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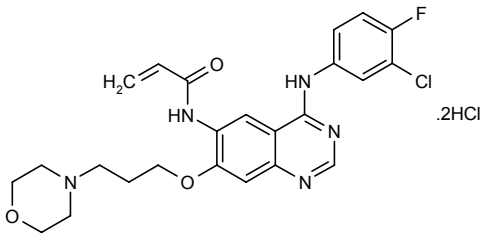
Canertinib Dihydrochloride

Prop INNM; USAN

Oncolytic Drug
HER (ErbB) Inhibitor

CI-1033
PD-183805 (as free base)

N-[4-(3-Chloro-4-fluorophenylamino)-7-[3-(4-morpholinyl)propoxy]quinazolin-6-yl]-2-propenamide dihydrochloride



$C_{24}H_{25}ClFN_5O_3.2HCl$
Mol wt: 558.8596
CAS: 289499-45-2
CAS: 267243-28-7 (as free base)
EN: 274534

Abstract

Dysfunction of intracellular signaling pathways has been implicated in the development and progression of cancer. Canertinib is an irreversible small-molecule tyrosine kinase inhibitor that blocks signal transduction through all four members of the ErbB (or epidermal growth factor [EGF]) family and is in development for the treatment of advanced nonhematological cancers. Canertinib inhibited ligand-dependent tyrosine phosphorylation in cell lines expressing ErbB receptor kinases. In mice bearing advanced human epidermoid A-431 xenografts, suppression of EGF receptor tyrosine phosphorylation correlated with antitumor activity. *In vitro* activity of canertinib was demonstrated against a range of tumor types, including glioblastoma, mesothelioma, non-small cell lung cancer, mammary tumors and orthotopic bladder tumors. A radiosensitizing effect was also demonstrated in a number of different carcinoma cell lines, as well as synergistic activity in combination with cisplatin, topotecan or gemcitabine. In clinical studies, canertinib has demonstrated an acceptable tolerability profile, with the most frequently reported toxicities being gastrointestinal symptoms and skin reactions. Although few objective responses were observed in these preliminary studies, a number of patients with different tumor types achieved stable disease for varying lengths of time and the clinical efficacy of canertinib is being evaluated in ongoing phase II studies.

Synthesis

Canertinib can be prepared by two related ways:

1) Condensation of 4-chloro-7-fluoro-6-nitroquinazoline (I) with 3-chloro-4-fluoroaniline (II) affords the 4-anilinoquinazoline (III), which by displacement of its activated fluorine with the potassium alkoxide of 3-(4-morpholinyl)-propanol (IV) gives the morpholinopropyl ether (V). Subsequent reduction of the nitro group of (V) using either iron dust and acetic acid or catalytic hydrogenation over Raney-Ni furnishes the aminoquinazoline (VI). Finally, compound (VI) is condensed with acrylic acid (VII) via activation as the mixed anhydride with isobutyl chloroformate or using EDC as the coupling reagent (1, 2). Scheme 1.

2) The reductocondensation of 3-chloro-4-fluoroaniline (II) with 3,4-dimethoxybenzaldehyde (VIII) by means of sodium triacetoxyborohydride in AcOH or isopropanol gives *N*-(3-chloro-4-fluorophenyl)-*N*-(3,4-dimethoxybenzyl)amine (IX), which is condensed with 4-chloro-7-fluoro-6-nitroquinazoline (I) – obtained by reaction of 7-fluoro-6-nitroquinazolin-4(3*H*)-one (X) with refluxing $SOCl_2$ – in refluxing isopropanol to yield the tertiary amine (XI). Condensation of amine (XI) with 3-(4-morpholinyl)-propanol (IV) by means of *t*-BuOK or *t*-BuONa in refluxing isopropanol or hot acetonitrile affords the aryl ether (XII). Reduction of the nitro group of (XII) by means of H_2 over Raney-Ni in THF provides the corresponding amino derivative (XIII), which is acylated with acryloyl chloride (VII) and TEA in methyl acetate to give the acrylamide derivative (XVI). Finally, the tertiary amino group of (XVI) is deprotected by means of refluxing TFA (3). Scheme 2.

Introduction

Intracellular signaling pathways control many key cellular functions, including proliferation, survival and apoptosis. Dysfunction of these pathways is implicated in many diseases, including cancer (4). The transmembrane receptor tyrosine kinases (RTKs) are a superfamily of receptors that mediate the internal transmission of extra-

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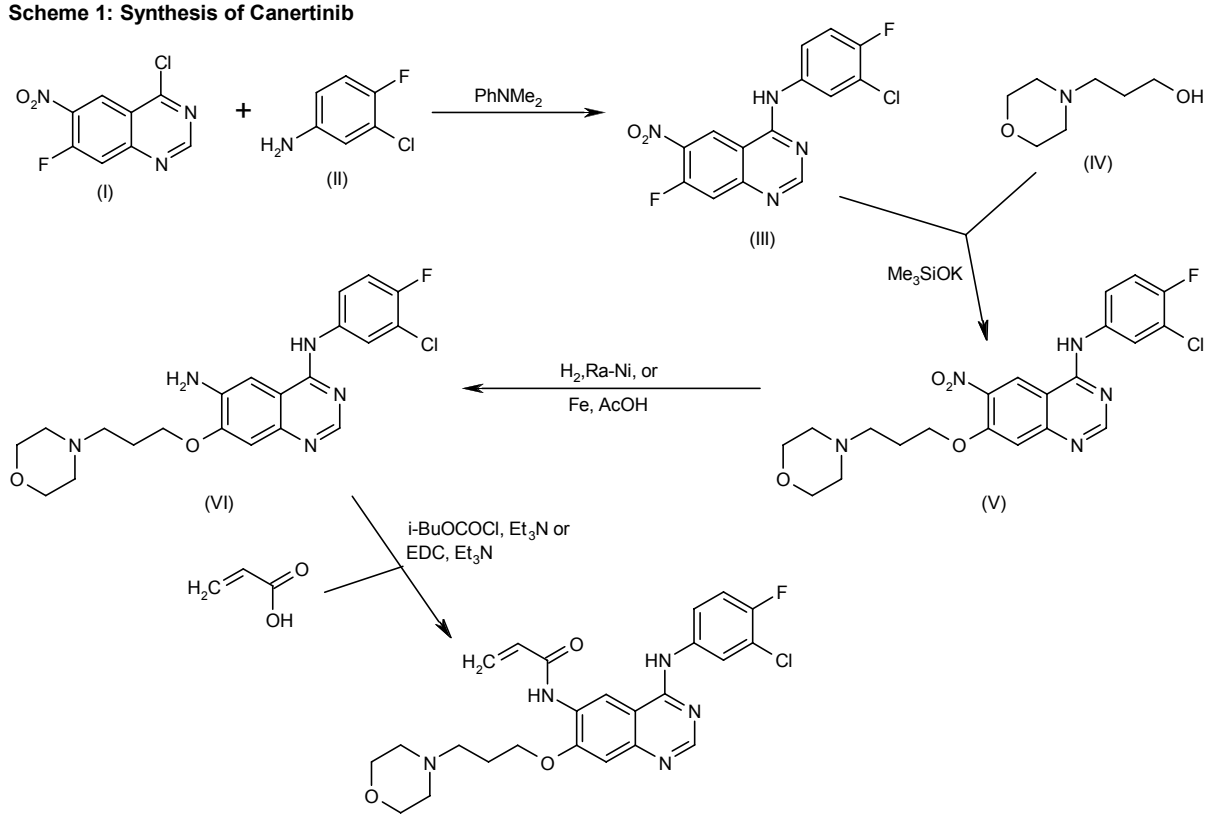
Scheme 1: Synthesis of Canertinib



cellular signals. Subclass I of this family consists of the ErbB, or epidermal growth factor (EGF), receptors, of which there are four members: EGFR/ErbB-1 (or HER1), ErbB-2 (or HER2/neu), ErbB-3 (or HER3) and ErbB-4 (or HER4). The receptors are expressed in various tissues of epithelial, mesenchymal and neuronal origin. Alterations in ErbB receptors have been identified in over 60% of human tumors and are often associated with more aggressive disease and poorer prognosis. Tyrosine kinase inhibitors that block ErbB receptors are therefore potential therapeutics for the treatment of cancer (5-10).

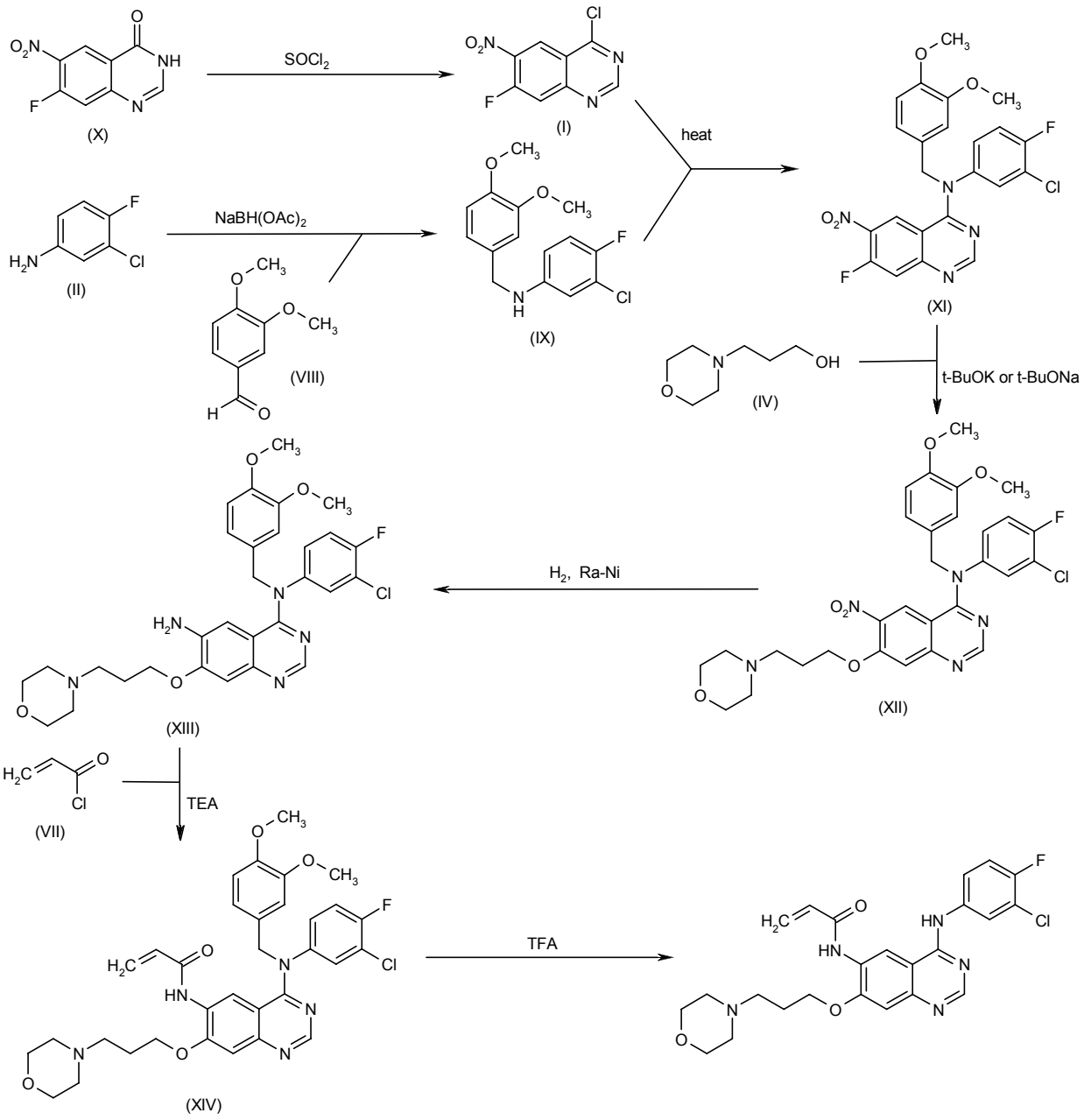
One group of small-molecule tyrosine kinase inhibitors that compete with ATP in the tyrosine kinase domain are the 4-anilinoquinazoline class of inhibitors, of which canertinib (CI-1033, PD-183805) is an irreversible inhibitor. It effectively blocks signal transduction through all four members of the ErbB family, unlike the majority of other ErbB receptor-targeted therapies that are approved or in clinical development (2, 11-13). It is currently in phase II development for the treatment of advanced non-hematological malignancies.

Pharmacological Actions

Canertinib is a potent and selective inhibitor of all three catalytically active members of the ErbB family –ErbB-1, ErbB-2 and ErbB-4– with IC_{50} values of 0.8, 19 and 7 nmol/l, respectively, in studies using purified enzymes. ErbB-3 does not have an active tyrosine kinase, but inhibition of signaling through this receptor was also effectively blocked due to inactivation of all the catalytically active heterodimerization partners. Canertinib inhibited ligand-dependent tyrosine phosphorylation in cell lines expressing either individual or multiple ErbB receptor kinases. Using the ligand EGF, which specifically activates ErbB-1, or heregulin, which activates ErbB-3 and ErbB-4, canertinib inhibited receptor activation with IC_{50} values in the range 5-31 nmol/l. Tyrosine phosphorylation was inhibited in both the MDA-MB-453 human breast carcinoma cell line, which expresses ErbB-2, ErbB-3 and ErbB-4, and in A-431 human epidermoid carcinoma cells overexpressing ErbB-1, with an IC_{50} of 5 nmol/l. In contrast, canertinib demonstrated little activity against other RTKs, including fibroblast growth factor receptor-1 (FGFR-1) and platelet-derived growth factor receptor (PDGFR) (14).

The activity of canertinib has also been demonstrated against the highly tumorigenic variant of ErbB-1 EGFRvIII, which is expressed in at least 60% of invasive breast carcinomas but not in normal tissues. Canertinib inhibited EGFRvIII-mediated 32D cell proliferation at concentrations of 0.01-1 nM, demonstrating 100-fold greater potency compared with other EGF family receptor-trans-

Scheme 2: Synthesis of Canertinib



fected 32D cells. Phosphorylation in the MCF7 human breast carcinoma cell line transfected with EGFRvIII was potently inhibited by canertinib, in addition to EGFRvIII-mediated cell proliferation and phosphorylation in NIH cells. Canertinib significantly inhibited NIH/EGFRvIII tumorigenicity in athymic nude mice (14, 15).

The correlation of the duration and extent of EGF receptor phosphorylation with antitumor activity was evaluated in mice bearing advanced A-431 xenografts. Tumor growth was completely suppressed in animals treated with canertinib 1, 2, 3 or 5 times weekly for 3 weeks over a dose range of 25-200 mg/kg/week. The maximum tumor growth delay was approximately 50 days. EGF receptor tyrosine phosphorylation was suppressed by more than 80% for over 72 h following single oral doses over the range 2.5-40 mg/kg, and this was correlated with the antitumor activity of canertinib (16).

Canertinib inhibited the secretion of vascular endothelial growth factor (VEGF) stimulated by transforming growth factor (TGF)-α, EGF and heregulin in A-431 cells and in human non-small cell lung cancer (NSCLC) NCI-H125 cells. In mice bearing established xenografts of these

tumor types, as well as glioblastoma SF-767 and mammary carcinoma MDA-MB-468 tumors, canertinib demonstrated significant antitumor activity, which was correlated with the reduction in plasma VEGF and IL-8 levels (17, 18). Furthermore, canertinib inhibited TGF-α-induced cellular proliferation and cell migration in a concentration-dependent manner in three malignant mesothelioma cell lines (19, 20). It also induced significant regression of orthotopic bladder tumors in athymic nude mice. In these studies, canertinib (10 mg/kg) was administered twice weekly for 4 weeks, resulting in inhibition of IL-8 and VEGF production, tumor cell proliferation and angiogenesis (21, 22). Moreover, canertinib significantly inhibited the growth of osteosarcoma cells at a concentration of 1 μM and induced apoptosis at concentrations of 1-5 μM (23, 24).

Canertinib was evaluated for its effect on the formation of tumor colony-forming units (CFU) using fresh human tumor specimens. An inhibitory response, defined as no more than 50% survival of colonies, was observed in 15%, 32% and 53%, respectively, of the specimens treated with 10, 100 and 1000 nM canertinib for 14 days. The responses at 100 and 1000 nM were significant compared to in untreated control plates ($p < 0.01$), and responses were observed in breast (67%), NSCLC (60%) and ovarian cancer specimens (25).

The ability of canertinib to induce apoptosis and growth arrest was examined in four human breast cancer cell lines overexpressing EGFR (SUM-149 and SUM-229) or expressing different levels of ErbB-2 (SUM-190 and SUM-225). The results showed that cell lines with greater overexpression of activated ErbB-2 responded to lower concentrations and shorter exposure periods of canertinib. SUM-225 cells, with higher levels of ErbB-2 than the SUM-190 cell line, achieved growth stasis with daily exposure to canertinib 0.1 μM, and apoptosis was seen after only 24-h exposure. At this concentration, phosphorylated ErbB-2 receptor remained detectable. In contrast, SUM-190 cells underwent apoptosis only after 72-h treatment with 1 μM canertinib. Cell lines overexpressing EGFR exhibited growth inhibition but not apoptosis after treatment with canertinib (26-28).

The radiosensitizing effect of canertinib was demonstrated in a number of different cancer cell lines. In a panel of breast cancer cell lines overexpressing ErbB, exposure to canertinib 1 μM and radiation (6 Gy) resulted in a 23-fold decrease in clonogenic survival compared with radiation alone. During multifraction (3 x 5 Gy) exposure, clonogenic survival was reduced 65-fold (29-31). In human bile duct carcinoma cells, treatment with canertinib alone significantly inhibited proliferation in a concentration-dependent manner but did not induce apoptosis. However, the sequential treatment of cells with canertinib followed by radiation induced apoptosis in 62.6% of cells (32, 33). A radiosensitizing effect for canertinib was also observed in human glioblastoma multiforme cell lines (34). The compound itself induced marked apoptosis in these cell lines (34, 35).

The radiosensitizing effect of canertinib was further investigated *in vivo*. In nude mice bearing human colon carcinoma LoVo and Caco-2 tumor xenografts, treatment with canertinib at a dose of 20 mg/kg/day and radiation (2 Gy daily) for 5 days each week for 3 weeks resulted in significant and prolonged suppression of tumor growth compared with either treatment alone. The increased efficacy of the combined treatment was associated with moderate but acceptable toxicity, *i.e.*, reversible weight loss. In other studies in tumor-bearing mice, the major toxicities were diarrhea, hair loss and skin sores. All treatment-related changes were reversible upon cessation of treatment (36, 37).

In addition to the radiosensitizing effects of canertinib, synergistic effects have been reported with chemotherapeutic agents, including with cisplatin in the A-431 human squamous cell line (38). Canertinib was synergistic when combined with topotecan or SN-38, the active metabolite of irinotecan, in glioblastoma or colorectal carcinoma cells (39, 40) and synergistic effects were observed with gemcitabine in breast cancer MDA-MB-453 cells (41, 42). In the studies combining canertinib with either radiation therapy or chemotherapy, the degree of synergy was affected by the order and timing of drug exposure, with maximum synergistic effects generally observed when canertinib was administered either immediately before or concomitantly with radiation or the cytotoxin.

Toxicity

In studies in rats, canertinib was administered orally on a daily or weekly basis for 7-28 days. The treatment was associated with diarrhea, hair loss and skin sores, as well as histopathological changes of gastrointestinal epithelial atrophy, corneal thinning, male mammary gland atrophy and ulcerative dermatitis. The severity of these effects increased proportionally with drug concentrations, dose frequency and duration of treatment, although all changes were reversible after treatment was discontinued (43).

Pharmacokinetics

A population pharmacokinetic analysis was conducted on 192 profiles from 72 patients with advanced solid tumors who received oral canertinib either once daily for 7 days every 21 days or once weekly for 3 weeks every 4 weeks. Doses ranging from 50 to 1000 mg were administered. C_{max} was proportional to dose and was reached 2-4 h after dosing. Mean plasma clearance was 266 l/h, with an apparent elimination half-life of 4 h. Canertinib did not accumulate with repeated dosing and the pharmacokinetics did not differ significantly for the daily and weekly regimens. The results supported dosing without the need for adjustment for body weight or surface area in adult patients (44).

Canertinib was administered orally to 68 patients with advanced solid tumors in escalating doses from 2 to 220 mg/day in 4 different schedules of on/off periods of treat-

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

Indication	Design	Treatments	n	Conclusions	Ref.
Cancer	Open	Canertinib, 250 mg p.o. o.d. 7x/14 d (n=15) Canertinib, 300 mg p.o. o.d. 7x/14 d (n=9)	24	Canertinib 250 mg p.o. was well tolerated and not associated with clinically relevant hypersensitivity or hematological toxicity in patients with cancer	48
Cancer	Open	Canertinib, 125 mg p.o. o.d. (n=6) Canertinib, 150 mg p.o. o.d. (n=7) Canertinib, 175 mg p.o. o.d. (n=6) Canertinib, 150 mg p.o. o.d. 21x/28 d [with or without food] (n=14)	33	The maximum tolerated dose for continuous oral administration of canertinib in patients with advanced solid tumors was established at 150 mg/d. No clinical responses were obtained, but 10 patients had disease stabilization for at least 8 weeks	49
Cancer	Open	Canertinib, 50-750 mg p.o. o.d. x 7 d 1x/21 d	53	Canertinib was generally well tolerated and induced responses or disease stabilization for more than 12 weeks in 14 patients with advanced solid tumors. Canertinib modulated the expression of ErbB-1, ErbB-2 and other tumor biomarkers in biopsy samples	50-52
Cancer	Open Multicenter	Canertinib, 300 mg p.o. o.d. x 14 d 1x/21 d [until progression or intolerable toxicity] (n=9) Canertinib, 350 mg p.o. o.d. x 14 d 1x/21 d [until progression or intolerable toxicity] (n=3) Canertinib, 450 mg p.o. o.d. x 14 d 1x/21 d [until progression or intolerable toxicity] (n=6) Canertinib, 500 mg p.o. o.d. x 14 d 1x/21 d [until progression or intolerable toxicity] (n=8) Canertinib, 560 mg p.o. o.d. x 14 d 1x/21 d [until progression or intolerable toxicity] (n=6)	32	Oral canertinib induced stable disease in 6 of 32 patients with advanced refractory solid tumors. The maximum tolerated dose was established at 450 mg once daily for 14 days in cycles of 21 days	53, 54
Cancer	Open	Canertinib, 10 mg i.v. over 30 min 3x/wk x 4 wks 1x/6 wks (n=5) Canertinib, 20 mg i.v. over 30 min 3x/wk x 4 wks 1x/6 wks (n=3) Canertinib, 30 mg i.v. over 30 min 3x/wk x 4 wks 1x/6 wks (n=6) Canertinib, 45 mg i.v. over 30 min 3x/wk x 4 wks 1x/6 wks (n=5) Canertinib, 67.5 mg i.v. over 30 min 3x/wk x 4 wks 1x/6 wks (n=3) Canertinib, 100 mg i.v. over 30 min 3x/wk x 4 wks 1x/6 wks (n=7) Canertinib, 150 mg i.v. over 30 min 3x/wk x 4 wks 1x/6 wks (n=8) Canertinib, 225 mg i.v. over 30 min 3x/wk x 4 wks 1x/6 wks (n=7) Canertinib, 337.5 mg i.v. over 30 min 3x/wk x 4 wks 1x/6 wks (n=7) Canertinib, 500 mg i.v. over 30 min 3x/wk x 4 wks 1x/6 wks (n=3)	53	Intravenous canertinib at doses up to 225 mg given 3 days per week was well tolerated and induced disease stabilization after 6-8 weeks of treatment in 19% of patients with advanced refractory solid tumors	55

ment, as follows: 14/7, 21/7, 28/7 or continuously. Plasma concentrations were dose-proportional without evidence of accumulation. Dose-limiting toxicity (DLT) of grade 3 stomatitis and rash was observed at the highest dose on the 28/7 schedule (45).

Clinical Studies

In a phase I study, oral canertinib was administered to patients with advanced-stage cancer once weekly at doses of 100, 200, 400, 500 and 560 mg for 3 weeks

every 28 days. Preliminary analysis of pharmacokinetic data for 19 patients showed a mean clearance of 241 l/h and a mean half-life of 6 h. The mean peak plasma concentration (C_{max}) at the 560-mg dose was 253 ng/ml. Reversible, dose-limiting hypersensitivity reactions occurred in 2 patients at 560 mg, but further dosing at this level was possible with oral diphenhydramine prophylaxis. The most frequently reported adverse events were emesis, diarrhea and acneiform rash. One patient demonstrated stable disease over 8 cycles of therapy (46).

In another study, 24 patients with advanced-stage nonhematological malignancies received 4-week courses of canertinib (250 and 300 mg orally) given for 7 days every other week. The dose of 250 mg was defined as the maximum tolerated dose (MTD) due to the incidence of DLT at the higher dose. Gastrointestinal (diarrhea, nausea and vomiting) and cutaneous adverse events were the most common toxicities reported during the study. Mean clearance was 280 l/h and C_{max} was reached in 2-4 h; C_{max} greatly exceeded the IC_{50} for prolonged inhibition of ErbB tyrosine kinase *in vitro*. The administration of a high-fat meal resulted in a small delay in C_{max}, but did not affect the extent of absorption of canertinib, as demonstrated by similar C_{max} and AUC values. Of the 14 patients evaluable for tumor response, 4 patients had stable disease as their best response, with a median time to progression of 6.5 months (47, 48). These results and those of the following clinical studies are summarized in Table I.

Another study in 14 patients with advanced solid tumors administered daily oral doses of canertinib of 150 mg for 21 days on a 28-day schedule also demonstrated a reduction in the rate of absorption following the ingestion of a high-fat meal, but no significant effect on the extent of absorption. A further 19 patients also received escalating doses of canertinib (125, 150 and 175 mg/day) according to the same schedule. Ten of the 33 total patients enrolled in both parts of the study had stable disease for at least 8 weeks as their best response. The MTD was determined to be 150 mg/day and the most frequently reported grade 1 and 2 toxicities included rash, diarrhea and stomatitis (49).

The effects of canertinib on tumor biomarkers were evaluated in a phase I study in 54 patients with solid tumors receiving escalating doses of 50-750 mg/day p.o. for 7 days every 3 weeks. The MTD was 750 mg/day. In tumor and skin specimens, cellular proliferation as measured by Ki67 immunohistochemistry was downregulated, and the cell cycle-inhibitory protein p27 was increased on day 8. There was a significant decrease in EGFR (ErbB-1) expression in tumor samples by day 8. A good correlation was observed between inhibition of ErbB-1 receptor phosphorylation and downregulation of Ki67. However, there was no relationship between dose level and the degree of modulation of biomarkers in tumor and skin. Evidence of clinically significant antitumor activity was obtained in 1 patient with locally advanced squamous cell skin cancer. The patient had significant tumor regression after 2 cycles of canertinib treatment (450 mg) and continued to receive treatment for 10 months, at which point there was no evidence of residual disease. The patient remained disease-free for nearly 1 year after discontinuing treatment. Biopsy of the tumor at baseline showed overexpression of both ErbB-1 and ErbB-2 receptors. Following treatment, at day 8, there was a 61% and 41% decrease in ErbB-1 and ErbB-2 phosphorylation, respectively, which was maintained for at least 7 days off treatment (74% and 43% decrease, respectively, on day 15). ErbB-3 and ErbB-4 expression was not assessed in this patient. Thirteen patients had stable disease for at least 12 weeks (50-52).

A further dose-escalating phase I study evaluated the tolerability and pharmacokinetics of canertinib administered orally over 14 consecutive days on a 21-day cycle. Thirty-two patients with advanced-stage nonhematological malignancies received doses of 300-560 mg/day. Dose-limiting toxicities were observed in 3 of the 6 patients at the highest dose level and the MTD was 450 mg. The most frequently reported adverse events were gastrointestinal symptoms and skin reactions. There were no complete or partial responses, but 6 patients had stable disease. Plasma concentrations were dose-proportional, with peak concentrations observed within 1-3 h of dosing. The study demonstrated that a 14 days on/7 days off schedule allowed greater drug exposure over time without a significant increase in toxicity compared with shorter dosing schedules (53, 54).

Intravenous dosing with canertinib was evaluated in a phase I study in 53 patients with advanced malignancies. Patients received infusions (10-500 mg/dose) on 3 days per week for either 4 weeks out of 6 or without interruption. The MTD was found to be 225 mg. Systemic exposure was dose-proportional and was not dependent on body weight or surface area. No confirmed objective responses were obtained, but 10 patients had stable disease at their first efficacy evaluation visit after 6-8 weeks of treatment (55).

Source

Pfizer, Inc. (US).

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