Role of protein tyrosine kinase inhibitors in cancer therapeutics

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Protein tyrosine kinases (PTKs) are critical in regulating cell growth and differentiation and are deeply involved in several cancers. PTK-inhibitors are mainly ATP-site directed and are finding use in the treatment of several cancers, and more than 30 such agents are now in phase I-III clinical trials. The present review focuses mainly on the development of PTK inhibitors in clinical trials, with special emphasis on imatinib mesylate, a rationally designed, potent oral anticancer agent and selective inhibitor for Abl tyrosine kinase, including Bcr-Abl, C-kit and platelet-derived growth factor-receptor tyrosine kinases, which has been implicated in several malignancies, including chronic myeloid leukemia and gastrointestinal stromal tumour.

Keywords: Protein tyrosine kinase, tyrosine kinase inhibitors, imatinib mesylate, chronic myeloid leukemia, acute myeloid leukemia, anticancer agent, gastrointestinal stromal tumor, epidermal growth factor receptor, vascular endothelial growth factor.

Introduction
Remarkable advances in recent years in the molecular biology of cancer have resulted in developing more rational and effective strategies for diagnosis and treatment of cancer. One such approach has been the use of protein kinases (PTKs) in cancer therapeutics. A PTK inhibitor, imatinib mesylate (originally called CGP-57-148 B), promises to be an important advance in the treatment of patients with chronic myeloid leukemia (CML) and gastrointestinal stromal tumour (GIST)\(^1\). PTKs play an important role in regulating cell growth and division and are often involved in tumor formation\(^2,3\). Role of PTK inhibitors in cancer therapeutics is an active area of research today. Several PTK inhibitors have been developed, which are now in pre-clinical and clinical studies (Table 1). The present review focuses mainly on the development of PTK inhibitors in clinical trials, with special emphasis on imatinib mesylate.

Tyrosine kinases are attractive anticancer targets
Approximately 2000 kinases are known and more than 90 PTKs have been found in human genome. PTKs can transfer a phosphate group from a donor molecule (usually ATP) to an amino residue of a protein. They are divided into two main classes, receptor- and non-receptor PTKs. Receptor PTKs possess an extracellular ligand binding domain and an intracellular catalytic domain with intrinsic tyrosine kinase activity and upon ligand binding, the receptor tyrosine kinase (RTK) triggers receptor dimerization and autophosphorylation. The autophosphorylation creates phosphotyrosine docking sites for proteins which transduce signals within the cell\(^4\). Non-receptor PTKs, which includes Abl, Fes/Fer, Syk/Zap70, Jak, Tec, Fak, Ack, Src and Csk participate in a variety of signalling processes, including mitogenesis, cell survival and immune response\(^5\).

In multicellular animals, Src-family of non-receptor PTKs, including v-Src/c-Src, Yes, c-Fgr, Lyn, Hck, Fyn, Blk and Trk are distinguished from other group of PTKs by the absence of extracellular or transmembrane domains. The Src-family of PTKs

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Abbreviations: CML, chronic myeloid leukemia; AML, acute myeloid leukemia; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; VEGF-R, vascular endothelial growth factor receptor; VEGF, vascular endothelial growth factor; PTK, protein tyrosine kinase; RTK, receptor tyrosine kinase; GIST, gastro-intestinal stromal tumor; RPTK, receptor PTK; Ph-chromosome, Philadelphia chromosome; Abl, Abelson tyrosine kinase; Bcr, breakpoint cluster region; KDR, vascular endothelial growth factor receptor 2; PDGF, platelet-derived growth factor; PDGF-R, platelet-derived growth factor receptor; SFK, Src family PTK; Csk, C-terminal Src family; C-kit, stem cell factor receptor
Table I—Protein kinase inhibitors in clinical trials

<table>
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<th>Inhibitor</th>
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<tr>
<td>Imatinib</td>
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<td>Abl (c-kit, PDGF-R)</td>
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<td>SU1248</td>
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<td>CEP-701</td>
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<td>TRA</td>
<td>Phase II</td>
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<td>ISIS 3521 (antisense)</td>
<td>ISIS</td>
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<td>Eisai</td>
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<td>UCN-01</td>
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<td>VEGF-R1/2, FGF-R, PDGF-R</td>
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<td>KDR (PDGF-R, FGF-R)</td>
<td>Phase I</td>
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<tr>
<td>ZD 6474</td>
<td>AstraZeneca</td>
<td>KDR and EGF-R</td>
<td>Phase I</td>
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<tr>
<td>PKC412</td>
<td>Novartis</td>
<td>Fl-3</td>
<td>Phase I for AML</td>
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Cdk, cyclin-dependent kinase; Chk1, checkpoint kinase 1; Cyc, cyclin epidermal growth factor; FGF-R, fibroblast growth factor receptor; KDR, kinase domain receptor (also called VEGF-R2); MEK, mitogen-activated protein kinase; PKA, protein kinase A; PKC, protein kinase C. *PTK787/ZK222584 currently in phase I/I tria1.²

serves as a central molecular switch that mediates the communication between an upstream extracellular signal and downstream intracellular events such as cell growth and differentiation, cell cycle progression and cell adhesion and movement.¹⁰,¹¹ They contain functional domains such as SH4 (Src homology 4), a unique domain, SH3, SH2, SH1 and a C-terminal regulatory region, which are responsible for their interaction with particular receptors and protein targets. The activity of several Src-family kinases have been found to be upregulated by gain of function mutations or over-expressions in several human leukemias, breast cancer, Hodgkins disease, Burkitt's lymphoma and melanoma. Therefore, the Src-family of PTKs is considered as attractive target for the drug design due to their involvement in several cancers.²

C-terminal Src kinase (Csk), a cytoplasmic PTK, consisting of an SH3, an SH2 and a kinase domain specifically phosphorylates Src-family kinases on a C-terminal Tyr and plays a pivotal role in various cell signalling. These domains have structural resemblance with other PTKs, relevant to their active site cleft, ATP and peptide-binding lobes (Fig. 1). PTKs play a crucial role in signal transduction pathway, known to regulate normal cellular functions. They are considered as attractive targets for the drug
design due to their ATP-binding property\textsuperscript{15,16}. PTK inhibitors are mainly selected as prime targets in various oncogenic complications.

PTK inhibitors of epidermal growth factor receptor (EGFR)

The EGFR receptors are small proteins consisting of an amino-terminal extracellular domain, a single transmembrane anchoring region and a carboxyterminal intracellular domain. These receptors are found on the surface of many types of cancer cells. EGFR binds exclusively to the growth factor, triggering production of tyrosine kinase, which in turn causes cell to grow and divide\textsuperscript{17,18}. EGFRs are a family of four receptors [Erb B1 (also termed EGFR and HER 1), Erb B2 (HER 2), Erb B3 (HER 3) and Erb B4 (HER 4)]. Inhibitors of EGFR are active after oral administration and are commonly referred to as EGFR tyrosine kinase inhibitors (EGFR-TKIs). They competitively inhibit binding of ATP to the tyrosine kinase domain of the receptor, thereby inhibiting EGFR autophosphorylation. EGFR and EGFR have demonstrated therapeutic benefit in several human tumors, including breast, lung, colorectal, ovarian, prostate, head and neck\textsuperscript{19-21}.

EGFR tyrosine kinase inhibitors ZD1839 and OSI-774

ZD1839 \{4-(3-chloro-4-fluroanilino)-7-methoxy-6-(3-morpholinopropoxy) quinazoline\}, a low molecular mass (447 kD) quinazoline derivative specifically inhibits Erb B1 and Erb B2 tyrosine kinase. It has demonstrated clinical antitumor activity in several tumors, including those derived from hormone-resistant prostate cancer, ovarian cancer, ductal carcinoma of the breast, colon, vulval, small cell and non-small-cell lung cancers\textsuperscript{22-24}.

OSI-774 \{6,7-bis-(2-methoxy-ethoxy)-quinazoline \(\text{A}_{\text{yl}}\)-[3-ethylphenyl]-amine\}, a low-molecular quinazoline derivative has a very similar pharmacological and physiological profile to ZD1839. It has shown activity in ovarian carcinoma, non-small cell lung cancer and head and neck cancers\textsuperscript{25,26}.

PTK inhibitors of vascular endothelial growth factor (VEGF)

VEGFs -C and -D are the ligands for VEGF receptors, VEGFR-2 (Flk-1/KDR) and VEGFR-3 (Flt-4). Signalling via VEGFR-3 is important for the maintenance and function of kidney glomeruli, ovarian corpus luteum angiogenesis, motor neurones of spinal cord and central nervous system, as well as hematopoietic stem cells\textsuperscript{27-29}. VEGF stimulates endothelial cells of blood vessels to proliferate and grow. This mechanism is called angiogenesis. As the uncontrolled growth of cancer cell is legendary, and requires a lot of blood flow to the cell area, if the blood vessel growth is restrained then in theory, cancer growth also can be restrained.

Inhibitors of VEGF and EGFR have found use in angiogenesis and play a pivotal role in several tumors, including thyroid carcinoma\textsuperscript{30,31}. Therefore, the VEGFR families of tyrosine kinases are considered to be attractive targets for rational drug design\textsuperscript{32,33}.

VEGF-tyrosine kinase inhibitors PTK787 and SU5416

PTK787 or ZK222584, a novel oral angiogenesis tyrosine kinase inhibitor was initially designed to

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Fig. 1—Structures of Csk catalytic domain and the substrate-docking site [(A): Ribbon structure of Csk with identified residues in the substrate-docking site shown in a ball-and-stick model. Several loop structures relevant to peptide substrate binding and catalysis are indicated by arrows; and (B) Surface structure of Csk (yellow) and the substrate-docking site (colored by electrostatic potential). The active site cleft is indicated\textsuperscript{13}].
inhibit VEGF signal transduction by binding directly to the ATP-binding sites of VEGFRs\textsuperscript{34}. It readily penetrates cells and inhibits VEGF-induced (VEGF-R2) autophosphorylation and mitogenesis and promotes endothelial cell apoptosis. Consistent with its anti-angiogenic activity, it reduces vascularity and blood flow in tumor tissues and does not have a direct effect on tumor cells. It inhibits the growth of thyroid cancer. It is now proceeding into large clinical studies and is looking more promising, both in terms of efficacy as well as tolerability\textsuperscript{35-38}.

\textbf{SU5416 compound (Z-3-[(2,4-dimethylpyrrol-5-yl)methylidencyl]-2-indolinone) is both lipophilic and potentially reactive in nature and is metabolized by the cytochrome P450 enzymes. It was found to be a potent inhibitor of the VEGF-R2, C-kit and Flk-2, and plays a role in tumor angiogenesis. It has shown anti-angiogenic activity in various cancers, including renal cell carcinoma, melanoma and multiple myeloma\textsuperscript{39-42}.}

\textbf{Imatinib, an Abl-specific tyrosine kinase inhibitor}

Chronic myeloid leukemia (CML) is characterized by the presence of an abnormally short chromosome 22 (Ph chromosome), resulting from a reciprocal translocation involving the long arms of chromosome 9 and 22\textsuperscript{43,44}. The disease progresses through three phases: chronic, accelerated and blast crisis. Chronic phase (CP) lasts for about 4-5 years. Initially, the patients usually have minor symptoms and the cancer is usually detected by routine blood tests. A characteristic elevation of white blood count and expression of Ph chromosome are evident. The treatment includes interferon therapy alone or with other drugs or bone marrow transplant. Accelerated phase (AP) is characterized by the increasing number of blasts in the peripheral blood and the median duration is usually of 6-9 months. Blast crisis phase (BC) is the most severe form of CML with an increasing number of blasts in the blood and bone marrow. Its duration is usually of 3-6 months\textsuperscript{45-47}.

Imatinib mesylate, chemically designated as 4-[(4-methyl-1-piperazinyl)methyl]-N-[4-methyl-3-[4-(3-pyridinyl)-2-pyrindimethyl]aminophenyl] benzamide methanesulfonate\textsuperscript{48} (Fig. 2) has recently been approved as a first-line therapy for patients with CML (May 2001) and gastro-intestinal stromal tumor (GIST) Feb 2002\textsuperscript{52,53}. It is considered as a milestone in cancer treatment, because it is generally well tolerated and causes fewer side effects\textsuperscript{54,55}.

\textbf{Mechanism of Imatinib}

The Bcr-Abl tyrosine phosphokinase enzyme is constitutively an active kinase which functions by binding ATP and transferring phosphate from ATP to tyrosine residues on various substrates, thereby transmitting intracellular signals independent of ligand binding to growth factor receptors (such as interleukin-3). Imatinib mesylate occupies the kinase pocket of Bcr-Abl protein, blocking access to ATP, and thus prevents phosphorylation of downstream effector molecules\textsuperscript{56,57} (Fig. 3).

\textbf{Clinical effectiveness}

Gastro-intestinal stromal tumors (GISTs) are the most common malignant form of sarcoma that arise in the gastrointestinal tract. In a phase I study, involving 40 patients (of whom 36 had GISTs), response was
monitored for 36 patients. They received imatinib mesylate orally with food, 400 mg once daily (n = 8), 300 mg twice daily (n = 8), 400 mg twice daily (n = 16) and 500 mg twice daily (n = 8). Patients were monitored for 3 months after initiation of treatment. An objective response was observed in 25 patients (69.4%), while stable disease was observed in 7 patients (19.4%) and disease progression was observed in 4 patients (11.1%)^{52,58}. In CML, 150 patients with chronic phase Ph-positive, in whom prior IFN-α therapy had failed, were selected for the study. Patients received 400 mg imatinib mesylate orally after breakfast. Dose was increased from 400 mg to 600 mg, in the absence of hematologic response. Patients exhibited the following effects: 44% complete cytogenic response, 22% partial cytogenic response and 8% minor cytogenic response at 12 months. The estimated progression free survival (PFS) was 89.2% at 12 months and 80.2% at 18 months^{59}.

**Dosing**

Although the recommended dosage of imatinib is 400 mg once daily for patients in chronic phase of CML and 600 mg once daily for patients in accelerated or blast crisis phase, but the dose may be increased (from 400 mg to 600 mg, or 600 mg to 800 mg), in the absence of significant toxicity, in the presence of disease progression, the failure to achieve response, or the loss of a previously achieved response. If the toxicity is resolved within two weeks, treatment should be resumed at the original dose of 400 mg/day and resolved after more than two weeks, or recurred after resuming therapy, the drug should be withheld, until the event has resolved by using guidelines for hematologic and nonhematologic toxicity. Treatment should be continued as long as the patient continues to benefit^{59,61}.

**Mechanism of relapse**

Several mechanisms of clinical resistance have been proposed. Some mechanisms involve alteration in Bcr-Abl itself, while others are Bcr-Abl independent, due to altered drug uptake. Mutations discovered within the PTK domains of Bcr-Abl gene itself has been characterized as a mechanism of resistance. At the time of disease relapse, mutations of amino acids (315 and 253, 255 and 351) were identified in 60% of patients with kinase domain mutations^{62-65}. To date, 18 different candidates of gleevec resistance mutations (GRMs) have been identified in the cells from patients, who became refractory to imatinib treatment at 14 different sites, all between Abl codons 244 and 396^{66,68}. Ber-Abl independent mechanisms, including imatinib sequestration via its binding to ε-1-acid glycoprotein, an acute phase serum protein may be a potential causative factor of resistance. There is still much to be learnt about imatinib resistance in long-term relapsed patients.

**Safety**

The most common frequent side effects include mild to moderate nausea, diarhoea, myalgias and peripheral edema and less being skin rashes and peripheral edema^{69}. Myelosuppression frequently and incidence was more common at higher doses (600 mg/day) in blast crisis and accelerated phase than in the chronic phase (400 mg/day) in CML^{48,70}. Hepatotoxicity was reported with severe elevation of transaminases or bilirubin, however, most abnormalities were managed with dosage reduction or interruption (median duration of episode 51 weeks), but may be fatal, though rarely, when co-administered with acetaminophen. Cytopenias, including neutropenia, thrombocytopenia and anaemia were consistently experienced in all studies^{59,71}.

**Contraindications**

Imatinib is metabolised in the liver by the cytochrome P450 system. It is contraindicated in patients with hypersensitivity to the drug. Patients should be cautioned to avoid or restrict the use of over-the-counter and prescription medications containing acetaminophen or any other ingredient in the product formulation, such as colloidal silicon dioxide, crospovidone, magnesium stearate, microcrystalline cellulose, gelatin, iron oxide, red (E172); iron oxide, yellow (E172) and titanium dioxide (E171)^{67,70}.

**Conclusion**

Role of various PTKs in signal transduction pathways has become clearly evident in a diverse array of cellular processes. The development of PTK inhibitors blocking signalling pathways is considered a promising approach for drug development. The clinical efficacy of imatinib has clearly demonstrated the potential of kinase inhibitors in CML and GIST. The studies of imatinib mesylate provide proof-of-principle for using aberrant kinases as therapeutic target in several cancers.
References


