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New Assignments for Multi-tasking Signal Transduction Inhibitors

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Running title:

Multi-tasking protein tyrosine kinase inhibitors

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CML, chronic myelogenous leukemia; PDGFR, platelet-derived growth factor receptor; SMC, smooth muscle cell; VSMC, vascular smooth muscle cell

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ABSTRACT

An article presented in this issue of Molecular Pharmacology (p. ...) provides an intriguing example of how tyrosine kinase inhibitors can be put to many uses. In this article, the action of dasatinib (BMS-354825) is contrasted to that of imatinib, a kinase inhibitor that is currently being used to treat chronic myelogenous leukemia and other disorders. Both pharmacologic inhibitors target several tyrosine kinases, including Bcr-Abl and the platelet-derived growth factor receptor (PDGFR). Up to this point, the PDGFR has not been a primary therapeutic target for this class of agents. The work of Chen and colleagues shows that dasatinib is a particularly potent inhibitor of PDGFR, and that the compound also targets Src kinase. The authors suggest that this combination of activities could be useful in the treatment of vascular obstructive diseases. While a lack of absolute specificity has classically been regarded as a pharmacologic drawback, this study exemplifies that drugs with multiple molecular targets can potentially provide a very beneficial spectrum of therapeutic activities in multiple disease states.

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Tyrosine kinase inhibitors have been considered as potential therapeutic agents in several disease states, and particularly in cancer. Over 100 gain-of-function oncogenes have been defined that can contribute to carcinogenesis (Blume-Jensen and Hunter, 2001). Since tyrosine kinases represent a large fraction of known dominant oncogenic proteins, they continue to be a prime target for the development of specific signal transduction inhibitors (Levitzki and Gazit, 1995; Blume-Jensen and Hunter, 2001). Protein tyrosine kinases catalyze the transfer of the γ phosphate of ATP to hydroxyl groups of tyrosines on target proteins. They are important regulators of intracellular signal transduction pathways mediating cell proliferation, differentiation, migration, metabolism, survival, and cell-cell communication (Hunter, 1998). The human genome encodes 518 serine/threonine and tyrosine kinases (Manning et al., 2002) , all of which bind ATP in highly conserved catalytic domains (Venter et al., 2001). It has long been recognized that protein kinase activities can be targeted by pharmacologic inhibitors. However, two potential problems presented themselves. First, the abundance of ATP in a cell raised a concern over the difficulty in developing inhibitors to be administered at concentrations that would effectively suppress particular kinases without cellular toxicity. Second, the high degree of commonality implied that it would be difficult to develop compounds that specifically inhibited particular protein kinases without having cross-reactivity toward others. The first concern has not presented a significant problem. The second concern has proven to be well founded. However, in some cases the lack of specificity is advantageous. The latter point is nicely demonstrated by the work of Chen and co-workers in this issue.

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Dozens of small molecule inhibitors have been identified that bind to the ATP site of tyrosine kinases with nanomolar or picomolar affinities and excellent specificity (Futreal et al., 2001; Davies et al., 2000). The recently marketed drug imatinib (STI-571, Gleevec) is a small molecular inhibitor that inhibits the Abl tyrosine kinases. Imatinib also inhibits the c-Kit (stem cell factor) and PDGFR tyrosine kinases (Buchdunger et al., 2000). Inhibition of Bcr-Abl is central to the therapeutic activity of imatinib in chronic myelogenous leukemia (CML). Imatinib appears to bind preferentially to the inactive conformation of Abl, thus blocking its activation (Schindler et al., 2000). It has been proposed that distinct structural features among tyrosine kinases in their inactive conformation may provide for the observed extent of drug-target selectivity. Nonetheless, lack of target selectivity has been observed, and this molecular "promiscuity" has resulted in broader therapeutic applications.

The breadth of action of imatinib has been used to advantage to expand its range of tumor targets. Treatment with this drug has shown remarkable clinical activity in gastrointestinal stromal tumors, which frequently contain activating mutations in the c-Kit tyrosine kinase (Heinrich et al., 2002). Preliminary clinical data suggest that imatinib is also active against leukemias expressing a fusion of the PDGFR with the Tel gene product (Sawyer, 2002). However, lack of target specificity can also lead to undesirable side effects. For example, there is a case report of cystoid macular edema (CME) occurring as a side effect of imatinib (Masood et al., 2005). The possible mechanism of this side effect may be mediated through inhibition of the PDGFR. The PDGFR is found in the retina (Robbins et al., 1994), where its down-regulation has been associated with the development of edema (Lindahl et al., 1997).

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Pathological changes observed in vascular remodeling include endothelial injury, proliferation, and hypercontraction of vascular smooth muscle cells (SMCs) (Humbert et al., 2004). Migration of medial SMCs and their proliferation in the intima contribute to thickening of injured and atherosclerotic vessels. These events are regulated, in part, by platelet-derived growth factor (PDGF) (Koyama et al., 1994; Balasubramaniam et al., 2003). PDGF consists of dimers that include two structurally similar polypeptides (A chain and B chain) that are encoded by separate genes (Heldin and Westermark, 1999; Raines et al., 1990). PDGF stimulates cell growth through the activation of cell surface receptors α and β (Heldin and Westermark, 1999; Raines et al., 1990). Recently, two additional PDGF genes were identified, encoding PDGF-C and PDGF-D polypeptides (Bergsten et al., 2001; Li et al., 2000). The PDGF receptors belong to a family of transmembrane receptor tyrosine kinases (RTKs) that include the epidermal growth factor receptor and vascular endothelial growth factor receptors. These receptors dimerize to bind the bivalent PDGF ligands. Formation of the PDGF-PDGFR results in an autophosphorylation of the RTK and increased kinase activity. *In vitro* studies suggest that PDGF-B has affinity for both α- and β-receptors, whereas PDGF-A binds only the α-receptor (Raines et al., 1990; Heldin and Westermark, 1999). PDGF and its receptors play a key role in embryonic development, as inactivation of the genes for PDGF and its receptors causes abnormal kidney, lung, cardiac, and vascular development (Heldin and Westermark, 1999; Leveen et al., 1994; Lindahl et al., 1998). Both receptors activate major mitogenic signaling transduction pathways, including Ras/MAPK, PI3K, and phospholipase Cγ (Heldin et al., 1998; Rosenkranz and Kazlauskas, 1999). Recently, upregulation of both PDGFRα and PDGFRß has been shown in lambs with chronic

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intrauterine pulmonary hypertension (Balasubramaniam et al., 2003). Pulmonary levels of the ligands PDGF-A or PDGF-B mRNA did not differ between pulmonary hypertensive and control animals. In lung biopsies from patients with severe pulmonary arterial hypertension (PAH), PDGF-A chain expression was significantly increased (Humbert et al., 1998).

Results presented in this issue by Chen and colleagues provide evidence for the inhibitory effect of a novel protein tyrosine kinase inhibitor, dasatinib (BMS-354825), on PDGF responses in vascular smooth muscle cells (VSMCs). In this study, the authors show that dasatinib inhibits the following PDGF-stimulated responses in rat VSMCs: 1) activation of PDGFR, STAT3, Akt, and Erk2, 2) migration, and 3) proliferation. Dasatinib also inhibits Src tyrosine kinase in VSMCs. Direct comparison of the actions of dasatinib and imatinib in VSMCs indicated that dasatinib is 67-fold more potent than imatinib in inhibiting PDGFR activation.

This study provides an excellent example of a multi-tasking signal transduction inhibitor. Dasatinib is an ATP-competitive, dual-specificity Src- and Abl-kinase inhibitor developed by Bristol–Myers Squibb (Princeton, USA) (Lombardo et al., 2004; Shah, et al., 2004). Src is an attractive target because Src activation may play a role in the development and progression of many tumors. Specifically, Src kinase modulates signal transduction through mutiple oncogenic pathways including PDGF receptor, vascular endothelial growth factor receptor, and others. Notably, dasatinib can also inhibit Bcr-Abl activation loop mutants that are found in some CML patients with acquired clinical resistance to imatinib (Shah et al., 2004). Dasatinib, which is structurally unrelated to imatinib, is 325-fold more potent than imatinib and is active against 18 of 19 Bcr-Abl

mutations found in patients who develop imatinib resistance (Shah et al., 2004; Hampton, 2006; O'Hare et al. 2005). Thus, dasatinib is currently being developed as an anti-cancer drug (Walz and Sattler, 2006). In this issue, Chen and co-workers demonstrate that dasatinib possesses potential novel therapeutic activity in cardiovascular diseases such as restenosis and stenosis. These conditions, which involve hyperproliferation of vascular cells, are very significant clinically and have therefore been the target of various pharmacologic approaches. Chen and colleagues suggest that the combination of activities (i.e., inhibition of both PDGFR and c-Src) observed for dasatinib could be useful in the treatment of vascular obstructive diseases.

The potential therapeutic applications of tyrosine kinase inhibitors in different disease states are being very actively investigated. With respect to the study by Chen and co-workers, issues that are worthy of further attention include: 1) the relative roles of PDGFR and c-Src in mediating VSMC migration, 2) further characterization of the downstream signaling steps most critical for PDGF-induced migration and proliferation (Bornfeldt et al., 1995), and 3) ability of dasatinib to inhibit restenosis in animal models and human clinical trials. New inhibitors often contribute to our understanding of complex cellular signal transduction pathways, unveiling new elements in pathophysiology. Combinations of the tyrosine kinase inhibitors with agents that inhibit downstream pathways should be explored as a novel multistep approach to treating human disease. We are approaching an age of maturity in pharmacology in which desired drug effects, as well as "side" effects, may be regarded as components of a therapeutic continuum that can be optimized to the treatment of specific disease states.

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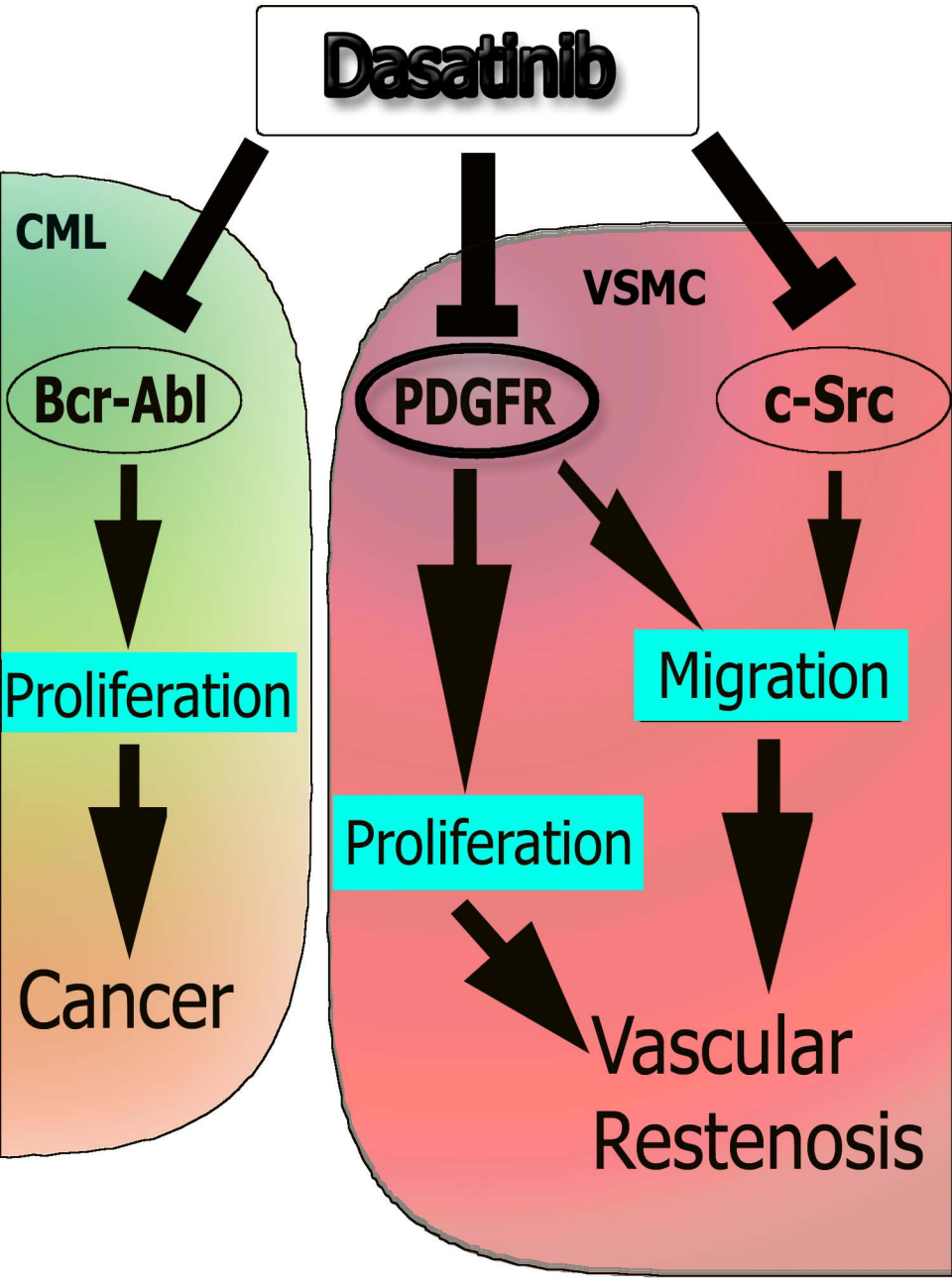
Figure Legend

Figure 1. Diagram depicting the inhibitory effects of dasatinib in chronic myelogenous leukemia (left) and vascular smooth muscle cells (right).


Dasatinib
CML
Bcr-Abl
Proliferation
Cancer
VSMC
PDGFR
c-Src
Migration
Proliferation
Vascular
Restenosis