Drugs of the Future 2002, 27(10) 923-934

CCC: 0377-8282/2002

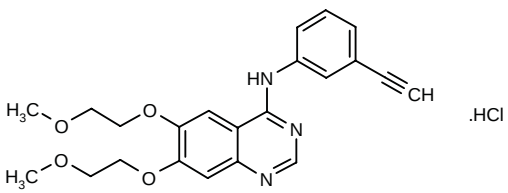
Erlotinib Hydrochloride

Prop INN; USAN

Oncolytic
EGF Receptor Inhibitor

CP-358774
NSC-718781
OSI-774
R-1415
Tarceva™

4-(3-Ethynylphenylamino)-6,7-bis(2-methoxyethoxy)quinazoline hydrochloride
N-[6,7-Bis(2-methoxyethoxy)quinazolin-4-yl)-N-(3-ethynylphenyl)amine hydrochloride



$C_{22}H_{23}N_3O_4$.HCl
Mol wt: 429.9016

CAS: 183319-69-9

CAS: 183321-74-6 (as free base)
EN: 250837

Abstract

The epidermal growth factor receptor (EGFR) is a type 1 receptor tyrosine kinase that is involved in the modulation of cellular differentiation and is overexpressed in many types of human cancers such as lung, pancreatic, ovarian, renal cell, gastric, hepatocellular and breast. Overexpression of EGFR is frequently correlated with increased tumor grade, increased metastatic potential and poor prognosis. Thus, inhibition of EGFR signaling is an attractive therapeutic option for the treatment of cancer. One method that can interfere with EGFR is the direct inhibition of EGFR tyrosine kinase activity. Several tyrosine kinase inhibitors have been developed and evaluated over the past 10 years of which the majority are reversible competitors with ATP for binding to the intracellular catalytic domain of the tyrosine kinase. One such EGFR tyrosine kinase inhibitor that has shown excellent antitumor activity is erlotinib hydrochloride, an oral quinazoline derivative that reversibly and selectively inhibits tyrosine kinase activity.

Synthesis

Erlotinib can be obtained by three related ways:

1) Alkylation of 3,4-dihydroxybenzoic acid ethyl ester (I) with 2-bromoethyl methyl ether (II) by means of K_2CO_3 and tetrabutylammonium iodide (TBAI) in refluxing acetone gives 3,4-bis(2-methoxyethoxy)benzoic acid ethyl ester (III), which is nitrated with HNO_3 in acetic acid to yield 4,5-bis(2-methoxyethoxy)-2-nitrobenzoic acid ethyl ester (IV). Reduction of ester (IV) with H_2 over PtO_2 in ethanol/HCl affords the corresponding aniline derivative (V), which is cyclized with ammonium formate (VI) in formamide at 165 °C to provide 6,7-bis(2-methoxyethoxy)-quinazolin-4(3*H*)-one (VII). Reaction of quinazoline (VII) with oxalyl chloride in refluxing chloroform gives the expected 4-chloroquinazoline derivative (VIII), which is finally condensed with 3-ethynylaniline (IX) in refluxing isopropanol containing pyridine (1-3). Scheme 1.

2) Reaction of the 4-chloroquinazoline derivative (VIII) with 4-(3-aminophenyl)-2-methyl-3-butyn-2-ol (X) in refluxing acetonitrile gives the secondary amine (XI), which is finally treated with anhydrous solid NaOH in refluxing either 1-butanol, 2-butanol, isopropanol or 2-methoxyethanol (3). Scheme 1.

3) Reaction of 3-bromonitrobenzene (XII) with trimethylsilylacetylene (XIII) by means of a Pd catalyst and Cu_2I in hot TEA gives 3-(trimethylsilylethynyl)nitrobenzene (XIV), which is reduced with H_2 over Pt/Al_2O_3 in isopropanol to provide 3-(trimethylsilylethynyl)aniline (XV). Condensation of the aniline (XV) with the quinazoline derivative (VIII) in refluxing isopropanol affords the silylated quinazoline derivative (XVI), which is finally deprotected with TBAF in THF (3). Scheme 1.

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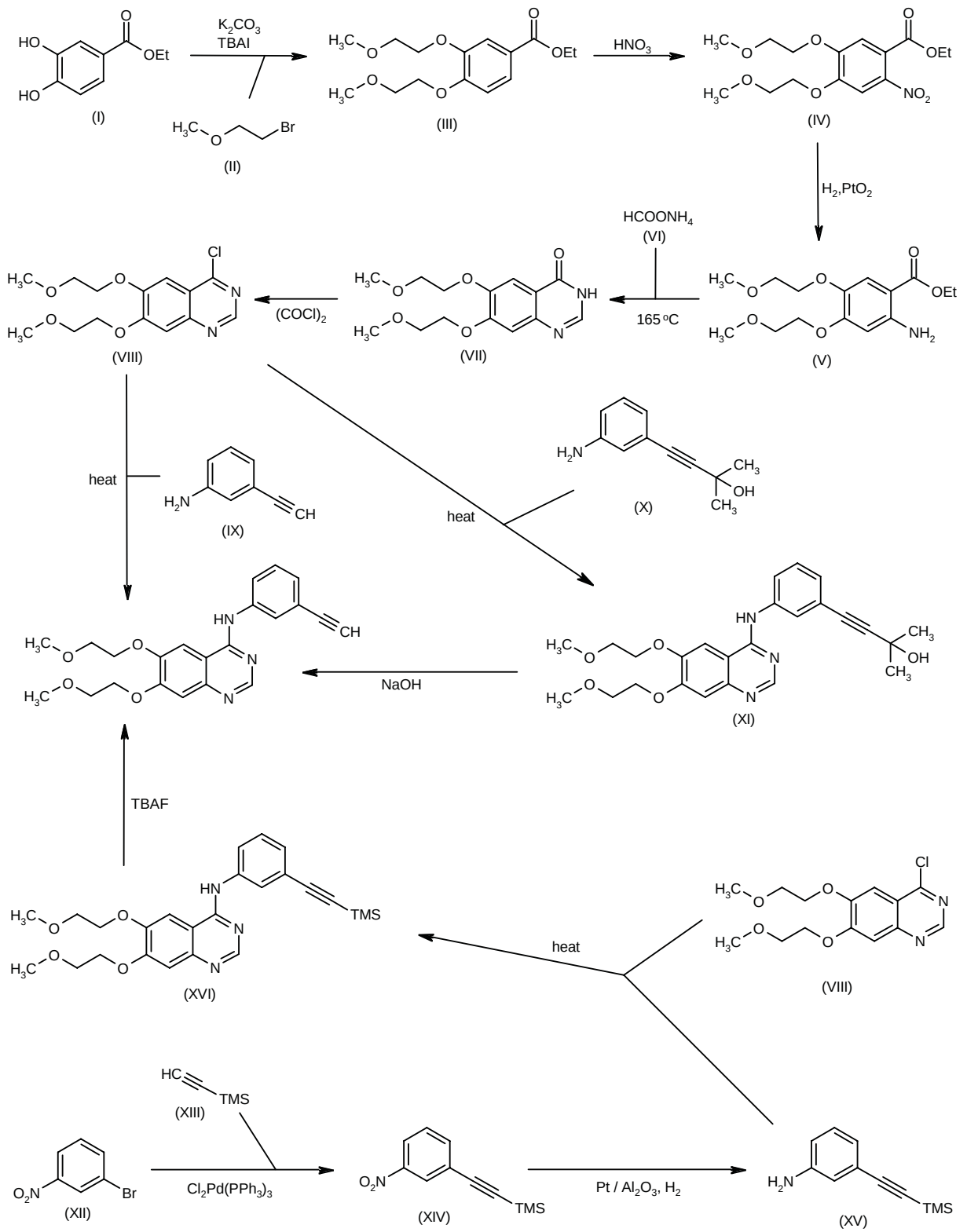
Scheme 1: Synthesis of Erlotinib



Introduction

Malignant neoplasms are characterized by autonomous cell growth, invasion of surrounding tissue and metastasis that are all the result of enhanced cellular proliferation and/or inhibition of apoptosis due to dysregulated cell signaling. Receptor tyrosine kinases are a class of signaling proteins which are frequently targeted through mutation or overexpression and thus are an attractive therapeutic target for the treatment of cancer. One such target is the epidermal growth factor receptor (EGFR, HER1, ErbB1), a type 1 receptor tyrosine kinase that is involved in the modulation of cellular differentiation and is overexpressed in many types of human cancers (4-8).

EGF is part of a family of growth factors that includes transforming growth factor (TGF), amphiregulin, heparin binding EGF and β-cellulin. TGFα is one growth factor that has been extensively studied and identified as playing a crucial role in cell proliferation of both normal and malignant epithelial cells. TGFα signaling occurs via binding to its specific cell membrane receptor, EGFR, which results in activation of EGFR tyrosine kinase activity, in turn triggering an intracellular signaling cascade. EGFR is a member of a subfamily of related receptors that exist as inactive monomers and include HER2/neu (ErbB2), HER3 (ErbB3) and HER4 (ErbB4). The EGFR extracellular binding domain contains 2 cysteine-rich regions that allows binding of EGF, TGFα, amphiregulin, heparin binding EGF as well as several virally encoded ligands. Following ligand binding, the receptors homodimerize or heterodimerize with another ligand-bound member of the Erb receptor family. Ligand binding induces activation of the tyrosine kinase intracellular domain via autophosphorylation which subsequently results in triggering of a cascade of intracellular signaling events (Fig. 1) (9). The intracellular signaling pathway involves activation of ras and mitogen-activated protein kinase (MAPK) which then activates various nuclear proteins. One such nuclear protein in particular is cyclin D1 which is necessary for cell cycle progression from the G_1 to S phase. EGF is not only essential for cell cycle progression, but is also a major component in the process of wound healing. Wound healing involves cell differentiation, mitogenesis, apoptosis, migration and angiogenesis, processes that are also critical for tumorigenesis (9-11).

In several tumor types such as pancreatic, lung, ovarian, renal cell, gastric, hepatocellular and breast, members of the EGFR family and their ligands can be overexpressed or expressed as an autocrine loop. Overexpression of EGFR is frequently correlated with increased tumor grade, increased metastatic potential and poor prognosis (12-15). Thus, inhibition of EGFR signaling appears to be an effective option in cancer therapy. There are currently several therapeutic approaches in this area that are under development. They include monoclonal antibodies to EGFR, small-molecule inhibitors of EGFR tyrosine kinase enzymatic activity,

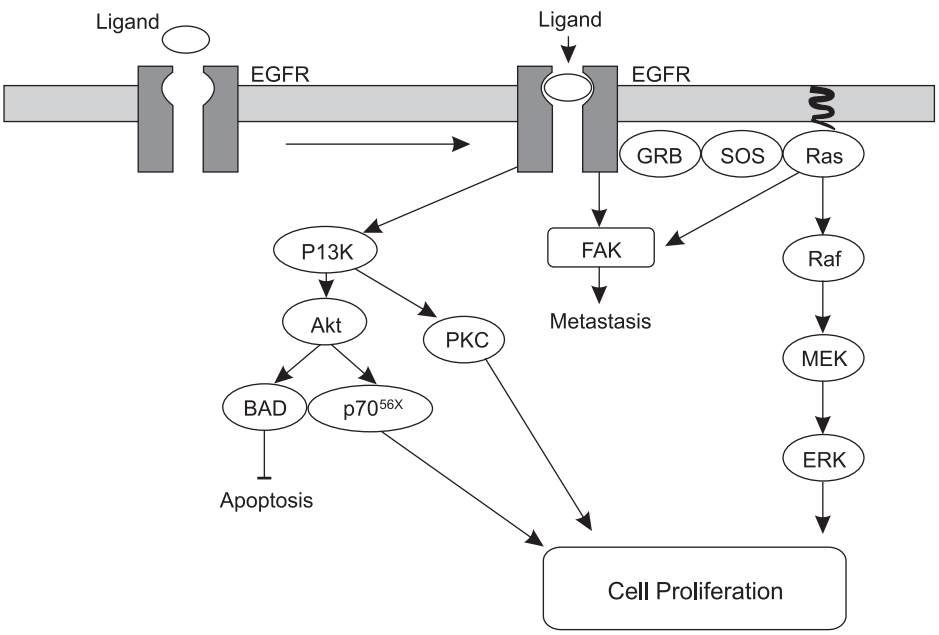
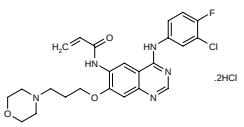
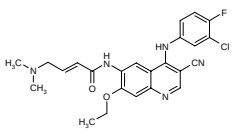
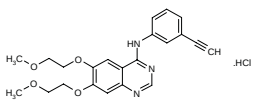
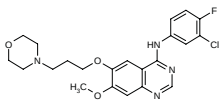
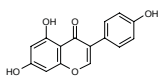
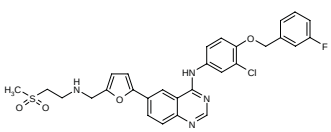
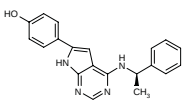


Fig. 1. A simplied schematic diagram of known pathways of EGFR signaling. The well-characterized Ras-Raf-MAPK pathway is activated downstream of EGFR activation. Cross-talk, however, existis and there is concurrent activation of the PI_3-kinase(Akt survival pathway. PI_3K = phosphoinositide-3-kinase; PKC = protein kinase C; FAK = focal adhesion kinase; MEK = mitogen-activated protein kinase kinase; ERK = extracellular signal-regulated kinase (from Adjei, A.A. *Epidermal growth factor receptor tyrosine kinase inhibitors in cancer therapy*. Drugs Fut 2001, 26(11): 1087-92).

Table I: Epidermal growth factor receptor tyrosine kinase inhibitors launched or under active development (from Prous Science Integrity®).

	Structure	EGFR inhibition IC_{50} (μM)	EGFR autophosphorylation inhibition IC_{50} (μM)	Cytotoxicity[a] CC_{50} (μM)
Canertinib .2HCl		0.002 (49, 50)	0.007 (49, 50)	0.080 (51)
EKB-569		0.0013-0.039 (53)	0.015 (53)	0.012-0.762 (53)
Erlotinib HCl (Tarceva™)		0.001-0.017 (18, 54)	0.02 (18)	0.10-5.10 (18, 54)
Gefitinib (Iressa®)		0.02 (10)	0.03 (55)	0.07-24.0 (56, 57)
Genistein		0.70-22 (58, 59)	2.59 (59)	8.5-77.0 (60-65)
GW-2016		0.011 (66, 67)	–	0.12-0.15 (66, 67)
PKI-166		0.0007 (52)	–	–

[a]Cytotoxicity against different human cancer cell lines. Reference numbers are shown in parentheses.

recombinant proteins containing TGFα or EGF fused to toxins (*e.g.*, Pseudomonas aeruginosa toxin), EGF conjugated to the natural, broad-spectrum tyrosine kinase inhibitor genistein, EGFR-directed vaccines and oligonucleotides to EGFR mRNA (10).

Hundreds of tyrosine kinase inhibitors have been developed and evaluated over the past 10 years and the majority of them, in general, are reversible competitors with ATP for binding to the intracellular catalytic domain of the tyrosine kinase. A number of potent and selective EGFR tyrosine kinase inhibitors have been designed using pharmacore modeling of the binding of compounds in the ATP pocket of tyrosine kinases (16, 17). Small-molecule EGFR tyrosine kinase inhibitors that have been launched or are currently under development are shown in Table I.

One such EGFR tyrosine kinase inhibitor that has shown antitumor potential is erlotinib hydrochloride (Tarceva™; OSI-774). Erlotinib is an oral quinazoline derivative that reversibly and selectively inhibits tyrosine kinase activity and has been chosen for further evaluation as an antineoplastic agent.

Pharmacological Actions

Erlotinib was shown to selectively and directly inhibit human purified tyrosine kinase (IC_{50} = 2 nM) and decrease EGFR autophosphorylation in intact human colorectal carcinoma (DiFi) and human breast cancer (MDA-MB-468) cells (IC_{50} = 20 nM). The agent was a potent inhibitor of the recombinant intracellular kinase domain of EGFR (IC_{50} = 1 nM). Treatment of DiFi cells in vitro with the agent inhibited proliferation (IC_{50} = 100 nM) and blocked the cell cycle at the G_1 phase; an accumulation of unphosphorylated retinoblastoma protein and p27kip1 was also seen, as well as formation of DNA fragments plus other properties indicative of apoptosis. Erlotinib (100 mg/kg) was also effective in vivo in athymic mice in preventing EGF-induced autophosphorylation of

EGFR in liver and in human head and neck tumor cell (HN5) xenografts (18).

The efficacy of erlotinib against several human glioblastoma multiforme cells which overexpress EGFR was shown *in vitro*. Treatment with a single 3 μM dose of erlotinib resulted in 50-65% inhibition of proliferation on day 10 as compared to controls. However, submicromolar concentrations of the agent were ineffective, indicating that this cancer type is less susceptible to erlotinib as compared to colon carcinoma (19).

The pharmacodynamics of erlotinib-induced inhibition of EGFR were further assayed in a study using human tumor tissue from s.c. HN5 xenografts in athymic mice. Oral administration of a single dose of the agent inhibited tyrosine phosphorylation with an ED_{50} of 10 mg/kg. The duration of the inhibition was prolonged so that a 70% reduction in EGFR-associated phosphotyrosine was observed over 24 h following administration of a single 100 mg/kg dose. This study also demonstrated the potent antitumor effects of erlotinib *in vivo* (5.7-92 mg/kg p.o. for 20 days starting 4 days posttransplantation) in athymic mice with EGFR-overexpressing human HN5 and human epidermoid (A431) s.c. xenografts. A more than 50% inhibition of HN5 tumor growth was observed with doses of 11-92 mg/kg/day and dose-dependent inhibition of A431 growth was seen with doses of 12.5-100 mg/kg/day. When cisplatin (maximum tolerated dose [MTD] = 10 mg/kg i.v.) was added to erlotinib therapy (9 mg/kg p.o. b.i.d. for 5 days), significant inhibition of HN5 tumor growth was observed that was greater than the inhibition seen with cisplatin alone (65-75 *vs.* 40%), regardless of the dosing sequence. No significant effects on body weight or lethal toxicity were observed with combination treatment (20).

An *in vitro* study demonstrated that erlotinib may be effective against certain cancers which express a mutant form of EGFR (EGFRvIII), such as breast, ovarian and glial tumors. Results from the study using CO12 cells (a NIH3T3 cell line transfected to overexpress wild-type EGFR) and HN5 cells showed that treatment with erlotinib induced dimerization of wild-type EGFR and inhibited growth. Moreover, when HC2 cells, a NIH3T3 cell line transfected to express EGFRvIII (a variant of the EGFR gene that results in constitutively active EGFR tyrosine kinase to levels similar to those seen in glioblastoma tumors), were treated with erlotinib, dose-dependent growth inhibition was observed (21).

An *in vitro* study examining the chemosensitivity of several anticancer agents in human cervical squamous carcinoma (ME180) and 2 resident subclones differing in EGFR expression (EGFR overexpressing ME180/TNF and non-EGFR-expressing ME180/Pt) to cisplatin, camptothecin and topotecan, showed that cotreatment including erlotinib (0.1 μM for 72 h) and cisplatin or camptothecin decreased the sensitivity of ME180/TNF cells to cisplatin and camptothecin to the levels of the parental ME180 cells and decreased the sensitivity of parental ME180 cells by 1.5- to 2-fold. Cellular sensitivity to cisplatin and camptothecin of ME180/Pt cells was unchanged when erlotinib was coadministered. It was concluded that in human cancers, the efficacy of platinum and topoisomerase I inhibitors may be dependent on the degree of EGFR expression (22).

However, a recent study provided evidence that the antitumor activity of erlotinib may not be due only to the levels of EGFR expression. In this study, erlotinib was shown to be very effective against tumors that are dependent on HER2/neu (ErbB2) activation for growth and survival such as mammary tumor cells derived from MMTV-ErbB2-transgenic mice. Erlotinib was found to inhibit p42 and p44 MAPKs in heregulin-stimulated ErbB2/ErbB3 expressing cells and TGFα-stimulated EGFR expressing cells with an IC_{50} of about 500 nM for all cells (23).

Erlotinib was effective in dose-dependently inhibiting growth of a human breast cancer cell line (MDA-MB-175) that coexpresses EGFR and ErbB2. In addition, combination treatment of these cells with suboptimal doses of a humanized recombinant monoclonal antibody directed against ErbB2 (0.74 μg/ml) and erlotinib resulted in inhibition of growth (up to 66%) that was 6 times greater than that seen with either agent alone. Thus, combination treatment with these two agents may be effective against tumors such as breast cancers which coexpress both receptors (24).

The broad-spectrum antitumor efficacy of erlotinib (0.2, 2 and 10 μM for 14 days) was shown in an *in vitro* study using freshly explanted tumor cells isolated from a total of 171 tumor specimens collected from patients undergoing tissue or fluid procurement procedures. Of the specimens, 51% were evaluable for positive or negative responses. Those types of tumor cells which had a good response (50% survival as compared to controls) were ovarian (79%), breast (67%), non-small cell lung (75%), renal cell (86%), colorectal (75%), non-Hodgkin's lymphoma (100%), gastric (50%), peritoneal (50%), pancreatic (100%) and bladder (100%). The inhibitory responses to erlotinib were significantly dose-dependent and were seen in 30, 54 and 68% of the cells treated with 0.2, 2 and 10 μM, respectively. The overall response rate was 57% (25).

The safety of oral erlotinib was demonstrated in mice, rats and dogs. Results *in vitro* showed that the agent did not induce microbial or mammalian cell mutations; no chromosomal aberrations were observed *in vitro* or *in vivo*. Studies *in vivo* using mice concluded that the agent had no proconvulsant or anticonvulsant activity. However, impaired coordination and loss of grip strength was observed in mice administered the high dose of 1000 mg/kg. The agent had no effects on renal, cardiovascular or cardiopulmonary function in rats or dogs. However, oral and i.v. administration of the agent resulted in an inhibition of gastric emptying in rats. Moreover, when rats were dosed for 1 month with 10 mg/kg/day, a reduction in food consumption and body weight gain was observed that was secondary to the inhibition in gastric emptying. Adverse events reported from studies in which dogs were treated for 1 month were emesis at doses greater than 50 mg/kg/day and an increase in regenerating renal proximal

tubule cells at 50 mg/kg/day. However, these adverse events were not seen in dogs at doses of 15 mg/kg/day given for 1 month or in rats treated with 5 mg/kg/day for 1 month (26).

Pharmacokinetics

The pharmacokinetics of i.v. erlotinib (> 1 mg/kg) were assessed in rats and dogs with results showing that total clearance decreased and AUC values increased supraproportionately possibly due to a saturation of clearance of the agent. The half-life after i.v. and p.o. dosing was 1-2 h in both species. The apparent oral bioavailability was 77% in rats administered 2 mg/kg and 45-88% in dogs given 0.5-10 mg/kg. Following dosing, several metabolites were identified, synthesized and found to have potent *in vitro* EGFR inhibitory activity. The major metabolite detected *in vivo* in rats and dogs after oral dosing was an *O*-desmethylated metabolite (OSI-420); the metabolite:parent compound AUC ratio was 1:4 in plasma (27).

The tissue distribution of erlotinib was elucidated in a study using athymic nude mice bearing HN5 tumors administered radiolabeled erlotinib. The agent was distributed uniformly throughout tumors and radioactivity was detected in tumors for at least 8 h postdosing. The radioactivity measured in tumors was lower than that found in other tissues except muscle and brain and tumor radioactivity half-life values were similar to those obtained in whole blood, myocardium and lung. The agent penetrated other tissues including pancreas and lung in a comparable manner with the exception of liver and kidney. From these results it was concluded that erlotinib uniformly penetrates HN5 tumors and desired target tissues (28).

The pharmacokinetics of erlotinib were reported from a phase I dose and schedule-duration escalation trial involving 27 patients with solid tumors. Patients were administered oral erlotinib in 1 of 2 schedules. Schedule 1 consisted of 25, 50 or 100 mg/day on 3 consecutive days/week for 3 weeks followed by 1 week of rest, and schedule 2 consisted of 50, 100, 150 or 200 mg/day or 100 mg b.i.d. on day 1 followed by a 2-day washout period and subsequent continuous dosing for 3 weeks with a 1-week rest period. An approximate 2-fold variation in exposure was observed between patients. Exposure increased in a dose-proportional manner. The average erlotinib concentrations (C_{avg}) following continuous daily dosing with 50, 100 and 200 mg/day were 432, 973 and 2120 ng/ml, respectively. The target C_{avg} value of 500 ng/ml was achieved with doses of 100 mg/day or greater. A dose-related accumulation in exposure was observed with a day 24:day 1 AUC_{0-24h} ratio of 1.2-4.8 obtained. Treatment was well tolerated. Dose-limiting grade 4 diarrhea was seen at 200 mg/day on schedule 2 and grade 1-2 acneiform upper body rashes with subepidermal neutrophilic infiltration and epidermal hyperproliferation were seen in 9 patients on schedule 2. Other adverse events seen were mild (headache, nausea, fatigue and transient increases in serum bilirubin and transaminases). Accrual is ongoing at 200 mg/day and 100 mg b.i.d. (29). The results of this study and some of those that follow are summarized in Table II.

A second phase I dose-escalation study involving 18 patients with advanced solid tumors (lung, prostate, colon, head and neck, breast, renal and liposarcoma) who had received a median of 2 prior chemotherapy regimens, also examined the pharmacokinetics and toxicity of oral erlotinib (100, 200, 400, 800, 1000 and 1200 mg once weekly for 3/4 weeks for up to 24 weeks). Treatment was well tolerated and no significant adverse events were observed at doses of 100 and 200 mg. Toxicities observed in subsequent cohorts were mild and included grade 2 headache, grade 1 mucositis, grade 2 acneiform rash, grade 2 nausea and grade 2 diarrhea. Grade 3 and grade 2 diarrhea developed in 1 and 2/3 patients given 1000 mg, respectively. The MTD has not yet been reached and accrual is ongoing at 1200 mg/week. Analysis of pharmacokinetics revealed a large inter- and intrapatient variability. However, pharmacokinetic parameters were relatively dose-proportional at 100-1000 mg/week. On day 1, the AUC_{0-24h}, C_{avg} and C_{max} values ranged from 21.3-116 μg·h/ml, 0.9-4.8 μg/ml and 1.5-7.1 μg/ml, respectively. The day 8:day 1 AUC_{0-24h} ratio ranged from 0.66-1.21 (30).

A phase I pharmacokinetic study has been completed involving 40 patients with advanced solid tumors. Patients were administered escalating doses of oral erlotinib in the following progressive longer treatment intervals: Group A, 25-100 mg once daily for 3 days/week every 4 weeks; Group B, 50-200 mg once daily for 3 weeks every 4 weeks; and Group C, 150 mg once daily (MTD from Group B) on an uninterrupted schedule. A total of 123 28-day courses were administered and treatment was generally well tolerated. No severe toxicities were observed in Group A. However, in Group B, doses greater than 150 mg/day resulted in a high incidence of severe diarrhea and/or cutaneous toxicities. The pharmacokinetics of erlotinib were dose-independent and no accumulation was observed with multiple daily dosing. At a dose of 150 mg/day, the minimum steady-state plasma concentrations, clearance rate, elimination half-life, volume of distribution and AUC value for the *O*-desmethylated metabolite (OSI-420) relative to erlotinib were 1.20 ± 0.62 μg/ml, 6.33 ± 6.41 l/h, 24.4 ± 14.6 h, 136.4 ± 93.1 l and 0.12 ± 0.12 μg/h/ml, respectively (31).

Clinical Studies

The EGFR inhibitory activity of erlotinib was characterized in 2 studies comparing tumor and normal skin biopsies from patients before and after 28 days of treatment with the agent (Study 1: 30 patients given 25-200 mg/day p.o.; Study 2: 15 patients with squamous cell carcinoma of the head and neck given 150 mg/day on an uninterrupted schedule). In the first study, examination of

Table II: Clinical studies of erlotinib hydrochloride (from Prous Science Integrity®).

Indication	Design	Treatments	n	Conclusions	Ref.
Cancer	Open	Erlotinib, 25 mg/d po 3x/wk (d1-d3) x 3 wk x 1x/28 d Erlotinib, 50 mg/d po 3x/wk (d1-d3) x 3 wk x 1x/28 d Erlotinib, 100 mg/d po 3x/wk (d1-d3) x 3 wk x 1x/28 d Erlotinib, 50 mg/d po x 1 d (d1) → Erlotinib, po x 3 wk x 1x/28 d Erlotinib, 100 mg/d po x 1 d (d1) → Erlotinib, po x 3 wk x 1x/28 d Erlotinib, 150 mg/d po x 1 d (d1) → Erlotinib, po x 3 wk x 1x/28 d Erlotinib, 200 mg/d po x 1 d (d1) → Erlotinib, po x 3 wk x 1x/28 d Erlotinib, 100 mg po bid x 1 d (d1) → Erlotinib, po x 3 wk x 1x/28 d	45	Erlotinib was well tolerated in patients with solid epidermal growth factor-positive tumors	29
Cancer	Open	Erlotinib, 100 mg po 1x/wk x 3 wk 1x/28 d x 24 wk (n=3) Erlotinib, 220 mg po 1x/wk x 3 wk 1x/28 d x 24 wk (n=3) Erlotinib, 400 mg po 1x/wk x 3 wk 1x/28 d x 24 wk (n=3) Erlotinib, 800 mg po 1x/wk x 3 wk 1x/28 d x 24 wk (n=3) Erlotinib, 1000 mg po 1x/wk x 3 wk 1x/28 d x 24 wk (n=6)	18	Erlotinib was well tolerated when administered weekly in doses up to 1000 mg/day in patients with advanced solid tumors	30
Cancer		Erlotinib, 25 mg po od x 3 d x 3 wk 1x/28 d (n=3) Erlotinib, 50 mg po od x 3 d x 3 wk 1x/28 d (n=3) Erlotinib, 100 mg po od x 3 d x 3 wk 1x/28 d (n=3) Erlotinib, 50 mg/d po od x 3 wk 1x/28 d (n=5) Erlotinib, 100 mg/d po od x 3 wk 1x/28 d (n=4) Erlotinib, 150 mg/d po od x 3 wk 1x/28 d (n=7) Erlotinib, 200 mg/d po od x 3 wk 1x/28 d (n=6) Erlotinib, 100 mg po bid od x 3 wk 1x/28 d (n=3) Erlotinib, 150 mg/d po od (n=15)	50	The recommended dose of erlotinib on an uninterrupted daily regimen was 150 mg. This regimen was well tolerated and demonstrated antitumor activity in patients with advanced solid epidermoid cancer	31
Cancer		Erlotinib, 25-100 mg/d po x 3 d 1x/4 wk Erlotinib, >/=50 mg/d po x 21 d 1x/4 wk Erlotinib, >/=50 mg/d po od	56	Treatment with erlotinib on a daily, uninterrupted schedule was feasible and demonstrated a modest antitumor effect in patients with cancer	34
Cancer	Open	Erlotinib, 150 mg/d po x 28 d	16	Erlotinib was well tolerated in patients with epidermal growth factor-positive tumors. FDG-positron emission tomography might prove to be predictive of standard response measures	35
Cancer	Open	Erlotinib, 100 mg po od + Docetaxel, 50 mg/m^2 iv 1x/3 wk (n=1) Erlotinib, 100 mg po od + Docetaxel, 60 mg/m^2 iv 1x/3 wk (n=8) Erlotinib, 100 mg po od + Docetaxel, 75 mg/m^2 iv 1x/3 wk (n=9) Erlotinib, 125 mg po od + Docetaxel, 75 mg/m^2 iv 1x/3 wk (n=5) Erlotinib, 125 mg po od + Docetaxel, 60 mg/m^2 iv 1x/3 wk (n=2) Erlotinib, 150 mg po od + Docetaxel, 75 mg/m^2 iv 1x/3 wk (n=2)	22	Erlotinib in combination with docetaxel showed antitumor activity in patients with NSCLC, head and neck cancer, ovarian cancer and other epithelial cancers	37

Continued

Table II (Cont.): Clinical studies of erlotinib hydrochloride (from Prous Science Integrity®).

Indication	Design	Treatments	n	Conclusions	Ref.
Cancer	Open	Erlotinib, 100 mg po od + Paclitaxel, 225 m/m^2 iv 1x/3 wk + Carboplatin, AUC 6 iv 1x/3 wk (n=6) Erlotinib, 150 mg po od + Paclitaxel, 225 mg/m^2 1x/3 wk + Carboplatin, AUC 6 iv 1x/3 wk (n=3)	9	Erlotinib in combination with paclitaxel and carboplatin showed antitumor activity in minimally treated or untreated cancer patients	38
Squamous cell carcinoma	Open, multicenter	Erlotinib, 150 mg/d po	114	Erlotinib was well tolerated and demonstrated evidence of antitumor activity in patients with advanced squamous cell carcinoma of the head and neck	39
Non-small cell lung cancer	Open, multicenter	Erlotinib, 150 mg/d po [until disease progression]	56	Erlotinib was well tolerated and had significant antitumor activity in patients with advanced epidermal growth factor-positive non-small cell lung cancer	40
Ovarian cancer	Open, multicenter	Erlotinib, 150 mg/d po	34	Erlotinib had objective antitumor activity in patients with advanced epidermal growth factor-positive ovarian carcinoma refractory to taxane and/or platinum regimens	41

biopsies from 30 patients revealed a significant increase in p27 and a significant decrease in activated phosphorylated (p-) ERK. In Study 2, 7 patients have completed 28 days of treatment and 5 pretreatment biopsies and 3 day 28 biopsies have been analyzed. p-EGFR, p-Akt and p-ERK were detected in 80% of the pretreatment biopsies. One of 3 posttreatment biopsies had a 60% decrease in p-EGFR and complete abolition of Akt activation. Another posttreatment biopsy had a 30% reduction in p-ERK. However, posttreatment biopsies from the third patient who developed rapid progressive disease revealed an 80% increase in p-EGFR and p-Akt and a 30% increase in p-ERK. Analysis of EGFR activation and downstream signaling from posttreatment samples suggest that erlotinib inhibited EGFR activation and signaling (32, 33).

The safety and efficacy of erlotinib was demonstrated in 2 studies involving a total of 40 patients with EGFR-positive cancers (Group A, 25-100 mg/day for 3 days every 4 weeks; Group B, 50 mg/day or greater for 21 days every 4 weeks; Group C, 50 mg/day or greater in an uninterrupted schedule; Group D, 150 mg/day in an uninterrupted schedule). In addition, the use of serial [^{18}FDG]-PET was shown to be effective in providing early evidence of antitumor activity or clinical benefit. Diarrhea and acneiform rash were the most common toxicities observed in Groups B and C. Only 1 of 12 patients in Group C had unacceptable toxicity. Toxicities reported from the 16 patients treated in Group D included grade 3 and 4 diarrhea (2 and 1 patients, respectively), grade 2 hyperbilirubinemia (1 patient) and grade 3 anemia (3 patients); grade 1 toxicities seen in this study were mucositis, neutropenia and headache. Responses from Groups A, B and C included complete regression of lung metastasis followed by resection of a residual primary renal cancer (20+ months) and a minor regression in a patient with colorectal cancer (11 months). Serial [^{18}FDG]-PETs from 2 patients with head/neck carcinoma in Group D showed a decrease in metabolic activity and a partial and minor response confirmed with CT/MRI at week 6. In addition, a marked decrease in p-EGFR was seen in 2/2 tumor biopsies (34, 35).

von Hippel-Lindau disease-associated neoplasmas are known to overexpress EGFR and TGF-α and the antitumor activity of erlotinib (150 mg/day p.o.) was shown against cerebellar hemangioblastomas in the case of a 37-year-old patient. Within 1 month of treatment, neurological symptoms improved; however, neurological examination was stable. A minor response of 2 cerebellar neoplasms was seen on MRI and cerebral spinal fluid white blood cells were normalized from 20 to 4 cells/mm^3. Mild toxicity of skin rash, loose stools and dry eyes were reported. The patient continued treatment for 9 months until progression occurred (36).

The efficacy of erlotinib in combination with other chemotherapeutic agents has been examined in several phase I trials. A study involving 16 patients with cancer showed activity of the agent (75 or 60 mg/day p.o.) in combination with docetaxel (100, 150 or 125 mg/m^2 i.v. every 3 weeks starting 3 days before erlotinib in course 1). To date, patients have received 62 courses. Of 6 patients receiving 75 mg erlotinib and 100 mg/m^2 docetaxel, 3 had dose-limiting toxicity (DLT) of febrile neutropenia. Because 2 of these patients were heavily pretreated, heavily pretreated and minimally pretreated patients were separated for analysis of results. The dose of erlotinib was reduced to 60 mg/m^2 in both heavily and minimally pretreated patients because minimally pretreated patients developed DLT of predominantly febrile neutropenia at erlotinib (mg)/docetaxel (mg/m^2) dose levels

of 75/150, 75/125 and 75/100. A dose level of 60/100 was well tolerated. To date, a near complete response was seen in a minimally pretreated patient with nasopharyngeal cancer and a minor response was observed in a minimally pretreated patient with non-small cell lung cancer (NSCLC). Stable disease for 4-7+ months has been observed in patients with bladder, ovary, stomach, skin and non-small cell lung cancers. Analysis of normal skin and tumor biopsies is under way and examination of the pharmacokinetics showed no apparent interactions between the agents (37).

Another phase I trial conducted in heavily and minimally pretreated cancer patients evaluated the efficacy of erlotinib (100 or 150 mg/day in an uninterrupted schedule) in combination with fixed-dose paclitaxel (225 mg/m^2 i.v.) + carboplatin (AUC_6 i.v. every 3 weeks) starting 3 days prior to erlotinib. Nine patients have been treated so far. DLTs of neutropenia and diarrhea developed in 2/6 patients receiving 100 mg erlotinib + paclitaxel/carboplatin and a DLT of grade 3 rash was seen in 2 patients administered 150 mg erlotinib + paclitaxel/carboplatin. Other toxicities included peripheral neuropathy, fatigue and diarrhea. A near complete response was observed in a patient with NSCLC and 2 minor responses in patients with NSCLC and penile carcinoma, respectively. Stable disease for 3-4+ months occurred in 2 patients with head/neck cancer and NSCLC, respectively. Analysis of normal skin and tumor biopsies is being performed and examination of the pharmacokinetics showed no apparent interactions between agents (38).

Erlotinib has also undergone phase II trials in patients with advanced, EGFR-expressing NSCLC, advanced squamous cell head and neck carcinoma and advanced ovarian cancer with promising results obtained.

The efficacy of erlotinib (150 mg/day p.o.) was examined in a multicenter, single arm, open label phase II trial involving 114 patients with bidimensionally measurable, advanced squamous cell carcinoma of the head and neck (EGFR-positive and -negative tumors; ECOG performance status = 0-2) who had recurrence following previous induction therapy. Treatment was well tolerated. The adverse events observed were acneiform rash affecting the face, upper torso and arms of 82/114 patients (mild, moderate and severe cases in 32, 39 and 9 patients, respectively) in addition to diarrhea, nausea, vomiting, headache and fatigue. One case of life-threatening cellulitis was reported although it appeared not to be related to erlotinib. Two patients discontinued due to rash and 24 others required dose reductions. No patient discontinued for diarrhea, which was managed with dose reductions or oral loperamide. Of the 78 patients evaluable for response, 10 had a partial response, 23 had stable disease and 45 had progressive disease (39).

Erlotinib (150 mg/day p.o. until disease progression) was shown to have significant antitumor activity in a phase II trial involving 56 patients suffering from stage IIIB/IV or recurrent metastatic NSCLC (ECOG performance status = 0-2) with 10% or more EGFR-positive tumors cells and disease progression or relapse after previous platinum-based therapy. Treatment was well tolerated. The most common adverse event was maculopapular acneiform rash seen in 44 patients, of whom 30, 13 and 1 had mild, moderate and severe cases, respectively; no patient discontinued for rash and dose reductions to 100 mg were required in only 2 patients. At 8 week, partial responses were seen in 7 patients, of which 6 were confirmed at 12 weeks. Stable disease was seen in 19 patients and 31 patients had disease progression. Responses and stable disease were not related to a higher number of EGFR-positive tumors cells (40).

A phase II trial involving 34 patients with bidimensionally measurable, advanced ovarian carcinoma (EGFR-positive tumors; elevated CA-125 levels of 49 units/ml or greater; ECOG performance status = 0-2) refractory to up to 3 previous taxane and/or platinum-based regimens, showed the antitumor efficacy of erlotinib (150 mg/day p.o.). The most common adverse events were acneiform rash on the face, upper torso and arms or other dermatological abnormalities affecting the majority (30/34) of patients, in addition to diarrhea, nausea, vomiting, headache and fatigue. Blinded third-party analysis of scans from 30 patients taken at 8 weeks indicated that 3 patients had objective partial responses. Of these responses, 2 lasted for 5 and 6 months, respectively; no follow-up scans were available for the third patient. Stable disease for 5, 5 and 6 months was observed in 3 patients, respectively; 12 other patients had stable disease at week 8, although follow-up scans were not available to determine duration (41).

Erlotinib alone or in combination with other chemotherapeutic regimens continues to undergo clinical evaluation as a treatment for several types of solid tumors. The antitumor efficacy of erlotinib (150 mg/day p.o.) is currently being examined in 2 phase III multicenter studies whose primary endpoint is overall survival. One study, expected to last 20 months, is a randomized, double-blind, placebo-controlled trial involving approximately 800 patients with stage IIIB/IV NSCLC who failed at least one previous chemotherapy regimen. The other is a randomized trial of an approximate 14-month duration involving about 470 patients with advanced or metastatic pancreatic cancer who are being treated with standard gemcitabine therapy alone or in combination with 100 mg/day erlotinib (42, 43).

A randomized, controlled, phase III trial is currently under way to evaluate the antitumor efficacy of erlotinib in combination with paclitaxel and carboplatin in approximately 1000 patients with NSCLC. The primary endpoint of this trial is improvement in patient survival. Another multicenter, randomized, controlled phase III trial has completed enrollment of around 1200 NSCLC patients and will assess the potential incremental survival benefit of 150 mg/day of erlotinib in combination with gemcitabine and cisplatin (44, 45).

A phase Ib trial has been initiated and involves patients with advanced breast cancer who relapsed after initial therapy. The study will evaluate the efficacy of erlotinib in combination with docetaxel and capecitabine (46).

Other phase III trials involving over 3000 patients have been planned to determine the efficacy of erlotinib as a treatment for ovarian, metastatic colorectal, head and neck, renal cell and pancreatic cancers. The FDA has granted erlotinib hydrochloride fast track designation for the treatment of chemotherapy-naive patients with stage III/IV NSCLC and as second- or third-line treatment of patients with incurable stage IIIB/IV NSCLC who have failed previous standard therapy (47, 48).

Sources

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