REVIEW

Src-family kinases in the development and therapy of Philadelphia chromosome-positive chronic myeloid leukemia and acute lymphoblastic leukemia

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Abstract

The BCR-ABL kinase inhibitor imatinib has shown significant efficacy in chronic myeloid leukemia (CML) and is the standard front-line therapy for patients in chronic phase. However, a substantial number of patients are either primarily refractory or acquire resistance to imatinib. While a number of mechanisms are known to confer resistance to imatinib, increasing evidence has demonstrated a role for BCR-ABL–independent pathways. The Src-family kinases (SFKs) are one such pathway and have been implicated in imatinib resistance. Additionally, these kinases are key to the progression of CML and Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph+ ALL). The dual SFK/BCR-ABL inhibitor dasatinib is now clinically available and has markedly greater potency compared with imatinib against native BCR-ABL and the majority of imatinib-resistant BCR-ABL mutants. Therefore, this agent, as well as other dual SFK/BCR-ABL inhibitors under development, could provide added therapeutic advantages by overcoming both BCR-ABL–dependent (i.e., BCR-ABL mutations) and –independent forms of imatinib resistance and delaying transition to advanced phase disease. In this review, we discuss the preclinical and clinical evidence demonstrating the involvement of SFKs in imatinib resistance and the progression of CML and Ph+ ALL, as well as the potential role of dual SFK/BCR-ABL inhibition in the management of these diseases.

Keywords: Src, leukemia, BCR-ABL, dasatinib, imatinib resistant

Introduction

The constitutively active BCR-ABL tyrosine kinase is the defining molecular abnormality in Philadelphia chromosome-positive (Ph+) chronic myeloid leukemia (CML) and acute lymphoblastic leukemia (ALL) [1–6]. The pathogenic role of BCR-ABL in CML and Ph+ ALL provided the rationale for therapeutic targeting of this signaling protein. Imatinib was the first available BCR-ABL targeted therapy and is currently the standard front-line therapy for CML in chronic phase (CP). However, despite the significant efficacy of this agent, a substantial number of patients are either primarily resistant to treatment or acquire resistance during the course of treatment [7–14]. Additionally, imatinib does not completely eradicate residual leukemic stem cells and progenitors [15,16], which present a persistent risk of disease relapse.

The Src-family kinases (SFKs) have been implicated in BCR-ABL signaling [17,18] and in the progression of CML and Ph+ ALL [19–27]. Furthermore, increasing evidence suggests that SFKs are involved in BCR-ABL-independent forms of imatinib resistance [26,27]. Here we will review the preclinical and clinical evidence demonstrating SFK involvement in BCR-ABL signaling, the transforming activity of BCR-ABL, progression of CML and Ph+ ALL, and imatinib resistance.

Oncogenic signaling pathways in CML and Ph+ ALL

BCR-ABL is a constitutively active, non-receptor tyrosine kinase [2,3,28]. The central role of this oncogenic kinase in the pathogenesis of CML has been well established [3,29]. BCR-ABL initiates
numerous signal transduction pathways that influence the growth and survival of hematopoietic cells and collectively induce leukemic transformation, such as STAT5, MEK1/2/ERK1/2, and NF-κB [30]. Several mechanisms have been implicated in the transforming activity of BCR-ABL, including constitutive mitogenic signaling [31] and reduced dependency on external growth factors [32], altered cell adhesion properties [33], and reduced apoptotic potential [34]. Additionally, evidence suggests that BCR-ABL disrupts the DNA repair response [35,36], which may play a role in disease progression by exacerbating genomic instability and promoting the accumulation of additional cytogenetic alterations.

Given the central role of BCR-ABL in the pathogenesis of CML, it is an attractive target for selective kinase inhibition. However, targeting BCR-ABL kinase activity alone may not be sufficient for the management of CML, as downstream pathways of BCR-ABL can be activated independently of BCR-ABL kinase activity [23], thereby leading to imatinib resistance. The SFKs are an example of such a downstream activator, and have been suggested to confer BCR-ABL independence. These non-receptor, intracellular tyrosine kinases regulate signal-transduction pathways involved in cell growth, differentiation, and survival [37–39] and are among the most extensively studied oncogenes in human cancers [40]. There are eight known SFK members (Src, Blk, Fgr, Fyn, Hck, Lck, Lyn, and Yes) with each comprising a unique domain and high-sequence homology in the four Src homology domains (SH1-4) [41]. SFKs exhibit a range of tissue expression patterns and several are primarily expressed in hematopoietic cells (Table I) [39,41].

Numerous studies have indicated an association between SFKs and myeloid and lymphoid leukemias [39]. Early research demonstrated the proleukemic potential of SFKs in a variety of hematopoietic cell lines [42–46]. Danhauser-Riedel et al. provided the first data demonstrating that the activity of the SFKs, Lyn and Hck, is increased in hematopoietic cells expressing BCR-ABL [18]. Activation of Hck or other SFK members has been suggested to be required for BCR-ABL–mediated transformation [20,47]. Expression of a kinase defective mutant of Hck blocked BCR-ABL–induced outgrowth of cytokine dependent leukemia cell lines [20]. Furthermore, pharmacologic inhibition of SFKs led to growth arrest and apoptosis in CML cell lines [48]. Recent SFK research has centered on the pathologic role of these signaling molecules in CML and Ph+ ALL, their involvement in disease progression and the development of imatinib resistance.

Cooperation between BCR-ABL and SFK signaling pathways
SFKs are collaborative oncogenic kinases in BCR-ABL–induced leukemias and may act to couple BCR-ABL to certain downstream signaling pathways involved in leukemic transformation (Figure 1) [17,18,20,47,48]. SFKs are activated through direct interaction with BCR-ABL [17,18,25], and likely involves the release of intramolecular, auto-inhibitory constraints [17,20,38]. In turn, the activity of BCR-ABL can be enhanced through SFK-mediated phosphorylation, which by Hck of tyrosine residues within the activation loop of ABL was found to increase ABL kinase activity [49]. In another study, it was demonstrated that Hck, Lyn, and Fyn phosphorylate multiple tyrosine residues within the SH3-SH2

![Figure 1. SFKs directly interact with BCR-ABL resulting in (1) activation of SFKs [17,18,23] and (2) augmentation of BCR-ABL kinase activity [49]. Activated SFKs work cooperatively with BCR-ABL in facilitating the growth and progression of leukemia [48,50,51]. Several downstream effectors of SFKs have been proposed to mediate the proleukemic effects, such as (3) STAT5 [50], which is known to activate genes involved in growth factor independence, differentiation, adhesion, and DNA repair [36,52–54] and (4) AKT [55], which is key in regulating cell proliferation and survival in BCR-ABL–dependent cells [56]. (5) Active SFKs also phosphorylate certain tyrosine residues on BCR-ABL to create a binding site for GRB-2. This adaptor protein may link the BCR-ABL pathway to Ras, which is known to activate the MEK/ERK oncogenic signaling cascade [17,48].](image-url)
region of BCR-ABL, and that these phosphorylations are required for full oncogenicity of BCR-ABL in myeloid cell lines. This impact on BCR-ABL function was suggested to occur through the release of an autoregulatory function that holds the kinase domain in an inactive state [47].

Role of SFKS in the progression of CML to blast crisis

Although BCR-ABL is considered the trigger for malignant transformation in CML, there is evidence of an important role for SFKS in disease progression. Studies from our laboratory demonstrated that the transition of CML to lymphoid blast crisis (LBC) in mice requires the presence of Lyn, Hck, and Fgr [23], and Donato et al. showed that overexpression and/or activation of Hck and Lyn occur during CML progression [21]. Downregulation of Lyn expression by RNAi was found to induce apoptosis in both myeloid and lymphoid blast cells, and this effect was more pronounced in the latter [24]. Although the biology of CML progression is not fully understood and likely involves multiple factors, the studies above clearly implicate SFKS in the development of advanced phase CML, particularly LBC. Furthermore, the involvement of SFKS in disease progression may partially explain the aggressive nature of advanced CML [21] and its relatively poor responsiveness to imatinib [12,57,58].

Role of SFKS in Ph+ ALL

SFKs also appear to play a significant role in the development of Ph+ ALL, which may be independent of BCR-ABL kinase activity. Data from our laboratory with mouse knockout models demonstrated that SFKS are required for induction of B-ALL by BCR-ABL [25]. We subsequently found that although imatinib had a weak effect on the survival of mice with B-ALL induced by BCR-ABL, coinhibition of BCR-ABL kinase activity and SFKS maintained long-term survival [23]. The independent role of SFKS in B-ALL is further supported by results demonstrating that a SFK inhibitor reduced the viability, and induced apoptosis, in pre-B leukemia cells expressing the imatinib-resistant T315I BCR-ABL mutant [25]. Moreover, while imatinib had no effect in mice with B-ALL induced by T315I BCR-ABL, treatment with the dual Src/ABL inhibitor dasatinib (which is also ineffective against the T315I BCR-ABL mutant) significantly prolonged survival. In addition, this improved survival correlated with SFK inhibition [23]. Collectively, these results indicate that SFKS play a role in B-lymphoid transformation that is not efficiently prevented by inhibiting BCR-ABL kinase activity with imatinib and that dual SFK/ABL inhibition may improve the overall treatment outcome of patients with this disease.

SFKS and resistance to imatinib

Resistance to imatinib develops rapidly in patients with advanced phase CML and Ph+ ALL [12,57,58]. A number of potential resistance mechanisms have been proposed including BCR-ABL kinase domain mutation, BCR-ABL overexpression, alterations in drug influx and efflux, and as mentioned previously, induction of BCR-ABL–independent pathways [59]. Increasing preclinical and clinical evidence implicates SFKS in imatinib resistance. Upregulation of Lyn and Hck was observed in blasts from patients with imatinib-resistant CML [21]. It was later found that while imatinib effectively reduced activation of BCR-ABL and downstream activation of SFKS in specimens derived from patients with imatinib-sensitive CML, this agent had no effect on SFK activation in samples from resistant patients despite BCR-ABL inhibition. In animal models, the antitumor activity of imatinib was significantly reduced upon loss of imatinib-mediated Lyn inhibition, but concomitant inhibition of SFKS and BCR-ABL recovered this activity [27]. These results further indicated that SFK activity becomes independent of BCR-ABL in progressive disease, thereby resulting in imatinib resistance. Other studies have provided similar evidence of SFK-mediated BCR-ABL independence. The SFKS Lyn and Hck were found to be overexpressed in CML cell lines with BCR-ABL–independent imatinib resistance, and coinhibition of SFKS and BCR-ABL in these cells resulted in an enhanced apoptotic response [21,26]. Dai et al. showed that imatinib-resistant cell lines demonstrated markedly increased expression of Lyn, and treatment with a specific Src inhibitor induced apoptosis in these cells. Furthermore, transfection of imatinib-sensitive cell lines with a constitutively active form of Lyn conferred resistance to imatinib [22].

SFKs may also mediate imatinib resistance by stabilizing the active form of BCR-ABL as imatinib is unable to bind this conformation [60,61]. As mentioned above, Meyn et al. suggested that SFK-mediated phosphorylations in the SH3-SH2 region of BCR-ABL promote the active conformation through disruption of an intramolecular regulatory component that holds the kinase domain in an inactive state [47]. Consistent with this, Azam et al. found that substitution of one of these SFK-phosphorylated tyrosine residues within the SH3 domain resulted in imatinib resistance [62]. Furthermore, Hck promotes the active conformation through the phosphorylation
of specific tyrosine residues within the ABL activation loop, which substantially decreases the sensitivity to imatinib [49,61].

**Dual SFK/BCR-ABL inhibition in CML and Ph+ ALL**

In CML and Ph+ ALL, treatment based exclusively on the inhibition of BCR-ABL kinase activity (e.g., imatinib and its analogs) will clearly not improve patient outcome if BCR-ABL–independent resistance occurs, and may select for resistant clones [23]. Additionally, imatinib is unable to eradicate BCR-ABL–expressing CD34+ cells [63], which will be necessary to achieve curative therapy. Given the role of SFKs in the development and progression of Ph+ ALL and CML and in BCR-ABL–independent imatinib resistance, dual inhibition of BCR-ABL and SFK will likely prove a more effective treatment strategy. Additionally, this strategy may also suppress the emergence of BCR-ABL–independent clones, delay and possibly prevent transition of CML to blast crisis (BC), and yield greater activity in advanced CML and Ph+ ALL [23].

As discussed above, preclinical studies have demonstrated that pharmacologic or genetic inhibition of SFKs induces apoptosis and growth arrest in BCR-ABL transformed cells [20,23–25,48] and may overcome imatinib resistance [22,40,64]. Moreover, dual inhibitors of BCR-ABL and SFKs may be less susceptible to conformational resistance than imatinib [65]. Although several such agents are currently in early stage clinical development [66], dasatinib is the most clinically advanced and is the only dual SFK/BCR-ABL inhibitor approved in the United States and Europe for the treatment of patients with imatinib-resistant or -intolerant CML and Ph+ ALL. This novel, orally available, tyrosine kinase inhibitor is structurally unrelated to imatinib, and capable of binding to the ABL kinase domain in multiple conformations [67–69]. This agent has demonstrated 325-fold greater activity against native BCR-ABL in vitro as compared with imatinib, and is active against all imatinib-resistant BCR-ABL mutations with the exception of T315I [67,68]. Additionally, dasatinib also has activity against other oncogenic tyrosine kinases such as c-Kit, platelet-derived growth factor-receptor (PDGFR), and ephrin A-receptor [70–73].

Numerous preclinical studies have indicated that dual SFK/BCR-ABL inhibition with dasatinib is advantageous in CML and Ph+ ALL. As previously mentioned, Donato et al. showed that dasatinib was able to recover the antitumor activity lost with imatinib treatment as a result of BCR-ABL–independent Lyn activation [27]. We recently reported that treatment with dasatinib induces complete remission of Ph+ ALL, and significantly prolongs survival of CML in mice [23]. It was also found that SFKs are required for the progression of CML to LBC, suggesting that treatment with dasatinib could potentially delay the transition of CML from CP to LBC. Additionally, although dasatinib does not kill leukemic stem cells, studies in B-ALL mice suggest that the cytostatic effects of dasatinib on this cell population could prevent leukemic transformation and afford long-term control of the disease [23].

Results from phase I and II trials showed that dasatinib induces rapid and deep responses in imatinib-resistant patients across all phases of CML and Ph+ ALL [74–79]. While it is clear that the more potent activity of dasatinib against native and mutant variants of BCR-ABL is, in part, responsible for its clinical efficacy in imatinib-resistant CML, BCR-ABL-independent effects appear to play a role as well. Dasatinib has shown activity in imatinib-resistant patients with no detectable mutations at baseline [80]. Furthermore, in a randomized clinical trial, dasatinib demonstrated superior efficacy compared to high-dose (HD) imatinib in patients with imatinib-resistant CP CML, and major cytogenetic responses (CyRs) were achieved in 55% (28/51) of patients without a BCR-ABL mutation at baseline compared with 34% (12/35) observed with HD imatinib [79]. A separate study showed that the clinical activity of dasatinib in patients with BCR-ABL–independent imatinib resistance correlated with inhibition of both BCR-ABL and SFKs in primary cell samples taken from the same patients [27]. Dasatinib was also found to be active in patients with CML after failure of imatinib and its analog nilotinib [81]. Notably, hematologic and CyRs were achieved with dasatinib in a substantial number of patients with advanced stage CML and Ph+ ALL [74,76–78]. This latter result is in marked contrast to the limited activity observed with imatinib and nilotinib in LBC CML and Ph+ ALL [66,82], further suggesting a potential role of SFKs in advanced disease. Collectively, the clinical data support the preclinical findings implicating SFKs in CML progression and imatinib resistance, and suggest that dual SFK/BCR-ABL inhibition may be more effective than inhibition of BCR-ABL alone.

**Most appropriate use of Src/ABL inhibitors**

Although dasatinib has demonstrated promising results in advanced phase patients, the outcome of treatment in this population is clearly inferior to that observed in CP CML. Additionally, it remains to be determined whether the responses generated in patients with BC CML and Ph+ ALL will be
durable. In the phase I trial of dasatinib responses in these patients were described as short-lived [74]; however, improved response durations were reported in phase II studies [77,78]. Moreover, many of these patients do not respond to treatment of any kind. This refractory nature is likely due to the accumulation of additional genetic abnormalities that occur with disease progression, conferring multiple and complex mechanisms of resistance which are not fully understood.

This raises the question of when it is most appropriate to begin treatment with dasatinib. The results in our mouse CML model demonstrating that SFKs are required for progression to BC and that dasatinib significantly prolonged survival compared to imatinib, suggest that early and continuous treatment with dasatinib in patients with CP CML may provide the greatest therapeutic benefit [23]. This strategy could more effectively prevent disease progression as well as the emergence of drug resistance, through suppression of leukemic stem cell transformation. Thus, while imatinib is currently the standard first-line therapy for newly diagnosed patients in CP, dasatinib should also be considered in these patients.

Earlier treatment with dasatinib may also prove beneficial given its greater potency, which could allow for more rapid achievement of the treatment goals, that is, complete CyR and major molecular response (MMR) [83]. Several studies have shown that both overall survival and progression-free survival is improved in patients who achieve a CyR at 3 or 6 months [8,84 – 88]. Similarly, early molecular responses are also associated with better outcome [89]. Indeed, dasatinib has shown impressive activity as front-line therapy in CP CML. Complete CyR rates at 3, 6, and 12 months were 77%, 92%, and 95%, respectively, and MMRs were achieved in 19% of patients at 6 months which increased to 32% at 12 months [90]. Long-term follow-up from this study and head-to-head studies evaluating dasatinib versus imatinib as first-line treatment are necessary to determine whether earlier treatment with dasatinib will improve the outcome of patients with CP CML.

Conclusions

Overall, preclinical and clinical evidence has demonstrated an important role for SFKs in the progression of CML and Ph+ ALL and the development of imatinib resistance. Compared with other BCR-ABL inhibitors, such as imatinib and nilotinib, the anti-SFK activity of dasatinib and other dual SFK/BCR-ABL inhibitors could provide added therapeutic advantages by overcoming both BCR-ABL-dependent and – independent imatinib resistance. Furthermore, long-term suppression of leukemic stem cells with dasatinib may reduce the emergence of resistant clones, translating to more durable responses and improved outcome. Long-term follow-up of dasatinib in imatinib-pretreated as well as early-CP patients will further elucidate the clinical benefit of inhibiting SFKs in CML and Ph+ ALL.

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References

1. Rowley JD. Letter: a new consistent chromosomal abnormality in chronic myelogenous leukemia identified by quinacrine fluorescence and Giemsa staining. Nature 1973; 243:290 – 293.
2. Heisterkamp N, Jenster G, Ten HJ, Zovich D, Pattengale PK, Groffen J. Acute leukemia in bcr/abl transgenic mice. Nature 1990;344:251 – 253.
3. Daley GQ, Van Etten RA, Baltimore D. Induction of chronic myelogenous leukemia in mice by the P210bcr/abl gene of the Philadelphia chromosome. Science 1990;247:824 – 830.
4. Pear WS, Miller JP, Xu L, Pui JC, Soffer B, Quackenbush RG, et al. Efficient and rapid induction of a chronic myelogenous leukemia-like myeloproliferative disease in mice receiving P210 bcr-abl-transduced bone marrow. Blood 1998;92: 3780 – 3792.
5. Kurzrock R, Gutterman JU, Talpaz M. The molecular genetics of Philadelphia chromosome-positive leukemias. N Engl J Med 1988;319:990 – 998.
6. Kantarjian HM, Giles F, Quintas-Cardama A, Cortes J. Important therapeutic targets in chronic myelogenous leukemia. Clin Cancer Res 2007;13:1089 – 1097.
7. Hochhaus A, Hughes T. Clinical resistance to imatinib: mechanisms and implications. Hematol Oncol Clin North Am 2004;18:641 – 656.
8. Gambacorti C, Talpaz M, Sawyers CL, Druker BJ, Hochhaus A, Schiffer C, et al. Five year follow-up results of a phase II trial in patients with late chronic phase (L-CP) chronic myeloid leukemia (CML) treated with imatinib who are refractory/intolerant of interferon-α. Blood 2005;106:1089.
9. Druker BJ, Guilhot F, O’Brien S, Larson N on behalf of the IRIS (International Randomized IF). Long-term benefits of imatinib (IM) for patients newly diagnosed with chronic myelogenous leukemia in chronic phase (CML-CP): the 5-year update from the IRIS study. J Clin Oncol 2006;24(Suppl 18S):S506.
10. O’Brien SG, Guilhot F, Larson RA, Gathmann I, Baccarani M, Cervantes F, 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.
11. Druker BJ, Guilhot F, O’Brien SG, Gathmann I, Kantarjian H, Gattermann N, et al. Five-year follow-up of patients receiving imatinib for chronic myeloid leukemia. N Engl J Med 2006;355:2408 – 2417.
12. Sawyers CL, Hochhaus A, Feldman E, Goldman JM, Miller CB, Ottmann OG, 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.
13. Talpaz M, Silver RT, Druker BJ, Goldman JM, Gambacorti-Passerini C, Guilhot F, 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.

14. Talpaz M, Goldman J, Sawyer C, Hochhaus A, Silver RT, Smith BD, et al. High dose imatinib (STI571, Gleevec) provides durable long-term outcomes for patients (Pts) with chronic myeloid leukemia (CML) in accelerated phase (AP) or myeloid blast crisis (BC): follow-up of the phase II studies. Blood 2003;102:3369.

15. Graham SM, Fjorgensen HG, Allan E, Pearson C, Alcorn MJ, Richmond L, et al. Primitive, quiescent, Philadelphia-positive stem cells from patients with chronic myeloid leukemia are insensitive to STI571 in vitro. Blood 2002;99:319 – 325.

16. Marley SB, Deininger MW, Davidson RJ, Goldman JM, Gordon MY. The tyrosine kinase inhibitor STI571, like interferon-α, preferentially reduces the capacity for amplification of granulocyte-macrophage progenitors from patients with chronic myeloid leukemia. Exp Hematol 2000;28:551 – 557.

17. Warmuth M, Bergmann M, Priess A, Hausmann K, Emmerich B, Hallek M. The Src family kinase Hck interacts with Bcr-Abl by a kinase-independent mechanism and phosphorylates the Grb2-binding site of Bcr. J Biol Chem 1997;272:33260 – 33270.

18. Danhauser-Riedl S, Warmuth M, Druker BJ, Emmerich B, Hallek M. Activation of Src kinases in p53/p56lck and p50lck by p210bcr/abl in myeloid cells. Cancer Res 1996;56:3589 – 3596.

19. Roginskaya V, Zuo S, Caudell E, Nambudiri G, Graker AJ, Corey SJ. Therapeutic targeting of Src-kinase Lyn in myeloid leukemic cell growth. Leukemia 1999;13:855 – 861.

20. Lionberger JM, Wilson MB, Smithgall TE. Transformation of myeloid leukemia cells to cytokine independence by Bcr-Abl is suppressed by kinase-defective Hck. J Biol Chem 2000;275:18581 – 18585.

21. Donato NJ, Wu JY, Stapley J, Gallicci G, Lin H, Arlinghaus R, et al. BCR-ABL independence and LYN kinase overexpression in chronic myelogenous leukemia cells selected for resistance to STI571. Blood 2003;101:690 – 698.

22. Dai Y, Rahmani M, Corey SJ, Dent P, Grant S. A Bcr-Abl independent, Lyn-dependent form of imatinib mesylate (STI571) resistance is associated with altered expression of Bcl-2. J Biol Chem 2004;279:34227 – 34239.

23. Hu Y, Swerdlov S, Duffy TM, Weimann R, Lee FY, Li S. Targeting multiple kinase pathways in leukemic progenitors and stem cells is essential for improved treatment of Ph+ leukemia in mice. Proc Natl Acad Sci USA 2006;103:16870 – 16875.

24. Prasznik A, Nakata Y, Kalota A, Emerson SG, Gewirtz AM. Short interfering RNA (siRNA) targeting the Lyn kinase induces apoptosis in primary, and drug-resistant, BCR-ABL(+) leukemia cells. Nat Med 2004;10:1187 – 1189.

25. Hu Y, Liu Y, Pelletier S, Buchdunger E, Warmuth M, Fabbro D, et al. Requirement of Src kinases Lyn, Hck and Fgr for BCR-ABL1-induced B-lymphoblastic leukemia but not chronic myeloid leukemia. Nat Genet 2004;36:453 – 461.

26. Donato NJ, Wu JY, Stapley J, Lin H, Arlinghaus R, Aggarwal BB, et al. Imatinib mesylate resistance through BCR-ABL independence in chronic myelogenous leukemia. Cancer Res 2004;64:672 – 677.

27. Donato NJ, Wu J, Kong LY, Meng F, Lee F, Talpaz M. Constitutive activation of SRC-family kinases in chronic myelogenous leukemia patients resistant to imatinib mesylate in the absence of BCR-ABL mutations: a rationale for use of SRC/ABL dual kinase inhibitor-based therapy. Blood 2005;106:1087.

28. Faderl S, Kantarjian HM, Thomas DA, Cortes J, Giles F, Pierce S, et al. Outcome of Philadelphia chromosome-positive adult acute lymphoblastic leukemia. Leuk Lymphoma 2000;36:263 – 273.

29. Lugo TG, Pendergast AM, Muller AJ, Witte ON. Tyrosine kinase activity and transformation potency of bcr-abl oncogene products. Science 1990;247:1079 – 1082.

30. Van Etten RA. Mechanisms of transformation by the BCR-ABL oncogene: new perspectives in the post-imatinib era. Leuk Res 2004;28(Suppl 1):S21 – S28.

31. Pui L, Liu J, Gish G, Mbanamu G, Bowtell D, Pelicci PG, et al. Bcr-Abl oncoproteins bind directly to activators of the Ras signalling pathway. EMBO J 1994;13:764 – 773.

32. Jiang X, Lopez A, Holyoke T, Eaves A, Eaves C. Autocrine production and action of IL-3 and granulocyte colony-stimulating factor in chronic myeloid leukemia. Proc Natl Acad Sci USA 1999;96:12804 – 12809.

33. Gordon MY, Dowdeng CR, Riley GP, Goldman JM, Greaves MF. Altered adhesive interactions with marrow stroma of haematopoietic progenitor cells in chronic myeloid leukaemia. Nature 1987;328:342 – 344.

34. Bedi A, Zehnbauer BA, Barber JP, Starkis SJ, Jones RJ. Inhibition of apoptosis by BCR-ABL in chronic myeloid leukemia. Blood 1994;83:2038 – 2044.

35. Deutsch E, Dugray A, AbdulKarim B, Marangoni E, Maggiorella L, Vagany S, et al. BCR-ABL down-regulates the DNA repair protein DNA-PKcs. Blood 2001;97:2084 – 2090.

36. Sliupianek A, Schmutter C, Tombline G, Nieborowska-Skorska M, Hoser G, Nowicki MO, et al. BCR/ABL regulates mammalian RecA homologs, resulting in drug resistance. Mol Cell 2001;8:795 – 806.

37. Abram CL, Courtenagde SA. Src family tyrosine kinases and growth factor signaling. Exp Cell Res 2000;254:1 – 13.

38. Boggon TJ, Eck MJ. Structure and regulation of Src family kinases. Oncogene 2004;23:7918 – 7927.

39. Li S. Src kinases as targets for B cell acute lymphoblastic leukaemia therapy. Expert Opin Ther Targets 2005;9:329 – 341.

40. Warmuth M, Damoiseaux R, Liu Y, Fabbro D, Gray N. Src family kinases: potential targets for the treatment of human cancer and leukemia. Curr Pharm Des 2003;9:2043 – 2059.

41. Lowell CA, Soriano P. Knockouts of Src-family kinases: stiff bones, wimpy T cells, and bad memories. Genes Dev 1996;10:1845 – 1857.

42. Overell RW, Watson JD, Gallis B, Weisser KE, Cosman D, Widmer MB. Nature and specificity of lymphokine independence induced by a selectable retroviral vector expressing v-src. Mol Cell Biol 1987;7:3394 – 3401.

43. Watson JD, Eszes M, Overell R, Conlon P, Widmer M, Gillis S. Effect of infection with murine recombinant retroviruses containing the v-src oncogene on interleukin 2- and interleukin 3-dependent growth states. J Immunol 1987;139:123 – 129.

44. Anderson SM, Carroll PM, Lee FD. Abrogation of IL-3 dependent growth requires a functional v-src gene product: evidence for an autocrine growth cycle. Oncogene 1990;5:317 – 325.

45. Engelmann A, Rosenberg N. bcr/abl and src but not myc and ras replace v-abl in lymphoid transformation. Mol Cell Biol 1990;10:4365 – 4369.

46. Keller G, Wagner EF. Expression of v-src induces a myeloproliferative disease in bone-marrow-reconstituted mice. Genes Dev 1989;3:827 – 837.

47. Meyn MA III, Wilson MB, Abdi FA, Fahey N, Schiavone AP, Wu J, et al. Src family kinases phosphorylate the Bcr-Abl SH3-H2 region and modulate Bcr-Abl transforming activity. J Biol Chem 2006;281:30907 – 30916.
48. Wilson MB, Schreiner SJ, Choi HJ, Kamens J, Smithgall TE. Selective pyrrolo-pyrimidine inhibitors reveal a necessary role for Src family kinases in Bcr-Abl signal transduction and oncogenesis. Oncogene 2002;21:8075 – 8088.

49. Tanis KQ, Veach D, Duewel HS, Bornmann WG, Koleske AJ. Two distinct phosphorylation pathways have additive effects on Abl family kinase activation. Mol Cell Biol 2003;23:3884 – 3896.

50. Klejman A, Schreiner SJ, Nieborowska-Skorska M, Slupianek A, Wilson M, Smithgall TE, et al. The Src family kinase Hck couples BCR/ABL to STAT3 activation in myeloid leukemia cells. EMBO J 2002;21:5766 – 5774.

51. Ptasznik A, Urbanowska E, Chinta S, Costa MA, Katz BA, Stanislaus MA, et al. Crosstalk between BCR/ABL oncoprotein and CXCR4 signaling through a Src family kinase in human leukemia cells. J Exp Med 2002;196:667 – 678.

52. Ilaria RL Jr, Hawley RG, Van Etten RA. Dominant negative mutants implicate STAT5 in myeloid cell proliferation and neutrophil differentiation. Blood 1999;93:4154 – 4166.

53. Nosaka T, Kawashima T, Misawa K, Ikuta K, Mui AL, Kitamura T. STAT5 as a molecular regulator of proliferation, differentiation and apoptosis in hematopoietic cells. EMBO J 1999;18:4754 – 4765.

54. Kieslinger M, Wildman I, Morogg R, Hofmann J, Marine JC, Ihle JN, et al. Antiapoptotic activity of Stat5 required during terminal differentiation of myeloid differentiation. Genes Dev 2000;14:232 – 244.

55. Warmuth M, Simon N, Mitina O, Mathes R, Fabbro D, Manley PW, et al. Dual-specific Src and Abl kinase inhibitors, PP1 and GCP76030, inhibit growth and survival of cells expressing imatinib mesylate-resistant Bcr-Abl kinases. Blood 2003;101:664 – 672.

56. Skorski T, Bellacosa A, Nieborowska-Skorska M, Majewski S, Martinez R, Choi JK, et al. Transformation of hematopoietic cells by BCR/ABL requires activation of a PI-3K/Akt-dependent pathway. EMBO J 1997;16:6151 – 6161.

57. Druker BJ, Sawyers CL, Kantarjian H, Resta DJ, Reese SF, Ford JM, et al. Activity of a specific inhibitor of the BCR-ABL tyrosine kinase in the blast crisis of chronic myeloid leukemia. Science 2000;289:1938 – 1942.

58. Majewski M, Martinez R, Choi JK, et al. Discovery of N-[2-chloro-6-methyl-phenyl]-2-[(6-(4-(2-hydroxyethyl)-piperazin-1-yl)-2-methylpyrimidin-4-ylamino)thiazole-5-carboxamide (BMS-345825), a dual Src/Abl kinase inhibitor with potent antitumor activity in preclinical assays. J Med Chem 2004;47:6658 – 6661.

59. Nam S, Kim D, Cheng JQ, Zhang S, Lee JH, Buettner R, et al. Action of the Src family kinase inhibitor, dasatinib (BMS-354825), on human prostate cancer cells. Cancer Res 2005;65:9185 – 9189.

60. Schittenhelm MM, Shiraga S, Schroeder A, Corbin AS, Griffith D, Lee FY, et al. Dasatinib (BMS-354825), a dual SRC/ABL kinase inhibitor, inhibits the kinase activity of wild-type, juxtamembrane, and activation loop mutant KIT isoforms associated with human malignancies. Cancer Res 2006;66:473 – 481.

61. Talpaz M, Shah NP, Kantarjian H, Donato N, Nicoll J, Paquette R, et al. Dasatinib in imatinib-resistant Philadelphia chromosome-positive leukemias. N Engl J Med 2006;354:2531 – 2541.

62. Baccarani M, Kantarjian HM, Apperley JF, Lipton JH, Druker B, Countouriotis A, et al. Efficacy of dasatinib (SPRYCEL) in patients (pts) with chronic phase chronic myelogenous leukemia (CP-CML) resistant to or intolerant of imatinib: updated results of the CA180013 START-C phase II study. Blood (Suppl.) 2006;108:164.

63. Cortes J, Kim DW, Guilhot F, Rosti G, Silver RT, Gollerkeri A, et al. Dasatinib (SPRYCEL) in patients (pts) with chronic myelogenous leukemia in accelerated phase (AP-CML) that is imatinib-resistant (im-r) or -intolerant (im-i): updated results of the CA180013 START-A phase II study. Blood (Suppl.) 2006;108:2160.

64. Martinelli G, Hochhaus A, Coutre S, Apperley JF, Shah N, Gollerkeri A, et al. Dasatinib (SPRYCEL) efficacy and safety in patients (pts) with chronic myelogenous leukemia in lymphoid (CML-LB) or myeloid blast (CML-MB) phase who are imatinib-resistant (im-r) or -intolerant (im-i). Blood (Suppl.) 2006;108:745.

65. Azam M, Daley GQ. Anticipating clinical resistance to target-directed agents: the BCR-ABL paradigm. Mol Diagn Ther 2006;10:67 – 76.

66. Kantarjian HM, Talpaz M, Giles F, O’Brien S, Cortes J. New insights into the pathophysiology of chronic myeloid leukemia and imatinib resistance. Ann Intern Med 2006;145:913 – 923.

67. O’hare T, Walters DK, Stoffregen EP, Jia T, Manley PW, Mestan J, et al. In vitro activity of Bcr-Abl inhibitors AMN107 and BMS-354825 against clinically relevant imatinib-resistant Abl kinase domain mutants. Cancer Res 2005;65:4500 – 4505.

68. Shah NP, Tran C, Lee FY, Chen P, Norris D, Sawyers CL. Overriding imatinib resistance with a novel ABL kinase inhibitor. Science 2004;305:399 – 401.

69. Tokarski JS, Newitt JA, Chang CY, Cheng JD, Wittekind M, Kiefer SE, et al. The structure of dasatinib (BMS-354825) bound to activated ABL kinase domain elucidates its inhibitory activity against imatinib-resistant ABL mutants. Cancer Res 2006;66:5790 – 5797.

70. Lee FY, Lombardo L, Camuso A, Castaneda S, Fager K, Fiebleh C, et al. BMS-354825 potently inhibits multiple selected oncogenic tyrosine kinases and possesses broad-spectrum antitumor activities in vitro and in vivo. Proc Am Assoc Cancer Res 2005;46:159 (abstr. 675).

71. Lombardo LJ, Lee FY, Chen P, Norris D, Barrish JC, Behnia K, et al. Discovery of N-[2-chloro-6-methyl-phenyl]-2-[(6-(4-((2-hydroxyethyl)-piperazin-1-yl)-2-methylpyrimidin-4-ylamino)thiazole-5-carboxamide (BMS-345825), a dual Src/Abl kinase inhibitor with potent antitumor activity in preclinical assays. J Med Chem 2004;47:6658 – 6661.

72. Slupianek A, Wilson M, Smithgall TE, et al. Action of the Src family kinase inhibitor, dasatinib (BMS-354825), on human prostate cancer cells. Cancer Res 2005;65:9185 – 9189.

73. Schittenhelm MM, Shiraga S, Schroeder A, Corbin AS, Griffith D, Lee FY, et al. Dasatinib (BMS-354825), a dual SRC/ABL kinase inhibitor, inhibits the kinase activity of wild-type, juxtamembrane, and activation loop mutant KIT isoforms associated with human malignancies. Cancer Res 2006;66:473 – 481.

74. Talpaz M, Shah NP, Kantarjian H, Donato N, Nicoll J, Paquette R, et al. Dasatinib in imatinib-resistant Philadelphia chromosome-positive leukemias. N Engl J Med 2006;354:2531 – 2541.

75. Baccarani M, Kantarjian HM, Apperley JF, Lipton JH, Druker B, Countouriotis A, et al. Efficacy of dasatinib (SPRYCEL) in patients (pts) with chronic phase chronic myelogenous leukemia (CP-CML) resistant to or intolerant of imatinib: updated results of the CA180013 START-C phase II study. Blood (Suppl.) 2006;108:164.

76. Cortes J, Kim DW, Guilhot F, Rosti G, Silver RT, Gollerkeri A, et al. Dasatinib (SPRYCEL) in patients (pts) with chronic myelogenous leukemia in accelerated phase (AP-CML) that is imatinib-resistant (im-r) or -intolerant (im-i): updated results of the CA180013 START-A phase II study. Blood (Suppl.) 2006;108:2160.

77. Martinelli G, Hochhaus A, Coutre S, Apperley JF, Shah N, Gollerkeri A, et al. Dasatinib (SPRYCEL) efficacy and safety in patients (pts) with chronic myelogenous leukemia in lymphoid (CML-LB) or myeloid blast (CML-MB) phase who are imatinib-resistant (im-r) or -intolerant (im-i). Blood (Suppl.) 2006;108:745.
79. Kantarjian H, Pasquini R, Hamerschlag N, Rousselot P, Holowiecki J, Joo tat S, et al. Dasatinib or high-dose imatinib for chronic-phase chronic myeloid leukemia after failure of first-line imatinib: a randomized phase 2 trial. Blood 2007;109:5143–5150.

80. Hochhaus A, Branford S, Radich J, Mueller MC, Shah N, Erben P, et al. Efficacy of dasatinib in chronic phase chronic myelogenous leukemia patients after imatinib failure according to baseline BCR-ABL mutations. J Clin Oncol 2007; 25(Suppl 18S):7023.

81. Quintas-Cardama A, Kantarjian H, Jones D, Nicaise C, O’Brien S, Giles F, et al. Dasatinib (BMS-354825) is active in Philadelphia chromosome-positive chronic myelogenous leukemia after imatinib and nilotinib (AMN107) therapy failure. Blood 2007;109:497–499.

82. Larson R, Ottmann O, Kantarjian H, le Coutre P, Baccarani M, Weitzman A, et al. A phase II study of nilotinib administered to imatinib resistant or intolerant patients with chronic myelogenous leukemia (CML) in blast crisis (BC) or relapsed/refractory Ph+ acute lymphoblastic leukemia (ALL). J Clin Oncol 2007;25(Suppl 18S):7040.

83. Druker BJ. Circumventing resistance to kinase-inhibitor therapy. N Engl J Med 2006;354:2594–2596.

84. Kantarjian HM, Talpaz M, O’Brien S, Smith TL, Giles FJ, Faderl S, et al. Imatinib mesylate for Philadelphia chromosome-positive, chronic-phase myeloid leukemia after failure of interferon-α: follow-up results. Clin Cancer Res 2002;8:2177–2187.

85. Kantarjian HM, Shan J, Smith T, Talpaz M, Kozuch P, Rios MB, et al. Response to therapy is independently associated with survival prolongation in chronic myelogenous leukemia in the blastic phase. Cancer 2001;92:2501–2507.

86. Marin D, Marktel S, Bua M, Seydlo RM, Franceschino A, Nathan I, et al. Prognostic factors for patients with chronic myeloid leukaemia in chronic phase treated with imatinib mesylate after failure of interferon α. Leukemia 2003;17:1448–1453.

87. Kantarjian H, O’Brien S, Cortes J, Giles F, Shan J, Rios MB, et al. Survival advantage with imatinib mesylate therapy in chronic-phase chronic myelogenous leukemia (CML-CP) after IFN-α failure and in late CML-CP, comparison with historical controls. Clin Cancer Res 2004;10:68–75.

88. Kantarjian H, Schiffer C, Sawyers CL, Hochhaus A, Guilhot F, Niederwieser DW, et al. Imatinib (Gleevec) maintains favorable long-term outcomes in chronic-phase chronic myeloid leukemia (CML) for patients failing interferon-α (IFN) follow-up of a phase II study. Blood 2003;102:3368.

89. Branford S, Rudzki Z, Harper A, Grigg A, Taylor K, Durrant S, et al. Imatinib produces significantly superior molecular responses compared to interferon α plus cytarabine in patients with newly diagnosed chronic myeloid leukemia in chronic phase. Leukemia 2003;17:2401–2409.

90. Quintas-Cardama A, Kantarjian H, O’Brien S, Jones D, Borthakur G, Nicaise C, et al. Dasatinib is safe and effective in patients with previously untreated chronic myelogenous leukemia (CML) in chronic phase (CML-CP). Haematologica 2007;92(Suppl 1):0360.