REVIEW ARTICLE

MECHANISMS OF DISEASE

# Tyrosine Kinases as Targets for Cancer Therapy

Daniela S. Krause, M.D., Ph.D., and Richard A. Van Etten, M.D., Ph.D.

From the Molecular Oncology Research Institute (D.S.K., R.A.V.) and the Division of Hematology–Oncology, Department of Medicine (R.A.V.), Tufts–New England Medical Center, Boston. Address reprint requests to Dr. Van Etten at Tufts–New England Medical Center, 750 Washington St., Box 5609, Boston, MA 02111, or at rvanetten@tufts-nemc.org.

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Protein tyrosine kinases (TKs) are enzymes that catalyze the transfer of phosphate from ATP to tyrosine residues in polypeptides. The human genome contains about 90 TK and 43 TK-like genes, the products of which regulate cellular proliferation, survival, differentiation, function, and motility. More than 25 years ago, TKs were implicated as oncogenes in animal tumors induced by retroviruses. However, they were largely ignored in drug development because of a paucity of evidence for a causative role in human cancer and concerns about drug specificity and toxicity. The landscape was changed radically by the success of imatinib mesylate, an inhibitor of the BCR-ABL TK in chronic myeloid leukemia (CML) — a result heralded as a proof-of-principle and a triumph of targeted cancer therapy. TKs are now regarded as excellent targets for cancer chemotherapy, but reality lies somewhere between the extremes of triumph and tribulation. In this article we will review mechanisms of aberrant TK signaling and strategies to inhibit TKs in cancer, summarize the status of TK-directed cancer therapies, and discuss challenges and prospects for the future.

## TK Regulation, Dysregulation, and Therapeutic Targeting

### Regulation of Normal TK Activity

TKs are divided into two main classes (Fig. 1). Receptor TKs are transmembrane proteins with a ligand-binding extracellular domain and a catalytic intracellular kinase domain, whereas nonreceptor TKs lack transmembrane domains and are found in the cytosol, the nucleus, and the inner surface of the plasma membrane. The enzymatic activities of both types of TK are under tight control, so that nonproliferating cells have very low levels of tyrosyl phosphorylated proteins. The kinase domains of all TKs have a bilobar structure, with an N-terminal lobe that binds ATP and magnesium, a C-terminal lobe containing an activation loop, and a cleft between the lobes to which polypeptide substrates bind.

In the absence of ligand, receptor TKs are unphosphorylated and monomeric, and the conformation of their kinase domains is inactive. In some receptor TKs, the cytoplasmic juxtamembrane region further inhibits the enzyme by interacting with the kinase domain.[2] Receptor TKs become activated when ligand binds to the extracellular domain, resulting in receptor oligomerization, disruption of the autoinhibitory juxtamembrane interaction, and autophosphorylation of a regulatory tyrosine within the activation loop of the kinase (Fig. 1). These changes reorient critical amino acid residues, thereby increasing the catalytic activity of the enzyme. After activation, autophosphorylation generates binding sites for signaling proteins, recruiting them to the membrane and activating multiple signaling pathways.[3]

The nonreceptor TKs, typified by c-ABL, are maintained in an inactive state by cellular inhibitor proteins and lipids and through intramolecular autoinhibition.[1] Nonreceptor TKs are activated by diverse intracellular signals through dissociation of

inhibitors, by recruitment to transmembrane receptors (causing oligomerization and autophosphorylation), and through *trans*-phosphorylation by other kinases (Fig. 1). TK signaling is terminated in part through the action of tyrosine phosphatases that hydrolyze tyrosyl phosphates and by the induction of inhibitory molecules.

### MECHANISMS OF TK DYSREGULATION IN CANCER

Given the multiple levels of regulation of TKs, it is not surprising that TKs are dysregulated in cancer cells in several ways (Fig. 2). A common mechanism of TK activation in hematologic cancers is the fusion of a receptor or nonreceptor TK with a partner protein, usually as a consequence of a balanced chromosomal translocation. A frequent feature of the partner protein is a domain that causes constitutive oligomerization of the TK in the absence of ligand binding or physiologic activating signals, thereby promoting autophosphorylation and activation. A primary example of this mechanism is BCR-ABL, the nonreceptor fusion TK in CML, in which a tetramerization domain in BCR overcomes autoinhibition of ABL catalytic activity through oligomerization and autophosphorylation.[4] With some receptor TKs, absence of the juxtamembrane inhibitory domain in the fusion protein contributes to activation.

A second important mechanism of TK dysregulation is a mutation that disrupts autoregulation of the kinase. Mutations in the Fms-like tyrosine kinase 3 (FLT3) receptor in acute myeloid leukemia (AML) render this TK active in the absence of ligand[5]; in another example, small deletions and point mutations in the kinase domain of epidermal growth factor receptor (EGFR) in a subset of non–small-cell lung cancers increase the sensitivity of the receptor to its ligand[6] and alter receptor signaling.[7,8] A third mechanism of TK dysregulation is increased or aberrant expression of a receptor TK, its ligand, or both. Examples include overexpression of the receptor TK ERBB2 (HER-2/neu) in breast cancer and overexpression of a mutant form of platelet-derived growth factor (PDGF), a receptor TK ligand, in dermatofibrosarcoma protuberans with t(11;17). Lastly, increased TK activity can result from a decrease in factors that limit TK activity, such as impaired tyrosine phosphatase activity or decreased expression of TK inhibitor proteins.[9] Aberrant TK activation can increase the survival, proliferation, and cytotoxic drug resistance of malignant cells, and in tumors it can increase angiogenesis, invasiveness, and metastatic potential.

### STRATEGIES TO TARGET TKs IN CANCER THERAPY

TKs can be inhibited pharmacologically through multiple mechanisms (Fig. 2). The idea behind much of anti-TK drug discovery is to find small molecules that directly inhibit the catalytic activity of the kinase by interfering with the binding of ATP or substrates. Other anti-TK drugs may inhibit activation of fusion TKs by blocking their dimerization. Antibodies against receptor TKs or their ligands interrupt TK signaling through neutralization of ligand, blockade of ligand binding, receptor internalization, and perhaps antibody-mediated cytotoxicity. The stability of some TKs is regulated by binding to heat-shock proteins (e.g., heat-shock protein 90 [Hsp90]), and inhibitors of Hsp90 can disrupt the binding of client proteins such as BCR-ABL and HER-2, causing their degradation. An important advantage of TK-directed therapy is that it is possible to perform pharmacodynamic studies that correlate inhibition of the targeted TK in cancer cells with clinical responses to the drug.

## TKs AS TARGETS IN THE TREATMENT OF MALIGNANT HEMATOLOGIC DISORDERS

### IMATINIB MESYLATE: THE FIRST SUCCESSFUL SMALL-MOLECULE TK INHIBITOR

The prime example of a dysregulated TK in the hematologic cancers (Table 1) is BCR-ABL, which has been implicated as the direct cause of CML.[41] Imatinib mesylate (Gleevec), a 2-phenylaminopyrimidine compound that is a specific inhibitor of several TKs — namely, ABL, ABL-related gene product (ARG), c-KIT, and PDGF receptor (PDGFR) — induces complete hematologic and cytogenetic remissions in most patients with chronic-phase CML[10] but is much less effective in the accelerated and blast-crisis phases of the disease.[11] ABL is also activated by fusion to nucleoporin 214 (NUP214) in 5 percent of T-cell acute lymphoblastic leukemias[15] and to ETV6 (also known as TEL) in rare cases of atypical CML and acute leukemia,[13] both potential targets for imatinib.[14]

Imatinib should also have activity in cancers caused by activated PDGFRs and c-KIT. PDGFR$\alpha$ is activated by cryptic interstitial chromosome 4 deletions that generate a FIP1L1–PDGFR$\alpha$ fusion TK in some patients with hypereosinophilic

syndrome[27] or systemic mastocytosis,[29] whereas PDGFR$\beta$ is activated in some patients with chronic myelomonocytic leukemia and balanced translocations that lead to fusion of PDGFR$\beta$ with one of several partner proteins[31] (Table 1). Imatinib induces dramatic clinical and molecular responses in both diseases.[32,42] c-KIT is activated by point mutations in many cases of systemic mastocytosis or mast-cell leukemia and less frequently in AML. The most common c-KIT mutation involves D816 in the activation loop of the kinase domain, but this mutant is not inhibited by imatinib.[43] The normal c-KIT receptor is expressed on most AML blasts and may be overexpressed and activated in some patients, but the responses of AML to imatinib are variable and do not correlate well with inhibition of KIT signaling.[44]

### FLT3: A MAJOR TK TARGET IN AML

FLT3 is a receptor TK expressed on blasts in most cases of AML, and it is activated by duplications within the juxtamembrane domain[5] in 25 to 30 percent of patients and point mutations[21] at D835 in 5 to 7 percent of patients (Table 1). Several inhibitors of FLT3 kinase activity are in various stages of development.[45] They inhibit growth and induce apoptosis in hematopoietic cell lines expressing activated FLT3 and have therapeutic efficacy in murine models of FLT3-induced leukemia.[46] In preliminary clinical trials, the drugs had few adverse effects and reduced circulating and bone marrow blasts in 20 to 50 percent of patients with relapsed or refractory AML.[22-24] Whether FLT3 inhibitors will benefit patients with AML whose blasts overexpress the normal FLT3 receptor is unclear. Such patients often do not have a response to FLT3 inhibitors, even though an autocrine FLT3 loop promotes the survival and growth of some AML blasts in vitro.[47,48]

### OTHER TK TARGETS IN MALIGNANT HEMATOLOGIC DISORDERS

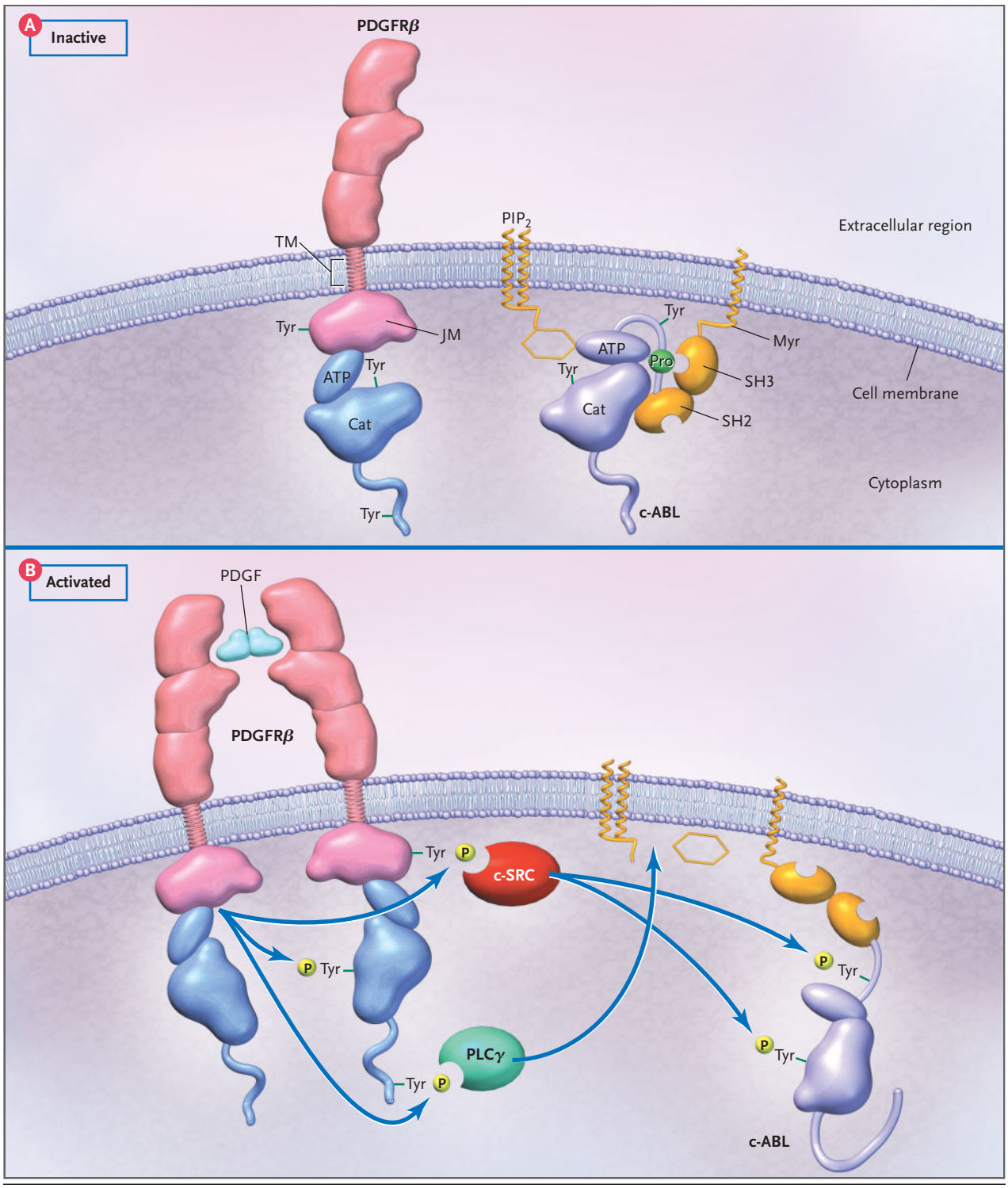
Other TKs have been implicated in hematologic cancers, but targeted therapeutics for these diseases are in their infancy. Fusion of the fibroblast growth factor receptor 1 (FGFR1) TK with one of several partners occurs in the 8p11 myeloproliferative syndrome,[18] and FGFR3 is mutated and overexpressed in multiple myeloma with t(4;14).[20] Translocations involving the anaplastic lymphoma kinase (*ALK*) gene are pathognomonic of anaplastic large-cell lymphoma and generate fusion of the ALK receptor TK with several partners.[16] Fusion of Janus kinase 2 (JAK2), a nonreceptor TK, with TEL or BCR has been described in cases of acute leukemia and atypical CML,[36] whereas an activating point mutation (V617F) in JAK2 is found in the majority of patients with polycythemia vera and in some patients with essential thrombocytosis and idiopathic myelofibrosis.[34,35] Preclinical studies suggest that many of these mutant TKs contribute to the pathogenesis of the disease.

**Figure 1 (facing page). Mechanisms of Activation of Normal TKs.**

A typical receptor TK (platelet-derived growth factor receptor $\beta$ [PDFGR$\beta$]) and nonreceptor TK (c-ABL) are depicted, with the ATP-binding (ATP) and catalytic (Cat) lobes of the kinase domains and the transmembrane (TM) region of PDGFR$\beta$ indicated. Panel A shows both kinases in their inactive states. Inactive PDGFR$\beta$ is monomeric and unphosphorylated, and the catalytic domain is inhibited by protrusion of a regulatory tyrosine (Tyr) in the activation loop into the substrate cleft and by an intramolecular interaction with the juxtamembrane (JM) domain. Inactive c-ABL is associated with the membrane through a covalent N-terminal myristate group (Myr) and is inhibited through intramolecular interaction of the Src homology-3 (SH3) domain with an adjacent proline (Pro) residue and by direct interaction of the catalytic domain with an inhibitory membrane lipid, phosphatidylinositol-4,5-bisphosphate ($PIP_2$). In Panel B, PDGFR$\beta$ is activated upon binding of the ligand (dimeric platelet-derived growth factor [PDGF]), which induces oligomerization of the receptor and intermolecular phosphorylation (P, in yellow) of the activation-loop tyrosine. This leads to a conformational change in the catalytic domain and increased enzymatic activity, while phosphorylation of other tyrosines within the intracellular domain of the receptor creates binding sites for SH2 domain–containing signaling proteins, including c-SRC (red oval) and phospholipase C$\gamma$ (PLC$\gamma$) (green oval). c-ABL is activated through the phosphorylation of two regulatory tyrosines, one in the activation loop and the other near the SH3 binding site, which can be phosphorylated by another TK, such as c-SRC. In addition, activated PLC$\gamma$ can hydrolyze and destroy the lipid inhibitor $PIP_2$. Further detail is provided in the review by Van Etten.[1]

### THE DRAMATIC RESPONSE OF CML TO KINASE INHIBITION: THE RULE OR AN EXCEPTION?

The excellent responses of chronic-phase CML and related syndromes to kinase-inhibitor therapy suggest that a dysregulated TK is the sole or predominant somatic genetic abnormality in the malignant cells. In contrast, one or more additional genetic abnormalities are required for the development of AML,[49] which may explain why the activity of FLT3 inhibitors in that disease is only moderate. FLT3 mutations may be acquired late in the




evolution of some cases of AML and therefore in some cases are not central to the origins of the disease.[50] The extreme sensitivity of some cancers to TK inhibition may reflect their absolute dependence on the targeted TK-signaling pathway for survival. This phenomenon of "oncogene addiction"[51] can be seen when imatinib suppresses myeloid colony formation in Philadelphia chromo-

some–positive bone marrow without affecting colonies from normal progenitors.[52]

The responsiveness of some hematologic cancers to kinase-inhibitor therapy may also be due to simultaneous inhibition of more than one TK target. Imatinib inhibits both BCR-ABL and c-KIT in hematopoietic progenitors, and this dual activity may in part account for its efficacy in CML.[53] Similarly, the drug SU5416 inhibits vascular endothelial growth factor receptors (VEGFR) in addition to FLT3 and c-KIT; among patients with c-KIT–positive AML, those who had a response to SU5416 had increased VEGF levels before treatment and decreased bone marrow microvasculature after treatment.[24] The response to this drug may thus be mediated both by inhibition of c-KIT signaling in the blasts and by blockade of VEGFR activation in the bone marrow microenvironment.

## TKs AS TARGETS FOR THERAPY OF SOLID TUMORS

### IMATINIB TARGETS IN SOLID TUMORS: GIST AND BEYOND

Considerable evidence points to the involvement of TKs in a variety of solid tumors (Table 2). Most gastrointestinal stromal tumors (GISTs) carry mutations in c-KIT that are associated with constitutive activation and receptor phosphorylation.[65,66] GISTs develop at high frequency in adults with germ-line *KIT* mutations, suggesting that c-KIT activation is insufficient for tumorigenesis.[91] Most patients with a GIST who do not have *KIT* mutations have mutations in *PDGFRα*.[85] Trials of imatinib in GIST that were prompted by these findings demonstrated partial responses in more than half the patients[67]; those with *KIT* mutations in exon 11 had the best response, whereas the few patients without either *KIT* or *PDGFRα* mutations did not have a response.[92] In contrast to patients with chronic-phase CML, complete responses to imatinib have not been observed in patients with GISTs,[67] and the varying responses of patients with different c-KIT mutations contrasts with the uniform in vitro sensitivity of these mutant receptors to the drug. Therefore, the dependence of GISTs on c-KIT signaling for survival and proliferation is probably incomplete and complex.

c-KIT and PDGFR have also been implicated in other solid tumors (Table 2), although evidence of the efficacy of kinase inhibitors in these diseases is limited. There are activating kinase mutations in c-KIT in some testicular seminomas[68] and c-KIT mutations or overexpression[69] in small-cell lung cancer, but a phase 2 trial of imatinib in small-cell lung cancer yielded equivocal results.[70] Some glioblastoma cell lines simultaneously express PDGF and its receptors and display evidence of autocrine PDGFR signaling.[81] Imatinib inhibits the growth of these cells[83] and increases their sensitivity to ionizing radiation.[93] Imatinib is also active in dermatofibrosarcoma protuberans,[87] in which platelet-derived growth factor B is fused to collagen type Iα1 and overexpressed.[86] Finally, PDGFRα or PDGFRβ is overexpressed in a subset of sarcomas[72,82,84] and chordomas,[94] some of which respond to imatinib (Table 2).

**Figure 2 (facing page). Mechanisms of TK Dysregulation and Therapeutic Targeting in Cancer.**

In each case, the TKs known to be activated through that mechanism are listed. Overexpression of a normal receptor TK (here, epidermal growth factor receptor [EGFR]), its ligand, or both is depicted in Panel A. In Panel B, mutations that render a receptor TK constitutively active in the absence of ligand are represented by internal tandem duplications (ITDs) in the juxtamembrane (JM) domain and point mutations (Asp835X) in the activation loop of Fms-like tyrosine kinase 3 (FLT3). In Panel C, BCR-ABL exemplifies the fusion of receptor and nonreceptor TKs to various N-terminal partner proteins as a consequence of chromosomal translocations and deletions. A common feature of the partner proteins is a domain that mediates oligomerization, such as the coiled-coil (CC) domain of BCR. Examples of therapeutic agents targeting TKs are listed in red. Small-molecule TK inhibitors usually act to block binding of ATP or substrate to the catalytic domain of the TK. BCR-ABL may also be targeted by compounds such as 17-allylamino-17-demethoxygeldanamycin (17-AAG) that interfere with binding to cellular chaperones such as Hsp90, by compounds that block oligomerization, by small interfering RNA (siRNA) that induces degradation of *BCR-ABL* mRNA, or by inhibitors of *BCR-ABL* gene transcription. None of these approaches have reached clinical development yet. Finally, receptor TKs and their ligands can be specifically targeted by monoclonal antibodies (top area of Panel A). PDGF denotes platelet-derived growth factor, EGF epidermal growth factor, KL KIT ligand, FL FLIT3 ligand, HGF hepatocyte growth factor, VEGF vascular endothelial growth factor, FGF fibroblast growth factor, PDGFR platelet-derived growth factor receptor, VEGFR vascular endothelial growth factor receptor, FGFR3 fibroblast growth factor receptor 3, ALK anaplastic lymphoma kinase, mRNA messenger RNA, and Ph Philadelphia chromosome.

### SMALL-MOLECULE INHIBITORS OF EGFR

EGFR is overexpressed, mutated, or both in many solid tumors (Table 2).[59] Gefitinib (Iressa) and er-

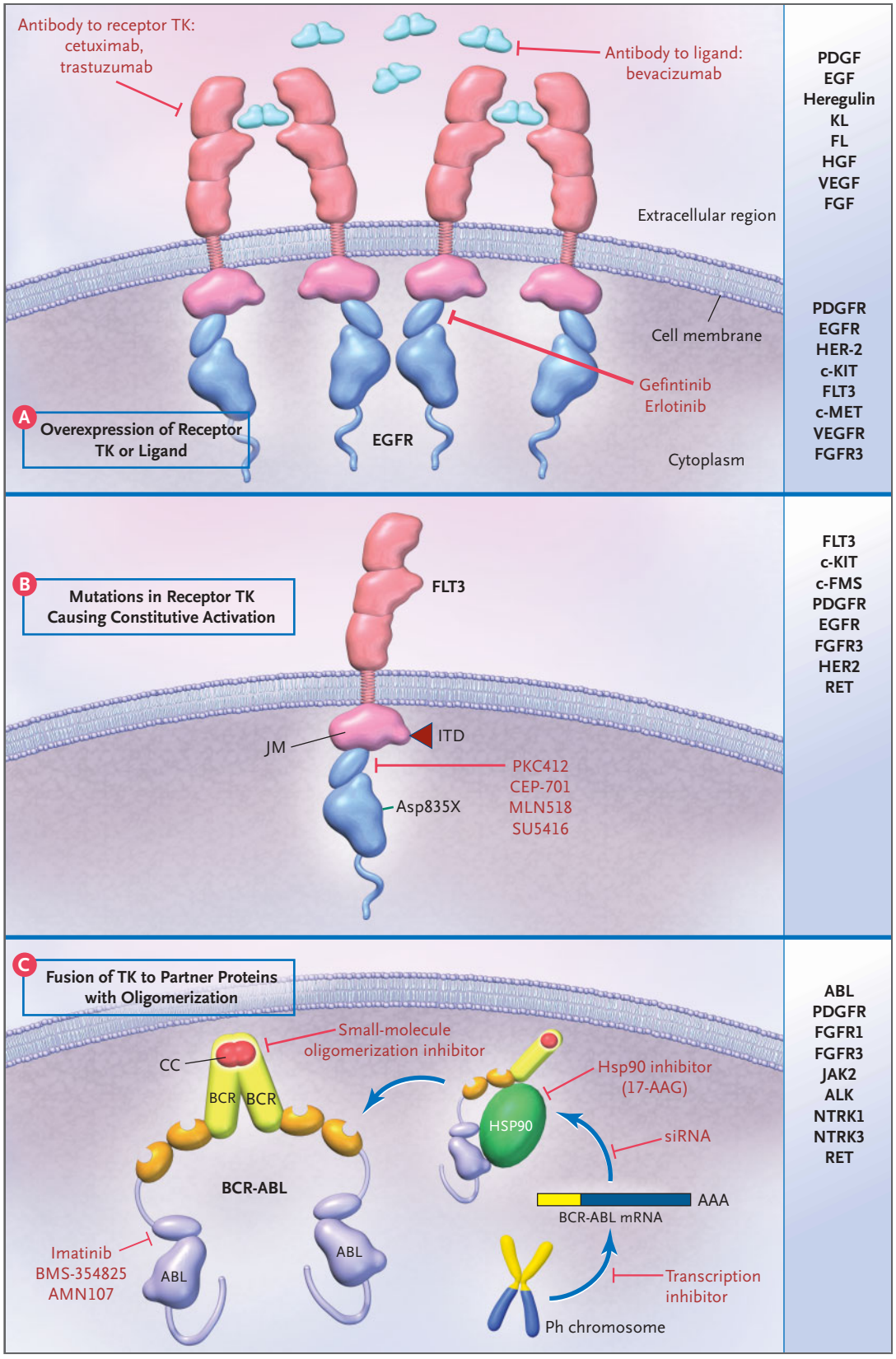


**Table 1. Tyrosine Kinase Targets in Malignant Hematologic Disorders.***

| Tyrosine Kinase† | Activating Mechanisms | Cancer | Targeted Therapy‡ | References |
|---|---|---|---|---|
| **ABL (9q34)** | | | | |
| BCR-ABL | t(9;22) | CML, ALL, AML | Imatinib (not for ALL), SMS-354825, | O'Brien et al.,[10] Druker et al.,[11] Shah et al.[12] |
| NUP214-ABL | Episomal fusion and amplification | T-ALL | Imatinib§ | Graux et al.[15] |
| **ALK (2p23)** | | | | |
| NPM-ALK | t(2;5) | ALCL | | Pulford et al.[16] |
| TPM3-ALK | t(1;2) | ALCL | | |
| ATIC-ALK | inv(2) | ALCL | | |
| CLTC-ALK | t(2;17) | ALCL | | |
| **ARG (1q25)** | | | | |
| TEL-ARG | t(1;12) | AML | Imatinib§ | Cazzaniga et al.[17] |
| **FGFR1 (8p11)** | | | | |
| ZNF198-FGFR1 | t(8;13) | EMS | PKC412, PD0173074§ | Macdonald et al.,[18] Chen et al.[19] |
| FOP-FGFR1 | t(6;8) | EMS | | |
| CEP110-FGFR1 | t(8;9) | EMS | | |
| HERVK-FGFR1 | t(8;19) | EMS | | |
| BCR-FGFR1 | t(8;22) | aCML | | |
| **FGFR3 (4p16)** | Overexpression with t(4;14); K650E | MM | PD0173074,§ SU5402§ | Chesi et al.[20] |
| TEL-FGFR3 | t(4;12) | T lymphoma | PD0173074,§ SU5402§ | |
| **FLT3 (13q12)** | Juxtamembrane internal tandem duplication; D835X; overexpression | AML | PKC412, MLN518, CEP-701, SU5416 | Nakao et al.,[5] Yamamoto et al.,[21] Stone et al.,[22] Smith et al.,[23] Fiedler et al.[24] |
| **c-FMS (5q33)** | L301F/S; Y969C | MDS, AML | | Ridge et al.[25] |
| **NTRK3 (15q25)** | | | | |
| TEL-NTRK3 | t(12,15) | AML | | Eguchi et al.[26] |
| **PDGFR$\alpha$ (4q12)** | | | | |
| FIP1L1-PDGFR$\alpha$ | Interstitial del(4q12) | HES, SM | Imatinib, PKC412 | Cools et al.,[27,28] Pardanani et al.[29] |
| BCR-PDGFR$\alpha$ | t(4;22) | aCML | Imatinib | Baxter et al.[30] |

lotinib (Tarceva) are anilinoquinazolines that are specific, competitive inhibitors of ATP binding by EGFR and were approved by the Food and Drug Administration (FDA) in 2004 for refractory locally advanced or metastatic non–small-cell lung cancer. Gefitinib led to partial responses in 11 to 19 percent of patients with refractory disease in phase 2 trials,[55] whereas erlotinib yielded partial responses in 9 percent of similar patients and improved overall and progression-free survival in a phase 3 trial.[56] Disappointingly, the addition of gefitinib or erlotinib to chemotherapy in the initial treatment of non–small-cell lung cancer did not yield any additional benefit.[95,96]

A small subgroup of patients with non–small-cell lung cancer who frequently had adenocarcinomas and most of whom were Asian, female, and nonsmokers had dramatic and sometimes durable responses to gefitinib monotherapy. Sequencing of the *EGFR* gene in tumor tissue from these patients revealed somatic gain-of-function mutations clustered around the ATP-binding pocket of the kinase domain of the EGFR protein in most cases.[6,7,54] Unlike c-KIT mutations in GIST, these EGFR mutations do not cause constitutive activation; rather, they enhance the responsiveness of the receptor to EGF ligand and increase its sensitivity to inhibition by gefitinib[6,7] and may preferentially activate antiapoptotic signaling pathways in the tumor cells.[8]

Paradoxically, in the Canadian randomized trial of erlotinib in non–small-cell lung cancer, tumor-cell expression of EGFR was significantly corre-

**Table 1. (Continued.)**

| Tyrosine Kinase† | Activating Mechanisms | Cancer | Targeted Therapy‡ | References |
|---|---|---|---|---|
| **PDGFRβ (5q33)** | | | | |
| TEL-PDGFRβ | t(5;12) | CMML | Imatinib | Golub et al.,[31] Apperley et al.[32] |
| HIP1-PDGFRβ | t(5;7) | CMML | Imatinib§ | |
| Rabaptin5-PDGFRβ | t(5;17) | CMML | Imatinib | Magnusson et al.[33] |
| H4-PDGFRβ | t(5;10) | aCML | Imatinib§ | |
| CEV14-PDGFRβ | t(5;14) | AML | Imatinib§ | |
| **JAK2 (9p24)** | V617F | Polycythemia vera, essential thrombocythemia openia, idiopathic myelofibrosis | | James et al.,[34] Kralovics et al.[35] |
| TEL-JAK2 | t(9;12) | AML, ALL | | Lacronique et al.[36] |
| BCR-JAK2 | t(9;22) | aCML | | |
| **c-KIT (4q11)** | D419X; V560X; D816X; overexpression | AML, SM | Imatinib (not for D816X), SU5416, PKC412 | Gari et al.,[37] Furitsu et al.,[38] Zermati et al.[39] |
| **SYK (9q22)** | | | | |
| TEL-SYK | t(9;12) | MDS | | Kuno et al.[40] |

* CML denotes chronic myeloid leukemia, ALL acute lymphoblastic leukemia, AML acute myeloid leukemia, aCML atypical chronic myeloid leukemia, T-ALL T-cell acute lymphoblastic leukemia, NPM nucleophosmin, ALCL anaplastic large-cell lymphoma, EMS 8p11 myeloproliferative syndrome, MM multiple myeloma, MDS myelodysplastic syndrome, HES hypereosinophilic syndrome, SM systemic mastocytosis, and CMML chronic myelomonocytic leukemia.
† Chromosomal locations are given in parentheses.
‡ Other than imatinib, the drugs listed have not been approved by the Food and Drug Administration.
§ A therapeutic response is predicted on the basis of the identity of the tyrosine kinase but has not been verified clinically.

lated with the response to kinase inhibitor therapy, but *EGFR* mutational status was not.[97] In contrast, studies from Asia, where *EGFR* mutant tumors are more prevalent, have found significant increases in response rates and overall survival among patients with *EGFR*-mutant tumors treated with gefitinib.[98,99] Additional prospective studies are needed to determine whether analysis of *EGFR* expression and mutation in tumors should be used to select patients with non–small-cell lung cancer for EGFR-inhibitor therapy.

EGFR kinase inhibitors are in phase 1 and 2 testing in a wide range of solid tumors.[100] EGFR is overexpressed in about 40 percent of glioblastomas and is activated by extracellular-domain deletions in a subset of these tumors,[57] but gefitinib had only minimal activity in a phase 2 trial in glioblastoma.[58]

### TARGETING RECEPTOR TKs AND THEIR LIGANDS WITH MONOCLONAL ANTIBODIES

Receptor TK signaling can also be inhibited by monoclonal antibodies against the receptor or its ligand (Fig. 2). The ERBB2 or HER-2 receptor TK is overexpressed through gene amplification in 20 to 25 percent of invasive primary and metastatic breast cancers and is associated with a poor prognosis.[61] Trastuzumab (Herceptin), a recombinant humanized monoclonal antibody against HER-2, increases response rates and improves survival when added to chemotherapy for metastatic HER-2–overexpressing breast cancer,[63] and in combination with adjuvant chemotherapy, it decreases recurrence in women who have early-stage breast cancer with HER-2 overexpression.[101] Another humanized monoclonal antibody, 2C4, blocks dimerization of HER-2 with other ErbB receptors and is in phase 1 studies.[102] HER-2 is mutated or overexpressed in other cancers,[62] which may also be candidates for anti–HER-2 therapy. A direct inhibitor of HER-2 kinase (CI-1033) is under development.

Several monoclonal antibodies against EGFR are also in development. Cetuximab (Erbitux) is a chimeric antibody against EGFR with activity in combination with chemotherapy in non–small-cell lung cancer, squamous-cell carcinoma of the

**Table 2. Tyrosine Kinase Targets in Solid Tumors.***

| Tyrosine Kinase† | Activating Mechanisms | Cancer | Targeted Therapy‡ | References |
|---|---|---|---|---|
| **ALK (2p23)** | | | | |
| TMP3- or TMP4-ALK | t(1;2) | IMT | | Pulford et al.[16] |
| CLTC-ALK | t (2;17) | IMT | | |
| CARS-ALK | t(2;11;2) | IMT | | |
| **ERBB1 (EGFR) (7p12)** | G719C/S; deletion $LREA_{747-750}$; L858R; L861Q | NSCLC | Gefitinib, erlotinib | Lynch et al.,[6] Pao et al.,[7] Paez et al.,[54] Kris et al.,[55] Shepherd et al.[56] |
| | Extracellular-domain deletions vI–vIII | Glioblastoma, NSCLC | Gefitinib, erlotinib§ | Frederick et al.,[57] Rich et al.[58] |
| | Overexpression | SCCHN, NSCLC, ovarian cancer, RCC, pancreatic cancer, colorectal cancer | Gefitinib,§ erlotinib,§ cetuximab, ABX-EGF | Mendelsohn and Baselga,[59] Cunningham et al. [60] |
| **ERBB2 (HER-2) (17q21)** | Overexpression; kinase-domain mutations | Breast cancer, lung cancer | Trastuzumab, 2C4, CI-1033 | Finn and Slamon,[61] Stephens et al.,[62] Slamon et al.[63] |
| **ERBB3 (12q13)** | Overexpression | Soft-tissue clear-cell sarcoma | | Schaefer et al.[64] |
| **c-KIT (4q11)** | Juxtamemebrane (exon 11) mutations: deletions, V560D/A; extracellular-domain (exon 9) mutations: $dup_{AY502-503}$; kinase domain (exon 13/17) mutations: K642E, D822K/H | GIST | Imatinib | Hirota et al.,[65] Rubin et al.,[66] Demetri et al.[67] |
| | D816V/H; N822K; Y823D/C | Seminoma | Imatinib§ (not D816X) | Kemmer et al.[68] |
| | Overexpression; extracellular-domain and juxtamembrane mutations | SCLC | Imatinib§ | Boldrini et al.,[69] Johnson et al.[70] |
| | Overexpression; extracellular-domain and juxtamembrane mutations | Sarcomas | Imatinib§ | Smithey et al.,[71] Tamborini et al.[72] |
| **c-MET (7q31)** | Overexpression; truncation | Musculoskeletal tumors | | Wallenius et al.[73] |
| | Juxtamembrane mutations: R988C, T1010I | SCLC | | Ma et al.[74] |
| | TPR-MET fusion | Gastric cancer | | Soman et al.[75] |
| | Kinase-domain mutations: M1268T, M1149T | Renal papillary carcinoma | | Schmidt et al.[76] |
| | Overexpression | Malignant melanoma | | Cruz et al.[77] |

head and neck, and colorectal cancer. In metastatic, EGFR-positive, chemotherapy-refractory colorectal cancer, cetuximab alone had minimal activity, but when combined with irinotecan it had a 22 percent response rate and modestly increased progression-free and overall survival,[60] leading to FDA approval for this indication in 2004. ABX-EGF is a humanized anti-EGFR monoclonal antibody with activity as a single agent in phase 2 trials in metastatic renal-cell and colorectal carcinoma.[103]

Vascular endothelial growth factor (VEGF) is essential for angiogenesis, and either it or its two receptor TKs (VEGFR-1 and VEGFR-2) are overexpressed in many non–small-cell lung cancers and breast, prostate, renal-cell, and colorectal cancers.[104] A pivotal phase 3 study in metastatic co-

**Table 2. (Continued.)**

| Tyrosine Kinase† | Activating Mechanisms | Cancer | Targeted Therapy‡ | References |
|---|---|---|---|---|
| **NTRK1 (1q21)** | | | | |
| Tropomyosin–NTRK1 | t(1;1) | PTC | | Greco et al.[78] |
| TPR-NTRK1 | t(1;1) | PTC | | |
| TFG-NTRK1 | t(1;3) | PTC | | |
| **NTRK3 (15q25)** | | | | |
| TEL-NTRK3 | t(12;15) | Congenital fibrosarcoma, mesoblastic nephroma, secretory breast carcinoma | | Knezevich et al.,[79] Tognon et al.[80] |
| **PDGFRα (4q12)** | Overexpression | Glioblastoma, osteosarcoma | Imatinib,§ CT52923 | Hermanson et al.,[81] McGary et al.,[82] Kilic et al.[83] |
| | Overexpression | PAIS | Imatinib§ | Zhao et al.[84] |
| | Kinase-domain mutations: D842V/Y, deletion $DIMN_{842-845}$; juxtamembrane mutations: deletion $RVIES_{560-564}$, V561D | GIST | Imatinib§ | Heinrich et al.[85] |
| | t(17;22) and overexpression of COLIα1–PDGFB ligand | DFSP | Imatinib | Simon et al.,[86] McArthur[87] |
| **RET (10q11)** | Cysteine point mutations in exons 7 and 8 | MEN-2A, FMTC | | Santoro et al.[88] |
| | Kinase-domain mutation: M918T | MEN-2B | | |
| | RET–PTC gene fusions | Radiation-associated PTC | | |
| **ROS (6q22)** | | | | |
| FIG-ROS | deletion(6)(q21;q21) | Glioblastoma, astrocytoma | | Charest et al.[89] |
| **VEGFR-1 and VEGFR-2** | Overexpression of VEGF ligand | NSCLC and breast, prostate, renal, colorectal cancers | Bevacizumab, anti-VEGFR, VEGFR inhibitor | Hurwitz et al.[90] |

* IMT denotes inflammatory myofibroblastic tumor, NSCLC non–small-cell lung cancer, SSCHN squamous-cell carcinoma of head and neck, RCC renal-cell carcinoma, GIST gastrointestinal stromal tumor, SCLC small-cell lung cancer, PTC papillary thyroid carcinoma, PAIS pulmonary artery intimal sarcoma, DFSP dermatofibrosarcoma protuberans, COLIα1 collagen type Iα1, MEN-2A and MEN-2B multiple endocrine neoplasia types 2A and 2B, FMTC familial medullary thyroid carcinoma, and VEGF vascular endothelial growth factor.
† Chromosomal locations are given in parentheses.
‡ Gefitinib, erlotinib, cetuximab, trastuzumab, imatinib, and bevacizumab have been approved by the Food and Drug Administration; the other drugs listed have not been approved.
§ A therapeutic response is predicted on the basis of the identity of the tyrosine kinase but has not been verified clinically.

lorectal cancer demonstrated that the addition of bevacizumab (Avastin), a humanized anti-VEGF monoclonal antibody, to irinotecan, fluorouracil, and leucovorin led to significant prolongation of survival.[90]

### OTHER TK TARGETS IN SOLID TUMORS

Several other TKs have been implicated in solid tumors, but targeted therapies against them are not yet available (Table 2). Activating point mutations and fusions of the receptor TK RET occur in multiple endocrine neoplasia and radiation-associated thyroid cancer.[88] Similarly, the receptor TK MET is overexpressed in melanoma and musculoskeletal tumors, activated by point mutations in small-cell lung cancer and renal papillary carcinoma, and dysregulated by fusion with TPR in gastric carcinoma. The receptor TKs ROS, ALK, NTRK1,

and NTRK3 are activated through the generation of fusion proteins in a diverse set of carcinomas and sarcomas. ALK and NTRK3 fusion proteins are also found in hematologic cancers. The type of disease is dictated by the tissue of expression; for example, TEL-NTRK3 fusions found in congenital fibrosarcoma can induce hematologic cancer when expressed directly in bone marrow.[105]

## CURRENT CHALLENGES AND FUTURE DIRECTIONS

### LIMITATIONS OF TK-TARGETED THERAPIES: TOXICITY AND RESISTANCE

Targeted cancer therapies should be less toxic than conventional chemotherapy because they are specific for tumor cells. Consistent with this expectation, a maximum tolerated dose for imatinib was never reached in the phase 1 trials of this agent. However, some toxic effects of TK-targeted therapies may be related to inhibition of TKs in normal tissues and therefore difficult to eliminate. Defects in cell-mediated immunity have been reported in patients with imatinib-treated CML and may be a consequence of blockade of c-ABL signaling in T lymphocytes.[106] The cardiotoxicity of trastuzumab may reflect a requirement for HER-2 signaling in cardiomyocytes, whereas the acneiform rash frequently seen in patients who have a response to gefitinib and cetuximab may correlate with inhibition of EGFR signaling in skin.

Resistance to TK-targeted therapies is a growing problem and may be due to any of several mechanisms (Fig. 3). Resistance to TK inhibitors was first identified in patients with advanced CML who had a relapse while receiving imatinib and was associated with point mutations that rendered the ABL kinase resistant to the drug or, less commonly, was associated with *BCR-ABL* gene amplification.[107] A long list of imatinib-resistant BCR-ABL mutants has been linked to drug resistance[108] and, in some cases, to disease progression.[109] Kinase mutations also play a role in acquired resistance to TK-inhibitor therapy in GIST and non–small-cell lung cancer.[110,111] A proportion of the most primitive cancer stem cells or initiating cells, including quiescent Philadelphia chromosome–positive CD34+ cells in CML,[112] may be resistant to TK-targeted therapy — a finding that may account for the relatively low frequency of imatinib-induced molecular remissions in CML.[113] Mechanisms of resistance to monoclonal-antibody therapies targeting receptor TKs are poorly understood, but they are thought to include receptor down-regulation and loss of TK-inhibitory pathways.[114]

Several strategies may prevent or overcome resistance to TK-targeted therapies. In CML, there are several second-generation ABL kinase inhibitors with increased potency and activity against most imatinib-resistant BCR-ABL mutants.[12] Drugs that irreversibly inactivate the kinase[115] or that block substrate binding[116] may overcome "gatekeeper" mutations (such as T315I in ABL), which cause resistance to all ATP-competitive inhibitors. Combinations of monoclonal antibodies against receptor TKs and small-molecule TK inhibitors of the receptor in solid tumors are under investigation. Another approach is to combine a TK inhibitor with cytotoxic chemotherapy or with drugs targeting other signaling pathways in the cancer cell. In colorectal cancer, cetuximab and bevacizumab show benefit only when combined with chemotherapy, but the mechanism is not understood. Finally, immune mechanisms may eradicate residual malignant disease, and trials of TK inhibitors in combination with adoptive immunotherapy or tumor-cell vaccines are warranted.

### STRATEGIES TO IDENTIFY NEW TK TARGETS IN CANCER

Constitutive activation of one or more TKs or downstream signaling pathways is likely in many if not most cancers, and we have only begun to identify them. Modern cytogenetics may be able to identify additional TKs activated through chromosomal translocations, rearrangements, and deletions in cancer cells. FISH analysis revealed *ABL1* gene amplification in a subset of T-cell acute lymphoblastic leukemia[117] and led to the identification of the NUP214–ABL fusion protein.[15] In the hypereosinophilic syndrome and glioblastoma, activated fusion proteins involving the PDGFR$\alpha$ and ROS receptor TKs are generated through interstitial chromosomal deletions too small to be detected by routine cytogenetics,[27,89] but they might be revealed through array comparative genomic hybridization. A serendipitous approach, illustrated by the discovery of the FIP1L1–PDGFR$\alpha$ kinase in hypereosinophilic syndrome,[42] is to test each new TK inhibitor in a wide range of patients with cancer and to investigate the identity of the sensitive kinase in any patient who has a response. Flow-cytometric tech-

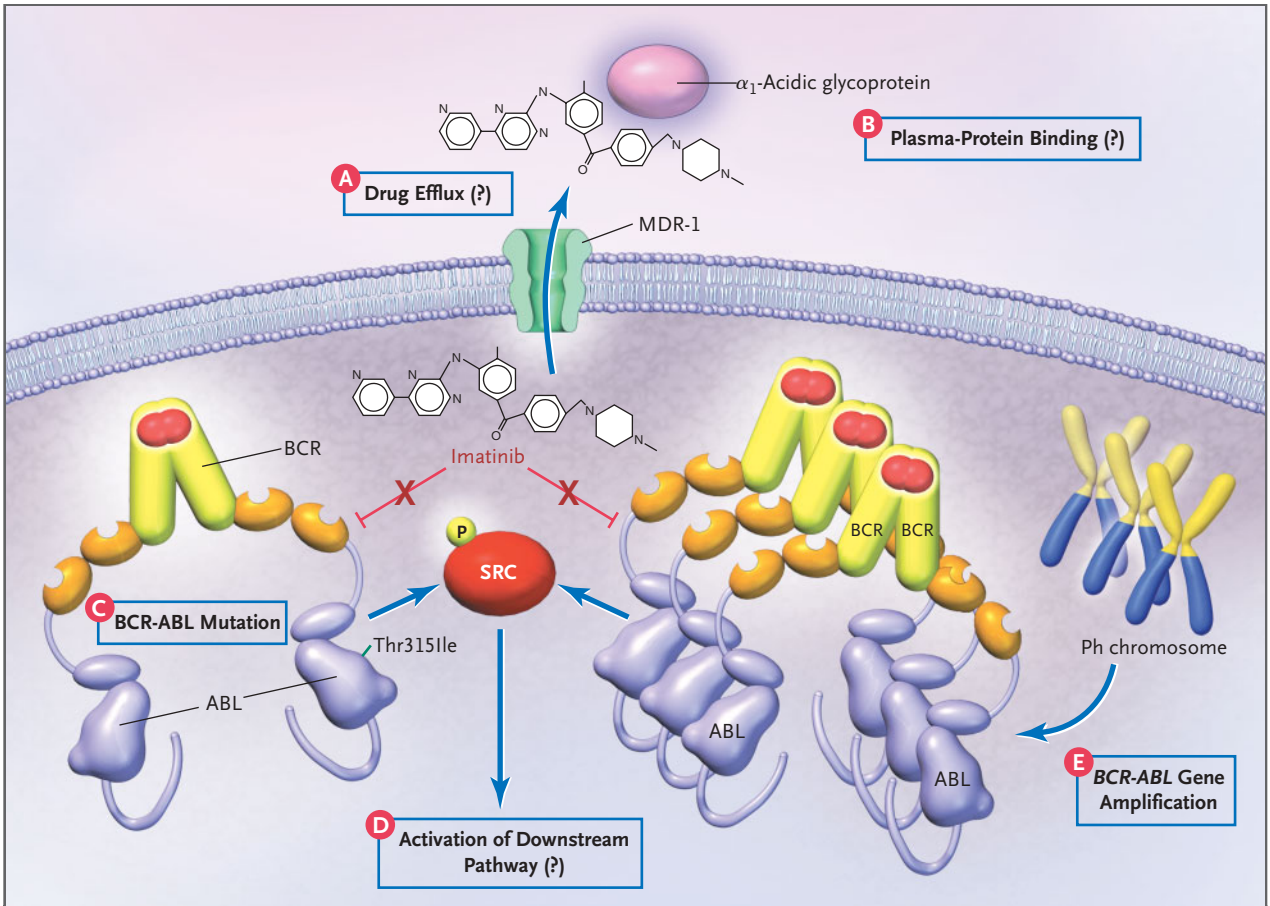



**Figure 3. Mechanisms of Resistance to TK-Inhibitor Therapy.**

Although causes of imatinib resistance in chronic myeloid leukemia are depicted here, these mechanisms are applicable to both small-molecule TK inhibitors and monoclonal antibodies against receptor TKs. Increased efflux of drug from the cancer cell, mediated by membrane transporters such as multidrug-resistance gene 1 protein (MDR-1), can decrease intracellular concentrations of the drug (mechanism A). Increased drug binding by plasma proteins such as $\alpha_1$-acidic glycoprotein can decrease the drug's effective concentration (mechanism B). Mutations in the BCR-ABL kinase domain, including Thr315Ile, can decrease or abolish the inhibitory effect of the drug (mechanism C). Constitutive activation of a signaling pathway downstream of a TK, such as a SRC family member, can alleviate the dependence of the cancer on the original TK target (mechanism D). Amplification of the *BCR-ABL* gene leading to overproduction of the TK can confer relative resistance to an inhibitor (mechanism E). For imatinib, mechanisms A, B, and D have not yet been identified in patients with drug-resistant CML.

niques to identify activated TKs and signaling pathways in primary cancer cells are showing promise.[118] Finally, a direct genomic approach can be pursued, where each exon of every TK is amplified by the polymerase chain reaction from tumor tissue and analyzed by DNA sequencing for potential activating mutations. This technique has identified several potential new activating TK mutations in colorectal cancer,[119] but it has a low yield. In cancers other than non–small-cell lung cancer, direct sequencing identified *ERBB2* and *EGFR* mutations in only 3 of 303 primary tumors and none of 203 tumor cell lines.[6,62]

## ARE CHANGES IN THE DRUG-DEVELOPMENT PROCESS NEEDED FOR TK-TARGETED THERAPIES?

The future of TK-targeted therapeutics in cancer is promising, but some changes in our approach to the development of these drugs may be indicated. Therapeutic agents targeting TKs do not fit well into traditional phase 1, 2, or 3 drug-development programs because their toxicity profile can be low and because the therapeutic responses may be limited to a small subgroup of patients with a given cancer.[120] In phase 2 or 3 studies, patients should be selected on the basis of evidence of activation of the TK target in their tumors, and such studies

should include pharmacodynamic analysis of target inhibition to avoid discarding a potentially valuable therapeutic agent because of perceived lack of efficacy.[121] The future of gefitinib is in jeopardy after a recent large phase 3 trial involving patients with refractory non–small-cell lung cancer showed that the drug conferred no survival benefit when compared with placebo[122] — a surprising finding, given that the expected frequency of tumors with responsive EGFR mutations was 8 to 10 percent and given that the study was powered to detect such differences.

Patient stratification will lead to smaller potential markets for each newly approved TK-targeted cancer therapy, a situation that may discourage the drug-development process at a very early stage. Clinical development of imatinib was nearly abandoned because the market for a drug for CML was perceived to be too small, but the high prevalence of the disease and the durability of clinical responses have driven annual sales to more than $1 billion. However, the prevalence of the other hematologic cancers caused by activated TKs (Table 1) is far lower, and strategies to encourage the development of therapeutic agents targeting uncommon subtypes of cancer are needed.

Finally, some compromise among the pharmaceutical industry, government, and third-party payers will be necessary with respect to the expense of TK-targeted therapies, each of which is priced to generate annual revenues of $20,000 to $30,000 per patient. Limiting targeted therapies to patients identified by molecular profiling as those who may have a response would help control costs, but ultimately we may not be able to afford a pharmacy of such drugs, which often must be combined with other expensive treatments and which in some patients with cancer may offer only modest survival benefits.

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