

## SMALL-MOLECULE KINASE INHIBITORS: FROM LAB BENCH TO CLINIC

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This review examines some of the kinase inhibitors as cancer treatments, their targets, how they work or sometimes fail to work and what researchers have learned from the first few years of targeted cancer therapeutics.

### Introduction

Thirty years after the 'war on cancer' was declared, cancer is still a major killer worldwide. According to the American Cancer Society's report "Cancer Facts and Figures, 2006", 1,399,790 new cancer cases are predicted in the US for 2006, with an estimated 564,000 deaths.

Despite discouraging predictions and statistics for cancer diagnoses and deaths, researchers have developed a new cancer therapeutic agent, the small-molecule kinase inhibitor. Small-molecule kinase inhibitors block kinase signaling and are rapidly moving from investigational status to FDA trials and onto pharmacy and infusion room shelves. Moreover, these therapeutics represent exciting progress in translation of research laboratory discoveries into life-saving drugs. The specificity of these drugs for their target molecules means they are less toxic than conventional chemotherapy, reducing the side effects suffered with conventional chemotherapeutic agents.

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### Elucidating Signaling Pathways

There are multiple signaling pathways, and their effect on cellular growth and differentiation has been an increasingly important and recognized area of cancer research. In March 2006, the Simulation Modeling of the MAP-kinase Pathway consortium (SIMAP) funded by the European Commission was chartered to develop a platform to simulate the MAP-kinase (MAPK) pathway, a signaling pathway associated with cancer and currently targeted by a number of cancer drugs and diagnostic tests. The goal of the consortium is to develop a comprehensive and robust simulation model of the

pathway that will incorporate data from the literature as well as experimental and clinical work.

Beyond the SIMAP consortium, research efforts have shown that various kinase molecules are activated and mutated in a variety of cancers. Dr. Charles Sawyers, researcher at the David Geffen School of Medicine, UCLA, concluded that using kinase inhibitor molecules works "consistently and reliably against cancers in which the kinase drug target is constitutively activated by gene mutation" (1). Sawyers recounted the success of the tyrosine kinase inhibitor Gleevec® (imatinib mesylate, also STI571) in treating chronic myeloid leukemia. The first small-molecule kinase inhibitor on the market, Gleevec® received FDA approval in May 2001.

However, optimism for the success of kinase inhibitors as anti-cancer agents cooled with the failure of the second tyrosine kinase inhibitor Iressa® (gefitinib) in 2002. Used in combination with chemotherapy, Iressa® failed to show superior results compared to standard treatments for lung cancer. The initial imatinib and gefitinib results highlight the 'opportunities and challenges' in developing kinase inhibitors as therapeutic agents for cancer (1).

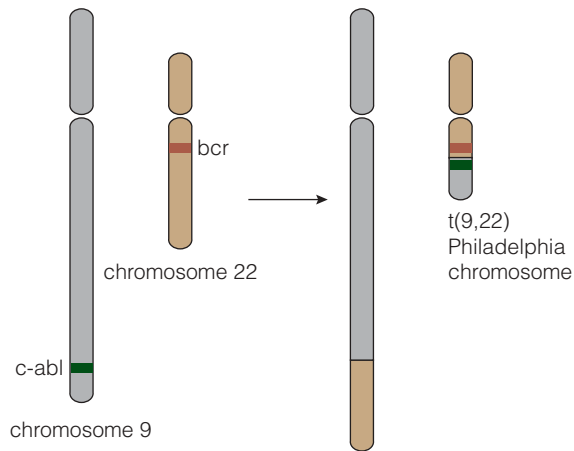
### Success with a Kinase Inhibitor

The first kinase inhibitor used in clinical trials was tested against CML (chronic myelogenous leukemia). CML is a bone marrow-derived blood cell cancer caused by activation of the Abl kinase after fusion of the Abl and Bcr genes (2). This fusion event, widely known as the Philadelphia chromosome translocation (Figure 1), occurs in a single bone marrow stem cell that undergoes clonal expansion over many years. At diagnosis, while frequently asymptomatic, patients regularly have peripheral blood counts 20-fold higher than normal, with as many as  $10^{12}$  tumor cells.

CML cells manage to maintain their ability to differentiate and function as normal blood cells. However, over time the disease advances to additional abnormalities, differentiation ceases and late-stage disease, known as blast crisis, develops. This disease stage can suddenly and quickly result in fatality.

The small-molecule kinase inhibitor imatinib competitively inhibits ATP binding to the kinase domain of target proteins. Imatinib was initially isolated during screening for compounds that block PDGFR (platelet-derived growth factor

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**Figure 1. Philadelphia Chromosome translocation.**

receptor), then was found to also inhibit Abl and Kit kinases (3,4). After a series of preclinical and clinical successes imatinib gained FDA approval for treatment of CML (5,6).

Response rates for imatinib in chronic phase CML are remarkable; peripheral blood counts return to normal in >90% of cases, and 50–70% of cases show no evidence of the Philadelphia chromosome translocation after 3–6 months of treatment. However, PCR studies show that nearly all patients have residual BCR-ABL-expressing blood cells, so imatinib may not be a cure for CML. In trials against late stage, blast crisis CML, imatinib showed early and impressive results. However, the positive results were quickly negated by emergence of drug resistance (7,8).

Although short-lived, excitement greeted the success of imatinib in blast crisis CML. Researchers looked for the cause of resistance as well as an understanding of why imatinib worked at all in late-stage disease, where multiple mutations are present. Studies have shown that resistance to imatinib is largely due to mutations in the BCR-ABL kinase domain, which alter binding (9–11). Additionally, molecular models and studies in mice (12,13), as well as the positive blast crisis CML/imatinib results, support the hypothesis that an initial kinase mutation is required for tumor maintenance, even in late stage disease when multiple genetic abnormalities are common. The glimmer of success with imatinib in blast crisis CML both supports the maintenance theory and brings hope for extending kinase inhibitors more widely, particularly in cases where the initiating kinase mutation can be identified and targeted with the proper inhibitor.

## Imatinib Use Beyond CML

Imatinib also has been used successfully in GIST (gastro-intestinal stromal tumors). These tumors of the stomach and small intestine express constitutively active c-kit tyrosine kinase receptor, with many containing activating point

mutations in the juxtamembrane region of c-kit (14). Imatinib therapy resulted in many complete or partial remissions (15,16). Furthermore, PET (positron emission tomography) studies showed that tracer uptake by cells, indicative of cancerous activity, declined markedly after initiation of imatinib treatment. Studies showed that tumors with c-kit mutations were more likely to respond, with the best response in cases where mutations occurred in the juxtamembrane region (17,18).

Imatinib failed to show activity against a c-kit mutation in the mast cell disorder systemic mastocytosis, probably due to an alteration of the drug-binding domain (19). On the other hand, some GIST respond to imatinib despite the absence of a c-kit mutation, explained by the discovery that a subset of GIST contain mutations in PDGFR $\alpha$ , a known imatinib target (20).

A high incidence of clinical response to imatinib has been seen in several additional cancers where kinase activation occurs because of DNA alterations in the kinase or its ligand, and the resulting constitutive activation is due to an imatinib-responsive target.

## Small-Molecule Inhibitors Beyond Imatinib

Other kinase inhibitors have been used as therapeutics for cancers resulting from constitutive kinase activation. Small percentages of AML (acute myeloid leukemia) and ALL (acute lymphoid leukemia) are known to have activating mutations in the Flt3 receptor tyrosine kinase (21,22). These mutations were inhibited by several kinase inhibitors, three of which showed activity in clinical trials (23–25).

In glioblastoma, EGFR (epidermal growth factor receptor) mutations, found in approximately 25% of cases, are often deletions in the extracellular domain. Such mutations induce a conformational change, resulting in constitutive activation of EGFR kinase. The small molecule EGFR inhibitor OSI-774 (Tarceva<sup>®</sup>; 26) brought clinical responses in approximately 20% of glioblastoma cases.

The gene encoding the kinase B-raf is a frequent site of mutations in melanoma; several types of B-raf mutations leading to constitutive B-raf activation have been reported. A few mutations have been noted to decrease B-raf kinase activity, while causing downstream activation of Erk kinases (27) suggesting a more complex twist in the effect of mutation on kinase activation. Raf inhibitors have been tested in clinical trials with melanomas, resulting in approval against human melanomas with B-raf mutations (28).

EGFR inhibitors were tested in phase I trials against a variety of tumor types, not necessarily just those showing EGFR dependency, with hints of clinical activity in lung and colon tumors (29,30). Further studies with gefitinib (Iressa<sup>®</sup>) in late-stage lung cancer provided dramatic results, albeit in low

numbers of cases (10%; 31,32). Studies of EGFR expression in these cases showed varying levels, but no absolute correlation to positive results (33). Similar results were seen with EGFR inhibitor OSI-774 (34). Although these results were disappointing, the lack of success could be attributed to poor patient criteria (selecting only those with predisposing EGFR lesions) for these trials. In addition, researchers point to the need for molecular data to better understand the results. These results made it clear that a pharmacogenomic approach might be necessary to identify the most appropriate inhibitor for individual mutations.

## Identifying Kinase-Dependent Lesions

The significance of kinases in melanoma and colon cancer (35) helped provide the kinase-cancer link and impetus for a comprehensive kinome sequencing project, completed in 2002 (36). Biological validation of the role of these mutations in cancer is ongoing, but research and results thus far point to those mutations that are found consistently in tumors as functional and important in kinase dependency.

Kinase dependency can occur without a corresponding kinase mutation, thus the need to also recognize tumors that are kinase-dependent without a kinase mutation. Such tumors are common in breast cancer, where overexpression of Her2/neu is common. Tools useful to find these mutations might include gene expression profiling using phospho-specific antibodies against kinase targets or substrates identified by immunohistochemical applications (37,38). Mass spectroscopy-based proteomics offers excellent sensitivity and specificity and could be performed on cell lysates after enrichment for kinase targets by immunoaffinity purification using antiphosphotyrosine antibodies. These techniques are commonly used in research laboratory settings (39), but application to and validation in clinical lab settings will require time, effort and expense.

Kinase dependency can also occur with the loss of a negative regulator, such as the PTEN tumor suppressor, which has been mutated or deleted in a variety of cancers. The loss of PTEN results in increased phosphoinositol-3-kinase (PI3K) activity and elevated downstream signaling through kinases such as Akt. Akt can regulate mTOR by phosphorylation, and data from studies using drugs that inhibit mTOR kinase show that indirect kinase activation has a similar effect to that of activating mutations (40-43). Tumors with mutations resulting in loss of PTEN function have constitutive activation of Akt as well as downstream kinases like mTOR. Such tumors have shown sensitivity to inhibitors of mTOR (40,41); thus loss of the negative regulator PTEN results in mTOR being required for tumor growth, although not for normal cell function. Akt and PI3-kinase are both essential to normal cell function and were thus initially considered unsafe as drug targets. However, knockout studies in mice have proven both to be potential targets for inhibitors (44,45). Most recently

oncogenic mutations of PI3K have been observed in many tumors, and mutations in the kinase domain cause cellular transformation, thus validating PI3K as an oncogene.

## Choosing Lead Compounds

While this is not an article on the art of drug screening and development, the story of small-molecule kinase inhibitors would be incomplete without noting one or two additional lessons learned from development of imatinib. Besides desirable properties of kinase inhibitors such as potency, selectivity, solubility, reasonable drug half-life and oral bioavailability, there are chemical structure issues of great importance in selecting a good kinase inhibitor. Imatinib binds the ABL kinase only when the activation loop is in the closed configuration (46). This selectivity explains imatinib's specificity for ABL and not SRC kinases. On the other hand, crystallographic studies show an ABL/SRC kinase inhibitor binds ABL in the open or closed configuration (47). Conformation issues affecting molecular folding and shape could explain why certain diseases responded to greatly decreased doses of imatinib (48). It is also possible that conformation-specific inhibitor binding could contribute to drug resistance, as noted earlier in blast crisis CML and other diseases. Recent data show that dasatinib (BMS-354825), a new kinase inhibitor, has excellent activity in CML cases that have developed resistance to imatinib. Dasatinib binds more loosely than imatinib, and to both Abl/Src. In June 2006 the FDA Drugs Advisory Committee voted to recommend accelerated approval of dasatinib for adults in all stages of CML with resistance to imatinib (49).

The kinase inhibitor imatinib has shown efficacy against at least three different cancers, with a respectable safety profile. Other small-molecule kinase inhibitors have demonstrated effectiveness, albeit in lesser amounts. Although perhaps not curative, kinase inhibitors show tremendous potential for saving lives and improving the quality of life for many with cancer. Data on the chemical structure of new lead compounds, together with the development and use of tools to identify kinase mutation types, can accelerate movement from lead identification to clinical trials and FDA approval by testing the compounds in cases most likely to show a response. ■

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