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BRIVANIB ALANINATE

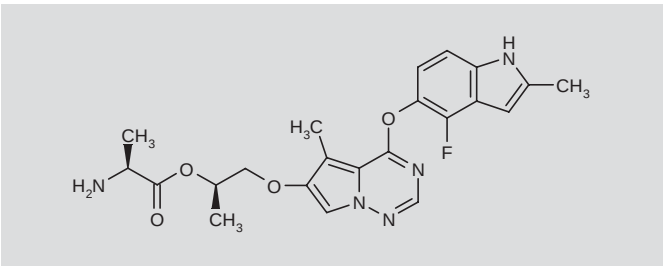
VEGFR/FGFR Inhibitor
Oncolytic

Prop INNM; USAN

BMS-582664

L-Alanine 2-[4-(4-fluoro-2-methyl-1*H*-indol-5-yloxy)-5-methylpyrrolo[2,1-*f*][1,2,4]triazin-6-yloxy]-1(*R*)-methylethyl ester

InChI=1S/C22H24FN5O4/c1-11-7-15-16(27-11)5-6-17(19(15)23)32-21-20-13(3)18(8-28(20)26-10-25-21)30-9-12(2)31-22(29)14(4)24/h5-8,10,12,14,27H,9,24H2,1-4H3/t12-,14+/m1/s1



$C_{22}H_{24}FN_5O_4$
Mol wt: 441.4555
CAS: 649735-63-7
EN: 396728

ABSTRACT

Brivanib alaninate has pharmacokinetic properties suitable for once- or twice-daily oral dosing and is completely hydrolyzed to the parent drug, brivanib, in vivo. Brivanib has a favorable cytochrome P450 profile and a low potential for drug-drug interactions. It inhibits growth of various solid carcinomas including hepatocellular carcinoma (HCC) xenografts in vivo. Brivanib-induced growth inhibition is associated with inhibition of angiogenesis and cell proliferation, and increased apoptosis. In phase I studies in patients with advanced or metastatic solid tumors, brivanib was well tolerated and had antitumor activity. In the phase II, open-label study, brivanib demonstrated promising efficacy and tolerability in patients with unresectable locally advanced or metastatic HCC as both first- and second-line treatment after exposure to prior therapy, including other antiangiogenics. As a result of these data, brivanib is in phase III development in HCC and in combination with other agents for treatment of various tumors, including colorectal cancer.

SYNTHESIS**

Brivanib alaninate can be synthesized as follows:
Deprotection of the 6-benzyloxypyrrolotriazine (I) by transfer hydrogenation with ammonium formate and Pd/C in DMF provides alcohol (II) (1, 2), which is condensed with (*R*)-propylene oxide (III) in the presence of triethylamine (1-3), and optionally LiCl (3), in ethanol at 70 °C to give the hydroxypropyl derivative brivanib (IV) (1-3). Subsequent coupling of alcohol (IV) with *N*-Cbz-L-alanine (V) by means of HATU in the presence of DIEA and DMAP in DMF or THF gives the *N*-protected alanine ester (VI), which is finally deprotected by treatment with ammonium formate and Pd/C in DMF (2, 3). Alcohol intermediate (II) is alternatively obtained by addition of methylmagnesium bromide to the 6-(ethoxycarbonyl)pyrrolotriazine derivative (VII) in THF to afford the tertiary alcohol (VIII), which undergoes oxidative cleavage to the key 6-hydroxypyrrolotriazine (II) in the presence of H_2O_2 and $BF_3{\cdot}Et_2O$ in dichloromethane (2, 3). Scheme 1.

The intermediates indolyloxy pyrrolotriazines (I) and (VII) can be prepared as follows. Cyclization of 2-oxobutyric acid (IX) with formamidine hydrochloride and hydrazine hydrate in the presence of AcOH in refluxing EtOH yields 6-ethyl-1,2,4-triazin-5-one (X). Subsequent condensation of triazinone (X) with 3-bromopyruvic acid (XI) in aqueous solution at 90 °C gives the pyrrolotriazine carboxylic acid (XII), which is esterified with refluxing EtOH/HCl to furnish ester (XIII) (4). Chlorination of hydroxypyrrolotriazine (XIII) with $POCl_3$, optionally in the presence of DIEA, in refluxing toluene affords the 4-chloro derivative (XIV) (2, 3), which is condensed with 4-fluoro-5-hydroxy-2-methylindole (XV) by means of K_2CO_3 in DMF to yield the indolyloxypyrrolotriazine adduct (VII) (2). Alternatively, displacement of chlorine in compound (XIV) with ethanolic NaOEt yields the 4-ethoxy derivative (XVI). Then, addition of methylmagnesium bromide to the ester group of (XVI) in THF leads to the tertiary alcohol (XVII), which undergoes oxidative cleavage to 4-ethoxy-6-hydroxy-5-methylpyrrolo[2,1-*f*]-1,2,4-triazine (XVIII) by treatment with H_2O_2 and $BF_3{\cdot}Et_2O$ in CH_2Cl_2. After protection of the hydroxyl group of (XVIII) with benzyl bromide and K_2CO_3 in DMF, selective cleavage of

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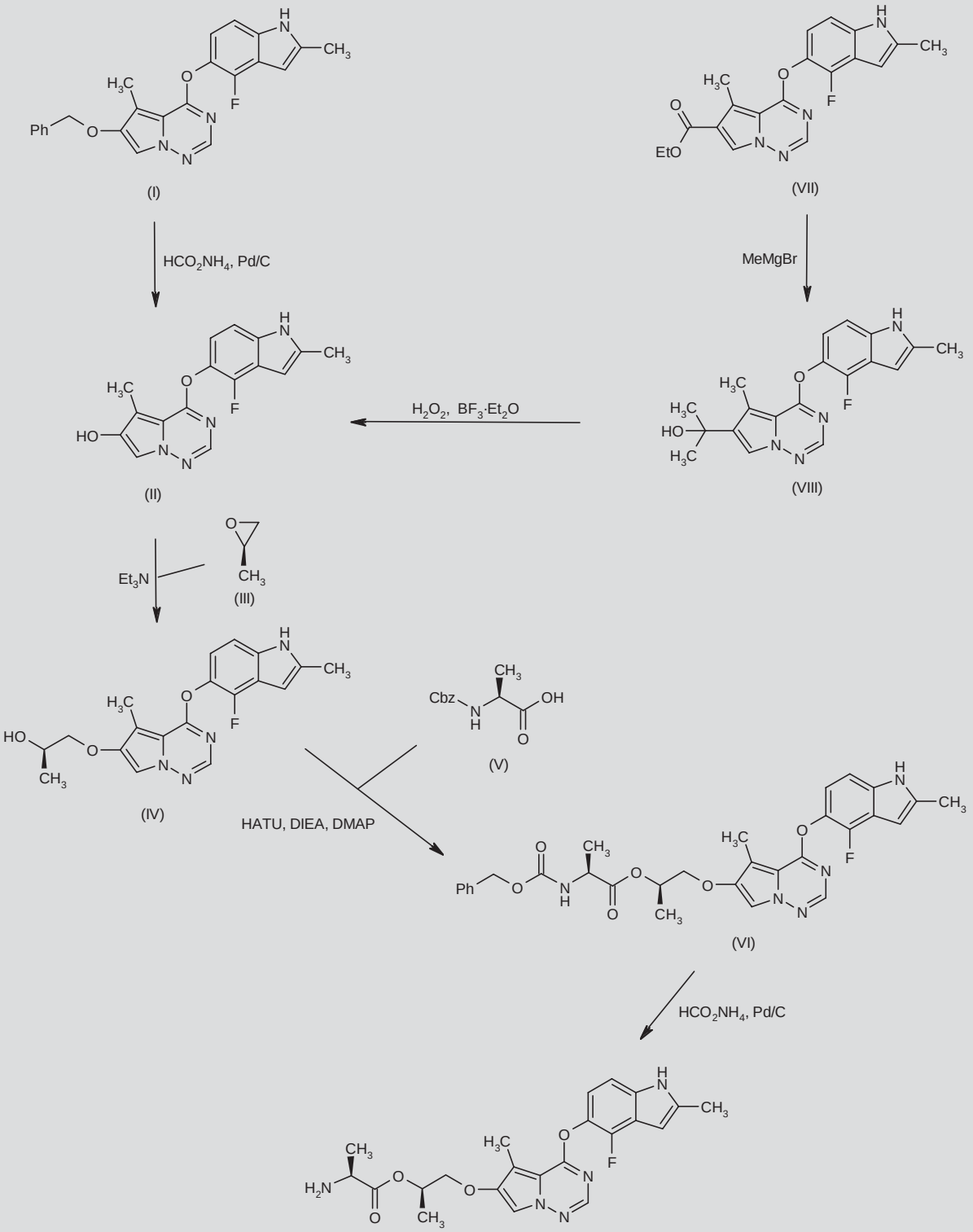
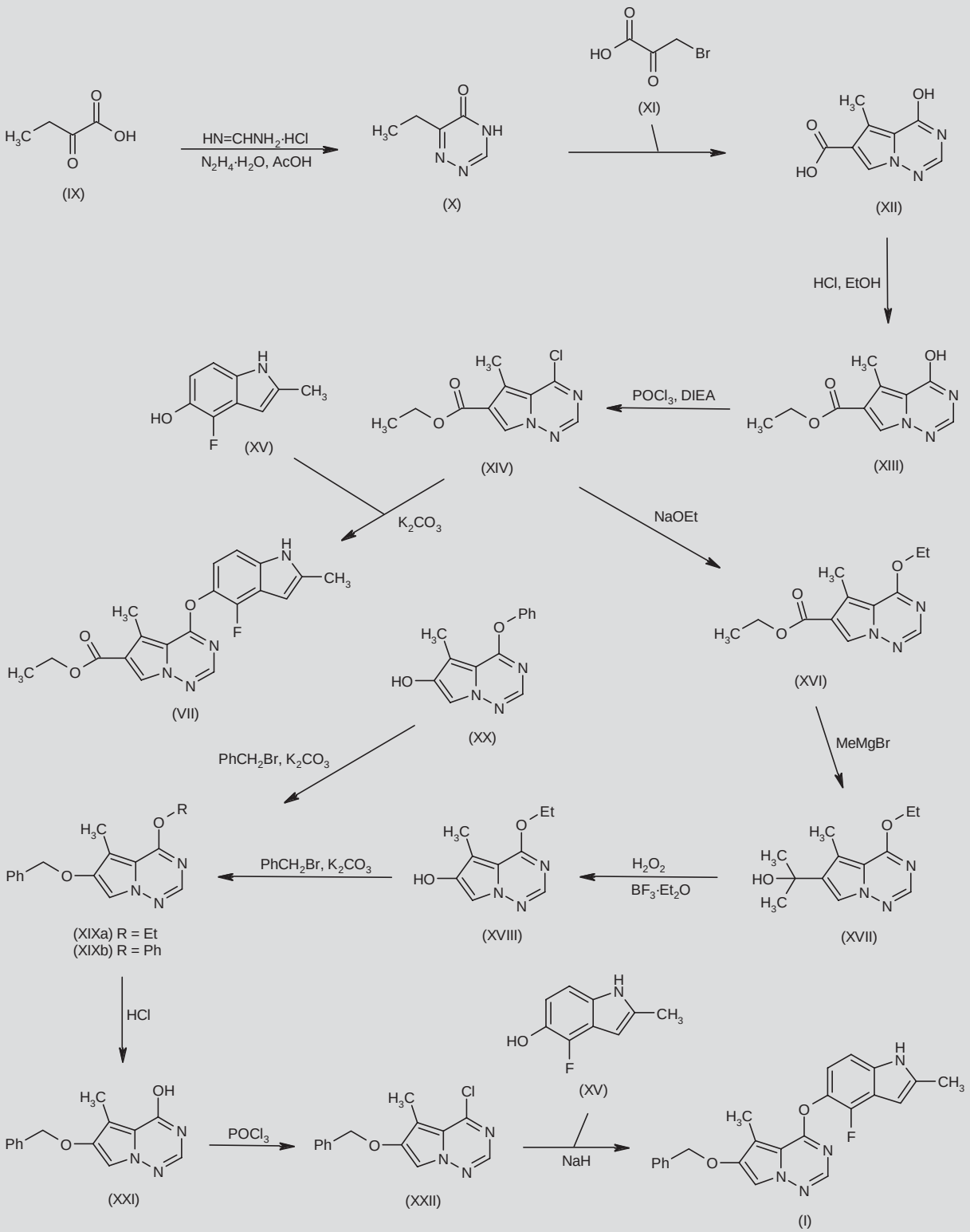
**Synthesis prepared by J. Bolós, R. Castañer. Thomson Reuters, Provenza 388, 08025 Barcelona, Spain.

Scheme 1. Synthesis of Brivanib alaninate



Scheme 2. Synthesis of Intermediates (I) and (VII)



the *O*-ethyl group in the resulting diether (XIXa) by means of HCl in hot ethanol provides 6-benzyloxy-4-hydroxy-5-methylpyrrolo[2,1-*f*]-1,2,4-triazine (XXI) (2). Similarly, the 4-phenoxy analogue (XX) is protected as the corresponding benzyl ether (XIXb), which undergoes *O*-phenyl group cleavage to (XXI) upon heating with HCl (1). Reaction of alcohol (XXI) with $POCl_3$ in refluxing toluene provides the 4-chloro derivative (XXII), which is then coupled with 4-fluoro-5-hydroxy-2-methylindole (XV) by means of NaH in DMF to provide intermediate (I) (1, 2). Scheme 2.

BACKGROUND

Angiogenesis, the development of new blood vessels from the pre-existing vasculature, is a critical process required for tumor growth and dissemination (5). This process is regulated by positive and negative factors that originate from cancer cells, endothelial cells, multiple types of stromal cells, blood and the extracellular matrix, although their relative contributions are thought to differ between tumor types and sites (6). These factors influence and are influenced by the tumor host microenvironment (7). However, when an imbalance of these factors is expressed in favor of angiogenic factors such as vascular endothelial growth factors (VEGFs), the 'dormant' tumor can undergo the so-called angiogenic switch, resulting in rapid tumor growth and an increased potential for metastasis (8). Inhibition of tumor-induced angiogenesis can theoretically not only prevent the growth of tumors but should also inhibit dissemination of tumor cells (9). It is apparent both preclinically and clinically that agents that inhibit the process of angiogenesis represent a promising therapeutic strategy for a variety of tumor types. Different types of tumors use distinct molecular strategies either alone or in combination to activate the angiogenic switch, suggesting that a selection of different therapeutic agents will need to be developed to treat all tumor types (10). Furthermore, it is known that as tumors grow they begin to produce a wider array of proangiogenic molecules, thereby 'escaping' blockade via one target. If only one proangiogenic molecule or pathway is blocked, tumors may switch to another or as is the case in many tumors, more than one target may be integral to angiogenesis. Therefore, a successful antiangiogenic and clinically effective approach will need to inhibit a number of molecular-targeted pathways.

Increased expression of VEGFs by tumor cells and VEGF receptor 2 (VEGFR-2) and VEGFR-1 in the tumor vasculature is a hallmark of a variety of human and animal tumors in vivo and correlates with tumor growth rate, microvessel density, tumor cell proliferation, tumor metastatic potential and poorer patient prognosis in a variety of malignancies (5, 11-16). VEGFR-2 is the principal receptor through which VEGFs exert their mitogenic, chemotactic and vascular permeabilizing effects on the host vasculature (12, 14-16). Evidence indicates that expression and signaling of VEGF are critical for tumor angiogenesis. Antibodies against VEGF (17) and VEGFR (18) as well as small molecule VEGFR inhibitors (19-21) have been shown to inhibit angiogenesis in a variety of tumor xenograft models based on decreased blood flow and blood vessel density as well as induction of tumor growth stasis. Although blocking the VEGF pathway has been shown to prevent tumor progression in the short term, eventual progression of disease in the presence of VEGF/VEGFR therapies has been observed in clinical studies (22-24). Recent evidence suggests that fibroblast growth factor (FGF), hepatocyte growth factor, placental growth factor, platelet-derived growth factor (PDGF) and insulin-like growth factor pathways, among an ever lengthening list, may have a role in angiogenic resistance. Acquired resistance to anti-VEGFR-2 antibodies was shown in one study to be caused by the redundancy of angiogenesis stimulators, demonstrated by upregulation of FGF in a pancreatic tumor after anti-VEGFR antibody treatment (25). FGF1 and basic FGF (FGF2) and their receptors promote autocrine and paracrine regulation of malignant tumors (26). FGF2 also has angiogenic activity in different experimental models in vivo and enhances the malignancy of a variety of cancers including hepatocellular carcinoma (HCC), pancreatic cancer, prostate cancer and bladder cancer (27). Endothelial cells express FGF receptor-1 (FGFR-1) and frequently FGFR-2, but have not been found to express FGFR-3 or FGFR-4 (28, 29). FGFs have a direct effect on tumor angiogenesis by promoting proliferation in FGFR-expressing endothelial cells (30). Several studies suggest the possibility that FGF2 induces neovascularization indirectly by activation of the VEGF/VEGFR pathway. In one study, simultaneous expression of FGF2 and VEGF resulted in rapid growing tumor xenografts in nude mice, which were characterized by high blood vessel density, patency and permeability (31). A further study demonstrated that VEGFR-2 antagonists inhibit both VEGF- and FGF2-induced angiogenesis in vitro and in vivo (32). In an implant mouse model of angiogenesis, VEGFR-2 inhibitors completely inhibited VEGF-induced growth of vascularized tissue and also FGF2-induced growth to some extent. In addition, the same VEGFR-2 inhibitors inhibited VEGF- and FGF2-induced bovine endothelial cell invasion. Both endogenous and exogenous FGF2 modulated VEGF expression in endothelial cells (7). FGF2 and PDGF together promoted tumor angiogenesis in mouse models, suggesting that two or more growth factors could act synergistically (33). Overall these effects may contribute to the rapid vascular regrowth that has been observed in tumors after removal of VEGF inhibition (34). Studies by Casanovas et al. (25) have indicated the importance of blocking the FGFR signaling pathway, via an FGF trap, following acquired resistance to VEGFR inhibition. Overall, these studies suggest that blocking multiple signaling pathways involved in tumor vascularization may provide a more effective and sustainable antitumor activity (35).

HCC is the fifth most frequent primary neoplasm, with approximately 660,000 deaths worldwide annually (36, 37). A number of chemotherapeutic agents have been evaluated for the treatment of HCC; however, no single or combination standard chemotherapy regimen has been particularly effective (38). Among the most prominent clinical features of HCC are hypervascularity, portal and hepatic vein invasion and early metastasis. Angiogenic factors such as angiopoietin, VEGF, PDGF and FGF2, released from the tumor itself, inflammatory cells and/or tumor stromal cells participate in the neovascularization of HCC (39-41). The *VEGF* gene and its protein are transcribed, expressed and secreted by hepatoma cells (39, 42, 43). VEGF expression was increased in several studies of surgical HCC specimens compared with adjacent nontumoral tissue (39, 42, 44-46). Plasma VEGF levels are significantly elevated in patients with HCC and distant HCC metastasis (47) and the increased expression of VEGF is correlated with histologic tumor grade (46) and tumor microvessel density (48). In addition to VEGF, high expression levels of FGF2 are also detected in patients with HCC (49). FGF2 stimulates the release and activity of collagenases, proteases and

integrins on the extracellular membrane to form nascent microvascular networks (50). The expression of heparanase in connection with FGF2 enhances growth, invasion and angiogenesis of the tumor (51). In a clinical study of patients undergoing resection of HCC, a high preoperative serum FGF2 level appeared to be predictive of invasive tumor and early postoperative recurrence (41). Furthermore, FGF2 has been shown to synergistically augment VEGF-mediated HCC development and angiogenesis (52). FGF2 functions as a mitogen for HCC cell proliferation via an autocrine mechanism and enhances the development and progression of HCC by binding to FGFR-1 (53).

In order to identify selective small molecule kinase inhibitors for antiangiogenic therapies, a new class of pyrrolo[2,1-*f*][1,2,4]triazine-based VEGFR-2 inhibitors was investigated, resulting in the discovery of BMS-540215 (brivanib) (1). Although brivanib demonstrated excellent kinase selectivity and potent in vivo efficacy against various tumor xenografts implanted in immunocompromised mice, oral administration of brivanib in mice as a micronized suspension produced significantly lower systemic exposure levels of brivanib than those obtained from solution formulations (Table I) (54). This is due to its low aqueous solubility (< 1 μg/mL at pH 6.5) (Fig. 1). This dissolution rate–limited absorption of brivanib, therefore, leads to the possibility of not achieving efficacious exposures in humans.

To improve the oral bioavailability and to increase drug plasma concentrations of brivanib, the prodrug strategy of incorporating a variety of amino acids onto the secondary alcohol group of brivanib through metabolically labeled ester linkage was developed. Details of the synthesis of a series of amino ester prodrugs of brivanib have been previously reported (3). Among the several prodrugs tested, BMS-582664 (brivanib alaninate) demonstrated good water solubility (73 mg/mL at pH 5.8) (Fig. 1) and efficient in vivo conversion to brivanib in various species, with the area under the curve (AUC) increasing proportionally with dose (Table II) (55).

Table I. *Comparison of pharmacokinetics of brivanib in mice as solution or micronized suspension (54).*

	Solution	Micronized suspension		
Dose, mg/kg	25	25	100	200
C_{max}, μM	6.4	0.55	2.05	1.71
AUC_{tot}, μM*hour	13.4	3.35	8.43	7.61

Vehicle for solution study was PEG400:ethanol:water (7:1:2) and for suspension study was 0.1% Tween 80 in water. C_{max}, maximum plasma concentration; AUC_{tot}, total area under the curve.

PRECLINICAL PHARMACOLOGY

Brivanib demonstrated potent and selective inhibition of VEGFR and FGFR tyrosine kinases (1) over a number of kinase families in vitro (Table III). Brivanib is an ATP competitive inhibitor of human VEGFR-2, with an IC_{50} of 25 nM and Ki of 26 nM. In addition, it inhibits VEGFR-1 (IC_{50} = 350 nM) and VEGFR-3 (IC_{50} = 10 nM). It also shows good selectivity for FGFR-1 (IC_{50} = 150 nM), FGFR-2 (IC_{50} = 125 nM) and FGFR-3 (IC_{50} = 68 nM). Consistent with its antiangiogenic activity, brivanib inhibits both VEGF- and FGF2-stimulated human umbilical vein endothelial cell (HUVEC) growth with

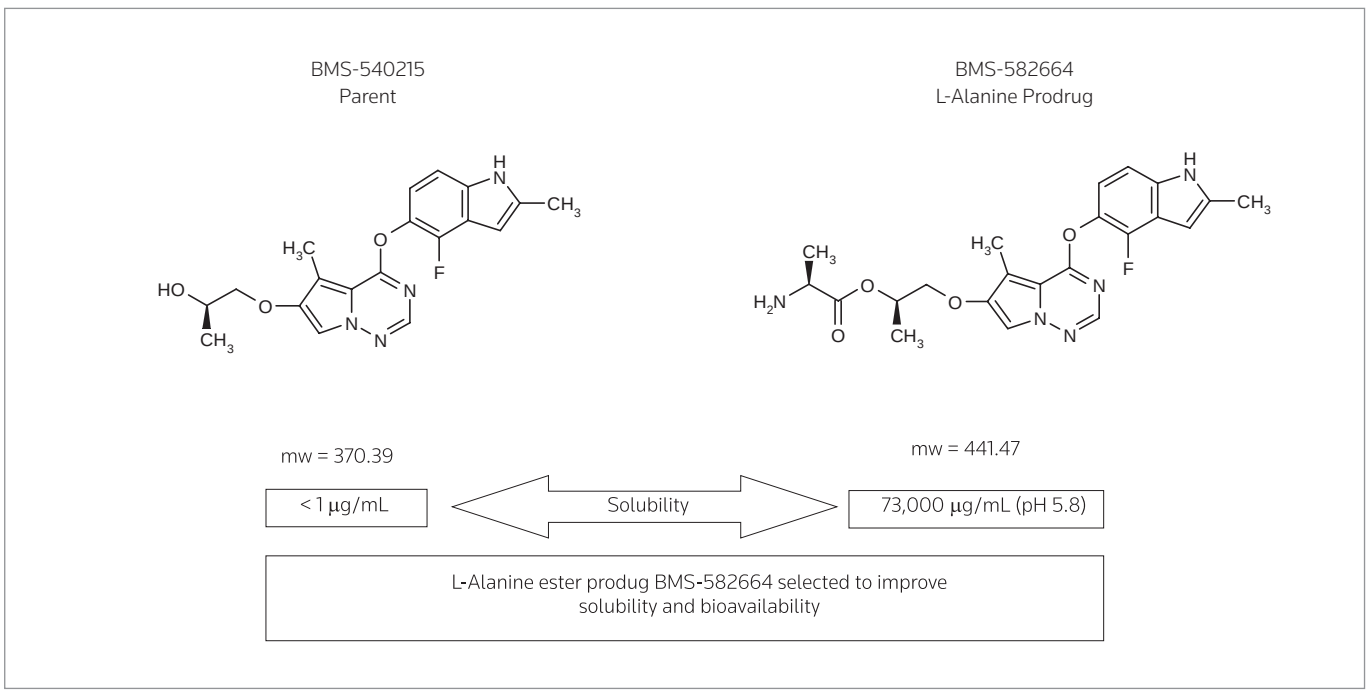


Figure 1. Chemical structure of brivanib (BMS-540215) and L-alanine prodrug (BMS-582664).

Table II. *Dose-related increase in exposure of brivanib after oral administration of brivanib alaninate in various species (55).*

	Dose of brivanib alaninate (mg/kg)	C_{max} (μM)	T_{max} (h)	AUC_{0-24} (μM/h)	Fold-change AUC
Rat	50	18 ± 6.1	1.3 ± 0.6	49 ± 6.0	-
	200	89 ± 24	3.0 ± 1.0	446 ± 188	8.9
Dog	4.5	3.7	0.6	15.5	-
	30	13.3	2.0	96.0	6.2
Monkey	5	6.7 ± 2.1	1.3 ± 0.7	32 ± 2.5	-
	30	23 ± 1.5	2.6 ± 3.0	290 ± 85	9.0

C_{max}, maximum plasma concentration; T_{max}, time of maximum plasma concentration; AUC_{0-24}, area under the curve, from time zero to 24 h.

Table III. *In vitro inhibitory activity of brivanib (BMS-540215) against various enzymes (1).*

Biochemical	IC_{50} (nM)	Cellular	IC_{50} (nM)
VEGFR-1	350	VEGF HUVEC	40
VEGFR-2	25	FGF HUVEC	276
VEGFR-3	10	EGF HUVEC	> 2,500
FGFR-1	150	L2987/H3396	> 10,000
FGFR-2	125		
FGFR-3	68		
FGFR-4	> 1,000		
Enzyme	**IC_{50} (nM)**	**Enzyme**	**IC_{50} (nM)**
HGFR	17,600	HER-1	1,970
PKC_{θ}	> 100,000	HER-2	> 10,000
PKCα	> 25,000	Lck	2,730
PKCβ	> 25,000	Syk	> 50,000
PKA	> 50,000	SRC	> 5,000
CDK2	> 50,000	CAMK II	> 50,000
EMT	> 50,000	GSK-3	> 50,000
IGF-1R	> 50,000	JAK-3	> 50,000
p38	> 30,000	PDGFRβ	7,460
AKT	> 50,000	MEK	> 25,000

CAMK, calmodulin-dependent kinase; CDK, cyclin-dependent kinase; EGF, epidermal growth factor; FGFR, fibroblast growth factor receptor; GSK, glycogen synthase kinase; HER, human epidermal growth factor receptor; HGFR, hepatocyte growth factor receptor; HUVEC, human umbilical vein endothelial cells; IGF, insulin-like growth factor; JAK, janus kinase; MEK, mitogen-activated protein kinase or extracellular signal-regulated kinase; PDGFR, platelet-derived growth factor receptor; PKA, protein kinase A; PKC, protein kinase C; VEGFR, vascular endothelial growth factor receptor.

IC_{50} values of 40 nM and 276 nM, respectively, but has no effect on EGF-stimulated HUVEC growth (1). Brivanib alaninate possesses high aqueous solubility (73 mg/mL at pH 5.8) and is highly hydrolyzed to the active moiety brivanib in vivo (3). The clearance of brivanib in humans is anticipated to be low to intermediate (hepatic extraction ratio < 0.7), while its volume of distribution is expected to be high. The minimum efficacious dose of brivanib alaninate was determined to be 60 mg/kg/day. Brivanib alaninate exhibits high solid stability (only 0.3% degradation at 50 °C with desiccant over a period of 12 weeks) and acceptable solution state stability up to pH 6.5. The compound is quite stable at pH 4.2 in buffer solution at 37 °C, with less than 1% of brivanib alaninate degrading to parent brivanib over a 24-hour period.

In multiple preclinical models of human xenograft tumors, brivanib alaninate demonstrated potent antitumor activity when dosed orally. Once-daily oral administration of brivanib alaninate for 14 consecutive days inhibited the growth of established tumors (80 - 100 mm^3) in a dose-dependent manner (Fig. 2) (56). The treatment/control ratios were 0.88, 0.40 and 0.28 at doses of 53 mg/kg, 80 mg/kg and 107 mg/kg daily for 14 days starting on day 17 postimplantation. The tumor growth inhibition values were 34%, 85% and 97%, respectively. At 80- and 107-mg/kg doses, essentially complete tumor stasis was observed throughout the dosing period (Fig. 2), with a percent tumor growth inhibition of 85% and 97%, respectively. No overt toxicity was observed in either cohort of animals, suggesting good safety margins in mice for this compound.

The antitumor activity of brivanib alaninate was further evaluated using mouse models of human HCC, which resemble many features of human HCC (57-59). In this experiment, patient-derived HCC

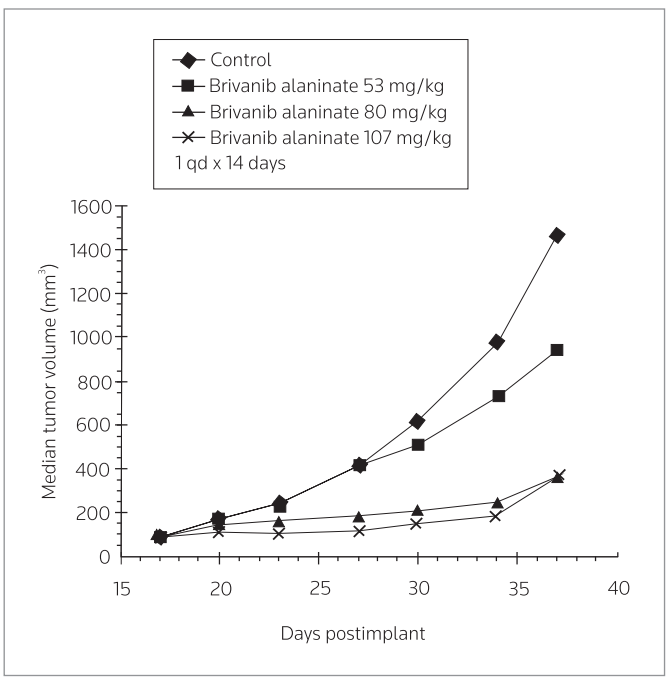


Figure 2. Brivanib alaninate inhibits growth of the human lung carcinoma xenograft, L2987 dosed orally on a once-a-day schedule (56). Adapted with permission from Bhide,. R.S., Lombardo, L.J., Hunt, J.T. et al. Mol Cancer Ther 2010, In press (Figure 1; Panel D).

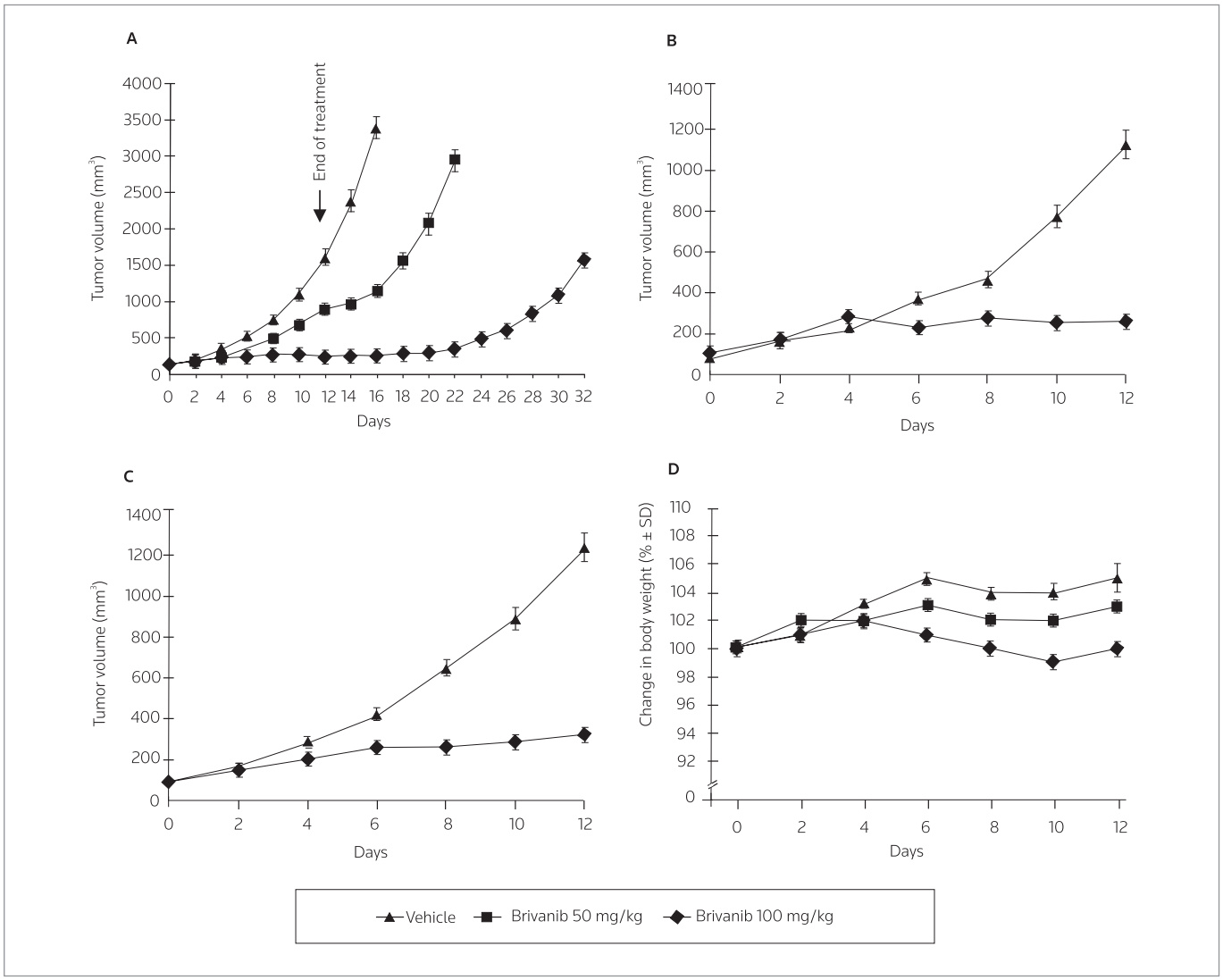


Figure 3. Effects of brivanib alaninate on growth rate of patient-derived hepatocellular carcinoma xenograft lines 06-0606, 2-1318 and 26-1004 (59). (A) Mice bearing xenograft line 06-0606 were treated with vehicle, 50 mg/kg brivanib alaninate or 100 mg/kg brivanib alaninate daily for 12 days. (B, C) Mice bearing xenograft lines 2-1318 and 26-1004 were treated with 100 mg/kg brivanib alaninate daily for 12 days. Each treatment arm involved 14 independent tumor-bearing mice representing the same xenograft line. Mean tumor volume ± SE at given time points are shown. Significant differences in tumor growth between vehicle- and brivanib alaninate-treated tumors were also observed as early as day 10 ($P < 0.01$). Note that brivanib alaninate had antitumor activity after cessation of treatment. (D) Percent change in body weight in mice treated with vehicle, 50 mg/kg brivanib alaninate and 100 mg/kg brivanib alaninate. Adapted with permission from Huynh, H., Ngo, V.C., Fargnoli, J. et al. Clin Cancer Res 2008, 14: 6146-53 (Figure 2).

xenografts (2-1318, 5-1318, 2006, 30-1004, 26-1004 and 06-0606) were subcutaneously implanted in male severe combined immunodeficiency disease mice aged 9 - 10 weeks. As shown in Figure 3A, the growth rate of tumors in mice with patient-derived xenograft line 06-0606 was decreased by brivanib alaninate treatment in a dose-dependent manner ($P < 0.01$). Tumor weights in mice treated with brivanib alaninate 50 mg/kg and 100 mg/kg by gavage daily for 12 days were approximately 55% and 13%, respectively, compared with vehicle-treated mice. Furthermore, brivanib alaninate, at a dose of 100 mg/kg, also inhibited tumor growth in mice with patient-derived xenograft lines 2-1318 (Fig. 3B) and 26-1004 (Fig. 3C) ($P < 0.01$). Maximal inhibition was seen at 100 mg/kg in all patient-derived xenograft lines (Table IV). The treatment/control ratios for xenograft lines 2-1318, 5-1318, 26-1004, 30-1004, 2006 and 06-0606 were 0.38, 0.40, 0.34, 0.60, 0.38 and 0.13, respectively, after 12 days' treatment. No overt toxicity, as defined by weight loss, was observed in either vehicle-treated or brivanib-treated mice during the course of treatment (Fig. 3D). Brivanib alaninate-induced growth inhibition was associated with a decrease in phosphorylated VEGFR-2 at Tyr1054/1059, increased apoptosis, reduced microvessel density, inhibition of cell proliferation and downregulation of cell-cycle regulators. The levels of FGFR-1 and FGFR-2 expression in these xenograft lines were positively correlated with their sensitivity to brivanib alaninate-induced growth inhibition (59).

Table IV. *Changes in body weight, tumor weight, microvessel density (CD-31), markers of cell proliferation (K_i-67) and apoptosis (cleaved caspase-3) in mice treated with either vehicle or brivanib alaninate (100 mg/kg) in six HCC xenograft lines (59).*

Xenograft line	Treatment	Body weight at sacrifice (g)	Tumor weight at sacrifice (mg)	Microvessel density*	K_i-67 index (%)	Cleaved caspase-3 (%)
2-1318	Vehicle	23.4 ± 1.7	950 ± 1.9	27 ± 5	20 ± 4.6	5.1 ± 3
	Brivanib alaninate	22.5 ± 1.4	360 ± 49†	6 ± 3†	4.5 ± 2.7†	12.2 ± 3†
5-1318	Vehicle	22.6 ± 1.1	880 ± 121	29 ± 7	19.8 ± 4	3.6 ± 1.8
	Brivanib alaninate	22.2 ± 0.6	350 ± 69†	8.6 ± 4†	5.1 ± 1.8†	12.4 ± 2.4†
26-1004	Vehicle	23.9 ± 1.3	1080 ± 13	27 ± 7	19.8 ± 6.1	3.5 ± 1.7
	Brivanib alaninate	21.8 ± 0.7	371 ± 69†	5.8 ± 3†	7.5 ± 2.9†	11.8 ± 2.1†
30-1004	Vehicle	25.0 ± 1.3	798 ± 65	15.4 ± 4	16.2 ± 4	2.7 ± 1.2
	Brivanib alaninate	24.2 ± 1.0	480 ± 54†	7.3 ± 3†	8.5 ± 2.1†	8.8 ± 1.8†
2006	Vehicle	18.6 ± 0.9	650 ± 83	18.4 ± 5	13.2 ± 2.9	3.1 ± 1.4
	Brivanib alaninate	18.9 ± 0.6	250 ± 57†	5.2 ± 3†	4.8 ± 1.2†	12.3 ± 2.1†
06-0606	Vehicle	23.8 ± 0.8	1369 ± 24	30.8 ± 8	27.9 ± 5	1.2 ± 0.5
	Brivanib alaninate	23.5 ± 0.9	184 ± 43†	7 ± 3†	6.4 ± 3.1†	18.9 ± 2.3†

*Mean microvessel density of 10 random 0.159 mm^2 fields at 100 × magnification; †$P < 0.001$ versus vehicle in same xenograft line.

Although antiangiogenic therapies improve survival in patients with colorectal cancer (CRC) and others, the survival benefits of antiangiogenic drugs have been rather modest, stimulating interest in developing more effective ways to combine antiangiogenic drugs with established chemotherapies. In preclinical studies, chemotherapeutics were most effective when given during a window of normalization created by antiangiogenic agents (60-62). Whether brivanib alaninate is able to enhance the antitumor activity of cyclophosphamide has been investigated. Using mouse models of HCC (58), a four-arm therapeutic study was run. Mice bearing 06-0606 or 10-0505 tumors (five per group) were allocated to the following groups: (a) control (vehicle only); (b) cyclophosphamide (100 mg/kg, once every 2 days for the first 7 days of the 14-day treatment cycle, i.p.); (c) brivanib alaninate (75 mg/kg/day for the first 7 days, then 7 days' drug holiday); and (d) cyclophosphamide plus brivanib alaninate. In this combined therapy, cyclophosphamide was given 1 day after brivanib alaninate because in mouse models VEGFR-2 and VEGF blockade or downregulation began to normalize the tumor vasculature by day 1 or 2, as detected by a significant decrease in mean vessel diameter and permeability (62-64). In both tumor types, combination of brivanib and cyclophosphamide showed superior antitumor activity compared with vehicle or the individual agents (unpublished data). This combination also resulted in minimal weight loss, suggesting that it did not produce marked normal tissue toxicity. These effects were accompanied by significantly increased survival in the combination groups compared with mice treated with a single agent. To determine whether the cyclophosphamide/brivanib alaninate combination is beneficial in patients with HCC, additional assessment and validation in future randomized studies is necessary.

PHARMACOKINETICS AND METABOLISM

Although brivanib alaninate demonstrates rapid conversion to the parent drug in human and mouse liver S9 fractions, it is essential to determine how those results translate to in vivo conditions. Data from pharmacokinetic evaluations of brivanib alaninate in rat, dog and monkey are shown in Table II (55). A dose-proportional increase in AUC after oral administration of brivanib alaninate was observed in dogs and somewhat higher increases were observed in rats and monkeys. No circulating concentrations of brivanib alaninate were detected in mouse, rat or monkey plasma after oral dosing and only very low levels (< 4.4 ng/mL up to 15 min) of brivanib alaninate were observed in dogs when a 30-mg/kg dose was orally administered (65).

The in vivo activity of brivanib alaninate was also assessed in tumor-bearing mice using dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) (66). Using this radiographic technique, blood flow, vessel surface area and permeability were evaluated indirectly by assessing uptake of a contrast agent. In this study, changes in tumor microcirculation resulting from the antiangiogenic effects of VEGFR-2 inhibition were measured in mice with human L2987 lung tumor xenografts by DCE-MRI (56). The contrast uptake in the tumor was measured as a pharmacodynamic (PD) marker of treatment response using the AUC for gadolinium for the first 60 seconds (AUC_{60}) after bolus injection of gadolinium DTPA using T1-weighted DCE-MRI. Reproducibility experiments conducted in the same mice 24 hours apart demonstrated that the 95% limit of change was -18% to +22% for a group of nine mice and the within-subject coefficient of variation was 24%. Mice dosed daily with oral brivanib alaninate had DCE-MRI assessments performed at 24 hours, 2 hours after the second dose and at 48 hours, 2 hours after the third dose. A dose-dependent response in blood flow and perfusion was observed at dose levels correlated with antitumor activity. Figure 4 shows DCE-MRI images of blood flow in tumor tissue after administration of brivanib alaninate at 36 mg/kg and 107 mg/kg, with higher contrast uptake (associated with higher blood flow) observed in tumor following treatment with the lower subefficacious dose of brivanib alaninate. There was a significant reduction in AUC_{60} of 54% and 64% at 24 hours and 48 hours, respectively, at the 107 mg/kg dose (n = 9), but no significant reduction was observed with the 36 mg/kg dose (Fig. 5).

Pharmacokinetic (PK) and PD studies of brivanib alaninate were performed in a phase I, open-label, dose-escalation study in 18 patients with advanced or metastatic solid tumors. Dose escalation starting at a dose of 180 mg in cohorts of patients (n = 3 or 6 for 800 mg

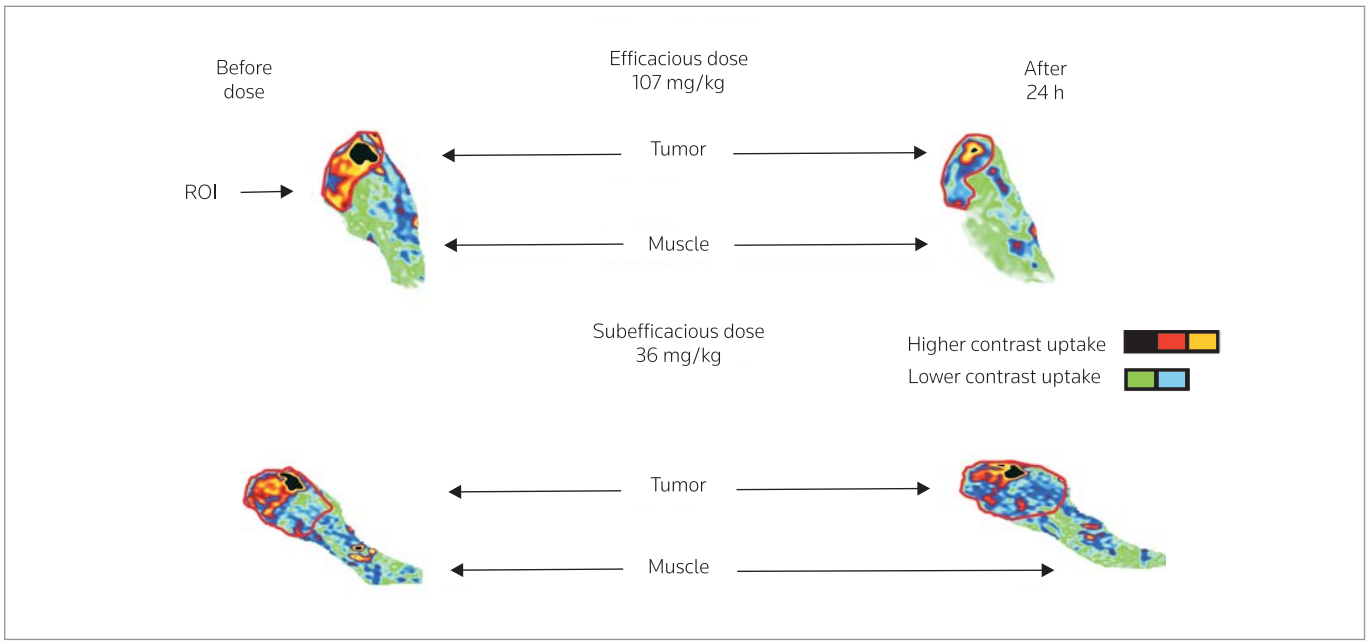


Figure 4. Effect of brivanib alaninate on dynamic contrast enhanced-magnetic resonance imaging after oral administration at efficacious and subefficacious dose levels (56). Brivanib alaninate in vivo activity is assessed pharmacodynamically in L2987 tumor-bearing animals using the imaging technique in order to measure contrast agent (Gd-DTPA) uptake, which is dependent on a combination of blood flow, vessel surface area and permeability parameters. Adapted with permission from Bhide, R.S., Lombardo, L.J., Hunt, J.T. et al. Mol Cancer Ther 2010, In press (Figure 6; Panel A).

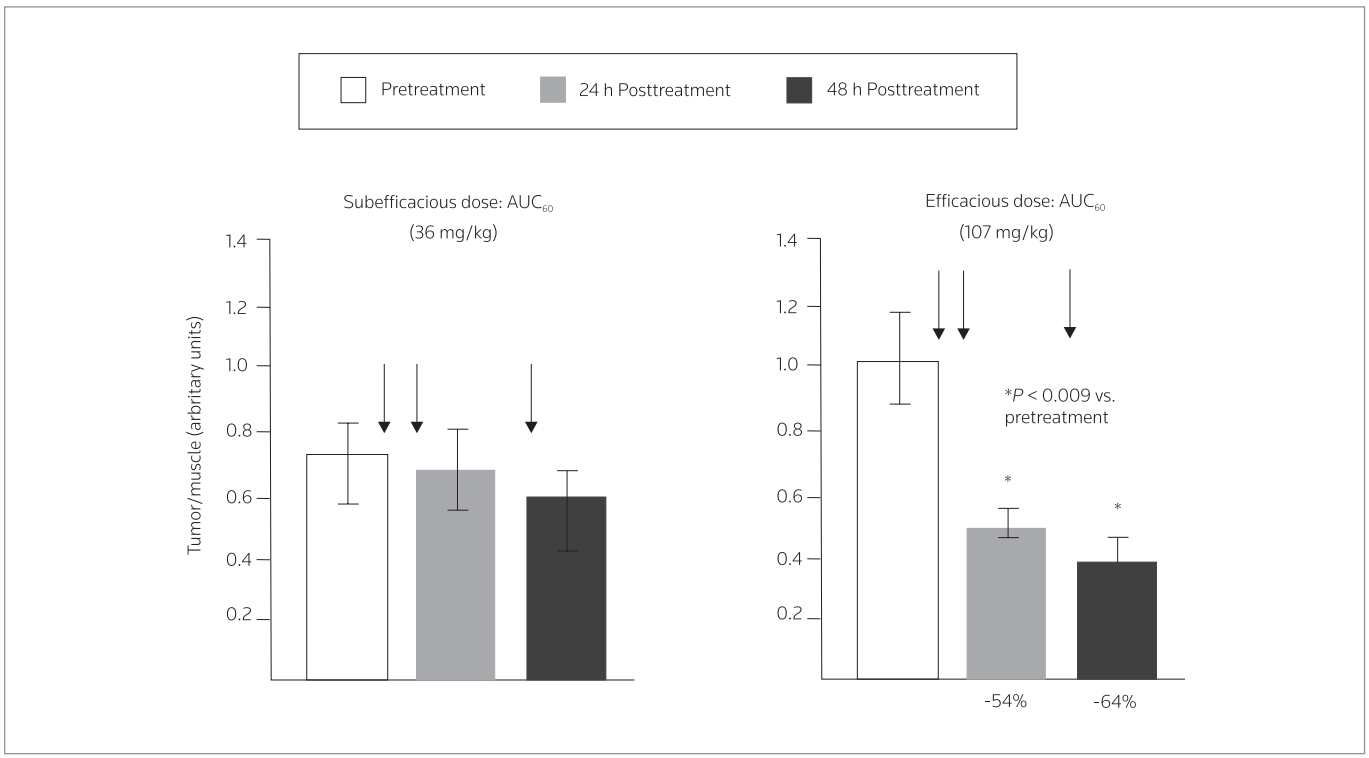


Figure 5. AUC over 60 s (AUC_{60}) values for tumor/muscle ratio by dynamic contrast enhanced-magnetic resonance imaging analysis (56). There was a significant reduction in AUC_{60} of 54% and 64% at 24 h and 48 h, respectively, at the 107 mg/kg dose (n = 9). In contrast, there was no significant difference at the subefficacious dose of 36 mg/kg (n = 10). Arrows indicate brivanib alaninate dosing time. Adapted with permission from Bhide, R.S., Lombardo, L.J., Hunt, J.T. et al. Mol Cancer Ther 2010, In press (Figure 6; Panel B).

dose) on a continuous daily schedule was run until dose-limiting toxicities were observed (67). This study revealed that the 800 mg continuous schedule resulted in the greatest change in transfer constant (Ktrans), AUC_{60} and serum markers at days 8 and 26. The 800 mg intermittent schedule resulted in some recovery day 2 to day 8 and a smaller change at day 26 than the 800 mg continuous schedule; within-subject coefficients of variation for Ktrans and AUC_{60} were 26% and 22%, respectively. The probability of DCE-MRI change increased with exposure (AUC_{tau}) to brivanib alaninate. Overall, brivanib alaninate demonstrated dose-proportional PK when dosed at 800 mg on a continuous schedule. PD changes were greatest on the 800 mg continuous schedule. Some recovery in DCE-MRI effect after a 2-day dosing break indicated that continuous dosing is preferable for biological activity (68).

In a continuation of this phase I study, the optimal dose or dose ranges for phase II studies from measurement of the effects of brivanib alaninate on the DCE-MRI parameters AUC_{60} and Ktrans on either continuous daily or intermittent schedules (320 mg, 800 mg continuous, 800 mg intermittent (5 days on, 2 days off) and 400 mg twice daily) were investigated in patients with CRC, renal cell carcinoma and HCC. The effect of brivanib alaninate on tumor perfusion as measured by DCE-MRI was related to dose and schedule of administration. The largest reduction in contrast agent occurred in patients receiving brivanib alaninate 800 mg daily and 400 mg twice daily. This reduction was sustained at trough on days 2 and 8; however, some recovery was observed with intermittent dosing, suggesting that continuous daily dosing is the optimum regimen. In addition to DCE-MRI, dose responses were also observed with brivanib alaninate at doses of 320 – 800 mg in the antiangiogenic PD markers collagen IV and soluble VEGFR-2 (68).

The PK, metabolism and elimination of brivanib alaninate in patients with advanced or metastatic solid tumors were analyzed via the administration of a single oral dose of 800 mg [^{14}C] brivanib alaninate containing 100 μCi of total radioactivity (0.125 μCi/mg) (69). Four patients were followed for a 10-day period following the single dose. After oral administration, brivanib alaninate was completely converted to the parent drug, brivanib, which was the major active circulating component in plasma. Brivanib absorption was > 86% when administered as brivanib alaninate, and multiple enzymes, including cytochrome P450 (CYP)1A2, CYP3A4 and multiple sulfotransferases, were found to be involved in the metabolism of brivanib, which was extensively metabolized in humans. Elimination was primarily via feces (unchanged brivanib represented < 7.5% of dose). In addition, renal excretion of brivanib was minimal, leading to the suggestion that renal impairment is unlikely to affect brivanib exposures.

The effects of subject demographics, particularly Asian race, on the brivanib alaninate PK profile were assessed in 125 patients (47 Asian; 78 non-Asian) with advanced and metastatic solid tumors (including HCC) enrolled in two clinical studies. In this population, the effect of body weight on clearance of brivanib was significant. However, there was substantial intersubject variability in relation to clearance (50%) after accounting for body weight, suggesting that a fixed-dose of brivanib alaninate is clinically viable. Race (Asian versus non-Asian), gender and age had no significant effect on clearance of brivanib, after accounting for body weight. In addition, there was substantial overlap in simulated steady-state brivanib AUC between Asian and non-Asian patients for the 800 mg daily dose of brivanib alaninate (70).

The effects of hepatic function on the PK profile of brivanib alaninate have also been evaluated in a recent phase I trial designed to compare patients with HCC and a defined level of hepatic impairment (Child-Pugh A, B or C) with patients with non-HCC solid malignancies and normal hepatic function. At present, data are available only for the cohort of HCC patients with mild hepatic function (Child-Pugh A). Overall, PK parameters (maximum plasma concentration, AUC extrapolated to infinity, AUC from time zero to 24 hours, time to maximum plasma concentration and terminal elimination half life) were similar for HCC patients with mild hepatic impairment and patients with non-HCC solid malignancies with normal hepatic function. The results, therefore, suggest that patients with HCC and Child-Pugh A can be treated with the same 800 mg daily dose as patients without hepatic impairment. Enrollment continues for the cohorts of HCC patients with Child-Pugh B and C (71).

DRUG INTERACTIONS

When evaluated in a CYP panel to assess the potential for drug–drug interactions, brivanib alaninate was found to be a potent inhibitor of these metabolizing enzymes in comparison with the parent brivanib (3). However, brivanib alaninate produces high exposure levels of parent brivanib, with the AUC increasing proportionally with dose in various species, demonstrating efficient in vivo conversion to brivanib (65). Moreover, no circulating brivanib alaninate was observed for brivanib alaninate in a mouse oral exposure assay (3) nor following oral dosing of brivanib alaninate in mice, rats and monkeys (65). Overall, brivanib alaninate has a favorable CYP inhibition profile. The risk of drug–drug interaction is further mitigated by the demonstration that brivanib alaninate is rapidly converted to brivanib in human-derived liver and intestinal microsomes and S9 (complete disappearance within 20 min of incubation) (65) and by the demonstration that brivanib alaninate was completely converted to brivanib in patients with advanced or metastatic solid tumors who received a single 800 mg oral dose (69).

BIOMARKER ANALYSES

At present, there is a lack of reliable and clinically predictive biomarkers for HCC (72). Serum alpha fetoprotein levels are often used as a tumor marker to detect and monitor HCC (73) and recent reports indicate that an alpha fetoprotein response may be a useful surrogate marker for clinical outcomes in HCC patients (74,75). Other biomarkers under investigation in HCC include VEGFR-2, VEGF, CD34, collagen IV and FGF2. The rationale for the investigation of collagen IV as a biomarker relates to evidence that plasma collagen IV levels increase during angiogenesis and the fact that they can be easily measured by enzyme-linked immunosorbent assay (76). A role for collagen IV as a biomarker is further supported by data from studies in xenograft models, which showed that as tumor growth slowed during brivanib treatment, collagen IV levels decreased (77). The rationale for investigating FGF2 as a biomarker stems primarily from the observation that a high preoperative serum FGF2 level appeared to be predictive of invasive tumor and early postoperative recurrence (41).

An exploratory goal of the phase I, open-label, dose-escalation study in patients with advanced or metastatic solid tumors was to perform PD and predictive analysis of selected biomarkers (78). For the PD analyses, the evaluated biomarkers were soluble VEGFR-2 and collagen IV; FGF2 was assessed for its potential to predict responsiveness to brivanib. In this study, brivanib alaninate treatment was associated with a decrease in soluble VEGFR-2; reductions with the 320 mg daily dose were small (median reduction < 15%), but higher with the 800 mg (800 mg daily or 400 twice daily) doses (maximum median reduction ≥ 20%). Brivanib alaninate treatment was also associated with reductions in collagen IV levels, again with small reductions observed for the 320 mg daily dose (median reduction < 10%), but higher reductions with the 800 mg (800 mg daily or 400 twice daily) doses (maximum median reduction ≥ 27%). The analyses also suggested that tissue FGF2 expression is predictive of responsiveness to brivanib alaninate treatment since patients with FGF-expressing tumors had more disease control and less tumor growth and showed a trend toward longer progression-free survival compared with patients with tumors not expressing FGF2. These data thus support earlier preclinical data in patient-derived HCC xenografts (59).

The potential for predicting response to treatment using biomarkers was also evaluated in patients with CRC enrolled in a phase I, open-label, dose-escalation/dose-expansion study of brivanib alaninate in combination with cetuximab for patients with advanced gastroduodenal malignancies who had failed prior therapy (79). Specifically, the analysis aimed to investigate an association of K-*ras* mutation status with clinical outcomes including progression-free survival and tumor response (80). A total of 48 patients with CRC were evaluable for K-*ras*; 44 patients were receiving an 800 mg dose of brivanib alaninate and four were receiving a 600 mg dose. K-*ras* mutations were detected in the tumors of 23 (48%) of these patients. Overall, patients with K-*ras* wild-type tumors had significantly longer progression-free survival, were more likely to show prolonged disease control and tended to show greater reductions in tumor volume compared with patients with K-*ras* mutant tumors. Nevertheless, brivanib alaninate appeared to have activity in patients with K-*ras* mutant tumors, as significant reductions in tumor volume were observed and disease stabilization was reported in 73% of these patients. Thus, it appears that brivanib is active in metastatic CRC independent of K-*ras* status.

CLINICAL STUDIES

Early on in the development of antiangiogenic therapy, minimal toxicity was predicted based on the concept that only newly forming blood vessels would be affected, such as those found in a growing tumor. However, recent clinical experience with the three approved antiangiogenic agents (bevacizumab, sorafenib and sunitinib) that inhibit the VEGFR signaling pathway in addition to other tyrosine kinases has demonstrated overlapping as well as distinct side effects. The most frequent side effects include hypertension, which can be managed by appropriate treatment, and possible increased risks of bleeding and thromboembolic events in patients with pre-existing cardiovascular disease (81). For both sorafenib and sunitinib, hand-foot syndrome is particularly prevalent and severe and needs to be aggressively managed. Other side effects include excessive bleeding in surgical patients and impaired wound healing but, as a class, relative to classical cytotoxic therapies, these agents are considered to be associated in general with manageable side effects and lower incidence of severe toxicities.

Phase I clinical studies

In a phase I, open-label, dose-escalation study of brivanib alaninate in patients with advanced or metastatic solid tumors, grade 3/4 adverse events at 320 mg, 800 mg intermittent and 800 mg continuous schedules, respectively, included fatigue (21%, 0%, 22%), elevated transaminase (8%, 11%, 17%), diarrhea (0%, 11%, 11%), hypertension (0%, 0%, 11%) and decrease in platelet count (8%, 0%, 11%) (67). The dose-limiting toxicities observed in two of three patients at a dose of 1000 mg were fatigue and dizziness, and altered mental status and dehydration in one patient each.

In the extension of this phase I study, continuous daily or intermittent schedules of brivanib alaninate (320 mg, 800 mg continuous, 800 mg intermittent (5 days on, 2 days off) and 400 mg twice daily) were investigated in patients with CRC, renal cell carcinoma and HCC (68). Of the 68 patients included in the study, 14, 19, 12 and 13 received brivanib alaninate at doses of 320 mg, 800 mg continuous, 800 mg intermittent and 400 mg twice daily, respectively. There was evidence of antitumor activity with two confirmed partial responses in patients receiving brivanib alaninate at ≥ 600 mg and six patients with stable disease at ≥ 6 months. The median time on study for all patients was 84 days. Furthermore, favorable changes in tumor size were observed in patients with a variety of tumors following brivanib alaninate treatment. Most adverse events were mild with the most frequently reported being gastrointestinal (nausea, vomiting and diarrhea), fatigue, hypertension and reversible transaminitis. The most frequently reported grade 3/4 toxicities were fatigue and reversible transaminitis. No grade 5 adverse events were reported, and no QT_c prolongation was observed across the 180- to 1000-mg brivanib dose range.

In a second phase I, open-label, dose-escalation study, brivanib alaninate was given in combination with cetuximab to patients with advanced gastroduodenal malignancies who had failed prior therapy (79). Brivanib alaninate was given orally on day 1 and daily from day 8, starting at a dose of 320 mg, and cetuximab was given iv on day 8 (400 mg/m^2), then weekly (250 mg/m^2). Dose escalation of brivanib alaninate continued to 800 mg daily when an expansion cohort for patients with CRC was opened for additional safety and efficacy. A total of 62 patients (59 CRC, 2 esophageal carcinoma and 1 HCC) have been treated with brivanib alaninate 320, 600 or 800 mg daily in combination with cetuximab. The best responses in the subset of 34 evaluable patients with CRC receiving brivanib alaninate 800 mg plus cetuximab were 6 partial responses (29%) and 11 stable disease (52%) in EGFR inhibitor-naïve patients; 5 partial responses (50%) and 5 stable disease (50%) in VEGF inhibitor-naïve patients; and 5 partial responses (56%) and 4 stable disease (44%) in EGFR and VEGF inhibitor-naïve patients. The best antitumor activity was seen in the subset of patients who had not received either EGFR or VEGF inhibitor therapy. In a single dose-limiting toxicity, bilateral pulmonary emboli occurred on day 28 in a patient receiving brivanib alaninate at the 320 mg dose level. There was no dose-limiting toxicity at the 800 mg dose. The combination of brivanib alaninate plus

cetuximab was tolerable; adverse events included fatigue, diarrhea, dermatitis acneiform, anorexia, pyrexia, vomiting and headache.

Phase II clinical studies

A phase II, open-label study conducted to evaluate the effects of brivanib alaninate 800 mg as either first-line or second-line therapy in patients with unresectable locally advanced or metastatic HCC has recently been completed. The study included two patient cohorts: cohort A included patients who had received no prior systemic therapy for HCC and cohort B included patients who progressed following receipt of one prior antiangiogenic agent. Overall, 55 patients were included in cohort A, of which 64% were Asian; 46 patients were included in cohort B, 43 (94%) of whom had failed sorafenib therapy and 3 (6%) of whom had failed therapy with thalidomide. Interim analyses from this study showed that 6 of 47 evaluable patients in cohort A had a partial response (2 unconfirmed), with a disease control rate of 60%. First-line treatment with brivanib alaninate was also associated with a median time to progression (TTP) of 2.8 months and a median overall survival of 10 months (82). In cohort B, 1 of 37 evaluable patients had a partial response and 16 had stable disease following second-line treatment with brivanib alaninate; the disease control rate was 46%. Second-line treatment with brivanib alaninate was also associated with a median investigator-assessed TTP of 2.7 months and a median overall survival of 9.8 months. To date, most safety data have been reported for the entire study population combining the two cohorts (N = 101). The most frequently reported grade 3/4 adverse events across the two cohorts were fatigue (10.9%), hypertension (9.9%) and diarrhea (5%). Serious adverse events included grade 3 encephalopathy in five patients; grade 3/4 abdominal pain in four patients; grade 3 diarrhea in three patients; grade 3 vomiting, asthenia and gait disturbance in two patients each; and grade 4 asthenia in one patient. Three patients (5.4%; grade 1/2 in two patients; grade 3 in one patient) in cohort A and five patients (10.9%; grade 1/2 in all five patients) in cohort B experienced hand-foot syndrome. Fifty-six patients died during the course of the study, but no deaths were considered treatment related.

Results of a subanalysis of data from cohort A, performed to evaluate the effects of Asian versus non-Asian race and geography, have also been reported (83). Following first-line treatment with brivanib alaninate, median overall survival was 10 months in Asian patients compared with 5.7 months in non-Asian patients. The subanalysis also showed that median TTP (2.7 months for Asian patients compared with 2.8 months for non-Asian patients) and the percentage of patients with a reduction in tumor volume (45% of Asian patients and 38% of non-Asian patients) was similar for both patient populations. Safety outcomes from this subanalysis indicated that overall tolerability following first-line treatment with brivanib alaninate was similar or slightly better in the Asian population compared with that of the non-Asian population.

Planned and ongoing clinical studies

The positive outcomes from the phase II study of brivanib alaninate for patients with HCC (82) have led to the planning and initiation of three phase III studies in the Brivanib Studies in HCC Patients at Risk (BRISK) clinical program.

- A phase III, randomized, double-blind, multicenter study of brivanib versus sorafenib as first-line treatment in patients with advanced HCC (BRISK-FL; ClinicalTrials.gov Identifier: NCT00858871). The objective of this study is to directly compare the efficacy and safety of brivanib alaninate as a first-line treatment in patients with HCC with sorafenib, which is currently the only antiangiogenic agent approved for the treatment of HCC. While both sorafenib and brivanib target VEGFR, the potential for additional clinical benefits of brivanib provided through FGFR inhibition will be investigated. The study also aims to evaluate the respective impact of the two treatments on health-related quality of life.
- A phase III, randomized, double-blind, multicenter study of brivanib plus best supportive care versus placebo plus best supportive care in subjects with advanced HCC who have failed therapy with or are intolerant to sorafenib (BRISK-PS; ClinicalTrials.gov Identifier: NCT00825955). The purpose of this study is to determine whether brivanib alaninate is an effective treatment for liver cancer in patients who have failed therapy with sorafenib or could not take the drug.
- A phase III, randomized, double-blind, multicenter study of brivanib versus placebo as adjuvant therapy to transarterial chemoembolization (TACE) in patients with unresectable HCC (BRISK-TA, ClinicalTrials.gov Identifier: NCT00908752). The primary aim of this study is to assess the benefits of using brivanib alaninate systemic therapy in combination with localized TACE therapy compared with TACE plus placebo.

In addition to the BRISK clinical trial program, several other studies are ongoing that will evaluate the efficacy, safety, PK/PD and/or potential for drug-drug interactions associated with brivanib alaninate treatment for a variety of cancers.

- A phase I, ascending, multiple-dose study of brivanib alaninate in combination with chemotherapeutic agents in patients with advanced cancers (ClinicalTrials.gov identifier: NCT00798252). The aims of this study are to determine safety and maximum tolerated dose of brivanib alaninate when administered in combination with capecitabine, doxorubicin and ixabepilone chemotherapy to subjects with advanced or metastatic solid tumors.
- A phase III randomized study of brivanib alaninate in combination with cetuximab versus placebo in combination with cetuximab in patients previously treated with combination chemotherapy for metastatic CRC (ClinicalTrials.gov identifier: NCT00640471). The aims of this study are to: (a) compare the overall survival of patients with previously treated metastatic CRC treated with brivanib alaninate in combination with cetuximab versus placebo in combination with cetuximab; (b) explore an association between FGF2, K-*ras* mutations, amphiregulin and epiregulin as determined from paraffin-embedded tumor specimens and the potential for clinical benefit from the addition of brivanib alaninate or placebo to cetuximab in terms of overall survival, progression-free survival and objective response rate compared with cetuximab alone; (c) explore an association with changes of collagen IV in the blood and the potential for clinical benefit from the addition of brivanib alaninate to cetuximab in terms of overall survival, progression-free survival and objective response rate compared with cetuximab alone.

Finally, a global epidemiology study, the BRIDGE HCC study, which has been designed to help develop a global understanding of HCC treatment and associated clinical outcomes, is underway (84). This multiregional longitudinal study will enroll more than 15,000 newly diagnosed HCC patients over a 7-year period (2005 – 2011) with patients followed from date of initial HCC diagnosis to death or to December 31, 2011, whichever comes first. The overall aims of the BRIDGE HCC study are to: (a) benchmark survival and other clinical parameters by treatment assignment; (b) determine unmet medical need with current therapy; and (c) contribute to global understanding of HCC, including assessment of treatment by geography and etiology, and associated clinical outcomes.

SUMMARY

In summary, brivanib alaninate demonstrates good water solubility, efficient in vivo conversion to brivanib and a low likelihood for drug-drug interaction. In preclinical studies, brivanib inhibited FGFR and VEGFR-2 signaling, both important targets in angiogenesis and tumorigenesis in several tumor types, including HCC and CRC. Brivanib has potent antitumor activity when dosed orally and is safely dosed in combination with cytotoxic drugs resulting in enhanced antitumor activity. Brivanib alaninate-induced growth inhibition was associated with inhibition of angiogenesis and inhibition of cell proliferation. In early clinical studies, brivanib was generally well tolerated, with manageable side effects, and showed evidence of antitumor activity in patients with advanced or metastatic solid tumors. Due to its tolerable and manageable safety profile and promising antitumor activity in a phase II trial, brivanib is undergoing further phase III investigations in several other studies in HCC and a variety of other tumor types such as CRC, both as a single agent and in combination regimens with standard chemotherapies.

SOURCE

Bristol-Myers Squibb Co. (US).

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DISCLOSURE

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