Antagonism of Platelet-Derived Growth Factor Receptor in Non–Small Cell Lung Cancer: Rationale and Investigations

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Abstract

Molecules that target growth and survival pathways in cancer cells have revolutionized the treatment of cancer. Imatinib mesylate is one such agent inhibiting the tyrosine kinase that results from the Bcr-Abl translocation. Imatinib is also a potent inhibitor of the platelet-derived growth factor receptor. The platelet-derived growth factor receptor is crucial in the regulation of interstitial fluid pressure, as well as in the function of pericytes. Increased interstitial fluid pressure is a common feature of solid tumors and is thought to impede transcapillary transport of chemotherapy. Preclinical data show that platelet-derived growth factor receptor antagonism decreases interstitial fluid pressure, augments intratumoral concentration of chemotherapy, and impairs tumor growth. Pericytes are important cells in the vascular support structure of tumors regulating endothelial cell survival and directing capillary growth. Preclinical data suggest that dual targeting of pericytes and endothelial cells is a more effective antiangiogenic strategy than antiendothelial monotherapy. Two phase II studies in advanced non–small cell lung cancer are currently under way with imatinib. The first trial evaluates the use of intermittent imatinib and weekly paclitaxel in elderly patients. The second trial evaluates a novel maintenance strategy of imatinib and the antivascular endothelial growth factor antibody bevacizumab after first-line chemotherapy with bevacizumab. These trials should indicate whether encouraging preclinical data can be translated into clinical benefit in non–small cell lung cancer.

Isoforms of platelet-derived growth factor receptor (PDGFR) and its ligand, PDGF, constitute a family of receptors and ligands involved in proliferative and prosurvival signaling within cells. PDGFR is a tyrosine kinase receptor with two structurally related forms, PDGFR-α and PDGFR-β (1). PDGF is a dimeric protein with four isoforms, AA, AB, BB, and CC, in which A and B chains share 60% homology (2). PDGF is also known as c-sis, the protooncogene for the viral oncogene causing simian sarcoma (c-sis; ref. 3). PDGF binds to the extracellular domain of PDGFR, causing autophosphorylation of the intracellular tyrosine kinase domain. Downstream mitogenic pathways are activated, including phosphoinositide 3-kinase and Ras, which induce cellular proliferation, survival, and migration (4). Important cellular targets of PDGF include fibroblasts, smooth muscle cells, and pericytes. In normal physiology, PDGF signaling plays an important role in embryonic connective tissue formation, wound healing, angiogenesis, and regulation of interstitial fluid pressure (IFP; ref. 3).

Given its progrowth role in cell signaling, PDGFR has been an attractive therapeutic target in a number of human malignancies. Imatinib mesylate, a synthetic tyrosine kinase inhibitor best known for its inhibition of Bcr-Abl in chronic myelogenous leukemia, is also a potent inhibitor of PDGFR. Imatinib has efficacy in two tumor types in which the PDGF axis is implicated in pathogenesis. In dermatofibrosarcoma protuberans, >90% of cases involve a 17;22 chromosomal translocation which results in a fusion oncogene. The posttranscriptional product is functional PDGF-B, a ligand for PDGFR-β also expressed by dermatofibrosarcoma protuberans tumor cells. Imatinib blocks this autocrine loop and is very effective in dermatofibrosarcoma protuberans manifesting this karyotype (5). In glioblastoma multiforme, PDGF and PDGFR are coexpressed, suggesting autocrine stimulation. As monotherapy or combined with hydroxyurea, imatinib has shown moderate clinical activity in this cancer (6, 7). In contrast, imatinib has shown no meaningful activity in prostate cancer despite coexpression of PDGF-A and PDGFR-α (8, 9). Thus, the presence of an autocrine loop insufficiently predicts efficacy of PDGFR blockade.

In non–small cell lung cancer (NSCLC), cytoplasmic PDGF expression by tumor is a negative prognostic indicator (10, 11), suggesting that the PDGF axis may be biologically relevant. The pattern of PDGF and PDGFR staining in human NSCLC was investigated by immunohistochemistry in 92 surgical specimens. PDGFR-β expression was universal but was restricted to tumor stroma. In contrast, PDGF-B reactivity was documented within tumor cells in 60% of the specimens (100% of large cell, 64% of squamous cell, and 55% of adenocarcinomas). PDGF-B
expression was associated with poor prognosis, independent of stage, age, and other prognostic indicators (11). A second study examined PDGF expression within human lung cancer cell lines, as well as surgical specimens. PDGF-A was expressed by both squamous and adenocarcinoma cell lines and seemed to tightly regulate expression of vascular endothelial growth factor (VEGF). In xenografted NSCLC tumors, tumor growth correlated with PDGF expression. Depletion of PDGF inhibited tumor growth, decreased VEGF, and decreased intratumoral microvessel density. Within human NSCLC specimens, detection of PDGF-A by immunohistochemistry correlated positively with tumor size and negatively with 5-year survival (10).

In NSCLC, the expression of PDGF by tumor cells and PDGFR by the tumor microenvironment suggests a paracrine loop, with potential for therapeutic exploitation. The correlation of PDGF expression with poor prognosis raises the question: how might PDGFR activation be relevant to tumor survival? Two hypotheses have been proposed: (a) PDGFR activation causes tumor interstitial hypertension and (b) PDGFR activation is proangiogenic. These hypotheses underlie two clinical trials in NSCLC being undertaken by our group at the University of Washington.

**PDGFR and Interstitial Hypertension**

A feature of solid tumor biology that frustrates local delivery of chemotherapy is elevated IFP (12). The interstitial compartment of a tumor or stroma is the space bordered by tumor vasculature and neoplastic cells. As in normal tissue, it is composed of an extracellular matrix of collagen and elastin suspended in a hyaluronate and proteoglycan gel (13). Dysfunctional attributes of neoplastic stroma contribute to the phenotype of interstitial hypertension, including leaky capillaries, absence of normal lymphatics, desmoplasia, and contraction of the interstitial matrix by stromal fibroblasts (14). In human breast cancer, desmoplasia seems to be a paracrine response to tumor PDGF expression (15). Interstitial hypertension has been documented in a variety of solid tumors, including breast, melanoma, head and neck, and colorectal cancers (14). In cervical cancer, interstitial hypertension is a strong independent predictor of survival (16).

IFP is one of Starling’s forces which control transport of solutes across the capillary wall. Starling’s forces determine net capillary filtration pressure and include the hydrostatic and osmotic pressures of both capillary and interstitial compartments (Fig. 1). In normal tissue with selectively permeable microvasculature, transcapillary transport occurs by both diffusion and convection. In general, low molecular weight compounds travel along concentration gradients (diffusion) and macromolecules are transported by convection (flow). In tumors, convection seems to play a larger role in transcapillary transport of small molecules, such as cytotoxic chemotherapy (13). Because Starling’s forces regulate convection-driven transport, high IFP is a barrier to the effective delivery of chemotherapy.

The IFP of normal tissue is actively regulated by signaling through the PDGFR. The central role of PDGF signaling was first shown in a rat model of anaphylaxis, in which PDGF-$\beta$ stimulation reversed interstitial hypotension induced by dextran (17). In rat dermis, increased IFP is mediated by stromal fibroblasts rich in PDGF-$\beta$, which exert contractile tension on the extracellular matrix (14). PDGF signaling also seems important in the interstitial hypertension of solid tumors. In the PR Ob rat colon carcinoma model, only the stroma and blood vessels of tumor express PDGFR and not the neoplastic cells. In this model, blockade of PDGFR with imatinib mesylate decreased mean tumor IFP by 34% and capillary-to-interstitium transport of the small molecule $^{51}$Cr-EDTA doubled (18).

The work described above provided the conceptual basis for targeting PDGFR to reduce tumor IFP, thereby increasing transcapillary delivery of chemotherapy. This concept has been tested in several tumor models. In severe combined immunodeficient mice with s.c. human anaplastic thyroid carcinomas (KAT-4 tumor model), PDGFR is restricted to stroma. In this model, blockade of PDGFR with imatinib decreased IFP by 60%, corresponding to a 2-fold to 4-fold increase in tumor uptake of the chemotherapy agent epothilone B. The therapeutic index of epothilone B increased 3-fold with concurrent imatinib (19). Augmented transport seemed specific to tumor, as imatinib did not significantly increase chemotherapy uptake into normal tissues. Combined therapy was well tolerated, as judged by absence of weight loss, compared with a 14% weight loss in a mouse population dosed at therapeutic equivalence with epothilone B alone. A follow-up study confirmed that the improved chemotherapeutic efficacy seen with imatinib relates to the PDGFR pathway. In another study in the same model, a high-affinity DNA-based aptamer to PDGF-B decreased tumor IFP by 50% and increased concentration of $[^{3}$H]paclitaxel by 2-fold to 4-fold. Parallel results were seen when imatinib was coadministered with $[^{3}$H]paclitaxel, with a 50% drop in tumor IFP, and lower tumor volume compared with $[^{3}$H]paclitaxel monotherapy (20).

The concept that PDGFR blockade with imatinib may increase tumoral delivery of chemotherapy is being investigated in NSCLC in a phase II study of first-line intermittent imatinib mesylate and weekly paclitaxel in 35 elderly patients with advanced NSCLC. Imatinib will be delivered in 4-day pulses at 600 mg daily. Each imatinib pulse brackets a paclitaxel infusion, dosed at 90 mg/m$^2$/week for 3 of the 4 weeks. The primary efficacy end point is response rate compared with historic controls. Secondary end points include overall survival and the safety profile of the regimen. Tumor specimens will be stained for PDGF for exploratory analysis of the relationships between PDGF expression, response, and survival.

**PDGFR and Angiogenesis**

In 1971, Judah Folkman hypothesized that solid tumors depend on neovascularization for growth (21). Beyond a tumor size of 2 to 3 mm, metabolic requirements for oxygen and nutrients would be insupportable by diffusion alone. Folkman proposed that blocking the formation of new tumor blood vessels or antiangiogenesis could be an effective strategy in cancer. Conceptually, antineoplastic therapy can be targeted against two compartments: the tumor cells and the tumor vasculature. Tumor cells are the target of conventional chemotherapy and, given a high endogenous mutation rate, frequently develop resistance. The cellular subunit of the capillary, the endothelial cell, is nonneoplastic with a low endogenous mutation rate. In theory, antiendothelial therapies may be given over a prolonged period of time without resistance arising in endothelial
cells. Thus, antiangiogenic strategies may prolong the period of stable disease.

To date, the key target of antiangiogenic therapy is the endothelial cell. In physiologic and pathologic angiogenesis, endothelial cells depend on signaling by VEGF (22). VEGF isoforms signal through VEGF tyrosine kinase receptors. VEGFR-2 is central in angiogenesis, mediating the mitogenic, chemotactic, and prosurvival signals provided to endothelial cells. Tumor cells are the major source of VEGF within the tumor microenvironment.

In vivo, dependence on VEGF signaling exists chiefly in the endothelial cells of newly formed blood vessels. In neonatal mice, VEGF blockade results in endothelial apoptosis, vascular disruption, and death; whereas in adult mice, no such changes are evident (23). In human glioma xenografts, VEGF blockade selectively obliterates immature blood vessels (24). Similarly, in prostate cancer, androgen deprivation before radical prostatectomy results in sharp reduction of VEGF expression within the prostatic specimen and concomitant ablation of immature vessels (24). Acquisition of pericyte coverage by the endothelial cell is the developmental feature associated with loss of VEGF dependence (25).

Pericytes are the primary endothelial support cell and are ubiquitous in tumor vasculature. Within the vascular compartment, pericyte cell bodies are found adjacent to capillaries, with cytoplasmic projections that parallel and encircle the capillary wall (Fig. 2). Pericytes deliver survival signals to endothelial cells, regulate capillary blood flow, and participate in capillary growth (26). Pericytes within tumors have multiple abnormalities, including bizarre morphology, disorganized cytoplasmic processes that extend away from the blood vessel wall into tumor tissue, and loose physical association with endothelial cells (27). Pericyte homeostasis is maintained through PDGFR signaling.

Thus, two interdependent cell types exist within the vascular compartment of tumors: endothelial cells and pericytes. Dual targeting of PDGFR and VEGF may represent a synergistic and more effective antiangiogenic strategy than anti-VEGF therapy alone. This theory has been investigated in a mouse model of pancreatic neuroendocrine cancer (28). In mice with established tumors, the use of SU5416, a VEGF receptor inhibitor, had little inhibitory effect over tumor growth. However, mice treated with imatinib and SU5416 had substantial decrease in tumor size. Histologic evaluation showed that combined treatment led to regression of tumor vasculature, distortion of pericytes, and increased apoptosis when compared with controls. Analysis of normal tissues did not show similar vascular disruption. The same mouse model was used to explore multitargeted approaches to angiogenesis inhibition (29). Unequivocally, combined targeting of PDGFR and VEGF receptor was more effective than anti-VEGF monotherapy. In a tumor regression trial, mice treated with dual inhibition had stable disease, whereas mice treated with only VEGF inhibition had progressive disease.

In humans, antiangiogenic therapy has resulted in a paradigm shift in NSCLC. ECOG 4599 defined the first effective antiangiogenic therapy for this malignancy: bevacizumab, a monoclonal antibody against VEGF. In this trial, patients were randomized between carboplatin/paclitaxel chemotherapy and the same treatment with bevacizumab. The addition of bevacizumab increased survival from 10.3 to 12.3 months (30). Of note, patients on the investigational arm who had not progressed after six cycles were treated with bevacizumab.
monotherapy until progression. A review of the presented data shows that nonprogressors who entered maintenance had a median progression-free survival of \(\sim 12\) weeks for that phase of the trial (30). Although a direct comparison is invalid, this is numerically superior to the progression-free survival of nonprogressors on the chemotheraphy only arm, who after the completion of therapy had a median progression-free survival of \(\sim 8\) weeks. In principle, bevacizumab maintenance may delay tumor progression due to continued blockade of VEGF-driven angiogenesis. However, this is short lived. A more effective maintenance strategy would be desirable in advanced NSCLC, in which time-to-symptom deterioration is rapid (31).

At the University of Washington, we will be conducting a novel maintenance trial designed to exploit the antiangiogenic synergy of VEGF and PDGFR antagonism in NSCLC. Eligible patients have completed four cycles of a platinum doublet plus bevacizumab without progression. Fifty patients will be enrolled and treated with a maintenance regimen of bevacizumab 15 mg/kg every 3 weeks and continuous daily imatinib at 400 mg twice daily. The primary end point is progression-free survival; secondary end points include overall survival and safety profile. Correlative work will include immunohistochemistry staining for VEGF and PDGFR in original tumor specimens, with exploratory analysis of how these markers predict duration of stable disease and survival.

**Conclusion**

In NSCLC, the era of molecular targeting has been ushered in by the successes of erlotinib and bevacizumab. Given the wealth of prospective targets in NSCLC, how are we to determine which hypotheses to translate to humans? Five criteria have been used to define a rational drug target in cancer (32): (a) high tissue expression, (b) a defined role in tumor pathogenesis or progression; (c) activation of the target coincides with function; (d) inhibition is possible with a safe candidate drug; and (e) the target lacks a vital role in normal adult physiology.

Antagonism of PDGFR in NSCLC is a rational translational strategy that seems to fulfill these criteria. PDGFR is expressed universally within the stroma of NSCLC, whereas the majority of tumor cells express its ligand, PDGF. Expression of PDGF correlates with poor survival, although its pathogenic role is still under investigation. PDGFR activation coincides with the phenotype of interstitial hypertension in solid tumors and is proangiogenic in NSCLC xenografts. Imatinib mesylate is a potent inhibitor of PDGFR, with a well-established safety profile in humans. Two phase II trials will investigate the potential efficacy of PDGFR antagonism in NSCLC. Strong efficacy signals will encourage further molecular and functional definition of this novel strategy.

**Open Discussion**

**Dr. Roman Perez-Soler:** Your work is very interesting. Now, you have a use for a PDGF inhibitor and you have convincingly explained the rationale. My question would be which is the best PDGF inhibitor that we have? Are you convinced that imatinib is the perfect PDGF inhibitor?

**Dr. Martins:** I cannot tell you the preclinical data comparing different platelet-derived growth factor inhibitors. I can tell you that imatinib looks pretty good in preclinical models, in achieving the same thing that an aptamer would do.

**Dr. Perez-Soler:** But sorafenib is also a PDGF inhibitor, correct?

**Dr. Heymach:** The IC\(_{50}\)s that are typically quoted for the PDGF receptor with imatinib are in the hundreds of nanomolar range; with drugs like sunitinib, we are down to less than 10 nanomolar. So there are certainly more potent inhibitors, and those will also hit VEGF receptor at the same time. Sorafenib is a little bit higher than sunitinib but better than imatinib. It may be better to test the hypothesis with a drug like sunitinib than imatinib, but the concept is certainly a reasonable one.

**Dr. Bruce Johnson:** When they moved imatinib into combination trials with etoposide/platinum and irinotecan/platinum, they ran into very severe problems of hematologic toxicity. This blocks c-kit, which is the receptor for stem cell factor. Unless you know that your schedule is going to obviate the hematologic toxicity, I would be very concerned about this, particularly in the elderly lung cancer population.

**Dr. Martins:** I showed you the phase I data where they used a higher dose of paclitaxel (100 mg) and a little higher dose of imatinib as well, and they did okay as far as hematologic toxicity. And we brought it down for this study, so we will see.

**Dr. John Heymach:** Severe immunosuppression was seen with irinotecan and imatinib in a small cell cancer trial by Bonnie Glisson, and with docetaxel and imatinib also.

**Dr. Johnson:** Both studies were stopped early.

**Dr. Martins:** Intermittent administration hopefully may play a role in avoiding that.

**Acknowledgments**

We thank Jeffrey Slater for his assistance with the manuscript.

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