INVITED REVIEW

Overview of fundamental study of pazopanib in cancerHong-Lin Zhao^{1*}, Fan Yang^{1*}, Xin Huang^{2,3} & Qing-Hua Zhou¹¹ Tianjin Key Laboratory of Lung Cancer Metastasis and Tumor Microenvironment, Tianjin Lung Cancer Institute, Tianjin Medical University General Hospital, Tianjin, China² Key Laboratory of Longevity and Aging-Related Diseases, Ministry of Education, Beijing, China³ Center for Translational Medicine, Guangxi Medical University, Nanning, Guangxi, China**Keywords**

Angiogenesis inhibitors; neoplasms; pazopanib.

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Received: 18 March 2014;

Accepted: 4 May 2014.

doi: 10.1111/1759-7714.12136

Thoracic Cancer 5 (2014) 487–493

Abstract

Angiogenesis is an indispensable process for tumor growth and metastasis. Anti-angiogenesis based therapy is one of the most promising treatments for inhibiting cancer progression. Through the exploration of inhibitors of vascular endothelial growth factor receptor (VEGFR)-2, deemed as the major angiogenesis pathway, pazopanib was found as a small molecular pan-VEGFR and pan-platelet-derived growth factor receptor (PDGFR) inhibitor, with suitable pharmacodynamic and pharmacokinetic parameters to be an oral drug. In an vitro study, pazopanib exerted anti-tumor effect through mechanisms including the Raf-MAPK/ERK (MEK)-extracellular signal-regulated kinase (ERK) pathway, and directly targeted on v-raf murine sarcoma viral oncogene homolog B (B-raf) as well. It inhibited the proliferation of cell lines, such as DU-145 and HRC-45 in hepatocellular carcinoma, through mechanisms like “cell cycle arrest.” In vivo xenograft studies and phase I/II clinical trials revealed a series of plasma cytokine and angiogenic factors, such as interleukin (IL)-6, IL-12, hepatocyte growth factor (HGF), and soluble VEGFR2, which have significant association with clinical curative effect. Pazopanib has been shown to be effective in solid tumors and some hematological malignancies. Future studies should focus on the exploration of biomarkers to screen sensitive patients and concomitant or metronomic dosage with other kinds of medicines.

Introduction

Cancer is one of the most tremendous threats to the well being of mankind, and the burden it creates continues to increase, because of the aging and growing global population, and lifestyle choices, such as smoking and a westernized diet.¹ Based on the statistics of GLOBOCAN 2008, there were nearly 12.7 million new cancer cases and 7.6 million cancer deaths in 2008.¹ Oncologists conclude that although the comprehensive therapy of cancer, including surgery, chemotherapy, and radiotherapy, has advanced significantly in the last 50 years, it has reached its plateau of efficacy.² Angiogenesis is a process of forming a new capillary. It is a key biological phenomenon of normal physiological process, such as wound healing and reproduction.³ Furthermore, it also plays a critical role in many aspects of tumorigenesis: from tumor formation, to invasion or even metastasis. In 1972, Folkman⁴ deemed that the growth of new capillary is mediated by a kind of diffusible factor, called the tumor-angiogenesis-factor (TAF), which can be isolated and induce proliferation of new

capillaries in places where TAF is injected. Combined with the evidence of lagging growth of unvascularized tumors, such as tumors in soft agar or tiny tumors implanted in the anterior chamber of the eye of animals, it can be inferred that, if TAF is indeed the mediator of angiogenesis, the blockade of TAF might lead to anti-angiogenesis. Actually, the so called “TAF” refers to the vascular endothelial growth factor (VEGF) family, platelet-derived growth factor (PDGF) family, and other angiogenesis-related growth factors, inflammatory mediators, enzymes, hormones, oligosaccharides, hematopoietic factors, and cell adhesion molecules.⁵ Tumor angiogenesis is the imbalanced outcome of these proangiogenic and antiangiogenic cytokines, as well as the involvement of various cell types, such as endothelial cells and pericytes.⁶ The process of angiogenesis has several different sequential steps, and each step is dependent upon specific factors, which also have some direct or indirect association with each other.

Among the factors, VEGF and PDGF are the two most indispensable. The VEGF family includes five members: VEGF (or VEGF-A), placenta growth factor (PlGF), VEGF-B,

VEGF-C, and VEGF-D.⁷ The VEGF ligands bind with differing specificities and affinities to three transmembrane tyrosine kinase receptors: VEGF receptor-1 (VEGFR-1)/fms-like tyrosine kinase 1 (Flt-1); VEGFR-2/human kinase insert domain receptor (KDR)/flk-1; and VEGFR-3/fms-like tyrosine kinase 4 (Flt4).⁷ Both VEGFR-1 and VEGFR-2 are essential during embryonic vasculature. Homozygous mutants of them are lethal.⁵ Further studies have confirmed that VEGF signaling through the VEGFR-2 pathway is fundamental to the initiation and promotion of angiogenesis.⁷ VEGFR-2 is confirmed to be the antagonistic receptor of VEGFR-1,^{7,8} while VEGFR-3, together with its ligand VEGF-C and VEGF-D, control lymphangiogenesis.⁷ The PDGF family includes five dimeric ligands: AA, BB, CC, DD, and AB.^{9,10} These isoforms exert their cellular effects through tyrosine kinase α - and β -receptors formed in homodimer or heterodimer, which play an important role in angiogenesis through recruitment of multiple cell types needed for angiogenesis.¹¹ The stem cell factor receptor (CSFR)/c-Kit is a tyrosine kinase that is involved in survival, self-renewal, and differentiation of hematopoietic stem cells. It has been reported that the CSFR/c-Kit participates in several types of malignancies, either through mutations that induce the receptor to remain constantly active, such as in mast cell leukemia, or through autocrine loops in which the tumor cells produce c-Kit, such as in small cell lung carcinomas and certain types of melanoma.¹²

Although there are many reviews on pazopanib, most of them focus on clinical studies or overall progression in preclinical and clinical investigation. Meanwhile, several teams focused on various fields of preclinical studies have found some exciting discoveries of pazopanib. Therefore, in this review, the preclinical studies of pazopanib are reviewed and reorganized, thus offering an integrated outline of these fundamental studies and guidance for further clinical study.

Chemistry and pharmacodynamics

Pazopanib (VOTRIENT, GW786034, GlaxoSmithKline), with the chemical name of 5-[[4-[(2,3-dimethyl-2H-indazol-6-yl)methylamino]-2-pyrimidinyl]amino]-2-methylbenzenesulfonamide monohydrochloride, is an orally bioavailable multi-targeted tyrosine kinase receptor inhibitor.¹³ The molecular structure of it is showed in Figure 1. Its benefits were discovered during screening for compounds that suppressed VEGFR-2.¹⁴ An adenosine triphosphate (ATP)-like component of the pazopanib structure forms hydrogen bonds with the tyrosine kinase receptors and competes with ATP for binding with the intracellular side of tyrosine kinase receptors, thus inhibiting ATP-induced activation.¹⁵ Pazopanib inhibits potent and specific VEGFR-1,-2,-3, PDGFR- α ,- β , the macrophage colony-stimulating factor (M-CSF) receptor/fms, and the CSFR/c-

Figure 1 Molecular structure of pazopanib.¹³

kit, with kinase IC_{50} 7, 15, 2, 73, 215, 48, and 6 nM, respectively.¹⁶ Investigators conducted an assay aimed at evaluating the inhibitory effect against wild-type VEGFR-2, v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog (c-Kit), PDGFR- β , and fms-related tyrosine kinase 3 (FLT-3) receptors in human umbilical vein endothelial cells (HUVEC), NCIH526, HFF, and RS4; 11 cells by Western blot. The IC_{50} toward each receptor of pazopanib was 8, 3, 2.6, and more than 1000 nM, separately. Therefore, the results of experiments in vivo were in accordance with that in vitro.

Pharmacokinetics

A phase I clinical trial of 63 patients with refractory malignancies revealed its pharmacokinetic and pharmacodynamic properties and evaluated its safety.¹⁷ Overall, the mean maximum plasma concentration (C_{max}) and area under the curve (AUC)₀₋₂₄ on the first day accelerated at the same pace as the escalation of the pazopanib dose, and the top value appeared in the 2000 mg group. Though the greatest mean exposure to pazopanib after a single dose was observed in the 2000 mg dose group, steady-state exposure to pazopanib seemed to plateau in the 800 mg once-daily dose group: the C_{max} in the 22nd day was similar to that of the 1000 mg once-daily, 1400 mg once-daily, and 2000 mg once-daily groups. It indicated that a dose of 800 mg once daily would be an adequate amount to achieve the greatest exposure.^{16,18} When comparing the pharmacokinetics of two different methods of administration (oral administration vs. continuous infusion), it can be concluded that the antitumor and antiangiogenic activity of pazopanib is dependent on steady-state concentration above a threshold.¹⁶ Crushing the tablet would greatly influence the pharmacokinetics of pazopanib: C_{max} increased 2-fold, while AUC increased and concentration of peak time (T_{max}) decreased by two hours.¹⁹ Therefore, it is recommended the tablet is taken as a whole, as its effect relies on the steady-state plasma concentration. Moreover, the mean of pazopanib C_{max} and AUC values is elevated twofold when pazopanib is taken with food, rather than in a fasting condition. Therefore, pazopanib is recommended to be administered on an empty stomach (at least 1 hour before or 2 hours after a meal).²⁰

The binding of pazopanib to human plasma protein *in vivo* was greater than 99% with no concentration dependence in the range of 10 to 100 $\mu\text{g/mL}$,¹³ which indicates a tremendous gap between the capability of inactivating the protein *in vitro* and inhibiting neoplasms *in vivo*.

Pazopanib is mainly metabolized by cytochrome P-450(CYP)3A4 in the liver, as well as by CYP1A2 and CYP2C8 in a low degree, and eliminated primarily by feces, with renal elimination accounting for <4% of the total dose.¹³ Elimination half-life on the first day and time to maximum observed plasma concentration on the first and 22nd day at the dose level of 800 mg once daily were 31.1, 3.5, and two hours, respectively. On the 22nd day at 800 mg in a once daily routine, the mean C_{24} was 24 $\mu\text{g/mL}$, while the clinical activity seemed to be related to the 22nd C_{24} values of at least 1524 $\mu\text{g/mL}$ in renal cell carcinoma (RCC) in preclinical studies.^{16,21}

Other fundamental study

In solid tumors

In 2011, it was discovered that pazopanib directly targets v-raf murine sarcoma viral oncogene homolog B (B-Raf) in an enzymatic assay.²² An *in vivo* xenograft study of pazopanib showed that pazopanib prevents the brain metastasis growth of human breast cancer cell line 231-BR-HER2, rather than through anti-angiogenic pathways. The 231-BR-HER2 cell harbors B-Raf mutations, meaning that the routine anti-proliferative pathway Raf-MAPK/ERK (MEK)-extracellular signal-regulated kinase (ERK) is blocked. The direct inhibition reveals a novel target, B-Raf, accounting for the angiogenesis effect. Moreover, independent enzymatic Ki studies demonstrated a 2.5-fold lower activity of pazopanib toward V600E B-Raf form.²² Pazopanib inhibition of tumor cell B-Raf is associated with its anti-angiogenic activity when quantified by vessel density and area.²³ Apart from the favorable B-Raf status, the expression of multiple targets, such as WM3918 cell lines expressing PDGFR β , VEGFR1, and VEGFR3, symbolizes a possible sensitivity toward pazopanib.²³ In 2013, it was found that a subpopulation of activated astrocyte in the subclinical phase of brain metastasis expressed p-PDGFR β and showed that they could be inhibited by pazopanib. Based on the hypothesis that p-PDGFR β expression of astrocyte is a hallmark in metastasis, this suggests a potential value in treatment for brain metastasis in breast cancer patients.²⁴

Interestingly, a study aimed at comparing the capability of inhibition between sunitinib and pazopanib among various RCC cell lines showed the distinctive mechanisms between pazopanib and sunitinib.²⁵

Eight kinds of RCC cell lines were treated with varying concentrations of sunitinib and pazopanib, and it was found that pazopanib had a comparably lower ability to inhibit

RCC. Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNNEL) assay showed that pazopanib failed to induce apoptosis in any tested RCC cell lines.²⁵ This, therefore, supported the view that pazopanib reacts upon neoplasms through a mechanism of "cell cycle arrest".⁶ The *in vitro* study conducted by Zhu *et al.* also uncovered that pazopanib possesses inhibiting activity toward the proliferation migration and invasion of hepatocellular carcinoma.²⁶

Another investigation also broadened our understanding of metronomic chemotherapy in ovarian cancer. Low dose agents, including oral cyclophosphamide, or injected irinotecan or paclitaxel, had no or only mild anti-tumor activity when taken together with pazopanib; however, the trial of low dose topotecan showed excellent anti-tumor activity.²⁷ Pazopanib only possesses a mild capability and exerts on mighty anti-tumor activity, given the evidence that all of the mice in the oral topotecan/pazopanib concurrent groups were still alive 180 days after initial treatment. The median survival rate of mice in the 25 mg/kg pazopanib group, 125 mg/kg pazopanib group, and oral topotecan group were 41 days, 41 days, and 90 days, respectively.²⁷

It has been confirmed that there is a similar synergic effect between pazopanib and topotecan upon various kinds of cell lines, including neuroblastoma, osteosarcoma, and rhabdomyosarcoma.^{28,29} *In vitro* study confirmed that, when used separately, pazopanib did not have any influence on sarcoma cell lines. However, the additional pazopanib led to a significant decrease of IC_{50} of topotecan from 65.0 ng/mL to 35.1 ng/mL in SK-N-BE(2) cells.²⁸ When it came to *in vivo*, a combination of pazopanib and topotecan showed significant antitumor and antiangiogenesis activity by evaluation of circulating angiogenic factors, such as circulating endothelial cells (CEC), circulating endothelial progenitor cells (CEP), and micro vessel densities, as well as enhanced survival. A further investigation examined the relationship between prolonged therapy with oral metronomic topotecan and pazopanib and acquired resistance tumor behavior in an SK-N-BE(2) xenograft model, the very cell line which was previously proven to have a higher sensitivity to topotecan when accompanied with pazopanib.³⁰ Although all three durations of combination therapies decreased the micro vessel densities compared to the control, the tumors treated 56 and 80 days had higher pericyte coverage, and lower proliferative and mitotic indices compared to 28 day therapies. The study also determined that Glut-1 and hexokinase II may be associated with acquired resistance, and concluded that the metabolic switch toward elevated glycolysis is a key mechanism for acquired resistance toward combined therapy.³⁰

Hematologic malignancies

Pazopanib not only has broad activity in solid tumors, but also in hematologic malignancies. In a series of *in vitro* and

in vivo studies of vatalanib and pazopanib, both inhibitors decreased the phosphorylation status of the VEGF receptor, downregulated antiapoptotic proteins, such as XIAP and MCL1, and induced dose-dependent and selective apoptosis in chronic lymphocytic leukemia (CLL) cells.³¹ In the analogous experiment with pazopanib, fludarabine showed significant additive effects when pazopanib was combined. Treatment of xenograft mice with 100 mg/kg of body weight for three weeks resulted in tumor inhibition rates of 77%, with general tolerance.³¹

The efficiency of pazopanib toward various malignancies is well reported, but there are contradictory outcomes on the issue of whether pazopanib could lead to apoptosis. A xenograft model study of pazopanib showed that the tumor growth in two treated mice groups was significantly delayed (in 30 mg/kg group) or even almost totally inhibited (in 100 mg/kg) compared with the control group.³² After statistical analysis, the mean overall survival figures of the 30 mg/kg and 100 mg/kg groups were 41 and 51 days, respectively, compared with the control group at 20 days, showing a significantly positive prolongation correlated with pazopanib concentration. However, further TUNNEL assays on tumor sections in treated groups versus the control group showed obvious apoptosis.

Nevertheless, this study revealed the synergistic effects of pazopanib together with routine and novel therapies.³² The tremendous gap between the remarkable antiangiogenic activity and disappointing clinical results when the antiangiogenic inhibitors were solely used, leads to new theories and trials about various methods of administration, such as combination chemotherapy.³³ Through the 3H[dT] uptake measurement of proliferation, immunomodulatory drugs, such as lenalidomide, actimid, and bortezomib, and low-dose DNA-damaging drugs such as melphalan, all had a CI <1, meaning an indicated synergism with pazopanib.³²

Biomarker analysis in phase I or II clinical trials

A multicenter, phase II, open-label, single-arm study enrolled patients with clinical stage I/II non-small-cell lung cancer (NSCLC) and assessed the efficacy of pazopanib based on tumor-volume change, as well as plasma cytokine and angiogenic factors (CAFs). The change in plasma CAFs could function as the markers of tumor response. Researchers identified eight CAFs associated with pazopanib therapy, among which soluble (s)VEGFR2 showed the largest decrease by 1.35-fold, placental growth factor demonstrated the largest increase by 18.04-fold, and interleukin (IL)-12 had the most significant correlation with tumor shrinkage. However, analysis of CAF changes in correlation with tumor shrinkage identified only sVEGFR2 to be a potentially useful biomarker of tumor response. Greater decreases in sVEGFR2 correlated with larger tumor shrinkage. On the other hand, baseline CAF levels could

act as predictors of tumor response. Baseline levels of 11 CAFs were statistically significantly related to tumor response, including: IL-12; hepatocyte growth factor (HGF); IL-16; IP-10; stromal cell-derived factor (SDF)-1 α ; IL-2R α ; IL-3; Interferon (IFN)- α 2; tumor necrosis factor-related apoptosis-inducing ligand (TRAIL); M-CSF; and PlGF. A later study confirmed that combining markers, IL-12 and HGF were more valuable for predictive ability compared to the individual markers, and were able to distinguish the responders and non-responders with an accuracy of 81% (27 of 33).³⁴

Another study of CAFs in a phase II RCC trial also confirmed the efficacy of sVEGFR2 in predicting the response of patients to pazopanib.³⁵ At the twelfth week, a decrease in sVEGFR2 compared with baseline was significantly correlated with tumor response ($P = 0.00002$). No significant correlation was observed between VEGF, or sVEGFR1. Because of the favorable anti-tumor activity of pazopanib in the interim analysis of this trial, it converted into an open-label, single-arm study, and then united with a phase III randomized, placebo-controlled trial. In a study by Tran *et al.*,³⁶ they first screened out five potential markers: IL-6; IL-8; HGF; tissue inhibitor of metalloproteinases-1 (TIMP-1); and E-selectin. This study confirmed the association between the seven CAFs and continuous tumor shrinkage or progression free survival (PFS) in patients treated with pazopanib, including five CAFs previously referred and two additional CAFs, VEGF and osteopontin, regarding their predictive and/or prognostic value in other studies. Finally, they validated that a high IL-6 level was a significant predictive marker for a PFS benefit from pazopanib, especially in patients pretreated with cytokines.

As the von Hippel-Lindau (VHL) protein, the key regulator of angiogenesis, is frequently functionally impaired in RCC, VHL gene variations (mutations or methylation) were observed in 90% (70/78) of patients.³⁵ However, the translational research suggested that the VHL status has no correlation with tumor response or PFS, which is the same result as using other factors such as sVEGFR1, or VEGF.^{35,37} This outcome negated the previous assumption that the VHL/HIF pathway element could be the biomarker to predict the clinical outcome to pazopanib therapy in RCC patients, and suggested that further studies are needed to explore suitable and effective ones.

Conclusion

The oral multitargeting tyrosine kinase receptor inhibitor pazopanib targets VEGFR-1,-2,-3, PDGFR- α ,- β , M-CSF receptor/fms, and SCFR/c-kit, as well as B-Raf, through enzymatic assay. So far, in vitro preclinical studies have indicated that pazopanib could inhibit tumor growth including human breast, prostate, renal, liver, lung, and ovarian cancer. In addition, pazopanib exerts an influence on the in vivo xenograft

Figure 2 Mechanism of pazopanib on angiogenesis pathways. Adapted from reference.¹¹ AKT/PKB, protein kinase B; ERK, extracellular signal-regulated kinase; FAK, focal adhesion kinase; mTOR, mammalian target of rapamycin; PDGFR, platelet-derived growth factor receptor; PKC, protein kinase C; PLC- γ , phospholipase C- γ ; VEGFR, vascular endothelial growth factor receptor; VHL, von Hippel-Lindau.

model including multiple myeloma, breast cancer, and synovial sarcoma.

Pre-clinical pharmacological studies profiled the basic features of pazopanib. The Phase I study on advanced cancer showed that the steady-state exposure to pazopanib reached a peak in the 800 mg once daily dose group, and another study suggested that pazopanib functioned better in the model of steady-state concentration above 40 mmol/L. Other fundamental studies suggested that pazopanib should be taken by whole, and not together with food.

Some clinical studies shed light upon the molecular mechanism of the antiangiogenic effect of pazopanib, as illustrated in Figure 2. Apart from previously discovered tyrosine

targets, B-Raf, a key element in the Raf-MEK-ERK signal pathway, is another key target. More attention has been focused on the exploration of biomarkers to predict the response to pazopanib therapy. Several phase II/III clinical trials have provided valuable data about alternatives of CAFs, and suggested that CAFs, such as IL-6, IL-12, HGF, and sVEGFR2, have a significant association with clinical curative effect and can act as biomarkers of efficacy or potential eligible patients.

Antiangiogenic therapy is a novel field of cancer therapy, compared with traditional chemotherapies. Although it could inhibit the growth of a primary tumor, whether this kind of remedy could aggravate the local hypoxia and boost

the growth of primary and metastatic tumors is still debated.³⁸ The gap between in vitro and in vivo studies, and the acquired resistance to the small molecular tyrosine kinase receptor inhibitor also calls for more innovative studies and treatment plans. The exploration of biomarkers is one direction, which could help screen sensitive patients and, in turn, enhance the input-output ratio on the health care system. Combination and metronomic therapies of pazopanib and other kinds of medicines are another direction. As discussed above, some medicines have emerged to have collaborative effects with pazopanib, and in vivo study has proved the efficacy of metronomic therapy with topotecan. More pre-clinical and clinical studies of cancers other than RCC are desperately needed for broadening the indication and increasing the clinical benefit. Only with further study and testing can improvements be made to avoid the shortcomings of single dose therapies and ensure that resources are used to their greatest benefit.

Acknowledgments

We thank for Dr Jian Zhang for helpful discussion, and Dr Yi Lu for editing.

Disclosure

No authors report any conflict of interest.

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