Therapeutically Targeted Anticancer Agents: Inhibitors of Receptor Tyrosine Kinases

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Abstract: The rationale to target receptor protein tyrosine kinases (RPTKs) as an approach to cancer chemotherapy has continued to become more compelling with time. Preclinical and clinical data strongly support the involvement of specific RPTKs in the formation and progression of a subset of solid and liquid tumors. The advances in our understanding of the oncogenic activation of these receptors have been matched by the identification of new structural classes of kinase inhibitors that exhibit enormous improvements with regard to potency, specificity and efficacy. This article summarizes current knowledge of the most promising RPTK inhibitors in clinical trials or known to be in late stage preclinical development.

Keywords: Angiogenesis, cancer therapy, signal transduction inhibitors, signaling pathways.

INTRODUCTION

Receptor protein tyrosine kinases (RPTKs) is a subclass of transmembrane-spanning proteins endowed with ligand-stimulatable kinase activity [1]. These enzymes are important regulators of intracellular signal transduction pathways involved in a number of cell functions, such as cell differentiation and proliferation [2]. The activity of RPTK is tightly controlled under normal physiological conditions, but many different tumor types have been shown to have dysfunctional RPTKs as a consequence of mutations or genetic alterations. Irrespective of the cause, this leads to enhanced or constitutive kinase activity and, in turn, to the aberrant and inappropriate post-receptor cellular signaling within the tumor cell. The RPTKs involved in oncogenic transformation are attractive targets for cancer drug discovery programs, and many efforts have focused in the last few years on preventing tyrosine phosphorylation by chemical inhibition of their kinase activity. Initially, inhibition of these RPTKs by ATP-site directed inhibitors was considered unlikely to succeed, but medicinal chemists have been able to impart potency and selectivity by modulating the interactions of the inhibitor with the ATP binding site of the selected RPTK (for reviews on this topic, see [3-12]). Parallel to these efforts, the number of RPTKs being used as therapeutic targets in oncology research have greatly increased [13], and it is impossible to capture all this in a brief review. Therefore, a set of RPTKs have been selected to individually illustrate the scientific rationale to target this class of enzymes, and to summarize current preclinical and clinical knowledge of their most promising inhibitors. Additional references have been included to guide the reader interested in more detailed information about a specific target or inhibitor.

REVERSIBLE AND IRREVERSIBLE KINASE INHIBITORS OF THE EGFR SYSTEM

The epidermal growth factor receptor (EGF) family is composed of four structurally related tyrosine kinases: EGFR (erbB-1, HER1); erbB-2 (HER2, Neu), erbB-3 (HER3), and erbB-4 (HER4) [14]. Activation of the kinase activity of these receptors triggers a network of signaling pathways that are involved in cellular proliferation, apoptosis, differentiation, angiogenesis, motility and invasion.

The EGFR system was first implicated in cancer when the avian erythroblastosis tumor virus was found to encode an aberrant form of the human EGFR. Further studies and clinical data have supported an important role for this receptor in the development and progression of different human tumors [15-19]. Overexpression of EGFR is a frequent genetic alteration in a large variety of epithelial cancers [20], and is associated with poor prognosis [16]. In a significant proportion of these tumors, gene amplification is accompanied by rearrangements that result in constitutively active receptors. This effect has also been observed in mutated forms of the EGFR. The most common mutation (EGFRvIII), which is found in gliomas and carcinomas, lacks part of the extracellular domain and, despite being unable to bind to the ligands, displays constitutive kinase activity [21]. These data led to the identification and development of inhibitors that selectively target the kinase activity of the EGFR in cancer cells [22-26] or antibodies that block signaling [27-28]. The EGFR kinase inhibitors undergoing clinical trials or known to be in late stage preclinical development are discussed in the following.

Gefitinib (iressa™, ZD-1839; AstraZeneca plc; **1**, Fig. (**1**)) is the first EGFR kinase inhibitor to be approved for marketing in any country. This 4-phenylamino-quinazoline derivative [29] is a potent ATP site-directed competitive inhibitor of the EGFR in biochemical assays (K_i= 2.1 nM on purified receptor; IC_{50}= 23 - 79 nM). By contrast, it shows minimal activity against other tyrosine and serine/threonine kinases, with IC_{50} values at least 100-fold higher than that for EGFR [30]. Gefitinib blocked EGFR autophosphorylation in a range of tumor cell lines [31] and potently inhibited the proliferation of cancer cells that overexpressed the EGFR (IC_{50}= 7 – 90 nM) [32]. Although gefitinib is a weak inhibitor of erbB-2, antiproliferative activity against cell lines that overexpress erbB-2 has also been reported [33]. Synergistic activity has been reported in

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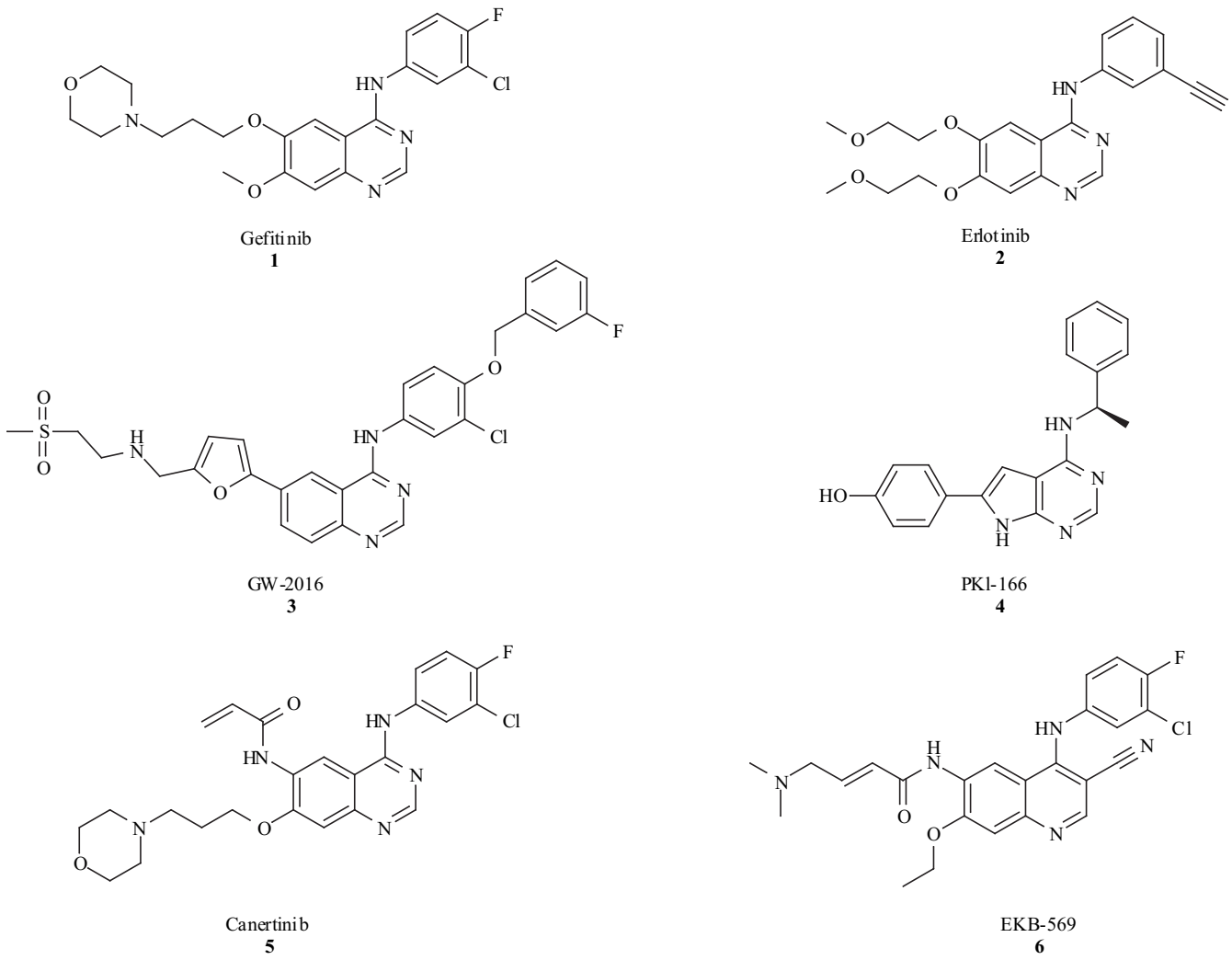

Fig. (1). Reversible and irreversible kinase inhibitors of the EGFR system.

combination studies with cytotoxic agents (e.g. doxorubicin, etoposide or cisplatin) [34].

Gefitinib demonstrated antitumor activity in human xenografts derived from A431, A549, KB, HT29, HX62, CR10, LoVo, MCF-7, and Du145 cell lines [35,36]. When administered orally at 10 mg/kg/day, a 50 % reduction in the growth of A431 tumor cells was observed, and complete regression of large tumors was obtained with 200 mg/kg/day for two weeks. Tumor growth was suppressed for as long as four months, but regrowth occurred when treatment was suspended. Encouraging results were also obtained in preclinical studies conducted in tamoxifen-resistant and sensitive MCF-7 cells [37] and colorectal LoVo tumors [38]. In this last study, the compound produced significant tumor growth delays *in vivo* when combined with either single or fractionated radiotherapy, compared with either treatment alone. The antitumor activity of gefitinib seems to be partly mediated by its antiangiogenic effects on EGFR expressing endothelial cells [39], and is accompanied by a decreased production of autocrine and paracrine pro-angiogenic factors [40].

The Japanese Ministry of Health, Labour and Welfare approved gefitinib for the treatment of inoperable or recurrent non-small cell lung cancer (NSCLC) in July 2002, making Japan the first country to register the drug. The approval was based on data from two pivotal Phase II studies (IDEAL 1 and 2). The results of these clinical trials showed that gefitinib provides clinically significant symptom relief for patients with extensively pre-treated advanced NSCLC (for additional information see http://www.prnewswire.com). This improvement in disease-related symptoms correlated with improved disease-free survival and tumor response [41]. The FDA granted fast track status for this compound in the USA, and AstraZeneca submitted the final documents of its rolling NDA for gefitinib to the FDA in August 2002, as monotherapy for patients with advanced NSCLC. The FDA Advisory Committee recommended accelerated approval for gefitinib as a third-lime treatment for NSCLC in September 2002. This vote was based on data from the phase II IDEAL studies and came as a surprise in light of the results obtained in the INTACT (Iressa™ NSCLC trials assessing combination therapy) trials. Despite the efficacy observed as a single agent, the compound failed to demonstrate a clear benefit in patient survival in NSCLC when given in combination with standard chemotherapy (e.g. carboplatin/doclitaxel or cisplatin/gemcitabin). The company has also submitted the drug for approval by European regulators this year to treat patients suffering from advanced NSCLC. Gefitinib will also be assessed in combination

with other agents in the treatment of patients with inoperable kidney, bladder, breast and ovarian cancer.

The clinical data reported to date on gefitinib indicate that it is well tolerated, with negligible dose limiting toxicities, even at doses of 800 mg/day. The most frequent side effects observed were follicular skin eruptions, diarrhoea, fatigue, gastrointestinal and cardiovascular disorders, and some events of liver toxicity [42,43]. AstraZeneca has strengthened recently its warning for gefitinib in Japan after cases of interstitial pneumonia and lung injury were reported.

Clear inhibition of EGFR activation and downstream-markers at doses below the MTD were observed by immunohistochemical evaluation of skin biopsies from Phase I patients using antibodies specific for the phosphorylated EGFR [44]. These results support the notion of pharmacodynamic assessments being required to select relevant doses and schedules instead of the classical maximally-tolerated dose for definitive efficacy and safety trials.

Erlotinib (tarceva™, CP-358774, OSI-774; OSI Pharmaceuticals Inc/Genentech Inc/Roche Holdings AG; **2**, Fig. (**1**)) is another example of an orally active inhibitor of EGFR being developed as a stand alone treatment for solid tumors and for use in combination with existing chemotherapy. The compound inhibits EGFR kinase activity in biochemical assays with an IC_{50} value of 1 - 2 nM, and shows high selectivity (ratio > 1000-fold) against other tyrosine kinases (e.g. Src, Abl, IGF-IR or InsR) [45,46]. In athymic nude mice bearing HN5 (head and neck tumor) xenografts, 50 % inhibition was observed after oral administration at 10 mg/kg/day. The inhibition of A431 derived tumors required a higher dose (200 mg/kg/day). Uniform distribution into HN5 tumors as well as other targets tissues was demonstrated using a radio-labeled compound. Long-lasting inhibition of EGFR autophosphorylation (70 % reduction over a period of 24 h) in tumor xenografts was observed ex-vivo after a single dose of 100 mg/kg. Combination experiments with cisplatin demonstrated additive antitumor activity and enhanced apoptosis with no observable effects on body weight or overt toxicity. The suppression of the PKB/Akt survival pathway by erlotinib seems to contribute to the induction of apoptosis [47,48].

Partial responses (14 to 16 %) were reported in Phase II clinical trials with NSCLC patients treated with erlotininb (150 mg/day orally). The median survival duration was 257 days and the one-year survival rate was estimated to be 48 %. In another Phase II study, oral administration of erlotinib to patients with advanced head and neck cancer showed three confirmed and two unconfirmed partial responses. Phase III trials in NSCLC, were initiated in 2001 and 2002 in combination with carboplatin and paclitaxel, or gemcitabine and cisplatin. A Phase III trial is also being conducted in patients with metastatic pancreatic cancer, and the TACTIX (tarceva™ in combination with taxotere and xeloda) trial started in October 2001 in patients with advanced breast cancer, who have relapsed following initial chemotherapy.

OSI Pharmaceuticals received fast track status from the FDA in September for erlotinib as a second- or third-line treatment for patients with incurable stage IIIB/IV NSCLC, who have failed to respond to standard therapy for advanced metastatic disease. Fast track status was also granted to erlotinib in May 2002 for the treatment of chemotherapy-naïve stage III/IV NSCLC.

Diarrhoea and acneiform rashes are the main adverse events observed in the clinical studies with erlotinib, and fatigue, headache, nausea and transient increases in serum bilirubin and transaminases have been reported as minor side effects [49,50] (see also the information provided in http://www.osip.com).

An alternative approach to block EGFR signaling has been the dual inhibition of EGFR and erbB-2 kinase activities. Although erbB-2 is an orphan receptor, it participates in receptor signaling by heterodimerization with the members of the EGFR family [51]. Dimerization causes activation of the kinase domain, leading to receptor transphosphorylation and initiation of signal transduction pathways linked to cell survival and division [52]. The epidemiological evidence implicating these two receptors in cancer patients (e.g. overexpression of erbB-2 occurs in around 30 % of breast cancers and co-expression of elevated levels of these two receptors has been observed in ovarian cancer patients) [53,54] suggested that a dual EGFR/erbB-2 inhibitor could provide a therapeutic opportunity in patients with tumors expressing either or both of these receptors. EGFR and erbB2 have homologous kinase domains and optimization of quinazoline and pyrido-[3,4-*d*]-pyrimidine derivatives [55] lead to GW-2016 (GlaxoSmithKline plc; **3**, Fig. (**1**)), which is indeed a potent inhibitor of both EGFR and erbB-2 (IC_{50} values 11 nM and 9.2 nM, respectively), while retaining selectivity against a range of other kinases [56]. The antiproliferative effects of the compound on EGFR/erbB-2 overexpressing tumor cell lines are in the 100 nM range and selectivity against normal cell lines is retained (IC_{50}= 10 μM). In preclinical studies, GW-2016 inhibited the growth of head and neck cancer xenografts at a dosage of 100 mg/kg po bid with no adverse effects [57]. In an animal model of human breast cancer, treatment with GW-2016 (100 mg/kg p.o. bid for 21 days) almost completely suppressed BT474 tumor growth [58]. GlaxoSmithKline is currently conducting Phase I trials with this compound.

PKI-166 (CGP-75166; Novartis AG; **4**, Fig. (**1**)) is a substituted pyrrolo[2,3-*d*]pyrimidine, which exhibits *in vitro* dual inhibition of EGFR and erbB-2 (IC_{50}= 1 and 11 nM, respectively) [59]. It has also some inhibitory activity in biochemical assays against Src, Abl, VEGFR-2 and flt-1 (103, 26, 327 and 962 nM, respectively), but shows good selectivity against receptor tyrosine kinases other than VEGFR, such as Met, Kit and Tek. EGF-mediated EGFR autophosphorylation and c-fos mRNA expression are inhibited in the nanomolar range (IC_{50}= 5 and 10 nM, respectively). At higher concentrations, the compound also inhibited cellular erbB2 autophosphorylation (IC_{50}= 0.1 – 1 μM).

PKI-166 has shown significant and dose-dependent *in vivo* antitumor activity in several EGFR expressing xenograft models in nude mice following oral administration of 10-100 mg/kg/day p.o. [60]. Complete and long-lasting inhibition of EGF-stimulated EGFR autophosphorylation in tumors was observed following administration of a single

100 mg/kg oral dose [61]. Several preclinical studies demonstrated enhanced antitumor effects of PKI-166 with cytotoxic agents. PKI-166 combined with gemcitabine can significantly reduce the growth and metastatic potential of highly metastatic human pancreatic tumors [61-63]. Similarly, PKI-166, alone or with paclitaxel, reduced the number of bone lesions by 40 – 60 % compared with controls or paclitaxel alone in a mouse model of metastatic human renal cell carcinoma [64]. Recently, it has been found that PKI-166 can also block the enzymatic activity of RET. Gain of function mutations of RET cause multiple endocrine neoplasia type 2 familial cancer syndrome and are also found in sporadic medullary thyroid carcinomas. Thus, targeting RET mutations with tyrosine kinase inhibitors might offer a potential treatment strategy for carcinomas sustaining oncogenic activation of RET (see also, ZD-6474 in the section on VEGFR inhibitors) [65].

The 450 mg daily cohort was identified as the MTD level in a Phase I dose-escalation study of oral PKI-166 administered to patients with advanced solid tumors. A confirmed partial response in a patient with NSCLC and several stable diseases were observed. Phamacodynamic analyses revealed qualitative decreases in immunohistochemical staining of phosphorylated and total EGFR in tumor and skin biopsies, as well as in hair follicles. The most frequent side-effects included rash, diarrhoea, fatigue and reversible elevated liver transaminases. The compound was in Phase II clinical trials when Novartis announced in October, 2002 that the development of PKI-166 was discontinued.

Contrary to the two approaches outlined before, a salient feature of some of the new inhibitors that target the EGFR system is the potency and selectivity obtained against erbB-2 [e.g. TAK-165, Takeda Chemical Industries Ltd. (structure not disclosed), IC_{50}= 6 nM for erbB2 *versus* IC_{50} >25 μM for EGFR]. Preclinical data suggest that these selective erbB2 inhibitors can also generate significant *in vivo* efficacy, but, as for any new therapeutic approach, the ultimate utility of these new agents can only be determined in clinical trials.

The potential utility of compounds that effectively block the function of the EGFR family but do not inhibit more structurally diverse tyrosine kinases has also being explored with pan-EGFR inhibitors [66,67]. Similar to the concept of dual EGFR/erbB-2 kinase inhibitors, the objective of this approach is to achieve greater efficacy and a broader spectrum of activity by blocking the kinase activity of all the members of the erbB family and the cross-talk between them [51]. One way to accomplish this is by using site-directed irreversible inhibitors [67]. These compounds contain Michael acceptor-type substituents and exploit the presence of a cysteine residue at the "sugar pocket" of the ATP binding site to establish the addition product when bound to the enzyme [68,69]. Cysteine-773, which is located on the extended coil stretch of the EGFR, is unique for the EGFR family of kinases providing selectivity (ratio of nearly 10^5 –fold) against other receptor or intracellular kinases. The prolonged suppression of kinase activity caused by these agents might be limited by the rate of receptor regeneration, which appears to be relatively rapid especially for the EGFR. The preclinical performance of this type of derivatives has improved to the point where several compounds are in clinical trials. Canertinib (CI-1033, PD-183805; Pfizer Inc; **5**, Fig. (**1**)) is an irreversible inhibitor of the kinase activity of EGFR (IC_{50}= 1.5 nM, *in vitro* enzyme assay) as well as that of erbB2 and erbB4 [70]. Inhibition of EGFR autophosphorylation in A431 cells was observed with an IC_{50} value of 7.4 nM. The compound was also highly effective *in vivo* in several EGFR- or erbB2-dependent xenograft models (e.g. A431, H125, BCA-1, SF767 or MCF-7). For example, it demonstrated optimal efficacy (T/C of 4 %) at 5 mg/Kg/day in A431 xenografts with a minimal weight loss (< 10 %) [70].

Several studies have shown that canertinib can synergize with a variety of cytotoxic agents and radiation. Treatment with canertinib of human breast MDA-MB-453 and BT474 cancer cell lines enhances the cytotoxicity of gemcitabine through inhibition of PKB/Akt and MAPK [71]. Furthermore, canertinib was synergistic with ionizing radiation [72] and cytotoxic agents [73]. In this last study, canertinib enhances the steady-state accumulation of a topoisomerase I inhibitor by blocking drug efflux by the breast cancer resistance protein transporters.

Canertinib has progressed through Phase I clinical studies using oral dosing, and acneiform rash, emesis, hematological and diarrhoea have been reported as the most common adverse events. In a Phase I study, the compound was administered daily for seven days every 21 days [74]. Of the 37 treated patients, there was a partial response in one patient with squamous cells carcinoma of the head and neck, and disease stabilization in ten patients. A second Phase I study administered the compound on days 1, 8, and 15 every 28 days, and one disease stabilization in a patient with osteosarcoma was documented out of 34 patients [75]. Ongoing clinical trials include a Phase II study in ovarian cancer.

EKB-569 (Howard Hughes Medical Institute/Wyeth Research; **6**, Fig. (**1**)) is a cyanoquinoline derivative that also binds covalently and irreversibly to the EGFR. It potently inhibits recombinant EGFR tyrosine kinase *in vitro* (IC_{50}= 1.3 nM) and its autophosphorylation in tumor cells (IC_{50}= 15 nM). In spite of its lower activity against erbB-2, EKB-569 is equipotent in inhibiting the proliferation of cells expressing EGFR or erbB-2, and 50-fold higher concentration is needed to inhibit cells that do not overexpress either receptor. Cell growth inhibition is associated with reversible cell cycle inhibition in G_0/G_1 [76], and 90 % inhibition of EGFR phosphorylation in subcutaneous implanted A431 xenografts in nude mice is observed within 90 min of oral administration of the compound (10 mg/kg).

EKB-569 either alone or in combination with the non-steroidal anti-inflammatory sulindac reduces the incidence of intestinal polyps in a murine model of human familial adenomatous polyposis [77]. Sulindac (5 mg/kg/day) had no effect on polyp formation, whereas EKB-559 (20 mg/Kg/day) reduced polyp formation by 87 % compared with controls. The combination therapy produced a 95 % reduction in polyp numbers, and 47 % of the treated mice had no evidence of tumors at all. No effect on the body weight or feeding habits in mice was observed when the

EGFR inhibitor was administered alone or in combination with sulindac. Histological examination of the entire gastrointestinal (GI) tract revealed no mucosal erosions, inflammation or other indications of GI toxicity. The preceding synergistic effect may be due to the convergence EGFR and cyclooxygenase-2 (COX-2) signaling, and point to the potential clinical use of EGFR plus COX-2 inhibitors in the prevention and treatment of cancers. Currently, EKB-569 in combination with sulindac is in Phase I trials in the US as a chemo preventative agent against colon cancer. Other Phase I studies are also underway in the USA in patients with a variety of cancers known to overexpress EGFR. Toxicity patterns in the clinical trials appear to be similar to other agents in this class and include diarrhoea and skin rash.

KINASE INHIBITORS OF THE VEGFR SYSTEM – BLOCKING TUMOR ANGIOGENESIS

To grow beyond a certain size, tumors must develop a network of blood vessels to supply nutrients and oxygen, and to remove waste products [78-80]. The formation of these new vessels is regulated through the production of several angiogenic factors –in particular, vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) [81-83] - that activate VEGFRs in endothelial cells [84-86]. Expression of VEGFRs is upregulated in activated endothelium and is high in vessels surrounding and invading growing tumor tissue [87]. Modulation of VEGFR signaling with kinase inhibitors was identified as an attractive approach to inhibit tumor angiogenesis, and several agents have proceeded into clinical trials in cancer indications [88-94] .

Three VEGFR kinase inhibitors from SUGEN Inc. (Pharmacia Corp) have progressed into clinical trials, SU-5416 (semaxanib; **7**, Fig. (**2**)), SU-6668 (**8**, Fig. (**2**)) and SU-11248 (**9**, Fig. (**2**)). The prototype compound, semaxanib [95,96], was for a long time the most extensively studied VEGFR kinase inhibitor, and its development proceeded into Phase I/II studies as a single agent [97,98], and later into Phase III combination studies. Clinical Phase II/III studies focused on metastatic colorectal cancer. This malignancy was selected on the basis of tumor microvessel density being a good prognostic indicator in these patients. Interim analysis of 355 patients showed no survival advantage of semaxanib in combination with 5-FU/leucovorin therapy and, consequently, the clinical development of the compound was discontinued.

SU-6668 (SUGEN Inc.; **8**, Fig (**2**)) is structurally related to SU-5416, but has a more favorable biopharmaceutical profile. The compound has a significantly lower K_i value for PDGFR (K_i= 0.008 μM) relative to VEGFR-2 (K_i= 2.1 μM) or FGFR-1 (K_i= 1.2 μM) in *in vitro* biochemical assays [99]. In cellular systems, SU-6668 inhibited VEGF-driven mitogenesis of HUVECs in a dose-dependent manner with a mean IC_{50} of 0.34 μM. In comparison, FGF-driven mitogenesis of HUVECs was inhibited with a mean IC_{50} of 9.6 μM. Oral (e.g. 4-200 mg/kg/day) or i.p. (e.g. 25 mg/kg/day) administration of the compound in athymic mice resulted in significant growth inhibition of a diverse panel of human tumor xenografts (e.g. A431, A375, Colo205, H460, and SF763T) [98,99], and increased the antitumor effects of irradiation [100]. Antiangiogenic together with antimetastatic activity has been demonstrated with SU-6668 (60 mg/kg/day i.p.) following injection of colon cancer cells into Balb/c mice [98]. The compound inhibited metastases (55.3 %), microvessel formation (36.2 %), and cell proliferation (27.3 %), and increased tumor cell (4.3-fold) and endothelial cell (81.4-fold) apoptosis. Using Western blot analyses, it has been recently proved that the *in vivo* antiangiogenic effects of the compound occur at doses similar to those required to inhibit VEGFR-2 and PDGFRβ phosphorylation in tumors [101].

In a Phase I study, SU-6668 was administered orally either once or twice-daily at doses of 100 to 2400 mg/m^2 to patients diagnosed as having advanced malignancies [102-104]. One minor response was seen in a patient with a desmoid tumor and stable disease was achieved in one patient with NSCLC and in another with a sarcoma. Mild-to-moderate side effects included nausea, vomiting, fatigue and tumor pain. The compound is now in Phase II clinical trials for the treatment of solid tumors.

SU-11248 (SUGEN Inc.; **9**, Fig (**2**)) is another indolin-2-one derivative that has recently entered Phase I clinical trials (see also the section on FTL3 inhibitors). The compound exhibits ATP-competitive inhibition against VEGFR-2, PDGFRβ and FGFR-1 with K_i values of 0.009, 0.008 and 0.83 μM, respectively [105]. The cellular activity of SU-11248 mirrors its biochemical profile. In ligand-dependent cell proliferation assays, SU-11248 potently inhibited VEGF- and FGF-induced proliferation of HUVECs (IC_{50}= 0.04 and 0.7 μM, respectively), and PDGF-induced proliferation of NIH-3T3 cells overexpressing PDGFRβ or PDGFRα (IC_{50}= 0.04 and 0.7 μM, respectively). The compound also shows good activity against c-Kit and FLT3 in biochemical and in cell based assays [106].

SU-11248 (80 to 20 mg/kg/day, p.o.) exhibited broad and potent *in vivo* antitumor activity using various human (e.g. HT-29, A431, Colo205, H460, SF763T, A375 and MDA-MB.435) or rat (e.g. C6) tumor xenografts in mouse [105]. Oral administration of SU-11248 (40 mg/kg) caused substantial inhibition of VEGF and PDGF receptor phosphorylation as shown by ex-vivo analysis of SF767T and A375 tumors. These studies show that constant inhibition of these receptors is not required for the compound to have potent antitumor activity.

Due to its broad activity and selectivity for the split kinase family of receptor tyrosine kinases, SU-11248 may have therapeutic potential for the treatment of malignancies that involved abnormal activation of c-Kit or FLT3 kinases (see also the following sections on these targets).

Vatalanib (PTK-787, CGP79787, ZK-222584; Novartis AG/Schering AG; 10, Fig. (**2**)), which is the first example of an inhibitor from the phthalazine class, inhibits VEGFR-1 and 2 (IC_{50}= 77 and 37 nM, respectively), and other kinases of the type III PDGFR family (IC_{50} values in the range of 100 - 1200 nM) [107]. The compound blocks the autophosphorylation of VEGFR in both HUVECs and VEGFR-2 transfected cells [108], and possesses good functional activity in cellular systems, inhibiting VEGF-mediated cell proliferation (IC_{50}= 16 nM), cell survival and

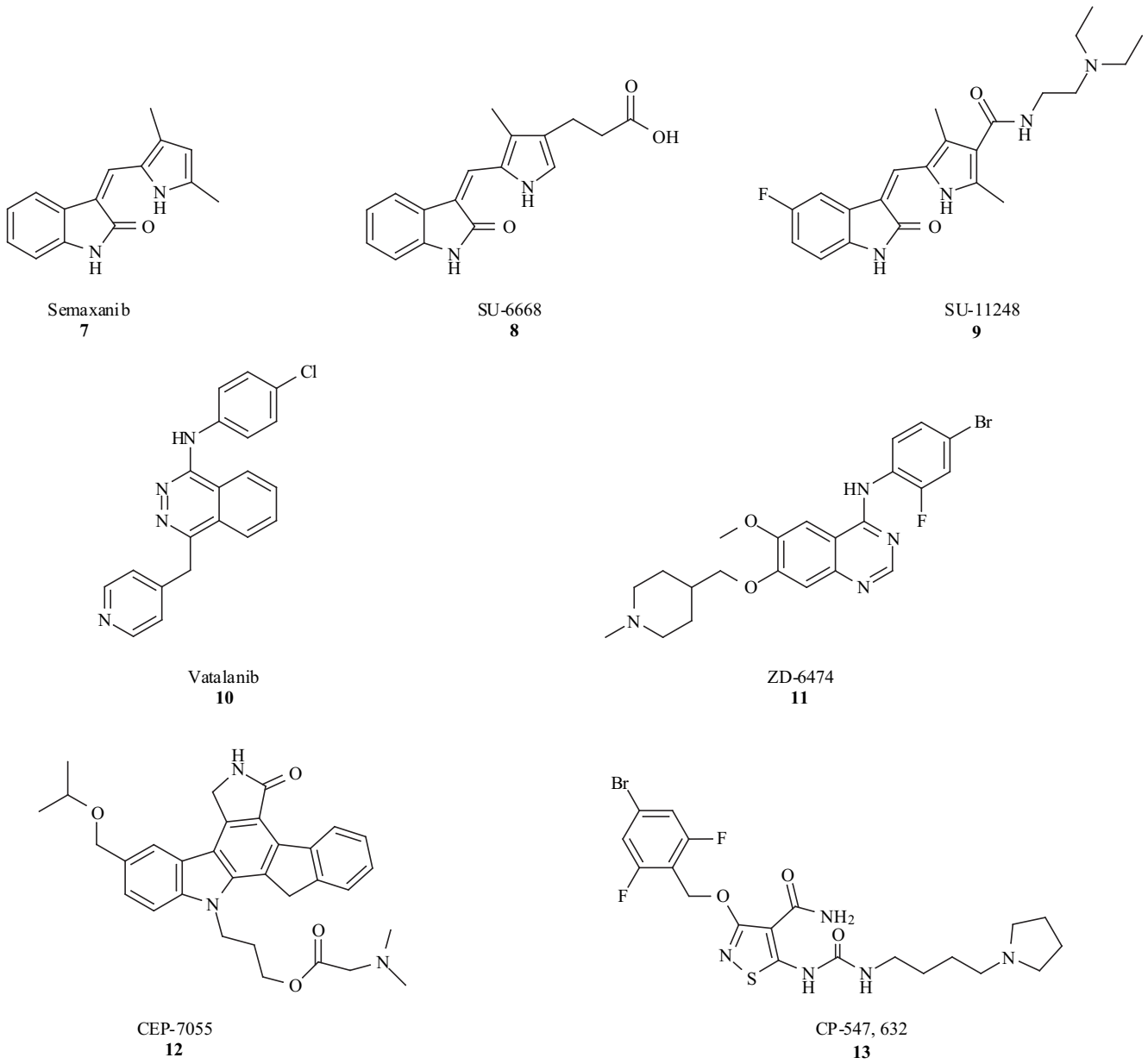

Fig. (2). Kinase inhibitors of the VEGFR system.

cell migration (IC_{50}= 58 nM) in HUVECs. In multiple myeloma cells, the compound enhanced the growth inhibitory effect of dexamethasone [109]. Antiangiogenic activity has been demonstrated *in vitro* using a capillary sprout formation assay with pieces of rat aorta grown in a fibrin gel (IC_{50}= 675 nM).

Following oral administration to mice (50 mg/kg), the compound reaches a peak plasma concentration of 30 μM and remains at > 1 μM at 8 h [108]. In accordance with this pharmacokinetic profile, the compound displays good *in vivo* antiangiogenic and antitumor activity by the oral route in several animal models [108,100]. Vatalanib (50 mg/kg/day orally) has also been shown to have antimetastatic activity in an orthotopic murine renal cell carcinoma model [111] and in a human pancreatic xenograft [112]. It acts as a radiation sensitizer in a nude mouse model with a radiation-resistant tumor [113].

Vatalanib was well-tolerated in animals bearing human tumors and did not impair wound healing. It had no significant effects on circulating blood cells or bone marrow leukocytes as might be expected from its c-Kit inhibitory activity [108].

In a Phase I dose escalating study (300 – 1200 mg/kg), vatalanib was rapidly absorbed and had a mean terminal half-life of 5.9 h. No dose-limiting toxicity was observed and the most frequent adverse events observed were nausea and vomiting. Using dynamic contrast enhanced magnetic resonance imaging of tumor vascular surface area and vessel permeability as surrogate markers for efficacy, dose-dependent effects of vatalanib were observed that correlated with clinical outcome [114]. A dose of 1250 mg once daily was selected for further trials, and vatalanib has proceeded to Phase II/III trials as a single agent and in combination treatment.

ZD-6474 (AZD-6474, AstraZeneca plc; **11**, Fig. (**2**)) is a quinazoline derivative that inhibits VEGFR-2 (IC_{50}= 40 nM) and has some additional in vitro activity against other kinase receptors (e.g. IC_{50}= 110 nM and 500 nM for VEGFR-3 and EGFR, respectively) [115,116]. The *in vitro* selectivity profile observed for ZD-6474 is reflected in the ability of the compound to inhibit growth-factor-stimulated HUVEC proliferation, with IC_{50} values of 60, 170 and 800 nM against VEGF, EGF and bFGF, respectively, while not affecting the normal growth of endothelial cells or tumor cell proliferation at concentrations < 3 μM. Recently, it has been shown that ZD-6474 can also block the enzymatic activity of RET-derived oncoproteins (IC_{50}= 100 nM) [117], and the signaling of an EGF activated EGFR/RET chimeric receptor (see also PKI-166 in the section on EGFR inhibitors).

ZD-6474 (12.5 to 100 mg/kg/day) dose-dependently inhibited the growth of a broad range of human tumor xenografts (e.g. MDA-MB-231, A549, CaLu-6, SKOV-3, PC-3 and A431) [115,118]. In an spontaneous metastatic model of breast cancer, the compound inhibited primary tumor growth (94% following 32 days; 100 mg/kg/day) and the formation of pulmonary metastases [119].

Inhibition of VEGF-mediated effects has also been reported in several animal models. ZD-6474 (50 mg/kg po) reduces the vascular permeability of prostate tumor xenografts (28 % as measured by GdDTPA contrast-enhanced MRI) in mice, and the VEGF-induced hypotension (67 % following 50 mg/kg po) and femoral growth in rats [120]. The compound (2.5 mg/kg, iv) reversed in anesthetized rats a hypotensive change only if this alteration was induced by VEGF (by 63 %) [115].

In Phase I clinical trials, no dose limiting toxicity was observed at doses ranging from 50 to 500 mg/day [121]. Adverse events included skin changes that were dose-dependent and reversible. Dose-limiting toxicities (e.g. diarrhoea and rash) were observed at the 600 mg/day, and asymptomatic QT prolongation occurred in 14 % of the patients [122]. The compound is now in Phase II trials in patients with solid tumors.

Among the most recently disclosed clinical angiogenesis inhibitors is CEP-7055 (Cephalon Inc/Sanofi-Synthélabo; **12**, Fig. (**2**)), an orally active *N,N*-dimethyl glycine ester pro-drug of CEP-5214 (Cephalon Inc) [123], which was reported to be a potent inhibitor of VEGFR-1, -2 and -3 kinases with IC_{50} values in the low nanomolar range (18 -12 nM) [124]. In mice, CEP-7055 inhibits the growth of a variety of subcutaneous tumor xenografts (50 – 80 %; e.g. A375, Calu-6, Aspc-1 and U251MG/U87MG) and the number of metastases (65 %, RENCA model) after oral administration (10 – 20 mg/kg, bid) [125]. CEP-7055 is currently in Phase I clinical trials. Pfizer has recently promoted CP-547632 (OSI Pharmaceuticals Inc./Pfizer Inc., **13**, Fig. (**2**)), a selective thiazole-based VEGFR (IC_{50}= 11 nM) and FGFR (IC_{50}= 9 nM) inhibitor, into Phase I/II clinical trials [126]. The compound exhibited dose-dependent pharmacokinetics in cancer patients and the target plasma concentrations were exceeded at daily dosages ≥ 160 mg. AG013736 (Agouron Inc./Pfizer Inc.) is a dual VEGFR/PDGR inhibitor that produced dose-dependent inhibition of MV533 colon xenografts and Lewis lung cancer tumors in mice (ED50= 1.2 mg/kg), and inhibited metastasis in an orthotopic melanoma model [127]. Other companies, e.g. Kirin (e.g. KRN633) [128,129], Merck Sharp & Dohme [130], Chiron (e.g. CHIR-200131) [131], and Boehringer Ingelheim (e.g. BIBF1000) [132], have also VEGFR kinases inhibitors in late preclinical development.

KINASE INHIBITORS OF KIT – A NEW TREATMENT FOR GASTROINTESTINAL STROMAL TUMORS

More than 30 gain-of-function mutations have been identified in the Kit receptor [13]. The transforming mechanism of these mutations (single or multiple amino acid changes) involves dimer formation resulting in constitutive ligand-independent kinase activation. Activation of the kinase activity of Kit by somatic mutations has been documented in a number of human malignancies, including gastrointestinal stromal tumors (GISTs), seminoma, acute myelogenous leukemia, and mastocytosis. Paracrine or autocrine activation of this receptor has also been postulated for small-cell lung cancer and ovarian cancer [133].

GISTs are the most common mesenchymal tumors of the gastrointestinal tract [134]. The constitutive Kit kinase activity observed in these tumors was hypothesized to provide growth and survival signal to GIST cells and to be crucial to the pathogenesis of this disease [133,135,136]. This hypothesis became testable with the identification and evaluation of Kit kinase inhibitors. Preclinical studies showed that imatinib (STI-571, CGP57148, Novartis AG; **14**, Fig. (**3**)), which was originally developed as a bcr-abl kinase inhibitor [137-140],* inhibited also c-Kit kinase activity *in vitro* (IC_{50}= 0.1 μM), blocked autophosphorylation of wild-type and activated mutant forms of Kit in different tumor cell lines, and decreased cellular proliferation of GIST cells [141-143]. These results provided the rationale to move forward with clinical testing of imatinib mesylate as an anticancer therapy for these chemotherapy-refractory tumors. In an open-label, randomized, multicenter trial, 147 Kit-positive GISTs patients received imatinib at oral daily doses of 400 or 600 mg/day. Overall, 59 patients (40.1 %) had a partial response, 61 patients (41.5 %) had stable disease, and, for technical reasons, response could not be evaluated in 7 patients (4.8 %). Early resistance to imatinib was noted in 20 patients (13.6 %) [144]. In another study, tolerable doses were found to be up to 800 mg/day. There was a partial remission rate of 36 %, a minor remission rate of 33 % and a stable disease rate of 19 % [145,146]. ^{18}F-FDG-PET studies seem to indicate that the compound causes inhibition of intratumoral metabolism and growth [146]. The most common adverse events observed with imatinib in patients with GSTIs were edema, fatigue, nausea and diarrhea. Gastrointestinal or abdominal hemorrhages occurred in some patients with large tumors [144].

Clinical responses in GIST patients following treatment with imatinib appear to be associated with the presence of activating mutations of Kit as patients expressing wild-type

* Imatinib (Gleevec™/Glivec™) received FDA approval on May 10, 2001 for the treatment of patients with chronic myeloid leukemia (CML) after failure of interferon-α therapy. For additional information on this compound, see: http://www.glivec.com or www.gleevec.com

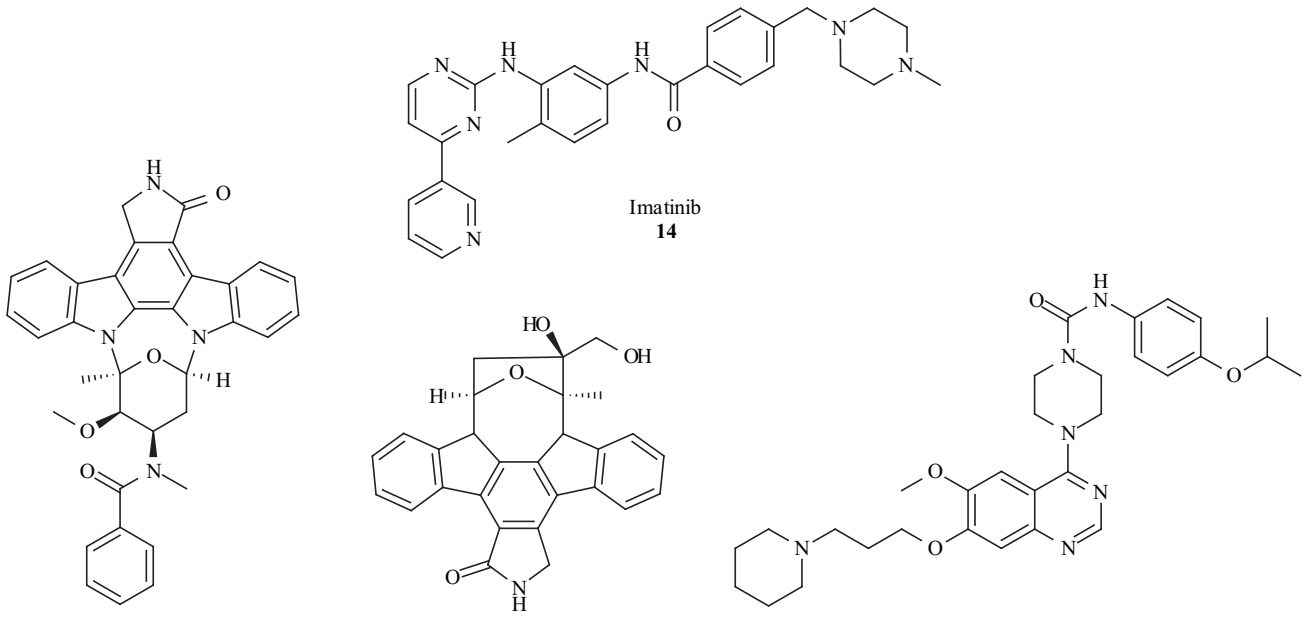

Fig. (3). Kinase inhibitors of c-kit and FLT3.

Kit had a significantly lower response [147]. The objective responses observed in the GSTIs clinical trails lead to approval of imatininb in February, 2002 by the FDA for the treatment of patients with Kit (CD117)-positive unresectable (inoperable) and/or metastatic malignant GISTs [147-149].

In addition to imatinib, other compounds in development inhibit Kit kinase activity in biochemical assays (e.g. SU-6668, SU-11248, PTK-787, ZD-6474 or PKC-412), but no selective inhibitor of Kit has been reported to date.

KINASE INHIBITORS OF FLT3 – A POTENTIAL TREATMENT FOR ACUTE MYELOID LEUKEMIA

The FLT3 receptor has been implicated in acute myeloid leukemia (AML) [150-153]. Constitutively activating internal tandem duplications (ITDs) in the juxtamembrane domain or mutations in the activation loop of the FLT3 receptor (e.g. missense mutation of D835) have been found in approximately 30 % of patients with AML [154,155]. These mutations confer a poor clinical prognosis and lower response rate in most retrospective studies suggesting that FLT3 may play a causative role in the progression of AML [154,155]. However, it has been recently reported that mutant FLT3 can only cause full-blown AML in conjunction with a second mutation affecting the differentiation of precursor cells [156]. Collectively, the data obtained with inhibitors that abrogate FLT3 kinase activity indicate that this receptor may be a viable therapeutic target for the treatment of AML.

To date, four inhibitors are in clinical trials in patients with AML harboring FLT3 activating mutations: i) midostaurin (PKC-412, CGP41251; Novartis AG; **15**, Fig. (**3**)), a staurosporine derivative with activity against PKC, VEGFR2, PDGFR, c-Kit, syk and FLT3 [157,158]; ii) CEP-701 (KT-5555, Cephalon Inc/Kyowa Hakko Kogyo Co Ltd; **16**, Fig. (**3**)), an indolocarbazole derivative reported to inhibit Trk and FLT3 [151] [159]; iii) SU-11248 (SUGEN Inc.; **9**, Fig. (**2**)), which inhibits PDGFR, VEGFR-2, FGFR-1, c-kit, and FLT3 [160,161]; and iv) MLN-518 (CT-53518, Millennium Pharmaceuticals Inc.; **17**, Fig. (**3**)) a quinazoline-piperazine derivative with activity against PDGFR, c-kit, and FLT3 [162]. In human FLT3-ITD-positive AML cell lines, these compounds induced apoptosis and inhibited ligand independent FLT3-ITD phosphorylation, cellular proliferation, and signaling through the PI3K and MAP kinase pathways. Administration of FLT3 kinase inhibitors to mice previously injected with cells carrying FLT3 mutations and developing a myelodysplastic syndrome significantly improved overall survival [150,151,157,162]. In addition to the preceding inhibitors, other compounds (e.g. indolinones and bis(1H-indolyl)-1-methanones) are able to inhibit the kinase activity of wild-type and mutant FLT3 *in vitro* and in cellular settings [163,164]. As for the c-Kit receptor, no selective inhibitor of FLT3 has been reported to date.

Due to the similarities between AML and blast crisis in chronic myeloid leukemia (CML), hematological response rates in AML patients treated with FLT3 kinase inhibitors should be similar to those found with imatinib in blast crisis CML [165,166]. Finally, as the imitanib experience has shown, resistance in relapsed AML patients is likely to occur following single-therapy with the FLT3 inhibitors.

ABBREVIATIONS

AML	=	acute myeloid leukemia
ATP	=	adenosine triphosphate
bFGF	=	basic fibroblast growth factor

bis = bis in die (twice a day)
c-Kit = receptor of stem cell growth factor
CML = chronic myeloid leukemia
COX-2 = cyclooxygenase-2
EGF = epidermal growth factor
EGFR = erbB-1, HER1, epidermal growth factor receptor
ErbB-2 = Her2, Neu, epidermal growth factor related receptor 2
ErbB-3 = HER3, epidermal growth factor related receptor 3
ErbB-4 = Her4, epidermal growth factor related receptor 4
FDA = Food and Drug Administration
FGF = fibroblast growth factor
FGFR = fibroblast growth factor receptor
^{18}F-FDG = 2-[fluorine-18]fluoro-2-deoxy-D-glucose
Flt-1 = fms-like tyrosine kinase 1
FLT3 = fms-like tyrosine kinase 3
5-FU = 5-fluorouracil
GI = gastrointestinal
GdDTPA = gadolinium (III)-diethyltriaminepentaacetic acid
GIST = gastrointestinal stromal tumor
INTACT = Iressa™ NSCLC trials assessing combination therapy
ITD = internal tandem duplications
HUVEC = human umbilical vein endothelial cell
IGF-IR = insulin-like growth factor-1 receptor
i.p. = intraperitoneal
InsR = insulin receptor
i.v. = intravenous administration
Met = hepatocyte growth factor receptor
MTD = maximum tolerated dose
MRI = magnetic resonance imaging
NDA = new drug application
NSCLC = non-small cell lung cancer
PDGF = platelet derived growth factor
PDGFR = platelet derived growth factor receptor
PET = positron emission tomography
PKB = Akt, protein kinase B
p.o. = per os (oral administration)
RET = rearranged during transfection
RNA = ribonucleic acid
RPTK = receptor protein tyrosine kinase
TACTIX = Tarceva™ in combination with taxotere and xeloda
Tek = tunica internal endothelial cell kinase 2
Trk = NTRK1/TPM3 chimeric oncogene
VEGF = vascular endothelial growth factor
VEGFR = vascular endothelial growth factor receptor
VEGFR-2 = KDR, vascular endothelial growth factor receptor 2

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