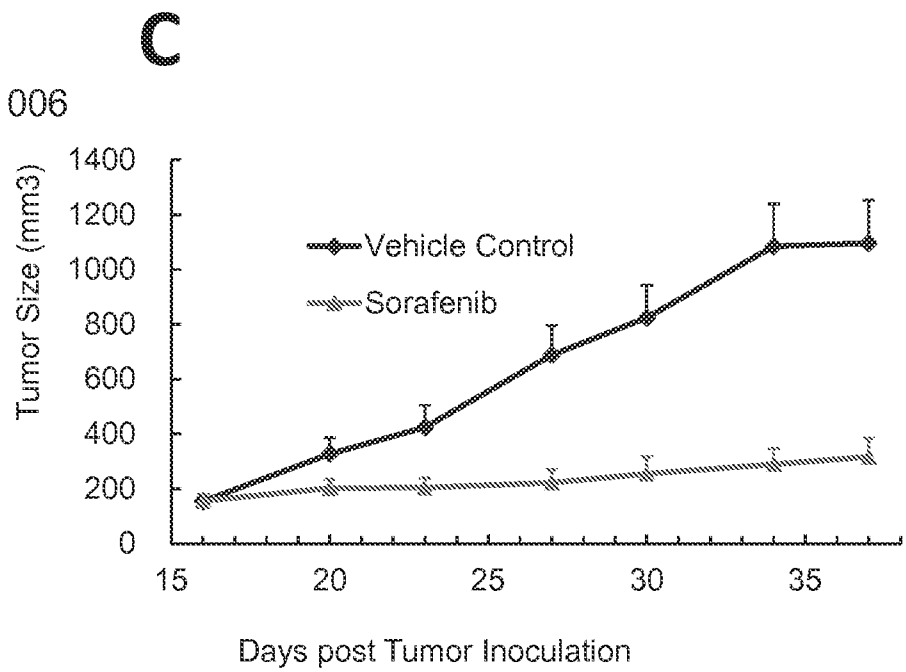

C
006
Tumor Size (mm3)
1400
1200
1000
800
600
400
200
0
15
20
25
30
35
Vehicle Control
Sorafenib
Days post Tumor Inoculation

FIG. 1D

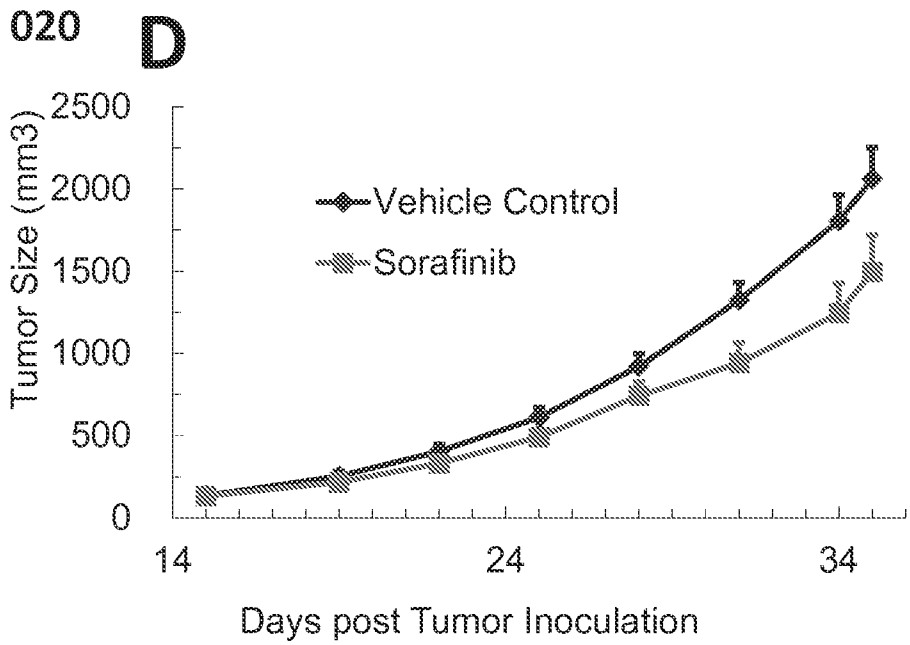
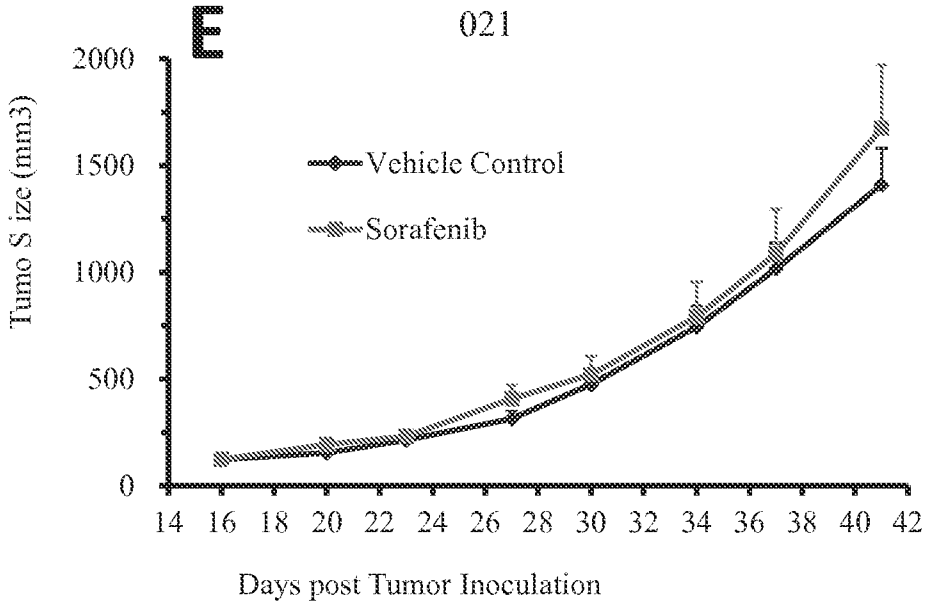
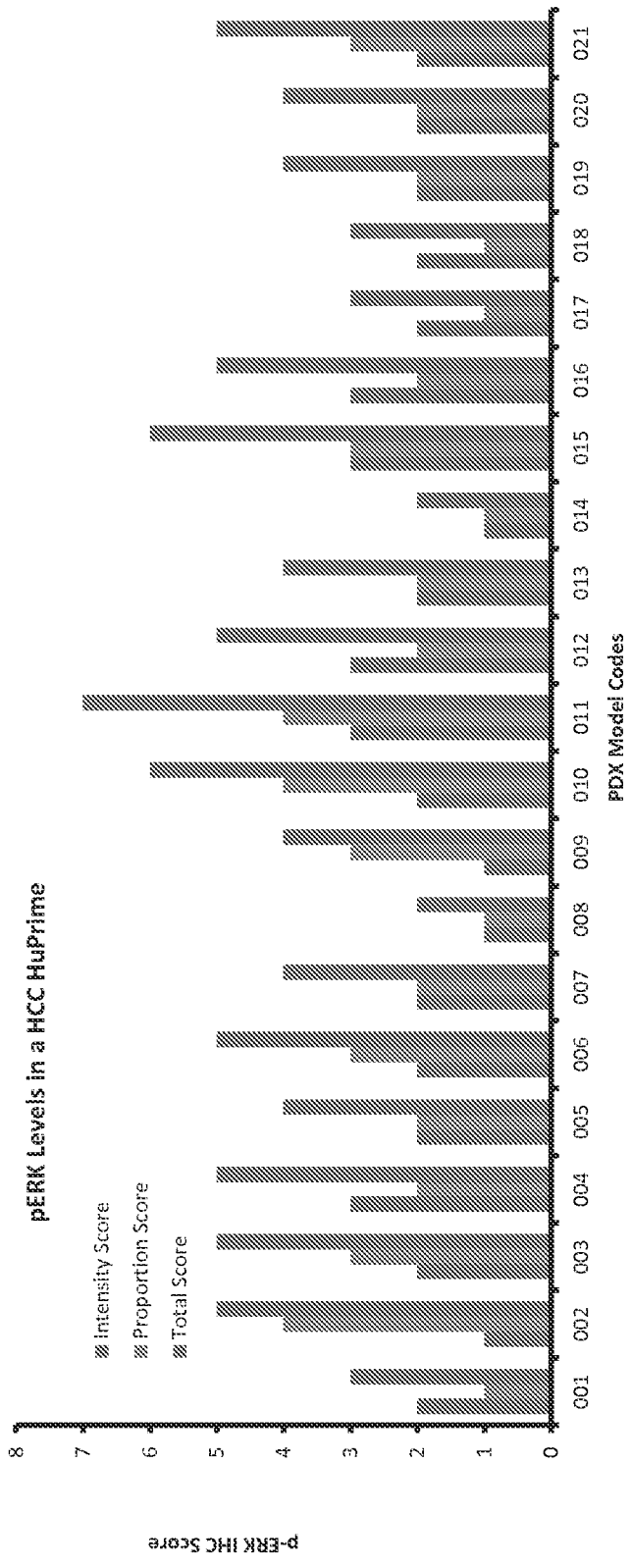

020
D
Tumor Size (mm3)
2500
2000
1500
1000
500
0
14
24
34
Vehicle Control
Sorafinib
Days post Tumor Inoculation

FIG. 1E


E
021
2000
1500
1000
500
0
Tumo S ize (mm3)
Vehicle Control
Sorafenib
14 16 18 20 22 24 26 28 30 32 34 36 38 40 42
Days post Tumor Inoculation


pERK Levels in a HCC HuPrime
Intensity Score
Proportion Score
Total Score
p-ERK IHC Score
0
1
2
3
4
5
6
7
8
001
002
003
004
005
006
007
008
009
010
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012
013
014
015
016
017
018
019
020
021
PDX Model Codes

FIG. 2

FIG. 3

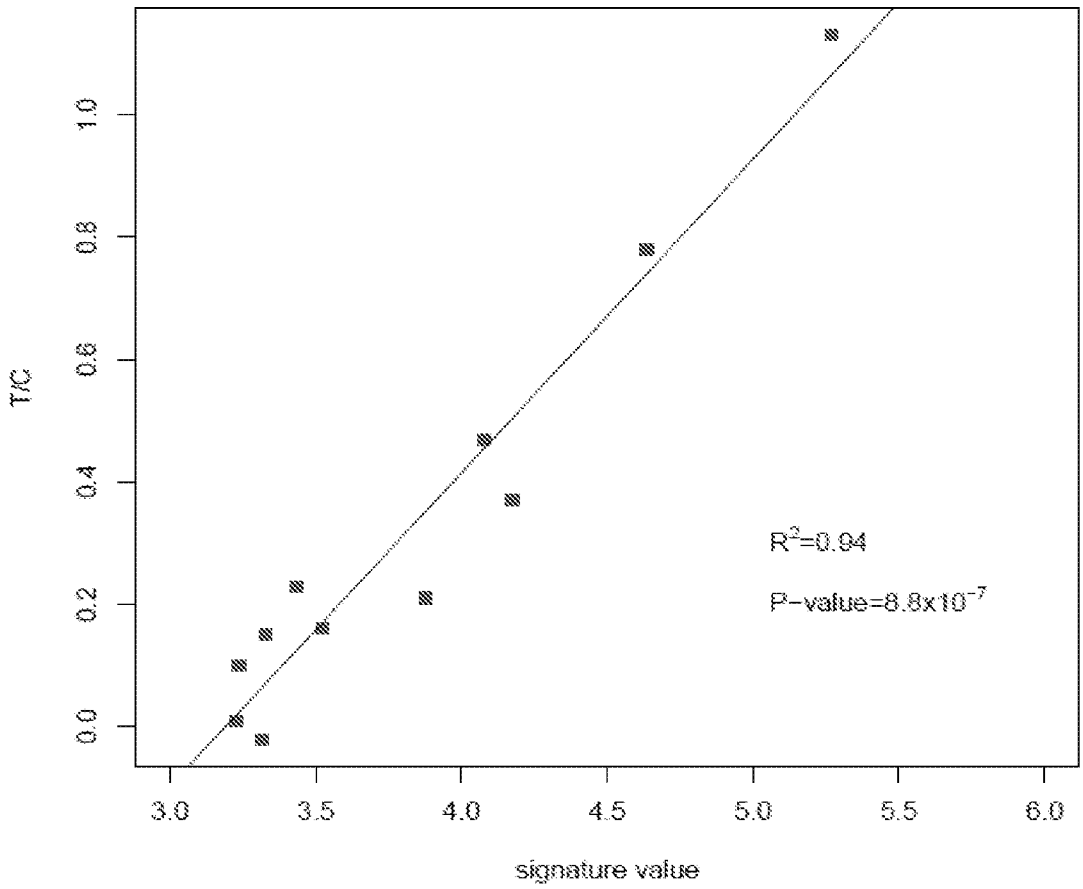
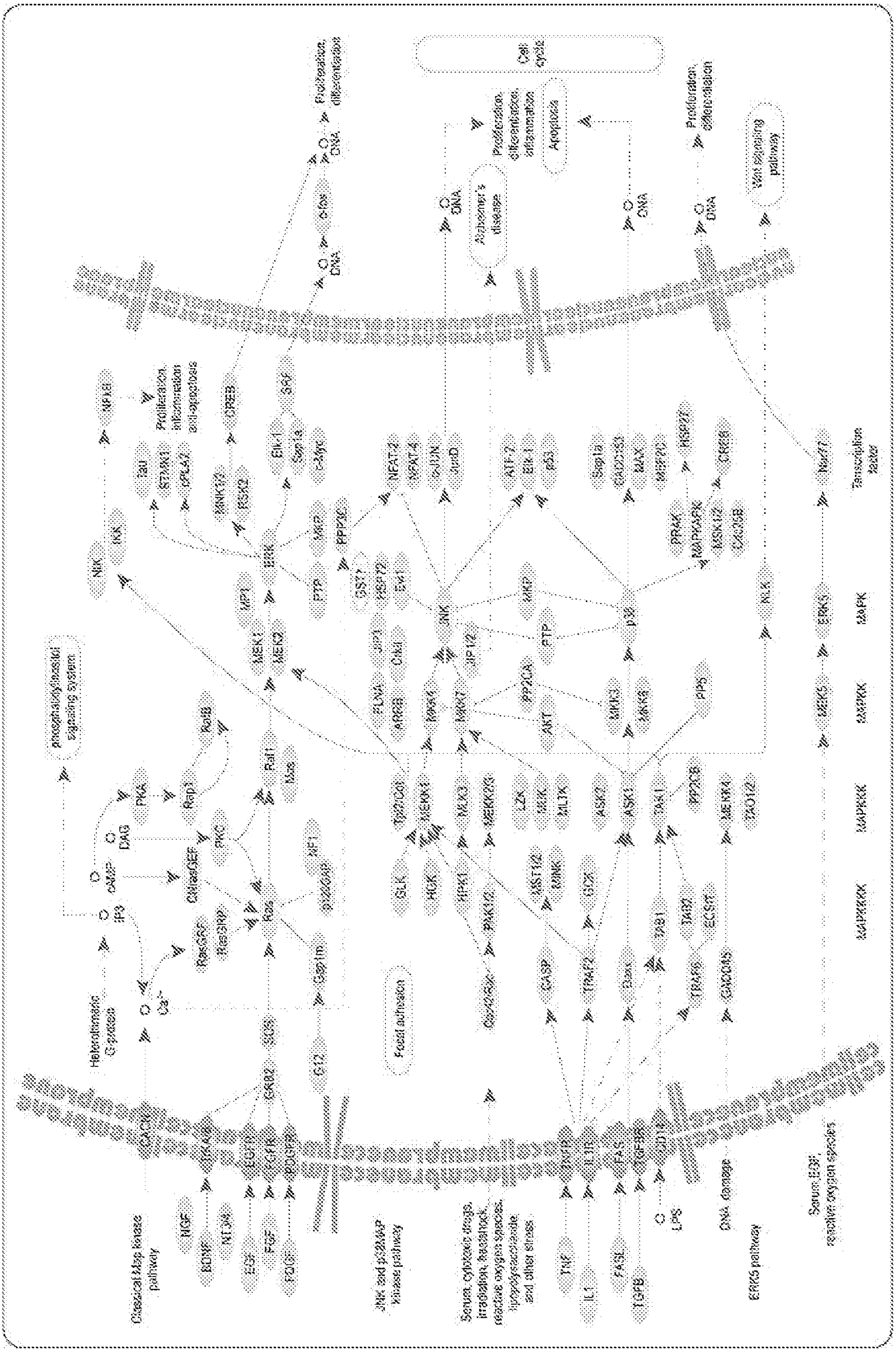

T/C
0.0
0.2
0.4
0.6
0.8
1.0
3.0
3.5
4.0
4.5
5.0
5.5
6.0
signature value
R^2=0.94
P-value=8.8x10^{-7}

FIG 4.



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2014/053142

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - G01N 33/48 (2014.01)
CPC - G01N 2570/00 (2014.11)
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) - A61K 39/00, 39/395, 45/00, 49/00; G01N 33/48 (2014.01)
CPC - G01N 2570/00, 33/50, 33/53, 33/5023, 33/6848, 33/6893 (2014.11)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
CPC (keyword delimited) - G01N 2570/00, 33/53, 33/50, 33/5023, 33/6848, 33/6893 (2014.11)
US Classes - 424/134.1; 435/6.11, 6.12, 6.15, 7.1, 7.23, 7.92; 436/501; 506/9

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

PatBase, Google Patents, Google, PubMed
Search terms used: kinase inhibitor, sorafenib, hepatocellular carcinoma, compare, assay, theranose, cancer, biomarker

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2013/022995 A2 (BROWN et al) 14 February 2013 (14.02.2013) entire document	1-19
A	US 2013/0122524 A1 (FANTL et al) 16 May 2013 (16.05.2013) entire document	1-19
A	US 2012/0245051 A1 (RIMM et al) 27 September 2012 (27.09.2012) entire document	1-19
A	ZHU et al. "Biomarkers for hepatocellular carcinoma: progression in early diagnosis, prognosis, and personalized therapy," Biomarker Research, 05 February 2013 (05.02.2013), Vol. 1, No. 10, Pgs. 1-8. entire document	1-19
T, Y	HARDING et al. "Predicting responsiveness to sorafenib: can the determination of FGF3/FGF4 amplifications enrich for clinical benefit?," Hepatobiliary Surgery and Nutrition, 01 August 2014 (01.08.2014), Vol. 3, No. 4, Pgs. 168-171. entire document	1-19

☐ 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
19 November 2014	17 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: Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2014/053142

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing filed or furnished:

   a. (means)

   ☐ on paper

   ☒ in electronic form

   b. (time)

   ☒ in the international application as filed

   ☐ together with the international application in electronic form

   ☐ subsequently to this Authority for the purposes of search

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

Specifically, SEQ ID NOs: 1 and 2 were searched.