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Axitinib

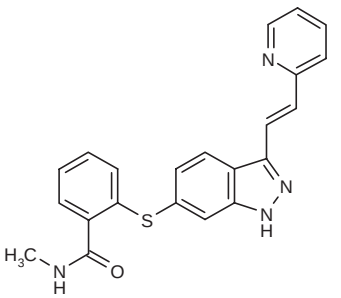
Rec INN; USAN

VEGFR/PDGFR Tyrosine Kinase Inhibitor
Antiangiogenic Agent

AG-013736
AG-13736

N-Methyl-2-[3-[2-(2-pyridyl)vinyl]-1*H*-indazol-6-ylsulfanyl]benzamide

InChI=1/C22H18N4OS/c1-23-22(27)18-7-2-3-8-21(18)28-16-10-11-17-19(25-26-20(17)14-16)12-9-15-6-4-5-13-24-15/h2-14H,1H3,(H,23,27)(H,25,26)/b12-9+



$C_{22}H_{18}N_4OS$
Mol wt: 386.4707

CAS: 319460-85-0

EN: 318296

Abstract

Targeting angiogenesis and vascular endothelial growth factor (VEGF) in particular as an approach to the treatment of cancer was validated several years ago with the approval of the anti-VEGF monoclonal antibody bevacizumab for the treatment of metastatic colon cancer, followed more recently by the launch of the small-molecule multitargeted (including VEGFR) tyrosine kinase inhibitors sunitinib and sorafenib. Axitinib is a new, orally bioavailable small molecule that inhibits several tyrosine kinases involved in angiogenesis, particularly VEGFR tyrosine kinases. The agent has demonstrated clinical activity, both alone and in combination with other chemotherapeutics, in several types of tumors, including non-small cell lung cancer (NSCLC), metastatic renal cell carcinoma (mRCC), metastatic breast cancer, pancreatic cancer and thyroid cancer, and clinical studies continue.

Synthesis

Axitinib can be prepared by several different methods. Iodination of 6-nitroindazole (I) utilizing iodine in the presence of NaOH provides 3-iodo-6-nitroindazole (II). Subsequent protection of indazole (II) with 2-(trimethylsilyl)ethoxymethyl chloride (SEMCl) under phase-transfer conditions, followed by Suzuki coupling of the SEM-protected compound (III) with styrylboronic acid, gives the styryl indazole (IV). The nitro group of (IV) is reduced by means of $SnCl_2$ to produce amine (V), which is then converted to the iodoindazole analogue (VI) by diazotization followed by reaction with I_2 and KI. Ozonization of the styryl moiety of (VI) and further reductive work-up leads to the aldehyde (VII), which is subjected to Wittig reaction with 2-picolyl triphenylphosphonium chloride (VIII) to produce the pyridylvinylindazole (IX). Displacement of the iodoindazole (IX) with methyl 2-mercaptobenzoate (X) in the presence of Cs_2CO_3 and palladium catalyst gives the thioether (XI). After alkaline hydrolysis of the methyl ester (XI), the resulting carboxylic acid (XII) is coupled with methylamine in the presence of HATU to furnish the *N*-methyl amide (XIII). Axitinib is then obtained by removal of the SEM protecting group of (XIII) by means of tetrabutylammonium fluoride and ethylenediamine (1). Scheme 1.

In an alternative method, reaction of 6-nitroindazole (I) with iodine and K_2CO_3 gives the 3-iodo derivative (II), which is protected as the 1-tetrahydropyranyl indazole (XIV) with dihydropyran and methanesulfonic acid. Palladium-catalyzed coupling of iodoindazole (XIV) with 2-vinylpyridine (XV), followed by reduction of the resulting adduct (XVI) with iron and NH_4Cl, provides the THP-protected 6-amino-3-(pyridylvinyl)indazole (XVII). After diazotization of aminoindazole (XVII) with $NaNO_2$/HCl in AcOH, treatment of the intermediate diazonium salt with I_2/KI yields the 6-iodoindazole (XVIII). The intermediate 2-mercapto-*N*-methylbenzamide (XXI) is prepared as follows. 2,2'-Dithiosalicylic acid (XIX) is chlorinated with $SOCl_2$, followed by condensation with methylamine, to give the dithiosalicylamide (XX), which is reductively

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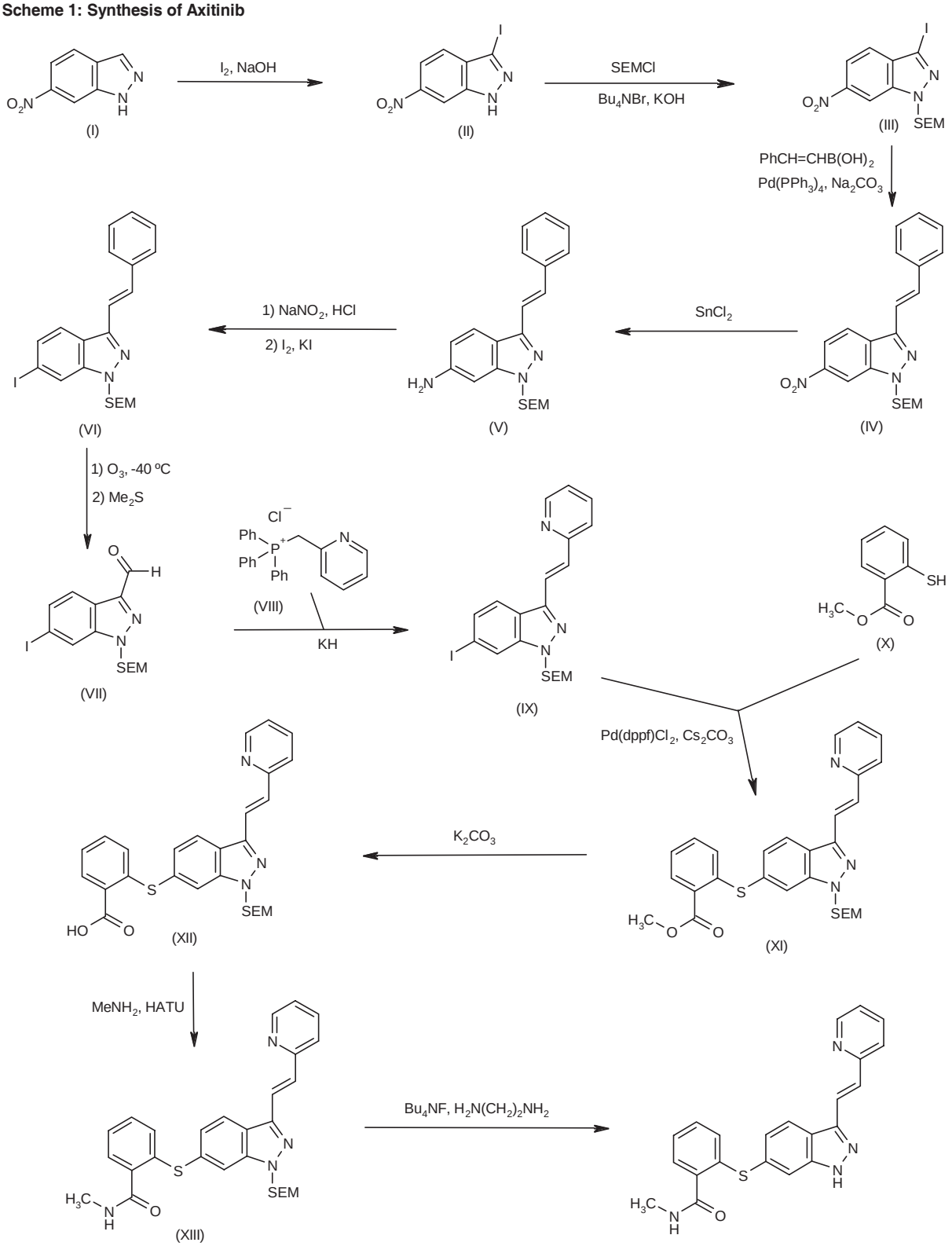
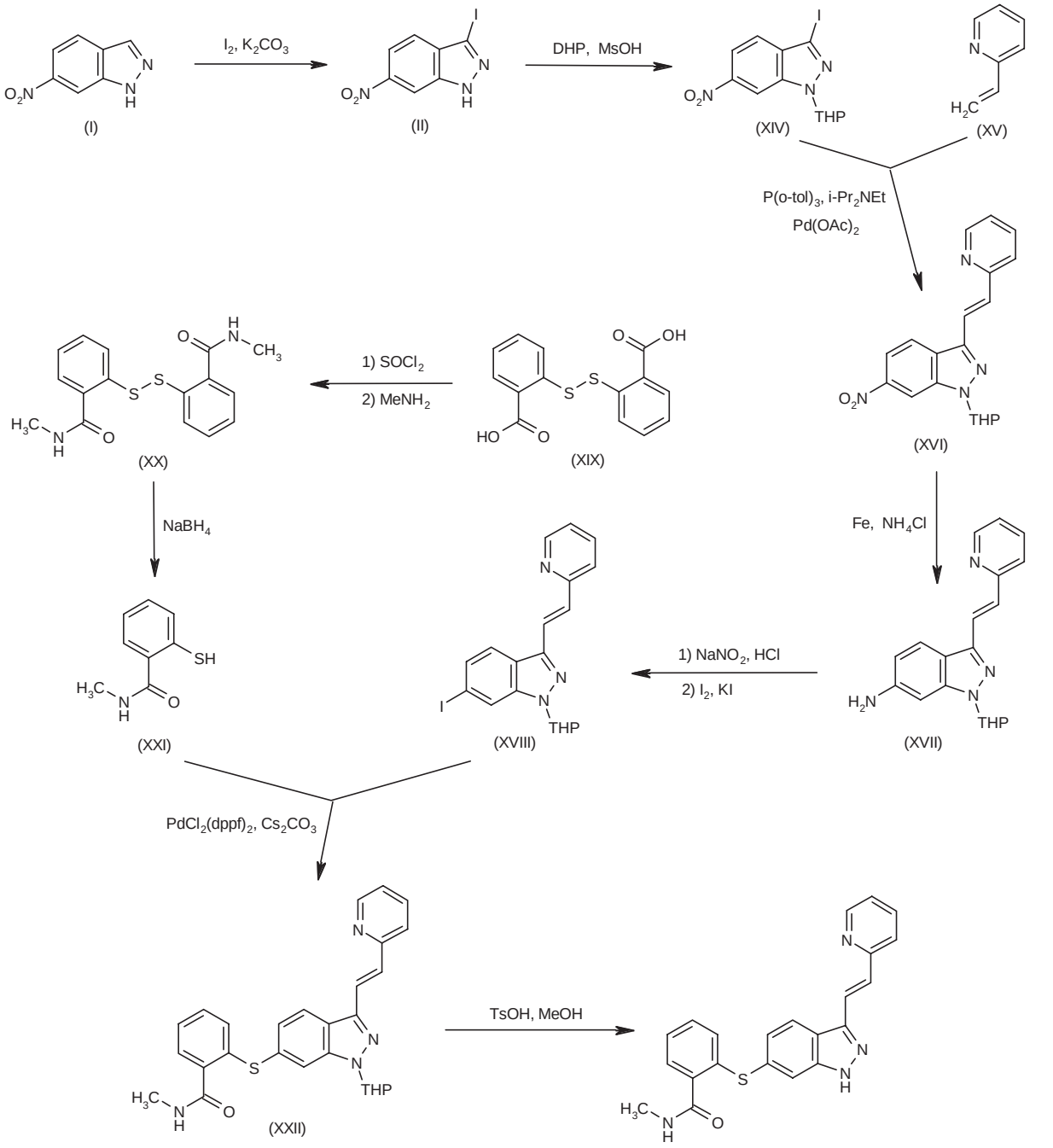
Scheme 1: Synthesis of Axitinib



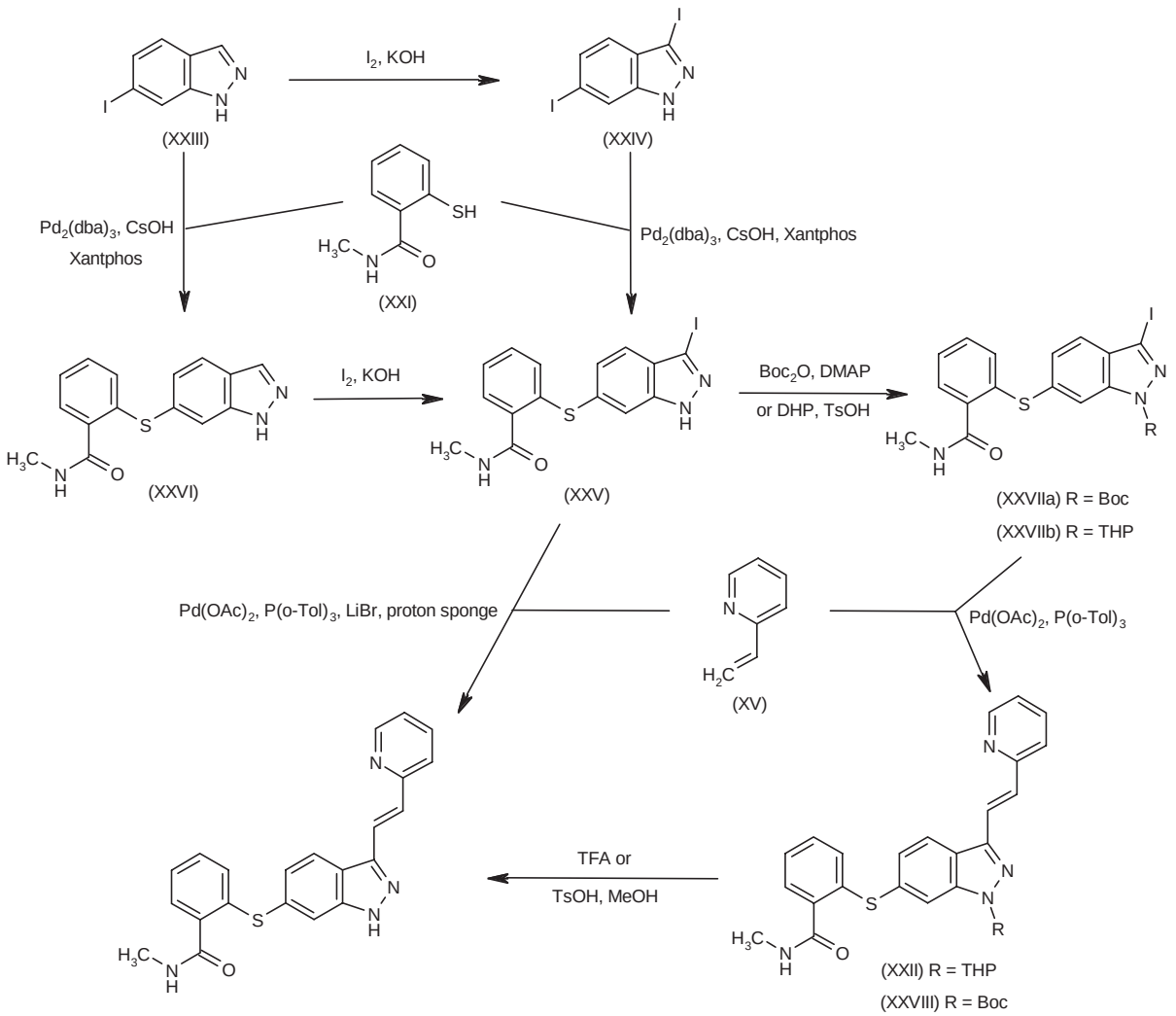
cleaved to the mercaptobenzamide (XXI) employing $NaBH_4$. Then, coupling between iodoindazole (XVIII) and 2-mercapto-*N*-methylbenzamide (XXI) by means of $PdCl_2(dppf)_2$ and Cs_2CO_3 affords the tetrahydropyranyl-protected axitinib (XXII), which is finally deprotected by treatment with *p*-toluenesulfonic acid in hot MeOH (2). Scheme 2.

A related procedure consists of the iodination of 6-iodoindazole (XXIII) with I_2 and KOH to give 3,6-diiodoindazole (XXIV), which is coupled with 2-mercapto-*N*-methylbenzamide (XXI) in the presence of $Pd_2(dba)_3$ and Xantphos diphosphine ligand to yield the diaryl thioether (XXV). Alternatively, intermediate (XXV) can be prepared by coupling of 6-iodoindazole (XXIII) with mer-

Scheme 2: Synthesis of Axitinib



Scheme 3: Synthesis of Axitinib



captobenzamide (XXI), followed by iodination of the resulting indazolyl thioether (XXVI). Optionally, the indazolyl derivative (XXV) is protected as the *N*-Boc (XXVIIa) or the *N*-tetrahydropyranyl derivative (XXVIIb) by treatment with Boc_2O and DMAP or with dihydropyran and *p*-TsOH, respectively. Palladium-catalyzed coupling of the protected iodoindazoles (XXVIIa) and (XXVIIb) with 2-vinylpyridine (XV) then leads to the respective *N*-protected axitinib derivatives (XXII) and (XXVIII), which are finally deprotected utilizing *p*-TsOH in MeOH or TFA, respectively. Alternatively, axitinib can be obtained by direct coupling of unprotected iodoindazole (XXV) with 2-vinylpyridine (XV) in the presence of palladium diacetate, tri-*o*-tolylphosphine, LiBr and 1,8-bis(dimethylamino)naphthalene (proton sponge) in hot NMP (3). Scheme 3.

Background

Tumor progression requires angiogenesis and blood vessel density has been shown to correlate with patient survival. Cancers are believed to lie dormant *in situ* until the fine balance between the production of angiogenesis-stimulatory (*e.g.*, vascular endothelial growth factor [VEGF], fibroblast growth factor [FGF], platelet-derived growth factor [PDGF]) and -inhibitory (*e.g.*, thrombospondins) factors has been disrupted, causing an angiogenic switch. Tumor cell lines secrete VEGF *in vitro* and VEGF mRNA is increased in most human tumors, while mRNA for VEGF receptors (VEGFRs) is upregulated in endothelial cells associated with tumors. Moreover, elevated serum levels of VEGF have been detected in individuals with several tumor types. Thus, interference

with VEGF activity represents an attractive target for inhibition of angiogenesis in cancer (4-8).

Targeting angiogenesis and VEGF in particular was validated with the FDA approval in 2004 of the first angiogenic agent, bevacizumab (Avastin™; Genentech, Roche), an anti-VEGF monoclonal antibody approved for the treatment of metastatic colon cancer (9). Other antiangiogenic strategies for the treatment of malignancies have emerged and are under active investigation, including blockade of matrix degradation, blockade of VEGF, FGF and PDGF receptor signaling, inhibition of normal endothelial cells and integrin antagonism. Because VEGF is a key mediator of neovascularization, research has focused on interfering with the VEGF/VEGFR system in order to modulate angiogenesis. The activity of VEGFs is mediated through binding to specific cell-surface receptors: VEGFR-1 (Flt-1), VEGFR-2 (KDR or FLK1) and VEGFR-3 (FLT4). VEGFR-1 and VEGFR-2 are expressed predominantly on vascular endothelial cells, while VEGFR-3 is expressed on lymphatic endothelium and is not as important for tumorigenesis. VEGF binding to its receptors induces homo- or heterodimerization of the ligand, which subsequently triggers intracellular autophosphorylation of their kinase domain. A cascade of signal transmission eventually leads to the growth message in the cell nucleus (Fig. 1) (10-14).

Small-molecule tyrosine kinase inhibitors that target VEGFRs are a particularly promising antiangiogenic approach and several such compounds have recently been introduced into clinical practice or are undergoing late-stage clinical evaluation, including sunitinib (Sutent®; Pfizer), launched in 2006, sorafenib (Nexavar®; Onyx, Bayer), launched in 2005, vatalanib, in phase III trials at Novartis and Bayer Schering Pharma, vandetanib and cediranib, in phase III development at AstraZeneca, and axitinib, also in phase III trials at Pfizer. Axitinib (AG-013736) is a small-molecule, orally bioavailable inhibitor of several tyrosine kinases involved in angiogenesis, particularly VEGFR tyrosine kinases. Several clinical trials testing it as a single agent or in combination with other therapies for the treatment of patients with refractory solid tumors, including renal cell carcinoma (RCC), thyroid cancer, non-small cell lung cancer (NSCLC), colorectal cancer, pancreatic cancer, melanoma and breast cancer, are currently in progress.

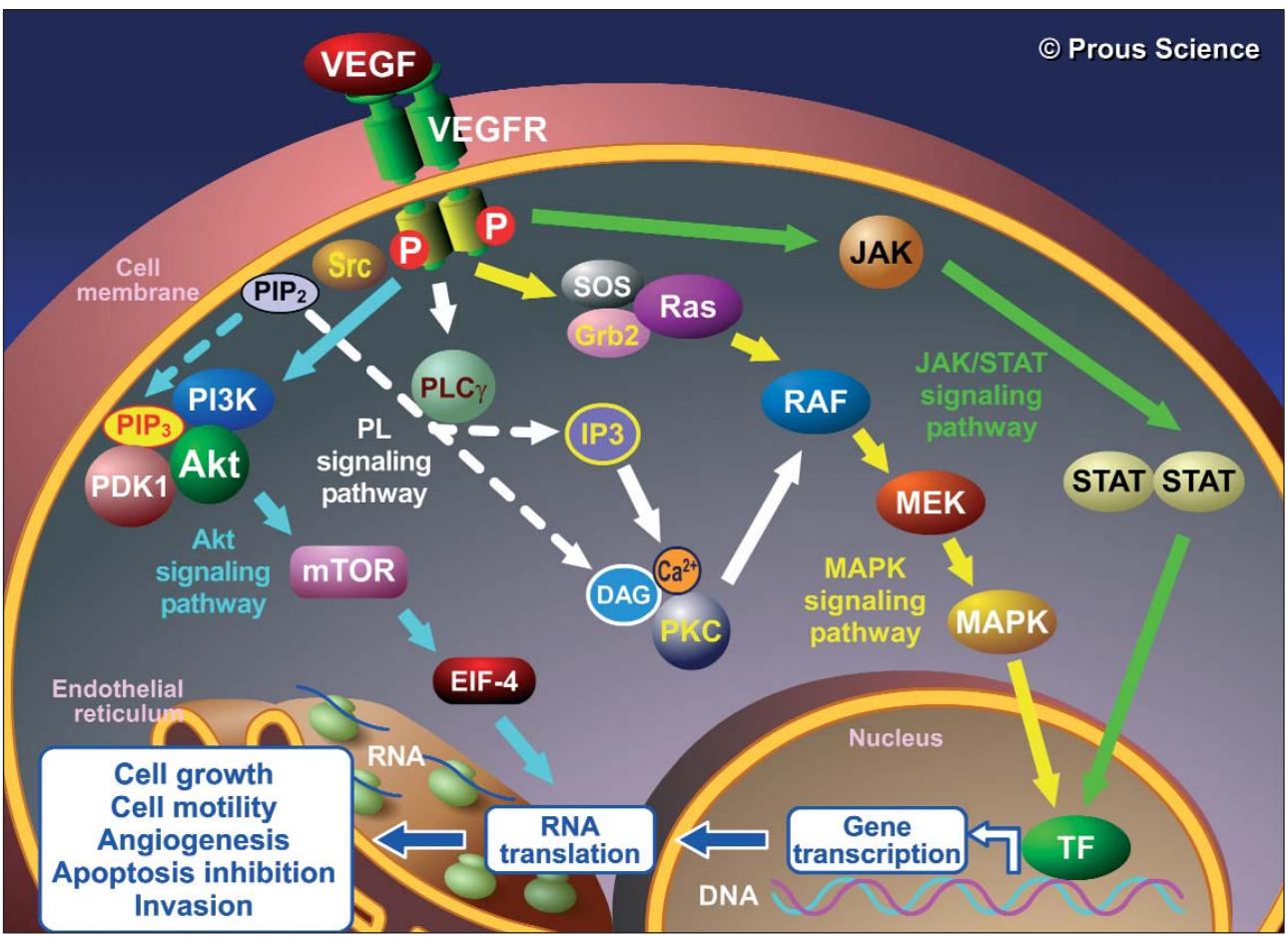


Fig. 1. VEGFR signaling pathways.

Preclinical Pharmacology

Axitinib inhibited VEGFR-1, -2 and -3 tyrosine kinases with respective IC_{50} values of 1.2, 0.25 and 0.29 nmol/l (15, 16). Axitinib was further demonstrated to potently and selectively inhibit VEGF-dependent receptor phosphorylation, downstream signal transduction and survival, proliferation and adhesion in human endothelial cells, whereas bFGF-stimulated survival of these cells was not affected. In angiogenesis models, the agent inhibited VEGF-stimulated skin vascular permeability in mice and receptor phosphorylation in retinal tissues of neonatal rats and mice bearing human tumor xenografts (17, 18).

Axitinib was effective in various human tumor xenograft models in mice following twice-daily oral administration. It inhibited the growth of subcutaneously implanted human colon tumors, which was accompanied by a decrease in microvessel density and increased necrosis in the tumor. Axitinib also produced a dose-dependent growth delay in the Lewis lung carcinoma model, and in an orthotopically implanted human melanoma model in SCID mice, it inhibited metastasis to the lung and lymph nodes (18, 19).

The changes in tumor vasculature were determined in axitinib-treated RIP-Tag2 transgenic mice developing spontaneous islet cell tumors and in mice bearing Lewis lung carcinoma. Within 24 h of initiating treatment in the RIP-Tag2 mice, endothelial fenestrations and sprouts almost completely disappeared, and patency and blood flow ceased in some vessels. By day 7, vascular density had decreased by almost 80%, and in surviving vessels pericytes had acquired a more normal phenotype. Reduced expression of VEGFR-2 and VEGFR-3 was seen in surviving endothelial cells, and cell-surface integrin expression was also markedly reduced in those cells following treatment with axitinib. Lewis lung tumors, which lack endothelial fenestrations, showed a decrease in vascular density of about 50% (20-22). Other experiments tested the effect of withdrawal following treatment with axitinib for 7 days. Treatment caused loss of 50-60% of the tumor vasculature. However, after treatment withdrawal, revascularization was complete by day 7. Importantly, subsequent rounds of treatment caused regression of the regrown vasculature to an extent similar to that seen after the first round, indicating that the tumor vasculature was still VEGF-dependent (23). Tumor vessels were found to be more sensitive to treatment than normal adult vessels (pancreas, thyroid, adrenal cortex, pituitary, choroid plexus, small intestine, adipose tissue, heart, lung, brain cortex, kidney, trachea), and no significant effects were observed on thyroid or renal function (16, 24).

In the human colorectal cancer MV522 xenograft model, bevacizumab alone resulted in 32% tumor growth inhibition at the maximum dose (5 mg/kg twice weekly x 3). In the same model, axitinib alone (at the ED_{80}; 30 mg/kg p.o. b.i.d.) produced 71% tumor growth inhibition and 67% tumor growth inhibition in mice that did not respond to bevacizumab. In the human melanoma M24met spontaneous metastasis model, axitinib was also more effective than bevacizumab in inhibiting metastasis, and the effect of their combination was no greater than that of axitinib alone (25).

Using the docetaxel-refractory murine Lewis lung carcinoma model, combination of docetaxel (40 mg/kg i.v. weekly) with axitinib (30 mg/kg p.o. b.i.d.) produced a tumor growth delay of 100% compared to 65% for axitinib alone and 9% for docetaxel alone. Survival was also prolonged by the combination treatment (86% compared to docetaxel alone). The mice tolerated the treatment well. Marked antitumor activity was also seen for the combination in other tumor xenograft models, including the metastatic human melanoma M24met model (26).

Axitinib in combination with radiotherapy produced substantially greater tumor growth delay than either treatment alone in the human prostate tumor DU 145 xenograft model in mice. This effect was accompanied by a decrease in tumor blood vessel count, but tumor hypoxia was not altered, indicating that oxygen delivery was not affected by the treatment (27).

In NOD/SCID mice injected with Namalwa lymphoma cells, daily oral treatment with axitinib significantly delayed the development of lymphoma and reduced lymphoma growth compared to untreated controls and animals treated with the maximum tolerated dose (MTD) of cyclophosphamide. Cyclophosphamide at the MTD delayed but did not prevent tumor growth and subsequent courses of cyclophosphamide treatment did not reduce tumor growth. High-dose cyclophosphamide was also associated with mobilization of circulating endothelial progenitor (CEP) cells (a marker of vasculogenesis). Subsequent treatment with axitinib downregulated CEP mobilization and reduced the viability of the CEP cells and lymphoma recurrence (28).

The effect of axitinib on tumor vasculature in the BT-474 human breast cancer model in mice was measured using dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI). Using the high-molecular-weight contrast agent albumin-$(GdDTPA)_{30}$ and the low-molecular-weight agent GdDTPA, tumor volume was found to be significantly decreased by axitinib (10-100 mg/kg/day p.o. for 3 weeks), which was associated with changes in tumor microvasculature (29-31).

Pharmacokinetics and Metabolism

In a multicenter, open-label phase I trial, 36 patients with refractory metastatic solid tumors (RCC, NSCLC, breast cancer, thyroid cancer, etc.) received axitinib (5-30 mg once or twice daily) on 28-day cycles. Pharmacokinetics were linear to dose. Axitinib was rapidly absorbed and eliminated, with a t_{max} of 2-6 h and a terminal plasma half-life of 2-5 h in the fed state. The rate and extent of absorption were greater in the fasted state, with higher C_{max} and $AUC_{0\text{-}24h}$ values and a shorter t_{max} (1-2 h) in most patients, but no significant difference in the half-life. Unchanged drug accounted for < 1% of the urinary excretion on days 1 and 29, indicating extensive metabo-

lism. Antacids had no clinically significant effect on the pharmacokinetic parameters (32-34).

Clinical Studies

The safety and efficacy of axitinib were also evaluated in the above study. Grade 3-4 toxicities included hypertension (11 patients with grade 3 hypertension, manageable by medication), stomatitis, diarrhea and elevated liver transaminases. The MTD was established as 5 mg b.i.d. in the fasted state. One of 8 patients treated at this dose experienced dose-limiting toxicity (DLT; grade 3 stomatitis). Two cases of fatal hemoptysis were also reported in patients with NSCLC. A durable partial response was obtained in 2 patients with RCC and 1 with adenoid cystic carcinoma, and several other patients also showed evidence of activity (32-34). DCE-MRI data demonstrated a dose-dependent decrease in tumor vascular function with increasing axitinib exposure (32, 34). The results from this and several of the following studies are summarized in Table I.

Two other phase I clinical trials are under way, one to study the effect of axitinib in Japanese patients with advanced solid tumors (35) and the other evaluating the effect of axitinib in combination with standard-of-care treatments for advanced solid tumors (36).

In a phase II trial, 52 treatment-experienced patients with cytokine-refractory mRCC received 5 mg axitinib b.i.d. in the fasted state for repeated 4-week cycles. Twenty-four patients (46%) achieved a partial response and 21 (40%) had stable disease, most of whom had tumor shrinkage. Responding patients showed reduced

Table I: Clinical studies of axitinib (from Prous Science Integrity®).

Drug	Design	Treatments	n	Conclusions	Ref.
Cancer	Open	Axitinib, 10 mg p.o. → [48 h later] 30 mg p.o. b.i.d. [fed] x 28 d (n=6) Axitinib, 2 mg p.o. b.i.d. [fasted] x 2 d → 5 mg p.o. b.i.d. [fasted] x 26 d (n=6) Axitinib, 5 mg p.o. b.i.d. [fed] x 28 d (n=6) Axitinib, 5 mg p.o. b.i.d. [fasted] x 28 d (n=8) Axitinib, 15 mg p.o. b.i.d. [fed] x 28 d (n=6) Axitinib, 20 mg p.o. b.i.d. [fed] x 28 d (n=4)	36	Axitinib 5 mg twice daily (in the fasting state) was associated with a reduction in tumor vascular parameters and antitumor activity in patients with advanced cancer and was the dose chosen for phase II studies according to conventional toxicity criteria	32, 34
Cancer	Open	Axitinib	12	A phase I study was initiated to evaluate the safety and best dose of axitinib in patients with advanced solid tumors	35
Cancer, kidney (renal cell carcinoma)	Open	Axitinib, 5 mg p.o. b.i.d. 1x/4 wks	52	Axitinib was well tolerated and demonstrated antitumor activity in patients with cytokine-refractory metastatic renal cell carcinoma	38
Leukemia, acute myeloid, Myelodysplasia	Open Multicenter	Axitinib, 5 mg b.i.d. p.o. x 56 [median] d	12	Axitinib was generally well tolerated but produced little evidence of efficacy in patients with acute myeloid leukemia or myelodysplastic syndrome	45
Cancer, pancreas	Open	Axitinib, 5 mg p.o. b.i.d. on d 3 + Gemcitabine, 1000 mg/m^2 infusion over 30 min on d 1, 8 & 15 1x/4 wks	8	Axitinib in combination with gemcitabine was safe, well tolerated and exhibited antitumor activity in patients with pancreatic cancer, with significant tumor regression observed in 2 patients	46
Cancer, pancreas	Randomized Open Multicenter	Axitinib + Gemcitabine Gemcitabine	102	This phase II study, which began in July 2005, will compare the tolerability and efficacy of gemcitabine alone or combined with axitinib on overall survival in patients with advanced cancer of the pancreas	47
Cancer, thyroid	Open	Axitinib, 5 mg p.o. b.i.d.	32	Axitinib was well tolerated and demonstrated significant antitumor activity in patients with advanced thyroid cancer	48, 49, 51
Cancer, thyroid	Open	Axitinib	100	Initiated in November 2006, this phase II study will evaluate the safety and efficacy of axitinib in patients with doxorubicin-refractory or -intolerant, locally advanced or metastatic thyroid cancer	52
Cancer, lung (non-small cell)	Randomized Open	CP-868596 + Docetaxel Axitinib + Docetaxel CP-868596 + Axitinib + Docetaxel Docetaxel	139	A phase II clinical study was initiated to study the efficacy of different combinations of axitinib, CP-868596 and docetaxel in the treatment of non-small cell lung cancer	53

tumor perfusion and decreased tumor perfusion was correlated with clinical response. Adverse events included grade 1-2 hypertension, diarrhea, fatigue, nausea and proteinurea. Grade 3-4 adverse events included hypertension (8 patients), diarrhea (4 patients) and fatigue (4 patients) (37-40).

A randomized, placebo-controlled phase I/II study examined axitinib in combination with docetaxel as first-line therapy for metastatic breast cancer. The phase II study was preceded by a phase I component in which 6 patients received docetaxel (80 mg/m^2 i.v. once every 3 weeks) plus axitinib (5 mg b.i.d. beginning on day 3), with the option to continue on axitinib treatment after stopping docetaxel. The plasma pharmacokinetic profiles indicated no drug interactions. Serious adverse events consisted of grade 3-4 neutropenia (3 patients), anemia (3 patients), febrile neutropenia (1 patient) and stomatitis (1 patient who stopped treatment due to the adverse effect); grade 2 hypertension was seen in 4 patients. Two patients had a partial response, 3 had stable disease and 1 had disease progression. Stable disease/partial response was maintained after a median of 14 weeks on axitinib as a single agent. The phase II component of the study had randomized 57 patients to docetaxel plus axitinib or placebo at the time of reporting (41, 42).

Twelve patients with poor-prognosis acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS) received axitinib (5 mg b.i.d. p.o.) for a median of 56 days in a phase II trial. Grade 3-4 toxicities included hypertension (5), mucositis (1) and deep venous thrombosis (1). No objective responses occurred and the study was stopped. Reduction in plasma soluble VEGFR-2 levels and increases in plasma VEGF and placental growth factor (PlGF) levels provided indirect evidence that axitinib had biological activity (43-45).

Axitinib was also evaluated in combination with gemcitabine as first-line therapy for patients with advanced pancreatic cancer. In the phase I portion of this phase I/II study, 8 patients were treated with gemcitabine (1000 mg/m^2 by 30-min infusion on days 1, 8 and 15 of a 4-week cycle) and axitinib (5 mg b.i.d. beginning on day 3). Grade 3-4 toxicities included anemia and thrombocytopenia in 1 patient, and neutropenia in another requiring dose reduction in cycle 3; grade 2 hypertension was observed in 3 patients. With a median of 3 cycles, 2 patients had a partial response and 4 had stable disease (46). The randomized phase II portion of this study is testing axitinib plus gemcitabine *versus* gemcitabine alone in patients with metastatic pancreatic cancer (47).

The results from a phase II study of axitinib in 32 patients with metastatic thyroid cancer refractory to or not suitable for radioactive iodine therapy have also been reported. Patients received axitinib (5 mg b.i.d.) for 6-469 days. The most common adverse events included proteinuria, fatigue, nausea, diarrhea, anorexia, vomiting, hypertension, weight loss, mucosal inflammation, abdominal pain, stomatitis and hoarseness. According to RECIST criteria, a partial response was obtained in 6 patients (19%), with an unconfirmed partial response in 5 additional patients. Two patients had a partial response lasting for > 12 months. Twelve patients discontinued treatment due to disease progression (6 patients), adverse events (3 patients) and other reasons (48-51). A phase II study is under way to study the effect of axitinib in patients with doxorubicin-refractory or intolerant locally advanced or metastatic thyroid cancer (52).

Several phase II studies are also in progress to evaluate the effect of axitinib either as a single agent or in combination with other therapeutics in patients with NSCLC (53, 54), sorafenib-refractory mRCC (55), metastatic colorectal cancer (56) and metastatic melanoma (57).

Source

Pfizer, Inc. (US).

References

1. Kania, R.S., Wallace, M.B., Borchardt, A.J. et al. (Agouron Pharmaceuticals, Inc.). *Indazole compounds and pharmaceutical compositions for inhibiting protein kinases, and methods for their use*. EP 1614683, JP 2003503481, JP 2006348043, WO 0102369.

2. Babu, S., Dagnino, R. Jr., Ouellette, M.A., Shi, B., Tian, Q., Zook, S.E. (Pfizer, Inc.). *Methods for preparing indazole compounds*. WO 2006048745.

3. Ewanicki, B.L., Flahive, E.J., Kasparian, A.J. et al. (Pfizer, Inc.; Agouron Pharmaceuticals, Inc.). *Methods of preparing indazole compounds*. US 2006094881, WO 2006048744.

4. Weidner, N., Semple, J.P., Welch, W.R. et al. *Tumor angiogenesis and metastasis: Correlation in invasive breast carcinoma*. N Engl J Med 1991, 324(12): 1-8.

5. Hanahan, D., Folkman, J. *Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis*. Cell 1996, 86(3): 353-64.

6. Salven, P., Manpaa, H., Orpana, A., Alitalo, K., Joensuu, H. *Serum vascular endothelial growth factor is often elevated in disseminated cancer*. Clin Cancer Res 1997, 3(5): 647-51.

7. Fontanini, G., Boldrini, L., Chine, S. et al. *Expression of vascular endothelial growth factor mRNA in non-small-cell lung carcinomas*. Br J Cancer 1999, 79(2): 363-9.

8. Mattern, J., Koomagi, R., Volm, M. *Association of vascular endothelial growth factor expression with intratumoral microvessel density and tumour cell proliferation in human epidermoid lung carcinoma*. Br J Cancer 1996, 73(7): 931-4.

9. Sorbera, L.A., Leeson, P.A., Bayes, M. *Bevacizumab*. Drugs Fut 2002, 27(7): 625-32.

10. Neufeld, G., Cohen, T., Gengrinovitch, S., Poitorak, Z. *Vascular endothelial growth factor (VEGF) and its receptors*. FASEB J 1999, 13(1): 9-22.

11. Gille, H., Kowalski, J., Li, B. et al. *Analysis of biological effects and signaling properties of Flt-1 (VEGFR-1) and KDR (VEGFR-2). A reassessment using novel receptor-specific vascular endothelial growth factor mutants*. J Biol Chem 2001, 276(5): 3222-30.

12. Hicklin, D.J., Ellis, L.M. *Role of the vascular endothelial growth factor pathway in tumor growth and angiogenesis*. J Clin Oncol 2005, 23(5): 1011-27.

13. Ferrara, N., Gerber, H.P., LeCouter, J. *The biology of VEGF and its receptors*. Nat Med 2003, 9: 669-76.

14. Ferrara, N. *Vascular endothelial growth factor: Basic science and clinical progress*. Endocr Rev 2004, 25: 581-611.

15. Lee, D., Heymach, J.V. *Emerging antiangiogenic agents in lung cancer*. Clin Lung Cancer 2006, 7(5): 304-8.

16. Kamba, T., Tam, B.Y., Hashizume, H. et al. *VEGF-dependent plasticity of fenestrated capillaries in the normal adult microvasculature*. Am J Physiol Heart Circ Physiol 2006, 290(2): H560-76.

17. Hu-Lowe, D., Hallin, M., Feeley, R. et al. *Characterization of potency and activity of the VEGF/PDGF receptor tyrosine kinase inhibitor AG013736.* Proc Am Assoc Cancer Res (AACR) 2002, 43: Abst 5356.

18. Wickman, G., Hallin, M., Amundson, K. et al. *Further characterization of the potent VEGF/PDGF receptor tyrosine kinase inhibitor AG-013736 in preclinical tumor models for its antiangiogenesis and antitumor activity*. 94th Annu Meet Am Assoc Cancer Res (AACR) (July 10-14, Washington, D.C.) 2003, Abst 3780.

19. Hu-Lowe, D., Heller, D., Brekken, J. et al. *Pharmacological activities of AG013736, a small molecule inhibitor of VEGF/PDGF receptor tyrosine kinases*. Proc Am Assoc Cancer Res (AACR) 2002, 43: Abst 5357.

20. Inai, T., Mancuso, M., Hashizume, H. et al. *Inhibition of vascular endothelial growth factor (VEGF) signaling in cancer causes loss of endothelial fenestrations, regression of tumor vessels, and appearance of basement membrane ghosts*. Am J Pathol 2004, 165(1): 35-52.

21. Nakahara, T., Norberg, S.M., Shalinsky, D.R., Hu-Lowe, D.D., McDonald, D.M. *Effect of inhibition of vascular endothelial growth factor signaling on distribution of extravasated antibodies in tumors*. Cancer Res 2006, 66(3): 1434-45.

22. Yao, V.J., Ozawa, M.G., Varner, A.S. et al. *Antiangiogenic therapy decreases integrin expression in normalized tumor blood vessels*. Cancer Res 2006, 66(5): 2639-49.

23. Mancuso, M.R., Davis, R., Norberg, S.M. et al. *Rapid vascular regrowth in tumors after reversal of VEGF inhibition*. J Clin Invest 2006, 116(10): 2610-21.

24. Baffert, F., Le, T., Sennino, B., Thurston, G., Kuo, C.J., Hu-Lowe, D., McDonald, D.M. *Cellular changes in normal blood capillaries undergoing regression after inhibition of VEGF signaling*. Am J Physiol Heart Circ Physiol 2006, 290(2): H547-59.

25. Hu-Lowe, D., Grazzini, M.L., Amundson, K. et al. *Antiangiogenic inhibitor axitinib (AG-013736) renders significant growth inhibition of bevacizumab-refractory xenograft tumors*. Eur J Cancer-Suppl [18th EORTC-NCI-AACR Symp Mol Targets Cancer Ther (Nov 7-10, Prague) 2006] 2006, 4(12): Abst 71.

26. Hu-Lowe, D.D., Grazzini, M.L. *Significant enhancement of anti-tumor efficacy of VEGF/PDGF receptor tyrosine kinase inhibitor AG-013736 in combination with docetaxel in chemorefractory and/or orthotopic xenograft tumor models in mice*. Proc Am Assoc Cancer Res (AACR) 2005, 46: Abst 2032.

27. Fenton, B.M., Paoni, S.F. *Combined effects of VEGF/PDGF receptor tyrosine kinase inhibitors plus fractionated radiation on DU145 prostate carcinoma vasculature and oxygenation*. Proc Am Assoc Cancer Res (AACR) 2006, 47: Abst 973.

28. Paul, S., Foutz, T.J., Calleri, A., Gobbi, A., Hu-Lowe, D., Shalinsky, D.R. *AG-013736, a potent VEGF/PDGF receptor tyrosine kinase inhibitor, is active against lymphoma growth and chemotherapy-induced vasculogenesis*. Blood 2003, 102(11, Part 1): Abst 2397.

29. Wilmes, L.J., Pallavicini, M.G., Fleming, L.M. et al. *AG-013736, a novel inhibitor of VEGF receptor tyrosine kinases, inhibits breast cancer growth and decreases vascular permeability as detected by dynamic contrast-enhanced magnetic resonance imaging*. Magn Reson Imaging 2007, 25(3): 319-27.

30. Wilmes, L.J., Hylton, N.M., Wang, D. et al. *AG-013736, a novel VEGFR TK inhibitor, suppresses tumor growth and vascular permeability in human BT474 breast cancer xenografts in nude mice*. Proc Am Assoc Cancer Res (AACR) 2003, 44: Abst 3772.

31. Li, K.L., Wilmes, L.J., Henry, R.G. et al. *Heterogeneity in the angiogenic response of a BT474 human breast cancer to a novel vascular endothelial growth factor-receptor tyrosine kinase inhibitor: Assessment by voxel analysis of dynamic contrast-enhanced MRI*. J Magn Reson Imaging 2005, 22(4): 511-9.

32. Herbst, R.S., Rugo, H.S., Liu, G. et al. *A phase I study of the VEGF/PDGF receptor tyrosine kinase inhibitor AG-013736 in patients with advanced solid tumors: Safety, pharmacokinetics, and dceMRI*. 15th AACR-NCI-EORTC Int Conf Mol Targets Cancer Ther (Nov 17-Nov 21, Boston) 2003, Abst C253.

33. Rugo, H.S., Herbst, R.S., Liu, G. et al. *Phase I trial of the oral antiangiogenesis agent AG-013736 in patients with advanced solid tumors: Pharmacokinetic and clinical results*. J Clin Oncol 2005, 23(24): 5474-83.

34. Liu, G., Rugo, H.S., Wilding, G. et al. *Dynamic contrast-enhanced magnetic resonance imaging as a pharmacodynamic measure of response after acute dosing of AG-013736, an oral angiogenesis inhibitor, in patients with advanced solid tumors: Results from a phase I study*. J Clin Oncol 2005, 23(24): 5464-73.

35. *A phase 1 study of AG-013736 (axitinib) in patients with advanced solid tumors (NCT00447005).* ClinicalTrials.gov Web site, May 20, 2007.

36. *Investigational agent AG-013736 in combinations with standard of care treatments for patient's with advanced solid tumor (NCT00454649).* ClinicalTrials.gov Web site, May 20, 2007.

37. *Anti-angiogenesis agent AG-013736 in patients with metastatic renal cell carcinoma (NCT00076011).* ClinicalTrials.gov Web site, May 20, 2007.

38. Rini, B.I., Rixe, O., Bukowski, R. et al. *AG-013736, a multitarget tyrosine kinase receptor inhibitor, demonstrates anti-tumor activity in a phase 2 study of cytokine-refractory, metastatic renal cell cancer (RCC)*. 41st Annu Meet Am Soc Clin Oncol (ASCO) (May 13-17, Orlando) 2005, Abst 4509.

39. Rixe, O., Meric, J., Bloch, J. et al. *Surrogate markers of activity of AG-013736, a multi-target tyrosine kinase receptor inhibitor, in metastatic renal cell cancer (RCC).* 41st Annu Meet Am Soc Clin Oncol (ASCO) (May 13-17, Orlando) 2005, Abst 3003.

40. George, D.J. *Phase 2 studies of sunitinib and AG013736 in patients with cytokine-refractory renal cell carcinoma*. Clin Cancer Res 2007, 13(2, Pt. 2): 753s-7s.

41. Rugo, H.S., Stopeck, A., Badorf, A., Pithavala, Y., Steinfeldt, H. *A phase I/II study of AG-013736, an oral anti-angiogenesis agent, in combination with docetaxel in patients (pts) with metastatic breast cancer (MBC)*. 28th Annu San Antonio Breast Cancer Symp (Dec 8-11, San Antonio) 2005, Abst 1067.

42. *AG-013736 in combination with docetaxel versus docetaxel alone for patients with metastatic breast cancer (NCT00076024)*. ClinicalTrials.gov Web site, May 20, 2007.

43. *Phase 2 study of AG-013736 in patients with poor prognosis acute myeloid leukemia (AML) or myelodysplastic syndrome (NCT00071006)*. ClinicalTrials.gov Web site, May 20, 2007.

44. Giles, F.J., Bellamy, W.T., Estrov, Z. et al. *The anti-angiogenesis agent, AG-013736, has minimal activity in elderly patients with poor prognosis acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS)*. Leuk Res 2006, 30(7): 801-811.

45. Giles, F.J., Steinfeldt, H., Bellamy, W.T. et al. *Phase 2 study of the anti-angiogenesis agent AG-013736 in patients with poor prognosis acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS)*. Blood 2004, 104(11, Part 1): Abst 1813.

46. Spano, J.P., Moore, M., Kim, S. et al. *A phase I study of axitinib (AG-013736), a potent inhibitor of VEGFRs, in combination with gemcitabine (GEM) in patients (pts) with advanced pancreatic cancer*. J Clin Oncol [42nd Annu Meet Am Soc Clin Oncol (ASCO) (June 2-6, Atlanta) 2006] 2006, 24(18, Suppl.): Abst 13092.

47. *AG-013736 in combination with gemcitabine versus gemcitabine alone for patients with metastatic pancreatic cancer (NCT00219557)*. ClinicalTrials.gov Web site, Mayj 20, 2007.

48. Cohen, E.E., Vokes, E.E., Rosen, L. et al. *A phase 2 study of axitinib (AG-013736), a potent inhibitor of VEGFRS, in patients with advanced thyroid cancer*. Ann Oncol [31st Eur Soc Med Oncol (ESMO) Congr (Sept 29-Oct 3, Istanbul) 2006] 2006, 17(Suppl. 9): Abst 579PD.

49. Kim, S., Rosen, L., Cohen, E.E. et al. *A phase II study of axitinib (AG-013736), a potent inhibitor of VEGFRs, in patients with advanced thyroid cancer*. J Clin Oncol [42nd Annu Meet Am Soc Clin Oncol (ASCO) (June 2-6, Atlanta) 2006] 2006, 24(18, Suppl.): Abst 5529.

50. *Study of the anti-angiogenesis agent AG-013736 in patients with metastatic thyroid cancer (NCT00094055)*. ClinicalTrials.gov Web site, May 20, 2007.

51. *Treatment for patients with metastatic thyroid cancer (NCT00176748)*. ClinicalTrials.gov Web site, May 20, 2007.

52. *Phase 2 study of AG-013736 in patients with doxorubicin-refractory or intolerant thyroid cancer (NCT00389441)*. ClinicalTrials.gov Web site, May 20, 2007.

53. *A phase 2 study in patients with advanced non-small cell lung cancer using new agents with and without docetaxel (NCT00386555)*. ClinicalTrials.gov Web site, May 20, 2007.

54. *Anti-angiogenesis agent AG-013736 in patients with advanced non-small cell lung cancer (NCT00094094)*. ClinicalTrials.gov Web site, May 20, 2007.

55. *Phase 2 study of AG-013736 in patients with refractory metastatic renal cell cancer (NCT00282048)*. ClinicalTrials.gov Web site, May 20, 2007.

56. *Phase 2 study with AG-013736 combined with chemotherapy and bevacizumab in patients with metastatic colorectal cancer (NCT00460603)*. ClinicalTrials.gov Web site, May 20, 2007.

57. *Anti-angiogenesis agent AG-013736 in patients with metastatic melanoma (NCT00094107)*. ClinicalTrials.gov Web site, May 20, 2007.