Inhibition of tumor angiogenesis by synthetic receptor tyrosine kinase inhibitors

Li Sun and Gerald McMahon

Protein tyrosine kinases have emerged as crucial targets for therapeutic intervention in cancer. More recently, growth factor ligands and their respective receptor tyrosine kinases (RTKs) have been shown to be required for tumor cell growth. This latter aspect includes tumor angiogenesis where the growth of tumors leads to compensatory effects on host cells in the tumor microenvironment leading to the growth of microvessels. The purpose of this review is to focus on synthetic chemical approaches to block RTKs associated with tumor angiogenesis as a means to limit the growth and spread of human tumors.

Angiogenesis involves the generation and growth of new blood vessels from pre-existing vasculature. The angiogenic process is defined by the activation of quiescent endothelial cells in pre-existing blood vessels followed by the growth and migration of endothelial cells leading to the dissolution of the vessel basement membrane. The migration and proliferation of endothelial cells then forms new capillary lumina and loops that lead to the formation of a new basement membrane and maturation of microvessels[1].

Under normal physiological conditions, angiogenesis has been regarded as a tightly controlled process that requires the balance of the anti-angiogenic and pro-angiogenic factors. In the absence of angiogenesis, the turnover rate of endothelial cells has been suggested to be in the order of years and would be expected to be enhanced during embryonic implantation and wound healing[2]. By contrast, pathological conditions in the adult would be predicted to lead to an angiogenic switch resulting in a shift in the balance between endogenous angiogenic inducers and inhibitors. Human diseases associated with angiogenesis include retinopathies as a consequence of diabetes or aging, rheumatoid arthritis, endometriosis, psoriasis, atherosclerosis and the growth of solid tumors[2].

Tumor angiogenesis

It has been well documented that angiogenesis plays a key role in the growth of solid tumors and their invasion and metastasis (Fig. 1)[3,4]. Angiogenesis has been shown to be a rate-limiting step in tumor development[5], and tumors have been limited to a growth of 1–2 mm^3 in the absence of blood supply. Beyond this minimum size, the growing tumor becomes necrotic and apoptotic[2]. As the tumor mass enlarges, hypoxic conditions in the center of the growing tumor prevail, resulting in the release of pro-angiogenic factors from tumor cells and supporting cells or stroma. As shown in Fig. 1, these angiogenic factors localize in the vicinity of pre-existing blood vessels leading to an initiation of the angiogenic process. The newly formed capillaries around the tumor mass have been suggested to provide three major functions during solid tumor development:

- Provision of nutrients for rapid tumor growth
- Removal of metabolic waste generated by tumor cells
- Transport of tumor cells to locations distal to the primary site.

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For these reasons, blockade of tumor angiogenesis has emerged as an attractive approach to devise novel therapies for the treatment of cancer[6]. In this regard, many therapeutic approaches and modalities have been shown to block tumor angiogenesis leading to a reduction in the growth of tumors in animal models[7,8]. As the angiogenesis process is relatively restricted to the growing tumor, the anti-angiogenic approach for cancer therapy would be predicted to have minimal target-directed side effects when compared with conventional chemotherapeutic agents. In addition, blockade of this process would have less chemoresistance, as non-tumor cells (i.e. endothelial cells) would be predicted to have less of a capacity to mutate and circumvent the blockade compared with anaplastic tumor cells. Several of these approaches are currently under clinical investigation including those to block the function of receptor tyrosine kinases (RTKs) or growth factor receptors[2].

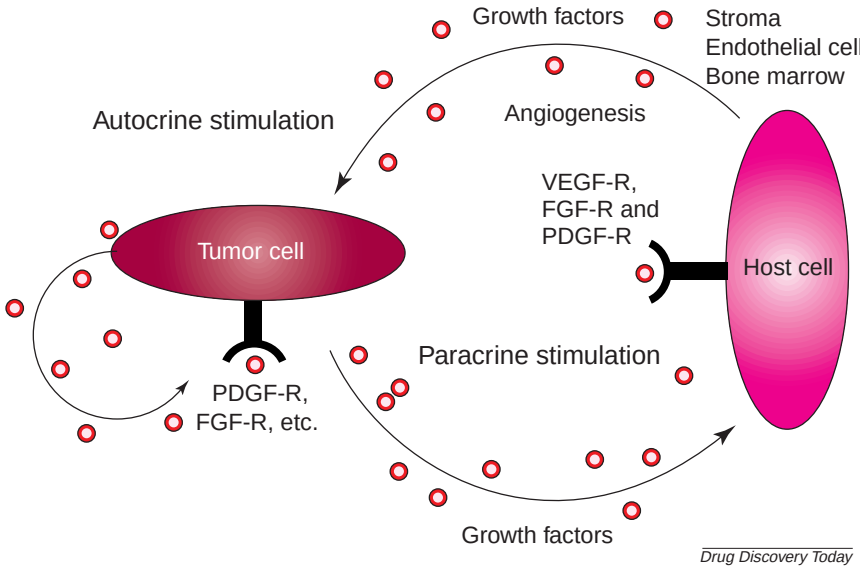

Figure 1. *Receptor tyrosine kinases involved in tumor growth. The growth of tumor cells is dependent on growth factors derived from the tumor (autocrine stimulation) or microenvironment (paracrine stimulation). Platelet-derived growth factor (PDGF) and fibroblast growth factor (FGF) are secreted from tumor cells and neighboring stromal and normal tissues (brain, liver, lung, bone) leading to enhanced growth and survival. Vascular endothelial growth factor (VEGF), FGF and PDGF stimulate the branching, extension and survival of endothelial cells resulting in the formation of new blood vessels during tumor angiogenesis.*

RTKs in tumor angiogenesis

Protein kinases comprise a family of enzymes that can transfer the terminal phosphate of ATP to protein substrates. In this regard, protein tyrosine kinases (PTKs) and serine/threonine kinases (STKs) play crucial roles in the proliferation, survival, differentiation and metabolism of cells. In the case of tyrosine kinases, RTKs traverse cellular membranes and bind to soluble and membrane-associated factors leading to a conformational change resulting in activation of the intracellular catalytic core. By contrast, in non-receptor TKs, the factor-binding extracellular domain is absent. In general, activation of receptors by formation of homo- or hetero-dimers (in the case of RTKs) or by tyrosine phosphorylation (in the case of non-receptor TKs) results in enzymatic activity catalyzing the rapid phosphorylation of tyrosine residues on the receptor or neighboring protein substrates.

RTKs can be grouped into several subfamilies depending on the extracellular and intracellular architecture and homology motifs. A large and related superfamily of RTKs is comprised of the platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), stem cell factor (SCF) and colony stimulating factor-1 (CSF-1) receptors. These receptors have been termed 'split' RTKs because of the presence of a unique insert region within a highly conserved catalytic intracellular core that is responsible for the enzymatic activity following ligand-induced receptor dimerization. As shown in Fig. 2, various RTKs are expressed on endothelial cells. However, the VEGF and angiopoietin-binding receptors exhibit a more restricted expression on endothelial cells compared with other cell types. For the purpose of this review, the discussion will focus on the split RTKs (VEGF-R, FGF-R and PDGF-R), which have been strongly implicated as therapeutic targets that influence the angiogenic process in growing tumors.

VEGFs and their receptors

VEGFs are secreted and expressed by tumor cells and surrounding tumor stromal cells in response to hypoxia. VEGFs transduce their biological activities following binding to RTKs such as VEGF-R2 (fetal liver tyrosine kinase 1/kinase insert domain-containing receptor; Flk-1/KDR) and VEGF-R1

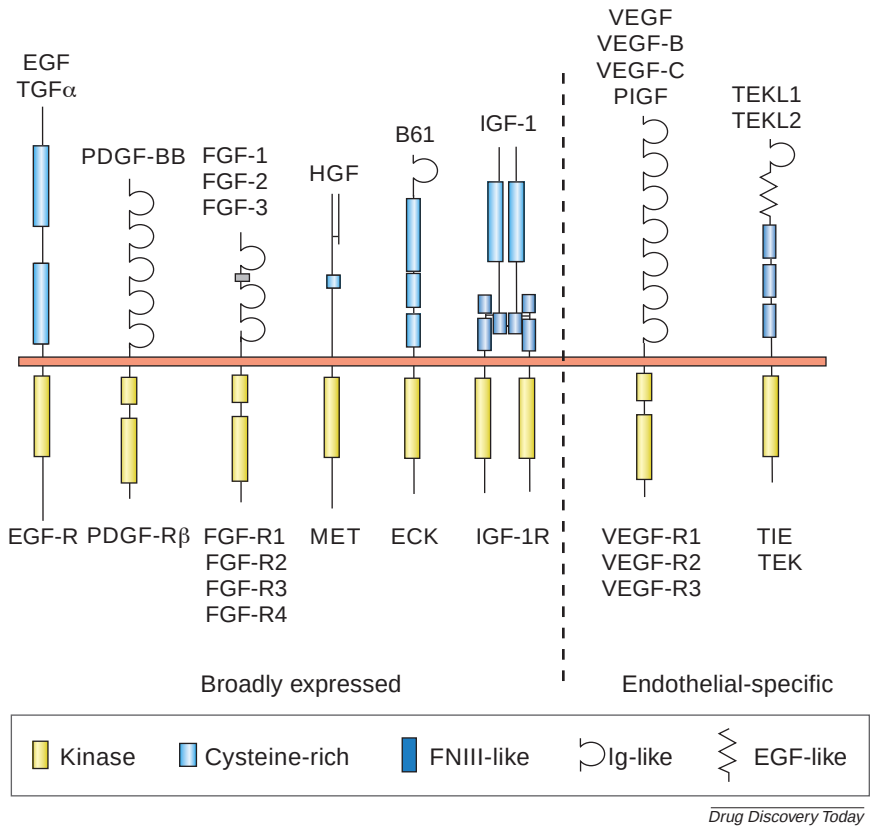

Figure 2. *Receptor tyrosine kinases (RTKs) expressed on endothelial cells. Several types of RTKs are expressed in endothelial cells. These receptors have been implicated in the growth, migration, survival, and differentiation of cells during the angiogenesis process. Vascular endothelial growth factors (VEGFs) and angiopoietins are factors that stimulate receptors expressed specifically on endothelial cells. Abbreviations: ECK, epithelial cell kinase; EGF, epidermal growth factor; FGF-R, fibroblast growth factor receptor; HGF, hepatocyte growth factor; IGF-1R, insulin-like growth factor 1 receptor; MET, hepatocyte growth factor/scatter factor receptor; PDGF-R, platelet-derived growth factor receptor; PIGF, placental growth factor; TEKL, tunica interna endothelial cell kinase ligands; TGF, transforming growth factor; TIE, tyrosine kinase with Ig and EGF homology domains.*

(Flt-1), which are localized to endothelial cells (Fig. 2). Flk-1/KDR is mitogenic for endothelial cells and is required for the development of mature endothelial cells[9,10]. Using various molecular validation approaches, FLK-1/KDR has been shown to be necessary for tumor angiogenesis and, hence, plays an important role in tumor growth, invasion and metastasis. These approaches have included the use of dominant-negative receptor mutants[11], germline disruption of the VEGF-R genes[12] and anti-VEGF monoclonal antibodies[13].

FGFs and their receptors

The FGF family of soluble growth factors is composed of a large number of structurally related proteins with a high heparin-binding affinity that exhibit a diversity of biological functions following binding to membrane receptors (FGF-Rs)[14]. FGF-Rs are expressed on many cell types and can be grouped into four subtypes (FGF-R1 to FGF-R4; Fig. 2). Importantly, endothelial cell culture and animal model systems have shown that FGF-R activation leads to angiogenesis[14]. It is of interest that FGF-Rs are also overexpressed, constitutively active and, in some cases, mutated (FGF-R3 and FGF-R4) in many tumor cells[14]. Active FGF-Rs might favor the angiogenic potential of tumor cells when compared with their normal cell counterparts where FGF-R activities are under tight regulation by local tissue constraints.

PDGFs and their receptors

As with FGF-Rs, PDGF-Rs are widely expressed in many adult tissues. PDGF-Rs promote tumor cell growth and survival by activation of autocrine loops in the tumor cell leading to the constitutive activation of PDGF-Rs (Fig. 1). Moreover, production of PDGFs by the surrounding tumor stroma or tissue bed (bone marrow, liver, lung) might contribute to growth and survival of tumor cells following spread from primary to metastatic sites. In addition, secretion of PDGFs by the growing tumor and surrounding stroma has been implicated in the endothelial cell-directed migration and proliferation of mesenchymal cells in the early stages of vessel formation[15]. PDGFs might also have growth stimulatory effects on pericytes[16] and the fibroblast-like cells that surround the endothelial cells[17], and could play an indirect role in angiogenesis by inducing VEGF secretion[18]. As with FGF-Rs, inhibition of PDGF-Rs would be predicted to block both angiogenic and tumor-specific mechanisms required for the growth and spread of cancers.

Synthetic RTK inhibitors

During the past decade, chemically diverse small-molecule protein kinase inhibitors have been discovered to be

potential therapeutic agents for various human diseases such as retinopathies, atherosclerosis, psoriasis, rheumatoid arthritis, endometriosis and solid tumor growth[19–22]. For example, inhibitors of the epidermal growth factor receptor (EGF-R) have included dianilinophthalimides[23], quinazolines[24,25], pyridopyrimidine[26–29], pyrrolopyrimidines[30], pyrazolopyrimidines[31], phenylaminopyrimidines[32] and isoflavones[19]. Importantly, it was deduced that many of these compounds compete for binding with ATP in the vicinity of the intracellular catalytic core of the EGF receptor, thereby leading to inhibition of tyrosine phosphorylation and, consequently, kinase activity of the EGF-R. Several of these EGF-R inhibitors are currently under clinical evaluation for the treatment of human cancers associated with overexpressed EGF receptors[33].

More recently, these chemical scaffolds and others have been shown to inhibit various other protein kinases viewed as molecular targets for therapeutic intervention for the treatment of human cancers[34]. These have included kinases such as the split RTKs [VEGF-R, PDGF-R, FGF-R, KIT (stem cell factor receptor)] and other RTKs [EGF-R, MET (hepatocyte growth factor/scatter factor receptor)], non-receptor TKs (c-Src, c-Abl), and the serine/threonine cyclin-dependent kinases (CDKs). As with the aforementioned EGF-R inhibitors, new ATP-mimetic chemical scaffolds were identified[34]. In addition, more than 30 published kinase co-crystallographic determinations have been made to date, involving kinase inhibitors or ATP (Refs 35–43). This information has been used to define molecular interactions for these inhibitors in the active site leading to the design of selective inhibitors for particular protein kinases.

Structure–activity relationships (SARs) were determined and molecular modeling studies further extended these observations to propose rules for particular chemical scaffolds that influence kinase selectivity due to substituent interactions around the scaffold core[34]. In this regard, quinazolines were synthesized and found to be inhibitors of EGF-R, Her-2, PDGF-R, VEGF-R, FGF-R and CSF-1R TKs. Pyridopyrimidines were found to inhibit EGF-R, FGF-R, PDGF-R and c-Src TKs, pyrazolo- and pyrrolo-pyrimidines have shown inhibitory activity against EGF-R, CSF-1R, FYN, LCK and c-Src TKs (Refs 30,31,34,44), and phenylaminopyrimidines are inhibitors of EGF-R, PDGF-R, c-Abl, PKC-α, CDK1 and CDK2 protein kinases[32]. In addition, the indolin-2-one ATP-mimetic scaffold emerged, which has inhibitory activity against Flk-1/KDR, FGF-R1, PDGF-R and other protein kinases[42,45,46]. Additionally, 3-phenyl-4(1*H*)-quinolones were identified and exhibited high potency and selectivity against EGF-R (Ref. 47).

Quinazolines as inhibitors of VEGF RTKs

The 4-phenylamido-quinazoline pharmacophore was first identified as a potent and selective inhibitor of EGF-R (Ref. 48). Recent studies for this class have revealed that modification of substituents around the core produces selective inhibitors of various PTKs including VEGF-Rs (Refs 49,50, Hennequin, L.F. *et al.* The design and synthesis of a novel, orally active VEGF-receptor tyrosine kinase inhibitor. *90th Annual Meeting of American Association for Cancer Research*, 10–14 April 1999, Philadelphia, PA, USA, Abstract 457). In this latter study, a series of quinazolines have been identified as potent inhibitors of both Flt-1 and Flk-1/KDR. A comparison of the VEGF-R inhibitors with the EGF-R inhibitors have revealed structural differences including the position of halogen substituents or the presence of small hydrophilic substituents (e.g. OH) on the aniline side chain.

From this series, the compound ZD4190 (Fig. 3) was identified and found to inhibit Flt-1 and Flk-1/KDR TKs, but was found to be inactive against the FGF-R TK. It was suggested that this compound would be ATP-competitive inhibitor as are the EGF-R quinazolines[48,51]. In cell-based assays, ZD4190 exhibited increased potency to inhibit VEGF-dependent human umbilical vein endothelial cell (HUVEC) proliferation when compared with FGF-dependent HUVEC proliferation. By contrast, the growth of tumor cells *in vitro* was not inhibited by ZD4190 (Refs 49,50).

Antitumor studies in mice demonstrated that ZD4190 was efficacious, through the use of a diverse panel of human xenografts including cells derived from the colon, lung, breast, prostate and ovarian origin[49]. In addition, acute effects of ZD4190 on PC3 (prostate cancer) vascular permeability was demonstrated using magnetic resonance imaging (Wedge, S.R. *et al.* Effect of the VEGF receptor tyrosine kinase inhibitor ZD4190 on vascular endothelial permeability. *90th Annual Meeting of American Association for Cancer Research*, 10–14 April 1999, Philadelphia, PA, USA, Abstract 2741). It was concluded that the antitumor properties of ZD4190 are mainly associated with its anti-angiogenic effects caused by its potent inhibitory activity against the VEGF-Rs.

In addition, ZD6474 (its structure has not been disclosed) has emerged as a PTK inhibitor with preferred pharmacokinetic properties (Wedge, S.R. *et al.* VEGF receptor tyrosine kinase inhibitors as potential anti-tumour agents. *91th Annual Meeting of American Association for Cancer Research*, 1–5 April 2000, San Francisco, CA, USA, Abstract 3610), as it is 1400-fold more soluble than ZD4190 in phosphate buffer with a pH of 7.4. The pharmacokinetic property of ZD6474 is compatible with once-daily oral

dosing and shows broad antitumor efficacy using this regimen. ZD6474 exhibits a nanomolar inhibition of KDR with sub-micromolar potencies against EGF-R, Flt-1 and FGF-R1, while in cell-based assays, ZD6474 inhibited VEGF-stimulated HUVEC proliferation with sub-micromolar inhibitory activity towards EGF- and bFGF (basic FGF)-stimulated HUVEC proliferation. In addition, ZD6474 has a broad antitumor spectrum in human tumor xenografts and, in some cases, can induce regression of established tumors. ZD6474 is currently in Phase I clinical trials.

Indolin-2-ones as kinase inhibitors of VEGF, FGF and PDGF receptors

3-Substituted indolin-2-ones were identified as inhibitors of the split RTKs including the receptors for VEGF, FGF and PDGF (Refs 42,45,46). These compounds inhibit the Flk-1/KDR TK, indicating their potential use in the treatment of angiogenesis-related disorders. SAR studies indicate that the selectivity of these compounds for particular RTKs depends on the substituents on the indolin-2-one core. Three 3-substituted indolin-2-ones have been co-crystallized with the catalytic domain of FGF-R1 (*flg*) and were found to localize in the ATP-binding site[35,42,43]. These structural studies identified these molecules as ATP-mimetic inhibitors and provided information for further chemical structural modification.

These studies also led to the identification of several classes of substituted indolin-2-ones that are selective inhibitors of different RTKs at the level of the enzyme and in cellular systems[42,45,46]. Two compounds of this type, SU5416 and SU6668 (Fig. 3), were derived from these studies and are currently in late (Phase III) and early clinical evaluations, respectively. In addition, both SU6668 and SU9902 (Fig. 4) were shown to be localized in the ATP-binding sites of the FGF-R1 catalytic core by co-crystallographic studies[42,43]. As with SU5402 (Ref. 35), SU9902 (Fig. 3) alters the conformation of the adenine binding pocket because of coordination of Asp568 to the propionic acid side chain substituent on the pyrrole ring (Fig. 4). This latter distortion of the nucleotide-binding loop by SU9902 might explain, in part, the increased potency of this compound for FGF-R1 compared with compounds such as SU5416 where such a substituent is absent.

SU5416 was found to be a potent and selective inhibitor of Flk-1/KDR when tested using both kinase and cellular assays[52]. It selectively inhibits VEGF-dependent mitogenesis and migration of human endothelial cells without inhibiting the growth of a variety of tumor cells in tissue culture systems. However, SU5416 significantly inhibited the growth of many tumor cell lines when grown as subcutaneous xenografts in mice, suggesting the involvement of a paracrine mechanism (Fig. 1) consistent with the angiogenesis mechanism.

In other studies, SU5416 altered tumor vascular density and vascular leakage after tumor implantation[52]. SU5416 also induced the regression of established tumors[53] and induced apoptosis of endothelial cells in a model of colon metastasis[54]. SU5416 is currently in late-stage clinical evaluation for the treatment of AIDS-related Kaposi's sarcoma, colorectal cancer and non-small cell lung cancer. In a dose-escalating Phase I study, SU5416 had significant biological activity in patients with AIDS-related Kaposi's sarcoma, a disease characterized by small tumors on the skin that are fueled by angiogenesis. The study also showed that SU5416 was well tolerated without effects on viral load or on the effects of other systemic HIV therapies. The maximum tolerated dose of 145 mg m^{-2} was the same as that established in patients with advanced solid tumors. In addition, a pilot study of SU5416 in combination with 5-fluorouracil and leucovorin is prepared for an ongoing Phase III trial for colorectal cancer.

SU6668 was identified as a TK inhibitor associated with Flk-1/KDR, FGF-R1 and PDGF-R (Ref. 43). By contrast to this inhibition, SU6668 lacks inhibitory activity towards the MET or EGF-R. Furthermore, by contrast to SU5416, SU6668 inhibited both VEGF- and FGF-dependent proliferation of HUVEC, which was consistent with its increased inhibitory activity at FGF-Rs. Furthermore, SU6668 induced tumor growth stasis or regression after oral administration to mice bearing a wide variety of tumor xenografts[43]. The anti-angiogenic properties of SU6668 were shown using intravital multi-fluorescence videomicroscopy in the dorsal skin chamber model, and the lack of blood vessels was shown using models of colon metastasis[54]. SU6668 is currently in early-stage clinical evaluation for the treatment of human cancers.

Pyridopyrimidines as inhibitors of FGF and VEGF TKs

As with 4-phenylamido-quinazolines, pyridopyrimidines were first discovered as highly selective and potent inhibitors of the EGF-R TK and were later found to inhibit other kinases including the PDGF-R, FGF-R and c-Src kinases by modification of the substituents around the core[29]. For instance, PD166285 (Fig. 3) exhibited nanomolar inhibition at the PDGF-R, FGF-R and c-Src (Ref. 55). Modification of the substituents on this core resulted in inhibitors with different kinase selectivity profiles. For example, PD173074 (Fig. 3) displayed potent and selective inhibitory activity towards FGF-R TK without affecting other kinase activities associated with PDGF-R, EGF-R and c-Src.

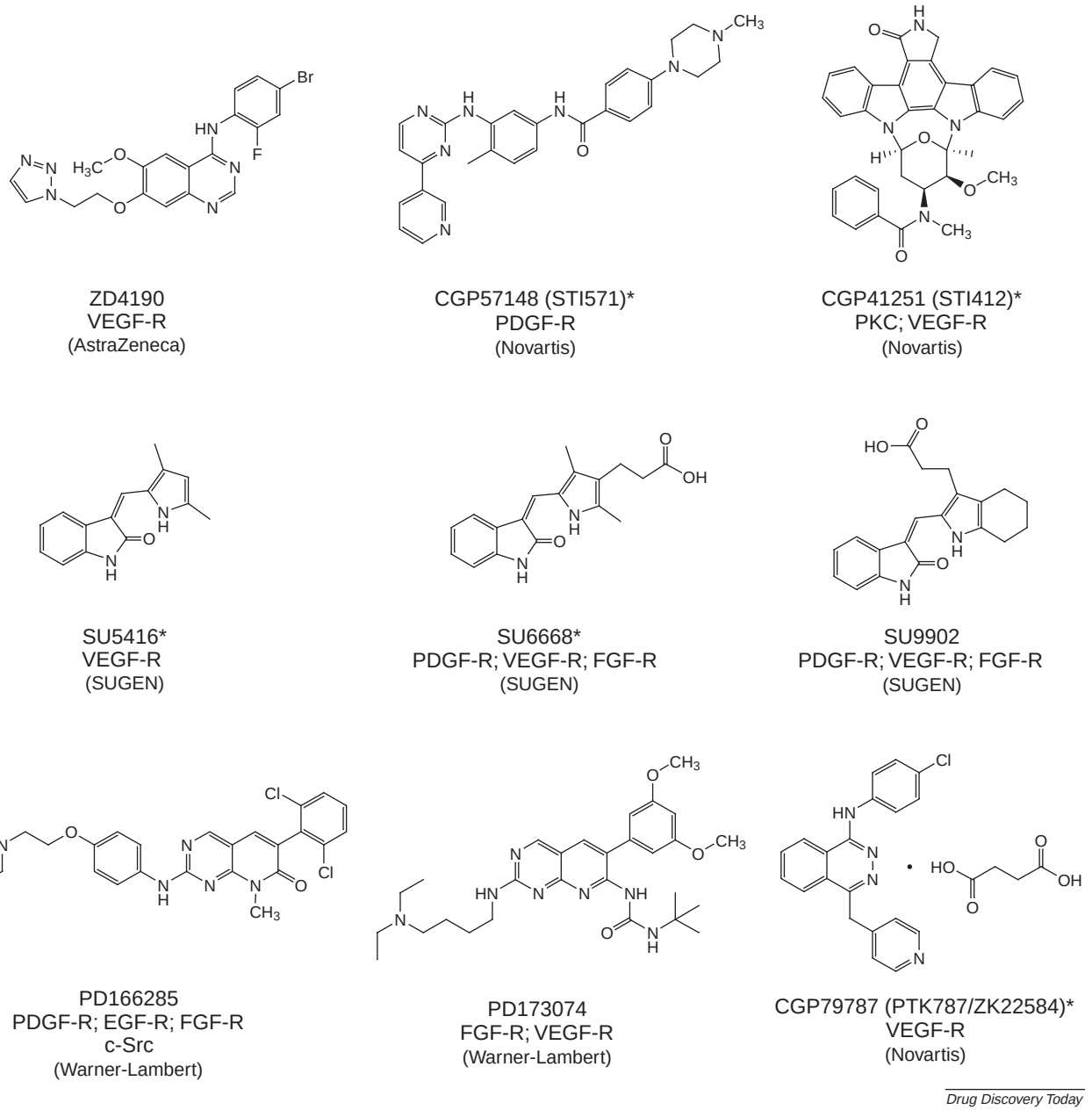

Figure 3. *Synthetic inhibitors of the vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF) and platelet-derived growth factor (PDGF) receptor tyrosine kinases. Shown are chemical structures relating to synthetic compounds that have inhibitory activities against the catalytic activities of the VEGF, FGF and PDGF receptor tyrosine kinases. Some of these inhibitors (*) are currently in clinical trials for the treatment of human diseases including cancers.*

To elucidate the mechanism of action of these compounds, PD173074 was co-crystallized within the catalytic domain of FGF-R1 (Ref. 56). The crystallographic analysis confirmed the ATP-dependent mechanism of inhibition for this structural class[56]. Both PD166285 and PD173074 showed potent inhibitory activity against the proliferation of HUVECs. PD166285 inhibited both VEGF- and FGF-stimulated HUVEC proliferation whereas PD173074 specifically inhibited FGF-stimulated HUVEC growth[56]. These compounds also inhibited the formation of microcapillaries on Matrigel-coated plastic and exhibited potent anti-angiogenic and antitumor activity. Additional preclinical studies

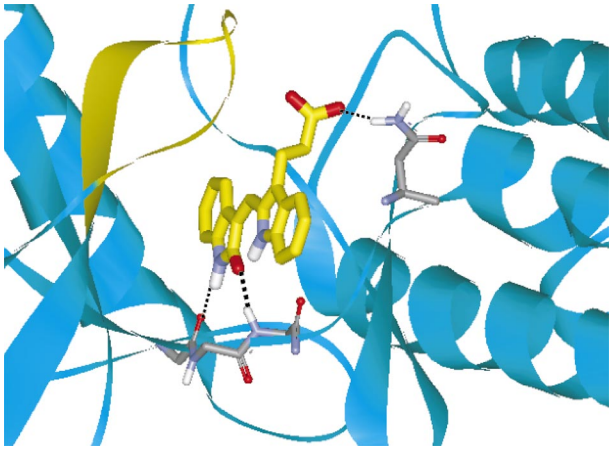

Figure 4. *Crystallographic analysis of the fibroblast growth factor receptor-1 (FGF-R1) tyrosine kinase inhibitor SU9902. SU9902 is shown localized in the ATP-binding pocket of the catalytic core of the FGF-R1 tyrosine kinase. Hydrogen bonds are delineated between SU9902 and the FGF-R1 adenine pocket. The nucleotide-binding loop (yellow) denotes a conformational pertubation in the binding pocket compared with crystallographic analysis of FGF-R1 in the presence of ATP. This change has been interpreted to reveal that the propionic acid of SU9902 might interact with Asn568 leading to increased inhibitory potency for this compound.*

are ongoing to provide rationales for further clinical development of these two compounds.

Phenylamino-pyrimidines as inhibitors of PDGF-R TK

Phenylamino-pyrimidines have been discovered and developed as selective inhibitors of various PTKs, including EGF-R, PDGF-R, Abl, PKC-α and CDKs (Ref. 32). Of particular interest is STI571 (CGP57148; Fig. 3), which is a potent ATP-competitive inhibitor of the *bcr*-Abl PTK (Ref. 57) and inhibits substrate phosphorylation by v-Abl, *bcr*-Abl and c-Abl TKs and PDGF-R and c-KIT (SCF-R) TKs in isolated enzyme or in cells[58,59]. By contrast, STI571 does not inhibit other PTKs or STKs. In addition, STI571 suppresses the proliferation of *bcr-abl*-expressing leukemic cells *in vivo*[58]. Such studies have provided the rationale for STI571 to enter clinical trials for the treatment of chronic myelogenous leukemias (CML), which contain activated forms of the *bcr*-Abl TK. In Phase I clinical trials, STI571 was administered at 25 and at 50 mg in CML patients who had failed IFN-α therapy and showed no serious side effects. In addition, at higher doses (>250 mg for up to 5 months), 100% of patients responded to the therapy. STI571 is currently in Phase II evaluation for the treatment of leukemias and other human cancers.

Staurosporine derivatives as inhibitors of PDGF and VEGF RTKs

STI412 (CGP41251; *N*-benzoyl staurosporine; Fig. 3) was originally identified as an inhibitor of protein kinase C (PKC) and later found to inhibit the VEGF-R TK (Ref. 60). STI412 selectively inhibits conventional calcium-dependent and diacylglycerol (DAG)-dependent PKCs, and both STI412 and its parent compound, staurosporine, are competitive with respect to ATP (Ref. 23). In cell-based assays, STI412 selectively inhibited PDGF-, VEGF- and SCF-induced autophosphorylation of their cognate receptors but did not affect the autophosphorylation of insulin-like growth factor receptor-1 (IGF-1) and EGF-R (Ref. 60). In addition, the inhibition of these RTKs correlated with inhibition of the downstream biological events such as mitogen-activated protein (MAP) kinase activation and induction of c-*fos* transcription[60]. STI412 inhibited the growth of various tumor cell lines *in vitro* with IC_{50} values of 0.04–1.94 μM.

In vivo studies indicated that STI412 exhibits dose-dependent tumor growth inhibition when various human tumor types were evaluated. In addition, STI412 inhibited angiogenesis stimulated by VEGF, but not by FGF. These studies have suggested that the antitumor properties of STI412 might be owing to both its anti-angiogenic effects on RTKs and its inhibitory activities on PKCs. A Phase I dose-escalation study in healthy male volunteers showed that STI412 was well tolerated up to 25 mg (Czendlik, C. and Graf, P. Safety, tolerability and pharmacokinetics of CGP-41251, a protein kinase C inhibitor (Phase I study). *NCI Eortc Symposium on New Drugs in Cancer Therapy*, 1996, Amsterdam, The Netherlands, Abstract 264]. An additional Phase I trial has been conducted on 26 patients with advanced solid tumors using doses ranging from 12.5 mg once-daily to 100 mg three times-a-day. No significant side effects were seen with doses up to 50 mg twice-a-day. STI412 is currently in early Phase II clinical evaluation for the treatment of various human cancers including colorectal cancer, breast cancer, chronic lymphocytic leukemia and non-Hodgkin's lymphoma[61].

Phthalazine derivatives as inhibitors of VEGF and PDGF RTKs

CGP79787, also known as PTK787 or ZK22584, is an analog of phthalazines (Fig. 3)[62]. Although there is no specific data published for CGP79787, it is known that these compounds inhibit both VEGF-R and PDGF-R RTKs in an ATP-competitive manner[62], but have no inhibitory effects on other receptor and non-receptor TKs including EGF-R, FGF-R, Tek, c-Src and v-Abl. In cell-based assays, these

phthalazine-based compounds inhibited VEGF-stimulated HUVEC proliferation at sub-micromolar concentrations. *In vivo* studies have revealed that selected compounds from this series exhibited dose-dependent inhibition of VEGF and PDGF-induced angiogenesis in mice[63,64]. CGP79787 is currently in Phase II clinical trials for the treatment of solid tumors. In addition, a Phase I trial of CGP79787 in patients with HIV-associated Kaposi's sarcoma is planned (National Cancer Institute: Angiogenesis inhibitors in clinical trials/Phase I study of PTK787/ZK222584 in patients with HIV-associated Kaposi's Sarcoma. http://cancertrials.nci.nih.gov/).

Conclusion

Since first proposed by Judah Folkman in 1971, angiogenesis has been shown to be important in the etiology of many human diseases. In cancer, an assessment of tumor angiogenesis has been viewed as a potential indicator of tumor aggressiveness and patient prognosis. Extensive studies in the field of tumor angiogenesis in the past two decades have validated various cancer targets including proteases and integrins resulting in the discovery of many new anti-angiogenic agents showing promise in animal models. Several of these inhibitors are currently in clinical evaluation[8]. It is important to note that many anti-angiogenesis therapies have been tested in preclinical models in combination with more traditional cytotoxic therapies. In these cases, such studies have suggested that angiogenesis inhibitors might be used in front-line, adjuvant, and even preventive settings for the emergence or regrowth of malignancies.

Many clinical studies are currently addressing the combined use of angiogenesis inhibitors with more conventional anticancer approaches (i.e. radiation or chemotherapy) to assess the contribution of the anti-angiogenic therapy to decrease disease progression and patient survival. It is also clear that approaches to inhibit RTKs and tumor angiogenesis might provide a novel method for the development of new agents for the treatment of human cancers and other diseases.

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