(54) Title: TREATING CANCER WITH AN HSP90 INHIBITORY COMPOUND

FIG. 1

(57) Abstract: Methods of treating cancer with ganetespib are disclosed. Also provided are methods of treating a cancer wherein the cancer has a high level of hypoxia, with ganetespib. Also provided are methods of treating a cancer with a mutation in KRAS, a mutation in EGFR, or a mutation in ALK, with ganetespib. Further provided are methods of treating tumor/cancer with a mutation in KRAS, a mutation in EGFR, or a mutation in ALK wherein the tumor/cancer also has certain level of LDH, with ganetespib.

**Declarations under Rule 4.17:**

- as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(iii))
- as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iv))

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TREATING CANCER WITH AN HSP90 INHIBITORY COMPOUND

RELATED APPLICATIONS

[0001] This application claims the benefit of priority to U.S. Provisional Patent Application Nos. 61/646,011, filed on May 11, 2012; 61/647,845, filed on May 16, 2012; and 61/815,082, filed on April 23, 2013. The contents of each of the above applications are incorporated herein by reference in their entireties.

FIELD OF THE INVENTION

[0002] This invention relates to the use of an Hsp90 inhibitor in treating humans with certain specific types of cancer. Regimens disclosed herein demonstrate potency against certain specific types of cancer, while showing optimal treatment effects.

BACKGROUND OF THE INVENTION

[0003] Although tremendous advances have been made in elucidating the genomic abnormalities that cause cancer, currently available chemotherapy remains unsatisfactory, and the prognosis for the majority of patients diagnosed with cancer remains poor. Many chemotherapeutic agents act on a specific molecular target thought to be involved in the development of the malignant phenotype. However, a complex network of signaling pathways regulate cell proliferation and the majority of malignant cancers are facilitated by multiple genetic abnormalities in these pathways. Therefore, it is less likely that a therapeutic agent that acts on one molecular target will be fully effective in curing a patient who has cancer.

[0004] Heat shock proteins (HSPs) are a class of chaperone proteins that are up-regulated in response to elevated temperature and other environmental stresses, such as ultraviolet light, nutrient deprivation and oxygen deprivation. HSPs act as chaperones to other cellular proteins (called client proteins), facilitate their proper folding and repair and aid in the refolding of misfolded client proteins. There are several known families of HSPs, each having its own set of client proteins. The Hsp90 family is one of the most abundant HSP families, accounting for about 1-2% of proteins in a cell that is not under stress,
increasing to about 4-6% in a cell under stress. Inhibition of Hsp90 results in the degradation of its client proteins via the ubiquitin proteasome pathway. Unlike other chaperone proteins, the client proteins of Hsp90 are mostly protein kinases or transcription factors involved in signal transduction, and a number of its client proteins have been shown to be involved in the progression of cancer.

**SUMMARY OF THE INVENTION**

[0005] A triazolone Hsp90 inhibitor is demonstrated herein to be particularly effective in specific dosing regimens for treating humans with cancer. It is also demonstrated herein that the Hsp90 inhibitor is particularly effective in treating certain specific types of cancer, including cancers having a mutation in one or more of KRAS, epidermal growth factor receptor (EGFR), or anaplastic lymphoma kinase (ALK); or cancers with certain level of hypoxia. The particular dosing regimens disclosed herein demonstrate potency against certain types of cancer, while showing optimal treatment effects.

[0006] The invention provides methods of treating cancer in a subject, comprising administering ganetespib or a pharmaceutically acceptable salt or a tautomer thereof wherein the subject has a cancer with a mutation in KRAS and a high level of hypoxia. In an embodiment, the method also includes using ganetespib in treating a subject with cancer having a mutation in KRAS or a high level of hypoxia. In another embodiment, the method further includes using ganetespib in treating a subject with cancer having a mutation in KRAS and/or having certain level of lactate dehydrogenase (LDH).

[0007] In certain embodiments, the cancer may be lung cancer, breast cancer, gastric cancer, colorectal cancer, pancreatic cancer, ocular melanoma, prostate cancer, gastrointestinal stromal tumors (GIST), advanced esophagogastric cancer, melanoma, hepatocellular cancer, solid tumor, liver cancer, head and neck cancer, colon cancer, small cell lung cancer, and non-small cell lung cancer (NSCLC), or any other type of cancer such as those provided herein. In certain embodiments, the NSCLC was previously treated and not responsive. In certain embodiments, the NSCLC was previously treated with crizotinib and is no longer responsive to the crizotinib treatment. In certain embodiments, the breast cancer is HER2 positive, and has been previously treated with trastuzumab. In certain
embodiments, the breast cancer is HER2 positive and trastuzumab refractory. In certain embodiments, the cancer is triple negative breast cancer. In certain embodiments, the cancer is metastatic hormone-resistant prostate cancer, or metastatic castration-resistant prostate cancer. In certain embodiments, the cancer is pancreatic cancer. In certain embodiments, the cancer comprises a tumor that has high level of hypoxia.

[0008] In certain embodiments, methods of the invention further include identifying a subject as having a cancer with a mutation in KRAS. In certain embodiments, methods of the invention further include identifying a subject as having a cancer having a high level of hypoxia. In certain embodiments, methods of the invention also include identifying a subject as having a cancer having a high level of LDH. In certain embodiments, methods of the invention further include identifying a subject as having a cancer with a mutation in KRAS and with a high level of hypoxia. In certain embodiments, methods of the invention further include identifying a subject as having a cancer with a mutation in KRAS and with a certain level of LDH.

[0009] The invention provides a method of treating cancer in a subject, comprising administering ganetespib or a pharmaceutically acceptable salt or a tautomer thereof, wherein the subject has a cancer with a mutation in ALK and with a high level of hypoxia. In an embodiment, the method also includes using ganetespib in treating a subject with cancer having a mutation in ALK and with a high level of hypoxia. In another embodiment, the method further includes using ganetespib in treating a subject with cancer having a mutation in ALK and with a certain level of LDH.

[0010] In certain embodiments, the cancer may be lung cancer, breast cancer, gastric cancer, colorectal cancer, pancreatic cancer, ocular melanoma, prostate cancer, gastrointestinal stromal tumors (GIST), advanced esophagogastric cancer, melanoma, hepatocellular cancer, solid tumor, liver cancer, head and neck cancer, colon cancer, small cell lung cancer, and non-small cell lung cancer (NSCLC), or any other type of cancer such as those provided herein. In certain embodiments, the NSCLC was previously treated and not responsive. In certain embodiments, the NSCLC was previously treated with crizotinib and is no longer responsive to the crizotinib treatment. In certain embodiments, the breast cancer is HER2 positive, and has been previously treated with trastuzumab. In certain
embodiments, the breast cancer is HER2 positive and trastuzumab refractory. In certain embodiments, the cancer is triple negative breast cancer. In certain embodiments, the cancer is metastatic hormone-resistant prostate cancer, or metastatic castration-resistant prostate cancer. In certain embodiments, the cancer is pancreatic cancer.

[0011] In certain embodiments, methods of the invention also include identifying a subject as having a cancer with a mutation in ALK. In certain embodiments, methods of the invention further include identifying a subject as having a cancer with a high level of hypoxia. In certain embodiments, methods of the invention also include identifying a subject as having a certain level of LDH. In certain embodiments, methods of the invention further include identifying a subject as having a cancer with a mutation in ALK and with a high level of hypoxia. In certain embodiments, methods of the invention further include identifying a subject as having a cancer with a mutation in ALK and with a certain level of LDH.

[0012] The invention provides methods for treating breast cancer in a subject, comprising administering ganetespib or a pharmaceutically acceptable salt or a tautomer thereof, wherein the breast cancer may be metastatic breast cancer, triple negative breast cancer, or HER2 positive breast cancer; and a high level of hypoxia.

[0013] In certain embodiments, the breast cancer is HER2 positive breast cancer, HER2 negative breast cancer, Basal A breast cancer, Basal B breast cancer, and inflammatory breast cancer, or any other type of cancer in which expression of ER, PR, and/or HER2 are relevant, e.g., ovarian cancer, prostate cancer, and other hormone sensitive cancers. In certain embodiments, the breast cancer is HER2 positive, and has been previously treated with trastuzumab. In certain embodiments, the breast cancer is HER2 positive and trastuzumab refractory. In certain embodiments, the method further includes identifying the subject as having a metastatic breast cancer. In certain embodiments, the method further includes identifying the subject as having a HER2+ breast cancer. In certain embodiments, the method further includes identifying the subject as having a triple negative breast cancer.

[0014] In certain embodiments, methods of the invention also include identifying a subject as having a breast cancer with a high level of hypoxia.
In certain embodiments, the methods include administration of ganetespib or a pharmaceutically acceptable salt or a tautomer thereof, at a dose of 2 mg/m² to 260 mg/m², or in any amount falling within that range.

In certain embodiments, the invention further includes administering one or more additional anticancer agents. The one or more additional anticancer agents may be BEZ-235, AZD6244, AZD8055, SN-38, gemcitabine, camptothecin, docetaxel, cisplatin, oxaliplatin, crizotinib, paclitaxel, trastuzumab, or pemetrexed.

In certain embodiments, the level of hypoxia, the marker levels, and mutations are detected in a subject sample, e.g., a tumor sample, and compared to an appropriate control.

In certain embodiments, the level of hypoxia is determined by detecting the activity level or expression level of one or more hypoxia modulated polypeptides or using detection methods selected from the group consisting of detection of activity or expression of at least one isoform or subunit of lactate dehydrogenase (LDH), at least one isoform or subunit of hypoxia inducible factor (HIF), at least one pro-angiogenic form of vascular endothelial growth factor (VEGF), phosphorylated VEGF receptor (pKDR) 1, 2, and 3; neurolipin 1 (NRP-1), pyruvate dehydrokinase (PDH-K), ornithine decarboxylase (ODC), glucose transporter-1 (GLUT-1), glucose transporter-2 (GLUT-2), tumor size, blood flow, EF5 binding, pimonidazole binding, PET scan, and probe detection of hypoxia level. In certain embodiments, the isoform or subunit of LDH comprises one or more selected from the group consisting of LDH5, LDH4, LDH3, LDH2, LDH1, LDHA and LDHB; or any combination thereof including total LDH. In certain embodiments, the isoform of HIF comprises one or more selected from the group consisting of HIF-1α, HIF-1β, HIF-2α, and HIF-2β; or any combination thereof including total HIF-1 and/or HIF-2. In certain embodiments, the pro-angiogenic isoform of VEGF is any VEGF-A isoform, or any combination of VEGF-A isoforms including total VEGF-A.

The invention provides methods for identifying a subject for treatment with ganetespib including, providing a subject sample from the subject, determining the level of hypoxia in a tumor from the subject, preferably in vitro, and determining, preferably in vitro if the subject has a tumor characteristic selected from the group consisting of: a mutation in
KRAS, a mutation in EGFR, a mutation in ALK, and triple negative breast cancer, wherein a high level of hypoxia in the sample and a mutation in KRAS, a mutation in EGFR, a mutation in ALK, or triple negative breast cancer, indicates the subject is likely to respond to therapy with ganetespib.

[0020] The invention provides methods for identifying a subject for treatment with ganetespib including, providing a subject sample from the subject, determining the level of hypoxia in a tumor from the subject, preferably in vitro, determining if the subject has metastatic breast cancer, wherein a high level of hypoxia in the sample and metastatic breast cancer indicates the subject is likely to respond to therapy with ganetespib.

[0021] The invention also provides kits to practice the methods of the invention. For example, a kit can include an instruction for administration of ganetespib to a subject having cancer with a high level of hypoxia and/or with a mutation in KRAS, or a mutation in EGFR, or a mutation in ALK. A kit can also include information on measuring the level of hypoxia and/or on determining a mutation in KRAS, or a mutation in EGFR, or a mutation in ALK.

BRIEF DESCRIPTION OF THE DRAWINGS

[0022] Figure 1 shows the activity of various chemotherapeutic agents in a 72 hr viability assay using MDA-MB-231 breast cancer cells.

[0023] Figure 2 shows the activity of ganetespib in a 24 hr viability assay using SUM149 inflammatory breast cancer (IBC) cells.

[0024] Figure 3 shows the activity of ganetespib in a viability assay in BT-474 breast cancer cells grown as mammospheres in Matrigel®. The cells were treated for 72 hr and analyzed by microscopy. ICso was determined by AlamarBlue®.

[0025] Figure 4A shows the activity of ganetespib in a single agent viability assay Detroit562 cells, a head and neck cancer cell line, exposed to various chemotherapeutic agents for 72 hr (left).

[0026] Figure 4B shows the expression of various proteins as determined by western blot of cell extracts from Detroit562 cells exposed to ganetespib for 24 hr (right).
Figure 5 shows a western blot of protein expression in cell extracts from Detroit562 head and neck cancer cells treated with 100 nM of ganetespib 24 hours prior to receiving the DNA damaging agent bleomycin (5 uM). Protein expression was measured at the indicated time points after bleomycin treatment. Bleomycin increased both Chkl and Chk2 phosphorylation, which was blocked when cells were treated first with ganetespib.

Figure 6 is a waterfall diagram showing the best percentage changes in size of target lesions responses according to ALK status after treatment with ganetespib. The y axis represents the percentage tumor volume change from baseline. For each patient (each bar) the percentage change in measurable tumor at best response was displayed by the genotype of the patient, i.e., ALK status. A subject was considered to be ALK+ (i.e., have an ALK mutation) if a mutation in ALK was detected using any of the methods.

Figure 7 shows a western blot of protein expression in BT-474 cells after treatment with ganetespib for 16 hours.

Figure 8 shows a graph of the average tumor volume over time in an MDA-MB-231 xenograft model in response to treatment with ganetespib.

Figure 9 is a waterfall diagram showing the best response in patients with metastatic breast cancer based on ER, PR, and HER2 marker status in a Phase II clinical trial of ganetespib.

Figure 10 shows a PET/CT scan of the lungs and bone before and after 19 days of treatment with ganetespib in a female patient with metastatic triple negative breast cancer. Arrows indicate the tumor mass.

Figure 11 shows a table of IC50 values for ganetespib in NSCLC cell lines with a KRAS mutation after treatment with ganetespib for 72 hr.

Figure 12 shows a graph of the results of treatment of various NSCLC cell lines with ganetespib, camptothecin, or a combination thereof for 72 hours.

Figure 13 shows a graph of the results of treatment of various NSCLC cell lines with NSCLC cells with ganetespib, pemetrexed, or a combination thereof for 72 hours.

Figure 14 shows a graph of the results of treatment of various NSCLC cell lines with ganetespib, gemcitabine, or a combination thereof for 72 hours.
Figure 15 shows a graph of the results of treatment of various NSCLC cell lines with ganetespib, certain platins, or a combination thereof for 72 hours.

Figure 16 shows a graph of the results of treatment of various NSCLC cell lines with ganetespib, SN-38, or a combination thereof for 72 hours.

Figure 17 shows a graph of the results of treatment of various NSCLC cell lines with ganetespib, docetaxel, or a combination thereof for 72 hours.

Figure 18 shows a graph of the results of treatment of various NSCLC cell lines with ganetespib, AZD6244, or a combination thereof for 72 hours.

Figure 19 shows a graph of the results of treatment of various NSCLC cell lines with ganetespib, BEZ235, or a combination thereof for 72 hours.

Figure 20 shows a graph of the results of treatment of mice with A549 NSCLC xenografts with ganetespib, BEZ-235, or a combination thereof.

Figures 21A and B show the activity of LDH5 as a percent of total LDH activity in serum samples from nude mice with (A) HCT116 tumors or (B) 786-0 tumors relative to tumor volume. Figures 21C and D show the protein levels of LDH5 as a percent of total LDH activity in serum samples from nude mice with (C) HCT116 tumors or (D) 786-0 tumors relative to tumor volume.

Figure 22 shows treatment with ganetespib for 24 hours decreases proliferation of Mia-PaCa2, HPAC and PANC-1 cells (p<0.001, one way ANOVA). These results were further confirmed by XTT assay.

Figure 23 shows Western blot for Mia-PaCa2, PANC-1 and HPAC cell lines treated with ganetespib for 24 hours. Results indicate decreased levels of HIF-1α and VEGF levels in pancreatic cancer cell lines.

Figure 24 shows ELISA assay demonstrates significant (p<0.001, one way ANOVA) down-regulation of VEGF secretion after treatment with ganetespib.

Figure 25 shows an Egg CAM assay - treatment with ganetespib for 24 hours in conditioned medium in three pancreatic cell lines. The conditioned medium was collected from control and treated cells. 100µl of conditioned medium, either control or treated, was
injected into fertilized chicken eggs. Eggs were incubated at 37°C for 15 days, dissected & membrane was photographed.

[0048] **Figure 26** shows that treatment with ganetespib significantly inhibits tumor growth and decreases angiogenesis in *in vivo* models of pancreatic cancer.

**DETAILED DESCRIPTION OF THE INVENTION**

[0049] The invention, in an embodiment, provides the use of an Hsp90 inhibitor, ganetespib, or a pharmaceutically acceptable salt or a tautomer thereof, for the treatment of certain types of cancer.

[0050] In an embodiment, the treatment method includes administering to a subject an effective amount of ganetespib from about 2 mg/m^2^ to about 260 mg/m^2^. In an embodiment, ganetespib is administered once weekly. In an embodiment, ganetespib is administered twice-weekly. In an embodiment, the twice-weekly administration is on two consecutive days. In an embodiment, ganetespib is administered for about 3 weeks. In another embodiment, the administration for 3 weeks is repeated after about 7 days dose-free. In an embodiment, the administration after 7 days dose-free is repeated at two or more times. In an embodiment, ganetespib is administered by intravenous infusion, such as peripheral intravenous infusion. In an embodiment, ganetespib is infused over 60 minutes.

[0051] The invention provides methods for the treatment of subjects with cancer having a high level of hypoxia. The invention also provides methods for the treatment of subjects with cancer with a mutation in KRAS, a mutation in EGFR, or a mutation in ALK. The invention further provides methods for the treatment of subjects with cancer with a mutation in KRAS, a mutation in EGFR, or a mutation in ALK and with a high level of hypoxia. The invention further provides methods of treatment of subjects with metastatic breast cancer with a high level of hypoxia. The invention also provides methods for treatment of subjects with triple negative breast cancer, *i.e.*, breast cancer that is estrogen receptor negative (ER-), progesterone receptor negative (PR-), and human epidermal growth factor receptor 2 negative (HER2-), with a high level of hypoxia. Cancers with a mutation in KRAS, EGFR, and ALK are difficult to treat, as are cancers in which the tumor is hypoxic. Metastatic breast cancer and triple negative breast cancer are also difficult to treat. The
methods provided herein allow for treatment of such cancers that are typically resistant to treatment.

[0052] Serum LDH level is well established as a prognostic factor associated with poor outcomes and large tumor burden in many tumor types. It is, therefore, interesting to note that a number of reports from large randomized phase 2 and phase 3 studies for several anti-cancer agents have shown a positive interaction between clinical outcomes and high baseline LDH levels. These include bevacizumab in pancreatic cancer (high LDH: OS HR=0.59, 95% CI 0.43-0.82; normal LDH: HR=0.98, 95% CI 0.78-1.24); bevacizumab in prostate cancer (high LDH: OS HR=0.80, P=0.029; normal LDH: OS HR=1.02, P=0.87); bevacizumab in melanoma (high LDH: OS HR=0.53, 95% CI 0.32-0.88; normal LDH: OS HR=1.25, 95% CI 0.73-1.25); temsirolimus in RCC (high LDH: OS HR=0.56, P=0.002; normal LDH: OS HR=0.90, P=0.51); vatalanib in colon cancer first line (high LDH: PFS HR=0.67, P=0.009; PFS HR=0.88, P=0.188); and vatalanib in colon cancer second line (high LDH: PFS HR=0.63, PO.001; PFS HR=0.83, P=.01).

[0053] The VEGF and mTOR signaling pathways are regulated by hypoxia, both at the transcriptional and translational level. The oxygen-sensitive transcription factor HIF-1α is one of the principal mediators of the hypoxic response in cancer cells, including the metabolic switch from oxidative phosphorylation to glycolysis. The hypoxia regulated LDHA gene is under transcriptional control of HIF-1. Therefore, serum LDH levels may in part reflect tumor oxygenation and metabolic status. This connection between tumor oxygenation and serum LDH levels may explain the enhanced activity seen in patients with high serum LDH levels for drugs that affect hypoxia-mediated signaling pathways, such as VEGF and mTOR inhibitors.

[0054] There is therefore evidence that both VEGF/mTOR inhibitors are sensitive to tumor oxygenation and metabolic status. Both classes of drugs seem to preferentially work in anaerobic tumor cells.

[0055] As demonstrated herein, ganetespib has an effect on hypoxia driven pathways including VEGF and mTOR. Several key elements of the VEGF and mTOR pathways are client proteins (VEGF, VEGFR1-3, IGF-1R, GLUT1-3, PI3K) that are downregulated. Therefore, without being bound by mechanism, ganetespib effects hypoxia driven pathways
and is demonstrated to be effective in treating tumors with mutations in KRAS, EGFR, and ALK, as well as in various breast cancer types including metastatic breast and triple negative breast cancer, wherein the tumor also has a high level of hypoxia.

[0056] In an embodiment, the method is used for treating a subject with NSCLC. In an embodiment, the NSCLC expresses wild-type EGFR and wild-type KRAS and has a high level of hypoxia. In an embodiment, the NSCLC has a mutation in EGFR and has a high level of hypoxia. In an embodiment, the NSCLC has a mutation in KRAS and has a high level of hypoxia. In an embodiment, the NSCLC has a mutation in EGFR and a mutation in KRAS and has a high level of hypoxia. In an embodiment, the NSCLC is ALK+ (i.e., has an ALK mutation) and has a high level of hypoxia. In an embodiment, the NSCLC is refractory. In an embodiment, the NSCLC was previously treated with other anticancer agents. In an embodiment, the NSCLC was treated with and became resistant to the crizotinib treatment. In an embodiment, the cancer is stage IIIIB or IV NSCLC.

[0057] In an embodiment, ganetespib is used for treating a subject with cancer with a high level of hypoxia. In an embodiment, the treatment method includes administering to the subject with a cancer with a high level of hypoxia an effective amount of ganetespib, or a pharmaceutically acceptable salt or a tautomer thereof. In an embodiment, ganetespib is used for treating a subject with a cancer with a high level of hypoxia in combination with one or more additional anticancer agents. In an embodiment, ganetespib is used for treating a subject with a high level of hypoxia in combination with one or more of BEZ235, AZD6244, AZD8055, SN-38, gemcitabine, camptothecin, docetaxel, cisplatin, oxaliplatin, crizotinib, paclitaxel, trastuzumab, and pemetrexed.

[0058] In an embodiment, ganetespib is used for treating a subject with cancer with a mutation in KRAS. In an embodiment, the treatment method includes administering to the subject with a cancer with a mutation in KRAS an effective amount of ganetespib, or a pharmaceutically acceptable salt or a tautomer thereof. In an embodiment, ganetespib is used for treating a subject with a cancer with a mutation in KRAS in combination with one or more additional anticancer agents. In an embodiment, ganetespib is used for treating a subject with a cancer with a mutation in KRAS in combination with one or more of BEZ235,
AZD6244, AZD8055, SN-38, gemcitabine, camptothecin, docetaxel, cisplatin, oxaliplatin, crizotinib, paclitaxel, trastuzumab, and pemetrexed.

[0059] In an embodiment, ganetespib is used for treating a subject with cancer with a mutation in KRAS and with a high level of hypoxia. In an embodiment, the treatment method includes administering to the subject with a cancer with a mutation in KRAS and with a high level of hypoxia an effective amount of ganetespib, or a pharmaceutically acceptable salt or tautomer thereof. In an embodiment, ganetespib is used for treating a subject with a cancer with a mutation in KRAS and with a high level of hypoxia in combination with one or more additional anticancer agents. In an embodiment, ganetespib is used for treating a subject with a cancer with a mutation in KRAS and with a high level of hypoxia in combination with one or more of BEZ235, AZD6244, AZD8055, SN-38, gemcitabine, camptothecin, docetaxel, cisplatin, oxaliplatin, crizotinib, paclitaxel, trastuzumab, and pemetrexed.

[0060] In an embodiment, ganetespib is used for treating a subject with NSCLC with a high level of hypoxia. In an embodiment, the treatment method includes administering to the subject with a NSCLC with a high level of hypoxia an effective amount of ganetespib, or a pharmaceutically acceptable salt or a tautomer thereof. In an embodiment, ganetespib is used for treating a subject with NSCLC with a high level of hypoxia in combination with one or more additional anticancer agents. In an embodiment, ganetespib is used for treating a subject with NSCLC with a high level of hypoxia in combination with one or more of BEZ235, AZD6244, AZD8055, SN-38, gemcitabine, camptothecin, docetaxel, cisplatin, oxaliplatin, crizotinib, paclitaxel, trastuzumab, and pemetrexed.

[0061] In an embodiment, ganetespib is used for treating a subject with NSCLC with a mutation in KRAS. In an embodiment, the treatment method includes administering to the subject with a NSCLC with a mutation in KRAS an effective amount of ganetespib, or a pharmaceutically acceptable salt or a tautomer thereof. In an embodiment, ganetespib is used for treating a subject with NSCLC with a mutation in KRAS in combination with one or more additional anticancer agents. In an embodiment, ganetespib is used for treating a subject with NSCLC with a mutation in KRAS in combination with one or more of BEZ235,
AZD6244, AZD8055, SN-38, gemcitabine, camptothecin, docetaxel, cisplatin, oxaliplatin, crizotinib, paclitaxel, trastuzumab, and pemetrexed.

[0062] In an embodiment, ganetespib is used for treating a subject with NSCLC with a mutation in KRAS and with a high level of hypoxia. In an embodiment, the treatment method includes administering to the subject with a NSCLC with a mutation in KRAS and with a high level of hypoxia an effective amount of ganetespib, or a pharmaceutically acceptable salt or a tautomer thereof. In an embodiment, ganetespib is used for treating a subject with NSCLC with a mutation in KRAS and with a high level of hypoxia in combination with one or more additional anticancer agents. In an embodiment, ganetespib is used for treating a subject with NSCLC with a mutation in KRAS and with a high level of hypoxia in combination with one or more of BEZ235, AZD6244, AZD8055, SN-38, gemcitabine, camptothecin, docetaxel, cisplatin, oxaliplatin, crizotinib, paclitaxel, trastuzumab, and pemetrexed.

[0063] In an embodiment, the method of treating a subject with a cancer with a high level of hypoxia, includes:

[0064] a) identifying a subject with cancer with a high level of hypoxia; and

[0065] b) administering to the subject an effective amount of ganetespib or a pharmaceutically acceptable salt or a tautomer thereof.

[0066] In an embodiment, the method further comprises administering one or more additional anticancer drugs. In an embodiment, the one or more drugs may be BEZ235, AZD6244, AZD8055, SN-38, gemcitabine, camptothecin, docetaxel, cisplatin, oxaliplatin, crizotinib, paclitaxel, trastuzumab, or pemetrexed.

[0067] In an embodiment, the method of treating a subject with a cancer with a mutation in KRAS, includes:

[0068] a) identifying a subject with cancer with a mutation in KRAS; and

[0069] b) administering to the subject an effective amount of ganetespib or a pharmaceutically acceptable salt or a tautomer thereof.

[0070] In an embodiment, the method further comprises administering one or more additional anticancer drugs. In an embodiment, the one or more drugs may be BEZ235,
AZD6244, AZD8055, SN-38, gemcitabine, camptothecin, docetaxel, cisplatin, oxaliplatin, crizotinib, paclitaxel, trastuzumab, or pemetrexed.

In an embodiment, the method of treating a subject with a cancer with a KRAS mutation and a high level of hypoxia, includes:

a) identifying a subject with a mutation in a KRAS gene and a high level of hypoxia; and

b) administering to the subject an effective amount of ganetespib or a pharmaceutically acceptable salt or a tautomer thereof.

In an embodiment, the method further comprises administering one or more additional anticancer drugs. In an embodiment, the one or more drugs may be BEZ235, AZD6244, AZD8055, SN-38, gemcitabine, camptothecin, docetaxel, cisplatin, oxaliplatin, crizotinib, paclitaxel, trastuzumab, or pemetrexed.

In an embodiment, the method of treating a subject with a NSCLC with a high level of hypoxia includes:

a) identifying a subject with a NSCLC with a high level of hypoxia; and

b) administering to the subject an effective amount of ganetespib or a pharmaceutically acceptable salt or a tautomer thereof.

In an embodiment, the method further comprises administering one or more additional anticancer drugs. In an embodiment, the one or more drugs may be BEZ235, AZD6244, AZD8055, SN-38, gemcitabine, camptothecin, docetaxel, cisplatin, oxaliplatin, crizotinib, paclitaxel, trastuzumab, or pemetrexed.

In an embodiment, the method of treating a subject with a NSCLC with a mutation in KRAS includes:

a) identifying a subject with a NSCLC with a mutation in KRAS; and

b) administering to the subject an effective amount of ganetespib or a pharmaceutically acceptable salt or a tautomer thereof.

In an embodiment, the method further comprises administering one or more additional anticancer drugs. In an embodiment, the one or more drugs may be BEZ235.
AZD6244, AZD8055, SN-38, gemcitabine, camptothecin, docetaxel, cisplatin, oxaliplatin, crizotinib, paclitaxel, trastuzumab, or pemetrexed.

[0083] In an embodiment, the method of treating a subject with a NSCLC with a mutation in KRAS and a high level of hypoxia includes:

[0084] a) identifying a subject with a mutation in a KRAS gene and a high level of hypoxia; and

[0085] b) administering to the subject an effective amount of ganetespib or a pharmaceutically acceptable salt or a tautomer thereof.

[0086] In an embodiment, the method further comprises administering one or more additional anticancer drugs. In an embodiment, the one or more drugs may be BEZ235, AZD6244, AZD8055, SN-38, gemcitabine, camptothecin, docetaxel, cisplatin, oxaliplatin, crizotinib, paclitaxel, trastuzumab, or pemetrexed.

[0087] In an embodiment, ganetespib is used for treating a subject with cancer with a mutation in ALK and a high level of hypoxia. In an embodiment, the treatment method includes administering to the subject with cancer with a mutation in ALK and a high level of hypoxia an effective amount of ganetespib, or a pharmaceutically acceptable salt or a tautomer thereof. In an embodiment, ganetespib is used for treating a subject with cancer with a mutation in ALK and a high level of hypoxia in combination with one or more additional anticancer agents. In an embodiment, ganetespib is used for treating a subject with cancer with a mutation in ALK and a high level of hypoxia in combination with one or more of BEZ235, AZD6244, AZD8055, SN-38, gemcitabine, camptothecin, docetaxel, cisplatin, oxaliplatin, crizotinib, paclitaxel, trastuzumab, and pemetrexed.

[0088] In an embodiment, ganetespib is used for treating a subject with NSCLC with a mutation in ALK and a high level of hypoxia. In an embodiment, the treatment method includes administering to the subject with NSCLC with a mutation in ALK and a high level of hypoxia an effective amount of ganetespib, or a pharmaceutically acceptable salt or a tautomer thereof. In an embodiment, ganetespib is used for treating a subject with NSCLC with a mutation in ALK and a high level of hypoxia in combination with one or more additional anticancer agents. In an embodiment, ganetespib is used for treating ALK+ NSCLC in combination with one or more of BEZ235, AZD6244, AZD8055, SN-38,
gemcitabine, camptothecin, docetaxel, cisplatin, oxaliplatin, crizotinib, paclitaxel, trastuzumab, and pemetrexed.

[0089] In an embodiment, the method of treating a subject with a cancer with a mutation in ALK and a high level of hypoxia includes:

[0090] a) identifying a subject with a mutation in an ALK gene and a high level of hypoxia; and

[0091] b) administering to the subject an effective amount of ganetespib or a pharmaceutically acceptable salt or a tautomer thereof.

[0092] In an embodiment, the method further comprises administering one or more additional anticancer drugs. In an embodiment, the one or more drugs may be BEZ235, AZD6244, AZD8055, SN-38, gemcitabine, camptothecin, docetaxel, cisplatin, oxaliplatin, crizotinib, paclitaxel, trastuzumab, or pemetrexed.

[0093] In an embodiment, the method of treating a subject with a NSCLC with a mutation in ALK and a high level of hypoxia includes:

[0094] a) identifying a subject with a mutation in an ALK gene and a high level of hypoxia; and

[0095] b) administering to the subject an effective amount of ganetespib or a pharmaceutically acceptable salt or a tautomer thereof.

[0096] In an embodiment, the method further comprises administering one or more additional anticancer drugs. In an embodiment, the one or more drugs may be BEZ235, AZD6244, AZD8055, SN-38, gemcitabine, camptothecin, docetaxel, cisplatin, oxaliplatin, crizotinib, paclitaxel, trastuzumab, or pemetrexed.

[0097] In an embodiment, the invention also provides a method for identifying a subject for treatment with ganetespib comprising:

   a) providing a subject sample from the subject;

   b) determining the level of hypoxia in a tumor from the subject in vitro;

   c) determining in vitro if the subject has a tumor characteristic of a mutation in KRAS, a mutation in EGFR, a mutation in ALK, or triple negative breast cancer; wherein a high
level of hypoxia in the sample and a tumor characteristic of a mutation in KRAS, a mutation in EGFR, a mutation in ALK, or triple negative breast cancer, indicates the subject is likely to respond to therapy with ganetespib.

[0098] In an embodiment, the invention also provides a method for identifying a subject for treatment with ganetespib comprising:

a) providing a subject sample from the subject;

b) determining the level of hypoxia in a tumor from the subject in vitro;

c) determining if the subject has metastatic breast cancer; wherein a high level of hypoxia in the sample and metastatic breast cancer indicates the subject is likely to respond to therapy with ganetespib.

head and neck (SCCHN), non-medullary thyroid carcinoma, neurofibromatosis type 1, CNS cancer, liposarcoma, leiomyosarcoma, salivary gland cancer, mucosal melanoma, acral/lentiginous melanoma, paraganglioma; pheochromocytoma, advanced metastatic cancer, solid tumor, squamous cell carcinoma, sarcoma, melanoma, endometrial cancer, head and neck cancer, rhabdomyosarcoma, multiple myeloma, gastrointestinal stromal tumor, mantle cell lymphoma, gliosarcoma, bone sarcoma, and refractory malignancy.

[00100] In some embodiments, the subject sample may be from tumor tissue, blood, serum, plasma, urine, stool, lymph, cerebrospinal fluid, circulating tumor cells, bronchial lavage, peritoneal lavage, exudate, effusion, or sputum.

[00101] In some embodiments, the level of hypoxia is determined by detecting an activity level or an expression level of one or more hypoxia modulated peptides. In some embodiments, the activity level or expression level of the one or more hypoxia modulated polypeptides are up regulated in the sample. In some embodiments, the level of hypoxia is determined by detecting the activity level or expression level of one or more hypoxia modulated polypeptides or using detection methods selected from the group consisting of detection of activity or expression of at least one isoform or subunit of lactate dehydrogenase (LDH), at least one isoform or subunit of hypoxia inducible factor (HIF), at least one pro-angiogenic form of vascular endothelial growth factor (VEGF), phosphorylated VEGF receptor (pKDR) 1, 2, and 3; neuropilin 1 (NRP-1), pyruvate dehydrokinase (PDH-K), ornithine decarboxylase (ODC), glucose transporter-1 (GLUT-1), glucose transporter-2 (GLUT-2), tumor size, blood flow, EF5 binding, pimonidazole binding, PET scan, and probe detection of hypoxia level.

[00102] In some embodiments, the isoform or subunit of LDH comprises one or more selected from the group consisting of, LDH5, LDH4, LDH3, LDH2, LDH1, LDHA and LDHB; or any combination thereof including total LDH. In some embodiments, the isoform of HIF is selected from the group consisting of HIF-1α, HIF-1β, HIF-2α, and HIF-2β; or any combination thereof including total HIF-1 and HIF-2. In some embodiments, the pro-angiogenic isoform of VEGF is any isoform of VEGF-A; or any combination thereof including total VEGF-A.
[00103] In some embodiments, detection of a high level of activity or expression of at least one LDH isoform or subunit comprises detection of an LDH activity or expression level of an LDH selected from the group consisting of total LDH, LDH5, LDH4; LDH5 plus LDH4; LDH5 plus LDH4 plus LDH3; and LDHA, wherein the activity level or expression level is 0.8 ULN or more. In some embodiments, detection of a high level of activity or expression of at least one LDH isoform or subunit comprises detection of an LDH activity or expression level of an LDH selected from the group consisting of total LDH, LDH5, LDH4; LDH5 plus LDH4; LDH5 plus LDH4 plus LDH3; and LDHA, wherein the activity level or expression level is 1.0 ULN or more.

[00104] In some embodiments, a high level of hypoxia is a change in a ratio or a ratio of normalized activity or expression levels of hypoxia modulated polypeptides. In some embodiments, a high level of hypoxia comprises a ratio or a normalized ratio of 1.0 or more of the ULN, wherein the ratio or normalized ratio is selected from the group consisting of the LDHA to LDHB, LDH5 or LDH4 to LDH1, LDH5 or LDH4 to total LDH, LDH5 and LDH4 to LDH1, LDH5 and LDH4 to total LDH, LDH5, LDH4, and LDH3 to LDH1, and LDH5, LDH4, and LDH3 to total LDH.

[00105] In certain embodiments, the methods are used for treating breast cancer, gastric cancer, colorectal cancer, pancreatic cancer, ocular melanoma, prostate cancer, melanoma, gastrointestinal stromal tumors (GIST), advanced esophagogastric cancer, hepatocellular cancer, solid tumor, small cell lung cancer, head and neck cancer, or hematological malignancies. In an embodiment, the subject was previously treated with another chemotherapeutic agent. In an embodiment, the breast cancer is triple negative breast cancer, invasive ductal carcinoma, or metastatic breast cancer. In an embodiment, the breast cancer is HER2 positive and trastuzumab refractory. In an embodiment, the breast cancer is HER2 positive and has been previously treated with trastuzumab. In an embodiment, the method is for treating triple negative breast cancer in combination with an additional anticancer agent. In an embodiment, the method is for treating triple negative breast cancer, or HER2 positive cancer in combination with BEZ235, AZD6244, AZD8055, SN-38, gemcitabine, camptothecin, docetaxel, cisplatin, oxaliplatin, crizotinib, paclitaxel, trastuzumab, or pemetrexed. In an embodiment, the method is for treating triple negative breast cancer or HER2 positive cancer in combination with trastuzumab.
In certain embodiments, the methods are used for treating ocular melanoma, pancreatic cancer, prostate cancer, solid tumor, hepatocellular cancer, colorectal cancer, or small cell lung cancer. In an embodiment, the ocular melanoma is metastatic. In an embodiment, the pancreatic cancer is metastatic. In an embodiment, the prostate cancer is metastatic hormone-resistant prostate cancer. In an embodiment, the prostate cancer is metastatic castration-resistant prostate cancer (CRPC). In an embodiment, the subject with prostate cancer was previously treated with docetaxel-based chemotherapy. In an embodiment, the cancer is a solid tumor. In an embodiment, the cancer is advanced hepatocellular cancer. In an embodiment, the colorectal cancer is refractory metastatic colorectal cancer. In an embodiment, the small cell lung cancer is relapsed or refractory.

In an embodiment, ganetespib is used for treating a subject with lung cancer with a high level of hypoxia in combination with an MEK inhibitor. In an embodiment, ganetespib is used for treating a subject with lung cancer with a mutation in KRAS, EGFR, or ALK in combination with an MEK inhibitor. In an embodiment, ganetespib is used for treating a subject with lung cancer with a mutation in KRAS, EGFR, or ALK, and a high level of hypoxia in combination with an MEK inhibitor. In an embodiment, ganetespib is used for treating a subject with lung cancer with a high level of hypoxia in combination with an MEK inhibitor and a PI3K/mTOR inhibitor. In an embodiment, ganetespib is used for treating a subject with lung cancer with a mutation in KRAS, EGFR, or ALK in combination with an MEK inhibitor and a PI3K/mTOR inhibitor. In an embodiment, ganetespib is used for treating a subject with lung cancer with a mutation in KRAS, EGFR, or ALK, and a high level of hypoxia in combination with an MEK inhibitor and a PI3K/mTOR inhibitor. In an embodiment, ganetespib is used for treating lung cancer in combination with a PI3K/mTOR inhibitor.

DEFINITIONS

Unless otherwise specified, the below terms used herein are defined as follows:

The terms "treat", "treatment" and "treating" include the reduction or amelioration of the progression, severity and/or duration of cancer, or the amelioration of one or more symptoms of cancer, resulting from the administration of ganetespib. The
terms "treat", "treatment" and "treating" also include the reduction of the risk of recurrence of cancer or the delay or inhibition of the recurrence of cancer. In an embodiment, the terms "treat", "treatment" and "treating" include the amelioration of at least one measurable physical parameter of cancer, such as growth of a tumor, not necessarily discernible by the patient. In other embodiments, the terms "treat", "treatment" and "treating" includes the inhibition of the progression of cancer either physically by the stabilization of a discernible symptom, physiologically by the stabilization of a physical parameter, or both. In another embodiment, the terms "treat", "treatment" and "treating" of cancer include the reduction or stabilization of tumor size or cancerous cell count, and/or delay of tumor formation.

[00110] The terms "cancer" or "tumor" are well known in the art and refer to the presence, e.g., in a subject, of cells possessing characteristics typical of cancer-causing cells, such as uncontrolled proliferation, immortality, metastatic potential, rapid growth and proliferation rate, decreased cell death/apoptosis, and certain characteristic morphological features.

[00111] Cancer cells are often in the form of a solid tumor. However, cancer also includes non-solid tumors, e.g., blood tumors, e.g., leukemia, wherein the cancer cells are derived from bone marrow. As used herein, the term "cancer" includes pre-malignant as well as malignant cancers. Cancers include, but are not limited to, acoustic neuroma, acute leukemia, acute lymphocytic leukemia, acute myelocytic leukemia (monocytic, myeloblastic, adenocarcinoma, angiosarcoma, astrocytoma, myelomonocytic and promyelocytic), acute T-cell leukemia, basal cell carcinoma, bile duct carcinoma, bladder cancer, brain cancer, breast cancer, bronchogenic carcinoma, cervical cancer, chondrosarcoma, chordoma, choriocarcinoma, chronic leukemia, chronic lymphocytic leukemia, chronic myelocytic (granulocytic) leukemia, chronic myelogenous leukemia, colon cancer, colorectal cancer, craniopharyngioma, cystadenocarcinoma, diffuse large B-cell lymphoma, Burkitt's lymphoma, dysproliferative changes (dysplasias and metaplasias), embryonal carcinoma, endometrial cancer, endotheliosarcoma, ependymoma, epithelial carcinoma, erythroleukemia, esophageal cancer, estrogen-receptor positive breast cancer, essential thrombocythemia, Ewing's tumor, fibrosarcoma, follicular lymphoma, germ cell testicular cancer, glioma, heavy chain disease, hemangioblastoma, hepatoma, hepatocellular cancer, hormone insensitive prostate cancer, leiomyosarcoma, liposarcoma, lung cancer,
lymphagioendotheliosarcoma, lymphangiosarcoma, lymphoblastic leukemia, lymphoma
(Hodgkin's and non-Hodgkin's), malignancies and hyperproliferative disorders of the
bladder, breast, colon, lung, ovaries, pancreas, prostate, skin, and uterus, lymphoid
malignancies of T-cell or B-cell origin, leukemia, lymphoma, medullary carcinoma,
medulloblastoma, melanoma, menigioma, mesothelioma, multiple myeloma, myelogenous
leukemia, myeloma, myxosarcoma, neuroblastoma, non-small cell lung cancer,
oligodendroglioma, oral cancer, osteogenic sarcoma, ovarian cancer, pancreatic cancer,
papillary adenocarcinomas, papillary carcinoma, pinealoma, polycythemia vera, prostate
cancer, rectal cancer, renal cell carcinoma, retinoblastoma, rhabdomyosarcoma, sarcoma,
sebaceous gland carcinoma, seminoma, skin cancer, small cell lung carcinoma, solid tumors
(carcinomas and sarcomas), small cell lung cancer, stomach cancer, squamous cell
carcinoma, synovioma, sweat gland carcinoma, thyroid cancer, Waldenstrom's
macroglobulinemia, testicular tumors, uterine cancer, and Wilms' tumor. Other cancers
include primary cancer, metastatic cancer, oropharyngeal cancer, hypopharyngeal cancer,
liver cancer, gall bladder cancer, bile duct cancer, small intestine cancer, urinary tract
cancer, kidney cancer, urothelium cancer, female genital tract cancer, uterine cancer,
gestational trophoblastic disease, male genital tract cancer, seminal vesicle cancer, testicular
cancer, germ cell tumors, endocrine gland tumors, thyroid cancer, adrenal cancer, pituitary
gland cancer, hemangioma, sarcoma arising from bone and soft tissues, Kaposi's sarcoma,
nerve cancer, ocular cancer, meningial cancer, glioblastomas, neuromas, neuroblastomas,
Schwannomas, solid tumors arising from hematopoietic malignancies such as leukemias,
metastatic melanoma, recurrent or persistent ovarian epithelial cancer, fallopian tube cancer,
primary peritoneal cancer, gastrointestinal stromal tumors, colorectal cancer, gastric cancer,
melanoma, glioblastoma multiforme, non-squamous non-small cell lung cancer, malignant
glioma, epithelial ovarian cancer, primary peritoneal serous cancer, metastatic liver cancer,
neuroendocrine carcinoma, refractory malignancy, triple negative breast cancer, HER2
amplified breast cancer, nasopharageal cancer, oral cancer, biliary tract, hepatocellular
carcinoma, squamous cell carcinomas of the head and neck (SCCHN), non-medullary
thyroid carcinoma, recurrent glioblastoma multiforme, neurofibromatosis type 1. CNS
cancer, liposarcoma, leiomyosarcoma, salivary gland cancer, mucosal melanoma, acral/
lentiginous melanoma, paraganglioma, pheochromocytoma, advanced metastatic cancer,
solid tumor, triple negative breast cancer, colorectal cancer, sarcoma, melanoma, renal carcinoma, endometrial cancer, thyroid cancer, rhabdomyosarcoma, multiple myeloma, ovarian cancer, glioblastoma, gastrointestinal stromal tumor, mantle cell lymphoma, and refractory malignancy.

"Solid tumor," as used herein, is understood as any pathogenic tumor that can be palpated or detected using imaging methods as an abnormal growth having three dimensions. A solid tumor is differentiated from a blood tumor such as leukemia. However, cells of a blood tumor are derived from bone marrow, therefore, the tissue producing the cancer cells is a solid tissue that can be hypoxic.

"Tumor tissue" is understood as cells, extracellular matrix, and other naturally occurring components associated with the solid tumor.

The term "EGFR" as used herein, refers to Epidermal Growth Factor Receptor (EGFR), a member of the type 1 subgroup of receptor tyrosine kinase family of growth factor receptors, which play critical roles in cellular growth, differentiation, and survival. Activation of these receptors typically occurs via specific ligand binding which results in hetero- or homodimerization between receptor family members, with subsequent autophosphorylation of the tyrosine kinase domain. Specific ligands which bind to EGFR include epidermal growth factor (EGF), transforming growth factor (TGF, amphiregulin and some viral growth factors. Activation of EGFR triggers a cascade of intracellular signaling pathways involved in both cellular proliferation (the ras/raf/MAP kinase pathway) and survival (the PI3 kinase/Akt pathway). Members of this family, including EGFR and HER2, have been directly implicated in cellular transformation (Accession Nos. NP_005219 and NP_004439).

A number of human malignancies are associated with aberrant (mutated) or overexpression of EGFR and/or overexpression of its specific ligands (Gullick, Br. Med. Bull. (1991), 47:87-98; Modijtahedi and Dean, Int. J. Oncol. (1994), 4:277-96; Salomon, et al., Crit. Rev. Oncol. Hematol. (1995);9:183-232, each of which is incorporated herein by reference). Aberrant or overexpression of EGFR has been associated with an adverse prognosis in a number of human cancers, including cancers of the head and neck, breast, colon, prostate, lung (e.g., NSCLC, adenocarcinoma and squamous lung cancer), ovaries, gastrointestinal
tract (gastric, colon, pancreatic), kidneys, bladder, central nervous system (e.g., glioma), prostate, and gynecological carcinomas. In some instances, overexpression of tumor EGFR has been correlated with both chemoresistance and a poor prognosis (Lei, et al., Anticancer Res. (1999), 29:221-8; Veale, et al., Br. J. Cancer (1993); 68:162-5. Oncogenic mutations of EGFR are known. Mutational analysis and expression analysis of EGFR are routinely assessed by clinical laboratories using standardized kits and reference levels. Such kits and methods are known in the art.

[00116] The term "EGFR inhibitor", as used herein, includes any compound that disrupts EGFR production within a cell or disrupts activation of EGFR signaling in the cell. Activation of EGFR leads to the Ras signaling cascade that results in uncontrolled cell proliferation. EGFR inhibitors include monoclonal antibodies that bind EGFR to inactivate it, and compounds that bind to the tyrosine kinase domain of EGFR to inhibit it. EGFR inhibitors include drugs such as erlotinib, gefitinib, and cetuximab. Particularly, erlotinib is described in US Patent Nos. 5,747,498, 6,900,221, 7,087,613, and RE41065. Trade names of certain EGFR inhibitors described herein include Tarceva®, Iressa®, and Erbitux®.

[00117] The KRAS oncogene (the cellular homolog of the Kirsten rat sarcoma virus gene, Accession No. NP_203524) is a critical gene in the development of a variety of cancers, and the mutation status of this gene is an important characteristic of many cancers. Mutation status of the gene can provide diagnostic, prognostic and predictive information for several cancers. The KRAS gene is a member of a family of genes (KRAS, NRAS and HRAS). KRAS is a member of the RAS family of oncogenes, a collection of small guanosine triphosphate (GTP)-binding proteins that integrate extracellular cues and activate intracellular signaling pathways to regulate cell proliferation, differentiation, and survival. Gain-of-function mutations that confer transforming capacity are frequently observed in KRAS, predominantly arising as single amino acid substitutions at amino acid residues G12, G13 or Q61. Constitutive activation of KRAS leads to the persistent stimulation of downstream signaling pathways that promote tumorigenesis, including the RAF/MEK/ERK and PI3K/AKT/mTOR cascades. In NSCLC, KRAS mutations are highly prevalent (20-30%) and are associated with unfavorable clinical outcomes. Mutations in KRAS appear mutually exclusive with those in EGFR in NSCLC tumors; more importantly, they can account for primary resistance to targeted EGFR TKI therapies. Mutations in the KRAS gene are
common in many types of cancer, including pancreatic cancer (-65%), colon cancer (-40%), lung cancer (-20%) and ovarian cancer (-15%).

Detection of KRAS mutations is routinely performed in clinical laboratories using routine methods and kits. Such kits and methods are well known in the art.

A variety of laboratory methods have been utilized to detect mutations in the KRAS gene. See, e.g., Jimeno et al. KRAS mutations and sensitivity to epidermal growth factor receptor inhibitors in colorectal cancer: practical application of patient selection. J. Clin. Oncol. 27, 1130-1135 (2009); Van Krieken et al. KRAS mutation testing for predicting response to anti-EGFR therapy for colorectal carcinoma: proposal for a European quality assurance program. Virchows Archiv. 453, 417-431 (2008). Most methods include the use of PCR to amplify the appropriate region of the KRAS gene, including exons 2 and 3, and then utilize different methods to distinguish wild-type from mutant sequences in key codons, such as 12 and 13. The detection methods include nucleic acid sequencing, allele-specific PCR methods, single-strand conformational polymorphism analysis, melt-curve analysis, probe hybridization and others. The main features for consideration for these molecular techniques are the ability to distinguish the appropriate spectrum of variants at the codons of interest and the sensitivity or limit of detection (LOD) for mutant alleles. Both of these parameters are important, given the fact that tumors may be very heterogeneous, both with regard to the percentage of tumor cells within a given tissue and the potential for genetic heterogeneity.

Moreover, many methods have also been developed for KRAS mutation analysis to address various specific issues, related to increased analytical sensitivity, and they include allele-specific PCR using amplification refractory mutation system (ARMS) technology or co-amplification at a lower denaturation temperature-PCR methods, pyrosequencing approaches and real-time PCR methods that use specific probe technologies, such as peptide nucleic acids. See, e.g., Pritchard et al. COLD-PCR enhanced melting curve analysis improves diagnostic accuracy for KRAS mutations in colorectal carcinoma. BMC Clin. Pathol. 10, 1-10 (2010); Weichart et al. KRAS genotyping of paraffin-embedded colorectal cancer tissue in routine diagnostics: comparison of methods and impact of histology. J. Mol. Diagn. 12, 35-42 (2010); Oliner et al. A comparability study of 5 commercial KRAS tests. Diagn.

[00121] There are several examples of laboratory-developed tests (LDTs) for detecting KRAS mutations, as well as a series of kits for research and for use in clinical diagnostics. For example, the TheraScreen® assay (DxS, Manchester, UK) is a CE-marked kit intended for the detection and qualitative assessment of seven somatic mutations in the KRAS gene, to aid clinicians in the identification of colorectal cancer patients who may benefit from anti-EGFR therapies, such as panitumumab and cetuximab. This assay uses an amplification refractory mutation system (ARMS), which is a version of allele-specific PCR; and detection of amplification products with Scorpion™ probes. TheraScreen® Package Insert, DsX, Manchester, UK (2009); Whitehall et al, A multicenter blinded study to evaluate KRAS mutation testing methodologies in the clinical setting. /Mol. Diagn. 11, 543-552 (2009); Oliner et al, A comparability study of 5 commercial KRAS tests. Diagn. Pathol. 5, 23-29 (2010).

[00122] In addition, the European Society of Pathology (ESP), to help evaluate the reliability of KRAS mutation testing, has established a quality-assurance program for KRAS mutation analysis in colorectal cancers at http://kras.equascheme.org.

[00123] The ALK (anaplastic lymphoma kinase) RTK (receptor tyrosine kinase, Accession No. NP_004295) was originally identified as a member of the insulin receptor subfamily of RTKs that acquires transforming capability when truncated and fused to NPM (nucleophosmin) in the t(2;5) chromosomal rearrangement associated with ALCL (anaplastic large cell lymphoma). To date, many chromosomal rearrangements leading to enhanced ALK activity have been described and are implicated in a number of cancer types. Recent reports of the EML4 (echinoderm microtubule-associated protein like 4)-ALK oncoprotein in NSCLC, together with the identification of activating point mutations in neuroblastoma, have highlighted ALK as a significant player and target for drug development in cancer. Representative ALK abnormalities (or "ALK+") include EML4-ALK fusions, KIF5B-ALK fusions, TGF-ALK fusions, NPM-ALK fusions, and ALK point mutations.

[00124] Mutational analysis and expression analysis of ALK are routinely assessed by clinical laboratories using standardized kits and reference levels. Such kits and methods are known in the art.
The following two assays are presented for general information about detection and identification of ALK alterations, mutations or rearrangements in an ALK gene or gene product. These types of assays were also used in obtaining the results in Examples 1 and 2 herein.

The EML4/ALK assay detects eight known fusion variants and other undefined variants, in conjunction with measuring expression of wild type EML4 and ALK 5' and 3'.

Lung cancer is the most common and deadly form of cancer in the USA, with a 5-year survival rate of approximately 15 percent. A subset of NSCLC patients have translocations which fuse the 5' end of the EML4 gene to the 3' end of the ALK gene creating an activated ALK oncogene. The incidence of ALK activation in NSCLC is low (2-7 percent), but it may be as high as 13 percent in patients with adenocarcinoma, no or a light history of smoking, younger age, and WT EGFR and KRAS genes. There are several other adenocarcinomas for which the ALK activation is relevant: breast, bladder, head & neck, and colon. Of particular interest, 5% of primary and metastatic melanoma patients harbor the translocation as well.

The EML4/ALK fusion protein displays constitutive ALK kinase activity, which can be targeted with ALK kinase inhibitors. The presence of an EML4/ALK translocation predicts a favorable response to ALK inhibitor therapy.

The quantitative Nuclease Protection Assay (qNPA™) is a multiplexed, lysis only assay of mRNA (53-58) that can also measure DNA and miRNA. What sets qNPA apart from other assays is that it does not require extraction of the DNA or RNA, but rather uses directly lysed samples. This permits high sample throughput, combined with the simultaneous measurement of DNA, mRNA and miRNA from the same lysate, and if necessary, on the same array.

qNPA also is very precise, with average whole assay CV's from tissues <10%, which means changes <1.2-fold can be detected, p<0.05. It is currently available as a low cost array plate-based assay measuring up to 47 genes / well.

Genetics: Multiple inversions on chromosome 2p generate in-frame fusions of the EML4 and ALK genes. While the breakpoints of EML4 can vary (fusion at exons 2, 6, 13, 14, 15, 18, and 20), the breakpoint of ALK occurs consistently at exon 20, 5' of the kinase.
domain. The majority (-70 percent) of translocations involve EML4 exon 13 (variant 1) or EML4 exon 6a/b (variant 3a/b). Due to close proximity of the EML4 and ALK genes, thus the small inversions, detection of some EML4/ALK variants is challenging with commercially available ALK break-apart FISH probes.

[00132] Product Format: The initial product is based upon the qNPA ArrayPlate format, either in 47 or 16 spot format as appropriate and dictated by the number of analytes to be tested with the ALK array.

[00133] Components: Kits are all inclusive with step-by-step instructions for ease of use.

[00134] Sample Type: Cell Lines, Blood, Purified RNA or FFPE

[00135] Intended Uses

[00136] The intended use for this product is to detect any of the specified expression wild types and fusion variants of ALK and EML4/ALK.

[00137] These are as follows:

| WT:     | ALK - 5' |
| WT:     | ALK - 3' |
| Fusion: | EML4/ALK - variant 1 |
| Fusion: | EML4/ALK - variant 2 |
| Fusion: | EML4/ALK - variant 3a |
| Fusion: | EML4/ALK - variant 3b |
| Fusion: | EML4/ALK - variant 4 |
| Fusion: | EML4/ALK - variant 5a |
| Fusion: | EML4/ALK - variant 5b |
| Fusion: | EML4/ALK - variant 6 |
| Fusion: | KIF5B-ALK |
| Fusion: | TFG-ALK |
| WT:     | EML4 - 5' |
| WT:     | KIF5B - 5' |
| WT:     | TFG - 5'' |

[00138] Insight ALK Screen is an RT-qPCR assay that detects the presence of ALK fusions and upregulation of ALK wild type (which is abnormal in adult tissue outside the central nervous system and can be indicative of ALK-driven disease). The assay uses a three tube reaction series (plus controls) to measure expression of the extracellular segment of ALK (ALK WT), ALK kinase domain expression (ALK Kinase), and expression of an internal
reference gene, Cytochrome c oxidase subunit 5B (COX5B). By focusing on relative expression of the ALK gene, Insight ALK Screen can more accurately detect the presence of ALK fusions than a variant-specific PCR approach that targets the 10+ unique 5' gene partners, such as EML4.

[00139] Methods and procedures for the detection of wild type ALK and NPM-ALK fusions can be found in U.S. Patent Nos. 5,529,925 and 5,770,421.

[00140] As used herein, a "subject with a mutation" in KRAS, ALK, EGFR, or other gene associated with cancer, or a "subject with a cancer with a mutation" in KRAS, ALK, EGFR, or other gene associated with cancer, and the like, are understood as a subject suffering from cancer, wherein the tumor has at least one alteration (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) in the indicated gene from the wild-type sequence in the gene and/or transcriptional, translational, and/or splicing control regions of the gene that result in the cell becoming cancerous, e.g., developing characteristics such as uncontrolled proliferation, immortality, metastatic potential, rapid growth and proliferation rate, decreased cell death/apoptosis, and certain characteristic morphological features. Mutations include, for example, insertions, deletions, truncations, point mutations, and translocations. Mutations within a gene product can result in constituent activation of the gene product. Mutations that include alterations in transcriptional, translational, or splicing control regions can result in aberrant expression, typically over-expression, of a wild-type gene product. It is understood that not all gene mutations, even in oncogenes, result in a cell becoming cancerous. Mutations that result in oncogenesis are well known in the art. Methods to test mutations for oncogenic activity are well known in the art.

[00141] A mutation can be detected using any of a number of known methods in the art. The specific method to detect the mutation will depend, for example, on the type of mutation to be detected. For example, alterations in nucleic acid sequences can be easily detected using polymerase chain reaction and fluorescence in situ hybridization methods (FISH). Protein expression levels can be detected, for example, using immunohistochemistry. An aberrant expression level of a wild-type protein can be used as a surrogate for detection of a mutation in a transcriptional, translational, and/or splicing control regions of the gene without direct detection of the specific genetic change in the
nucleic acid in the subject sample. The specific method of detection of the mutation is not a limitation of the invention. Methods to compare protein expression levels to appropriate controls are well known in the art.

[00142] In a preferred embodiment, when multiple tests are used to detect a mutation and one is positive, the mutation is considered to be present. The methods do not require that multiple assays be performed to detect a mutation.

[00143] As used herein, and in the art, an "ALK+" tumor or cancer is understood as a tumor or cancer that has a mutation such that ALK is overexpressed and/or has a mutation that causes a cancerous phenotype in the cell.

[00144] Moreover, it is generally recognized that once a mutation in EGFR is detected in a cancer, the mutation in KRAS will be eliminated in the same cancer. Put reversely, if a mutation in KRAS is positively identified in a cancer from a subject, it is then not necessary to engage in any further EGFR related identification. Similar principle can be applied to a mutation in ALK in a cancer. That is if there is a mutation in ALK detected in a cancer, it is extremely rare that a mutation in EGFR or KRAS will be implicated. Stated another way, once a mutation in ALK is positively identified in a cancer, no further identification is necessary either for a mutation in EGFR or for a mutation in KRAS in the same cancer.

[00145] As used herein, a subject with a "wild-type" KRAS, ALK, EGFR, or other gene associated with cancer, or a "subject with a cancer with a wild-type" KRAS, ALK, EGFR, or other gene associated with cancer, and the like, are understood as a subject suffering from cancer, wherein the tumor does not have any significant alterations (i.e., alterations that result in a change of function) in the indicated gene from the native sequence in the gene and/or transcriptional, translational, and/or splicing control regions of the native gene that result in the cell becoming cancerous, e.g., developing characteristics such as uncontrolled proliferation, immortality, metastatic potential, rapid growth and proliferation rate, decreased cell death/apoptosis, and certain characteristic morphological features. As used herein, a "wild-type" gene is expressed at a level that does not result in the cell becoming cancerous.
[00146] As used herein, and in the art, a "HER2 positive" tumor or cancer is a tumor or cancer that expresses a wild-type level of HER2. Loss of HER2 expression (HER2-) is associated with a cancer phenotype.

[00147] As used herein, and in the art, an "estrogen receptor positive" or "ER positive" tumor or cancer is a tumor or cancer that expresses a wild-type level of estrogen receptor (ER). Loss of ER expression (ER-) is associated with a cancer phenotype.

[00148] As used herein, and in the art, a "progesterone receptor positive" or "PR positive" tumor or cancer is a tumor or cancer that expresses a wild-type level of progesterone receptor (PR). Loss of PR expression (PR-) is associated with a cancer phenotype.

[00149] Expression levels of HER2, ER, and PR that are considered to be positive or negative for expression are well known in the art. Assessments for HER2, ER, and PR status are routinely made in the art.

[00150] As used herein, the term "lactate dehydrogenase" refers to an enzyme that interconverts pyruvate and lactate with concomitant interconversion of NADH and NAD+. Under conditions of hypoxia, the reaction favors the conversion of pyruvate to lactate. Under conditions of normoxia, or low levels of hypoxia, the reaction favors the conversion of lactate to pyruvate. Functional lactate dehydrogenase are homo or hetero tetramers composed of M and H protein subunits encoded by the LDHA and LDHB genes respectively: LDH-1 (4H) is the predominant form found, for example, in the heart and red blood cells (RBCs); LDH-2 (3H1M) is the predominant found, for example, in the reticuloendothelial system; LDH-3 (2H2M) is the predominant form found, for example, in the lungs; LDH-4 (1H3M) is the predominant form found, for example, in the kidneys, placenta and pancreas; and LDH-5 (4M) is the predominant form found, for example, in the liver and striated muscle. Typically, multiple forms of LDH are found in these tissues. Lactate dehydrogenase is classified as (EC 1.1.1.27). The specific ratios tested may be tumor-type specific.

[00151] As used herein, the terms "hypoxia" and "hypoxic" refer to a condition in which a cancer or a tumor has a low oxygen microenvironment or a less well-oxygenated microenvironment. Hypoxia occurs when tumor growth exceeds new blood vessel
formation, and a tumor must undergo genetic and adaptive changes to allow them to survive and proliferate in the hypoxic environment. The development of intratumoral hypoxia is a common sign of solid tumors. When a tumor microenvironment is less well-oxygenated, there is a greater dependency on oxygen-sensitive pathways, including but not limited to HIFα pathways, VEGF pathways, and mTOR pathways. These pathways facilitate crucial adaptive mechanisms, such as angiogenesis, glycolysis, growth-factor signaling, immortalization, genetic instability, tissue invasion and metastasis, apoptosis, and pH regulation (see, e.g., Harris, Nature Reviews, 2:38-47, 2002). These pathways may also facilitate invasion and metastasis. Accordingly, the treatment of a subject with a cancer or tumor with ganetespib is more effective when the subject has a tumor that exhibits a modulated level of hypoxia, e.g., a high level of hypoxia. As the level of hypoxia in the tumor can be determined by obtaining a sample from a site other than the tumor, as used herein, the subject can be stated to demonstrate a modulated level of hypoxia when it is the tumor present in the subject that demonstrates a modulated level of hypoxia. As used herein it is understood that the subject with a modulated level of hypoxia is typically not suffering from systemic oxygen imbalance or ischemic disease at a site remote from the tumor.

[00152] As used herein, the term "level of hypoxia" is understood as the amount of one or more markers indicative of a low oxygen level, or cells having characteristics and/or employing biological pathways characteristic of cells with a low oxygen level, e.g., due to the Warburg effect. Such markers include, but are not limited to, lactate dehydrogenase (LDH), at least one isoform or subunit of hypoxia inducible factor (HIF), at least one pro-angiogenic form of vascular endothelial growth factor (VEGF), phosphorylated VEGF receptor (pKDR) 1, 2, or 3; neurolipin 1 (NRP-1), pyruvate dehydrokinase (PDH-K), and ornithine decarboxylase (ODC). Tumor size can also be correlated with a level of hypoxia. A level of hypoxia can also be determined by PET scan. LDH can be one or more isoforms or subunits of LDH such as LDH5, LDH4, LDH3, LDH2, LDH1, LDHM (also known as LDHA) and LDHH (also known as LDHB). In one embodiment, LDH can be a total sample of all LDH isoforms or subunits.
"Hypoxia inducible factors" or "HIFs" are transcription factors which respond to changes in available oxygen in a cellular environment. HIF1α is a master regulator of hypoxic gene expression and oxygen homeostasis. HIF can be one or more subunits or isoforms of HIF including HIF-1α, HIF-1β, HIF-2α, and HIF-2β. VEGF can be one or more of the various splice forms of VEGF including pro-angiogenic VEGF-A and antiangiogenic VEGF-B.

As used herein, the term "level of LDH" refers to the amount of LDH present in a sample which can be used to indicate the presence or absence of hypoxia in the tumor in the subject from whom the sample was obtained. LDH enables the conversion of pyruvate to lactate and is a critical component of glycolysis under hypoxic conditions. LDH can be total LDH or one or more isoforms or subunits of LDH such as LDH5, LDH4, LDH3, LDH2, LDH1, LDH4M (also known as LDHA) and LDH4H (also known as LDHB). A modulated level of LDH can refer to a high level of LDH or a low level of LDH. In one embodiment, a PET scan (which is positive when aerobic glycolysis is active) is an indicator of a high level of LDH. In another embodiment, a PET scan (which is negative when aerobic glycolysis is inactive) is an indicator of a low level of LDH. In one embodiment, a high level of LDH is at least 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3, 4, 5, 6, 7, 8, 9, or 10 times the value of normal level of LDH. In another embodiment, a low level of LDH is 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2, or 0.1 times the value of a normal level of LDH. A normal level of LDH, or any other marker, can be defined as any value within the range of normal, or the upper limit of the normal value, or the lower limit of the normal value.

Assays for determining the level of LDH in a sample are well known in the art and provided herein.

In another embodiment, the level of LDH can be understood to be a change in the relative levels of protein or activity of LDH isoforms or the ratio of LDH isoforms. Preferably, the ratios are the ratios of normalized values, e.g., the level of the LDH subunit or isoform is normalized to the ULN, the LLN, or a median value. A change of the relative levels of the isoforms can be indicative of the level of hypoxia. For example, an increase in the level of LDHA relative to LDHB can be indicative of an increase in hypoxia.

Alternatively, an increase in the level of LDH5 and/ or LDH4, either individually or in total,
relative to the level of LDH1 or total LDH can be indicative of an increase in hypoxia. The relative levels can be compared to relative levels in an appropriate control sample from normal subjects, e.g., subjects without cancer or ischemic disease. That is, the ratios are the ratios of normalized values, e.g., the level of the LDH subunit or isoform is normalized to the ULN, the LLN, or a median value. The normal levels can be considered to be a range with an upper level of normal and a lower level of normal. In certain embodiments, a high level of LDH can be understood as an increase in the normalized level of LDHA or LDH5 and/or LDH4 relative to the normalized level of LDHB or LDH1 or total LDH, respectively, or to total LDH of at least 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3.4, 5, 6, 7, 8, 9, or 10 times the value of normalized level of LDHA or LDH5 and/or LDH4 relative to the normalized level of LDHB or LDH1 or total LDH, respectively. In another embodiment, a low level of LDH is a ratio of 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2, or 0.1 of the normalized value of LDHA or LDH5 and/or LDH4 relative to the normalized level of LDHB or LDH1 or total LDH, respectively.

[00156] As used herein, a "normalized ratio" is understood as a proportion of two values that have been compared to a standard, either an external (e.g., population control level) or an internal (e.g., level from a normal tissue, level from an earlier time point, level of one or more isoforms) control to allow for comparison of samples between individuals. For example, the ratio of normalized levels of hypoxia modulated polypeptides can be determined by determining a ratio of two normalized levels of two isoforms or subunits of LDH or total LDH by comparing the level of a first isoform or subunit of LDH in the sample relative to a control sample to provide a first normalized level, and the level of a second isoform or subunit of LDH or total LDH relative to a control sample to provide a second normalized level, and calculating a ratio of the first normalized level and the second normalized level to provide a normalized ratio of LDH isoforms or subunits, wherein at least one of the first level and the second level are not total LDH. In certain embodiments, a low level of hypoxia is a normalized ratio of the ULN of LDHA to LDHB of 1.0 or less, or a normalized ratio of the ULN of LDH5 and/or LDH4 to LDH1 or total LDH of 1.0 or less.

[00157] Assays for determining the level of LDH in a sample are well known in the art. See, e.g., U.S. Publication Nos. 2010/0178283 and 2008/0213744 and U.S. Patent Nos. 4,250,255
and 6,242,208, the entire contents of each of which are expressly incorporated herein by reference. LDH sequences are further provided in public databases (e.g., at http://blast.ncbi.nlm.nih.gov/Blast.cgi).

[00158] It is also understood that levels of the various markers can include the level of a post-translationally modified marker, e.g., the total amount of an isoform of HIF may remain the same, but the amount of the hydroxylated version of the HIF may increase. In addition, it is noted that HIF and other hypoxia modulated polypeptides can be upregulated by a number of conditions other than hypoxia, e.g., pH change, changes in levels of O2' or H2O2, etc. Accordingly, although the term "level of expression," as used herein, is intended to encompass all hypoxia responsive factors, a change in their level of expression may or may not actually directly reflect the amount of oxygen available to the tumor.

[00159] Methods to detect the levels of markers of hypoxia are well known in the art. Antibodies against and kits for detection of hypoxia modulated polypeptides can be purchased from a number of commercial sources. Alternatively, using routine methods known in the art (e.g., immunization of animals, phage display, etc.) antibodies against one or more hypoxia modulated polypeptides or subunits or isoforms thereof can be made and characterized. Antibodies can be used for the detection of levels of hypoxia using ELISA, RIA, or other immunoassay methods, preferably automated methods, for the quantitative detection of proteins in samples of bodily fluids or homogenized solid samples. Hypoxia can be detected by enzyme activity assays (e.g., LDH activity, kinase activity) including in gel assays to resolve the activity of various isoforms of proteins. Alternatively, immunohistochemical methods can be used on tumor samples and tissue sections. Antibodies against prodrugs that localize in hypoxic regions (e.g., EF5, pimonidazole, etc.) can also be used to detect hypoxia. Functional imaging measuring blood flow in the tumor can be used as an indicator of hypoxia in the tissue. Direct measurement of hypoxia can be performed by inserting a sensor into the tumor. Qualitative scoring methods and scanning methods to detect staining are known in the art. When qualitative scoring methods are used, it is preferred that two independent, blinded technicians, pathologists, or other skilled individuals analyze each sample with specific methods for resolving any significant disagreement in scoring, e.g., a third individual reviews the tissue sample.
Alternatively, nucleic acid-based methods of detection of levels of hypoxia are also well known in the art. Methods of designing primers and probes for quantitative reverse transcription real time (rt) PCR are known in the art. Methods for performing northern blots to detect RNA levels are known in the art. Nucleic acid detection methods can also include fluorescence in situ hybridization (FISH) and in situ PCR. Qualitative scoring methods and scanning methods to detect staining are known in the art. When qualitative scoring methods are used, it is preferred that two independent, blinded technicians, pathologists, or other skilled individuals analyze each sample with specific methods for resolving any significant disagreement in scoring, e.g., a third individual reviews the tissue sample.

"Baseline" refers to the level of hypoxia or the level of LDH upon patient entrance into the study and is used to distinguish from levels of hypoxia or levels of LDH the patient might have during or after treatment.

"Elevated" or "lower" refers to a patient's value relative to the upper limit of normal ("ULN") or the lower limit of normal ("LLN") which are based on historical normal control samples. Since the level of the hypoxic marker present in the subject will be a result of the disease and not a result of treatment, typically not a control, a sample obtained from the patient prior to onset of the disease will not likely be available. Because different labs may have different absolute results, LDH values are presented relative to that lab's upper limit of normal value (ULN). LDH can be expressed in IU/ml (International Units per milliliter). An accepted ULN for LDH is 234 IU/ml, however, this value is not universally accepted or applicable to all methods of detection of LDH in all samples.

The specific value for ULN and LLN will also depend, for example, on the type of assay (e.g., ELISA, enzyme activity, immunohistochemistry, imaging), the sample to be tested (e.g., serum, tumor tissue, urine), and other considerations known to those of skill in the art. The ULN or LLN can be used to define cut-offs between normal and abnormal. For example, a low level of a marker (e.g., LDH) can be defined as a marker level less than or equal to the ULN for that marker, with a high level being all values greater than the ULN. Cut-offs can also be defined as fractional amounts of the ULN. For example, a low level of a marker can be understood to be a level of about 0.5 ULN or less, 0.6 ULN or less, 0.7 ULN or
less, 0.8 ULN or less, 0.9 ULN or less, 1.0 ULN or less, 1.1 ULN or less, 1.2 ULN or less, 1.3 ULN or less, 1.4 ULN or less, 1.5 ULN or less, 1.6 ULN or less, 1.7 ULN or less, 1.8 ULN or less, 1.9 ULN or less, 2.0 ULN or less, 2.5 ULN or less, 3.0 ULN or less, or 4.0 ULN or less, with the corresponding high level of the marker being a value greater than the low level. In certain embodiments, the presence of a low level of a marker in a subject sample as defined above can be indicative that a subject will or will not respond to a particular therapeutic intervention. In certain embodiments, the presence of a high level of a marker in a subject sample as defined above can be indicative that a subject will or will not respond to a particular therapeutic intervention.

[00164] Marker levels can also be further stratified, for example, into low, intermediate, and high based on the ULN value. For example, the presence of a low level of a marker in a subject sample as defined above can be indicative that a subject will or will not respond to a particular therapeutic intervention. An intermediate level of a marker, e.g., a range bracketed by any range within the values of 0.5 ULN, 0.6 ULN, 0.7 ULN, 0.8 ULN, 0.9 ULN, 1.0 ULN, 1.1 ULN, 1.2 ULN, 1.3 ULN, 1.4 ULN, 1.5 ULN, 1.6 ULN, 1.7 ULN, 1.8 ULN, 1.9 ULN, and 2.0 ULN, can be considered an intermediate range wherein the level of the marker may be indeterminate that a subject will or will not respond to a particular therapeutic intervention. A high level, greater than the intermediate level, would be indicative that a subject will or will not respond to a particular therapeutic intervention.

[00165] Similarly, cut-offs of ratios of LDH subunits or isoforms comparing the ULN, the LLN, or the median values to differentiate between high and low levels of hypoxia can be defined as any value or range bracketed by the values 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, or higher.

[00166] The "normal" level of expression of a marker is the level of expression of the marker in cells of a subject or patient not afflicted with cancer. In one embodiment, a "normal" level of expression refers to the level of expression of the marker under normoxic conditions.

[00167] An "over-expression" or "high level of expression" of a marker refers to an expression level in a test sample that is greater than the standard error of the assay employed to assess expression, and is preferably at least 1.1, 1.2, 1.3, 1.4, 1.5, .16, 1.7, 1.8, 1.9,
2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3, 4, 5, 6, 7, 8, 9, or 10 times the expression level of the marker in a control sample (e.g., sample from a healthy subject not having the marker associated disease, i.e., cancer). In one embodiment, expression of a marker is compared to an average expression level of the marker in several control samples.

[00168] A "low level of expression" or "under-expression" of a marker refers to an expression level in a test sample that is less than at least 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2, or 0.1 times the expression level of the marker in a control sample (e.g., sample from a healthy subjects not having the marker associated disease, i.e., cancer). In one embodiment, expression of a marker is compared to an average expression level of the marker in several control samples.

[00169] As used herein, the term "oxygen-sensitive pathway" is a cellular signaling pathway which is activated by hypoxia. Oxygen-sensitive pathways may be up-regulated by hypoxia. Alternatively, an oxygen-sensitive pathway may be down-regulated by hypoxia. Oxygen-sensitive pathways include, but are not limited to, HIF pathways (such as HIF1α pathways), VEGF pathways, and mTOR pathways. As used herein, the term "hypoxia-modulated gene" or "hypoxia-modulated polypeptide" refers to a gene or protein which is up-regulated or down-regulated by hypoxia.

[00170] As used herein, the term "HIF pathway" and "HIF pathway members" as used herein, describe proteins and other signaling molecules that are regulated by HIF-1 and HIF-2. Hypoxia-Inducible Factor 1 (HIF-1) is a transcription factor that has been shown to play an essential role in cellular responses to hypoxia. Upon hypoxic stimulation, HIF-1 has been shown to activate genes that contain Hypoxic Response Elements (HREs) in their promoters, and thus up-regulate a series of gene products that promote cell survival under conditions of low oxygen availability. The list of known HIF-responsive genes includes glycolytic enzymes (such as lactate dehydrogenase (LDH), enolase-1 (ENO-1), and aldolase A, glucose transporters (GLUT 1 and GLUT 3), vascular endothelial growth factor (VEGF), inducible nitric oxide synthase (NOS-2), and erythropoietin (EPO). The switch of the cell to anaerobic glycolysis, and the up-regulation of angiogenesis by VEGF is geared at maximizing cell survival under conditions of low oxygen tension by reducing the requirement for oxygen, and increasing vasculature to maximize oxygen delivery to tissues. The HIF-1 transcription
complex has recently been shown to comprise a heterodimer of two basic helix-loop-helix proteins, HIF-1α and HIF-1β (also known as ARNT, Aryl Hydrocarbon Receptor Nuclear Translocator).

**[00171]** HIF-1α is a member of the basic-helix-loop-helix PAS domain protein family and is an approximately 120 kDa protein containing two transactivation domains (TAD) in its carboxy-terminal half and DNA binding activity located in the N-terminal half of the molecule. HIF-1α is constitutively degraded by the ubiquitin-proteosome pathway under conditions of normoxia, a process that is facilitated by binding of the von Hippel-Lindau (VHL) tumor suppressor protein to HIF-1α. Under conditions of hypoxia, degradation of HIF-1α is blocked and active HIF-1α accumulates. The subsequent dimerization of HIF-1α with ARNT leads to the formation of active HIF transcription complexes in the nucleus, which can bind to and activate HREs on HIF-responsive genes.

**[00172]** As used herein, the term "VEGF pathway" and "VEGF pathway members" as used herein, describe proteins and other signaling molecules that are regulated by VEGF. For example, VEGF pathway members include VEGFR1, 2, and 3; PECAM-1, LacCer synthase, and PLA2.

**[00173]** As used herein, the term "mTOR pathway" and "mTOR pathway members" as used herein, describe proteins and other signaling molecules that are regulated by mTOR. For example, mTOR pathway members include SK6, PDCD4, eIF4B, RPS6, eIF4, 4E-BP1, and eIF4E.

**[00174]** The term "expression" is used herein to mean the process by which a polypeptide is produced from DNA. The process involves the transcription of the gene into mRNA and the translation of this mRNA into a polypeptide. Depending on the context in which used, "expression" may refer to the production of RNA, or protein, or both.

**[00175]** The terms "level of expression of a gene" or "gene expression level" refer to the level of mRNA, as well as pre-mRNA nascent transcript(s), transcript processing intermediates, mature mRNA(s) and degradation products, or the level of protein, encoded by the gene in the cell.
[00176] As used herein, "level of activity" is understood as the amount of protein activity, typically enzymatic activity, as determined by a quantitative, semi-quantitative, or qualitative assay. Activity is typically determined by monitoring the amount of product produced in an assay using a substrate that produces a readily detectable product, e.g., colored product, fluorescent product, radioactive product. For example, the isoforms of LDH in a sample can be resolved using gel electrophoresis. Lactate, nicotinamide adenine dinucleotide (NAD+), nitroblue tetrazolium (NBT), and phenazine methosulphate (PMS) can be added to assess LDH activity. LDH converts lactate to pyruvate and reduces NAD+ to NADH. The hydrogens from NADH are transferred by PMS to NBT reducing it to a purple formazan dye. The percentage of each LDH isoenzyme activity as well as the relative amount of each isoenzyme to the other isoforms or total LDH can be determined, for example, by densitometry.

[00177] Mutations, protein expression levels, and hypoxia are preferably detected in a subject sample from the cancer tissue or tumor tissue, e.g., cells, extracellular matrix, and other naturally occurring components associated with the tumor. The mutation or expression level can be detected in a biopsy sample or in a surgical sample after resection of the tumor. The term "sample" as used herein refers to a collection of similar fluids, cells, or tissues isolated from a subject. The term "sample" includes any body fluid (e.g., urine, serum, blood fluids, lymph, gynecological fluids, cystic fluid, ascetic fluid, ocular fluids, and fluids collected by bronchial lavage and/or peritoneal rinsing), ascites, tissue samples (e.g., tumor samples) or a cell from a subject. Other subject samples include tear drops, serum, cerebrospinal fluid, feces, sputum, and cell extracts. In an embodiment, the sample is removed from the subject. In a particular embodiment, the sample is urine or serum. In an embodiment, the sample comprises cells. In another embodiment, the sample does not comprise cells. In certain embodiments, the sample can be the portion of the subject that is imaged. Samples are typically removed from the subject prior to analysis, however, tumor samples can be analyzed in the subject, for example, using imaging or other detection methods (e.g., using a PET scan, a functional imaging method such as MRI to detect blood flow) or tested to determine level of hypoxia (e.g., tumor tissue assayed for level of hypoxia using a probe).
In some embodiments, only a portion of the sample is subjected to an assay for determining the level of hypoxia or the level of the tumor using any method provided herein. In certain embodiments, the level of hypoxia is indicated by the level of an isoform or subunit of lactate dehydrogenase (LDH) or any combination of subunits or isoforms including total LDH, or various portions of the sample are subjected to various assays for determining the level of hypoxia or the level of an isoform or subunit of LDH. Also, in many embodiments, the sample may be pre-treated by physical or chemical means prior to the assay. For example, samples, for example, blood samples, can be subjected to centrifugation, dilution and/or treatment with a solubilizing substance prior to assaying the samples for the level of hypoxia or LDH. Such techniques serve to enhance the accuracy, reliability and reproducibility of the assays of the present invention.

As used herein, the terms "identify" or "select" refer to a choice in preference to another. In other words, to identify a subject or select a subject is to perform the active step of picking out that particular subject from a group and confirming the identity of the subject by name or other distinguishing feature. With respect to the instant invention, it is understood that identifying a subject or selecting a subject as having one or more mutations in one or more genes of interest, having a wild-type gene, having a change in the expression level of a protein, or having a high level of hypoxia, and can include any of a number of acts including, but not limited to, performing a test and observing a result that is indicative of a subject having a specific mutation or a high level of hypoxia; reviewing a test result of a subject and identifying the subject as having a specific mutation or a high level of hypoxia; reviewing documentation on a subject stating that the subject has a specific mutation or a high level of hypoxia and identifying the subject as the one discussed in the documentation by confirming the identity of the subject e.g., by an identification card, hospital bracelet, asking the subject for his/her name and/or other personal information to confirm the subjects identity.

As used herein, the term "refractory" cancer or tumor is understood as a malignancy which is either initially unresponsive to chemo- or radiation therapy, or which becomes unresponsive over time. A cancer refractory to an intervention may not be refractory to all interventions. A refractory cancer is typically not amenable to treatment with surgical interventions.
As used herein, "relapse" is understood as the return of a cancer or the signs and symptoms of a cancer after a period of improvement.

The articles "a", "an" and "the" are used herein to refer to one or to more than one (i.e. to at least one) of the grammatical object of the article unless otherwise clearly indicated by contrast. By way of example, "an element" means one element or more than one element.

The term "including" is used herein to mean, and is used interchangeably with, the phrase "including but not limited to".

The term "or" is used herein to mean, and is used interchangeably with, the term "and/or," unless context clearly indicates otherwise.

The term "such as" is used herein to mean, and is used interchangeably, with the phrase "such as but not limited to".

Unless specifically stated or obvious from context, as used herein, the term "about" is understood as within a range of normal tolerance in the art, for example within 2 standard deviations of the mean. About can be understood as within 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, 0.1%, 0.05%, or 0.01% of the stated value. Unless otherwise clear from context, all numerical values provided herein can be modified by the term about.

The recitation of an embodiment for a variable or aspect herein includes that embodiment as any single embodiment or in combination with any other embodiments or portions thereof.

Any compositions or methods provided herein can be combined with one or more of any of the other compositions and methods provided herein.

As used herein, the term "subject" refers to human and non-human animals, including veterinary subjects. The term "non-human animal" includes all vertebrates, e.g., mammals and non-mammals, such as non-human primates, mice, rabbits, sheep, dog, cat, horse, cow, chickens, amphibians, and reptiles. In a preferred embodiment, the subject is a human and may be referred to as a patient.
Methods of Detection of Mutations


[00191] Methods of detection of expression levels of ER, PR, and HER2 are well known in the art. Thresholds of expression to that constitute ER, PR, and HER2 status are also well known in the art.

Methods of Detection of Hypoxia

[00192] The instant invention provides methods of identifying a subject who will likely respond favorably to treatment with a selected agent by determining the level of hypoxia in a tumor, either by looking directly at markers within the tumor tissue or looking at markers in a peripheral sample from the subject, e.g., a bodily fluid such as blood, serum, plasma, lymph, urine, cerebrospinal fluid, or fecal matter, for the presence of one or more indicators of the level of hypoxia in the tumor.

[00193] The specific subject sample analyzed will depend, for example, on the site of the tumor. It is known that hypoxia drives angiogenesis in tumors, resulting in leaky blood vessels resulting in the presence of markers in circulation. Further, tumor growth and hypoxia are typically associated with necrosis and cell breakdown, resulting in cellular material in other bodily fluids or wastes. These readily accessible subject samples allow for
the monitoring of the subject for the presence, or absence, of markers for hypoxia prior to and during the course of treatment.

[00194] Biopsies are routinely obtained for the purpose of cancer diagnosis, and solid tumors are frequently further resected prior to initiation of chemotherapy which also can be used for analysis to determine the level of hypoxia. Biopsy samples and resected tumor samples typically include at least some normal tissue adjacent to the tumor that can be used as a control.

[00195] In one embodiment of the invention, the modulated level of hypoxia is a high level of hypoxia. In one embodiment of the invention, the modulated level of hypoxia is a high level of LDH.

[00196] In one embodiment, the level of hypoxia is determined by detecting the level of one or more hypoxia-modulated polypeptides or using one or more methods such as imaging methods. In one embodiment, a hypoxia-modulated polypeptide is at least one isoform or subunit of lactate dehydrogenase (LDH), at least one isoform or subunit of hypoxia inducible factor (HIF), at least one pro-angiogenic form of vascular endothelial growth factor (VEGF), phosphorylated VEGF receptor (pKDR), neurolipin 1 (NRP-1), pyruvate dehydrokinase (PDH-K), and ornithine decarboxylase (ODC). In one embodiment, the isoform or subunit of LDH is LDHH, LDH5, LDH4, LDH3, LDH2, LDH1 or LDHM, or any combination thereof. In another embodiment, the isoform or subunit of LDH is LDH5. In another embodiment, the level of hypoxia is determined by determining the ratio of two or more forms of LDH, e.g., the ratio of LDH5:LDH1. In another embodiment, the isoform of HIF is HIF-1α, HIF-1β, HIF-2a, and HIF-2β. In another embodiment, the pro-angiogenic isoform of VEGF is any one or a combination of VEGF-A splice variants. Antibodies against prodrugs that localize in hypoxic regions (e.g., EF5, pimonidazole, etc.) can also be used to detect hypoxia. Tumor size can also be correlated with a level of hypoxia. A level of hypoxia can also be determined by PET scan. Functional imaging measuring blood flow in the tumor can be used as an indicator of hypoxia in the tissue. Direct measurement of hypoxia can be performed by inserting a sensor into the tumor.

[00197] Methods to detect the protein or activity levels of markers of hypoxia, or hypoxia modulated polypeptides, are well known in the art. Antibodies against and kits for
detection of hypoxia modulated polypeptides can be purchased from a number of commercial sources. Alternatively, using routine methods known in the art (e.g., immunization of animals, phage display, etc.) antibodies against one or more hypoxia modulated polypeptides or subunits or isoforms thereof can be made and characterized. Antibodies can be used for the detection of levels of hypoxia using ELISA, RIA, or other immunoassay methods, preferably automated methods, for the quantitative detection of proteins in samples of bodily fluids or homogenized solid samples. Alternatively, immunohistochemical methods can be used on tumor samples and tissue sections.

Qualitative scoring methods and scanning methods to detect staining are known in the art. When qualitative scoring methods are used, it is preferred that two independent, blinded technicians, pathologists, or other skilled individuals analyze each sample with specific methods for resolving any significant disagreement in scoring, e.g., a third individual reviews the tissue sample. Many markers of hypoxia, including LDH, are enzymes. Enzymatic activity can be assayed in total, or for individual isoforms, for example, using in gel assays.

Alternatively, nucleic acid based methods of detection of levels of hypoxia are also well known in the art. Methods of designing primers and probes for quantitative reverse transcription real time (rt) PCR are known in the art. Methods for performing northern blots to detect RNA levels are known in the art. Nucleic acid detection methods can also include fluorescence in situ hybridization (FISH) and in situ PCR. Qualitative scoring methods and scanning methods to detect staining are known in the art.

In another aspect, the present invention provides methods for the preselection of a subject for therapeutic treatment with ganetespib, wherein the subject has previously been found to have a high level of hypoxia. The invention also provides methods for the preselection of a subject for therapeutic treatment with ganetespib by evaluating the results of an assessment of a sample from the subject for a high level of hypoxia.

Such determinations can be made based on a chart review of the level of hypoxia of the tumor of the subject. Inclusion criteria can include information being available regarding the cancer type, the specific treatment regimen with ganetespib.
In another aspect, the present invention provides methods for treating a cancer with ganetespib in a subject having a high level of hypoxia and a mutation in KRAS, EGFR, ALK, or in a subject who has HER2+ breast cancer, or in a subject with triple negative breast cancer. thereby treating the cancer. In certain embodiments, the subject has previously been treated with an additional chemotherapeutic agent.

Other methods include methods of treating a subject who has cancer by prescribing to the subject an effective amount of ganetespib, wherein the subject has previously been found to have a high level of hypoxia and a mutation in KRAS, EGFR, ALK, or in a subject who has HER2+ breast cancer, or in a subject with triple negative breast cancer. As used herein, the term "prescribing" is understood as indicating a specific agent or agents for administration to a subject. Furthermore, the present invention also includes methods of increasing the likelihood of effectively treating a subject having cancer by administering a therapeutically effective amount of ganetespib to the subject, wherein the subject has previously been found to have a modulated level of hypoxia and a mutation in KRAS, EGFR, ALK, or in a subject who has HER2+ breast cancer, or in a subject with triple negative breast cancer.

Cancers that may be treated or prevented using the methods of the invention include, for example, acoustic neuroma, acute leukemia, acute lymphocytic leukemia, acute myelocytic leukemia (monocytic, myeloblasts, adenocarcinoma, angiosarcoma, astrocytoma, myelomonocytic and promyelocyte), acute T-cell leukemia, basal cell carcinoma, bile duct carcinoma, bladder cancer, brain cancer, breast cancer, bronchogenic carcinoma, cervical cancer, chondrosarcoma, chordoma, choriocarcinoma, chronic leukemia, chronic lymphocytic leukemia, chronic myelocytic (granulocytic) leukemia, chronic myelogenous leukemia, colon cancer, colorectal cancer, craniopharyngioma, cystadenocarcinoma, diffuse large B-cell lymphoma, dysproliferative changes (dysplasias and metaplasias), embryonal carcinoma, endometrial cancer, endothelial sarcoma, ependymoma, epithelial carcinoma, erythroleukemia, esophageal cancer, estrogen-receptor positive breast cancer, essential thrombocytemia, Ewing's tumor, fibrosarcoma, follicular lymphoma, germ cell testicular cancer, glioma, heavy chain disease, hemangioblastoma, hepatoma, hepatocellular cancer, hormone insensitive prostate cancer, leiomyosarcoma,
liposarcoma, lung cancer, lymphagioendotheliosarcoma, lymphangiosarcoma, lymphoblastic leukemia, lymphoma (Hodgkin's and non-Hodgkin's), malignancies and hyperproliferative disorders of the bladder, breast, colon, lung, ovaries, pancreas, prostate, skin and uterus, lymphoid malignancies of T-cell or B-cell origin, leukemia, lymphoma, medullary carcinoma, medulloblastoma, melanoma, meningioma, mesothelioma, multiple myeloma, myelogenous leukemia, myeloma, myxosarcoma, neuroblastoma, non-small cell lung cancer, oligodendroglioma, oral cancer, osteogenic sarcoma, ovarian cancer, pancreatic cancer, papillary adenocarcinomas, papillary carcinoma, pinealoma, polycythemia vera, prostate cancer, rectal cancer, renal cell carcinoma, retinoblastoma, rhabdomyosarcoma, sarcoma, sebaceous gland carcinoma, seminoma, skin cancer, small cell lung carcinoma, solid tumors (carcinomas and sarcomas), small cell lung cancer, stomach cancer, squamous cell carcinoma, synovioma, sweat gland carcinoma, thyroid cancer, Waldenstrom's macroglobulinemia, testicular tumors, uterine cancer and Wilms' tumor. Other cancers include primary cancer, metastatic cancer, oropharyngeal cancer, hypopharyngeal cancer, liver cancer, gallbladder cancer, small intestine cancer, urinary tract cancer, kidney cancer, urothelium cancer, female genital tract cancer, uterine cancer, gestational trophoblastic disease, male genital tract cancer, seminal vesicle cancer, testicular cancer, germ cell tumors, endocrine gland tumors, thyroid cancer, adrenal cancer, and pituitary gland cancer, hemangiomas, sarcomas arising from bone and soft tissues; Kaposi's sarcoma, nerve cancer, ocular cancer, and meningial cancer, glioblastomas, neuromas, Schwannomas, solid tumors arising from hematopoietic malignancies such as leukemias, metastatic melanoma, recurrent or persistent ovarian epithelial cancer, fallopian tube cancer, primary peritoneal cancer, gastrointestinal stromal tumors, colorectal cancer, gastric cancer, melanoma, glioblastoma multiforme, non-squamous non-small-cell lung cancer, malignant glioma, epithelial ovarian cancer, primary peritoneal serous cancer, metastatic liver cancer, neuroendocrine carcinoma, refractory malignancy, triple negative breast cancer, HER2 amplified breast cancer, squamous cell carcinoma of the head and neck (SCCHN), nasopharyngeal cancer, oral cancer, biliary tract, hepatocellular carcinoma, non-medullary thyroid carcinoma, recurrent glioblastoma multiforme, neurofibromatosis type 1, CNS cancer, liposarcoma; leiomyosarcoma; salivary gland cancer, mucosal melanoma; acral/ lentiginous melanoma, paraganglioma, and pheochromocytoma.
[00204] It is understood that diagnosis and treatment of a complex disease such as cancer is not performed by a single individual, test, agent, or intervention. For example, a subject may meet with a primary care physician to express a concern and be referred to an oncologist who will request tests that are designed, carried out, and analyzed by any of a number of individuals, but not limited to, radiologists, radiology technicians, physicists, phlebotomists, pathologists, laboratory technicians, and radiation, clinical, and surgical oncologists. Selection, dosing, and administration of agents to a subject diagnosed with cancer will be performed by any of a number of individuals including, but not limited to, radiologists, radiology technicians, physicists, pathologists, infusion nurses, pharmacists, and radiation, clinical, and surgical oncologists. Therefore, it is understood that within the terms of the invention, identifying a subject as having a specific level of hypoxia and a mutation in KRAS, EGFR, ALK, or a HER2+ breast cancer, or a triple negative breast cancer can include any of a number of acts including, but not limited to, performing a test and observing a result that is indicative of a subject having a specific level of hypoxia and a mutation in KRAS, EGFR, ALK, or a HER2+ breast cancer, or a triple negative breast cancer; reviewing a test result of a subject and identifying the subject as having a specific level of hypoxia and a mutation in KRAS, EGFR, ALK, or a HER2+ breast cancer, or a triple negative breast cancer; reviewing documentation on a subject stating that the subject has a specific level of hypoxia and a mutation in KRAS, EGFR, ALK, or a HER2+ breast cancer, or a triple negative breast cancer and identifying the subject as the one discussed in the documentation by confirming the identity of the subject, e.g., by an identification card, hospital bracelet, asking the subject for his/her name and/or other personal information to confirm the subjects identity.

[00205] Similarly, administering an agent can be performed by a number of people working in concert. Administering an agent includes, for example, prescribing an agent to be administered to a subject and/or providing instructions, directly or through another, to take a specific agent, either by self-delivery, e.g., as by oral delivery, subcutaneous delivery, intravenous delivery through a central line, etc.; or for delivery by a trained professional, e.g., intravenous delivery, intramuscular delivery, intratumoral delivery, etc.
As used herein, "detecting", "detection" and the like are understood as an assay performed for identification of a specific analyte in a sample, e.g., a gene or gene product with a mutation, or the expression level of a gene or gene product in a sample, or the hypoxia level in a sample, typically as compared to an appropriate control cell or tissue. The specific method of detection used is not a limitation of the invention. The detection method will typically include comparison to an appropriate control sample.

The term "control sample," as used herein, refers to any clinically relevant comparative sample, including, for example, a sample from a healthy subject not afflicted with cancer, a sample from a subject having a less severe or slower progressing cancer than the subject to be assessed, a sample from a subject having some other type of cancer or disease, a sample from a subject prior to treatment, a sample of non-diseased tissue (e.g., non-tumor tissue), a sample from the same origin and close to the tumor site, and the like. A control sample can be a purified sample, protein, and/or nucleic acid provided with a kit. Such control samples can be diluted, for example, in a dilution series to allow for quantitative measurement of analytes in test samples. A control sample may include a sample derived from one or more subjects. A control sample may also be a sample made at an earlier time point from the subject to be assessed. For example, the control sample could be a sample taken from the subject to be assessed before the onset of the cancer, at an earlier stage of disease, or before the administration of treatment or of a portion of treatment. The control sample may also be a sample from an animal model, or from a tissue or cell lines derived from the animal model, of the cancer. The level of signal detected or protein expression in a control sample that consists of a group of measurements may be determined, e.g., based on any appropriate statistical measure, such as, for example, measures of central tendency including average, median, or modal values.

Chemotherapeutic Agents

In certain embodiments, ganetespib is administered with one or more additional chemotherapeutic agents.
The taxanes are anti-cancer agents that include paclitaxel (Taxol®) and docetaxel (Taxotere®). Both drugs have proved to be effective in the treatment of a variety of solid tumors including breast, ovarian, lung, and bladder cancers. Thus, the term "paclitaxel analog" is defined herein to mean a compound which has the basic paclitaxel skeleton and which stabilizes microtubule formation. Many analogs of paclitaxel are known, including docetaxel. In addition, a paclitaxel analog can also be bonded to or be pendant from a pharmaceutically acceptable polymer, such as a polyacrylamide. The term "paclitaxel analog", as it is used herein, includes such polymer linked taxanes.

The term "vascular endothelial growth factor inhibitor" or "VEGF inhibitor" includes any compounds that disrupt the function of vascular endothelial growth factor A (VEGF) production within a cell. VEGF inhibitors are another class of anticancer agents. VEGF inhibitors include drugs such as bevacizumab (Avastin®), sunitinib (Sutent®), and sorafenib (Nexavar®). Examples of VEGF receptor inhibitors include sunitinib and sorafenib. Monoclonal antibody therapies, such as bevacizumab, that block VEGF are described in U.S. Patent Nos. 6,884,879, 7,060,269, and 7,297,334.

The dosages of other anti-cancer agents, which have been or are currently being used to prevent, treat, manage, or ameliorate disorders, such cancer, or one or more symptoms thereof can be used in the combination therapies of the invention. Preferably, dosages lower than those which have been or are currently being used to prevent, treat, manage, or ameliorate cancer, or one or more symptoms thereof, are used in the combination therapies of the invention. The recommended dosages of agents currently used for the prevention, treatment, management, or amelioration of cancer, or one or more symptoms thereof, can be obtained from any reference in the art including, but not limited to, Hardman et al., eds., 1996, Goodman & Gilman's The Pharmacological Basis Of Basis Of Therapeutics 9th Ed, Mc-Graw-Hill, New York; Physician's Desk Reference (PDR) 57th Ed., 2003, Medical Economics Co., Inc., Montvale, NJ.

An "effective amount" is that amount sufficient to treat a disease in a subject. A therapeutically effective amount can be administered in one or more administrations.

The term "effective amount" includes an amount of ganetespib which is sufficient to treat the cancer, to reduce or ameliorate the severity, duration, or progression of
cancer, to retard or halt the advancement of cancer, to cause the regression of cancer, to delay the recurrence, development, onset, or progression of a symptom associated with cancer, or to enhance or improve the therapeutic effect(s) of another therapy. For example, an effective amount can induce, for example, a complete response, a partial response, or stable disease; as determined, for example, using RESIST criteria.

[00214] An "effective amount" of a therapeutic agent produces a desired response. Having a positive response to treatment with a therapeutic agent is understood as having a decrease in at least one sign or symptom of a disease or condition (e.g., tumor shrinkage, decrease in tumor burden, inhibition or decrease of metastasis, improving quality of life ("QOL"), delay of time to progression ("TTP"), increase of overall survival ("OS"), etc.), or slowing or stopping of disease progression (e.g., halting tumor growth or metastasis, or slowing the rate of tumor growth or metastasis). It is understood that an "effective amount" need not be curative.

[00215] An effective amount of ganetespib is understood as an amount of ganetespib to improve outcome relative to an appropriate control group, e.g., an untreated group, a group treated with a combination of therapies not including ganetespib. Methods to select appropriate control groups and perform comparative analyses are within the ability of those of skill in the art.

[00216] The precise amount of compound administered to provide an "effective amount" of ganetespib to the subject will depend on the mode of administration, the type and severity of the cancer and on the characteristics of the subject, such as general health, age, sex, body weight and tolerance to drugs. The skilled artisan will be able to determine appropriate dosages depending on these and other factors. When administered in combination with other therapeutic agents, e.g., when administered in combination with an anti-cancer agent, an "effective amount" of any additional therapeutic agent(s) will depend on the type of drug used. Suitable dosages are known for approved therapeutic agents and can be adjusted by the skilled artisan according to the condition of the subject, the type of condition(s) being treated and the amount of a compound of the invention being used by following, for example, dosages reported in the literature and recommended in the

The dosage of an individual agent used in combination therapy may be equal to or lower than the dose of an individual therapeutic agent when given independently to treat, manage, or ameliorate a disease or disorder, or one or more symptoms thereof. In one embodiment, the disease or disorder being treated with a combination therapy is a triple-negative breast cancer.

In an embodiment, the amount of ganetespib administered is from about 2 mg/m² to about 500 mg/m², for example, from about 100 mg/m² to about 500 mg/m², from about 125 mg/m² to about 500 mg/m², from about 150 mg/m² to about 500 mg/m² or from about 175 mg/m² to about 500 mg/m². In an embodiment, the amount of ganetespib administered is about 100 mg/m² to about 300 mg/m², from about 125 mg/m² to about 300 mg/m², from about 150 mg/m² to about 300 mg/m² or from about 175 mg/m² to about 300 mg/m². In some embodiments, the amount of ganetespib administered is about 2 mg/m², 4 mg/m², about 7 mg/m², about 10 mg/m², about 14 mg/m², about 19 mg/m², about 23 mg/m², about 25 mg/m², about 33 mg/m², about 35 mg/m², about 40 mg/m², about 48 mg/m², about 49 mg/m², about 50 mg/m², about 65 mg/m², about 75 mg/m², about 86 mg/m², about 100 mg/m², about 110 mg/m², about 114 mg/m², about 120 mg/m², about 144 mg/m², about 150 mg/m², about 173 mg/m², about 180 mg/m², about 200 mg/m², about 216 mg/m² or about 259 mg/m².

The language "twice-weekly" includes administration of ganetespib two times in about 7 days. For example, the first dose of ganetespib is administered on day 1, and the second dose of ganetespib may be administered on day 2, day 3, day 4, day 5, day 6 or day 7. In some embodiments, the twice-weekly administration occurs on days 1 and 2, days 1 and 3 or days 1 and 4.

In some embodiments, ganetespib is cyclically administered twice-weekly. For example, ganetespib is administered for a first period of time, followed by a "dose-free" period, then administered for a second period of time. The language "dose-free" includes the period of time in between the first dosing period and the second dosing period in which no ganetespib is administered to the subject. A preferred cycle is administering ganetespib at a dose described above two times during the week for three consecutive weeks followed by one dose-free week. This cycle is then repeated, as described below.
The language "one cycle" includes the first period of time during which ganetespib is administered, followed by a dose-free period of time. The dosing cycle can be repeated and one of skill in the art will be able to determine the appropriate length of time for such a cyclical dosing regimen. In an embodiment, the cycle is repeated at least once. In an embodiment, the cycle is repeated two or more times. In an embodiment, the cycle is repeated 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or more times, or as many times as medically necessary as determined by one of skill in the art, e.g., as long as the subject exhibits a response with no dose limiting toxicities. In an embodiment, the cycle is repeated until the patient has been determined to be in partial remission (e.g., 50% or greater reduction in the measurable parameters of tumor growth) or complete remission (e.g., absence of cancer). One of skill in the art can determine a patient's remission status using routine methods well known in the art.

The language "pharmaceutically acceptable salt" includes salts prepared from ganetespib by reacting the phenolic functional groups and a pharmaceutically acceptable inorganic or organic base. Suitable bases include hydroxides of alkali metals such as sodium, potassium, and lithium; hydroxides of alkaline earth metal such as calcium and magnesium; hydroxides of other metals, such as aluminum and zinc; ammonia, and organic amines, such as unsubstituted or hydroxy-substituted mono-, di-, or trialkylamines; dicyclohexylamine; tributyl amine; pyridine; N-methyl,N-ethylamine; diethylamine; triethylamine; mono-, bis-, or tris-(2-hydroxy-lower alkyl amines), such as mono-, bis-, or tris-(2-hydroxyethyl)amine, 2-hydroxy-tert-butylamine, or tris-(hydroxymethyl)methylamine, N,N-di-lower alkyl-N-(hydroxy lower alkyl)-amines, such as N,N-dimethyl-N-(2-hydroxyethyl)amine, or tri-(2-hydroxyethyl)amine; N-methyl-D-glucamine; and amino acids such as arginine, lysine, and the like. A pharmaceutically acceptable salt can also be formed by reacting the amine functional groups and a pharmaceutically acceptable inorganic or organic acid. Suitable acids include hydrogen sulfate, citric acid, acetic acid, oxalic acid, hydrochloric acid (HQ), hydrogen bromide (HBr), hydrogen iodide (HI), nitric acid, hydrogen bisulfide, phosphoric acid, isonicotinic acid, oleic acid, tannic acid, pantothenic acid, saccharic acid, lactic acid, salicylic acid, tartaric acid, bitartratic acid, ascorbic acid, succinic acid, maleic acid, besylic acid, fumaric acid, gluconic acid, and other pharmaceutically acceptable acids known in the art.
acid, glucaronic acid, formic acid, benzoic acid, glutamic acid, methanesulfonic acid, ethanesulfonic acid, benzenesulfonic acid, palmoic acid and p-toluenesulfonic acid.

[00223] The language "tautomer of ganetespib" includes all tautomeric forms of ganetespib, or pharmaceutically acceptable salts thereof.

[00224] As used herein, the term "in combination" refers to the use of more than one therapeutic agent (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, or more). The use of the term "in combination" does not restrict the order in which the therapeutic agents are administered to a subject afflicted with cancer. A first therapeutic agent, such as a compound described herein, can be administered prior to (e.g., 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks before), concomitantly with, or subsequent to (e.g., 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks after) the administration of a second therapeutic agent or treatment, such as an anti-cancer agent, to a subject with cancer. In certain embodiments, one agent may be administered more frequently than the other agent such that multiple doses of one agent are administered for each dose of the other agent(s).

[00225] In an embodiment, the method comprises administering to the subject with cancer with a mutation in KRAS and a high level of hypoxia an effective amount of a combination of ganetespib, or a tautomer or a pharmaceutically acceptable salt thereof, and one or more of BEZ235, AZD6244, AZD8055, SN-38, gemcitabine, camptothecin, docetaxel, cisplatin, oxaliplatin, crizotinib, paclitaxel, trastuzumab, and pemetrexed. In an embodiment, the method is in combination with BEZ235. In an embodiment, the combination is with AZD6244. In an embodiment, the combination is with AZD8055. In an embodiment, the combination is with SN-38. In an embodiment, the combination is with gemcitabine. In an embodiment, the combination is with camptothecin. In an embodiment, the combination is with docetaxel. In an embodiment, the combination is with cisplatin. In an embodiment, the combination is with oxaliplatin. In an embodiment, the combination is with crizotinib. In an embodiment, the combination is with trastuzumab. In an embodiment, the combination is with pemetrexed.
In an embodiment, ganetespib may be used in combination with one or more additional anti-cancer agents for treatment of a subject with a NSCLC with a mutation in KRAS and a high level of hypoxia. In an embodiment, the method comprises administering to the subject with NSCLC with a mutation in KRAS and a high level of hypoxia an effective amount of a combination of ganetespib, or a tautomer or a pharmaceutically acceptable salt thereof, and one or more of BEZ235, AZD6244, AZD8055, SN-38, gemcitabine, camptothecin, docetaxel, cisplatin, oxaliplatin, crizotinib, paclitaxel, trastuzumab, or pemetrexed. In an embodiment, the combination is with BEZ235. In an embodiment, the combination is with AZD6244. In an embodiment, the combination is with AZD8055. In an embodiment, the combination is with SN-38. In an embodiment, the combination is with gemcitabine. In an embodiment, the combination is with docetaxel. In an embodiment, the combination is with cisplatin. In an embodiment, the combination is with oxaliplatin. In an embodiment, the combination is with crizotinib. In an embodiment, the combination is with trastuzumab. In an embodiment, the combination is with pemetrexed.

In an embodiment, the method comprises administering to the subject with a cancer with a mutation in ALK and a high level of hypoxia an effective amount of a combination of ganetespib, or a tautomer or a pharmaceutically acceptable salt thereof, and one or more of BEZ235, AZD6244, AZD8055, SN-38, gemcitabine, camptothecin, docetaxel, cisplatin, oxaliplatin, crizotinib, paclitaxel, trastuzumab, or pemetrexed. In an embodiment, the combination is with BEZ235. In an embodiment, the combination is with AZD6244. In an embodiment, the combination is with AZD8055. In an embodiment, the combination is with SN-38. In an embodiment, the combination is with gemcitabine. In an embodiment, the combination is with docetaxel. In an embodiment, the combination is with cisplatin. In an embodiment, the combination is with oxaliplatin. In an embodiment, the combination is with crizotinib. In an embodiment, the combination is with trastuzumab. In an embodiment, the combination is with pemetrexed.

In an embodiment, ganetespib may be administered for treating NSCLC with a mutation in ALK and a high level of hypoxia in a subject in combination with one or more additional anti-cancer agents. In an embodiment, the method comprises administering to
the subject with NSCLC with a mutation in ALK and a high level of hypoxia an effective amount of a combination of ganetespib, or a tautomer or a pharmaceutically acceptable salt thereof, and one or more of BEZ235, AZD6244, AZD8055, SN-38, gemcitabine, camptothecin, docetaxel, cisplatin, oxaliplatin, crizotinib, paclitaxel, trastuzumab, or pemetrexed. In an embodiment, the combination with BEZ235. In an embodiment, the combination is with AZD6244. In an embodiment, the combination is with AZD8055. In an embodiment, the combination is with SN-38. In an embodiment, the combination is with gemcitabine. In an embodiment, the combination is with camptothecin. In an embodiment, the combination is with docetaxel. In an embodiment, the combination is with cisplatin. In an embodiment, the combination is with oxaliplatin. In an embodiment, the combination is with crizotinib. In an embodiment, the combination with trastuzumab. In an embodiment, the combination is with pemetrexed.

[00229] In an embodiment, the one or more additional anti-cancer agents include one or more of VEGF inhibitors (e.g., bevacizumab, sunitinib, or sorafenib), EGFR inhibitors (e.g., erlotinib, gefitinib or cetuximab), tyrosine kinase inhibitors (e.g., imatinib), proteosome inhibitors (e.g., bortezomib), taxanes (e.g., paclitaxel and paclitaxel analogues), and ALK inhibitors (e.g., crizotinib). In an embodiment, the additional anticancer drug is trastuzumab.

[00230] Ganetespib and optionally, one or more additional anti-cancer agents, can be administered to a subject by routes known to one of skill in the art. Examples of routes of administration include, but are not limited to, parenteral, e.g., intravenous, intradermal, subcutaneous, oral, intranasal (e.g., inhalation), transdermal, topical, transmucosal, and rectal administration. The agents can be administered by different routes of administration.

[00231] Ganetespib, and optionally, one or more additional anti-cancer agents, may be formulated with a pharmaceutically acceptable carrier, diluent, or excipient as a pharmaceutical composition. Pharmaceutical compositions and dosage forms of the invention comprise one or more active ingredients in relative amounts and formulated in such a way that a given pharmaceutical composition or dosage form can be used to treat cancer. Administration in combination does not require co-formulation.
[00232] A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. In an embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous, subcutaneous, intramuscular, oral, intranasal, or topical administration to human beings. In some embodiments, ganetespib is formulated at a concentration of 8 mg/mL in 90% v/v PEG 300 and 10% v/v Polysorbate 80 for intravenous administration.

[00233] In certain embodiments, the invention also provides the use of ganetespib or a pharmaceutically acceptable salt thereof for the manufacture of a medicament for the treatment of a subject with a high level of hypoxia. In certain embodiments, the invention further provides the use of ganetespib or a pharmaceutically acceptable salt thereof for the manufacture of a medicament for the treatment of a subject with a cancer with a high level of hypoxia in combination with one or more of BEZ235, AZD6244, AZD8055, SN-38, gemcitabine, camptothecin, docetaxel, cisplatin, oxaliplatin, crizotinib, paclitaxel, trastuzumab, and pemetrexed.

[00234] In certain embodiments, the invention also provides the use of ganetespib or a pharmaceutically acceptable salt thereof for the manufacture of a medicament for the treatment of a subject with a cancer with a mutation in KRAS. In certain embodiments, the invention further provides the use of ganetespib or a pharmaceutically acceptable salt thereof for the manufacture of a medicament for the treatment of a subject with a cancer with a mutation KRAS in combination with one or more of BEZ235, AZD6244, AZD8055, SN-38, gemcitabine, camptothecin, docetaxel, cisplatin, oxaliplatin, crizotinib, paclitaxel, trastuzumab, and pemetrexed.

[00235] In certain embodiments, the invention also provides the use of ganetespib or a pharmaceutically acceptable salt thereof for the manufacture of a medicament for the treatment of a subject with a cancer with a mutation in KRAS and with a high level of hypoxia. In certain embodiments, the invention further provides the use of ganetespib or a pharmaceutically acceptable salt thereof for the manufacture of a medicament for the treatment of a subject with a cancer with a mutation in KRAS and a high level of hypoxia in combination with one or more of BEZ235, AZD6244, AZD8055, SN-38, gemcitabine,
camptothecin, docetaxel, cisplatin, oxaliplatin, crizotinib, paclitaxel, trastuzumab, and pemetrexed.

[00236] In certain embodiments, the invention also provides the use of ganetespib or a pharmaceutically acceptable salt thereof for the manufacture of a medicament for the treatment of a subject with a NSCLC with a high level of hypoxia. In certain embodiments, the invention further provides the use of ganetespib or a pharmaceutically acceptable salt thereof for the manufacture of a medicament for the treatment of a subject with a NSCLC with a high level of hypoxia in combination with one or more of BEZ235, AZD6244, AZD8055, SN-38, gemcitabine, camptothecin, docetaxel, cisplatin, oxaliplatin, crizotinib, paclitaxel, trastuzumab, and pemetrexed.

[00237] In certain embodiments, the invention also provides the use of ganetespib or a pharmaceutically acceptable salt thereof for the manufacture of a medicament for the treatment of a subject with a NSCLC with a mutation in KRAS. In certain embodiments, the invention further provides the use of ganetespib or a pharmaceutically acceptable salt thereof for the manufacture of a medicament for the treatment of a subject with a NSCLC with a mutation in KRAS in combination with one or more of BEZ235, AZD6244, AZD8055, SN-38, gemcitabine, camptothecin, docetaxel, cisplatin, oxaliplatin, crizotinib, paclitaxel, trastuzumab, and pemetrexed.

[00238] In certain embodiments, the invention also provides the use of ganetespib or a pharmaceutically acceptable salt thereof for the manufacture of a medicament for the treatment of a subject with a NSCLC with a mutation in KRAS and with a high level of hypoxia. In certain embodiments, the invention further provides the use of ganetespib or a pharmaceutically acceptable salt thereof for the manufacture of a medicament for the treatment of a subject with a NSCLC with a mutation in KRAS and a high level of hypoxia in combination with one or more of BEZ235, AZD6244, AZD8055, SN-38, gemcitabine, camptothecin, docetaxel, cisplatin, oxaliplatin, crizotinib, paclitaxel, trastuzumab, and pemetrexed.

[00239] In certain embodiments, the invention also provides ganetespib or a pharmaceutically acceptable salt thereof for use in treating a subject with a cancer with a high level of hypoxia. In certain embodiments, the invention further provides ganetespib or
a pharmaceutically acceptable salt thereof for use in treating a subject with a cancer with a high level of hypoxia in combination with one or more of BEZ235, AZD6244, AZD8055, SN-38, gemcitabine, camptothecin, docetaxel, cisplatin, oxaliplatin, crizotinib, paclitaxel, trastuzumab, and pemetrexed.

[00240] In certain embodiments, the invention also provides ganetespib or a pharmaceutically acceptable salt thereof for use in treating a subject with a cancer with a mutation in KRAS. In certain embodiments, the invention further provides ganetespib or a pharmaceutically acceptable salt thereof for use in treating a subject with a cancer with a mutation in KRAS in combination with one or more of BEZ235, AZD6244, AZD8055, SN-38, gemcitabine, camptothecin, docetaxel, cisplatin, oxaliplatin, crizotinib, paclitaxel, trastuzumab, and pemetrexed.

[00241] In certain embodiments, the invention also provides ganetespib or a pharmaceutically acceptable salt thereof for use in treating a subject with a cancer with a mutation in KRAS and a high level of hypoxia. In certain embodiments, the invention further provides ganetespib or a pharmaceutically acceptable salt thereof for use in treating a subject with a cancer with a mutation in KRAS and a high level of hypoxia in combination with one or more of BEZ235, AZD6244, AZD8055, SN-38, gemcitabine, camptothecin, docetaxel, cisplatin, oxaliplatin, crizotinib, paclitaxel, trastuzumab, and pemetrexed.

[00242] In certain embodiments, the invention also provides ganetespib or a pharmaceutically acceptable salt thereof for use in treating a subject with a NSCLC with a high level of hypoxia. In certain embodiments, the invention further provides ganetespib or a pharmaceutically acceptable salt thereof for use in treating a subject with NSCLC with a high level of hypoxia in combination with one or more of BEZ235, AZD6244, AZD8055, SN-38, gemcitabine, camptothecin, docetaxel, cisplatin, oxaliplatin, crizotinib, paclitaxel, trastuzumab, and pemetrexed.

[00243] In certain embodiments, the invention also provides ganetespib or a pharmaceutically acceptable salt thereof for use in treating a subject with a NSCLC with a mutation in KRAS. In certain embodiments, the invention further provides ganetespib or a pharmaceutically acceptable salt thereof for use in treating a subject with NSCLC with a mutation in KRAS in combination with one or more of BEZ235, AZD6244, AZD8055, SN-38,
gemcitabine, camptothecin, docetaxel, cisplatin, oxaliplatin, crizotinib, paclitaxel, trastuzumab, and pemetrexed.

[00244] In certain embodiments, the invention also provides ganetespib or a pharmaceutically acceptable salt thereof for use in treating a subject with a NSCLC with a mutation in KRAS and a high level of hypoxia. In certain embodiments, the invention further provides ganetespib or a pharmaceutically acceptable salt thereof for use in treating a subject with NSCLC with a mutation in KRAS and a high level of hypoxia in combination with one or more of BEZ235, AZD6244, AZD8055, SN-38, gemcitabine, camptothecin, docetaxel, cisplatin, oxaliplatin, crizotinib, paclitaxel, trastuzumab, and pemetrexed.

[00245] In certain embodiments, the invention also provides the use of ganetespib or a pharmaceutically acceptable salt thereof for the manufacture of a medicament for the treatment of a subject with a cancer with a mutation in EGFR and a high level of hypoxia. In certain embodiments, the invention further provides the use of ganetespib or a pharmaceutically acceptable salt thereof for the manufacture of a medicament for the treatment of a subject with a cancer with a mutation in EGFR and a high level of hypoxia in combination with one or more of BEZ235, AZD6244, AZD8055, SN-38, gemcitabine, camptothecin, docetaxel, cisplatin, oxaliplatin, crizotinib, paclitaxel, trastuzumab, and pemetrexed.

[00246] In certain embodiments, the invention also provides the use of ganetespib or a pharmaceutically acceptable salt thereof for the manufacture of a medicament for the treatment of a subject with a NSCLC with a mutation in EGFR and a high level of hypoxia. In certain embodiments, the invention further provides the use of ganetespib or a pharmaceutically acceptable salt thereof for the manufacture of a medicament for the treatment of a subject with a NSCLC with a mutation in EGFR and a high level of hypoxia in combination with one or more of BEZ235, AZD6244, AZD8055, SN-38, gemcitabine, camptothecin, docetaxel, cisplatin, oxaliplatin, crizotinib, paclitaxel, trastuzumab, and pemetrexed.

[00247] In certain embodiments, the invention also provides ganetespib or a pharmaceutically acceptable salt thereof for use in treating a subject with a cancer with a mutation in EGFR and a high level of hypoxia. In certain embodiments, the invention also
provides ganetespib or a pharmaceutically acceptable salt thereof for use in treating a subject with a cancer with a mutation in EGFR and a high level of hypoxia in combination with one or more of BEZ235, AZD6244, AZD8055, SN-38, gemcitabine, camptothecin, docetaxel, cisplatin, oxaliplatin, crizotinib, paclitaxel, trastuzumab, and pemetrexed.

[00248] In certain embodiments, the invention also provides ganetespib or a pharmaceutically acceptable salt thereof for use in treating a subject with a NSCLC with a mutation in EGFR and a high level of hypoxia. In certain embodiments, the invention also provides ganetespib or a pharmaceutically acceptable salt thereof for use in treating a subject with a NSCLC with a mutation in EGFR and a high level of hypoxia in combination with one or more of BEZ235, AZD6244, AZD8055, SN-38, gemcitabine, camptothecin, docetaxel, cisplatin, oxaliplatin, crizotinib, paclitaxel, trastuzumab, and pemetrexed.

[00249] In certain embodiments, the invention also provides the use of ganetespib or a pharmaceutically acceptable salt thereof for the manufacture of a medicament for the treatment of a subject with cancer with a mutation in ALK and with a high level of hypoxia. In certain embodiments, the invention further provides the use of ganetespib or a pharmaceutically acceptable salt thereof for the manufacture of a medicament for the treatment of a subject with a cancer with a mutation in ALK and with a high level of hypoxia in combination with one or more of BEZ235, AZD6244, AZD8055, SN-38, gemcitabine, camptothecin, docetaxel, cisplatin, oxaliplatin, crizotinib, paclitaxel, trastuzumab, and pemetrexed.

[00250] In certain embodiments, the invention also provides the use of ganetespib or a pharmaceutically acceptable salt thereof for the manufacture of a medicament for the treatment of a subject with NSCLC cancer with a mutation in ALK and with a high level of hypoxia. In certain embodiments, the invention further provides the use of ganetespib or a pharmaceutically acceptable salt thereof for the manufacture of a medicament for the treatment of a subject with an ALK+ NSCLC in combination with one or more of BEZ235, AZD6244, AZD8055, SN-38, gemcitabine, camptothecin, docetaxel, cisplatin, oxaliplatin, crizotinib, paclitaxel, trastuzumab, and pemetrexed.

[00251] In certain embodiments, the invention also provides ganetespib or a pharmaceutically acceptable salt thereof for use in treating a subject with cancer with a
mutation in ALK and with a high level of hypoxia. In certain embodiments, the invention also provides ganetespib or a pharmaceutically acceptable salt thereof for use in treating a subject with cancer with a mutation in ALK and with a high level of hypoxia in combination with one or more of BEZ235, AZD6244, AZD8055, SN-38, gemcitabine, camptothecin, docetaxel, cisplatin, oxaliplatin, crizotinib, paclitaxel, trastuzumab, and pemetrexed.

[00252] In certain embodiments, the invention also provides ganetespib or a pharmaceutically acceptable salt thereof for use in treating a subject with a cancer with a mutation in ALK and with a high level of hypoxia. In certain embodiments, the invention also provides ganetespib or a pharmaceutically acceptable salt thereof for use in treating a subject with NSCLC with a mutation in ALK and with a high level of hypoxia in combination with one or more of BEZ235, AZD6244, AZD8055, SN-38, gemcitabine, camptothecin, docetaxel, cisplatin, oxaliplatin, crizotinib, paclitaxel, trastuzumab, and pemetrexed.

[00253] In certain embodiments, the invention also provides the use of ganetespib or a pharmaceutically acceptable salt thereof for the manufacture of a medicament for the treatment of a subject with triple negative breast cancer and with a high level of hypoxia. In certain embodiments, the invention further provides the use of ganetespib or a pharmaceutically acceptable salt thereof for the manufacture of a medicament for the treatment of a subject with triple negative breast cancer and a high level of hypoxia in combination with one or more of BEZ235, AZD6244, AZD8055, SN-38, gemcitabine, camptothecin, docetaxel, cisplatin, oxaliplatin, crizotinib, paclitaxel, trastuzumab, and pemetrexed.

[00254] In certain embodiments, the invention also provides ganetespib or a pharmaceutically acceptable salt thereof for use in treating a subject with triple negative breast cancer and with a high level of hypoxia. In certain embodiments, the invention also provides ganetespib or a pharmaceutically acceptable salt thereof for use in treating a subject with triple negative breast cancer and with a high level of hypoxia in combination with one or more of BEZ235, AZD6244, AZD8055, SN-38, gemcitabine, camptothecin, docetaxel, cisplatin, oxaliplatin, crizotinib, paclitaxel, trastuzumab, and pemetrexed.
The invention is illustrated by the following examples, which are not intended to be limiting in any way.

EXAMPLES

Example 1—A Phase I dose escalation study of ganetespib in twice-weekly administration in patients with solid tumors

An open-label Phase I dose-escalation study in subjects with solid tumors was performed. The first cohort consisted of three subjects who received 2 mg/m\(^2\) of ganetespib during a 1-hour infusion 2 times per week (e.g., [Monday, Thursday] or [Tuesday, Friday]) for three consecutive weeks followed by a 1 week dose-free interval. The first infusion for the first three subjects was staggered by a minimum of 5 days between subjects. This staggered enrollment scheme was followed for the first cohort only. Subjects tolerating ganetespib continued treatment past week 8 until disease progression as long as the re-treatment criteria continued to be met.

Subsequent cohorts were originally planned to receive escalating doses of 4, 7, 10, 14, 19, 25, 33, 40 and 48 mg/m\(^2\), provided that the previous dose was well-tolerated during cycle 1 (week 1–4). The dose escalation scheme was updated to 25, 50, 75 and 100 mg/m\(^2\), provided that the previous dose was well-tolerated during cycle 1. Following the completion of enrollment at 50 mg/m\(^2\) twice per week, subsequent cohorts were to be treated at 100 mg/m\(^2\) (100% increase above prior dose) and 120 mg/m\(^2\) (20% increase above prior dose), with further dose increments to be approximately 20% over the previous dose level, until the maximum tolerated dose (MTD) was determined. Enrollment was completed at 100 mg/m\(^2\) and the next doses planned were 120 mg/m\(^2\) and 144 mg/m\(^2\).

There had to be at least three evaluable subjects in a cohort before dose escalation could occur. An evaluable subject was defined as one who had received at least 5 of 6 doses of ganetespib during cycle 1 and had a subsequent follow up visit or experienced a dose limiting toxicity (DLT) after any dose. Once a subject experienced a DLT the cohort was expanded to six subjects. If only 1 of 6 subjects experienced a DLT, further dose escalation was allowed. However, if 2 of 3 or 2 of 6 subjects experienced a DLT, dose escalation terminated.
A subject's duration of participation included a 2-week screening period and two 4-week treatment cycles totaling approximately 10 weeks. However, at the investigator's discretion, subjects tolerating ganetespib continued treatment past week 8 until disease progression.

The subjects in this study had histologically- or cytologically-confirmed non-hematological malignancy that was metastatic or unresectable. The subjects were documented to be refractory to, or were not candidates for, current standard therapy.

Ganetespib was formulated using 90% v/v PEG 300 and 10% v/v Polysorbate 80 at a concentration of 8 mg/mL and was packaged in a Type I glass amber vial, stoppered with a Flurotec®-coated stopper, and sealed. Each vial had a deliverable volume of 12.5 mL (equivalent to 100 mg/vial). The formulation was further diluted with 5% dextrose for injection in infusion container (DEHP-free 500mL) to a concentration range of 0.02 to 1.2 mg/mL and administered via infusion tubing (DEHP-free) with a 0.22 micron end filter over an hour to the patient. The dosing solution once prepared was administered within 3 hours. Eligible subjects received the drug during a 1-hour infusion 2 times per week for 3 consecutive weeks followed by a 1-week dose-free interval. The amount of ganetespib administered depended upon the cohort to which the subject was assigned and the subject's body surface area (BSA). This cycle was repeated for subjects tolerating ganetespib who did not experience disease progression.

Forty-one of 54 enrolled patients were assessable for response. A total of 13 patients discontinued prior to the Week 8 response assessment. Confirmed partial responses included 1 patient with melanoma and 1 patient with triple negative breast cancer. Fifteen (15) patients achieved stable disease.

Example 2 - Efficacy of ganetespib in the treatment of triple negative breast cancer

Triple-negative breast cancer (TNBC) represents 10-20% of all diagnosed breast cancer cases and tests negative for the presence of estrogen receptor (ER), progesterone receptor (PR), and the human epidermal growth factor receptor 2 (HER2). Therefore, this breast cancer subtype does not respond to hormonal therapy used to treat breast cancer, such as tamoxifen or aromatase inhibitors, or therapies that target HER2 receptors, such as...
Herceptin®. Triple-negative breast cancer is characterized as more aggressive than other breast cancer subtypes, disproportionately affects younger women, and is associated with a poorer 5-year survival rate of 77%, as compared to the 93% survival rate for other cancers. Triple-negative breast cancer is typically treated with a combination of therapies such as surgery, radiation therapy, and chemotherapy, however, early relapse and metastasis is common.

[00264] A 39-year old white female with triple negative breast cancer (with Stage III invasive ductal carcinoma) enrolled in a Phase I dose escalation study of ganetespib described in Example 1. The patient's disease had progressed on 7 prior chemotherapeutical regimens. The patient was administered 144 mg/m² of ganetespib twice-weekly for 3 weeks, followed by 1 week dose-free. After 2 cycles, she demonstrated stable disease per Response Evaluation Criteria in Solid Tumors (RECIST). Figure 10 shows a PET/CT scan of the subject.

[00265] After 4 cycles, there was a documented 31% reduction in target lesion size (partial response). The treatment was interrupted due to brain metastases treated with whole brain radiation, but treatment with ganetespib resumed in cycle 5. The patient tolerated the treatment well with mild/moderate toxicities.

Example 3 - Efficacy of ganetespib in the treatment of non-small cell lung cancer (NSCLC) by KRAS, EGFR, and ALK mutation status

[00266] A Phase 2 clinical trial was performed to determine the efficacy of ganetespib in the treatment of NSCLC.

[00267] Patients with advanced NSCLC who failed prior treatments received 200 mg/m² of ganetespib as a 1-hr infusion once weekly for 3 of a 4-week cycle in a Simon two-stage study design assessing primary endpoint of PFS rate at 16 wks. Initial cohorts were defined by mutation status: A) EGFR mutation, KRAS wild-type B) EGFR wild-type, KRAS mutation C) EGFR wild-type, KRAS wild-type (WT). If ≥2/14 patients in A, B or C were progression-free at 16 weeks, enrollment increased to 23 patients for that cohort. Tumor response was assessed every 8 weeks. Cohort D was added to include 35 additional EGFR wild-type and KRAS wild-type patients with adenocarcinoma histology. Additional mutational analysis of
BRAF, PIK3CA, ERBB2 and MET, as well as FISH analysis for ALK translocation, were performed for Cohorts C and D.

[00268] There were 73 patients in the study (31 M, 42 F; median age 62 years, range 28-82; ECOG 0-1; prior therapies range 1-10) that received a median of 2 cycles (range 1-12) of ganetespib in cohorts A (14), B (17), and C+D (42). Adverse events reported in ≥20% of patients included diarrhea, fatigue, nausea, anorexia, constipation, and dyspnea and were generally grade 1-2.

[00269] In Cohort B, subjects with a wild-type EGFR and a KRAS mutation, greater than 60% of patients with NSCLC exhibited tumor shrinkage at 8 weeks, indicating that ganetespib is useful in the treatment of NSCLC with a KRAS mutation.

[00270] Expansion criteria were achieved for cohort C, including a durable partial response (PR) and seven patients with prolonged stable disease (>16 weeks).

[00271] Of the 23 patients of cohorts C and D (EGFR wild-type, KRAS wild-type) in the Phase 2 trial tested for ALK translocation or rearrangement (ALK+), eight patients were ALK+ in at least one assay. Six of these eight ALK+ patients (75%) showed tumor shrinkage in target lesions. One ALK+ patient showed no change in tumor size, and one ALK+ patient achieved stable disease (tumor growth <20%). The disease control rate in this population was 7/8 (88%), and the objective response rate (complete response (CR) + partial response (PR)) was 4/8 (50%) (See Figure 6).

[00272] In summary, ganetespib administered as a single-agent was well-tolerated in patients with NSCLC at 200 mg/m² once weekly without severe liver, ocular, cardiovascular or renal toxicity. Clinical activity was observed in patients with advanced NSCLC tumors with both a wild-type EGFR and a KRAS mutation; a wild-type EGFR and a wild-type KRAS. Clinical activity was observed in patients with ALK+ NSCLC tumors (i.e., tumors with an ALK mutation). This demonstrates the utility of ganetespib for the treatment of NSCLC with various mutations.
Example 4 — Efficacy of ganetespib in a Phase 2 study for the treatment of gastrointestinal stromal tumors (GIST)

[00273] A gastrointestinal stromal tumor (GIST) is a type of cancer that occurs in the gastrointestinal (GI or digestive) tract, including the esophagus, stomach, gall bladder, liver, small intestine, colon, and rectum. The American Cancer Society estimates 4,500 to 6,000 GIST cases are diagnosed each year in the United States. Although these tumors can start anywhere in the GI tract, they occur most often in the stomach (50% to 70%) or the small intestine (20% to 30%). Gastric cancer is second to lung cancer as the most lethal cancer worldwide, with 5-year survival rates in the range of 10% to 15%.

[00274] Patients with advanced (e.g., metastatic or unresectable) GIST following failure of prior therapy, e.g., imatinib or sunitinib, received ganetespib (200 mg/m\(^2\)) as a 1 hour IV infusion once per week for 3 weeks of a 28 day cycle. GIST status was assessed at 8 weeks per RECIST, until progression. In this Simon's 2 stage study design, if \( \geq 4/23 \) patients in Stage 1 had clinical benefit (CR + PR + stable disease (SD) \( \geq 16 \) weeks) enrolment would continue with Stage 2. Hsp90 client protein levels were analyzed in biopsies pre-therapy and 24-48 h post-treatment with ganetespib in a subset of patients.

[00275] There were 26 patients (15 M, 11 F; median age 53 years, range 33-67; ECOG status 0-1; median 5 prior therapy regimens, range 3-12, wild-type platelet derived growth factor receptor alpha (PDGFR\(A\)) that received a median of 2 cycles of ganetespib (range 1-8). Adverse events reported in \( \geq 20\% \) of patients were generally NCI CTC grade 1-2 and included diarrhea, fatigue, nausea, vomiting, increased alkaline phosphatase, headache, insomnia, and abdominal pain. 12/23 evaluable patients had SD (4 SD \( \geq 16 \) weeks, 8 SD \( \geq 8 \) wks), meeting formal criteria to enroll Stage 2. However, analysis of client proteins in paired tumor biopsies from 4 patients did not show prolonged inhibition of activated KIT or its downstream pathways.

[00276] In summary, ganetespib given by once-weekly dosing was well-tolerated in patients with heavily pre-treated advanced GIST, with no evidence of severe liver, ocular, cardiac or renal toxicity. Disease stabilization was seen in a subset of patients. These results demonstrate the utility of ganetespib in the treatment of GIST.
Example 5-- Efficacy of ganetespib in a Phase 2 study for the treatment of solid tumors

[00277] A phase 2 study of ganetespib was performed to determine its efficacy in the treatment of solid tumors.

[00278] Patients with solid tumors who had exhausted standard treatment options received ganetespib as a 1 hr infusion twice-weekly for 3 weeks of a 28 day cycle until disease progression. Serial PK and pharmacodynamic samples were obtained during cycle 1. Safety assessments included frequency and grade of adverse events (AEs), laboratory parameters and ECG changes.

[00279] Data were presented for 49 patients (22 M, 27 F; median age 55 years, range 32-81; ECOG status range 0-2) treated at doses from 2-144 mg/m². Patients received a median of 2 (range 1-12) cycles of ganetespib. AEs reported in ≥20% of patients treated at doses from 2-120 mg/m² were fatigue, diarrhea, nausea, anemia, abdominal pain, constipation, anorexia, vomiting, and headache; the majority of events were mild to moderate in severity with absence of severe liver, ocular, cardiac and renal toxicity. Two DLTs (elevated transaminases) were reported in the 10 and 144 mg/m² cohorts. Ganetespib showed linear PK, rapid distribution, a mean terminal half-life of 10-14 hours, a volume of distribution greater than total body water and no accumulation in plasma. A confirmed durable PR by RECIST was seen in a patient with metastatic melanoma. Additionally, 2 NSCLC patients who received 6 months of treatment had durable SD, with tumor shrinkage.

[00280] In summary, ganetespib was well-tolerated administered twice-weekly. Preliminary safety profile, activity signals and differences in client protein kinetics warrant continued evaluation of ganetespib using a twice-weekly dosing regimen.

Example 6-- A Phase 2 trial of ganetespib: efficacy and safety in patients with metastatic breast cancer (MBC)

[00281] A phase 2 trial was performed to determine the safety and efficacy of ganetespib in the treatment of subjects with metastatic breast cancer.

[00282] Patients with locally advanced or MBC were treated with single agent of ganetespib at 200mg/m³ on a cycle of once weekly for 3 weeks, one week off, on a 28 day cycle.
cycle. The primary endpoint of the trial was overall response rate using RECIST 1.1. Patients with HER2+ breast cancer were required to have received prior therapy with trastuzumab. No more than 3 lines of chemotherapy in the metastatic setting were permitted, but there was no limit on prior lines of hormone therapy. Patients were evaluated for response after 2 cycles. The trial used a Simon two-stage design requiring at least 3 responses among the first 22 patients, to allow expansion to a total of 40 patients.

A total of 22 patients were treated with a median age of 51 years (38 to 70) and the following subtypes: 13 HER2+ (10 ER+/HER2+; 3 ER-/HER2+), 6 ER+/HER2-, and 3 ER-/PR-/HER2- (TNBC).

Prior treatment regimens are summarized as follows:

<table>
<thead>
<tr>
<th>Prior lines of chemotherapy in metastatic setting</th>
<th>Number of Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>2</td>
<td>9</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Prior lines of trastuzumab in metastatic setting</th>
<th>Number of Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
</tr>
</tbody>
</table>

The responses of the subjects by ER, PR, and HER2 status are summarized in the table below.

<table>
<thead>
<tr>
<th>Response</th>
<th>Total (N=22)*</th>
<th>ER+/HER2- (N=6)</th>
<th>HER2+ (N=13)*</th>
<th>TNBC (N=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ORR</td>
<td>2 (9%)</td>
<td>0</td>
<td>2 (9%)</td>
<td>0</td>
</tr>
<tr>
<td>CR</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>PR</td>
<td>2 (9%)</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>SD</td>
<td>7 (32%)</td>
<td>0</td>
<td>6 (27%)</td>
<td>1 (5%)</td>
</tr>
<tr>
<td>CBR*</td>
<td>2 (9%)</td>
<td>0</td>
<td>2 (9%)</td>
<td>0</td>
</tr>
</tbody>
</table>

*(CR+PR+SD > 6 months)
These were the first data showing an objective anti-tumor response with single agent Hsp90 inhibitor therapy in patients with advanced breast cancer. Additionally, these were the first data to show anti-tumor activity for an Hsp90 inhibitor in TNBC. In this study, the single agent of ganetespib was well tolerated, with expected GI toxicity that was mild in nature and manageable in all patients.

Example 7—Ganetespib displays activity across breast cancer subtypes

[00285] Breast cancer is a heterogeneous disease historically broken down into 4 subtypes. Various compounds were tested for their effects in cell viability assays using various breast cancer cell lines. Cellular viability was assessed using the CellTiter-Glo® Luminescent Cell Viability Assay (Promega, Madison, WI, USA) according to the manufacturer’s protocol. KRAS mutant NSCLC cell lines were seeded into 96-well plates based on optimal growth rates determined empirically for each line. Twenty-four hours after plating, cells were dosed with graded concentrations of ganetespib for 72 h. CellTiter-Glo® was added (50% v/v) to the cells, and the plates incubated for 10 min prior to luminescent detection in a SpectraMax® Plus 384 microplate reader (Molecular Devices, Sunnyvale, CA, USA). Data were normalized to percent of control and IC50 values used to determine the sensitivity of each line. For the comparative analysis study with MEK and PI3K/mTOR inhibitors, A549, H2009, Calu-1, and H358 cells were treated with graded concentrations of ganetespib, AZD6244, or BEZ235 for 72 h and cell viability measured as above.

[00286] Shown in Figure 1, ganetespib showed potency across all 4 subtypes (luminal HER2 +, luminal HER2 -, Basal A, Basal B) of breast cancer cells, grown as a monolayer in vitro. The IC50s of the various compounds and the ER, PR, and HER2 status are provided in the table below.
Breast cancer, cell lines, marker status, and IC50 in response to ganetespib at 72 hr (viability by CTG).

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Subtype</th>
<th>ER</th>
<th>PR</th>
<th>HER2</th>
<th>IC50, nM</th>
</tr>
</thead>
<tbody>
<tr>
<td>OCUB-M</td>
<td>Basal</td>
<td>--</td>
<td>--</td>
<td></td>
<td>39</td>
</tr>
<tr>
<td>MDA-MB-468</td>
<td>Basal A</td>
<td>--</td>
<td>--</td>
<td></td>
<td>27</td>
</tr>
<tr>
<td>HCC70</td>
<td>Basal A</td>
<td>--</td>
<td>--</td>
<td></td>
<td>114</td>
</tr>
<tr>
<td>MDA-MB-231</td>
<td>Basal B</td>
<td>--</td>
<td>--</td>
<td></td>
<td>24</td>
</tr>
<tr>
<td>SK-BR-3</td>
<td>Luminal</td>
<td>--</td>
<td>--</td>
<td>+</td>
<td>10</td>
</tr>
<tr>
<td>BT-474</td>
<td>Luminal</td>
<td>+</td>
<td>+</td>
<td></td>
<td>13</td>
</tr>
<tr>
<td>MCF7</td>
<td>Luminal</td>
<td>+</td>
<td>+</td>
<td></td>
<td>25</td>
</tr>
</tbody>
</table>

[00287] Basal breast cancer is a subtype believed to be more stem like and less differentiated than luminal breast cancer, and therefore more aggressive with limited treatment options. Comparison was made for the anticancer activity of ganetespib versus MEK and mTOR inhibitors in the basal line MDA-MB-231, using lapatinib as a control since these cells were HER2 negative. Shown in Figure 1, ganetespib was highly potent, killing all the cells as opposed to the weak activity of the mTOR and MEK inhibitors.

[00288] Ganetespib was assayed in inflammatory breast cancer (IBC), a rare but aggressive form of breast cancer distinct from the subtypes presented above. Shown in Figure 2, ganetespib displayed considerable anticancer activity against SUM149 cells 24 hr after exposure.

[00289] BT-474 HER2+ luminal cells were cultured as mammospheres in Matrigel® and exposed to ganetespib for 72 hr. As shown in Figure 3, ganetespib was fully capable of killing cells organized into spheroids, with an IC50 (20 nM) nearly identical to that observed in 2D (13 nM), demonstrating that ganetespib retained its activity in breast cancer cells grown in three dimensions.
Example 8—Expression of proteins in BT-474 HER2+ luminal breast cancer cells after treatment with ganetespib

[00290] Expression of various proteins in the BT-474 HER2+ luminal breast cancer cells after exposure to ganetespib was assessed by western blot using routine methods. Briefly, following treatment, tumor cells were disrupted in lysis buffer (CST) on ice for 10 min. Lysates were clarified by centrifugation and equal amounts of proteins resolved by SDS-PAGE before transfer to nitrocellulose membranes (Invitrogen, Carlsbad CA). Membranes were blocked with 5% skim milk in TBS with 0.5% Tween and immunoblotted with the indicated antibodies. Antibody-antigen complexes were visualized using an Odyssey system (LI-COR, Lincoln, NE). Figure 7 is a western blot showing expression of various proteins in the BT-474 HER2+ cells at various time points after treatment with ganetespib.

Example 9—Treatment of breast cancer with ganetespib and BEZ235 in a mouse xenograft tumor model

[00291] Female immunodeficient CD-1 (nude) mice (Charles River Laboratories, Wilmington, MA) were maintained in a pathogen-free environment, and all in vivo procedures were approved by the Synta Pharmaceuticals Corp. Institutional Animal Care and Use Committee. A549 NSCLC cells (7.5 x 10⁶) were subcutaneously implanted into the animals. Mice bearing established tumors (100-200 mm³) were randomized into treatment groups of 8 and i.v. dosed via the tail vein with either vehicle, ganetespib formulated in 10/18 DRD (10% DMSO, 18% Cremophor RH 40, 3.6% dextrose, 68.4% water) or p.o. dosed with BEZ235 formulated in PEG300/NMP (90% PEG300, 10% N-Methylpyrrolidone). Animals were treated with ganetespib at 50 mg/kg weekly or BEZ235 at 10 mg/kg 5 times a week, either alone or in combination. Tumor growth inhibition was determined as described previously. See Proia et al. PLoS One. 2011;6(4):el8552. The results are shown in Figure 8.

[00292] As shown in Figure 8, average tumor volume was significantly reduced in mice treated with ganetespib and BEZ235 as compared to vehicle, particularly at later time points. The efficacy of ganetespib and BEZ235 were about the same, and average tumor volume was
reduced in mice treated with ganetespib and BEZ235 as compared to vehicle control, particularly at later time points.

[00293] In summary, ganetespib displayed anticancer activity in all four breast cancer subtypes, as well as inflammatory breast cancer. Importantly, ganetespib was equally effective in killing cells grown as three dimensional spheres compared to cells grown in monolayer, as well as *in vivo*.

**Example 10 – Ganetespib displays activity across GIST subtypes**

[00294] Many of the oncoproteins associated with gastric cancer and Hsp90 client proteins including HER2, MET, RAS and the FGFR family. Ganetespib was evaluated for its effect on the growth of AGS (wt-p53 and mut-KRAS) and MKN45 (wt-p53, wt-KRAS, MET amplified) gastric cancer cells. Cells were treated for 72 hr and viability determined by CTG (upper) or Syto60 (lower). Most gastric cell lines displayed low nanomolar IC₅₀ with ganetespib, as shown in the Table below.

<table>
<thead>
<tr>
<th>IC₅₀ (nM)</th>
<th>AGS</th>
<th>MKN45</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ganetespib</td>
<td>1.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Docetaxel</td>
<td>0.3</td>
<td>0.1</td>
</tr>
<tr>
<td>17-AAG</td>
<td>50</td>
<td>1.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Ganetespib IC₅₀ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGS</td>
<td>5</td>
</tr>
<tr>
<td>SNU-1</td>
<td>6</td>
</tr>
<tr>
<td>GT3TKB</td>
<td>6</td>
</tr>
<tr>
<td>MKN-45</td>
<td>8</td>
</tr>
<tr>
<td>GCIY</td>
<td>9</td>
</tr>
<tr>
<td>HGC-27</td>
<td>11</td>
</tr>
<tr>
<td>ECC12</td>
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</tr>
<tr>
<td>SNU-5</td>
<td>31</td>
</tr>
<tr>
<td>Hs746T</td>
<td>384</td>
</tr>
</tbody>
</table>

[00295] Ganetespib was also evaluated for its effects on Hsp90 client proteins in AGS gastric cancer cells by western blot. Ganetespib abolished the expression of EGFR, IGF-IR, C-RAF and their down-stream effectors PI3K/AKT and MAPK, resulting in PARP cleavage
and increased levels of p-Histone H2X (Ser139), a marker for DNA fragmentation during apoptosis. Similar to the observation in melanoma cells, exposure to ganetespib enhanced B-RAF expression. Without being bound by mechanism, it is suggested that a decrease in phosphorylation of CDK1 by ganetespib may be due to the loss of WEE1 expression, an important regulatory kinase for CDK1. In MKN45 cells, exposure of ganetespib led to the complete degradation of MET and IGF-IR, followed by inactivation of AKT.

In summary, ganetespib displayed potent anticancer activity with low nanomolar IC50s in gastric cancer cell lines. Without being bound by mechanism, it is suggested that the activity is at least, in part, a result of widespread degradation of client proteins essential for cell growth, proliferation and survival including MET, IGF-IR, EGFR, WEE1 and CDK1.

Example 11—Ganetespib displays efficacy in head and neck cancer subtypes

Head and neck (H/N) cancer refers to a group of biologically similar cancers originating from the upper autodigestive tract. First line therapies include EGFR inhibitors and platins. Modulation of EGFR and other client proteins by ganetespib was investigated in Detroit 562 H/N cancer cells. As shown in Figure 4B, ganetespib led to the depletion of EGFR and JAK2, resulting in the inactivation of several key effectors including AKT, STAT3, p70S6, and ERK followed by cleaved PARP. Single agent viability analyses were then performed and it was found that the IC50 of ganetespib (42 nM) correlated with initiation of client protein degradation (Figure 4A). A fraction of cells remained viable after a 72 hr exposure to ganetespib, in contrast to the platins which completely killed the cells.

Western blot of protein expression is shown in Figure 5. Cell extracts from Detroit 562 head and neck cancer cells were treated with 100 nM of ganetespib 24 hours prior to receiving the DNA damaging agent bleomycin (5 μM). Protein expression was measured at the indicated time points after bleomycin treatment. Bleomycin increased both Chkl and Chk2 phosphorylation, which was blocked when cells were treated first with ganetespib.
Example 12—Ganetespib in combination with standard of care chemotherapies displays efficacy in NSCLC cancer subtypes with mutations in KRAS

[00299] Mutant KRAS is detected in 20-25% of non-small cell lung carcinomas (NSCLC) and represents one of the most common oncogenic drivers of this disease. NSCLC tumors with oncogenic KRAS respond poorly to currently available therapies necessitating the pursuit of new treatment strategies. Recent results from a Phase 2 trial with ganetespib revealed that >60% of patients with NSCLC having a mutation in KRAS exhibited tumor shrinkage at 8 weeks, indicating that ganetespib is useful in the treatment of this disease.

[00300] To further understand the actions of ganetespib in NSCLC tumors having a mutation in KRAS, studies were executed in a diverse panel of KRAS mutant NSCLC cell lines to investigate whether ganetespib is effective in suppressing critical cell signaling nodes responsible for KRAS-driven NSCLC cell survival and to assess whether ganetespib can synergize with both clinical agents targeted against these signaling nodes and standard of care chemotherapies.

[00301] For combinatorial analysis, cells were seeded in 96-well plates at a predetermined, optimum growth density for 24 h prior to the addition of drug or vehicle to the culture medium. Drug combinations were applied at a non-constant ratio over a range of concentrations for 72 or 96 hours. For each compound tested, a 7 point dose range was generated based on 1.5 fold serial dilutions using IC₅₀ values set as the mid-point. Cell viability was assessed by either AlamarBlue® (Invitrogen, Carlsbad, CA) or CellTiter-Glo® assays and normalized to vehicle controls. For each combination study, the level of growth inhibition (fraction affected) is plotted relative to vehicle control. Data are presented as one relevant combination point and the corresponding single agent data for each cell line tested.

[00302] Ganetespib displayed potent anticancer activity across 15 KRAS mutant NSCLC cell lines assayed in vitro, with an average IC₅₀ of 24 nM. Combining ganetespib with anti-mitotics, alkylating agents or topoisomerase inhibitors resulted in an increase in cell death of up to 44, 61 and 26%, respectively, versus monotherapy. At the molecular level, ganetespib induced the destabilization of several KRAS substrates, including BRAF and CRAF, leading to inactivation of their downstream effectors followed by programmed cell death. Ganetespib effectively suppressed the growth of human KRAS mutant NSCLC
tumor xenografts in vivo; however, ganetespib did not induce tumor regression. In light of this, we sought to investigate whether inhibitors targeting KRAS driven signaling nodes would confer greater sensitivity to ganetespib. In vitro, combinations of low dose of ganetespib with either MEK or PI3K/mTOR inhibitors consistently resulted in greater activity than monotherapy, up to 77% and 42%, respectively. Furthermore, ganetespib suppressed activating feedback loops that occur in response to MEK and PI3K/mTOR inhibition, providing a rationale for the enhanced combinatorial activity. To validate these results, in vivo combinations were performed with ganetespib and a PI3K/mTOR inhibitor in KRAS mutant NSCLC xenografts. While both agents promoted tumor shrinkage on their own, considerable improvement in tumor growth inhibition was observed in the combination arm.

[00303] More particularly, ganetespib elicited promising activity against mutant KRAS NSCLC tumor cells (Figure 11). In order to further identify feasible strategies to enhance the anti-tumor activity of ganetespib, combination studies were performed with standard of care chemotherapies in mutant KRAS NSCLC cell lines. Combining low nanomolar concentrations of ganetespib with the topoisomerase I inhibitor camptothecin resulted in a 1.5, 3.4, and 1.4 fold increase in cytotoxicity for H2009, H2030, and H358 cells, respectively (Figure 12). Similar results were observed for SN-38, another topoisomerase I inhibitor (Figure 16). It was also found that combining ganetespib with the antimetabolite pemetrexed enhanced cell death by 2.4 and 1.5 fold for H2030 and H2009 cells, respectively, while a marginal increase in cytotoxicity was observed for A549 and H358 cells (Figure 13). Ganetespib in combination with the nucleoside analog, gemcitabine, increased cell death 2.3 and 1.4 fold for H2009 and A549 cells, respectively, and no benefit was observed for H358 cells (Figure 14).

[00304] More combination data are presented in Figures 15-20. These results highlight the heterogeneity in response to various targeted agents and chemotherapies as well as the variability in benefit achieved when these agents are combined with ganetespib. Taken together, these results suggest that chemotherapies currently used for the treatment of NSCLC may enhance the antitumor activity of ganetespib.
Without being bound by mechanism, it is suggested that ganetespib promotes destabilization of multiple oncogenic signaling proteins and is potently cytotoxic in KRAS mutant NSCLC cells and simultaneously disrupts multiple nodes of KRAS driven signaling resulting in enhanced apoptosis compared to MEK or PI3K/mTOR inhibitors. Combining ganetespib with MEK or mTOR inhibitors blocks feedback induced accumulation of activated MEK and ERK contributing to enhanced cytotoxicity in vitro and in vivo. Common standard of care chemotherapeutics utilized in the treatment of NSCLC enhance the activity of ganetespib.

In summary, ganetespib, a potent inhibitor of Hsp90, has shown encouraging evidence of clinical activity, including tumor shrinkage in patients with KRAS mutant NSCLC. In vitro, ganetespib exhibited potent anticancer activity in NSCLC cells with a diverse spectrum of KRAS mutations due in part to degradation and inactivation of critical KRAS signaling effectors. Combination with targeted therapies that overlap with these signaling nodes led to enhanced anticancer activity in vitro and in mouse models of KRAS mutant NSCLC. Taken together, these results demonstrate clinical utility of ganetespib in patients with KRAS mutant NSCLC.

Standard of care chemotherapeutics utilized in KRAS mutant NSCLC show activity with ganetespib in vitro. Camptothecin, pemetrexed and gemcitabine showed up to 4 fold increases in cell death when combined with ganetespib. None of the agents antagonized the anticancer activity of ganetespib.

Example 13—Phase 1 trial of the combination of ganetespib and docetaxel in the treatment of solid tumors.

A Phase 1 study of ganetespib in combination with docetaxel in solid tumors has been performed.

A trial to evaluate three dose-level combinations of docetaxel and ganetespib, administered on a three-week cycle, with the primary objective of determining an optimal dose for future clinical trials was performed. Docetaxel was administered as a one hour IV infusion on day 1 and ganetespib was administered as a one hour IV infusion on days 1 and 15. The dose level combinations evaluated were 150 mg/m² and 60 mg/m², 150 mg/m² and 77.
75 mg/m^2; and 200 mg/m^2 and 75 mg/m^2 for ganetespib and docetaxel respectively. The standard of care dose level for docetaxel was 75 mg/m^2. A total of 19 patients received at least one dose of study treatment at the cut-off time. The median number of cycles of treatment was 4, with a range of 1 to 11 cycles of treatment. No prophylactic treatment for neutropenia was used. The combination of ganetespib at 150 mg/m^2 and docetaxel at 75 mg/m^2 was selected as the recommended dose.

**[00310]** It was observed that a patient responded with over 50% shrinkage of target tumor lesions on the trial diagnosed with cancer of the parotid gland, the largest of the salivary glands. The patient did not respond to prior treatment regimens including carboplatin, cetuximab, and methotrexate.

**[00311]** The most common adverse event was neutropenia (67%), including four patients (22%) who reported febrile neutropenia. Neutropenia, a known effect of docetaxel treatment, was commonly observed at approximately 8 days following dosing and typically resolved spontaneously within 7 days. Serious adverse events were reported in a total of nine patients (50%) including two reports of pneumonia and one report each of chest pain, chills, dyspnea, fatigue, mucosal inflammation, neutropenia, pneumothorax, pulmonary embolism, rib fracture, transient ischaemic attack, and vomiting.

**[00312]** Pharmacokinetic data indicate a pharmacokinetic similarity between ganetespib administered alone and ganetespib administered prior to docetaxel. There was no effect of ganetespib on docetaxel pharmacokinetics.

**[00313]** These results support the use of ganetespib at a dose of 150 mg/m^2 in combination with docetaxel at a dose of 75 mg/m^2 for treating NSCLC and other solid cancers.

**Example 14 — Method of evaluating activity levels of LDH isoforms in subject samples**

**[00314]** Human tumor cell lines HCT116 (ATCC #CRL-247; Schroy PC, et al. Cancer 76: 201-209, 1995) and 786-0 (ATCC #CRL-1932; Williams RD, et al. In vitro 12: 623-627, 1976), were obtained from the American Type Culture Collection (Manassus, Virginia, USA) were cultured using routine methods until a sufficient number of cells were obtained for
implantation. Studies were conducted on animals between 7 and 12 weeks of age at implantation. To implant HCT116 tumor cells into nude mice, the cells were trypsinized, washed in PBS and resuspended at a concentration of 75 x 10⁶ cells/ml in McCoy's modified medium with 50% of BD Matrigel® Basement Membrane Matrix (BD Biosciences®, Bedford, Massachusetts, USA). To implant 786-0 tumor cells into nude mice, the cells were trypsinized as above, washed in PBS and resuspended at a concentration of 75 x 10⁶ cells/ml in RPMI 1640 medium with 50% of BD Matrigel® Basement Membrane Matrix. Using a 27 gauge needle and 1 cc syringe, 0.1 ml of the cell suspension was injected into the corpus adiposum of nude mice. The corpus adiposum is a fat body located in the ventral abdominal vicera in the right quadrant of the abdomen at the juncture of the os coxae (pelvic bone) and the os femoris (femur). The location permits palpation and measurement of the tumors using external calipers. Tumor volumes (V) were calculated by caliper measurement of the width (W), length (L) and thickness (T) of tumors using the following formula: \( V = 0.5236 \times (L \times W \times T) \). Animals were randomized into treatment groups so that the average tumor volumes of each group were similar at the start of dosing.

[00315] Blood was collected from the tumor bearing mice at appropriate time points, serum was prepared, and the serum frozen for later analysis. On the same days as blood collection, tumor volumes (V) were calculated by caliper measurement of the width (W), length (L) and thickness (T) of tumors using the following formula: \( V = 0.5236 \times (L \times W \times T) \). After collection of the serum samples was completed, serum samples were resolved by gel electrophoresis. Following electrophoresis, the bands for the five isoenzymes were visualized by an enzymatic reaction using an in-gel assay. Lactate, nicotinamide adenine dinucleotide (NAD+), nitroblue tetrazolium (NBT), and phenazine methosulphate (PMS) were added to assess LDH activity. LDH converts lactate to pyruvate and reduces NAD+ to NADH. The hydrogens from NADH are transferred by PMS to NBT reducing it to a purple formazan dye. The percentage of each LDH isoenzyme activity as well as the relative amount of LDH5 was determined by densitometry (Beckman Appraise densitometer, Beckman Coulter Inc. or Sebia (GELSCAN, Sebia Inc). The percent of LDH5 protein and LDH5 activity relative to the total LDH present (i.e., the amount of LDH5, LDH5, LDH3, LDH2, and LDH1 combined) was calculated and graphed against tumor volume. The results are shown in Figures 21A-D.
Figures 21A and 21B show the amount of LDH5 activity as a percent of total LDH activity as determined by the in-gel assay. As shown, the HCT116 tumors had a substantially greater percent to LDH5 activity relative to total LDH activity as compared to the 7860 tumors. Figures 21C and 21D demonstrate that despite the difference in the relative activity of LDH5 that is observed, the amount of LDH5 protein present relative to total LDH is about the same for both tumor types.

Example 15 — Characterization of treatment outcomes to demonstrate improved efficacy of ganetespib in subjects with solid tumors with a high level of LDH

Clinical trials have been performed to demonstrate the efficacy of ganetespib in the treatment of cancer provided in the foregoing examples.

Preliminarily, subjects within each of the groups, or at least the groups in which subjects were treated with ganetespib, are divided into high and low LDH level based on the upper limit of normal (ULN) for the site where the testing is done. A value equal to or less than the ULN is considered as low. Values greater than the ULN are considered high. Alternatively, low LDH can be considered as levels up to and including 0.8 ULN with high LDH being considered all values above 0.8 ULN. Alternatively, low LDH can be considered as levels up to and including 1.2 or 1.5 ULN with high LDH being considered all values above 1.2 or 1.5 ULN, respectively. It may be possible to further stratify the high and low ULN groups to provide further predictive power of the LDH level in predicting the response of a subject to treatment with ganetespib, e.g., assigning those with an LDH level of 1 to <2 times, or 1 to <3 times, etc. the ULN as having an intermediate or slightly elevated LDH level. Ratios of LDH isoforms or subunits, e.g., ratios of the ULN values of LDHA to LDHB or LDH4 and/or LDH5 to LDH1 or total LDH can also be used to determine high and low levels of hypoxia. Other cut-off values such as those provided in the instant application can also be selected. Statistical analysis can be used to select appropriate cut-offs. Subjects are further characterized for KRAS, EGFR, and ALK mutation status. Subjects with breast and ovarian cancer are also tested for ER, PR, and HER2 status. Mutations and receptor status are detected using routine clinical methods.
Subjects with a high level of hypoxia and/or a mutation in KRAS, a mutation in EGFR, or a mutation in ALK are selected for treatment with ganetespib as likely to benefit from such treatment. Subjects with metastatic breast cancer and a high level of hypoxia, or subjects with triple negative breast cancer with a high level of hypoxia are selected for treatment with ganetespib as likely to benefit from such treatment.

Example 16 – Characterization of treatment outcomes to demonstrate improved efficacy of ganetespib in subjects with other cancers with a high level of LDH

Multiple Phase 1 and 2 clinical trials have been and are being performed to demonstrate the efficacy of ganetespib in non-small cell lung cancer, gastrointestinal stromal tumors, colorectal cancer, gastric cancer, small cell lung cancer, and melanoma as discussed in the previous example.

A chart review is performed to determine if levels of one or more hypoxic markers, particularly LDH, is analyzed for the subjects prior to, and optionally during treatment with ganetespib. If no information is available regarding the levels of hypoxic markers, serum samples retained from the study subjects are analyzed for LDH level and outcomes are analyzed in view of the LDH level.

The chart review is performed to further determine KRAS, EGFR, and ALK mutational status. If the subject has breast or ovarian cancer, the ER, PR, and HER2 status are also determined during the chart review.

If the hypoxia and marker status are not available during a chart review, tumor samples can be assayed to determine hypoxia and biomarker status of the tumor.

Preliminarily, subjects within each of the groups, or at least the groups in which subjects were treated with ganetespib, are divided into high and low LDH level based on the upper limit of normal (ULN) for the site where the testing is done. A value equal to or less than the ULN is considered as low. Values greater than the ULN are considered high. Alternatively, low LDH can be considered as levels up to and including 0.8 ULN with high LDH being considered all values above 0.8 ULN. Alternatively, low LDH can be considered as levels up to and including 1.2 or 1.5 ULN with high LDH being considered all values
above 1.2 or 1.5 ULN, respectively. It may be possible to further stratify the high and low ULN groups to provide further predictive power of the LDH level in predicting the response of a subject to treatment with ganetespib, e.g., assigning those with an LDH level of 1 to <2 times, or 1 to <3 times, etc. the ULN as having an intermediate or slightly elevated LDH level. Ratios of LDH isoforms or subunits, e.g., ratios of the ULN values of LDHA to LDHB or LDH4 and/or LDH5 to LDH1 or total LDH can also be used to determine high and low levels of hypoxia. Other cut-off values such as those provided in the instant application can also be selected. Statistical analysis can be used to select appropriate cut-offs.

[00326] Subjects are further characterized for KRAS, EGFR, and ALK mutation status. Subjects with breast and ovarian cancer are also tested for ER, PR, and HER2 status. Mutations and receptor status are detected using routine clinical methods.

[00327] Subjects with a high level of hypoxia and/or a mutation in KRAS, a mutation in EGFR, or a mutation in ALK are selected for treatment with ganetespib as likely to benefit from such treatment. Subjects with metastatic breast cancer and a high level of hypoxia, or subjects with triple negative breast cancer with a high level of hypoxia are selected for treatment with ganetespib as likely to benefit from such treatment.

Example 17 – Trial to demonstrate improved efficacy of ganetespib in subjects with various cancer types with a high level of LDH

[00328] Subjects are identified as having one of advanced solid tumor malignancies including metastatic or unresectable malignancy with evidence of progression, non-small cell lung cancer, gastrointestinal stromal tumors, colorectal cancer, gastric cancer, small cell lung cancer, melanoma, refractory malignancy. A subject is selected as being candidate for treatment with ganetespib. Routine assessments are made prior to treatment to characterize the disease state of the subject including, but not limited to, imaging studies, hematological studies, and physical examination. Additionally, coded serum sample from the subject is tested to determine the LDH level; KRAS, EGFR, and ALK mutation status, and ER, PR, and HER2 status. The results from the LDH level determination are not matched to the subject until the end of the treatment period. However, samples can be tested to allow sufficient
numbers of subjects with low and high LDH levels to be recruited to provide sufficient power to the study.

[00329] Subjects are treated with the standard dose of ganetespib, either alone or in combination with other agents, e.g., using the regimens presented in the prior examples. At predetermined regular or irregular intervals, subjects are assessed for specific outcomes including, but not limited to, overall survival, progression free survival, time to progression, and adverse events. Treatment is continued for as long as the subject responds positively to treatment with bevacizumab and there are no limiting adverse events.

[00330] Upon conclusion of the study, the results from the LDH level analysis and mutational analyses are unblinded and matched to the subjects. As specific methods of testing are available, the amount of LDH is scored as being low or high based on the upper limit of normal (ULN) for the site where the testing is done. A value equal to or less than the ULN is considered as low. Alternatively, low LDH can be considered as levels up to and including 0.8 ULN with high LDH being considered all values above 0.8 ULN. Alternatively, low LDH can be considered as levels up to and including 1.2 or 1.5 ULN with high LDH being considered all values above 1.2 or 1.5 ULN, respectively. It may be possible to further stratify the high and low ULN groups to provide further predictive power of the LDH level in predicting the response of a subject to treatment with ganetespib, e.g., assigning those with an LDH level of 1 to <2 times, or 1 to <3 times, etc. the ULN as having an intermediate or slightly elevated LDH level. Ratios of LDH isoforms or subunits, e.g., ratios of the ULN values of LDHA to LDHB or LDH4 and/ or LDH5 to LDH1 or total LDH can also be used to determine high and low levels of hypoxia. Other cut-off values such as those provided in the instant application can also be selected. Statistical analysis can be used to select appropriate cut-offs.

[00331] Subjects are further characterized for KRAS, EGFR, and ALK mutation status. Subjects with breast and ovarian cancer are also tested for ER, PR, and HER2 status. Mutations and receptor status are detected using routine clinical methods.

[00332] Subjects with a high level of hypoxia and a mutation in KRAS, a mutation in EGFR, or a mutation in ALK are selected for treatment with ganetespib as likely to benefit from such treatment. Subjects with metastatic breast cancer and a high level of hypoxia, or
subjects with triple negative breast cancer with a high level of hypoxia are selected for
treatment with ganetespib as likely to benefit from such treatment.

Example 18 _ Selection of subjects with lung cancer and a high level of LDH for
treatment with ganetespib

[00333] Subject is identified as having lung cancer, either small cell or non-small cell lung
cancer, or other cancer type known to be or suspected to be susceptible to treatment with
ganetespib, and being candidate for treatment with ganetespib. A serum sample from the
subject is tested to determine the LDH level; KRAS, EGFR, and ALK mutation status, and
ER, PR, and HER2 status. The amount of LDH is scored as being low or high based on the
upper limit of normal (ULN) for the site where the testing is done. A value equal to or less
than the ULN is considered as low. A value greater than the ULN is considered to be high.
Alternatively, low LDH can be considered as levels up to and including 0.8 ULN with high
LDH being considered all values above 0.8 ULN. Alternatively, low LDH can be considered
as levels up to and including 1.2 or 1.5 ULN with high LDH being considered all values
above 1.2 or 1.5 ULN, respectively. It may be possible to further stratify the high and low
ULN groups to provide further predictive power of the LDH level in predicting the response
of a subject to treatment with temsirolimus, e.g., assigning those with an LDH level of 1 to <2
times, or 1 to <3 times, etc. the ULN as having an intermediate or slightly elevated LDH
level. Ratios of LDH isoforms or subunits, e.g., ratios of the ULN values of LDHA to LDHB
or LDH4 and/or LDH5 to LDH1 or total LDH can also be used to determine high and low
levels of hypoxia. Other cut-off values such as those provided in the instant application can
also be selected.

[00334] If the subject has a high LDH level and/or a mutation in KRAS, a mutation in
EGFR, or a mutation in ALK are selected for treatment with ganetespib as likely to benefit
from such treatment.
Example 24—Antiangiogenic activity of ganetespib in pancreatic cancer models

[00335] Pancreatic cancer is the fourth most common cause of cancer related mortality in US. In the year 2012 alone, approximately 43,900 new cases of pancreatic cancer are estimated in the US.

[00336] It is postulated that functional inhibition of Hsp90 by ganetespib can inhibit angiogenesis and growth in vitro and in vivo models of pancreatic cancer. PANC-1 and HPAC cell lines were treated with vehicle or G (50nM) for 24h and lysates were analyzed by Western blot. Egg CAM and matrigel plug assays were performed to quantify the effects of ganetespib on angiogenesis. Efficacy of ganetespib (100mg/kg) was assessed in mice bearing HPAC and ASPC-1 xenograft. Western blot analyses demonstrated a significant reduction in intracellular HIF-1α and VEGF protein levels in PANC-1 and HPAC cells treated with G. Results from ELISA assays showed that ganetespib reduced VEGF secretion in the culture medium from both pancreatic lines. Treatment of ganetespib reduced angiogenesis compared to vehicle in all three models. Animals with human pancreatic tumor xenografts treated with ganetespib had significant tumor growth delay and inhibition of angiogenesis. The preclinical data demonstrates that ganetespib can inhibit pancreatic cancer growth and angiogenesis, suggesting that targeting Hsp90 is a rational new approach to pancreatic cancer therapy to be explored in clinical trials.

Materials and Methods

[00337] Cell lines: Mia-PaCa2, PANC-1, HPAC and ASPC-1 cell lines (ATCC, Manassas, VA) were cultured according to the ATCC manual. Medium was supplemented with 10% fetal bovine serum (Invitrogen Corporation, Carlsbad, CA), 50 units/ml penicillin, and 50μg/ml streptomycin (Life Technologies, Inc., Frederick, MD). Cells were incubated at 37°C in a humidified 5% CO2 atmosphere.

[00338] Chemicals and antibodies: Primary antibodies specific to Hsp90, HIF-1α, VEGF, Actin and HRP conjugated secondary antibodies (Santa Cruz Biotechnology, California and Cell Signaling Technology, USA) were used for Western blot. Ganetespib was provided by Synta Pharmaceuticals, Lexington, MA and Matrigel was purchased from BD Biosciences.
VEGF levels: VEGF concentration in the conditioned medium (from control and treated cells) was determined using a commercial Human VEGF Quantikine ELISA kit (R&D systems, Minneapolis, MN) as per manufacturer's instructions.

Western blotting: Cells were harvested at the end of treatment and lysed in the RIPA protein extraction buffer (Sigma-Aldrich, Saint Louis, Missouri, USA) containing the protease inhibitor cocktail. Equal amounts of protein fractions of lysates were resolved over SDS-PAGE and transferred on to PVDF membrane. Membranes were incubated with primary antibodies followed by HRP-conjugated secondary antibodies. Bound antibodies were visualized using enhanced chemiluminescence. To confirm equal loading, membranes were verified and re-probed with an antibody specific for the housekeeping gene, anti-[3-actin.

Egg CAM assay: Mia-PaCa2, PANC-1 and HPAC cells were treated with Ganetespib (50nM) or control for 24 hours, the conditioned medium was harvested and 100µl of conditioned or control medium was injected into fertilized chicken eggs (Avian Vaccine Service center, North Franklin, CT). Eggs were incubated at 37°C for 15 days and dissected. Chorioallantoic membrane was photographed.

In vivo tumor growth delay and angiogenic assay: Five-week-old SCID mice were divided into 4 groups with 10 animals in each group. First two groups received 100 µl of Ice cold matrigel medium containing ASPC-1 (1 X 106 cells/100µl) and other two groups received HPAC cell lines subcutaneously. Once the tumor reached 100-120 mm3, the groups 2 and 4 received ganetespib (100 mg/kg body weight) IV once a week for three weeks. None of the animals died from the treatment. Every other day, tumor was measured using vernier caliper scale for a total of five weeks, when the animals were sacrificed. Skin around the implanted matrigel tumor was removed carefully and the tumor with its surrounding was photographed under visible light.

Conclusions

In summary, growth inhibition and anti-angiogenic effects of ganetespib was observed in pancreatic cancer cell lines, and ganetespib decreased HIF-1α and VEGF
expression which results in decrease in VEGF secretion and inhibition of angiogenesis in vivo.

[00344] All publications cited herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples throughout the specification are illustrative only and not intended to be limiting in any way. In addition, this application is related to PCT application Nos. PCT/US2011/061440 and PCT/US2011/061446, both filed on November 18, 2011; and to PCT application No. PCT/US12/37564, filed on May 11, 2012, claiming priority to U.S. Provisional Patent Application Nos. 61/484,988 and 61/484,992, filed on May 11, 2011; 61/489,867, filed on May 25, 2011; 61/493,063; filed on June 3, 2011; 61/498,966, filed on June 20, 2011; 61/504,417, filed on July 5, 2011; 61/538,400, filed on September 23, 2011; 61/565,126, filed on November 30, 2011; 61/567,942, filed on December 7, 2011; 61/578,459, filed on December 21, 2011; and 61/583,773, filed on January 6, 2012. The contents of each of these applications are incorporated herein by reference in their entirety.
What is claimed is:

1. A method of treating cancer in a subject, comprising administering ganetespib or a pharmaceutically acceptable salt or a tautomer thereof, wherein the subject has a cancer with a high level of hypoxia and/or with a mutation in KRAS.

2. The method of claim 1, wherein the cancer is selected from the group consisting of lung cancer, breast cancer, gastric cancer, colorectal cancer, pancreatic cancer, ocular melanoma, prostate cancer, gastrointestinal stromal tumors (GIST), advanced esophagogastric cancer, melanoma, hepatocellular cancer, solid tumor, liver cancer, head and neck cancer, colon cancer, small cell lung cancer, and non-small cell lung cancer (NSCLC).

3. The method of claim 1 or 2, wherein the cancer is NSCLC or pancreatic cancer.

4. The method of claim 3, wherein the NSCLC was previously treated and not responsive.

5. The method of claim 3 or 4, wherein the NSCLC was previously treated with crizotinib and is no longer responsive to the crizotinib treatment.

6. The method of claim 1 or 2, wherein the cancer is breast cancer.

7. The method of claim 6, wherein the breast cancer is HER2 positive, and has been previously treated with trastuzumab.

8. The method of claim 6 or 7, wherein the breast cancer is HER2 positive and trastuzumab refractory.

9. The method of claim 6, wherein the cancer is triple negative breast cancer.
10. The method of claim 2, wherein the prostate cancer is metastatic hormone-resistant prostate cancer, or metastatic castration-resistant prostate cancer.

11. The method of any one of claims 1 to 10, further comprising identifying a subject as having a cancer with a mutation in KRAS.

12. A method of treating cancer in a subject, comprising administering ganetespib or a pharmaceutically acceptable salt or a tautomer thereof, wherein the subject has a cancer with a mutation in ALK and with a high level of hypoxia.

13. The method of claim 12, wherein the cancer is selected from the group consisting of lung cancer, breast cancer, gastric cancer, colorectal cancer, pancreatic cancer, ocular melanoma, prostate cancer, gastrointestinal stromal tumors (GIST), advanced esophagogastric cancer, melanoma, hepatocellular cancer, solid tumor, liver cancer, head and neck cancer, colon cancer, small cell lung cancer, and non-small cell lung cancer (NSCLC).

14. The method of claim 12 or 13, wherein the cancer is NSCLC or pancreatic cancer.

15. The method of claim 14, wherein the NSCLC was previously treated and not responsive.

16. The method of claim 14 or 15, wherein the NSCLC was previously treated with crizotinib and is no longer responsive to the crizotinib treatment.

17. The method of claim 12 or 13, wherein the cancer is breast cancer.

18. The method of claim 17, wherein the breast cancer is HER2 positive, and has been previously treated with trastuzumab.
19. The method of claim 17 or 18, wherein the breast cancer is HER2 positive and trastuzumab refractory.

20. The method of claim 17, wherein the cancer is triple negative breast cancer.

21. The method of claim 13, wherein the prostate cancer is metastatic hormone-resistant prostate cancer, or metastatic castration-resistant prostate cancer.

22. The method of any one of claims 12 to 21, further comprising identifying a subject as having a cancer with a mutation in ALK.

23. A method of treating breast cancer in a subject, comprising administering ganetespib or a pharmaceutically acceptable salt or a tautomer thereof, wherein the breast cancer is selected from the group consisting of metastatic breast cancer, triple negative breast cancer, and HER2 positive breast cancer; and with a high level of hypoxia.

24. The method of claim 23, wherein the breast cancer is HER2 positive breast cancer, HER2 negative breast cancer, Basal A breast cancer, Basal B breast cancer, or inflammatory breast cancer.

25. The method of claim 23 or 24, wherein the breast cancer is HER2 positive, and has been previously treated with trastuzumab.

26. The method of any one of claims 23 to 25, wherein the breast cancer is HER2 positive and trastuzumab refractory.

27. The method of any one of claims 23 to 26, further comprising identifying the subject as having a metastatic breast cancer, or having a HER2+ breast cancer, or having a triple negative breast cancer.
28. The method of any one of claims 1-27, wherein ganetespib or a pharmaceutically acceptable salt or a tautomer thereof is administered at a dose of from about 2 mg/m² to about 260 mg/m².

29. The method of any one of claims 1-28, wherein the method further comprises administering one or more additional anticancer agents.

30. The method of claim 29, wherein the one or more agents are selected from the group consisting of BEZ-235, AZD6244, AZD8055, SN-38, gemcitabine, camptothecin, docetaxel, cisplatin, oxaliplatin, crizotinib, paclitaxel, trastuzumab, and pemetrexed.

31. The method of any one of claims 1-30, wherein the amount of ganetespib administered is from about 75 mg/m² to about 260 mg/m².

32. The method of any one of claim 1-31, wherein the amount of ganetespib administered is from about 125 mg/m² to about 260 mg/m².

33. The method of any one of claim 1-32, wherein the amount of ganetespib administered is from about 175 mg/m² to about 260 mg/m².

34. The method of any one of claims 1-30, wherein ganetespib administered is about 75 mg/m², about 85 mg/m², about 100 mg/m², about 110 mg/m², about 115 mg/m², about 120 mg/m², about 145 mg/m², about 150 mg/m², about 175 mg/m², about 180 mg/m², about 200 mg/m², about 215 mg/m² or about 260 mg/m².

35. The method of any one of claims 1-34, wherein ganetespib is administered by intravenous infusion.

36. The method of claim 35, wherein the infusion is a peripheral intravenous infusion.

37. The method of claim 35, wherein ganetespib is infused over 60 minutes.
38. A kit to practice the method of any one of claims 1 to 37.
SUM149 Basal Inflammatory Breast Cancer Cells

Fraction affected @ 24 hr

FIG. 2
FIG. 5
### FIG. 7

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Baseline FDG PET/CT
Arrow: Right lower lobe lung metastases

FDG PET/CT Day 19
after ganetespib therapy

FIG. 10
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**FIG. 11**
Compound (I) + camptothecin

Fraction of cells affected

comp (I) (17 nM) combination
comp (I) (17 nM) combination
comp (I) (11 nM) combination
comp (I) (20 nM) combination
comp (I) (20 nM) combination

H2030 H2009 H358 A549

FIG. 12
Compound (I) + pemetrexed

Fraction of cells affected

comp (I) (17 nM) combination
pemetrexed (500 nM)

comp (I) (25 nM) combination
pemetrexed (135 nM)

comp (I) (11 nM) combination
pemetrexed (148 nM)

comp (I) (25 nM) combination
pemetrexed (304 nM)

H2030
H2009
A549
H358

FIG. 13
Compound (I) + platins

Fraction of cells affected

comp (I) (25 nM) combination
oxaliplatin (439 nM)
comp (I) (25 nM) combination
cisplatin (5 μM)

Calu-1

FIG. 15
FIG. 16

Compound (I) + SN-38

Fraction of cells affected

comp (I) (10 nM) SN-38 (10 nM) combination
comp (I) (11 nM) SN-38 (22 nM) combination
comp (I) (38 nM) SN-38 (75 nM) combination
comp (I) (34 nM) SN-38 (7 nM) combination
comp (I) (23 nM) SN-38 (15 nM) combination

H23    H2030    H727    H441    H2122
FIG. 17
FIG. 22

Relative cell viability to control

PANC-1
HPAC
Mia-PaCa2

0.0 0.2 0.4 0.6 0.8 1.0 1.2

10nM 25nM 50nM 100nM 200nM
Western blot for Mia-PaCa2, PANC-1 and HPAC cell lines treated with Ganetespib for 24 hours. Results indicate decreased levels of HIF-1α and VEGF levels in treated cells.

**FIG. 23**
FIG. 24
FIG. 25
INTERNATIONAL SEARCH REPORT

International application No
PCT/US2013/040594

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61P35/00 A61K31/41 A61K31/4196

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)
A61P A61K

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, CHEM ABS Data, EMBASE, WPI Data, BEI LSTEIN Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevan to claim No.</th>
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<td>X</td>
<td>FOLEY KEVIN P ET AL: &quot;Hsp90 inhibits Hi alpha degradation in the hypoxic regions of solid tumors&quot;, PROCEEDINGS OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH ANNUAL MEETING, vol. 51, April 1 (2010-04), page 640, XP002698942, &amp; 101ST ANNUAL MEETING OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH; WASHINGTON, DC, USA; APRIL 17-21, 2010 ISSN: 0197-Q16X</td>
<td>1-4,38</td>
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Further documents are listed in the continuation of Box C. See patent family annex.

* Special categories of cited documents:
  "X" 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 has been established that the publication date of another cited document 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

A document member of the same patent family

Date of the actual completion of the international search
26 June 2013

Date of mailing of the international search report
05/07/2013

Name and mailing address of the ISA/
European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016

Authorized officer
Garabatos-Perera, J
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<td>W. YING et al.: &quot;Ganetespi b, a Unique Triazolone-Containing Hsp90 Inhibitor, Exhibits Potent Anti tumor Activity and a Superior Safety Profile for Cancer Therapy&quot;, MOLECULAR CANCER THERAPEUTICS, vol. 11, no. 2, 1 February 2012 (2012-02-01), pages 475-484, XP055034243, ISSN: 1535-7163, DOI: 10.1158/1535-7163.MCT-11-0755</td>
<td>1-4, 12, 13, 23, 24, 28-35, 38</td>
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<td>Y</td>
<td>abstract page 477, right-hand column, paragraph 2; table SI page 478, right-hand column - page 479, left-hand column, paragraph 2; figure 3 page 479, right-hand column, paragraph 3 - page 480, left-hand column, line 7; figure 5 page 480; figure 4 -----</td>
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<td>HEMCHANDRA MAHASETHI et al.: &quot;Abstract 2326: Anti angiogenic effects associated with the inhibition of HSP90 in colorectal cancer&quot;, AMERICAN ASSOCIATION FOR CANCER RESEARCH, PROCEEDINGS, AMERICAN ASSOCIATION FOR CANCER RESEARCH, US, vol. 72, no. 8, Suppl. 1, 15 April 2012 (2012-04-15), XP008163018, ISSN: 0569-2261, DOI: 10.1158/1538-7445.AM2012-2326</td>
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<td>Y</td>
<td>WANG YISONG ET AL: &quot;STA-9090, a small-mol ecule Hsp90 inhibitor for the potential treatment of cancer &quot;. CURRENT OPINION IN INVESTIGATIONAL DRUGS, THOMSON REUTERS (SCI ENTI FIC) LTD, LONDON, UK, vol. 11, no. 12, 1 December 2010 (2010-12-01) , pages 1466-1476, XP009159004, ISSN: 2040-3429 the whole document</td>
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**DOCUMENTS CONSIDERED TO BE RELEVANT**

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<td>A</td>
<td>X. LU ET AL: &quot;In vivo Dynamics and Distinct Functions of Hypoxia in Primary Tumor Growth and Organotropic Metastasis of Breast Cancer&quot;, CANCER RESEARCH, vol. 70, no. 10, 4 May 2010 (2010-05-04), pages 3905-3914, XP055066747, ISSN: 0008-5472, DOI: 10.1158/0008-5472.CAN-09-3739</td>
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INTERNATIONAL SEARCH REPORT

Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

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

1. □ Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:

2. □ Claims Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. □ Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

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

see additional sheet

1. □ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. X As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. □ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. □ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

□ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

□ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

□ No protest accompanied the payment of additional search fees.
This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: ll(completely) ; 1-10, 12-36 (partially)

   Ganetespi b for use in the treatment of a cancer with a high level of hypoxia and with a mutation in KRAS

2. claims: 12-36(partially)

   Ganetespi b for use in the treatment of a cancer with a high level of hypoxia and with a mutation in ALK, wherein said cancer is not covered by any previous invention

3. claims: 23, 24, 27-36(all partially)

   Ganetespi b for use in the treatment of a metastatic breast cancer with a high level of hypoxia, wherein said cancer is not covered by any previous invention

4. claims: 23, 24, 27-36(all partially)

   Ganetespi b for use in the treatment of a triple negative breast cancer with a high level of hypoxia, wherein said cancer is not covered by any previous invention

5. claims: 23-36(partially)

   Ganetespi b for use in the treatment of a HER2 positive breast cancer with a high level of hypoxia, wherein said cancer is not covered by any previous invention

6. claims: l-10(partially)

   Ganetespi b for use in the treatment of a cancer with a high level of hypoxia, wherein said cancer is not covered by any previous invention

7. claims: l-10(partially)

   Ganetespi b for use in the treatment of a cancer with a mutation in KRAS, wherein said cancer is not covered by any previous invention
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<td>WO 2012037072 A1</td>
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