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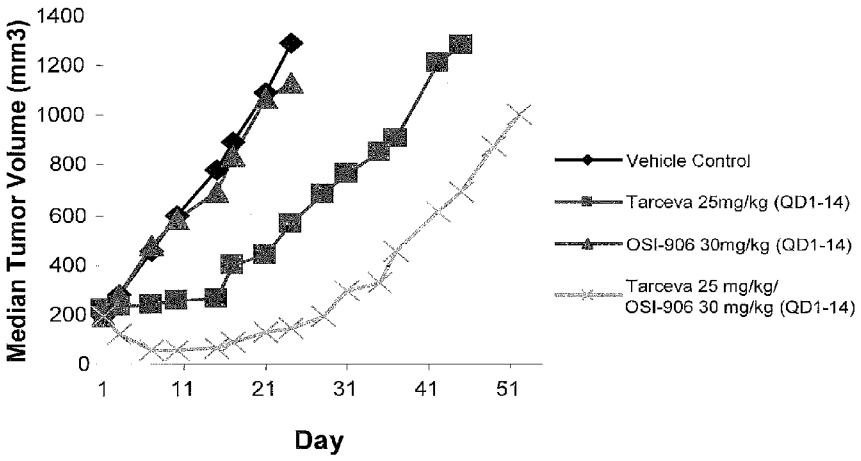
(54) **Title**: NSCLE COMBINATION THERAPY

Fig. 6



(57) **Abstract**: A method of treating NSCLC comprising in some aspects administering OSI-906 and an EGFR inhibitor to a patient having NSCLC having an activating EGFR mutation, as disclosed herein.

WO 2012/129145 A1

NSCLC COMBINATION THERAPY

FIELD AND BACKGROUND

The present invention pertains in some aspects to treating non-small cell lung cancer (NSCLC), and to treating NSCLC with combination drug therapy, including combinations comprising EGFR (epidermal growth factor receptor) and insulin-like growth factor receptor (IGF-1R) inhibitors, and including treating NSCLC in which there is an activating mutation in the EGFR kinase domain.

Over-expression of EGFR kinase, or its ligand TGF-α, is frequently associated with many cancers, including breast, lung, colorectal, head and neck cancers (Salomon D.S., et al. (1995) Crit. Rev. Oncol. Hematol. 19:183–232; Wells, A. (2000) Signal, 1:4-11), glioblastomas, and astrocytomas, and is believed to contribute to the malignant growth of these tumors. A specific deletion-mutation in the EGFR gene has also been found to increase cellular tumorigenicity (Halatsch, M-E. et al. (2000) J. Neurosurg. 92:297-305; Archer, G.E. et al. (1999) Clin. Cancer Res. 5:2646-2652). Activation of EGFR stimulated signaling pathways promote multiple processes that are potentially cancer-promoting, *e.g.*, proliferation, angiogenesis, cell motility and invasion, decreased apoptosis and induction of drug resistance.

The development for use as anti-tumor agents of compounds that directly inhibit the kinase activity of the EGFR, as well as antibodies that reduce EGFR kinase activity by blocking EGFR activation, are areas of intense research effort (de Bono J.S. and Rowinsky, E.K. (2002) Trends in Mol. Medicine 8:S19-S26; Dancey, J. and Sausville, E.A. (2003) Nature Rev. Drug Discovery 2:92-313).

Erlotinib (also known as OSI-774 or 774 and marketed in the product Tarceva®) is an orally available inhibitor of EGFR kinase. In vitro, erlotinib has demonstrated substantial inhibitory activity against EGFR kinase in a number of human tumor cell lines, including colorectal and breast cancer (Moyer J.D. et al. (1997) Cancer Res. 57:4838), and preclinical evaluation has demonstrated activity against a number of EGFR-expressing human tumor xenografts (Pollack, V.A. et al (1999) J. Pharmacol. Exp. Ther. 291:739). In a phase III trial, erlotinib monotherapy significantly prolonged survival, delayed disease progression and delayed worsening of lung cancer-related symptoms in patients with advanced, treatment-refractory NSCLC (Shepherd, F. et al. (2005) N. Engl. J. Med. 353(2):123-132). In November 2004 the U.S. Food and Drug Administration (FDA) approved Tarceva® for the treatment of patients with locally advanced or metastatic NSCLC after failure of at least one prior chemotherapy regimen.

It has been found that deletions in exon 19 of EGFR or EGFR L858R missense substitutions are present in more than 80% of NSCLC patients who respond to erlotinib treatment. Sequist et al., J. Clin. Oncol., 25, 587-595 (2007); 33d EMSO Congress: Abstract

230 PD (2008). See also Pao et al., PNAS, 101(36), 13306-311 (2004). However, the majority of responders eventually acquire resistance. Yao et al., *infra*.

F. Capuzzo et al., describe the SATURN erlotinib trial, including EGFR status stratification. EGFR mutation analyses were done using DNA lysates from tissue samples, with exons 18-21 amplified by PCR. Mutations had to be confirmed on both strands of at least two PCR products. *The Lancet*, 11, 521-529 (2010). US7294468 describes methods of identifying somatic mutations in the kinase domain of EGFR, in particular, in-frame deletion or substitution in exon 18, 19, 20, or 21 (*e.g.*, Cols. 1-12). H. Cheng et al. review EGFR mutation testing. *Curr. Oncol. Rep.*, 12, 335-348 (2010).

Yao et al. report that a subpopulation of NSCLC cells is intrinsically resistant to erlotinib and display features suggestive of epithelial-mesenchymal-transition (EMT). PNAS, 107(35), 15535-15540 (2010).

The IGF-1 pathway has an important role in human tumor development. IGF-1R overexpression is frequently found in various tumors (breast, colon, lung, sarcoma) and is often associated with an aggressive phenotype. High circulating IGF1 concentrations are strongly correlated with prostate, lung and breast cancer risk. Furthermore, IGF-1R is required for establishment and maintenance of the transformed phenotype *in vitro* and *in vivo* (Baserga R. *Exp. Cell. Res.*, 1999, 253, 1-6). The kinase activity of IGF-1R is essential for the transforming activity of several oncogenes: EGFR, PDGFR, SV40 T antigen, activated Ras, Raf, and v-Src. The expression of IGF-1R in normal fibroblasts induces neoplastic phenotypes, which can then form tumors *in vivo*. IGF-1R expression plays an important role in anchorage-independent growth. IGF-1R has also been shown to protect cells from chemotherapy-, radiation-, and cytokine-induced apoptosis. Conversely, inhibition of endogenous IGF-1R by dominant negative IGF-1R, triple helix formation or antisense expression vector has been shown to repress transforming activity *in vitro* and tumor growth in animal models. The IGF-1R signaling pathway also appears to be a robust target in colorectal cancer (CRC), based upon data demonstrating overexpression of the receptor and ligands in CRC, association with a more malignant phenotype, chemotherapy resistance, and correlation with a poor prognosis (Saltz, L.B., et al. J Clin Oncol 2007;25(30): 4793-4799; Tripkovic I., et al. Med Res. 2007 Jul;38(5):519-25. Epub 2007 Apr 26; Miyamoto S., et al. Clin Cancer Res. 2005 May 1;11(9):3494-502; Nakamura M., et al. Clin Cancer Res. 2004 Dec 15;10(24):8434-41; Grothey A, et al. J Cancer Res Clin Oncol. 1999;125(3-4):166-73).

US2006/0235031 refers to 6,6-bicyclic ring substituted heterobicyclic protein kinase inhibitors as IFG-1R inhibitors and uses thereof, including for treating cancer. Disclosed therein is OSI-906, which is a selective dual inhibitor of IR and IGF-1R in Phase III development. US2003/0114467; US2003/0153752; and US2005/0037999 refer to pyrazolo- and pyrrolo-pyrimidines and uses thereof, including for cancer treatment, and generally refer to various

combinations with other anticancer agents. US2005/0153966 refers to heterocyclic compounds said to be kinase inhibitors and uses thereof, including for cancer treatment. US2004/0180911 refers to pyrimidine derivatives and uses thereof, including for tumors and proliferative diseases, and states that the compounds can be used in combination with other chemotherapy drugs. WO2004/056830 refers to pyrrolopyrimidine derivatives and uses thereof, including for cancer treatment, and states that the compounds can be used in combination with other anticancer agents. Valeriote et al., *Cancer Chemotherapy Reports*, 59(5), 895-900 (1975), states that "extensive literature describing additivity and synergism in anticancer agents exists." US2004/0106605 is entitled "Synergistic Methods and Compositions for Treating Cancer," and generally refers to combinations of IGF1R inhibitors with EGFR inhibitors.

Growth factors acting through receptor tyrosine kinases (RTKs) drive tumor initiation and progression by accelerating cell proliferation and promoting cell survival. The RTKs for epidermal growth factor (EGF) and insulin-like growth factor (IGF) contribute to tumorigenesis for a multitude of tumor types including non-small cell lung cancer (NSCLC), colorectal, pancreatic, and breast tumors (Holbro, T., and Hynes, N. E. (2004). ErbB receptors: directing key signaling networks throughout life. Annu Rev Pharmacol Toxicol *44*, 195-217; Kurmasheva, R. T., and Houghton, P. J. (2006). IGF-I mediated survival pathways in normal and malignant cells. Biochim Biophys Acta *1766*, 1-22; Levitzki, A. (2003). EGF receptor as a therapeutic target. Lung Cancer *41 Suppl 1*, S9-14; Roskoski, R., Jr. (2004). The ErbB/HER receptor protein-tyrosine kinases and cancer. Biochem Biophys Res Commun *319*, 1-11.) Tumor cells can exhibit redundancy surrounding RTKs that contributes to *de novo* resistance to a single RTK inhibitor, and crosstalk between RTKs can confer *acquired* resistance whereby the inhibition of one RTK is compensated by enhanced activity through an alternative RTK.

It has been shown that IGF-1R signaling is associated with acquired resistance of cancer cells to chemo or radiation therapies, and molecular targeted therapies including epidermal growth factor receptor (EGFR) inhibition. Indeed, it has recently been shown that in several different cancer types the efficacy of EGFR and ErbB2 signal transduction inhibitors could be acutely attenuated by IGF-1R activation of the PI3-kinase/Akt pathway (Chakravarti, A., Loeffler, J. S., and Dyson, N. J. (2002). Insulin-like growth factor receptor I mediates resistance to anti-epidermal growth factor receptor therapy in primary human glioblastoma cells through continued activation of phosphoinositide 3-kinase signaling. Cancer research 62, 200-207; Jones, H. E., Goddard, L., Gee, J. M., Hiscox, S., Rubini, M., Barrow, D., Knowlden, J. M., Williams, S., Wakeling, A. E., and Nicholson, R. I. (2004). Insulin-like growth factor-I receptor signaling and acquired resistance to gefitinib (ZD1839; Iressa) in human breast and prostate cancer cells. Endocr Relat Cancer 11, 793-814; Lu, Y., Zi, X., Zhao, Y., Mascarenhas, D., and Pollak, M. (2001). Insulin-like growth factor-I receptor signaling and resistance to trastuzumab (Herceptin). Journal of the National Cancer Institute 93, 1852-1857; Nahta, R., Yuan, L. X.,

Zhang, B., Kobayashi, R., and Esteva, F. J. (2005). Insulin-like growth factor-I receptor/human epidermal growth factor receptor 2 heterodimerization contributes to trastuzumab resistance of breast cancer cells. Cancer research 65, 11118-11128). For instance, IGF-1R activation correlates with acquired resistance of breast and prostate cancer cells to EGFR inhibition (Jones et al., 2004). IGF-1R has also been shown to mediate resistance to anti-EGFR therapies in glioblastoma, colorectal, and NSCLC tumor cells (Chakravarti et al., 2002; Liu et al., 2001; Jones et al., 2004; Morgillo et al., 2006; Hurbin et al., 2003; Knowlden et al., 2005).

Several reports have stated that some EGFR kinase inhibitors can improve tumor cell or neoplasia killing when used in combination with certain other anti-cancer or chemotherapeutic agents or treatments (*e.g.*,. Raben, D. et al. (2002) Semin. Oncol. 29:37-46; Herbst, R.S. et al. (2001) Expert Opin. Biol. Ther. 1:719-732; Magne, N et al. (2003) Clin. Can. Res. 9:4735-4732; Magne, N. et al. (2002) British Journal of Cancer 86:819-827; Torrance, C.J. et al. (2000) Nature Med. 6:1024-1028; Gupta, R.A. and DuBois, R.N. (2000) Nature Med. 6:974-975; Tortora, et al. (2003) Clin. Cancer Res. 9:1566-1572; Solomon, B. et al (2003) Int. J. Radiat. Oncol. Biol. Phys. 55:713-723; Krishnan, S. et al. (2003) Frontiers in Bioscience 8, e1-13; Huang, S et al. (1999) Cancer Res. 59:1935-1940; Contessa, J. N. et al. (1999) Clin. Cancer Res. 5:405-411; Li, M. et al. Clin. (2002) Cancer Res. 8:3570-3578; Ciardiello, F. et al. (2003) Clin. Cancer Res. 9:1546-1556; Ciardiello, F. et al. (2000) Clin. Cancer Res. 6:3739-3747; Grunwald, V. and Hidalgo, M. (2003) J. Nat. Cancer Inst. 95:851-867; Seymour L. (2003) Current Opin. Investig. Drugs 4(6):658-666; Khalil, M.Y. et al. (2003) Expert Rev. Anticancer Ther.3:367-380; Bulgaru, A.M. et al. (2003) Expert Rev. Anticancer Ther.3:269-279; Dancey, J. and Sausville, E.A. (2003) Nature Rev. Drug Discovery 2:92-313; Kim, E.S. et al. (2001) Current Opinion Oncol. 13:506-513; Arteaga, C.L. and Johnson, D.H. (2001) Current Opinion Oncol. 13:491-498; Ciardiello, F. et al. (2000) Clin. Cancer Res. 6:2053-2063; US2003/0108545; US2002/0076408; US2003/0157104; WO99/60023; WO01/12227; WO02/055106; WO03/088971; WO01/34574; WO01/76586; WO02/05791; and WO02/089842).

US2004/0106605 is entitled "Synergistic Methods and Compositions for Treating Cancer," and generally refers to combinations of IGF-1R inhibitors with EGFR inhibitors. US2008/0267957 and US2008/0014200 disclose methods of treatment that include administering both an IGF-1R inhibitor and an EGFR inhibitor. These publications are incorporated herein in their entireties for all purposes, including the particular IGF-1R inhibitors, EGFR inhibitors, underlying mechanistic information, and methods of treatment.

Also noted are Harris et al., *Diseases of the Breast*, p. 1193 (2005); Ueda et al., *Modern Path.*, 19, 788-796 (2006); Wilsbacher et al., *J. Biol. Chem.*, 283, 35, 23721-30 (2008); *Science Daily* June 25, 2008 (www.sciencedaily.com/releases/2008/06/ 080624135934.htm; accessed Jan. 13, 2009); Takahari et al., *Oncology*, 76, 42-48 (2009); Erlotinib With or Without IMC-A12

(clinicaltrials.gov/ct2/show/NCT00778167?show_desc=Y; accessed Jan. 13, 2009); Riely et al., *Clin. Cancer Res.*, 13(17) (Sept. 2007); In vitro studies have been presented to support the hypothesis that an EGFR and IGF-1R inhibitor combination could synergistically inhibit proliferation and potentially drive apoptosis in early stage tumors with an epithelial phenotype - Barr et al., *Clin. Exp. Metastasis*, 25:685-693 (2008); M. Höpfner, Free University Berlin Dissertations Online (2007) (www.diss. fu-berlin.de/diss/ receive/fudiss_thesis_ 000000002588?lang=en; accessed Jan. 13, 2009).

US 2008/0267957 discloses cancer treatments comprising treating with a combination of erlotinib and OSI-906. WO 2010/107968 discloses methods of using OSI-906 in combination with erlotinib in a treatment beyond progression setting.

Clinicaltrials.gov, accessed on March 1, 2011, refers to a planned Phase II clinical study of erlotinib in combination with OSI-906 in patients with advanced NSCLC with activating EGFR mutations.

All of the foregoing are incorporated herein by reference in their entireties.

Unfortunately, not all subjects respond to Tarceva®/erlotinib or other EGFR inhibitors. Moreover, most responders eventually progress after a period of treatment. Standard protocol may call for cessation of EGFR inhibitor treatment when progression occurs. Thus, there is a need for improved cancer treatments, including treatments through which the effectiveness duration of an EGFR inhibitor is prolonged or extended, such as by increasing the time of progression-free survival on the EGFR inhibitor, such as by the addition of another small molecule therapeutic agent to the treatment regimen.

SUMMARY

The invention includes a method of treating NSCLC comprising administering an effective regimen comprising OSI-906 and an EGFR kinase inhibitor to a patient having an NSCLC tumor or tumor metastasis having an activating EGFR mutation and expressing a mutant EGFR.

The invention includes identifying a NSCLC patient having an activating EGFR mutation. In some embodiments, the invention includes treating a patient with advanced Stage IIIB or IV NSCLC.

The invention includes treating an NSCLC patient whose cancer has become resistant to an EGFR inhibitor.

The invention includes administering the EGFR inhibitor before or after taking food and/or administering OSI-906 with food.

The invention is further described herein and not limited by this summary.

FIGURES

FIG. 1. Analysis of pRTK signaling in NSCLC EGFR mutant cell lines.

FIGS. 2A, 2B. Phenotypic effects of erlotinib + OSI-906 combination in PC3E NSCLC cells.

FIG. 3. Effects of erlotinib + OSI-906 combination on intracellular signaling events in PC3E NSCLC cells.

FIG. 4. Cell killing with combination of erlotinib and OSI-906 in PC3E NSCLC cells.

FIG. 5. *In vivo* efficacy of erlotinib + OSI-906 combination in H1650 NSCLC model.

FIG. 6. *In vivo* efficacy of erlotinib + OSI-906 combination in H1650 NSCLC model. Synergistic inhibition of tumor growth is observed.

FIG. 7. *In vivo* efficacy of erlotinib + OSI-906 combination in PC-14 NSCLC xenografts.

FIG. 8. *In vivo* efficacy of erlotinib + OSI-906 combination in PC-14 NSCLC xenografts.

DETAILED DESCRIPTION

In some embodiments, the invention includes a method of treating NSCLC in a patient having an NSCLC tumor or tumor metastasis having an activating EGFR mutation and expressing a mutant EGFR, comprising administering to the patient an effective regimen comprising OSI-906 and an EGFR tyrosine kinase inhibitor. An activating mutation is one or more mutations causing deregulation of EGFR, and can include, for example, any of the activating mutations described in Sharma et al., *Nature Reviews Cancer*, 7, 169-181 (2007), which is incorporated herein in its entirety by this reference.

In some embodiments, the mutation comprises an in-frame exon 19 deletion. In some embodiments, the mutation comprises an exon 21 point mutation. In some embodiments, the exon 21 mutation is L858R. In some embodiments, the mutation comprises an exon 20 in-frame duplication or insertion. In some embodiments, the mutation comprises a mutation exemplified herein.

In some embodiments, the NSCLC does not exhibit PTEN loss. In some embodiments, the NSCLC does not include an activating KRas mutant.

In some embodiments, cells of the NSCLC have an epithelial phenotype. Phenotype can determined according to the art, such as according to ecadherin, ErbB3 or claudin 4.

In some embodiments, the NSCLC is advanced stage IIIB or stage IV.

In some embodiments, the NSCLC has acquired resistance to EGFR inhibitor treatment, such as by IGF-1R activation. In some embodiments, the NSCLC does not have a primary resistance to EGFR inhibitor treatment. See, *e.g.*, Sequist et al., *Sci. Transl. Med.*, 3, 75ra26, 1-12 (2011), which is incorporated herein in its entirety by this reference.

NSCLC tumor cells harboring primary activating mutations in EGFR exhibit elevated sensitivity to the EGFR tyrosine kinase inhibitors erlotinib and gefitinib, however, acquired

resistance following prolonged exposure to an EGFR TKI can be associated with increased dependence on MET. This mode of resistance to EGFR inhibitors is especially prominent for tumors exhibiting genetic amplification of the MET loci. In tumors expressing wild-type (WT) EGFR, IGF-1R/IR signaling can confer acquired resistance, where prolonged exposure to agents targeting either EGFR or IGF-1R/IR may be associated with an increased dependence on signaling through the reciprocal receptor. For a number of tumor types, including NSCLC, dependence on either EGFR or IGF-1R/IR appears to be greater in epithelial tumor cell lines compared to those that have undergone an epithelial-mesenchymal-transition (EMT), and crosstalk between EGFR and IGF-1R/IR is commonly observed in tumor cells with an epithelial EMT status.

In some embodiments, the EGFR inhibitor comprises erlotinib. In some embodiments, the erlotinib is administered at about 50-300 mg QD. In some embodiments, the erlotinib is administered at about 150 mg QD. In some embodiments, the erlotinib is administered about one hour before or about two hours after food.

In some embodiments, the OSI-906 is administered at about 75-300 mg BID. In some embodiments, the OSI-906 is administered at about 150 mg BID. In some embodiments, the OSI-906 is administered at or around meal times. For example, administered with food or less than about two hours after food consumption or less than about one half hour before food consumption. In some embodiments, this is applicable generally to the administration of OSI-906, regardless of disease setting or single agent or combination agent approach.

In some embodiments, the patient survives at least about one year from starting the method. In some embodiments, the patient experiences stable disease or partial response for at least about one half year from starting the method.

In some embodiments, the OSI-906 and the EGFR inhibitor together produce a synergistic anti-tumor effect.

The invention includes the use of an IGFR-1R/IR inhibitor such as OSI-906 and an EGFR inhibitor such as erlotinib in the manufacture of a medicament for any of the uses and treatments described.

NSCLC

The invention can be used to treat non-small cell lung cancer (NSCLC), which includes squamous cell carcinoma, adenocarcinoma, and large cell carcinoma. In some embodiments, the NSCLC is advanced stage IIIB or stage IV. In some embodiments, the NSCLC has acquired resistance to EGFR inhibitor treatment.

In some embodiments, the patient is screened for an activating EGFR mutation. A fresh biopsy or archival tumor sample can used to sequence the EGFR gene. *See, e.g.*, US7294468, Capuzzo et al.; Pao et al., *supra*.

In some embodiments, the mutation comprises an in-frame exon 19 deletion, or the mutation comprises an exon 21 point mutation. In some embodiments, the exon 21 mutation is L858R. In some embodiments, the mutation comprises an exon 20 in-frame duplication or insertion.

In some embodiments, cells of the NSCLC have an epithelial phenotype.

EGFR Inhibitor

The EGFR inhibitor can be any effective EGFR tyrosine kinase inhibitor, preferably having government marketing approval presently or at any time. It can be a small molecule or other molecule that binds to the intracellular receptor domain (HER-1) to inhibit its kinase activity. In some embodiments, the EGFR inhibitor can comprise a known compound of the quinazoline, pyridopyrimidine, or pyrrolopyrimidine structural classes. *See, e.g.*, de Bono et al., *Trends Mol. Med.*, 8, S19-26 (2002). In some embodiments, the EGFR inhibitor comprises erlotinib. Erlotinib may be referred to as Tarceva, OSI-774, or 774. *See, e.g.*, US 2008/0267957. In some embodiments, the EGFR inhibitor comprises gefitinib. Other examples are set forth hereinbelow. The skilled artisan has sufficient knowledge to select any appropriate agent.

IGF-1R/IR Inhibitor

Any suitable IGF-1R or IGF-1R/IR inhibitor can be used, preferably having government marketing approval presently or at any time. The invention includes administering OSI-906 (*cis*-3-[8-amino-1-(2-phenylquinolin-7-yl)-imidazo[1,5-*a*]pyrazin-3-yl]-1-methylcyclobutanol). The small-molecule selective IGF-1R/IR inhibitor OSI-906 is known to the skilled artisan and is disclosed in US7534797 (Example 31). Physical forms of OSI-906 are disclosed in US Appl. No. 61/357688 (filed June 23, 2010). OSI-906 is currently in Phase II/III development.

According to the invention, additional active agent(s) may be utilized as appropriate.

COMPOSITION

The invention includes pharmaceutical compositions comprising one or more compounds or pharmaceutically acceptable salt thereof for use in the invention, formulated for a desired mode of administration with or without one or more pharmaceutically acceptable and useful carriers.

The pharmaceutical compositions of the present invention comprise a compound (or a pharmaceutically acceptable salt thereof) as an active ingredient, optional pharmaceutically acceptable carrier(s) and optionally other therapeutic ingredients or adjuvants. The compositions include compositions suitable for oral, rectal, topical, and parenteral (including subcutaneous, intramuscular, and intravenous) administration, although the most suitable route

in any given case will depend on the particular host, and nature and severity of the conditions for which the active ingredient is being administered. The pharmaceutical compositions may be conveniently presented in unit dosage form and prepared by any of the methods well known in the art of pharmacy.

Compounds of the invention can be combined as the active ingredient in intimate admixture with a pharmaceutical carrier according to conventional pharmaceutical compounding techniques. The carrier may take a wide variety of forms depending on the form of preparation desired for administration, *e.g.*, oral or parenteral (including intravenous). Thus, the pharmaceutical compositions of the present invention can be presented as discrete units suitable for oral administration such as capsules, cachets or tablets each containing a predetermined amount of the active ingredient. Further, the compositions can be presented as a powder, as granules, as a solution, as a suspension in an aqueous liquid, as a non-aqueous liquid, as an oil-in-water emulsion, or as a water-in-oil liquid emulsion. In addition to the common dosage forms set out above, the compound may also be administered by controlled release means and/or delivery devices. The compositions may be prepared by any of the methods of pharmacy. In general, such methods include a step of bringing into association the active ingredient with the carrier that constitutes one or more necessary ingredients. In general, the compositions are prepared by uniformly and intimately admixing the active ingredient with liquid carriers or finely divided solid carriers or both. The product can then be conveniently shaped into the desired presentation.

The pharmaceutical carrier employed can be, for example, a solid, liquid, or gas. Examples of solid carriers include lactose, terra alba, sucrose, talc, gelatin, agar, pectin, acacia, magnesium stearate, and stearic acid. Examples of liquid carriers are sugar syrup, peanut oil, olive oil, and water. Examples of gaseous carriers include carbon dioxide and nitrogen.

A tablet containing the composition of this invention may be prepared by compression or molding, optionally with one or more accessory ingredients or adjuvants. Compressed tablets may be prepared by compressing, in a suitable machine, the active ingredient in a free-flowing form such as powder or granules, optionally mixed with a binder, lubricant, inert diluent, surface active or dispersing agent. Molded tablets may be made by molding in a suitable machine, a mixture of the powdered compound moistened with an inert liquid diluent. Each tablet preferably contains from about 0.05 mg to about 5 g of the active ingredient and each cachet or capsule preferably containing from about 0.05 mg to about 5 g of the active ingredient. A formulation intended for the oral administration to humans may contain from about 0.5mg to about 5g of active agent, compounded with an appropriate and convenient amount of carrier material which may vary from about 5 to about 95 percent of the total composition. Unit dosage forms will generally contain between from about 1mg to about 2 g of the active ingredient,

typically 50 mg, 100 mg, 150 mg, 200 mg, 250 mg, 300 mg, 400 mg, 500 mg, 600mg, or 800 mg.

Pharmaceutical compositions of the present invention suitable for parenteral administration may be prepared as solutions or suspensions of the active compounds in water. Pharmaceutical compositions of the present invention suitable for injectable use include sterile aqueous solutions or dispersions. Pharmaceutical compositions of the present invention can be in a form suitable for topical use such as, for example, an aerosol, cream, ointment, lotion, dusting powder, or the like. Pharmaceutical compositions of this invention can be in a form suitable for rectal administration wherein the carrier is a solid. It is preferable that the mixture forms unit dose suppositories.

Treatment, Dosing, and Results

In some embodiments, erlotinib is administered at about 50-300 mg QD, or about 150 mg QD. In some embodiments, erlotinib is administered about one hour before or about two hours after patient food consumption.

In some embodiments, OSI-906 is administered at about 75-300 mg BID, or about 150 mg BID. In some embodiments, OSI-906 is administered with food or less than about two hours after food consumption or less than about one half hour before food consumption.

In some embodiments, OSI-906 is administered on every day of the method. In some embodiments, OSI-906 is administered only on the first 3, 4, 5, 6, or 7 days of each 14 day period (or other treatment cycle period).

In some embodiments, the OSI-906 and the EGFR inhibitor together produce a synergistic anti-tumor effect.

Results, including response and progression, can be evaluated using objective criteria such as RECIST criteria. For example, RECIST version 1.1 (2009) can be used.

In some embodiments, the patient survives at least about one month, two months, four months, six months, eight months, one year, sixteen months, two years, or four years from starting the method.

In some embodiments, the patient experiences progression-free survival for at least about one month, two months, four months, six months, eight months, one year, sixteen months, or two years, from starting the method.

In some embodiments, the patient experiences stable disease or at least partial response for at least about one month, two months, four months, six months, eight months, one year, sixteen months, or two years, from starting the method.

EXPERIMENTAL

I. Data suggest and indicate that the combination of erlotinib and OSI-906 is more efficacious in NSCLC models expressing a mutant EGFR compared to the actions of each single agent. This is manifested in an enhanced inhibition of the PI3K/AKT pathway with the combination treatment.

Characterization of NSCLC Cell lines Harboring EGFR Mutations

A panel of NSCLC cell lines was shown to harbor a mutation in the EGFR kinase domain. The cell lines H1650, HCC2935, HCC4006, PC3E and HCC827 harbored a deletion mutation in exon19, whereas H1975 harbored the double point mutation L858R/T790M. Analysis of the signaling pathways active within these cells by pRTK analysis is shown in Fig 1. All of the cell lines showed high levels of pEGFR, suggesting this to be the dominant active RTK in these cells.

Low levels of pIGF1R/IR in H1975, HCC2935, HCC4006 and PC3E cells and significant levels of pMET and pcRET in the HCC827 cell line were detected.

Sensitivity to either erlotinib or OSI-906 in 3 day cell proliferation assays was analyzed, with the results shown in Table 1.

Table 1

Cell Line	Mutation	Erlotinib Sensitivity (EC50)	OSI-906 Sensitivity (EC50)
H1650	delE746-A750	>10 μM	>10 μM
H1975	L858R/T790M	>10 μM	>10 μM
HCC827	delE746-A750	0.007 μM	>10 μM
HCC4006	del L747 - E749, A750P	0.026 μM	>10 μM
HCC2935	del E746 - T751	0.033 μM	>10 μM
$PC3_E$ (JCP-3)	L747-E749del, A750P	0.016 μM	>10 μM

HCC827, HCC4006, HCC2935 and PC3E cells were hypersensitive to erlotinib treatment with IC50's below 50 nM. H1650 cells showed limited sensitivity, as has been reported in the literature even though they express a mutant EGFR; however the mechanism of resistance is currently unclear. H1975 cells harbor the secondary resistance mutation T790M rendering them resistant to the actions of erlotinib *in vitro*. None of the six cell lines in Table 1 were sensitive to treatment with OSI-906 alone.

Combination of Erlotinib and OSI-906 in EGFR Mutant Cells

EGFR mutant cells were treated with a combination of erlotinib and OSI-906 for either 48hr apoptosis or 72 hr proliferation assays and synergy analyzed by BLISS methodology (Table 2: Key: -, no effect; +(A), additivity; (S), synergy).

Table 2

		Erlotinib	Erlotinib Combination Activity with OSI-906	
Cell Line	**Mutation**	**EC50**	**Proliferation**	**Apoptosis**
H1650	delE746-A750	>10 μM	-	-
H1975	L858R/T790M	>10 μM	-	-
HCC827	delE746-A750	7 nM	-	+ (A)
HCC4006	delE746-A749,A750P	26 nM	-	+ (A)
HCC2935	delE746-T751	33 nM	-	+ (A)
$PC3_E$ (JCP-3)	L747-E749del,A750P	15 nM	+ (S)	+ (S)

Synergy was observed in both the apoptosis and proliferation assays with the erlotinib plus OSI-906 combination in the PC3E EGFR mutant cell line. Additivity was observed in apoptosis assays with the drug combination in HCC827, HCC4006 and HCC2935 cells but did not translate into an effect in the proliferation assay.

Analysis of the PC3E EGFR Mutant Model

PC3E cells were treated with the combination of erlotinib and OSI-906 for effects on apoptosis and cellular proliferation (Figure 2A and B).

PC3E cells were hyper-sensitive to erlotinib but OSI-906 treatment had no discernable effect upon cell growth. However, the combination of erlotinib and OSI-906 showed a greater effect than the theoretically predicted effects if the two agents were only additive in nature (Figure 2A). Similarly, a synergistic induction in caspase 3/7 activity was observed (a measure of cell apoptosis) with the combination (Figure 2B).

Effects on cell signaling were analyzed to gain an understanding of the mechanism responsible for the synergistic effects in this EGFR mutant cell line. PC3E cells were treated with either erlotinib (1uM), OSI-906 (1uM), or a combination of the two agents for 2 hours and the effects on intracellular signaling cascades analyzed (Figure 3). Both agents inhibited their targets, based on analysis of the phosphorylation status of EGFR, IR, and IGF1R on phosphor-

receptor tyrosine kinase arrays. Erlotinib treatment reduced the phosphorylation of EGFR and OSI-906 reduced the phosphorylation of IGF1R and IR. The combination of erlotinib and OSI-906 resulted in a decrease in the phosphorylation of EGFR, IR, and IGF1R. As to effects on ERK and PI3K/AKT: Treatment with erlotinib caused a reduction in the levels of pERK but only a modest effect on the phosphorylation of PRAS40 (a substrate of AKT). Treatment with OSI-906 had no effect on pERK signaling but had a modest effect on pPRAS40. But the combination of the two agents produced an enhanced effect on the inhibition of PRAS40 compared to each single agent alone. In addition, there was an enhanced increase in the levels of cleaved PARP (cPARP) protein with the combination, suggestive of increased apoptosis and consistent with the previously described caspase 3/7 assay.

To study whether treatment with the combination of erlotinib and OSI-906 in this model would induce complete cell killing, PC3E cells were treated with erlotinib (1uM), OSI-906 (1uM) or a combination of the two agents for 9 days (Figure 4). Analysis of the cell culture dishes after this treatment revealed that OSI-906 alone had little effect on cell number. Treatment with erlotinib caused almost complete cell death, however small colonies of cells that appeared to be resistant to the effects of erlotinib treatment were observed. No such cells were observed in the dish treated with the combination of erlotinib and OSI-906.

To study whether the cells remaining were still viable, drug treatments were removed, and cell culture was continued in drug-free media for a further 10 days. The cells treated with erlotinib alone grew to confluency after withdrawal of drug for 10 days. In contrast, the dish of cells treated with the combination of erlotinib + OSI-906 failed to show any cell growth during the period of drug removal. These results suggest that there is a small population of EGFR mutant cells that are resistant to the effects of erlotinib alone but sensitive to the combination of erlotinib plus OSI-906.

In Vivo efficacy of Erlotinib + OSI-906 Combination in EGFR Mutant NSCLC

Cell line H1650 was used in a mouse xenograft model. Treatment of the tumors for 14 days with erlotinib caused significant tumor regressions, whereas treatment with OSI-906 had no effect (Figure 5). The combination of erlotinib (100mg/kg) and OSI-906 (10mg/kg) had no significant additional effect over erlotinib alone during the dosing period, however when drug was removed and the tumors allowed to grow out, there was a reduction in the growth rate of tumors treated with the combination compared to those treated with erlotinib alone.

The data suggest possible synergism in this model. However, as erlotinib was extremely effective at the dose used (100mg/kg) the additional effect of OSI-906 could have been masked. Thus, the experiment was repeated using doses of erlotinib that induced tumor stasis (25mg/kg) rather than regression (100mg/kg). In addition, the amount of OSI-906 used was increased and confirmed no drug-drug interaction that would potentially confound the

interpretation of the experiment (Figure 6). At these concentrations of drug, the combination of erlotinib + OSI-906 for 14 days induced dramatic tumor regressions compared to the tumor stasis observed with upon treatment with erlotinib alone.

Cell line PC-14 was used in a mouse xenograft model. (Figures 7 and 8 and Table 3). In 14 daily doses, treatment with OSI-906 alone had no effect. Treatment with 25 mg/kg erlotinib had no effect. Treatment with 100 mg/kg erlotinib alone caused significant tumor regressions similar to the combination of erlotinib 25 mg/kg and OSI-906 30 mg/kg. However, a marked improvement was achieved in the combination of erlotinib 100 mg/kg and OSI-906 10 mg/kg. T-C indicates the time for a treatment group to reach 400% tumor size minus the time for the control group tumors to reach the same size. The T-C value for 25 mg/kg erlotinib + 30 mg/kg OSI-906 was 32 days, whereas the T-C values for erlotinib and OSI-906 alone were both 2 days, indicating potential synergy (Fig. 8; Table 3).

Table 3

Group	Dose (mg/kg)	%TGI	T-C	% Regression	%BW	% Mortality
Control	NA	NA	NA	none	105.7	0
erlotinib	25	88	2	none	97.4	0
OSI-906	30	28	2	none	93.6	0
erlotinib + OSI-906	25+30	100	32	87	88.7	0

Overall, the data demonstrate enhanced efficacy with the combination of erlotinib and OSI-906, compared to single agent treatment in a NSCLC EGFR mutant background. In vitro data demonstrated that in PC3E cells, combined treatment with erlotinib and OSI-906 resulted in a synergistic inhibition of cell growth and induction of apoptosis. This was linked to an enhanced inhibition of signaling through the PI3K/AKT pathway with the combination compared to either single agent. In vivo data demonstrated a synergistic effect on tumor growth using the combination of erlotinib and OSI-906 in the EGFR mutant cell model H1650.

II. Role of EMT

EMT may influence the sensitivity to erlotinib of NSCLC expressing mutant EGFR. Mutant EGFR cell lines could be induced to undergo EMT by stimulation with TGF-β resulting in a reduction of their sensitivity to erlotinib. In addition, cell lines were generated with acquired resistance to erlotinib and analysis of these cells indicated that they had undergone EMT. The resistant cells had down-regulated epithelial markers such as E-cadherin and ErbB3 and up-

regulated mesenchymal markers such as vimentin and fibronectin. The cells exhibited a gene expression profile consistent with an EMT and also had taken on a more migratory and invasive phenotype.

A panel of mutant EGFR NSCLC cell lines (H1650, HCC827, HCC4006, HCC2935, H1975, and PC3E) was analyzed to determine their EMT status. All of the EGFR mutant cell lines were E-cadherin positive and vimentin negative and displayed a cuboid phenotype, suggesting that they were all epithelial in nature. Each of these cell lines was treated with TGF-β for 7 days to determine whether they had the capacity to undergo EMT. Four of the six cell lines (HCC827, HCC4006, HCC2935 and H1975) underwent a phenotype and marker change consistent with EMT. The cells exhibited an elongated and scattered morphology and had down regulated E-cadherin and up regulated vimentin. These data are consistent with the hypothesis that mutant EGFR expressing NSCLC cells lines have the capacity to undergo an EMT.

The effect of TGF-β treatment had on the erlotinib sensitivity of the transitioned cells was analyzed. HCC827 untreated cells, those treated with TGF-β for 14 days and those that had undergone a reversion after TGF-β removal were treated with increasing concentrations of erlotinib and cell number assayed after 3 days. The parental cells were hypersensitive to the treatment of erlotinib, however those treated with TGF-β show a marked reduction in drug sensitivity. In addition, those cells that had undergone a reversion to an epithelial phenotype also regained sensitivity to erlotinib treatment. Taken together these data suggest that the EMT status of EGFR mutant NSCLC cell lines can influence their sensitivity to erlotinib.

HCC4006 cells were cultured *in vitro* with increasing concentrations of erlotinib over a 6 month period. We isolated 15 different erlotinib-resistant clones and analyzed the sensitivity of these cells to erlotinib treatment in 3 day proliferation assays. Erlotinib treatment of the parental HCC4006 cells results in significant cell growth inhibition and apoptosis. In contrast, none of the erlotinib-resistant clones showed any signs of sensitivity to erlotinib over the duration of this assay, confirming their resistance to the actions of EGFR inhibition.

The parental cell lines and the resistant clones were treated with erlotinib for 2 hours and measured the effect on pEGFR levels. Treatment with erlotinib decreased the level of pEGFR in the parental line and all the resistant clones, suggesting that the drug was able to able to enter the cells and inhibit the EGFR target. Thus the mechanism of resistance to erlotinib was not due to an inability of the drug to inhibit its target.

Although erlotinib was able to inhibit phosphorylation of EGFR in the parental and erlotinib-resistant clones it was only able to inhibit downstream AKT signaling (as measured by pPRAS40 levels) in the parental line. This suggests that the erlotinib-resistant lines control activation of the PI3K/AKT pathway in an EGFR-independent manner and potentially provides an explanation for their resistance to erlotinib.

The expression of the epithelial marker E-cadherin and the mesenchymal marker vimentin were assayed. The parental HCC4006 line was E-cadherin positive but vimentin negative. In contrast all of the erlotinib-resistant clones had lost the expression of E-cadherin and gained expression of vimentin. This data indicated the erlotinib-resistant clones had undergone a protein marker change consistent with EMT.

Taken together these observations provide definitive evidence that an EGFR mutant expressing NSCLC cell line can acquire resistance to erlotinib treatment by undergoing an EMT.

The acquisition of morphology and marker changes consistent with EMT in the erlotinib-resistant cells led to testing whether these clones had acquired phenotypic properties associated with mesenchymal cells. One major property of mesenchymal cells is their enhanced migratory and invasive capacity in comparison to epithelial cells. The migratory potential of the parental HCC4006 cells and one of the erlotinib-resistant clones were studied using the ORIS migration assay. Cells were plated and assayed for migration into the center of the well after 24 hours by either DAPI or falloidin staining. The erlotinib-resistant clone 4R14 showed a clear migration into the center of the well after 24 hours. This was in contrast to the minimal migration observed with the parental HCC4006 cells. The enhanced migration of the erlotinib-resistant lines was consistent with the mesenchymal gene and protein expression pattern in these cells.

III. Food Effect Study

An open label, randomized, two-way crossover study in patients with advanced solid tumors (n=12) investigated the safety and PK of OSI-906 administered with a high fat meal (containing 50 to 60% fat, 30% carbohydrates and 15% protein) and under fasted conditions. Patients received 300 mg of OSI-906 once-daily on days 1 to 3 every 14 days. Blood samples were collected on days 1 and 15. Plasma concentrations of OSI-906 were determined by using a validated LC-MS/MS method. Results: The 300 mg OSI-906 once-daily treatment for 3 days in every 14 days under both fed and fasted conditions was well tolerated by all patients. The point estimates of geometric mean ratio (fed/fasted) for C_{max}, and AUC demonstrated 16 to 38% higher exposure of OSI-906 with high fat meal. The 90% confidence intervals of Cmax and AUC were 80.5 -167 and 97.5-163, respectively. The T_{max} was delayed in presence of high fat meal (median 4 vs.1 hr). The median terminal half-life was similar for both treatments (4.38 vs. 4.02 hr). Conclusions: The exposure of OSI-906 was higher in the presence of a high fat meal.

DEFINITIONS

The language and terms herein are to be given their broadest meaning accepted by the skilled artisan, unless otherwise specified.

The term "cancer" in an animal, including human, refers to the presence of cells possessing characteristics typical of cancer-causing cells, such as uncontrolled proliferation, immortality, metastatic potential, rapid growth and proliferation rate, and certain characteristic morphological features. Often, cancer cells will be in the form of a tumor, but such cells may exist alone within an animal, or may circulate in the blood stream as independent cells, such as leukemic cells.

"Cell growth", as used herein, for example in the context of "tumor cell growth", unless otherwise indicated, is used as commonly used in oncology, where the term is principally associated with growth in cell numbers, which occurs by means of cell reproduction (i.e. proliferation) when the rate of the latter is greater than the rate of cell death (e.g. by apoptosis or necrosis), to produce an increase in the size of a population of cells, although a small component of that growth may in certain circumstances be due also to an increase in cell size or cytoplasmic volume of individual cells. An agent that inhibits cell growth can thus do so by either inhibiting proliferation or stimulating cell death, or both, such that the equilibrium between these two opposing processes is altered.

"Tumor growth" or "tumor metastases growth", as used herein, unless otherwise indicated, is used as commonly used in oncology, where the term is principally associated with an increased mass or volume of the tumor or tumor metastases, primarily as a result of tumor cell growth.

"Abnormal cell growth", as used herein, unless otherwise indicated, refers to cell growth that is independent of normal regulatory mechanisms (e.g., loss of contact inhibition). This includes the abnormal growth of: (1) tumor cells (tumors) that proliferate by expressing a mutated tyrosine kinase or over-expression of a receptor tyrosine kinase; (2) benign and malignant cells of other proliferative diseases in which aberrant tyrosine kinase activation occurs; (4) any tumors that proliferate by receptor tyrosine kinases; (5) any tumors that proliferate by aberrant serine/threonine kinase activation; and (6) benign and malignant cells of other proliferative diseases in which aberrant serine/threonine kinase activation occurs.

As used herein, the term "patient" refers to a human in need of treatment with an anti-cancer agent for any purpose, and more preferably a human in need of such a treatment to treat cancer, or a precancerous condition or lesion.

The term "treating" as used herein, unless otherwise indicated, means reversing, alleviating, inhibiting the progress of, or preventing, either partially or completely, the growth of tumors, tumor metastases, or other cancer-causing or neoplastic cells in a patient. The term "treatment" as used herein, unless otherwise indicated, refers to the act of treating.

The phrase "a method of treating" or its equivalent, when applied to, for example, cancer refers to a procedure or course of action that is designed to reduce or eliminate the number of cancer cells in an animal, or to alleviate the symptoms of a cancer. "A method of

treating" cancer or another proliferative disorder does not necessarily mean that the cancer cells or other disorder will, in fact, be eliminated, that the number of cells or disorder will, in fact, be reduced, or that the symptoms of a cancer or other disorder will, in fact, be alleviated. Often, a method of treating cancer will be performed even with a low likelihood of success, but which, given the medical history and estimated survival expectancy of an animal, is nevertheless deemed an overall beneficial course of action.

As used herein, "agent" or "biologically active agent" refers to a biological, pharmaceutical, or chemical compound or other moiety. Non-limiting examples include simple or complex organic or inorganic molecule, a peptide, a protein, an oligonucleotide, an antibody, an antibody derivative, antibody fragment, a vitamin derivative, a carbohydrate, a toxin, or a chemotherapeutic compound. Various compounds can be synthesized, for example, small molecules and oligomers (e.g., oligopeptides and oligonucleotides), and synthetic organic compounds based on various core structures. In addition, various natural sources can provide compounds for screening, such as plant or animal extracts, and the like. A skilled artisan can readily recognize that there is no limit as to the structural nature of the agents of the present invention.

The term "agonist" as used herein refers to a compound having the ability to initiate or enhance a biological function of a target protein, whether by inhibiting the activity or expression of the target protein. Accordingly, the term "agonist" is defined in the context of the biological role of the target polypeptide. While preferred agonists herein specifically interact with (e.g. bind to) the target, compounds that initiate or enhance a biological activity of the target polypeptide by interacting with other members of the signal transduction pathway of which the target polypeptide is a member are also specifically included within this definition.

The terms "antagonist" and "inhibitor" are used interchangeably, and they refer to a compound having the ability to inhibit a biological function of a target protein, whether by inhibiting the activity or expression of the target protein. Accordingly, the terms "antagonist" and "inhibitors" are defined in the context of the biological role of the target protein. While preferred antagonists herein specifically interact with (e.g. bind to) the target, compounds that inhibit a biological activity of the target protein by interacting with other members of the signal transduction pathway of which the target protein is a member are also specifically included within this definition. A preferred biological activity inhibited by an antagonist is associated with the development, growth, or spread of a tumor, or an undesired immune response as manifested in autoimmune disease.

The term "effective amount" or "therapeutically effective amount" refers to that amount of a compound described herein that is sufficient to effect the intended application including but not limited to disease treatment, as defined below. The therapeutically effective amount may vary depending upon the intended application (in vitro or in vivo), or the subject and disease

condition being treated, e.g., the weight and age of the subject, the severity of the disease condition, the manner of administration and the like, which can readily be determined by one of ordinary skill in the art. The term also applies to a dose that will induce a particular response in target cells, e.g. reduction of platelet adhesion and/or cell migration. The specific dose will vary depending on the particular compounds chosen, the dosing regimen to be followed, whether it is administered in combination with other compounds, timing of administration, the tissue to which it is administered, and the physical delivery system in which it is carried.

The term "selective inhibition" or "selectively inhibit" as applied to a biologically active agent refers to the agent's ability to selectively reduce the target signaling activity as compared to off-target signaling activity, via direct or interact interaction with the target.

For purposes of the present invention, "co-administration of" and "co-administering" an refer to any administration of the two active agents, either separately or together, where the two active agents are administered as part of an appropriate dose regimen designed to obtain the benefit of the combination therapy. Thus, the two active agents can be administered either as part of the same pharmaceutical composition or in separate pharmaceutical compositions.

The terms "responsive" or "responsiveness" when used herein in referring to a patient's reaction to administration of an active agent, refers to a response that is positive or effective, from which the patient is likely to benefit.

The term "method for manufacturing a medicament" or "use of for manufacturing a medicament" relates to the manufacturing of a medicament for use in the indication as specified herein, and in particular for use in tumors, tumor metastases, or cancer in general. The term relates to the so-called "Swiss-type" claim format in the indication specified.

As used herein, the term "EGFR kinase inhibitor" refers to any EGFR kinase inhibitor that is currently known in the art or that will be identified in the future, and includes any chemical entity that, upon administration to a patient, results in inhibition of a biological activity associated with activation of the EGF receptor in the patient, including any of the downstream biological effects otherwise resulting from the binding to EGFR of its natural ligand. Such EGFR kinase inhibitors include any agent that can block EGFR activation or any of the downstream biological effects of EGFR activation that are relevant to treating cancer in a patient. Such an inhibitor can act by binding directly to the intracellular domain of the receptor and inhibiting its kinase activity. Alternatively, such an inhibitor can act by occupying the ligand binding site or a portion thereof of the EGF receptor, thereby making the receptor inaccessible to its natural ligand so that its normal biological activity is prevented or reduced. Alternatively, such an inhibitor can act by modulating the dimerization of EGFR polypeptides, or interaction of EGFR polypeptide with other proteins, or enhance ubiquitination and endocytotic degradation of EGFR. EGFR kinase inhibitors include but are not limited to small molecule inhibitors, antibodies or antibody fragments, peptide or RNA aptamers, antisense constructs, small

inhibitory RNAs (i.e. RNA interference by dsRNA; RNAi), and ribozymes. In a preferred embodiment, the EGFR kinase inhibitor is a small organic molecule or an antibody that binds specifically to the human EGFR.

EGFR kinase inhibitors include, for example quinazoline EGFR kinase inhibitors, pyrido-pyrimidine EGFR kinase inhibitors, pyrimido-pyrimidine EGFR kinase inhibitors, pyrrolo-pyrimidine EGFR kinase inhibitors, pyrazolo-pyrimidine EGFR kinase inhibitors, phenylamino-pyrimidine EGFR kinase inhibitors, oxindole EGFR kinase inhibitors, indolocarbazole EGFR kinase inhibitors, phthalazine EGFR kinase inhibitors, isoflavone EGFR kinase inhibitors, quinalone EGFR kinase inhibitors, and tyrphostin EGFR kinase inhibitors, such as those described in the following patent publications, and all pharmaceutically acceptable salts and solvates of said EGFR kinase inhibitors: International Patent Publication Nos. WO 96/33980, WO 96/30347, WO 97/30034, WO 97/30044, WO 97/38994, WO 97/49688, WO 98/02434, WO 97/38983, WO 95/19774, WO 95/19970, WO 97/13771, WO 98/02437, WO 98/02438, WO 97/32881, WO 98/33798, WO 97/32880, WO 97/3288, WO 97/02266, WO 97/27199, WO 98/07726, WO 97/34895, WO 96/31510, WO 98/14449, WO 98/14450, WO 98/14451, WO 95/09847, WO 97/19065, WO 98/17662, WO 99/35146, WO 99/35132, WO 99/07701, and WO 92/20642; European Patent Application Nos. EP 520722, EP 566226, EP 787772, EP 837063, and EP 682027; U.S. Patent Nos. 5,747,498, 5,789,427, 5,650,415, and 5,656,643; and German Patent Application No. DE 19629652. Additional non-limiting examples of small molecule EGFR kinase inhibitors include any of the EGFR kinase inhibitors described in Traxler, P., 1998, Exp. Opin. Ther. Patents 8(12):1599-1625.

Specific preferred examples of small molecule EGFR kinase inhibitors that can be used according to the present invention include [6,7-bis(2-methoxyethoxy) -4-quinazolin-4-yl]-(3-ethynylphenyl) amine (also known as OSI-774, erlotinib, or TARCEVA® (erlotinib HCl); OSI Pharmaceuticals/Genentech/ Roche) (U.S. Pat. No. 5,747,498; International Patent Publication No. WO 01/34574, and Moyer, J.D. et al. (1997) Cancer Res. 57:4838-4848); CI-1033 (formerly known as PD183805; Pfizer) (Sherwood et al., 1999, Proc. Am. Assoc. Cancer Res. 40:723); PD-158780 (Pfizer); AG-1478 (University of California); CGP-59326 (Novartis); PKI-166 (Novartis); EKB-569 (Wyeth); GW-2016 (also known as GW-572016 or lapatinib ditosylate; GSK); and gefitinib (also known as ZD1839 or IRESSA™; Astrazeneca) (Woodburn et al., 1997, Proc. Am. Assoc. Cancer Res. 38:633). A particularly preferred small molecule EGFR kinase inhibitor that can be used according to the present invention is [6,7-bis(2-methoxyethoxy)-4-quinazolin-4-yl]-(3-ethynylphenyl) amine (i.e. erlotinib), its hydrochloride salt (i.e. erlotinib HCl, TARCEVA®), or other salt forms (e.g. erlotinib mesylate).

EGFR kinase inhibitors also include, for example multi-kinase inhibitors that have activity on EGFR kinase, i.e. inhibitors that inhibit EGFR kinase and one or more additional kinases. Examples of such compounds include the EGFR and HER2 inhibitor CI-1033 (formerly

known as PD183805; Pfizer); the EGFR and HER2 inhibitor GW-2016 (also known as GW-572016 or lapatinib ditosylate; GSK); the EGFR and JAK 2/3 inhibitor AG490 (a tyrphostin); the EGFR and HER2 inhibitor ARRY-334543 (Array BioPharma); BIBW-2992, an irreversible dual EGFR/HER2 kinase inhibitor (Boehringer Ingelheim Corp.); the EGFR and HER2 inhibitor EKB-569 (Wyeth); the VEGF-R2 and EGFR inhibitor ZD6474 (also known as ZACTIMA™; AstraZeneca Pharmaceuticals), and the EGFR and HER2 inhibitor BMS-599626 (Bristol-Myers Squibb).

Antibody-based EGFR kinase inhibitors include any anti-EGFR antibody or antibody fragment that can partially or completely block EGFR activation by its natural ligand. Non-limiting examples of antibody-based EGFR kinase inhibitors include those described in Modjtahedi, H., et al., 1993, Br. J. Cancer 67:247-253; Teramoto, T., et al., 1996, Cancer 77:639-645; Goldstein et al., 1995, Clin. Cancer Res. 1:1311-1318; Huang, S. M., et al., 1999, Cancer Res. 15:59(8):1935-40; and Yang, X., et al., 1999, Cancer Res. 59:1236-1243. Thus, the EGFR kinase inhibitor can be the monoclonal antibody Mab E7.6.3 (Yang, X.D. et al. (1999) Cancer Res. 59:1236-43), or Mab C225 (ATCC Accession No. HB-8508), or an antibody or antibody fragment having the binding specificity thereof. Suitable monoclonal antibody EGFR kinase inhibitors include, but are not limited to, IMC-C225 (also known as cetuximab or ERBITUX™; Imclone Systems), ABX-EGF (Abgenix), EMD 72000 (Merck KgaA, Darmstadt), RH3 (York Medical Bioscience Inc.), and MDX-447 (Medarex/ Merck KgaA).

EGFR kinase inhibitors for use in the present invention can alternatively be peptide or RNA aptamers. Such aptamers can for example interact with the extracellular or intracellular domains of EGFR to inhibit EGFR kinase activity in cells. An aptamer that interacts with the extracellular domain is preferred as it would not be necessary for such an aptamer to cross the plasma membrane of the target cell. An aptamer could also interact with the ligand for EGFR (e.g. EGF, TGF-α), such that its ability to activate EGFR is inhibited. Methods for selecting an appropriate aptamer are well known in the art. Such methods have been used to select both peptide and RNA aptamers that interact with and inhibit EGFR family members (e.g. see Buerger, C. et al. et al. (2003) J. Biol. Chem. 278:37610-37621; Chen, C-H. B. et al. (2003) Proc. Natl. Acad. Sci. 100:9226-9231; Buerger, C. and Groner, B. (2003) J. Cancer Res. Clin. Oncol. 129(12):669-675. Epub 2003 Sep 11.).

EGFR kinase inhibitors for use in the present invention can alternatively be based on antisense oligonucleotide constructs. Anti-sense oligonucleotides, including anti-sense RNA molecules and anti-sense DNA molecules, would act to directly block the translation of EGFR mRNA by binding thereto and thus preventing protein translation or increasing mRNA degradation, thus decreasing the level of EGFR kinase protein, and thus activity, in a cell. For example, antisense oligonucleotides of at least about 15 bases and complementary to unique regions of the mRNA transcript sequence encoding EGFR can be synthesized, e.g., by

conventional phosphodiester techniques and administered by e.g., intravenous injection or infusion. Methods for using antisense techniques for specifically inhibiting gene expression of genes whose sequence is known are well known in the art (e.g. see U.S. Patent Nos. 6,566,135; 6,566,131; 6,365,354; 6,410,323; 6,107,091; 6,046,321; and 5,981,732).

Small inhibitory RNAs (siRNAs) can also function as EGFR kinase inhibitors for use in the present invention. EGFR gene expression can be reduced by contacting the tumor, subject or cell with a small double stranded RNA (dsRNA), or a vector or construct causing the production of a small double stranded RNA, such that expression of EGFR is specifically inhibited (i.e. RNA interference or RNAi). Methods for selecting an appropriate dsRNA or dsRNA-encoding vector are well known in the art for genes whose sequence is known (e.g. see Tuschi, T., et al. (1999) Genes Dev. 13(24):3191-3197; Elbashir, S.M. et al. (2001) Nature 411:494-498; Hannon, G.J. (2002) Nature 418:244-251; McManus, M.T. and Sharp, P. A. (2002) Nature Reviews Genetics 3:737-747; Bremmelkamp, T.R. et al. (2002) Science 296:550-553; U.S. Patent Nos. 6,573,099 and 6,506,559; and International Patent Publication Nos. WO 01/36646, WO 99/32619, and WO 01/68836).

Ribozymes can also function as EGFR kinase inhibitors for use in the present invention. Ribozymes are enzymatic RNA molecules capable of catalyzing the specific cleavage of RNA. The mechanism of ribozyme action involves sequence specific hybridization of the ribozyme molecule to complementary target RNA, followed by endonucleolytic cleavage. Engineered hairpin or hammerhead motif ribozyme molecules that specifically and efficiently catalyze endonucleolytic cleavage of *EGFR* mRNA sequences are thereby useful within the scope of the present invention. Specific ribozyme cleavage sites within any potential RNA target are initially identified by scanning the target molecule for ribozyme cleavage sites, which typically include the following sequences, GUA, GUU, and GUC. Once identified, short RNA sequences of between about 15 and 20 ribonucleotides corresponding to the region of the target gene containing the cleavage site can be evaluated for predicted structural features, such as secondary structure, that can render the oligonucleotide sequence unsuitable. The suitability of candidate targets can also be evaluated by testing their accessibility to hybridization with complementary oligonucleotides, using, e.g., ribonuclease protection assays.

Both antisense oligonucleotides and ribozymes useful as EGFR kinase inhibitors can be prepared by known methods. These include techniques for chemical synthesis such as, e.g., by solid phase phosphoramadite chemical synthesis. Alternatively, anti-sense RNA molecules can be generated by in vitro or in vivo transcription of DNA sequences encoding the RNA molecule. Such DNA sequences can be incorporated into a wide variety of vectors that incorporate suitable RNA polymerase promoters such as the T7 or SP6 polymerase promoters. Various modifications to the oligonucleotides of the invention can be introduced as a means of increasing intracellular stability and half-life. Possible modifications include but are not limited to

the addition of flanking sequences of ribonucleotides or deoxyribonucleotides to the 5' and/or 3' ends of the molecule, or the use of phosphorothioate or 2'-O-methyl rather than phosphodiesterase linkages within the oligonucleotide backbone.

In the context of this invention, the sensitivity of tumor cell growth is defined as high ("sensitive") if the tumor cell is inhibited with an EC_{50} (half-maximal effective concentration) of less than 1 μM, and low (*i.e.*, resistant) if the tumor cell is inhibited with an EC_{50} of greater than 10 μM. Sensitivities between these values are considered intermediate.

The term EC_{50} (half maximal effective concentration) refers to the concentration of agent that induces a response halfway between the baseline and maximum for the specified exposure time, and is used as a measure of the compound's potency.

CLAIMS

1. A method of treating NSCLC in a patient having an NSCLC tumor or tumor metastasis having an activating EGFR mutation and expressing a mutant EGFR, comprising administering to the patient a therapeutically effective regimen comprising OSI-906 and an EGFR tyrosine kinase inhibitor.

2. The method of Claim 1, wherein the NSCLC has an acquired resistance to EGFR inhibitor treatment.

3. The method of Claim 2, wherein cells of the NSCLC have an epithelial phenotype.

4. The method of Claim 3, wherein the EGFR inhibitor comprises erlotinib.

5. The method of Claim 4, wherein the NSCLC is in advanced stage IIIB or stage IV.

6. The method of Claim 4, wherein the erlotinib is administered at about 50-300 mg QD.

7. The method of Claim 4, wherein the erlotinib is administered at about 150 mg QD.

8. The method of any one of Claims 4-7, wherein the erlotinib is administered about one hour before or about two hours after the patient eats food.

9. The method of any one of Claims 1-7, wherein the OSI-906 is administered at about 75-300 mg BID.

10. The method of any one of Claims 1-7, wherein the OSI-906 is administered at about 150 mg BID.

11. The method of any one of Claims 1-7, wherein the OSI-906 is administered with food or less than about two hours after food consumption or less than about one half hour before food consumption by the patient.

12. The method of any one of Claims 1-7, wherein the patient survives at least about one year from starting the method.

13. The method of any one of Claims 1-7, wherein the patient experiences stable disease or partial response for at least about one half year from starting the method.

14. The method of any one of Claims 1-7, wherein the OSI-906 and the EGFR inhibitor together produce a synergistic anti-tumor effect.

Fig. 1

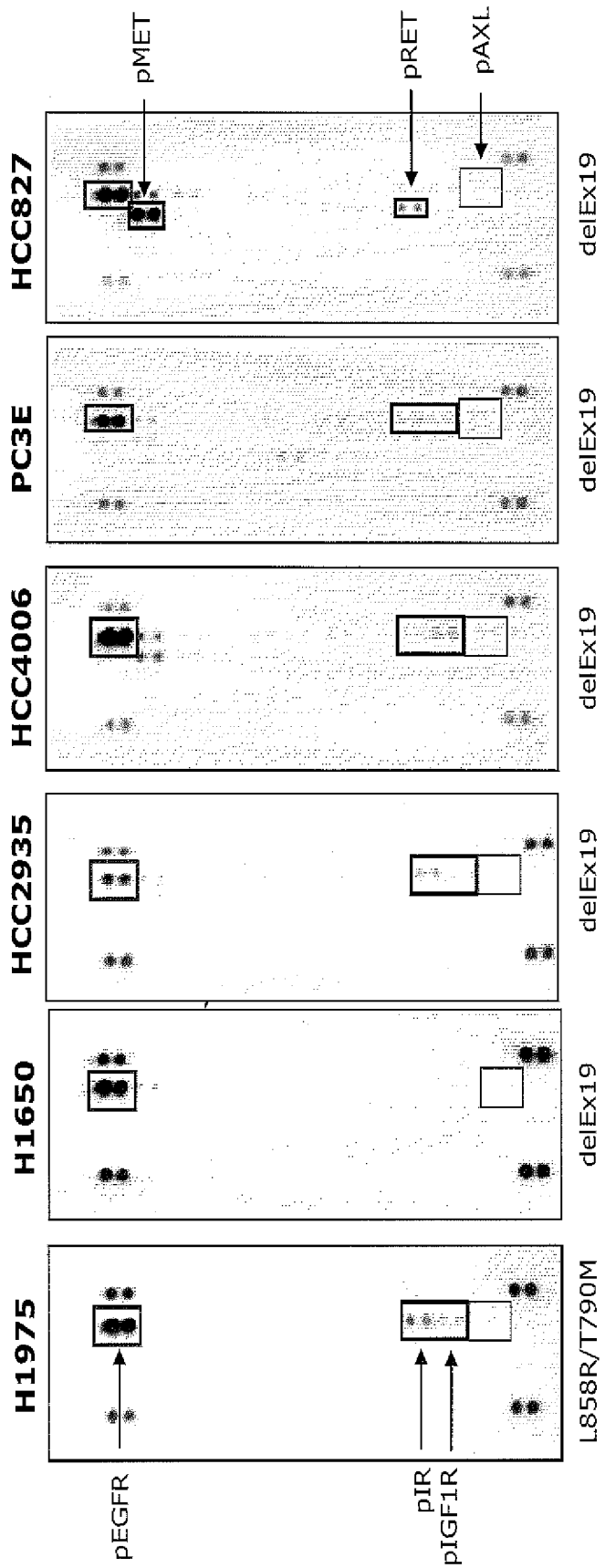

pMET
pRET
pAXL
HCC827
PC3E
HCC4006
HCC2935
H1650
H1975
delEx19
delEx19
delEx19
delEx19
delEx19
L858R/T790M
pEGFR
pIR
pIGF1R

Fig. 2A

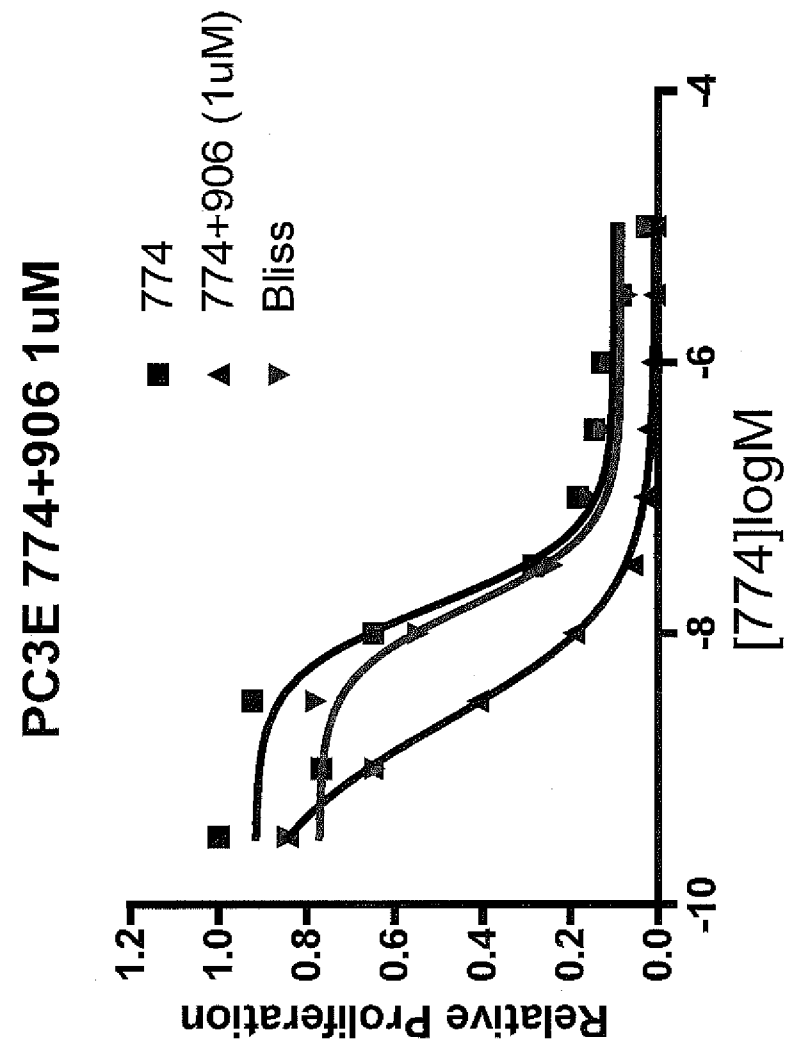

PC3E 774+906 1uM
774
774+906 (1uM)
Bliss
Relative Proliferation
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-10
-8
-6
-4
[774]logM

Fig. 2B

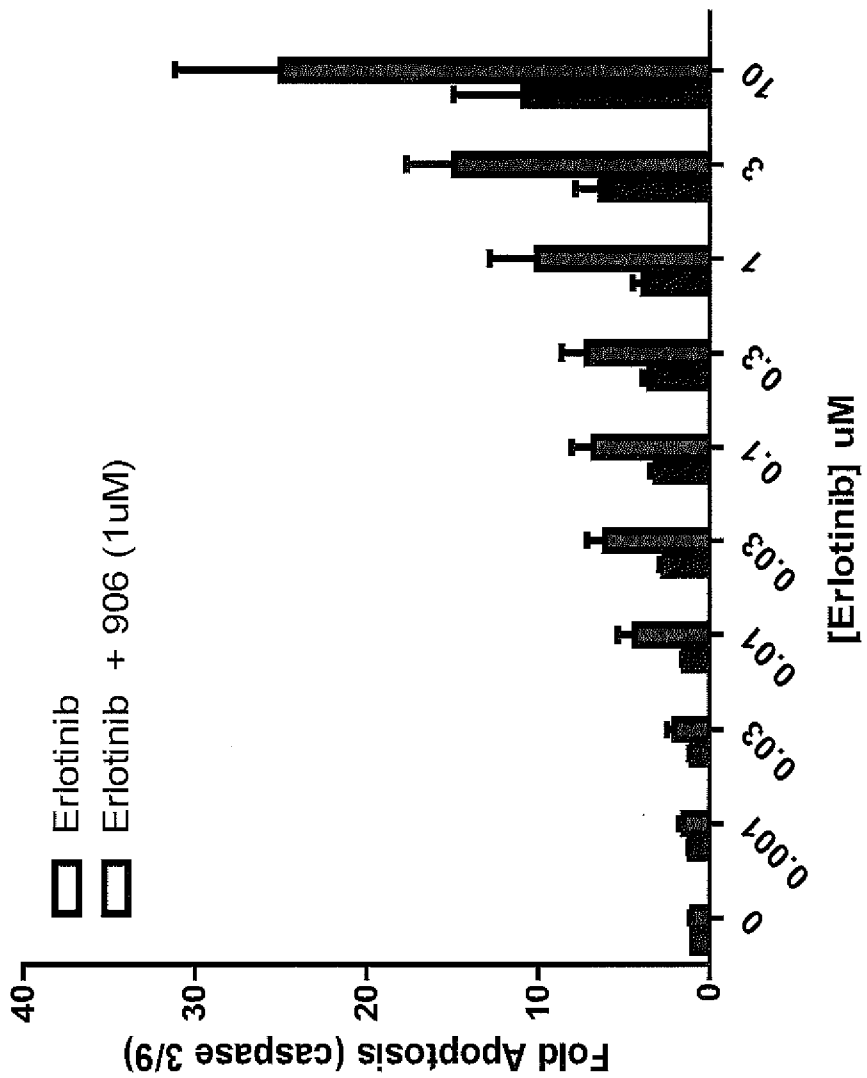
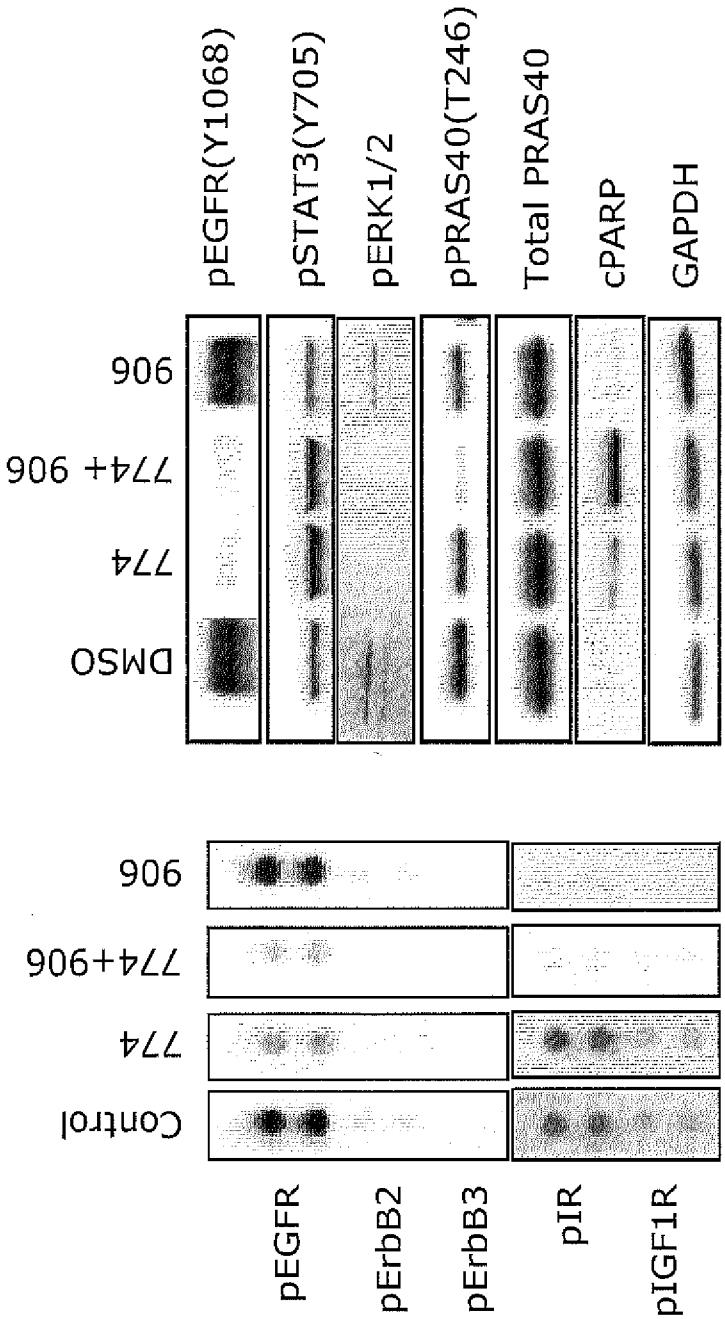

Erlotinib
Erlotinib + 906 (1uM)
Fold Apoptosis (caspase 3/9)
0
10
20
30
40
[Erlotinib] uM
0
0.001
0.03
0.01
0.03
0.1
0.3
1
3
10

Fig. 3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pSTAT3(Y705)
pERK1/2
pPRAS40(T246)
Total PRAS40
cPARP
GAPDH
DMSO
774
774+ 906
906
pEGFR
pErbB2
pErbB3
pIR
pIGF1R
Control
774
774+906
906

Fig. 4

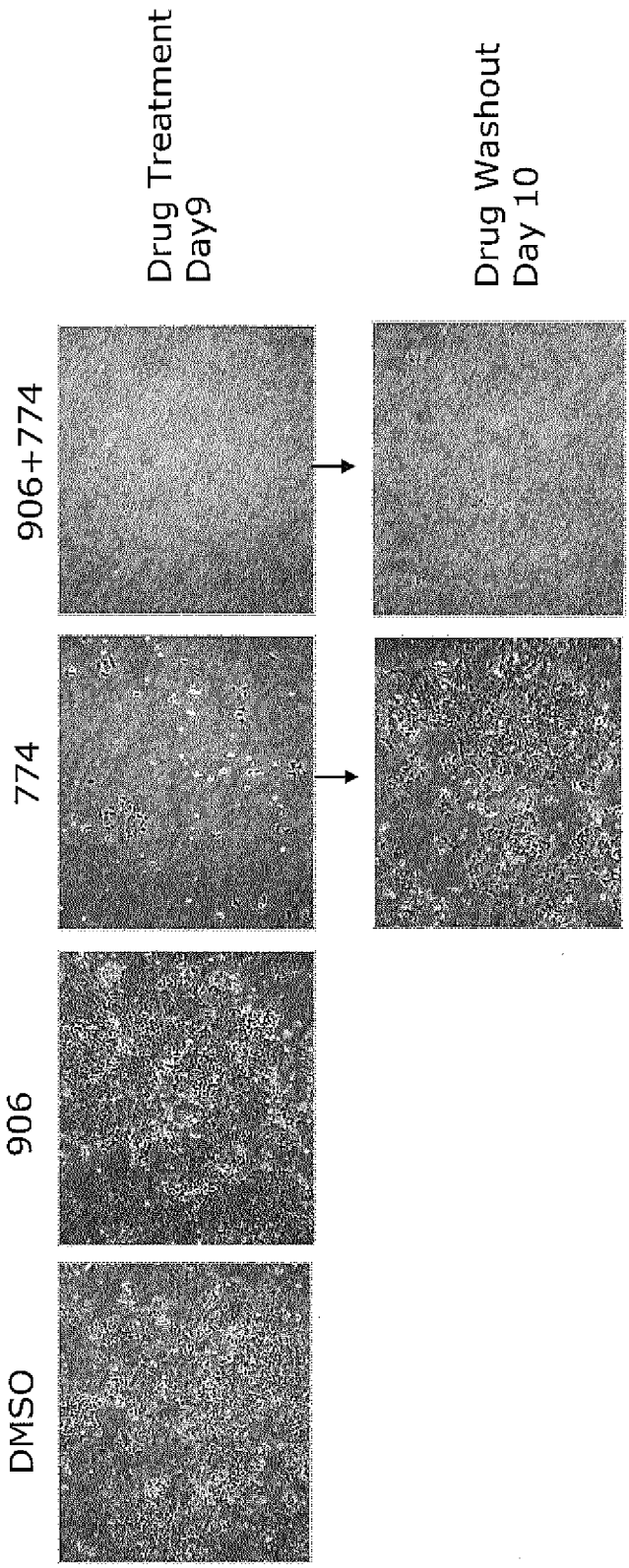


Fig. 5

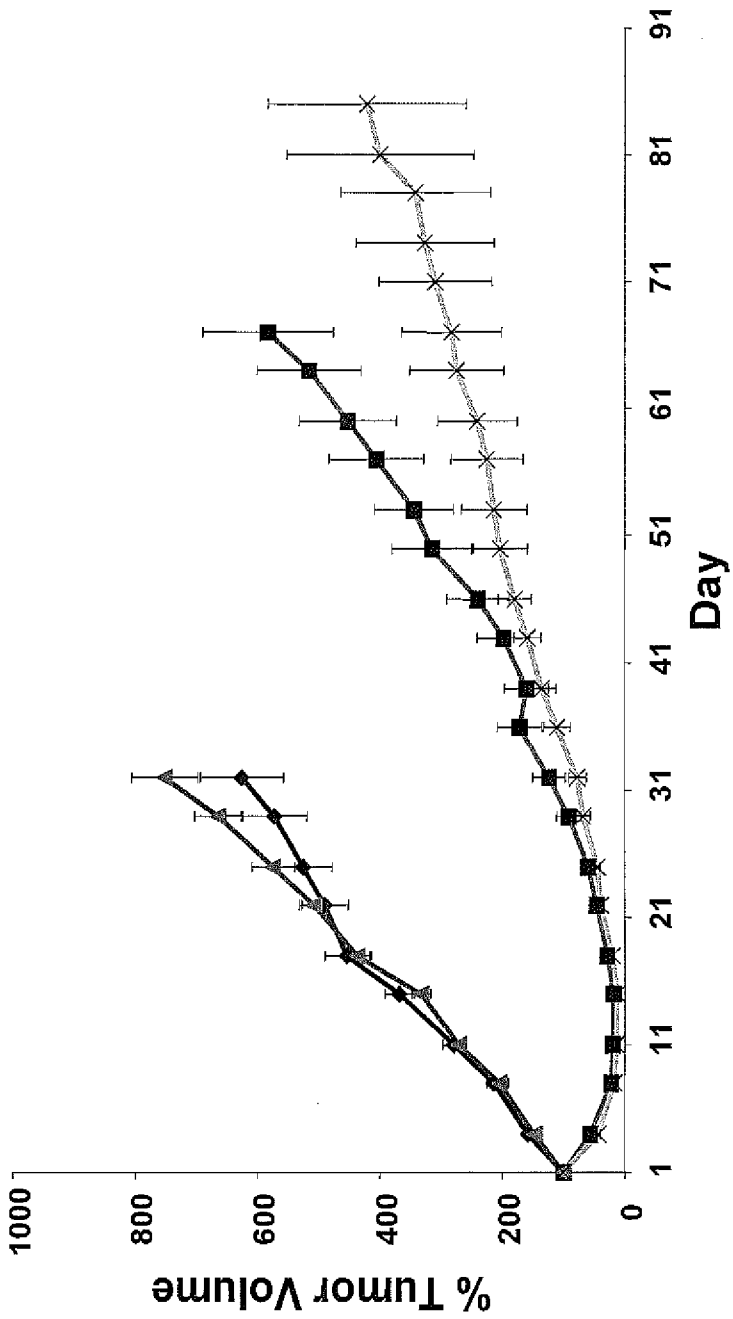

Vehicle Control
Tarceva 100mg/kg (QD1-14)
OSI-906 50mg/kg (QD1-14)
Tarceva 100 mg/kg / OSI-906 10 mg/kg (QD1-14)
% Tumor Volume
1000
800
600
400
200
0
1
11
21
31
41
51
61
71
81
91
Day

Fig. 6

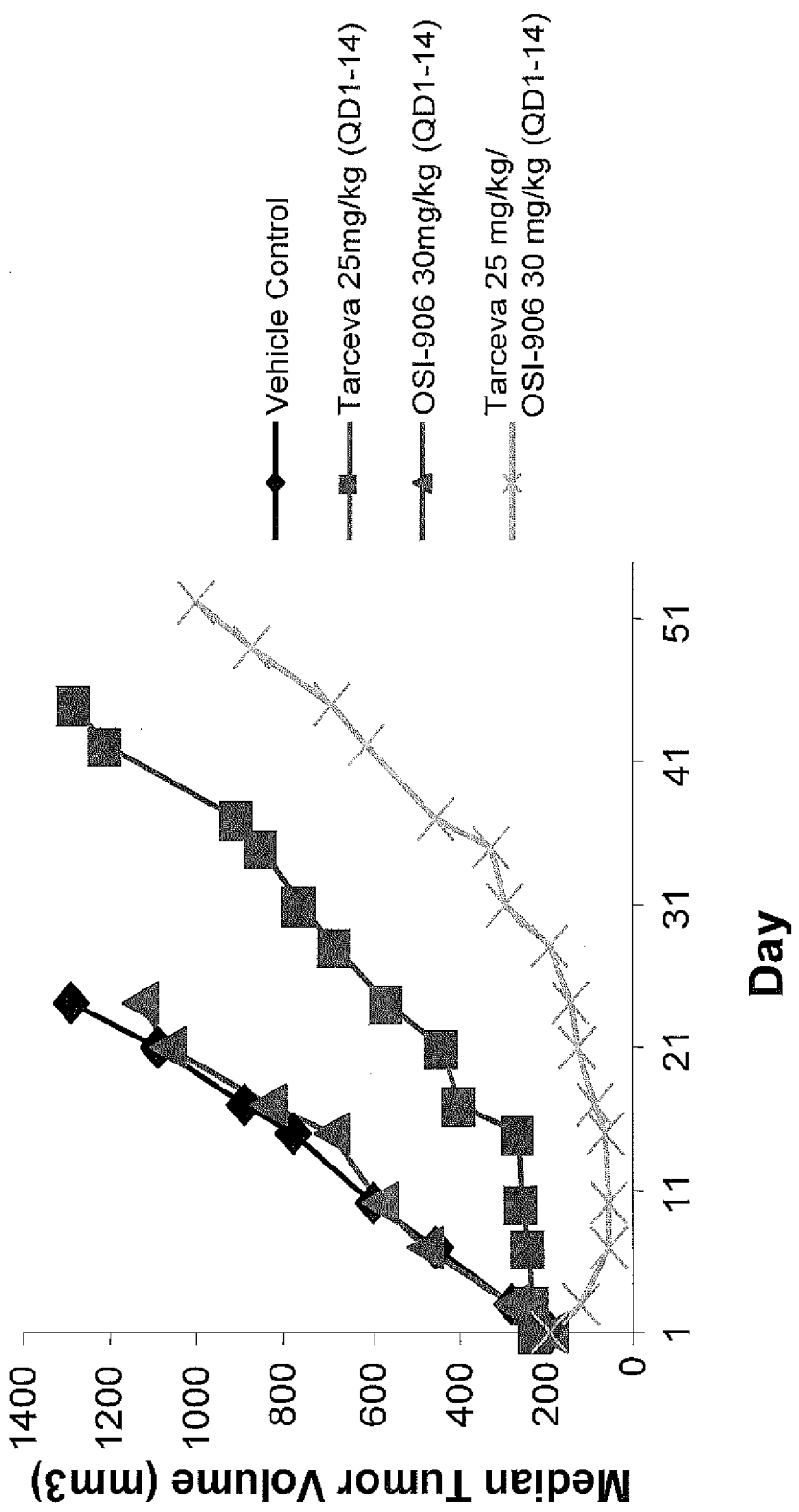

Vehicle Control
Tarceva 25mg/kg (QD1-14)
OSI-906 30mg/kg (QD1-14)
Tarceva 25 mg/kg/
OSI-906 30 mg/kg (QD1-14)
Median Tumor Volume (mm3)
1400
1200
1000
800
600
400
200
0
1
11
21
31
41
51
Day

Fig. 7

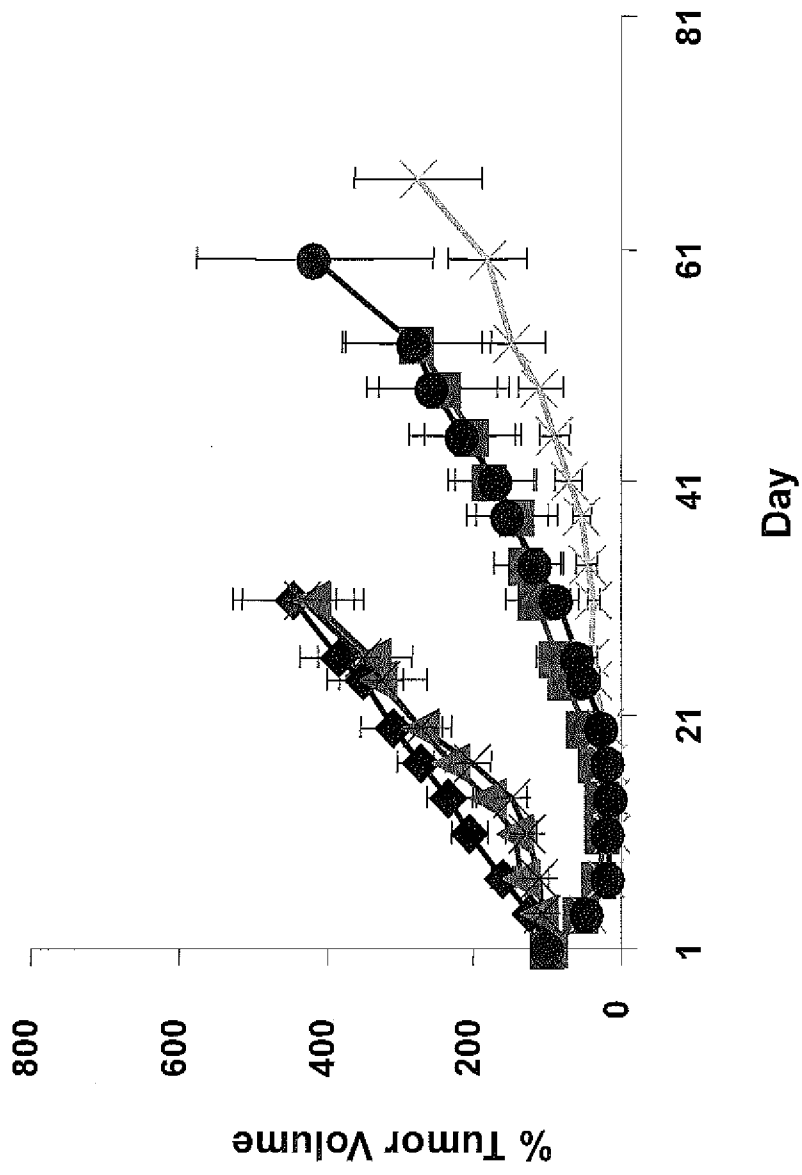
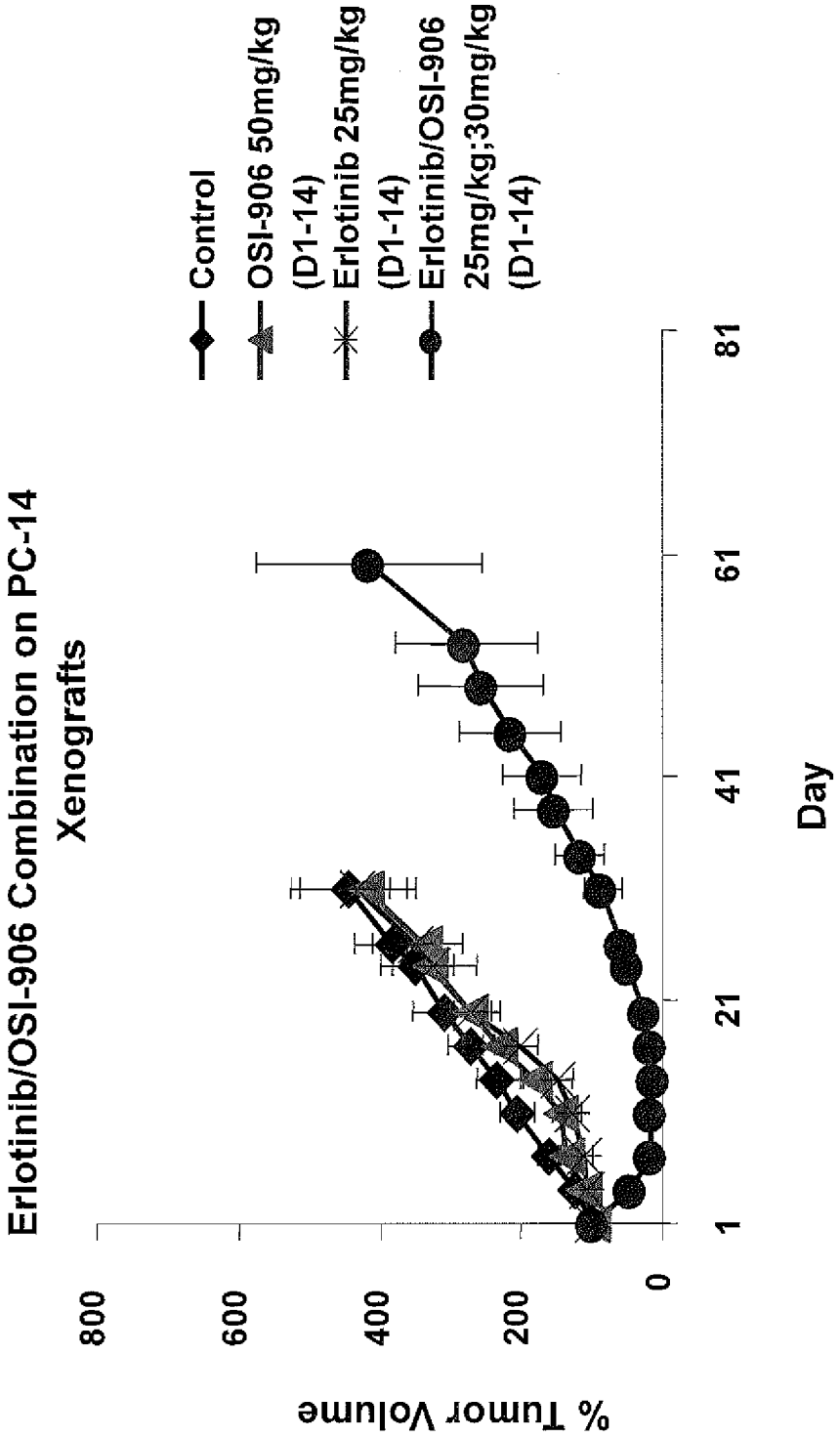

Erlotinib/OSI-906 Combination on PC-14 Xenografts
Control
Erlotinib 100mg/kg (D1-14)
OSI-906 50mg/kg (D1-14)
Erlotinib/OSI-906 100mg/kg;10mg/kg (D1-14)
Erlotinib 25mg/kg (D1-14)
Erlotinib/OSI-906 25mg/kg;30mg/kg (D1-14)
% Tumor Volume
800
600
400
200
0
1
21
41
61
81
Day

Fig. 8


Erlotinib/OSI-906 Combination on PC-14 Xenografts
Control
OSI-906 50mg/kg (D1-14)
Erlotinib 25mg/kg (D1-14)
Erlotinib/OSI-906 25mg/kg;30mg/kg (D1-14)
% Tumor Volume
800
600
400
200
0
1
21
41
61
81
Day

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2012/029598

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/4985 A61K31/517 A61K45/06 A61P35/00
ADD.

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)
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, SCISEARCH, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ClinicalTrials.gov, US National Institute of Health: "Study of Erlotinib (Tarceva®) in Combination With OSI-906 in Patients With Advanced Non-small Cell Lung Carcinoma (NSCLC) With Activating Mutations of the Epidermal Growth Factor Receptor (EGFR) Gene", OSI Pharmaceuticals , 22 February 2011 (2011-02-22), XP002677837, Retrieved from the Internet: URL:http://clinicaltrials.gov/archive/NCT01221077/2011_02_22 [retrieved on 2012-06-14] cited in the application	1,9-14
Y	page 1, lines 8-11 page 2, lines 29-34 page 2, lines 39-42	5
	-/--	

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 June 2012	27/06/2012

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

1

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	page 3, lines 7-8 page 3, lines 43-44 -----	
X	WO 2010/107968 A1 (OSI PHARM INC [US]; FRANKLIN MARYLAND [US]; WILD ROBERT C [US]) 23 September 2010 (2010-09-23) cited in the application	1-4,6-8, 12-14
Y	page 13, lines 13-15 page 14, line 18 - page 18, line 2 claims 1-2, 5, 7, 11, 15 -----	5
X	BROWN ERIC ET AL: "Combination treatment strategy of an IGF-1R kinase inhibitor, OSI-906, in models of progressive disease following initial response to EGFR tyrosine kinase inhibitor therapy", PROCEEDINGS OF THE ANNUAL MEETING OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH; 100TH ANNUAL MEETING OF THE AMERICAN-ASSOCIATION-FOR-CANCER-RESEARCH; DENVER, CA, USA; APRIL 18 -22, 2009, AMERICAN ASSOCIATION FOR CANCER RESEARCH, US, vol. 50, 1 April 2009 (2009-04-01), page 419, XP001539735, ISSN: 0197-016X	1-4, 12-14
Y	the whole document -----	5
A	WILLIAM PAO ET AL: "Rational, biologically based treatment of EGFR-mutant non-small-cell lung cancer", NATURE REVIEWS CANCER, vol. 10, no. 11, 22 October 2010 (2010-10-22), pages 760-774, XP55029993, ISSN: 1474-175X, DOI: 10.1038/nrc2947 abstract figure 5 -----	1-14
A	M. N. BALAK ET AL: "Novel D761Y and Common Secondary T790M Mutations in Epidermal Growth Factor Receptor-Mutant Lung Adenocarcinomas with Acquired Resistance to Kinase Inhibitors", CLINICAL CANCER RESEARCH, vol. 12, no. 21, 1 November 2006 (2006-11-01), pages 6494-6501, XP55029967, ISSN: 1078-0432, DOI: 10.1158/1078-0432.CCR-06-1570 abstract table 2 -----	1-14
	-/--	

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INTERNATIONAL SEARCH REPORT

International application No
PCT/US2012/029598

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	SHARMA S V ET AL: "Epidermal growth factor receptor mutations in lung cancer", NATURE REVIEWS. CANCER, NATUR PUBLISHING GROUP, LONDON, GB, vol. 7, 1 March 2007 (2007-03-01), pages 169-181, XP002556732, ISSN: 1474-175X, DOI: 10.1038/NRC2088 cited in the application abstract page 170, right-hand column, paragraph 2 figure 1	1-14
A	THOMSON S ET AL: "Epithelial to mesenchymal transition is a determinant of sensitivity of non-small-cell lung carcinoma cell lines and xenografts to epidermal growth factor receptor inhibition", CANCER RESEARCH, AMERICAN ASSOCIATION FOR CANCER RESEARCH, US, vol. 65, no. 20, 15 October 2005 (2005-10-15), pages 9455-9462, XP002391562, ISSN: 0008-5472, DOI: 10.1158/0008-5472.CAN-05-1058 abstract page 9456, right-hand column, paragraph 2; table 1	1-14
A	US 2008/267957 A1 (ARNOLD LEE D [US] ET AL) 30 October 2008 (2008-10-30) cited in the application paragraphs [0642] - [0644]	1-14

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2012/029598

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2010107968 A1	23-09-2010	EP 2408479 A1	25-01-2012
		US 2012064072 A1	15-03-2012
		WO 2010107968 A1	23-09-2010
US 2008267957 A1	30-10-2008	NONE	