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MINIREVIEW

Clinical Targeting of Mutated and Wild-Type Protein Tyrosine Kinases in Cancer

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Clinical therapies for cancer have evolved from toxic, nontargeted agents to manageable, highly targeted therapies. Protein tyrosine kinases are a family of signaling molecules implicated in nearly every cancer type and are the foundation for the development of modern targeted agents. Recent genomic analyses have identified activating mutations, translocations, and amplifications of tyrosine kinases. Selective targeting of these genetically altered tyrosine kinases has resulted in significant clinical advances, including increased patient survival. This indicates that altered protein tyrosine kinases are the main drivers of many different cancers. However, lost during analyses of genetic lesions are the contributions of activated, wild-type kinases on tumor-dependent pathways. New approaches in phosphoproteomic technologies have identified several wild-type tyrosine kinase activation states, suggesting that non-genetically altered kinases can be essential "nodes" for signal transduction. Here, we summarize the evidence supporting the common mechanisms of protein tyrosine kinase activation in cancer and provide a personal perspective on the kinases BCR-ABL and BTK, as well as nonmutated kinase targets in prostate cancer, through our work. We outline the mechanisms of tyrosine kinase activation in the absence of direct mutation and discuss whether non-genetically altered tyrosine kinases or their associated downstream signaling pathways can be effectively targeted.

The seminal finding of BCR-ABL tyrosine kinase activity (1) and the subsequent development of the small-molecule inhibitor imatinib (2) have pioneered an era of therapies targeted toward mutated tyrosine kinases in cancer. Advancements in genomic sequencing and in our understanding of tumor signaling pathways have revealed several mutated and nonmutated pathway-activated tyrosine kinases. In this minireview, we will describe the different mechanisms of tyrosine kinase activation and how therapies tailored to these kinases have transformed disease outcomes. In particular, we will highlight two main examples of tyrosine kinases investigated in our lab, BCR-ABL and Bruton's tyrosine kinase (BTK). We will also examine a shifting paradigm in which nonmutated tyrosine kinases are being considered primary targets for tyrosine kinase inhibitor (TKI) therapy in cancer. Finally, we will discuss how phosphoproteomic approaches can identify activation of nonmutated kinase pathways and the challenges that we face by clinically targeting activated wild-type tyrosine kinases.

TYROSINE KINASE SIGNALING

Since the discovery of v-SRC and v-ABL tyrosine phosphorylation 35 years ago (3, 4), considerable progress has been made in understanding how tyrosine phosphorylation contributes to normal cellular homeostasis and disease. Tyrosine phosphorylation is regulated by a family of enzymes known as tyrosine kinases. Tyrosine kinases are crucial mediators of normal cellular signal transduction functions, including cell proliferation, survival, migration, and apoptosis. Humans express at least 90 tyrosine kinases, including 58 receptor tyrosine kinases (RTKs) (5). RTKs are activated through binding of their extracellular domain to ligands, such as growth factors and cytokines. This ligand binding results in RTK dimerization/oligomerization (6) and subsequent tyrosine phosphorylation. The N-lobe of the tyrosine kinase domain adopts a conformational change upon activation, initiating 2 phases of tyrosine phosphorylation (7). The first phase of tyrosine autophosphorylation occurs in *trans*, and phosphotyrosine residues are phosphorylated within the activation loop of the tyrosine kinase domain (in a precise order) or juxtamembrane domain (7). The second phase of tyrosine autophosphorylation occurs on phosphotyrosines that are recognized by signaling molecules containing Src homology 2 (SH2) or phosphotyrosine binding (PTB) domains (8). The recruitment of these signaling molecules then initiates a cascade of RTK-specific pathways that regulates cell fate. Hence, the abundance of signaling molecules recruited by activated RTKs renders these kinases crucial nodes for transmission of cellular information.

Despite the large number of signaling molecules recruited by RTKs, regulation of tyrosine kinases is tightly controlled to prevent aberrant cellular activities, usually through receptor-mediated ubiquitylation and degradation (9). However, genetically altered mutations or pathway-activated mechanisms of tyrosine kinases can transform a cell from a normal state into a cancerous one. Over 50% of the 90 tyrosine kinases identified have been implicated in cancer despite the fact that tyrosine phosphorylation represents only 1% of the total phosphoproteome (10). The disproportionate contribution of tyrosine kinases to cancer further highlights the need to understand the signaling networks initiated by tyrosine kinases as well as how to effectively target them.

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TABLE 1 Activating genetic alterations of tyrosine kinases and FDA-approved targeted inhibitors in human malignancies[a]

Genetic alteration	Kinase	Disease(s)	FDA-approved inhibitor(s)
Mutation(s)			
Exons 19, 21	EGFR	NSCLC	Erlotinib, afatinib
Exons 8, 9, 11, 13, 17	c-KIT	GIST	Imatinib, sunitinib
Exons 12, 14, 18	PDGFRα	GIST	Imatinib, sunitinib
Exons 10, 11, 13, 14	RET	Medullary thyroid carcinoma	Vandetinib, cabozantinib
V617F	JAK2	Myeloproliferative neoplasms	Ruxolitinib
Translocation(s)			
BCR-ABL1 fusion	ABL1	CML	Imatinib, dasatinib, nilotinib, bosutinib, ponatinib
RET/PTC family fusions	RET	PTC	Vandetinib
EML4-ALK fusion	ALK	NSCLC	Crizotinib
Amplification(s): increased copy no. and/or overexpression	ERBB2	Breast cancer, gastric cancer	Trastuzumab, lapatinib, ado-trastuzumab emtansine, pertuzumab
Pathway activation			
B-cell receptor signaling	BTK	CLL B-cell non-Hodgkin's lymphoma	Ibrutinib
Angiogenesis	VEGFR	CCRCC, soft-tissue carcinoma	Sorafenib, sunitinib, pazopanib, axitinib

[a] Abbreviations: NSCLC, non-small-cell lung cancer; GIST, gastrointestinal stromal tumor; CML, chronic myelogenous leukemia; PTC, papillary thyroid carcinoma; CCRCC, clear cell renal cell carcinoma; CLL, chronic lymphocytic leukemia.

MAJOR MECHANISMS FOR GENETICALLY ALTERED AND NONMUTATED TYROSINE KINASE ACTIVATION IN CANCER

Constitutive signals from either mutated or wild-type tyrosine kinases result in hyperactive pathways leading to continued cancer cell growth and survival. This reliance on the hyperactive pathway, known as pathway addiction, has provided unique opportunities for targeted inhibition of tyrosine kinases and has resulted in clinical successes (Table 1). Genetically altered tyrosine kinases can act as drivers in cancer through activating mutations (11–14), DNA translocations (15–17), or DNA amplifications (18) (Fig. 1A to C). However, wild-type or nonmutated tyrosine kinases can also function as critical nodes for pathway activation (19–22) (Fig. 1D and E).

Somatic activating mutations usually occur within the activation loop of the kinase domain (13, 14, 23). These mutations serve to stabilize the kinase in an active confirmation and to destabilize *cis*-inhibitory interactions. However, mutations of other domains within the tyrosine kinase can elicit constitutive activity. These include the juxtamembrane domain of KIT (13) or the cysteine-rich extracellular domain of RET (23). The cysteine-rich extracellular domain of RET forms intramolecular disulfide bonds with other cysteines in the kinase. Mutations in these cysteine residues increase aberrant disulfide bonding between partner RET molecules, resulting in dimerization and constitutive activity (24). Sequences within the juxtamembrane domain of KIT interact with several parts of the tyrosine kinase domain, maintaining the kinase in an inhibitory state. Mutations within the juxtamembrane domain of KIT disrupt these autoinhibitory interactions, resulting in a constitutively active kinase (13). Clinically, the different locations of the mutations for a particular kinase can dramatically influence patient survival (25). For example, in gastrointestinal stromal tumors (GIST), c-KIT mutations occur in several different exons (exon 9, 11, 13, or 17), and patients with mutations in exon 11 (juxtamembrane domain) respond favorably to imatinib therapy, with a longer median survival than that of patients with mutations in exon 9 (extracellular domain) (26). While the mechanism is not clear, the evidence paints a clear picture that segregating patients based on mutational status may be an important strategy for therapy decisions.

DNA translocations predominantly result in the molecular deregulation of a gene at one of the breakpoints or by creating a gene fusion. These chromosomal rearrangements include deregulation of two main classes of proteins: transcriptional regulators (frequency, 38 to 44%), such as MYC, ETV1, ERG, and RUNX1, and tyrosine kinases (frequency, 5 to 7%), including ABL, fibroblast growth factor receptor 1 (FGFR1), RET, and ALK (27, 28). Although several different tyrosine kinases are involved in gene fusions, a common feature is to maintain the catalytically active tyrosine kinase domain while removing the upstream inhibitory domains (28). Further, the breakpoint region is often conserved for each tyrosine kinase and usually located within one to three introns upstream of the tyrosine kinase domain (28). Finally, the same tyrosine kinase can have multiple fusion partners even within the same disease. These fusion partners contain coiled-coil or leucine zipper domains that result in dimerization or oligomerization of the rearranged kinase, leading to ligand-independent activation (28). However, other mechanisms, such as 5′ regulatory sequences that drive tyrosine kinase expression (29) or removal of the N-terminal myristoylation site of the BCR-ABL fusion, an important autoinhibitory element in ABL kinase (30), may also occur.

DNA amplifications, as observed with *ERBB2* (*HER2/neu*) in breast cancer (18) or *MET* in lung cancer (31) and gastric cancer (32), result in constitutive kinase activity by increasing its dimerization at the cell surface. *ERBB2* amplification/overexpression is found in 25 to 30% of breast cancers and is a significant

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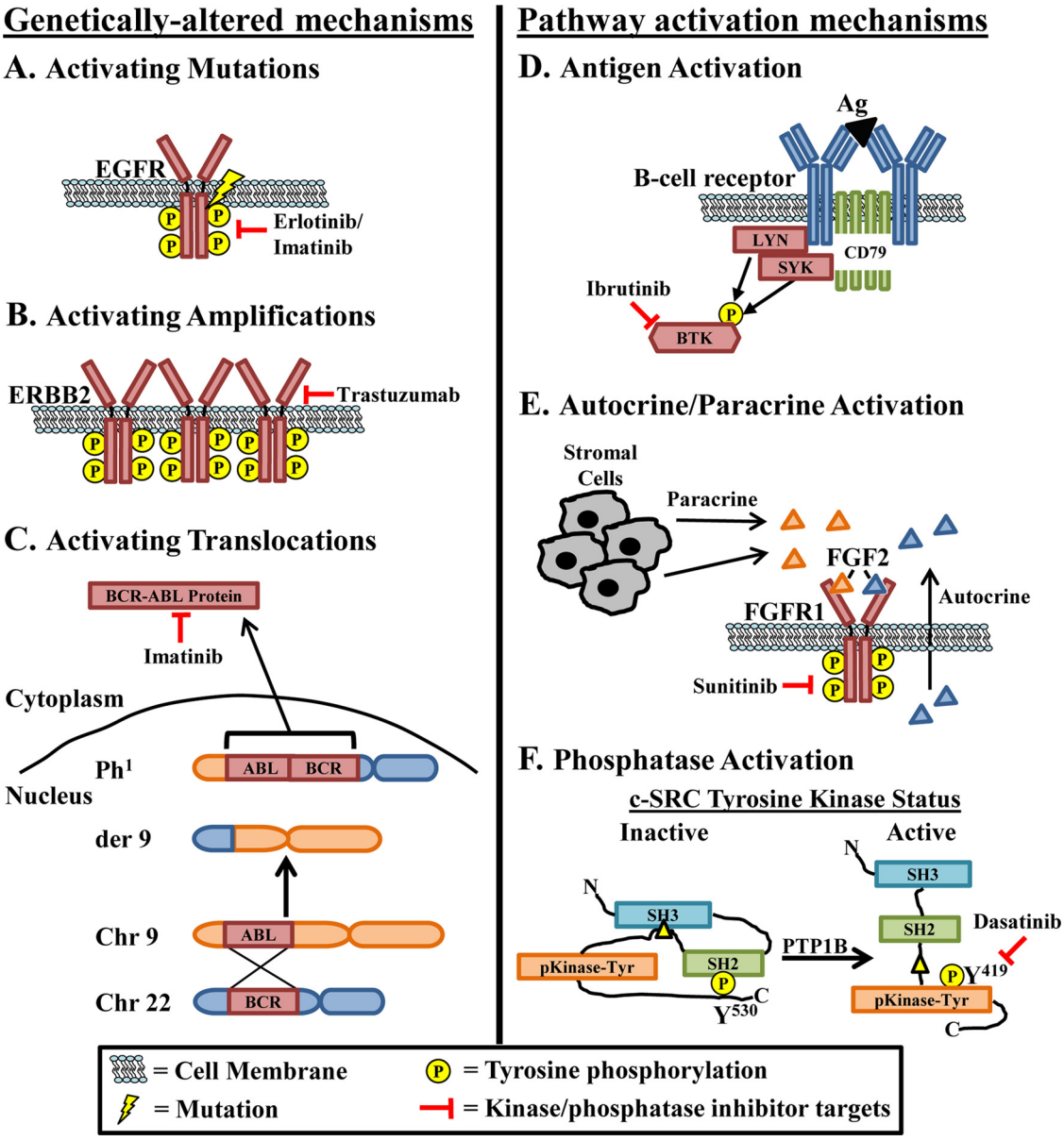

FIG 1 Mechanisms of mutated and wild-type tyrosine kinase activation in cancer. Three prototypical mechanisms of genetically altered tyrosine kinase activation as evidenced through genetic alterations. Shown are examples of mutations (A), amplifications (B), or translocations (C) that render a kinase constitutively active and thus contribute to the addiction of tumor cells on pathways driven by the genetically altered kinase. In the absence of genetic alterations (D to F), nonmutated tyrosine kinases can be activated via many different mechanisms and contribute to pathway signaling. In all cases, this pathway addiction leads to increased tumor cell proliferation and survival. In some cases, highly potent therapies that inhibit (red) the altered protein kinase have resulted in robust clinical outcomes. RTK, receptor tyrosine kinase; Chr, chromosome; Ph^1, Philadelphia chromosome; BCR, breakpoint cluster region; Ag, antigen; BTK, Bruton's tyrosine kinase; PTP1B, protein tyrosine phosphatase 1B.

predictor of overall survival and time to relapse in patients with breast cancer (18). In one study of younger women, *ERRB2* amplification as measured by fluorescent *in situ* hybridization (FISH) was highly predictive of recurrence (33). Patients with low- or high-level amplifications demonstrated a 54% or 86% recurrence rate, respectively, compared to 17% in patients without amplification. Furthermore, *ERRB2* amplification status was a stronger indicator of breast cancer recurrence risk than were classical clinical predictors such as lymph node status or tumor size. The identification of *ERRB2* as a clinical biomarker and the elucidation of its role in the pathogenesis of a subset of breast cancers subsequently led to the development of the ERBB2-targeted antibody trastuzumab (Herceptin). A pivotal trial in patients with *ERRB2*-amplified metastatic breast cancer demonstrated that the addition of trastuzumab to chemotherapy was associated with a higher response rate, longer disease-free survival, increased median survival, and a 20% reduction in disease-related death (34).

An alternative mechanism of tyrosine kinase activation involves nonmutated pathway activation. Activation of wild-type tyrosine kinases includes BTK in hematopoietic malignancies (35)

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and vascular endothelial growth factor receptors 1 and 2 (VEGF1/2), SRC, and FGFR1 in adenocarcinomas (20–22, 36). Since genetic mutations of these kinases are not frequently observed in these tumors, other mechanisms are postulated to regulate tyrosine kinase activity independently of constitutive, cell-autonomous activation. These include positive feedback loops whereby downstream mitogenic pathways positively influence tyrosine kinase activity (37); paracrine or autocrine stimulation by receptor tyrosine kinase ligands (21); increased or decreased phosphatase activity targeting pathway regulators or dephosphorylating the inhibitory sites of tyrosine kinases, resulting in activation (38); and decreased proteosomal degradation by stabilizing tyrosine kinase levels through heat shock protein 90 (HSP90) scaffold binding (39). While not exhaustive, these mechanisms shed light on several different ways that nonmutated tyrosine kinases can elicit pathway activation and emphasize the possibility that targeting these kinases or pathway components in cancer would be as beneficial as targeting mutated kinases in the clinical setting.

GENETICALLY ALTERED (BCR-ABL) AND WILD-TYPE (BTK) TYROSINE KINASES AS THERAPEUTIC TARGETS

From work done in our laboratory (1, 35, 40–49), two well-characterized but distinct examples of tyrosine kinase activity in hematopoietic malignancies are the BCR-ABL activating translocation in chronic myelogenous leukemia (CML) and acute lymphoblastic leukemia (ALL) with the Philadelphia chromosome and pathway activation through BTK in B-cell malignancies. Below, we compare and contrast these two kinases and point out the crucial observation that tyrosine kinase activation drives cancer progression via either genetic alterations (i.e., mutations) or pathway activation (i.e., wild-type kinases).

THE DUAL FUNCTION OF TYROSINE KINASE c-ABL IN CELLULAR GROWTH AND TRANSFORMATION IS DEPENDENT ON ITS CELLULAR LOCALIZATION AND PHOSPHORYLATION STATUS

The ABL nonreceptor tyrosine kinases include ABL1 (c-ABL) and ABL2 (ARG). Both of these kinases have distinct and similar functions as indicated through genetic inactivation in mice (50–52). Double knockout (*ABL/ARG*$^{-/-}$) day 10.25 embryos displayed delayed closure of the neural tube as well as severe defects in the apical network of the actin cytoskeleton (50). These mice often die by embryonic day 10.5 with hemorrhage into the peritoneum or pericardial space (50). Further, global knockout of *ABL* in C57BL/6J mice revealed severe cardiac hypertrophy due to increased proliferation and perinatal lethality (53). Recently, c-ABL and ARG were found to play an important role in vascular function (54). A mouse expressing the endothelial tissue-specific knockout of the ABL gene was crossed with a global *ARG*$^{-/-}$ background, resulting in lethality at late embryonic and perinatal stages of development (54). This was accompanied by regions of vascular loss as evidenced by increased endothelial cell apoptosis, diminished TEK (TIE2) signaling, and loss of ANGPT1-mediated survival (54).

The structural organization of c-ABL is highly similar to that of SRC family kinases. They both include an SH3 domain, an SH2 domain, and a catalytic core. However, c-ABL has a unique C terminus relative to SRC family kinases and includes F- and G-actin binding domains and nuclear export, proline-rich, nuclear localization, and DNA binding sequences (55). c-ABL activity is regulated by intramolecular interactions primarily between the SH2 and SH3 domains, which maintain c-ABL in a closed, inactive conformation (56). c-ABL continuously shuttles between the nucleus and cytoplasm. The equilibrium between these subcellular locations dictates the biological function of c-ABL. Nuclear c-ABL functions as a regulator of gene expression and inhibitor of cellular growth, while cytosolic c-ABL is involved in cellular signaling and localization to cellular adhesion complexes (57–59).

Interestingly, overexpression of c-ABL does not transform cells (60). However, the transformation capacity of c-ABL can be enhanced by several distinct mechanisms, including mutations in the kinase (61) or SH3 (62) domains, deletions of the SH3 domain (60), and translocations (47), which all maintain the tyrosine kinase activity of c-ABL (1). Tyrosine phosphorylation within the activation loop of c-ABL is critical for cellular localization and kinase activity driving cellular growth and transformation. Activation loop tyrosine phosphorylation can be triggered by activation of SRC family kinases (SFK) (63), epidermal growth factor receptor (EGFR) family members (64, 65), or insulin-like growth factor 1 receptor (IGF1R) (64), which then signals through downstream effectors such as extracellular signal-regulated kinase 5 (ERK5) (63) or Jun N-terminal protein kinase (JNK) or STAT3 (64), leading to increased tumor growth and proliferation. Indeed, tyrosine kinase inhibitors such as imatinib bind the c-ABL activation loop in its inactive conformation, thereby blocking the ability of the activation loop to bind ATP productively and rendering the kinase inactive (66).

CLINICAL TARGETING OF BCR-ABL GENE FUSION IN LEUKEMIA

The discovery of the Philadelphia (Ph) chromosome by Nowell and Hungerford provided evidence of a consistent genetic alteration in CML (67). This chromosomal abnormality mapped the cellular oncogene c-*ABL* from chromosome 9 to the breakpoint cluster region (*BCR*) of chromosome 22, creating the *BCR-ABL* fusion gene (68, 69). Phosphorylation of ABL kinase is ensured via translocation of BCR-ABL to the cytosol, which has constitutive kinase activity (1). Autophosphorylation and activation of the tyrosine kinase domain of BCR-ABL are accomplished by the formation of homotetrameric complexes mediated by the BCR coiled-coil domain (70). In addition to eliciting kinase activity, BCR-ABL also has kinase-independent functions that involve phosphorylation of Y^{177} in the BCR domain. This phosphorylation can act as a scaffold and recruits GRB2 to initiate downstream signaling pathways (71). Indeed, other kinases such as ERBB3 and PEAK1 (SGK269) function in a similar context, exhibiting elevated tyrosine phosphorylation in cancer cells behaving more as a scaffold rather than stimulating constitutive kinase activity (72, 73). However, it was the constitutive kinase activity of BCR-ABL that made it a very attractive target for inhibition. The chemical compound CGP 57148 was screened *in vitro* against a panel of kinases and was found to inhibit v-ABL with nanomolar affinity (74). This lead compound (now named imatinib [Gleevec]) was later shown to inhibit BCR-ABL tyrosine kinase activity (75) and was approved as a new treatment for patients harboring the BCR-ABL mutation in CML.

In CML, the severity of this disease can be classified into 3 phases: chronic, accelerated, and blast (or blast crisis) (76). Most patients are diagnosed in the chronic phase, are minimally symptomatic, and respond well to standard therapies. Over the course

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of 3 to 5 years, progression to the accelerated or blast phase occurs. Prior to 2001, the median survival rates were 3 to 5 years from the time of diagnosis and a bone marrow transplant was the only potentially curative treatment option. The introduction of imatinib in CML therapy has led to a major transformation in the natural history of the disease. In a 5-year follow-up of the initial phase III IRIS study, 93% of patients in chronic phase receiving imatinib did not progress to the accelerated phase or blast crisis, with an estimated overall survival rate near 90% (77). Based on a review of the outcomes associated with a diagnosis of CML since 1965 at the M. D. Anderson Cancer Center, the substantial impact of imatinib therapy on CML therapy is evident particularly in the chronic and accelerated phases of the disease (78). The 8-year survival rate of patients in chronic phase has climbed from 6% (before 1975) to 87% (since 2001). In addition, the 8-year survival rate of patients in accelerated phase has increased from <20% (before 1990) to 75% (since 2001). Furthermore, a recent study demonstrated that CML patients who are being treated with imatinib and have achieved a complete cytogenetic remission after 2 years have a 1% CML-related 8-year mortality rate that is not statistically different from the mortality rate of the general population (79).

The clinical success of imatinib in CML also brought into focus patients who were refractory to or who relapsed during imatinib therapy. Primary resistance to imatinib in chronic-phase CML is uncommon, with over 98% of patients demonstrating a complete hematologic response by 60 months in the IRIS trial (77). However, secondary resistance is problematic, as the failure rate at 60 months for patients receiving imatinib is 17%, with the majority of failures occurring within the first few years of treatment. Sequencing analysis of the blood and marrow samples from patients who relapsed after imatinib therapy demonstrated that the majority exhibited ABL kinase domain mutations that either disrupt amino acids in contact with imatinib or preclude the inactive conformational state of BCR-ABL necessary for imatinib interaction (80). Additional mechanisms of resistance have been identified and include the overexpression or amplification of BCR-ABL, activation of BCR-ABL-independent pathways, and alterations in drug influx/efflux (81, 82).

To overcome imatinib resistance, additional ABL kinase inhibitors, such as dasatinib, nilotinib, and bosutinib, were also developed as ATP-competitive inhibitors and showed high efficacy in first-line therapy and second-line therapy after failure of imatinib (83–86). However, one clinically challenging mutation has been the T315I "gatekeeper mutation," which confers resistance to nearly all BCR-ABL tyrosine kinase inhibitors (TKIs). Thr315 resides at the back of the ATP pocket, and replacement with a bulky hydrophobic isoleucine causes activation of the kinase through stabilization of the hydrophobic spine and increased interaction with ATP (87). A novel inhibitor named ponatinib that circumvents the T315I mutation and other mutations through structure-guided drug design has now been approved (88). Ponatinib forms a complex network of distributed binding interactions over multiple residues of the ABL kinase domain, which renders the complex less prone to disruption from point mutations (89). In a phase II trial in heavily pretreated patients with CML, ponatinib was highly active, with one-third of the patients demonstrating a major molecular response, indicating a multiple-log decrease in *BCR-ABL* transcript level (83). In addition, no single BCR-ABL mutation was identified that conferred resistance to ponatinib (83). Ponatinib received accelerated approval by the FDA for resistant CML but was subsequently placed on a partial hold due to significantly increased risks of serious venous and arterial thromboses appreciated after an additional 24-month clinical follow-up of the trial. However, given the therapeutic risk-to-benefit ratio, particularly for patients resistant to other TKI therapies, the sale and development of ponatinib were reauthorized with an amended boxed warning for patients.

ROLE OF BTK IN B-CELL LINEAGE DEVELOPMENT

The nonreceptor tyrosine kinase BTK is important for B-cell lineage development. A wide array of hereditary missense and nonsense mutations, insertions, and deletions spanning all domains of BTK lead to human X-linked agammaglobulinemia (XLA), which results in the arrest of early B-lymphoid development in humans (49, 90, 91). Patients with these mutations are susceptible to bacterial infections and enteroviral disease with a loss of plasma cells and reduction of serum IgG, IgM, and IgA (92). Gamma globulin therapy and antibiotics are mainstays in the treatments of these patients (92).

Analogous to human XLA, deletion or mutation of BTK in the CBA/N mouse leads to X-linked immunodeficiency (XID) (93, 94). The XID mutation results in a reduction of B-cell numbers to 30 to 50% of normal with the remaining B cells expressing an unusual IgM-high and IgD-low phenotype (95). Further, these mice have lower serum levels of IgM and IgG3, do not enter S phase after anti-IgM stimulation, and lack $CD5^+$ B-1 B cells (95, 96). As a result, B cells do not respond appropriately to a variety of activation signals, including interleukin 5 (IL-5), IL-10, and CD40 (97–99), and also fail to make antibodies to T-cell-independent type 2 antigens (95). Overall, the XID phenotype typically results in a milder form of XLA despite the observation of identical mutations (R28 residue) in patients with classical XLA (100). However, there are examples where patients display mild or asymptomatic XLA and yet harbor the R28 mutation (101, 102). This suggests that the genetic background of an individual is important in determining the severity of the XLA phenotype.

The evidence for BTK in B-cell lineage development indicates that this tyrosine kinase is critical for B-cell maturation and activation. Activation of wild-type BTK is triggered by B-cell receptor cross-linking, leading to the membrane localization of a fraction of BTK molecules (103). This translocation results in a 2-step process of BTK tyrosine phosphorylation, first by SRC family kinases followed by autophosphorylation within the SH2 domain of BTK (35, 40). Phosphorylation of BTK by SYK or LYN tyrosine kinases results in the phosphorylation and subsequent activation of phospholipase Cγ2 (PLCγ2) (104). PLCγ2 phosphorylation ultimately regulates downstream mitogen-activated protein kinase (MAPK)- and phosphoinositol-3-kinase (PI3K)-dependent signaling networks (105). These downstream signaling networks result in a diverse array of protumorigenic processes, including increased gene expression, cell proliferation, and survival (105).

WILD-TYPE BTK ACTIVATION AND THERAPEUTIC TARGETING IN LYMPHOMAS

B-cell receptor activation and subsequent signaling through BTK appear to be critical to the pathogenesis of certain B-cell malignancies. In chronic lymphocytic leukemia (CLL), autoantigen binding and activation of the B-cell receptor induce the prolifer-

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ation of leukemic clones (106). More-convincing genetic evidence of the importance of chronic active B-cell receptor signaling has been delineated in the activated B-cell-like (ABC) subtype of diffuse large B-cell lymphoma (DLBCL), in which BTK and other B-cell receptor pathway components are essential for the survival of the lymphoma (107).

The importance of BTK function in B-cell malignancies has led to the development of the tyrosine kinase inhibitor ibrutinib (PCI-32765). This drug has received three Breakthrough Therapy designations from the FDA due to its activity and promise in treating multiple B-cell malignancies, including mantle cell lymphoma (MCL) (108), Waldenstrom's macroglobulinemia (109), and CLL (110).

Ibrutinib initially received accelerated approval in the United States in November 2013 for the indication of relapsed or refractory MCL based on a phase II study that demonstrated a 68% overall response rate and estimated progression-free survival of 13.9 months (108). Notably, few grade 3 or 4 toxicities were reported, indicating that the drug was well tolerated by patients. The high activity of ibrutinib has also been reported in patients with Waldenstrom's macroglobulinemia. In a phase II study of patients with relapsed or refractory Waldenstrom's macroglobulinemia, the overall response rate was 81%, paralleled by significant improvements in red blood cell indices and reductions in serum IgM levels (109). Perhaps the greatest overall impact of ibrutinib may be seen in CLL. In a phase Ib/II trial evaluating ibrutinib in patients with relapsed or refractory CLL, the overall response rate was 71% with minimal grade 3 or 4 toxicities (110). Strikingly, the subgroup of patients with the adverse 17p13.1 deletion, wherein loss of the TP53 locus confers resistance to cytotoxic chemotherapy and portends a poor prognosis, demonstrated a 68% overall response rate with ibrutinib therapy.

PHOSPHOPROTEOMIC AND KINOME siRNA SCREENING METHODS TO DETECT AND ASSESS FUNCTION OF ACTIVATED, WILD-TYPE TYROSINE KINASES AS THERAPEUTIC TARGETS IN CANCER

Clinically, tumors are primarily assessed using genetic sequencing technologies, therefore missing pathway-activated, nonmutated tyrosine kinases. However, the lack of predominant activating tyrosine kinase mutations in some epithelial cancers makes it difficult to pinpoint essential pathway drivers, and new approaches are needed to identify nonmutated, pathway-activated tyrosine kinases. To assess these kinases, phosphoproteomic enrichment strategies coupled with highly sensitive quantitative mass spectrometry (MS) and kinome-wide small interfering RNA (siRNA) screens have been utilized (37, 111–125).

Kinome-wide siRNA screens have been used on numerous cancer cell lines to assess the behavior and effect of targeting each individual kinase within a controlled system. These screens have identified kinases that behave as regulators of hormonal signaling (122), survival and proliferation (120), mechanisms of escape during hormonal depletion (126, 127), regulators of downstream pathway activation (119, 123), or mechanisms of drug resistance (118, 121).

Phosphoproteomics provides valuable information about the activation states of tyrosine kinases independent of genomic alterations, thereby uncovering tyrosine kinases that may be driving essential pathways in cancers that next-generation whole-genome or exome sequencing may miss (117, 124, 125). A pioneering study investigating the phosphoproteomic landscape in non-small-cell lung cancer (NSCLC) tissues and cell lines identified over 50 different kinases and 2,500 downstream substrates (116). The authors further show that cell lines expressing heightened levels of the phosphorylated receptor tyrosine kinase (RTK) platelet-derived growth factor receptor alpha (PDGFRα) were sensitive to imatinib treatment while resistant cell lines were negative for PDGFRα expression. This study provides a proof of principle that kinase profiling may predict the sensitivity of tumors to selected TKIs based on RTK activation patterns. Since this publication, phosphoproteomics analyses have been performed on numerous epithelial and hematogenous cancer tissues and cell lines, including lung cancer (113, 115, 116), breast cancer (117, 128, 129), colorectal cancer (130), renal cancer (131), melanoma (132), prostate cancer (111, 125, 133, 134), CML (37), and acute myeloid leukemia (AML) (114).

We have implemented phosphoproteomics to investigate tyrosine kinase activity in both mouse models of prostate tumorigenesis where we express nontyrosine kinase oncogenes (111) and human metastatic castration-resistant prostate cancer (CRPC) tissues obtained at rapid autopsy (125). Despite the low overall mutation rates and rare events of activating mutations of tyrosine kinases in prostate cancer (135, 136), we observed numerous activated tyrosine kinases, including SRC, EGFR, ABL, and JAK2, as well as tyrosine phosphorylation of MAPK1/3 and STAT3 (111, 125). Our phosphoproteomic profiling analysis also revealed tyrosine phosphorylation signatures to be strikingly different between prostate cancer cell line-derived xenografts, treatment-naive prostate cancers, and metastatic CRPC (125, 133). This finding is interesting, as it would suggest that cell lines (at least in prostate cancer) do not accurately reflect the phosphorylation states of prostate cancer tissues isolated from patients. Most phosphoproteomic studies analyze cell lines perturbed by wild-type or mutated oncogenes implicated in the cancer type of interest and therefore limit analysis to a finite set of kinase alterations. While valuable, these analyses would not be useful for cancers that lack activating mutations of tyrosine kinases, and increased emphasis should be placed on analyzing patient tumors.

The biggest challenge arising from this and other phosphoprofiling studies will be to determine if the activated tyrosine kinases identified via phosphoproteomics are drivers of cancer in a fashion similar to that of genetically altered tyrosine kinases seen in other cancer types. In prostate cancer, the need for relevant *in vivo* models to assess the function of nonmutated activated tyrosine kinases is crucial to understanding the signaling pathways and nodes for appropriate therapy in this disease. In addition, the promiscuity of kinase inhibitors such as cabozantinib (VEGFR2, MET, or RET inhibitor) or sorafenib (RAF or VEGFR inhibitor) confounds the exact contribution of specific driver kinases in regulating survival and growth. However, this may be of therapeutic benefit, as multiple targets can be simultaneously inhibited with these agents, as suggested in CML (137). Finally, the evaluation of mechanisms of resistance to activated tyrosine kinases should also be assessed to predict combination or sequential therapies that could be tested clinically. This has been realized in certain cancers, such as BCR-ABL in CML, where sequential administration of new therapies circumvents common resistance mechanisms (83, 86).

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CONCLUDING REMARKS

As we move toward the era of personalized medicine, the reliance on state-of-the-art technologies such as next-generation sequencing and phosphoproteomics will dramatically advance our knowledge base of activated kinases in cancer. Already, this has led to dramatic improvements in cancer therapy for diseases such as breast cancer, NSCLC, CML, and B-cell malignancies. Tyrosine kinase inhibitors are also less toxic and more potent than, and highly specific compared to, previous toxic, nonspecific chemotherapies such as alkylating agents, mitotic agents, or topoisomerase inhibitors. Combined, these methodologies will present a clearer picture of the activation states of genetically altered or nonmutated kinases for targeted therapy and will enable the systematic evaluation of resistance mechanisms during cancer treatment.

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