Development of an Effective Therapy for Chronic Myelogenous Leukemia

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Abstract: Targeted small-molecule drugs have revolutionized treatment of chronic myeloid leukemia (CML) during the last decade. These agents interrupt a constitutively active BCR-ABL, the causative agent for CML, by interfering with adenosine 5′ triphosphate–dependent ABL tyrosine kinase. Although the efficacy of tyrosine kinase inhibitors (TKIs) has resulted in overall survival of greater than 90%, TKIs are not curative. Moreover, no currently approved TKIs are effective against the T315I BCR-ABL variant. However, a new generation of TKIs with activity against T315I is on the horizon. We will highlight the clinical utility of historical CML therapeutics, those used today (first- and second-generation TKIs), and discuss treatment modalities that are under development. Recent advances have illuminated the complexity of CML, especially within the marrow microenvironment. We contend that the key to curing CML will involve strategies beyond targeting BCR-ABL because primitive human CML stem cells are not dependent on BCR-ABL. Ultimately, drug combinations or exploiting synthetic lethality may transform responses into definitive cures for CML.

Key Words: Chronic myeloid leukemia, BCR-ABL, tyrosine kinase inhibitors, drug resistance, synthetic lethality

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Chronic myeloid leukemia (CML) is one of the most extensively studied cancers, and a highly treatable disease with overall survival greater than 90% using current therapies.[1–3] Chronic myelogenous leukemia results from a reciprocal transl ocation between chromosomes 9 and 22 [t(9;22)(q34;q11)], which is thought to occur in a hematopoietic stem cell. The derivative chromosome 22, originally believed to be a shortened 22, is commonly referred to as Philadelphia chromosome (Ph). As a result of the translocation, fusions are formed between the breakpoint cluster region (BCR) gene on chromosome 22 and the Abelson oncogene (ABL) on chromosome 9. BCR-ABL, which resides on Ph, is critical to disease pathogenesis, whereas its reciprocal ABL-BCR does not seem to play any major role.[4,5] The BCR-ABL protein, a constitutively active tyrosine kinase, drives survival and growth of CML cells.[6] This tyrosine kinase activity was subsequently exploited for targeted CML therapy with the development of the first successful tyrosine kinase inhibitor (TKI) imatinib.[7] Although CML accounts for only 20% of all adult and 2.6% of childhood leukemias in the United States,[8] it has become a paradigm of successful cancer therapy based on a rational treatment approach.

Patients are typically diagnosed in the chronic phase of CML (CP-CML) and usually present with constitutional symptoms, splenomegaly, and left-shifted neutrophilic leukocytosis. However, at least in developed countries, the disease is frequently discovered when an abnormal "routine" blood count leads to a diagnostic workup. The chronic phase is characterized by an expansion of the myeloid cell compartment, with preserved terminal differentiation. In the absence of efficient therapy, there is inexorable progression to accelerated phase (AP) and blastic phase/blast crisis (BP or BC), which are characterized by a gradual or sudden loss of differentiation capacity, poor response to treatment, and short survival.[9]

During the first half of the 20th century, treatment was largely limited to splenic irradiation, which offered pain control but no survival benefit. Effective drug therapy for CML began in 1953 with oral busulfan, an alkylating agent. Busulfan's use was limited by significant myelosuppression, marrow fibrosis, and prolonged aplasia but remained the preferred therapy for almost 20 years and is still in use as part of conditioning regimens in allogeneic stem cell transplantation.[10] Hydroxyurea, an inhibitor of ribonucleotide reductase, was introduced into CML therapy in 1972 and improved median survival rates over busulfan from 44 to 58 months; however, neither therapy prevented progression to BC-CML.[11–13] Allogeneic hematopoietic stem cell transplant (allo-SCT), pioneered by the Seattle group in the mid-1970s, was the first therapy known to induce a state of Ph-negativity and is still considered the only therapy with the potential of curing CML. Incremental improvements to transplant technology, such as better supportive care and high-resolution HLA typing, led to greatly improved outcomes.[14] Today, treatment algorithms reserve allografting for patients with progression to AP/BC.[15–17]

Interferon α (IFN-α) entered the therapeutic space in the mid-1980s and was the first drug that induced a cytogenetic response.[18] The exact mechanism for the antileukemic effect is not known but may involve enhanced immune surveillance, modulation of hematopoiesis, and/or interleukin signaling, resulting in selective toxicity to the leukemic clone.[19,20] In randomized controlled trials, the 6-year survival for patients on IFN therapy was 50%, much superior to chemotherapeutics (29% at 6 years with either busulfan or hydroxyurea).[21,22] Subsequent studies explored the combination of IFN with cytarabine, which had previously shown some activity as a single-agent for CML. On the basis of a randomized comparison, this combination advanced to standard-of-care drug therapy in the mid-1990s.[23] Still, only a minority of patients achieved durable responses, and most patients eventually progressed to BC. Therefore, the treatment algorithm was to offer an allogeneic stem cell transplant to all eligible patients, leaving the majority—those without a suitable donor or with prohibitive comorbidities—with IFN as their best option.[24,25]

With the advent of imatinib and the second-generation TKIs dasatinib and nilotinib, small-molecule drugs have become the mainstay for first-line CML management.[26–29] The success

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of TKI therapy has drastically improved patient survival, and projections indicate that CML prevalence will continue to increase as a result. In fact, it has been estimated that there may be up to 250,000 CML patients in the United States in 2040.[30]

Tyrosine kinase inhibitors are very effective inhibitors of BCR-ABL kinase activity; second-generation agents are more potent, and they have expanded inhibition against various BCR-ABL mutants resistant to the first-generation drug, imatinib.[31] As we mark a decade of imatinib use, we have developed an understanding of disease response to these targeted agents, although many questions still remain. Will long-term BCR-ABL inhibition by TKIs eradicate all disease-causing cells, at least in some patients? If not, how can this be accomplished? Will it be possible for one compound to completely inhibit all BCR-ABL variants, including the T315I gatekeeper mutant? This review will discuss currently approved standard-of-care drugs and highlight promising novel agents. In addition, we will cover therapeutic roadblocks, such as targeting the bone marrow microenvironment and BCR-ABL–independent survival of CML stem cells.

FOOD AND DRUG ADMINISTRATION–APPROVED FIRST-LINE TKIS

Measuring Response

Disease stage is monitored using peripheral blood and marrow differentials, marrow cytogenetics, BCR-ABL detection by fluorescence in situ hybridization (FISH), and BCR-ABL copy number surveillance by quantitative real-time polymerase chain reaction (PCR). Normalization of blood counts and spleen size is termed *complete hematologic remission* (CHR) and is the earliest measure of response. Cytogenetic response is measured as the percentage of Ph+ karyotypes in 20 bone marrow metaphases. Zero Ph metaphases constitutes a complete cytogenetic response (CCyR); 1% to 35%, a partial response (PCyR); 30% to 65%, a minor response; and 66% to 95%, a minimal response.[32] Major cytogenetic response (MCyR) includes both CCyR and PCyR. A major molecular response is defined as a 3-log reduction of BCR-ABL messenger RNA compared with a standardized baseline as measured by quantitative real-time PCR.[33] For an excellent perspective on response to TKI therapy, please see the recent review by Radich.[34]

Imatinib

Imatinib mesylate (STI571/Gleevec; Novartis) is a competitive inhibitor of the adenosine 5′ triphosphate (ATP)–binding site of the BCR-ABL tyrosine kinase. Its development is regarded as a prototype for structure-based design of specifically targeted inhibitors.[35] Preclinical efficacy was described first in patient-derived BCR-ABL–expressing cells and finally in a mouse model expressing BCR-ABL–positive cells.[36] A phase I trial included an initial cohort of 83 patients. Despite dose escalation up to 1000 mg daily, the maximum tolerated dose was not achieved, and 400 mg/d was selected as an effective dose.[7] Clinical efficacy (phase II) studies were conducted for each disease phase (CP, AP, and BC) enrolling more than 1000 patients. Impressively, these studies confirmed or surpassed the efficacy seen in phase I but also confirmed that responses in AP/BC are less frequent and less durable.[37–39] The phase III International Randomized Study of Interferon and STI571 (IRIS) study demonstrated clear superiority of imatinib over IFN plus low-dose cytarabine for CP-CML. Specifically, at 18 months, freedom from progression to AP/BC was 96.7% in the imatinib group and 91.5% in the IFN group ($P < 0.001$), with a CCyR of 76.2% compared with 14.5%.[40] On the basis of the efficacy seen in these studies, imatinib gained approval from the US Food and Drug Administration (FDA) for the treatment of patients who had failed IFN (2001) and for newly diagnosed patients in 2003. Subsequent updates of the IRIS study at 60 months confirmed these results. Overall survival in the patients treated with first-line imatinib was 89%, a revolutionary improvement over previous IFN-based regimens. No survival difference was demonstrated compared with the IFN/cytarabine arm because most IFN patients crossed over to imatinib for intolerance or lack of efficacy.[41]

Single-center studies had suggested that increasing imatinib from 400 to 800 mg/d could improve response rates. However, randomized comparisons failed to confirm these initial results.[42] More recently, the German CML IV study showed a significant difference in the rate of mismatch repair (MMR) in favor of higher doses of imatinib. It has been suggested that the more flexible dosing regimen in this study led to an overall higher dose intensity and a superior result.[43] At this point, the standard dose of imatinib for newly diagnosed patients remains 400 mg daily, and the drug remains a viable option for newly diagnosed patients in the chronic phase.[42] Imatinib, however, falls short of effectively treating most patients in AP/BC.

Dasatinib

Inhibitors targeting Src kinases were the goal of Lombardo et al[44] when they discovered a dual-Src/ABL kinase inhibitor initially referred to as BMS-354825 and now known as dasatinib (Sprycel; Bristol-Myers Squibb). Dasatinib binds with high affinity to both ABL and the SRC kinase in the ATP-binding site, translating to an ABL inhibition potency 300 times that of imatinib in biochemical and cell proliferation assays. In addition to SRC-family kinases, c-KIT, PDGFR-α/β, and the ephrin receptor kinases are also inhibited by dasatinib.[45] Uniquely, this TKI binds ABL in both the active and inactive states, leading to a more complete inhibition regardless of protein confirmation.[46]

Dasatinib dose escalation studies were conducted in a cohort of 84 patients across all CML disease phases including a minority with Ph+ acute lymphoblastic leukemia (ALL). A maximum tolerated dose for dasatinib was not determined, but importantly, patients who enrolled after previous imatinib intolerance showed no similar toxicities.[47] Efficacy of this phase I trial established 70 mg twice daily as the optimal dose for further studies. The phase II trials for Src/ABL Tyrosine kinase inhibition Activity Research Trials of dasatinib (START) were conducted separately for each disease phase. Dasatinib demonstrated a robust and durable response in CP (CHR, 87%; MyCR, 52%) and a progression-free survival of 92% at 8 months.[48] Impressive responses were seen in AP (MCyR, 33%) and BC (MCyR, 31% myeloid and 50% lymphoid); however, these responses were much less durable than those in CP.[49,50] In 2006, the FDA granted approval of dasatinib at 70 mg twice daily for refractory CML patients. Further dose optimization studies led recommendations of 100 mg once daily for CP-CML,[51,52] whereas 70 mg twice daily remained the dose for advanced CML.[53]

Nilotinib

To overcome imatinib resistance, nilotinib (AMN107/Tasigna; Novartis) was rationally designed based on a thorough analysis of the ABL-imatinib complex to increase binding affinity. Nilotinib is more selective than imatinib, favoring ABL inhibition over the 2 other target kinases KIT and PDGFR.[54] Nilotinib is 10 to 50 times more potent than imatinib and is an inhibitor of many BCR-ABL mutants that are resistant to imatinib.[54,55] Phase I studies for nilotinib in imatinib-resistant CML or Ph+ ALL patients revealed significant activity in the chronic

phase (CHR, 92%; CCyR, 35%) and acceptable responses in the accelerated phase, whereas results in the blastic phase were disappointing, recapitulating the imatinib experience.[56] An administration of 400 mg twice daily emerged as the phase II dose. Subsequent phase II studies in CP and AP reported MCyR of 48% and 29%, respectively.[57,58] Nilotinib was approved in 2007 for CP and AP-CML. Recent follow-up of these patients indicates that nilotinib provides a rapid and durable response in these disease phases, especially in patients with prior suboptimal response to imatinib.[27,59]

Resistance to Currently Approved TKIs

Despite the promise of TKIs in treating CML, drug resistance does occur. Resistance can be primary (failure of a newly diagnosed patient to achieve satisfactory response to drug) or secondary/acquired (failure of a patient on treatment who initially responded to maintain this response). Tyrosine kinase inhibitor failure has been linked to mutations in the ABL kinase domain that impair drug binding, increased BCR-ABL expression, and changes in drug efflux transporters that result in low intracellular drug concentrations, particularly with imatinib.[60,61] These changes can occur during progression to advanced disease phases, but they do not, in and of themselves, cause progression.[1] In vitro mutagenesis screens have been used to profile TKIs. These studies revealed the broadest activity for dasatinib, followed by nilotinib, whereas imatinib has extensive gaps in coverage, consistent with clinical data.[62,63] On the basis of in vitro profiles, we and others have developed heat maps of predicted in vivo activity.[64] However, it is important to note that the in vivo response is more complex, involving additional parameters such as plasma protein binding and plasma peak and trough drug concentrations.[65] As a result, the correlation between in vitro predictions and clinical responses is relatively weak,[66,67] with the notable exception of the T315I mutant, which is resistant to all currently approved TKIs. This poses a significant challenge to therapy because the T315I mutation is reported to represent 15% to 20% of all mutations.[68]

Tyrosine kinase inhibitors have transformed a previously fatal disease into a manageable chronic condition, but drug discontinuation usually results in disease recurrence, even in patients with profound responses such as MMR or "PCR-undetectable" CML, although rare exceptions may exist.[69,70] Thus, drug treatment must continue indefinitely, a significant drawback to current TKI therapy. Consistent with these clinical observations, there is evidence that all 3 agents fail to eliminate primitive CML cells and that the bone marrow environment is a potential safe haven for these cells.[71] Taken together, this suggests that minimal residual disease may be beyond the reach of our current TKI-based therapeutic arsenal. This is often referred to as disease persistence.

Second-Generation TKIs in First-Line Therapy

Treatment advantages of second-generation TKIs over imatinib were suggested during phase II studies; additional trials comparing these inhibitors were quickly planned and executed. The phase III trial, Evaluating Nilotinib Efficacy and Safety in Clinical Trials—Newly Diagnosed Patients, compared nilotinib 300 or 400 mg twice daily and imatinib (400 mg once daily). After 1 year, MMR for either nilotinib dose (43%–45%) was nearly double that of imatinib, and CCyR was significantly higher in the nilotinib cohorts (78%–80% vs 65%).[28] In addition, nilotinib was superior in progression-free survival. As a result, the FDA granted accelerated approval of nilotinib in June 2010 for newly diagnosed CML patients.[72]

The Dasatinib versus Imatinib Study in Treatment-Naive CP-CML Patients (DASISION) trial tested dasatinib at 100 mg daily versus imatinib 400 mg daily in newly diagnosed chronic phase patients. This report indicated a comparable advantage as seen in the Evaluating Nilotinib Efficacy and Safety in Clinical Trials—Newly Diagnosed Patients trial regarding MMR for dasatinib over imatinib (46% vs 28%) and CCyR (77% vs 66%).[26] Progression-free survival was also improved, although the difference failed to reach statistical significance. Regulatory approval of dasatinib for newly diagnosed CP-CML patients was granted in October 2010.

Adverse Effects of Currently Approved TKIs

A comprehensive appreciation of TKI-related toxicities is beyond the scope of this review. Hematologic toxicity is common and correlates with disease state, being more frequent in patients with advanced disease compared with newly diagnosed patients. It is generally believed that this reflects the more limited reserve of normal hematopoiesis in patients with long-standing or more aggressive CML. Nonhematologic toxicity is diverse and dependent on the specific TKI. The good news is that these toxicities are largely nonoverlapping, which implies that cross-intolerance to all 3 approved TKIs is rare. For a comprehensive and detailed review of toxicity, the reader is referred to a recent review.[73] Importantly, annual updates of the IRIS study, as well as independent studies, confirmed the safety of long-term imatinib therapy in the sense that grades 3 to 4 toxicities are rare, and no new and unexpected adverse effects became apparent with longer follow-up.[41,74] The body of data available for dasatinib and nilotinib is more limited, and it will be important to remain vigilant as therapeutic time increases for these drugs.

NOVEL AGENTS

ATP-Competitive ABL Inhibitors Without Activity Against T315I

Several TKIs have been developed that exhibit a target spectrum similar to the approved drugs, although they are distinct in off-target effects. The most advanced of these drugs is bosutinib (SKI-606; Wyeth), originally developed as a Src kinase inhibitor.[75] Bosutinib has shown inhibitory activity in CML cell lines and primary cells and has demonstrated tumor regression in CML xenograft models. Unlike approved TKIs, bosutinib does not inhibit c-Kit or PDGFR.[76] Phase I and II studies revealed drug activity in patients who failed imatinib. However, as expected, efficacy in patients who failed a second-generation TKI was lacking. A phase III study did not meet the primary end point (ie, superior rates of CCyR at 12 months in comparison with imatinib 400 mg daily). Current speculation attributes lack of efficacy to insufficient dose intensity triggered by dose interruptions due to diarrhea, a common, but transient adverse effect that should have been managed with supportive care. Bosutinib could possibly add to the therapeutic armamentarium as another drug with a unique adverse effect profile. However, it does not address the problems of the T315I mutant and BCR-ABL–independent resistance. Overall, the future of bosutinib is unclear.[77]

T315I Active Inhibitors

The most advanced third-generation inhibitor of BCR-ABL is ponatinib (AP24534; Ariad).[78] Unlike all approved TKIs, ponatinib is effective against the T315I mutant as well as a large sample of other mutants previously detected in patients with clinical TKI resistance.[68] In vitro screens revealed no mutational vulnerabilities in BCR-ABL, suggesting that ponatinib may be

the first true "pan-BCR-ABL" TKI. This drug also inhibits other kinases including FLT3, FGFR, vascular endothelial growth factor receptor (VEGFR), c-Kit, and PDGFR.[79,80] Ponatinib showed significant activity in a phase I study of patients with Ph+ leukemia, mostly CML, who had failed other TKIs. Interestingly, responses were most impressive in patients with the T315I mutation, turning a poor prognostic factor into a favorable one.[81] Ponatinib is currently in phase II clinical trials (PACE trial, Ponatinib Ph+ ALL and CML Evaluation). PACE is a global, single-arm clinical study including patients in all disease phases of CML and Ph+ ALL. Given its activity against the T315I mutant, ponatinib may well replace nilotinib and dasatinib in salvage therapy. A phase III study for ponatinib in first-line therapy is in the planning stage.

Aurora kinases are serine/threonine kinases known to regulate mitosis.[82] Because of their role in cell cycle progression and the fact that they are overexpressed in leukemias and solid tumors,[83] aurora kinases make attractive targets in CML therapeutic development. Several compounds with activity against ABL mutants, including T315I, were developed and have entered clinical trials. Among these, the most tested candidate is AT9283 (Astex Therapeutics) with activity against ABL, as well as Aurora A/B kinases, and Janus kinases 2/3 (JAK2 and JAK3).[84] Preclinical efficacy was demonstrated in mouse models leading to initiation of clinical trials.[84] Phase I and IIa clinical trials were completed in October 2010, and a recommended phase II dose was determined (NCT00522990). Danusertib, another Aurora kinase inhibitor, is currently in phase I studies in patients with refractory Ph+ leukemias.[85] Results have not yet been published. Two other Aurora kinase inhibitors with activity against T315I mutant ABL, MK-0457 and XL228, failed in clinical trials (NCT00464113) for various reasons, including toxicity.[86] The clinical efficacy of compounds inactive against T315I, but which inhibit other pathways (like the Src-family kinases) remains to be determined. Table 1 provides an overview of new compounds in development for Ph+ leukemias.

Allosteric/Non-ATP Competitive Inhibitors

DCC-2036 (Deciphera) is an inhibitor of BCR-ABL that forces a conformational change of ABL on drug binding. ABL can exist in either an active (type I) or inactive (type II) conformation based on phosphorylation status. Structure-based design of DCC-2036 elucidated a "switch-pocket" in ABL, inducing a stable and inactive state.[87] DCC-2036 inhibits ABL in a non-ATP competitive manner; it also inhibits Src, Lyn, Fgr, Hck, Flt3, and Tie2 but spares Kit. Based on efficacy in preclinical studies, a phase I trial has been initiated and is currently recruiting.

An allosteric, non-ATP competitive inhibitor of BCR-ABL is GNF-2 (Genomics Novartis Foundation), which was discovered during kinase activity screening.[88] GNF-2 is hypothesized to bind at the myristoyl binding cleft of BCR-ABL, distant from

TABLE 1. Drugs Developed for CML Therapy With Activity Against ABL-Kinase and Other Kinases Listed

Novel ABL Inhibitors				
Inhibitor	**Non-ABL Kinase Target(s)**	**T315I**	**Status**	**References**
DCC-2036	Src, Lyn, Fgr, Hck, Flt3, Tie2	Active	Phase I/II	NCT00827138
GNF compounds	ABL only	Active	Preclinical	*
ON012380	ABL only	Active	Preclinical	†
PPY-A	ABL only	Active	Preclinical	‡,§
SGX393	ABL only	Active	Preclinical	¶
XL228	Aurora A/B, FAK, Src	Active	Phase I—terminated	NCT00464113
MK-0457	Aurora A-C, Flt3	Active	Phase II—terminated	NCT00405054
AT9283	Aurora A/B, JAK2/3	Active	Phase I/II	NCT00522990
Danusertib	Aurora A-C, Ret, Trk-A, FGFR-1	Active	Phase II	NCT00335868
Ponatinib	Flt3, FGFR, VEGFR, c-kit, PDGFR	Active	Phase II	NCT01207440
Bafetenib	Lyn	NA	Phase I—development unlikely	NCT00352677
AP23464	Src family	Active	Preclinical	‖
Bosutinib	Src, TEC, STE20, CAMK2G	NA	Phase I/II/III	NCT00811070, NCT00261846
DSA compounds	Src	Active	Preclinical	**
PD166326	Src	NA	No trials or recent reports	††
Saracatinib	Src family	NA	Not in trials for CML	‡‡
HG-7-85-01	Src, PDGFR, VEGFR, Flt3, Ret, Tie2, Kit, DDR1, b-raf	Active	Preclinical	§§

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†*Proc Natl Acad Sci U S A*. 2005;102:1992–1997.

‡*Chem Biol Drug Des*. 2007;70:171–181.

§*Med Res Rev*. 2011;31:1–41.

¶*Proc Natl Acad Sci U S A*. 2008;105:5507–5512.

‖*Chem Biol Drug Des*. 2010;75:223–227.

***Cancer Res*. 2009;69:2384–2392.

††*Blood*. 2005;105:3995–4003.

‡‡*Expert Opin Investig Drugs*. 2010;19:931–945.

§§*Blood*. 2010;115:4206–4216.

NA indicates not active.

the active site of BCR-ABL. GNF-2 has exceptional specificity for BCR-ABL, does not inhibit c-Kit, PDGFR, or other kinases (63 tested), and is nontoxic toward non–BCR-ABL–expressing cells.[88] GNF-2 has been found to enhance imatinib activity against BCR-ABL, whereas a GNF-2 analog (21a-I) was found to synergize with dasatinib against the T315I mutant.[89] Other GNF analogues are in development,[90,91] but none are currently in clinical trials.

The Essential BCR Coiled Coil

Oligomerization of BCR-ABL through the coiled-coil domain (Fig. 1) is essential for oncogenicity,[92,93] making this region an attractive target for therapeutic development.[94] Non-small-molecule inhibitors targeting the BCR coiled-coil are exciting alternatives that disrupt BCR-ABL oligomerization and activation. We have recently reported the disruption of BCR-ABL via a rationally designed mutant coiled-coil peptide.[95] Such peptides may reduce the risk of acquired resistance due to the numerous contact points between the coiled-coil and the protein or because peptides are not typical substrates for drug efflux transporters whose overexpression may lead to resistance.[85] Delivery strategies for peptide therapeutics to the CML cell are a current focus of our laboratory.

Degrading BCR-ABL

A natural compound in vegetables, PEITC, was found to kill T315I harboring cells in culture and from patient samples.[96] PEITC induces oxidative stress in CML cells leading to degradation of BCR-ABL. Another degradation strategy involves a novel ubiquitin cycle inhibitor, WP1130, reported to rapidly induce ubiquitination of BCR-ABL resulting in protein relocation into aggresomes, rendering it inactive. Both imatinib-sensitive and -resistant CML cells initiated apoptosis in response to WP1130.[97]

Hsp90 (heat shock protein 90) inhibitors geldanamycin and 17-AAG were shown to induce degradation of BCR-ABL protein in vitro.[98,99] Mechanistically, after dissociation of Hsp-90 from client proteins, Bag1 (B-cell lymphoma-2 [Bcl-2]–associated athanogene-1) mediates BCR-ABL localization to the proteasome and stimulates its degradation via an E3-ligase–dependent mechanism.[100] However, clinical trials in CML were disappointing.

Immunotherapy

In addition to small molecules, immunologic targeting of BCR-ABL, rather than kinase inhibition, may be effective. Interferon may function by inducing cytotoxic T-cell responses against myeloid antigens.[101] A more specific approach is vaccines targeting the BCR-ABL junction.[102,103] Despite some encouraging results, the efficacy of this approach remains unproven in the absence of a prospective randomized trial. Antibodies to the BCR-ABL junction have also been produced.[104,105] Updates to these are smaller fragments of antibodies such as iDabs,[106] including those specific to BCR-ABL,[107] and small

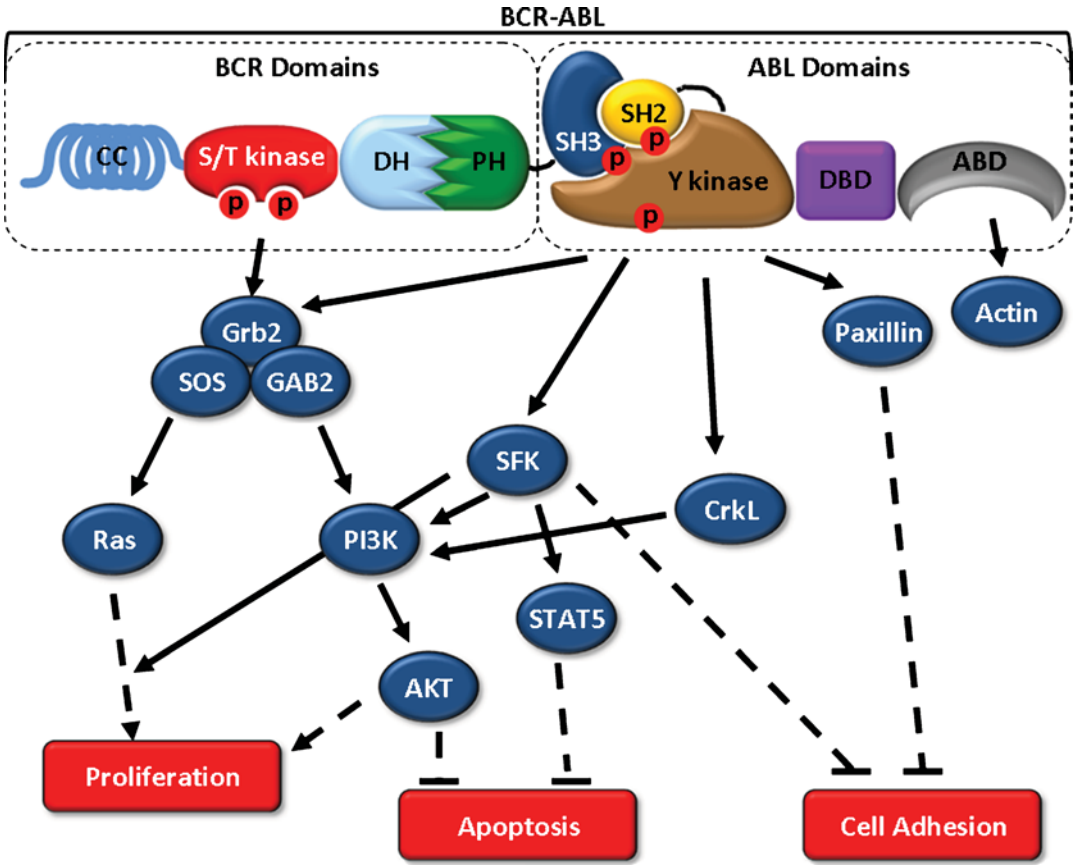

FIGURE 1. p210 BCR-ABL functional domains and effects of downstream signaling. BCR-ABL signaling leads to enhanced proliferation, reduced apoptotic potential, and altered cell adhesion. Contributions from both BCR and ABL domains on downstream signaling are illustrated. Dashed lines indicate additional intermediate signaling steps not detailed in this figure. ABD indicates actin binding domain; CC, coiled-coil; DBD, DNA binding domain; DH, Dbl homology; PH, Pleckstrin homology; S/T kinase, serine/threonine kinase; SH2 or SH3, Src homology 2/3; Y kinase, tyrosine kinase. Figure courtesy of Andrew Dixon.

antibody mimics or monobodies.[108] The clinical utility of these antibodies is unclear.

TARGETING CML STEM CELLS AND THEIR MICROENVIRONMENT

Stem Cell Niche

In vitro, TKIs are known to have antiproliferative effects on primitive CML cells, but they do not induce apoptosis. This may explain why TKIs fail to eliminate CML stem cells in vivo, evident by disease persistence and the inability to discontinue therapy. We have reported that primitive human CML stem cells are not dependent on BCR-ABL, suggesting that, on TKI challenge, CML stem cells rely on survival signals other than BCR-ABL. It is likely that these signals are provided by the microenvironment. It follows that therapies that only biochemically target BCR-ABL will be unable to eliminate CML stem cells.[71] Cytokines, chemokines, and the extracellular matrix, collectively referred to as the microenvironment, may activate signaling pathways involved in survival. Therapeutic strategies that target stem cells within this context hold promise to eliminate residual leukemia, including cytokine antagonists, adhesion molecule antagonists, and inhibitors of survival and self-renewal.[109]

The Hedgehog (Hh) signaling pathway has been implicated in hematopoietic stem cell renewal. Consistent with a critical role of Hh for CML pathogenesis, lack of Smoothened, an essential component of the pathway, was shown to attenuate CML in murine models.[110] Similarly, the Hedgehog inhibitor LDE225 in combination with nilotinib resulted in elimination of CML stem and progenitor cells.[111] Several Hedgehog inhibitors, including PF-04449913, for hematologic malignancies are also in clinical development.[112] Wnt/β-catenin signaling has also been shown to play a critical role in hematopoietic stem cell self-renewal and may offer therapeutic opportunities.[113]

AKT, a well-established downstream target of BCR-ABL, phosphorylates the Foxo3a transcription factor, leading to its exclusion from the nucleus and suppression of transcription. Despite this, Foxo3a is nuclear in primitive CML cells. Recent data have suggested that TGF-β signaling may be responsible for this unexpected finding, and it has been inferred that this may allow CML stem cells to remain in a quiescent state, despite BCR-ABL activity. If so, this would suggest that inhibiting TGF-β may push the critical cells into cycle, thereby rendering them susceptible to BCR-ABL inhibition. Efficient depletion of CML in vivo was found with a combination treatment using imatinib, a TGF-β inhibitor, and Foxo3a depletion.[114]

Yet another strategy is to interfere with stem cell homing. For example, CXCR4 is a receptor for the chemokine SDF-1 (stromal-derived factor 1), and plays a role in homing

TABLE 2. A Summary of Current Combination Therapies to Improve CML Treatment Outcomes in Clinical Trials

Combination Therapies for CML				
TKI	**Combination Second/Third Drug**	**Function of Non-TKI**	**Stage**	**Reference**
Any TKI	Arsenic trioxide	Multiple*	Phase I	NCT01397734
BOS/DAS	PF-04449913	Hh inhibitor	Phase I	NCT00953758
DAS	BMS-833923	smo inhibitor	Phase I/II	NCT01218477
DAS	Vorinostat	HDAC inhibitor	Phase I	NCT00816283
IM	Cytarabine or IFN	DNA synthesis or multiple†	Phase III	NCT00219739
IM	IFN	Multiple†	Phase II/IV	NCT00573378, NCT00390897
IM	IFN/granulocyte macrophage colony stimulating factor (GM-CSF)	Multiple†/GM differentiation	Unknown	NCT00050531
IM	Valproic acid	HDAC inhibitor	Phase II	NCT01011998
IM	Homoharringtonine (HHT)	Protein synthesis inhibitor	Phase II	NCT00114959
IM	Vatalanib (PTK 787)	VEGF, c-KIT, PDGFR inhibitor	Phase I/II	NCT00088231
IM	Zileuton	Alox5 inhibitor	Phase I	NCT01130688
IM	NIL	BCR-ABL	Phase II	NCT00769327
IM	Arsenic trioxide	Multiple*	Phase II	NCT00250042
IM	Lonafarnib	Farnesyl-OH-transferase inhibitor	Phase I	NCT00047502
IM	Tipifarnib	Farnesyltransferase inhibitor	Phase I	NCT00040105
IM	Vincristine/dexamethasone	Microtubule inhibitor/immunosuppressant	Phase II	NCT00763763
IM	GM-K562 - biologic	Immune surveillance initiation	Phase II	NCT00363649
IM	Everolimus	mTOR inhibitor	Phase I/II	NCT00093639
IM	Hydroxychloroquine	Lysosomal acidification/autophagy inhibitor	Phase II	NCT01227135
IM	TALL-104 - biologic	Modified therapeutic T cell	Phase II	NCT00415909
NIL	IFN	Multiple†	Phase I/II	NCT01220648, NTC01294618

*Proapoptotic/antiproliferative.

†Inhibits angiogenesis migration and proliferation.

BOS indicates bosutinib; DAS, dasatinib; GM, granulocyte and macrophage; HDAC, histone deacetylase; Hh, hedgehog; IM, imatinib; mTOR, molecular target of rapamycin; NIL, nilotinib; PDGFR, platelet-derived growth factor receptor; smo, smoothened; TKI, tyrosine kinase inhibitor; VEGF, vascular endothelial growth factor.

of CD34+ stem cells to the bone marrow microenvironment. Imatinib inhibition of BCR-ABL restores the CXCR4 interaction with SDF-1, leading to the migration and attachment of CML cells to the bone marrow microenvironment. However, a CXCR4 antagonist, AMD3465, partially inhibited cell migration to mesenchymal cells in coculture conditions. Similar results were seen with QLT0267, an integrin signaling inhibitor.[115]

Drug Combinations and Synthetic Lethality

Although stem cells express, but are not addicted to, BCR-ABL it may still be possible to manipulate other pathways which assume an essential role in response to ABL inhibition. This idea of synthetic lethality for cancer therapy is not new but has recently received more attention in the CML field propelled by emerging data demonstrating BCR-ABL–independent disease persistence on TKI therapy. In an RNAi-based screen for dysregulated genes in response to imatinib therapy, the Wnt pathway emerged as the viable target for a second hit.[116] Other critical pathways involved in disease progression or leukemic cell function have become attractive targets to augment BCR-ABL inhibition. For example, inhibition of ATG7,[117] MUC1,[118] Alox5,[119] and mTOR[120] have all been investigated in preclinical studies because they do not cause loss of hematopoietic stem cell function but instead target the leukemic clone in combination with TKIs. A list of recent clinical trials for combination therapies can be found in Table 2.

Finally, transcription factors such as STAT5 can mediate resistance to TKIs.[121] Some patients in BC-CML have significant down-regulation of signal transducers and activators of transcription (STAT) inhibitor proteins, potentiating cell survival and residual disease.[122] A new STAT5 inhibitor, pimozide, is able to decrease STAT5 and its target genes, resulting in growth inhibition of Ph+ patient samples independently of ABL mutations.[123] The precise mechanism of action of this compound is not known. For a comprehensive discussion on other signal transduction pathways in CML, the reader is referred to the referenced chapter.[124]

CONCLUSIONS

The rational design of drugs targeting BCR-ABL has made CML a manageable disease, resulting in prolonged survival for most patients. Mutations resulting in resistance to imatinib have driven development of the second-generation TKIs nilotinib and dasatinib. These inhibitors are active against a broad spectrum of BCR-ABL mutants, with the notable exception of the T315I "gatekeeper" mutant, which, in turn, has led to third-generation inhibitors. The most advanced of these is ponatinib, which has been termed a *pan-BCR-ABL inhibitor*, as it does not have identifiable gaps in BCR-ABL coverage. As complete ablation of BCR-ABL activity becomes a reality, the question arises whether we will see BCR-ABL–independent resistance emerge as a unifying feature of TKI failure. As the field has focused on the role of kinase domain mutations, relatively little is known about these mechanisms.

On the other side of the response spectrum is minimal residual leukemia despite prolonged TKI therapy. Although the relapse rate in this population of patients is very low, the need for continued treatment has major health and economic implications, and it remains possible that we will see unexpected late adverse effects in patients after decades of TKI therapy. Recent evidence suggests that primitive CML cells survive despite inhibition of BCR-ABL, suggesting a biologic barrier to disease eradication by TKIs.[71] We contend that eradicating CML will require targeting the stem cell niche. Several pathways have emerged as potential targets, and a clear winner has not yet been identified. In many respects, CML has served as a paradigm for cancer therapy, and it is likely that this will continue to be the case as we start to transform profound responses into definitive "cures."

REFERENCES

1. Kantarjian H, O'Brien S, Jabbour E, et al. Impact of treatment end point definitions on perceived differences in long-term outcome with tyrosine kinase inhibitor therapy in chronic myeloid leukemia. *J Clin Oncol*. 2011;29:3173–3178.
2. Signorovitch JE, Wu EQ, Betts KA, et al. Comparative efficacy of nilotinib and dasatinib in newly diagnosed chronic myeloid leukemia: a matching-adjusted indirect comparison of randomized trials. *Curr Med Res Opin*. 2011;27:1263–1271.
3. Tauchi T, Kizaki M, Okamoto S, et al. Seven-year follow-up of patients receiving imatinib for the treatment of newly diagnosed chronic myelogenous leukemia by the TARGET system. *Leuk Res*. 2011;35:585–590.
4. Bartram CR, de Klein A, Hagemeijer A, et al. Translocation of c-*ab1* oncogene correlates with the presence of a Philadelphia chromosome in chronic myelocytic leukaemia. *Nature*. 1983;306:277–280.
5. Rowley JD. Letter: a new consistent chromosomal abnormality in chronic myelogenous leukaemia identified by quinacrine fluorescence and Giemsa staining. *Nature*. 1973;243:290–293.
6. Anafi M, Gazit A, Gilon C, et al. Selective interactions of transforming and normal abl proteins with ATP, tyrosine–copolymer substrates, and tyrphostins. *J Biol Chem*. 1992;267:4518–4523.
7. Druker BJ, Talpaz M, Resta DJ, et al. Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. *N Engl J Med*. 2001;344:1031–1037.
8. National Cancer Institute, National Institutes of Health. Surveillance Epidemiology and End Results Web site. Available at: http://seer.cancer.gov/statfacts/html/cmyl.html. Accessed July 14, 2011.
9. Vardiman JW, Harris NL, Brunning RD. The World Health Organization (WHO) classification of the myeloid neoplasms. *Blood*. 2002;100:2292–2302.
10. Wite LJ, Blackburn EK, Callender ST, et al. Chronic granulocytic leukaemia: comparison of radiotherapy and busulphan therapy. Report of the Medical Research Council's working party for therapeutic trials in leukaemia. *Br Med J*. 1968;1:201–208.
11. Hehlmann R, Heimpel H, Hasford J, et al. Randomized comparison of busulfan and hydroxyurea in chronic myelogenous leukemia: prolongation of survival by hydroxyurea. The German CML Study Group. *Blood*. 1993;82:398–407.
12. Rushing D, Goldman A, Gibbs G, et al. Hydroxyurea versus busulfan in the treatment of chronic myelogenous leukemia. *Am J Clin Oncol*. 1982;5:307–313.
13. Richards SM. Hydroxyurea versus busulphan for chronic myeloid leukaemia: an individual patient data meta-analysis of three randomized trials. Chronic myeloid leukemia trialists' collaborative group. *Br J Haematol*. 2000;110:573–576.
14. Pavlu J, Szydlo RM, Goldman JM, et al. Three decades of transplantation for chronic myeloid leukemia: what have we learned? *Blood*. 2011;117:755–763.
15. Breccia M, Palandri F, Iori AP, et al. Second-generation tyrosine kinase inhibitors before allogeneic stem cell transplantation in patients with chronic myeloid leukemia resistant to imatinib. *Leuk Res*. 2010;34:143–147.
16. Oyekunle A, Klyuchnikov E, Ocheni S, et al. Challenges for allogeneic hematopoietic stem cell transplantation in chronic myeloid leukemia in the era of tyrosine kinase inhibitors. *Acta Haematol*. 2011;126:30–39.
17. Venepalli N, Rezvani K, Mielke S, et al. Role of allo-SCT for CML in 2010. *Bone Marrow Transplant*. 2010;45:1579–1586.
18. Talpaz M, Kantarjian HM, McCredie K, et al. Hematologic remission and cytogenetic improvement induced by recombinant human interferon alpha A in chronic myelogenous leukemia. *N Engl J Med*. 1986;314:1065–1069.

19. Dowding C, Gordon M, Guo AP, et al. Potential mechanisms of action of interferon-alpha in CML. *Leuk Lymphoma*. 1993;11(1 suppl):185–191.
20. Guilhot F, Lacotte-Thierry L. Interferon-alpha: mechanisms of action in chronic myelogenous leukemia in chronic phase. *Hematol Cell Ther*. 1998;40:237–239.
21. Ohnishi K, Ohno R, Tomonaga M, et al. A randomized trial comparing interferon-alpha with busulfan for newly diagnosed chronic myelogenous leukemia in chronic phase. *Blood*. 1995;86:906–916.
22. Tura S, Baccarani M, Zuffa E, et al. The Italian Cooperative Study Group on Chronic Myeloid Leukemia. Interferon alfa-2a as compared with conventional chemotherapy for the treatment of chronic myeloid leukemia. *N Engl J Med*. 1994;330:820–825.
23. Beck JR, Guilhot J, Giles FJ, et al. Cytarabine added to interferon improves the cost-effectiveness of initial therapy for patients with early chronic phase chronic myelogenous leukemia. *Leuk Lymphoma*. 2001;41:117–124.
24. Silver RT, Woolf SH, Hehlmann R, et al. An evidence-based analysis of the effect of busulfan, hydroxyurea, interferon, and allogeneic bone marrow transplantation in treating the chronic phase of chronic myeloid leukemia: developed for the American Society of Hematology. *Blood*. 1999;94:1517–1536.
25. Silberman G, Crosse MG, Peterson EA, et al. Availability and appropriateness of allogeneic bone marrow transplantation for chronic myeloid leukemia in 10 countries. *N Engl J Med*. 1994;331:1063–1067.
26. Kantarjian H, Shah NP, Hochhaus A, et al. Dasatinib versus imatinib in newly diagnosed chronic-phase chronic myeloid leukemia. *N Engl J Med*. 2010;362:2260–2270.
27. Nicolini FE, Turkina A, Shen ZX, et al. Expanding Nilotinib Access in Clinical Trials (ENACT): an open-label, multicenter study of oral nilotinib in adult patients with imatinib-resistant or imatinib-intolerant Philadelphia chromosome–positive chronic myeloid leukemia in the chronic phase. *Cancer*. 2011.
28. Saglio G, Kim DW, Issaragrisil S, et al. Nilotinib versus imatinib for newly diagnosed chronic myeloid leukemia. *N Engl J Med*. 2010;362:2251–2259.
29. Deininger M, O'Brien SG, Guilhot F, et al. International Randomized Study of Interferon Vs STI571 (IRIS) 8-year follow up: sustained survival and low risk for progression or events in patients with newly diagnosed chronic myeloid leukemia in chronic phase (CML-CP) treated with imatinib. *ASH Annual Meeting Abstracts*. 2009;114:1126.
30. Corm S, Micol J, Leroyer A, et al. Kinetic of chronic myeloid leukaemia (CML) prevalence in Northern France since the introduction of imatinib. *J Clin Oncol*. 2008;26(20 suppl):Abstract 7088.
31. Hantschel O, Rix U, Superti-Furga G. Target spectrum of the BCR-ABL inhibitors imatinib, nilotinib and dasatinib. *Leuk Lymphoma*. 2008;49:615–619.
32. Baccarani M, Castagnetti F, Gugliotta G, et al. Response definitions and European Leukemianet Management recommendations. *Best Pract Res Clin Haematol*. 2009;22:331–341.
33. Hughes TP, Kaeda J, Branford S, et al. Frequency of major molecular responses to imatinib or interferon alfa plus cytarabine in newly diagnosed chronic myeloid leukemia. *N Engl J Med*. 2003;349:1423–1432.
34. Radich JP. Measuring response to BCR-ABL inhibitors in chronic myeloid leukemia. *Cancer*. [published online ahead of print June 29, 2011] doi:10.1002/cncr.26280.
35. Druker BJ, Lydon NB. Lessons learned from the development of an abl tyrosine kinase inhibitor for chronic myelogenous leukemia. *J Clin Invest*. 2000;105:3–7.
36. Druker BJ, Tamura S, Buchdunger E, et al. Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr-Abl positive cells. *Nat Med*. 1996;2:561–566.
37. Talpaz M, Silver RT, Druker BJ, et al. Imatinib induces durable hematologic and cytogenetic responses in patients with accelerated phase chronic myeloid leukemia: results of a phase 2 study. *Blood*. 2002;99:1928–1937.
38. Sawyers CL, Hochhaus A, Feldman E, et al. Imatinib induces hematologic and cytogenetic responses in patients with chronic myelogenous leukemia in myeloid blast crisis: results of a phase II study. *Blood*. 2002;99:3530–3539.
39. Kantarjian H, Sawyers C, Hochhaus A, et al. Hematologic and cytogenetic responses to imatinib mesylate in chronic myelogenous leukemia. *N Engl J Med*. 2002;346:645–652.
40. O'Brien SG, Guilhot F, Larson RA, et al. Imatinib compared with interferon and low-dose cytarabine for newly diagnosed chronic-phase chronic myeloid leukemia. *N Engl J Med*. 2003;348:994–1004.
41. Druker BJ, Guilhot F, O'Brien SG, et al. Five-year follow-up of patients receiving imatinib for chronic myeloid leukemia. *N Engl J Med*. 2006;355:2408–2417.
42. Cortes JE, Baccarani M, Guilhot F, et al. Phase III, randomized, open-label study of daily imatinib mesylate 400 mg versus 800 mg in patients with newly diagnosed, previously untreated chronic myeloid leukemia in chronic phase using molecular end points: tyrosine kinase inhibitor optimization and selectivity study. *J Clin Oncol*. 2010;28:424–430.
43. Baccarani M, Cortes J, Pane F, et al. Chronic myeloid leukemia: an update of concepts and management recommendations of European LeukemiaNet. *J Clin Oncol*. 2009;27:6041–6051.
44. Lombardo LJ, Lee FY, Chen P, et al. Discovery of *N*-(2-chloro-6-methylphenyl)-2-(6-(4-(2-hydroxyethyl)- piperazin-1-yl)-2-methylpyrimidin-4-ylamino)thiazole-5-carboxamide (BMS-354825), a dual Src/Abl kinase inhibitor with potent antitumor activity in preclinical assays. *J Med Chem*. 2004;47:6658–6661.
45. Lindauer M, Hochhaus A. Dasatinib. Recent results. *Cancer Res*. 2010;184:83–102.
46. Burgess MR, Skaggs BJ, Shah NP, et al. Comparative analysis of two clinically active BCR-ABL kinase inhibitors reveals the role of conformation-specific binding in resistance. *Proc Natl Acad Sci U S A*. 2005;102:3395–3400.
47. Talpaz M, Shah NP, Kantarjian H, et al. Dasatinib in imatinib-resistant Philadelphia chromosome–positive leukemias. *N Engl J Med*. 2006;354:2531–2541.
48. Hochhaus A, Kantarjian HM, Baccarani M, et al. Dasatinib induces notable hematologic and cytogenetic responses in chronic-phase chronic myeloid leukemia after failure of imatinib therapy. *Blood*. 2007;109:2303–2309.
49. Guilhot F, Apperley J, Kim DW, et al. Dasatinib induces significant hematologic and cytogenetic responses in patients with imatinib-resistant or -intolerant chronic myeloid leukemia in accelerated phase. *Blood*. 2007;109:4143–4150.
50. Cortes J, Rousselot P, Kim DW, et al. Dasatinib induces complete hematologic and cytogenetic responses in patients with imatinib-resistant or -intolerant chronic myeloid leukemia in blast crisis. *Blood*. 2007;109:3207–3213.
51. Shah NP, Kantarjian HM, Kim DW, et al. Intermittent target inhibition with dasatinib 100 mg once daily preserves efficacy and improves tolerability in imatinib-resistant and -intolerant chronic-phase chronic myeloid leukemia. *J Clin Oncol*. 2008;26:3204–3212.
52. Porkka K, Khoury HJ, Paquette RL, et al. Dasatinib 100 mg once daily minimizes the occurrence of pleural effusion in patients with chronic myeloid leukemia in chronic phase and efficacy is unaffected in patients who develop pleural effusion. *Cancer*. 2010;116:377–386.
53. Apperley JF, Cortes JE, Kim DW, et al. Dasatinib in the treatment of chronic myeloid leukemia in accelerated phase after imatinib failure: the START a trial. *J Clin Oncol*. 2009;27:3472–3479.
54. Weisberg E, Manley PW, Breitenstein W, et al. Characterization of AMN107, a selective inhibitor of native and mutant Bcr-Abl. *Cancer Cell*. 2005;7:129–141.
55. Weisberg E, Manley P, Mestan J, et al. AMN107 (nilotinib): a novel and selective inhibitor of BCR-ABL. *Br J Cancer*. 2006;94:1765–1769.
56. Kantarjian H, Giles F, Wunderle L, et al. Nilotinib in imatinib-resistant CML and Philadelphia chromosome–positive ALL. *N Engl J Med*. 2006;354:2542–2551.
57. le Coutre P, Ottmann OG, Giles F, et al. Nilotinib (formerly AMN107), a highly selective BCR-ABL tyrosine kinase inhibitor, is active in patients with imatinib-resistant or -intolerant accelerated-phase chronic myelogenous leukemia. *Blood*. 2008;111:1834–1839.

58. Kantarjian HM, Giles F, Gattermann N, et al. Nilotinib (formerly AMN107), a highly selective BCR-ABL tyrosine kinase inhibitor, is effective in patients with Philadelphia chromosome–positive chronic myelogenous leukemia in chronic phase following imatinib resistance and intolerance. *Blood*. 2007;110:3540–3546.
59. Koren-Michowitz M, le Coutre P, Duyster J, et al. Activity and tolerability of nilotinib: a retrospective multicenter analysis of chronic myeloid leukemia patients who are imatinib resistant or intolerant. *Cancer*. 2010;116:4564–4572.
60. Hochhaus A, Kreil S, Corbin AS, et al. Molecular and chromosomal mechanisms of resistance to imatinib (STI571) therapy. *Leukemia*. 2002;16:2190–2196.
61. Gromicho M, Dinis J, Magalhaes M, et al. Development of imatinib and dasatinib resistance: dynamics of expression of drug transporters ABCB1, ABCC1, ABCG2, MVP, and SLC22A1. *Leuk Lymphoma*. 2011;52: 1980–1990.
62. Bradeen HA, Eide CA, O'Hare T, et al. Comparison of imatinib mesylate, dasatinib (BMS-354825), and nilotinib (AMN107) in an *N*-ethyl-*N*-nitrosourea (ENU)–based mutagenesis screen: high efficacy of drug combinations. *Blood*. 2006;108:2332–2338.
63. von Bubnoff N, Manley PW, Mestan J, et al. Bcr-Abl resistance screening predicts a limited spectrum of point mutations to be associated with clinical resistance to the Abl kinase inhibitor nilotinib (AMN107). *Blood*. 2006;108:1328–1333.
64. O'Hare T, Eide CA, Deininger MW. Bcr-Abl kinase domain mutations, drug resistance, and the road to a cure for chronic myeloid leukemia. *Blood*. 2007;110:2242–2249.
65. Laneuville P, Dilea C, Yin OQ, et al. Comparative in vitro cellular data alone are insufficient to predict clinical responses and guide the choice of BCR-ABL inhibitor for treating imatinib-resistant chronic myeloid leukemia. *J Clin Oncol*. 2010;28:e169–e171; author reply e72.
66. Branford S, Fletcher L, Cross NC, et al. Desirable performance characteristics for BCR-ABL measurement on an international reporting scale to allow consistent interpretation of individual patient response and comparison of response rates between clinical trials. *Blood*. 2008;112:3330–3338.
67. Hughes T, Saglio G, Branford S, et al. Impact of baseline BCR-ABL mutations on response to nilotinib in patients with chronic myeloid leukemia in chronic phase. *J Clin Oncol*. 2009;27:4204–4210.
68. Burke AC, Swords RT, Kelly K, et al. Current status of agents active against the T315I chronic myeloid leukemia phenotype. *Expert Opin Emerg Drugs*. 2011;16:85–103.
69. Mahon FX, Rea D, Guilhot J, et al. Discontinuation of imatinib in patients with chronic myeloid leukaemia who have maintained complete molecular remission for at least 2 years: the prospective, multicentre Stop Imatinib (STIM) trial. *Lancet Oncol*. 2010;11:1029–1035.
70. Deininger M. Hematology: curing CML with imatinib—a dream come true? *Nat Rev Clin Oncol*. 2011;8:127–128.
71. Corbin AS, Agarwal A, Loriaux M, et al. Human chronic myeloid leukemia stem cells are insensitive to imatinib despite inhibition of BCR-ABL activity. *J Clin Invest*. 2011;121:396–409.
72. Giles FJ, Rosti G, Beris P, et al. Nilotinib is superior to imatinib as first-line therapy of chronic myeloid leukemia: the ENESTnd study. *Expert Rev Hematol*. 2010;3:665–673.
73. Mauro MJ, Deininger MW. Management of drug toxicities in chronic myeloid leukaemia. *Best Pract Res Clin Haematol*. 2009;22:409–429.
74. Gambacorti-Passerini C, Antolini L, Mahon FX, et al. Multicenter independent assessment of outcomes in chronic myeloid leukemia patients treated with imatinib. *J Natl Cancer Inst*. 2011;103:553–561.
75. Boschelli DH, Ye F, Wang YD, et al. Optimization of 4-phenylamino-3-quinolinecarbonitriles as potent inhibitors of Src kinase activity. *J Med Chem*. 2001;44:3965–3977.
76. Puttini M, Coluccia AM, Boschelli F, et al. In vitro and in vivo activity of SKI-606, a novel Src-Abl inhibitor, against imatinib-resistant Bcr-Abl+ neoplastic cells. *Cancer Res*. 2006;66:11314–11322.
77. Redaelli S, Piazza R, Rostagno R, et al. Activity of bosutinib, dasatinib, and nilotinib against 18 imatinib-resistant BCR/ABL mutants. *J Clin Oncol*. 2009;27:469–471.
78. Huang WS, Metcalf CA, Sundaramoorthi R, et al. Discovery of 3-[2-(imidazo[1,2-b]pyridazin-3-yl)ethynyl]-4-methyl-*N*-{4-[(4-methylpiperazin-1-yl)methyl]-3-(trifluoromethyl)phenyl}benzamide (AP24534), a potent, orally active pan-inhibitor of breakpoint cluster region-abelson (BCR-ABL) kinase including the T315I gatekeeper mutant. *J Med Chem*. 2010;53:4701–4719.
79. O'Hare T, Shakespeare WC, Zhu X, et al. AP24534, a pan-BCR-ABL inhibitor for chronic myeloid leukemia, potently inhibits the T315I mutant and overcomes mutation-based resistance. *Cancer Cell*. 2009; 16:401–412.
80. Gozgit JM, Wong MJ, Wardwell S, et al. Potent activity of ponatinib (AP24534) in models of FLT3-driven acute myeloid leukemia and other hematologic malignancies. *Mol Cancer Ther*. 2011;10:1028–1035.
81. Cortes J, Talpaz M, Bixby D, et al. A phase 1 trial of oral ponatinib (AP24534) in patients with refractory chronic myelogenous leukemia (CML) and other hematologic malignancies: emerging safety and clinical response findings. *ASH Annual Meeting Abstracts*. 2010;116:210.
82. Carmena M, Earnshaw WC. The cellular geography of aurora kinases. *Nat Rev Mol Cell Biol*. 2003;4:842–854.
83. Howard S, Berdini V, Boulstridge JA, et al. Fragment-based discovery of the pyrazol-4-yl urea (AT9283), a multitargeted kinase inhibitor with potent aurora kinase activity. *J Med Chem*. 2009;52:379–388.
84. Tanaka R, Squires MS, Kimura S, et al. Activity of the multitargeted kinase inhibitor, AT9283, in imatinib-resistant BCR-ABL–positive leukemic cells. *Blood*. 2010;116:2089–2095.
85. Balabanov S, Gontarewicz A, Keller G, et al. Abcg2 overexpression represents a novel mechanism for acquired resistance to the multikinase inhibitor Danusertib in BCR-ABL–positive cells in vitro. *PLoS One*. 2011;6:e19164.
86. Vertex's Collaborator Merck Suspends Patient Enrollment in Clinical Trials of MK-0457 (VX-680) Pending Full Analysis of Clinical Data. 2007. Available at: www.drugs.com/clinical_trials/vertex-s-collaborator-merck-suspends-patient-enrollment-clinical-trials-mk-0457-vx-680-pending-full-2764.html. Accessed July 31, 2011.
87. Chan WW, Wise SC, Kaufman MD, et al. Conformational control inhibition of the BCR-ABL1 tyrosine kinase, including the gatekeeper T315I mutant, by the switch-control inhibitor DCC-2036. *Cancer Cell*. 2011;19:556–568.
88. Adrian FJ, Ding Q, Sim T, et al. Allosteric inhibitors of Bcr-Abl–dependent cell proliferation. *Nat Chem Biol*. 2006;2:95–102.
89. Deng X, Okram B, Ding Q, et al. Expanding the diversity of allosteric bcr-abl inhibitors. *J Med Chem*. 2010;53:6934–6946.
90. Zhang J, Adrian FJ, Jahnke W, et al. Targeting Bcr-Abl by combining allosteric with ATP-binding-site inhibitors. *Nature*. 2010;463:501–506.
91. Iacob RE, Zhang J, Gray NS, et al. Allosteric interactions between the myristate- and ATP-site of the Abl kinase. *PLoS One*. 2011;6:e15929.
92. McWhirter JR, Galasso DL, Wang JY. A coiled-coil oligomerization domain of Bcr is essential for the transforming function of Bcr-Abl oncoproteins. *Mol Cell Biol*. 1993;13:7587–7595.
93. Zhao X, Ghaffari S, Lodish H, et al. Structure of the Bcr-Abl oncoprotein oligomerization domain. *Nat Struct Biol*. 2002;9:117–120.
94. Beissert T, Puccetti E, Bianchini A, et al. Targeting of the N-terminal coiled coil oligomerization interface of BCR interferes with the transformation potential of BCR-ABL and increases sensitivity to STI571. *Blood*. 2003;102:2985–2993.
95. Dixon AS, Pendley SS, Bruno BJ, et al. Disruption of Bcr-Abl coiled coil oligomerization by design. *J Biol Chem*. 2011;286:27751–27760.
96. Zhang H, Trachootham D, Lu W, et al. Effective killing of Gleevec-resistant CML cells with T315I mutation by a natural compound PEITC through redox-mediated mechanism. *Leukemia*. 2008;22:1191–1199.
97. Sun H, Kapuria V, Peterson LF, et al. Bcr-Abl ubiquitination and Usp9x inhibition block kinase signaling and promote CML cell apoptosis. *Blood*. 2011;117:3151–3162.
98. Gorre ME, Ellwood-Yen K, Chiosis G, et al. BCR-ABL point mutants isolated from patients with imatinib mesylate-resistant chronic myeloid leukemia remain sensitive to inhibitors of the BCR-ABL chaperone heat shock protein 90. *Blood*. 2002;100:3041–3044.

99. Isaacs JS, Xu W, Neckers L. Heat shock protein 90 as a molecular target for cancer therapeutics. *Cancer Cell*. 2003;3:213–217.
100. Tsukahara F, Maru Y. Bag1 directly routes immature BCR-ABL for proteasomal degradation. *Blood*. 2010;116:3582–3592.
101. Burchert A, Wolfl S, Schmidt M, et al. Interferon-alpha, but not the ABL-kinase inhibitor imatinib (STI571), induces expression of myeloblastin and a specific T-cell response in chronic myeloid leukemia. *Blood*. 2003;101:259–264.
102. Bocchia M, Gentili S, Abruzzese E, et al. Effect of a p210 multipeptide vaccine associated with imatinib or interferon in patients with chronic myeloid leukaemia and persistent residual disease: a multicentre observational trial. *Lancet*. 2005;365:657–662.
103. Cathcart K, Pinilla-Ibarz J, Korontsvit T, et al. A multivalent bcr-abl fusion peptide vaccination trial in patients with chronic myeloid leukemia. *Blood*. 2004;103:1037–1042.
104. Telegeev GD, Dubrovska AN, Nadgorna VA, et al. Immunocytochemical study of Bcr and Bcr-Abl localization in K562 cells. *Exp Oncol*. 2010;32:81–83.
105. van Denderen J, Hermans A, Meeuwsen T, et al. Antibody recognition of the tumor-specific Bcr-Abl joining region in chronic myeloid leukemia. *J Exp Med*. 1989;169:87–98.
106. Perez-Martinez D, Tanaka T, Rabbitts TH. Intracellular antibodies and cancer: new technologies offer therapeutic opportunities. *Bioessays*. 2010;32:589–598.
107. Tse E, Lobato MN, Forster A, et al. Intracellular antibody capture technology: application to selection of intracellular antibodies recognising the BCR-ABL oncogenic protein. *J Mol Biol*. 2002;317:85–94.
108. Wojcik J, Hantschel O, Grebien F, et al. A potent and highly specific FN3 monobody inhibitor of the Abl SH2 domain. *Nat Struct Mol Biol*. 2010;17:519–527.
109. Konopleva MY, Jordan CT. Leukemia stem cells and microenvironment: biology and therapeutic targeting. *J Clin Oncol*. 2011;29:591–599.
110. Zhao C, Chen A, Jamieson CH, et al. Hedgehog signalling is essential for maintenance of cancer stem cells in myeloid leukaemia. *Nature*. 2009;458:776–779.
111. Irvine DA, Zhang B, Allan EK, et al. Combination of the hedgehog pathway inhibitor LDE225 and nilotinib eliminates chronic myeloid leukemia stem and progenitor cells. American Society of Hematology Annual Meeting; December 5, 2009: New Orleans, LA.
112. Sheridan C. Genentech obtains proof of concept for hedgehog inhibition. *Nat Biotech*. 2009;27:968–969.
113. Zhao C, Blum J, Chen A, et al. Loss of beta-catenin impairs the renewal of normal and CML stem cells in vivo. *Cancer Cell*. 2007;12:528–541.
114. Naka K, Hoshii T, Muraguchi T, et al. TGF-beta–FOXO signalling maintains leukaemia-initiating cells in chronic myeloid leukaemia. *Nature*. 2010;463:676–680.
115. Jin L, Tabe Y, Konoplev S, et al. CXCR4 up-regulation by imatinib induces chronic myelogenous leukemia (CML) cell migration to bone marrow stroma and promotes survival of quiescent CML cells. *Mol Cancer Ther*. 2008;7:48–58.
116. Gregory MA, Phang TL, Neviani P, et al. Wnt/Ca^{2+}/NFAT signaling maintains survival of Ph+ leukemia cells upon inhibition of Bcr-Abl. *Cancer Cell*. 2010;18:74–87.
117. Bellodi C, Lidonnici MR, Hamilton A, et al. Targeting autophagy potentiates tyrosine kinase inhibitor–induced cell death in Philadelphia chromosome–positive cells, including primary CML stem cells. *J Clin Invest*. 2009;119:1109–1123.
118. Brossart P, Schneider A, Dill P, et al. The epithelial tumor antigen MUC1 is expressed in hematological malignancies and is recognized by MUC1-specific cytotoxic T-lymphocytes. *Cancer Res*. 2001;61:6846–6850.
119. Chen Y, Hu Y, Zhang H, et al. Loss of the *Alox5* gene impairs leukemia stem cells and prevents chronic myeloid leukemia. *Nat Genet*. 2009;41:783–792.
120. Mancini M, Corradi V, Petta S, et al. mTOR inhibitor RAD001 (Everolimus) enhances the effects of imatinib in chronic myeloid leukemia by raising the nuclear expression of c-ABL protein. *Leuk Res*. 2010;34:641–648.
121. Warsch W, Kollmann K, Eckelhart E, et al. High STAT5 levels mediate imatinib resistance and indicate disease progression in chronic myeloid leukemia. *Blood*. 2011;117:3409–3420.
122. Melo JV, Barnes DJ. Chronic myeloid leukaemia as a model of disease evolution in human cancer. *Nat Rev Cancer*. 2007;7:441–453.
123. Nelson EA, Walker SR, Weisberg E, et al. The STAT5 inhibitor pimozide decreases survival of chronic myelogenous leukemia cells resistant to kinase inhibitors. *Blood*. 2011;117:3421–3429.
124. Melo JV, Goldman JM, Deininger M. Signal transduction inhibitors in chronic myeloid leukemia. In: *Myeloproliferative Disorders*. Berlin, Germany: Springer; 2007:75–102.