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Stem cell and kinase activity-independent pathway in resistance of leukaemia to BCR-ABL kinase inhibitors

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Abstract

BCR-ABL tyrosine kinase inhibitors, such as imatinib (Gleevec) are highly effective in treating human Philadelphia chromosome-positive (Ph^+) chronic myeloid leukaemia (CML) in chronic phase but not in terminal acute phase; acquired drug resistance caused mainly by the development of BCR-ABL kinase domain mutations prevents cure of the leukaemia. In addition, imatinib is ineffective in treating Ph^+ B-cell acute lymphoblastic leukaemia (B-ALL) and CML blast crisis, even in the absence of the kinase domain mutations. This type of drug resistance that is unrelated to BCR-ABL kinase domain mutations is caused by the insensitivity of leukaemic stem cells to kinase inhibitors such as imatinib and dasatinib, and by activation of a newly-identified signalling pathway involving SRC kinases that are independent of BCR-ABL kinase activity for activation. This SRC pathway is essential for leukaemic cells to survive imatinib treatment and for CML transition to lymphoid blast crisis. Apart from BCR-ABL and SRC kinases, stem cell pathways must also be targeted for curative therapy of Ph^+ leukaemia.

Keywords: BCR-ABL • Ph^+ leukaemia • leukaemic stem cells • kinase inhibitors • SRC kinases • drug resistance

Introduction

The translocation between chromosomes 9 and 22 gives rise to the human Philadelphia chromosome, and results in formation of the chimeric BCR-ABL tyrosine kinase, a constitutively activated, oncogenic tyrosine kinase. Chronic myeloid leukaemia (CML) and B-cell acute lymphoblastic leukaemia (B-ALL) are both philadelphia chromosome-positive (Ph^+) leukaemias induced by the BCR-ABL oncogene.

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CML often initiates in a chronic phase and eventually progresses to a terminal blastic phase, in which either acute myeloid or acute B-lymphoid leukaemia develops. Some Ph^+ leukaemia patients, however, have B-ALL as their first clinical appearance. Because B-ALL is similar pathologically to acute B-lymphoid leukaemia in the blastic phase of CML, Ph^+ leukaemia may present as CML or B-ALL, and successful treatment of Ph + leukaemia requires management of both diseases induced by BCR-ABL. It is commonly believed that shutting down the kinase activity of BCR-ABL will completely inhibit its functions, leading to inactivation of its downstream signalling pathways. Therefore, current therapeutic efforts for Ph^+ leukaemia have focused on targeting BCR-ABL kinase activity using kinase inhibitors.

The BCR-ABL tyrosine kinase inhibitor imatinib mesylate (Gleevec) is the standard of care for Ph^+ leukaemia. Imatinib has been shown to induce a complete haematologic response in chronic phase CML patients [1]. However, imatinib has been unable to completely eliminate BCR-ABL-expressing leukaemic cells [2, 3], and patients frequently present with cellular and clinical drug resistance [4–10]. We and others have shown that imatinib prolongs survival of mice with BCR-ABL-induced CML [11, 12], but does not cure the disease [11]. Recently, three newly developed BCR-ABL kinase inhibitors, dasatinib [13], AP23464 [14] and AMN107 [15], have been shown to inhibit almost all imatinib-resistant BCR-ABL mutants in cell culture and animal studies; the exception is the BCR-ABL-T315I mutant, which is present in 15–20% of patients resistant to imatinib therapy. Besides its anti-BCR-ABL kinase activity, dasatinib is also a potent inhibitor of SRC family kinases, but the role of the anti-SRC activity of this compound in Ph^+ leukaemia therapy has not been studied [13]. For unknown reasons, imatinib is much less effective in treating CML blastic phase patients and patients with Ph^+ B-ALL [16, 17], which has not been shown to be related to the BCR-ABL kinase domain mutations, the most common type of imatinib-resistance. Because imatinib is a strong inhibitor of BCR-ABL kinase activity, the inability of imatinib to cure CML and B-ALL in mice [11] suggests that inactivation of BCR-ABL kinase activity alone is insufficient to control the disease.

We have previously shown that the three SRC family kinases LYN, HCK and FGR are activated by BCR-ABL in pre-B leukaemic cells and are required for the development of B-ALL [11]. We have also shown that imatinib does not cure mice with B-ALL, consistent with a study using human leukaemic cells, in which cells from patients resistant to imatinib expressed an activated form of LYN [18]. Recently, we have demonstrated that inhibition of SRC kinases and BCR-ABL by dasatinib is effective in controlling B-ALL in mice, but leukaemic stem cells in B-ALL or CML mice are insensitive to treatment with dasatinib or imatinib [19]. In this review, we focus on discussion of imatinib-resistant mechanisms that are not associated with mutations in BCR-ABL kinase domain because we believe that targeting these BCR-ABL kinase activity-independent pathways is critical to curative therapy of Ph^+ leukaemia.

Ph^+ leukaemia

The *BCR-ABL* oncogene is the cause of Ph^+ leukaemias. The *BCR* gene, on chromosome 22, breaks either at exon 1, exon 12/13 or exon 19 and fuses to the *c-ABL* gene on chromosome 9 to form, respectively, three types of BCR-ABL chimeric gene: P190, P210 or P230. Each of the three forms of the *BCR-ABL* oncogene is associated with a distinct type of human leukaemia. The P190 form is most often present in B-ALL but only rarely in CML [20], whereas P210 is mainly involved in CML and in some acute lymphoid [20] and myeloid leukaemias in CML blast crisis. P230 is found in a very mild form of CML [21]. Ph^+ B-ALL and lymphoid blast crisis of CML account for 20% of adults and 5% of children with acute B-lymphoid leukaemia. Among those patients with BCR-ABL-induced B-ALL, 50% of adults and 20% of children carry P210 form of *BCR-ABL* and the rest of the patients carry the P190 form [16, 20, 22].

Chronic phase CML responds to imatinib therapy [1]. The disease can progresses from chronic phase to accelerated phase or blast crisis, and the transition from chronic phase to blast crisis results in loss of imatinib response in Ph^+ leukaemia patients. Although the mechanism underlying the disease progression is not fully understood, additional genetic alterations are believed to play a role in this process. Mutations of tumour suppressor genes, such as the retinoblastoma gene (Rb), p16 and p53 appear to be associated with CML blast crisis patients [23–25]. However, it is still not known how BCR-ABL-expressing

cells acquire these additional genetic lesions. An increase in genetic instability caused by BCR-ABL is one plausible mechanism, as BCR-ABL deregulates the functions of DNA repair-related genes according to several studies. For example, BCR-ABL down-reg-ulates expression of the DNA repair enzyme DNA-PKcs [26]. BCR-ABL may interact with the xeroderma pigmentosum group B protein, which could lead to the impairment of DNA repair function [27]. Expression of two other genes related to genetic stability, *BRCA-1* and *RAD51*, is also deregulated by BCR-ABL [28, 29]. BCR-ABL can also cause overexpression and increased activity of the error-prone polymerase β, leading to an increased mutagenesis [28]. A recent study showed that BCR-ABL associates with rad 3-related protein (ATR), which is involved in DNA repair, and inhibits activation of ATR following DNA damage, leading to alteration of cellular responses to DNA damage [30]. Although BCR-ABL is a primary driver for growth of leukaemic cells [31], it is believed that the concomitant effect of BCR-ABL on cell survival and DNA double strand break repair may lead to the acquisition of additional genetic lesions contributing to progression of CML [32]. These studies demonstrate that disruption of DNA repair mechanisms by BCR-ABL may lead to progression of chronic phase CML to more advance disease.

Leukaemic stem cells

A key characteristic of stem cells is the ability for self-renewal. Only long-term and short-term self-renewing haematopoietic stem cells (HSCs) have the ability to renew themselves, although other multi-potent progenitors in the haematopoietic system proliferate and differentiate to become more mature blood cells. [33]. Some multi-potent progenitors that are not normally self-renewing can aberrantly acquire self-renewing capacity during leukaemogenesis to become leukaemic stem cells. For example, granulocyte-macrophage progenitors have been identified as potential leukaemic stem cells for human CML myeloid blast crisis, and β-catenin that is involved in self-renewal of normal HSCs [34, 35] is also activated in granulocyte-macrophage progenitors [36], which appear to have acquired the potential for self-renewal through activation of β-catenin. It is still an open question whether cancer stem cells exist in all types of tumours; however, it is convincing that the cells capable of initiating human AML in non-obese diabetic mice with severe combined immunodeficiency disease (NOD/SCID) are exclusively $CD34^+CD38^-$ cells [37], which is characteristic of normal HSCs. This hints that leukaemic stem cells and normal HSCs may share mechanisms for regulation of self-renewal. This is supported by the studies using *Bim-1*-deficient mice, in which *Bim-1* is required for maintenance of self-renewal of normal HSCs [38, 39] and stem cells for AML induced co-operatively by the *Hoxa9* and *Meis1a* genes [38]. Evidence that Bim-1-related pathways play roles in the repopulating ability of the leukaemic stem cells is provided by the finding that $Bim1^{-/-}$ bone marrow cells from AML mice are incapable of re-producing the disease in secondary recipients [38]. However, failure to re-populate malignant diseases to secondary recipients does not exclude the possibility that the transferred cancer stem cells with self-renewal capability did not engraft due to complex mechanisms related to the donor–recipient interactions. This interaction between stem cells and their specific bone marrow microenvironment is critical for regulating the balance between self-renewal and differentiation of HSCs [40]. A new way of understanding physiopathology of human haematologic malignancies is to fully understand how leukaemic stem cells communicate with bone marrow microenvironment.

Identification of CML stem cells in mice

CML is believed to be a stem cell disease. BCR-ABL induces CML in mice [41–43], but mouse CML stem cells were not identified and characterized until last year. We first tested whether BCR-ABL-expressing HSCs function as the stem cells in mice. When C57BL/6 (B6) bone marrow cells transduced with BCR-ABL retrovirus were sorted into two separate populations ($Sca\text{-}1^-$ or $Sca\text{-}1^+$), only BCR-ABL-transduced $Sca\text{-}1^+$ cells transferred lethal CML to secondary B6 recipient mice [19], suggesting that early bone marrow progenitors contain CML stem cells. To narrow down the specific cell lineages that function as CML stem cells, HSCs ($Lin^-c\text{-}kit^+Sca\text{-}1^+$) were thought to be likely candidate population. Indeed, BCR-ABL-expressing HSCs ($GFP^+Lin\text{-}c\text{-}Kit^+Sca\text{-}1^+$)

isolated from bone marrow cells of primary CML mice induces CML in B6 recipient mice, indicating that BCR-ABL-expressing HSCs function as CML stem cells [19]. It is still to be tested whether other cell lineages serve as CML stem cells.

BCR-ABL kinase inhibitors prolong survival of CML mice, but do not completely eradicate CML stem cells

BCR-ABL kinase inhibitors are effective in treating CML, but are unlikely to provide a cure for the disease, as imatinib does not effectively kill BCR-ABL-expressing primitive human $CD34^+$ cells [2] and cure CML mice [11]. When a more potent BCR-ABL kinase inhibitor dasatinib [13] is used to treat CML mice, the mice lived significantly longer than those treated with imatinib, but eventually still died of this disease, indicating that, like imatinib [44], dasatinib may not eradicate leukaemic stem cells in CML mice because CML in mouse leukaemia model originates from multi-lineage repopulating cells [45]. When dasatinib is tested for killing BCR-ABL-expressing haematopoieticHSCs *in vivo*, BCR-ABL-expressing HSCs (GFP^+CD34^-c-Kit^+ Hoe^-) in the side population (SP) [46] of BM cells from the dasatinib-treated CML mice were not completely eradicated by dasatinib treatment [19]. This and other results suggest that neither dasatinib nor imatinib will cure CML and that targeting at least one additional component of leukaemic stem cells is required for curing the disease. The inability of dasatinib or possibly other kinase inhibitors to completely eradicate CML stem cells may be attributed to failure of the kinase inhibitors to access the stem cells. However, this is not the case, as dasatinib inhibited BCR-ABL phosporylation in the stem cells *in vivo* [19], indicating the drug entered and functioned in CML stem cells. The observed dasatinib-resistance of CML stem cells is not attributed to appearance of BCR-ABL-T315I (insensitive to dasatinib) clone in the mice, as sequencing analysis of genomic DNA from BM cells of these dasatinib-treated CML mice did not reveal the T315I mutation in the BCR-ABL kinase domain (data not shown). Wong *et al.* [47] show that c-kit is involved in CML development, however, the failure of imatinib and dasatinib to eradicate leukaemic stem cells is not related to c-kit function, as both drugs inhibit c-kit [48]. Together, these results demonstrate that inhibition of BCR-ABL kinase activity alone is insufficient to eradicate CML stem cells, leading to cure of the disease.

B-ALL stem cells and their sensitivity to kinase inhibitors

Although it is unclear what the stem cells for Ph^+ B-ALL are, Ph^+ B-ALL and CML could develop from a common stem cell, as chronic phase CML progresses to acute lymphoid leukaemia and Ph^+ B-ALL often coexists with CML [49]. This idea is supported by the observation that the same antiserum recognizes both B-ALL cells and cells from CML patients [50]. Furthermore, lymphoid and myeloid leukaemias induced by BCR-ABL originate from the same progenitor cells in mice [45]. On the other hand, Ph^+ B-ALL induced by P190 form of BCR-ABL is rarely present in CML [20], suggesting a possibility that early lymphoid progenitors become the stem cells for Ph^+ B-ALL. We have noticed that the majority of residual cells in dasatinib-treated B-ALL mice are BCR-ABL-expressing $B220^+/CD43^+$ and $B220^+/CD43^-$ pro-/pre-B cells, which may have acquired self-renewal capacity to function as B-ALL stem cells. We indeed observed that the $B220^+/CD19^+/GFP^+$ cells sorted from bone marrow of B-ALL mice transferred lymphoid leukaemia in lethally irradiated syngeneic mice [19], showing that BCR-ABL-expressing pro-B cells can function as B-ALL stem cells.

It is critical to test whether B-ALL is sensitive to treatment with BCR-ABL kinase inhibitors. Imatinib is weakly effective in treating B-ALL [11, 19]. Although dasatinib remarkably prolongs survival of B-ALL mice, there were still small amount of leukaemic cells remaining in peripheral blood of these mice, even after 3 months of treatment; when dasatinib treatment was stopped, B-ALL mice relapsed and died if not treated [19]. The relapsed B-ALL mice remained sensitive to dasatinib treatment, and the disease could be controlled by continuous use of this drug [19]. These results indicate that continuous dasatinib treatment could prevent the residual leukaemic cells

(presumably B-ALL stem cells) from developing into fatal B-ALL, although this drug did not completely eradicate B-ALL stem cells.

Compared with dasatinib-treated CML mice that survived much longer than untreated mice, dasatinib-treated B-ALL mice survived continuously as long as the treatment continued [19]. The more superb therapeutic effect of dasatinib on B-ALL than on CML could be due to the activity of dasatinib against SRC kinases. As mentioned above, the pathways that regulate self-renewal of stem cells for haematologic malignancies involve the Bim-1 [38] and Wnt/β-catenin [36] pathways, and SRC kinases may be involved in regulation of the β-catenin pathway [51]. In addition, v-SRC has been shown to activate β-catenin-LEF/TCF (lymphoid enhancer factor/T-cell factor)-mediated transcription through the mitogen-activated protein kinase (MAPK) pathway [52]. A possible role of SRC kinases in self-renewal of Ph^+ leukaemia needs to be further studied.

SRC kinase may impact B-ALL stem cell functions through activation of the downstream signalling molecule β-catenin

Activation of β-catenin in the Wnt signalling pathway has been shown to play a role in self-renewal of HSCs and proliferation of tumour cells [33, 34, 53]. As mentioned above, this molecule is activated in granulocyte-macrophage progenitors of advanced phase CML patients and is involved in self-renewal ability of these cells *in vitro* [36]. The striking therapeutic effect of dasatinib, but not imatinib, on B-ALL [19] suggests that inhibition of SRC kinases not only played a key role in killing the highly proliferating leukaemic cells, but also may have an inhibitory effect on leukaemic stem cells, which would diminish the contribution of these stem cells to the disease. Therefore, we investigated whether SRC kinases are involved in activation of β-catenin in BCR-ABL-expressing cells. We observed that β-catenin is activated in the BCR-ABL-expressing mouse pre-B cell line (BaF/3) (Fig. 1A). To test whether activated SRC kinases directly activate β-catenin, we expressed v-SRC in BaF/3 cells. v-SRC activated β-catenin (Fig. 1B), suggesting that normal SRC kinases activated by BCR-ABL may activate β-catenin directly. To provide supporting evidence for this hypothesis, we first compared the levels of β-catenin expression in BCR-ABL-expressing pre-B leukaemic cells in the presence and absence of the SRC kinases Lyn, Hck and Fgr. We have previously shown that, while the lack of Lyn, Hck and Fgr causes a severe defect in the development of B-ALL, BCR-ABL-transduced *Lyn*$^{-/-}$*Hck*$^{-/-}$*Fgr*$^{-/-}$ bone marrow cells can grow under Whitlock-Witte culture conditions at high cell density [11]. Using these conditions to test the role of the three SRC kinases in β-catenin activation, we found that the level of β-catenin activation in *Lyn*$^{-/-}$*Hck*$^{-/-}$*Fgr*$^{-/-}$ leukaemic cells was lower than that in wild-type leukaemic cells (Fig. 1C). To test whether inhibition of SRC kinases by dasatinib down-regulates β-catenin activation, we treated BCR-ABL-T315I-expressing pre-B leukaemic cells with dasatinib. β-catenin activation was inhibited by dasatinib at 100 nM, and this was associated with complete inhibition of SRC activation (Fig. 1D). These results link SRC kinases to β-catenin activation, implying that Src kinase may impact B-ALL stem cell functions through activation of the downstream signalling molecule β-catenin.

Activation of SRC kinases by BCR-ABL is independent of its kinase activity

BCR-ABL activates SRC kinases in myeloid cells, and SRC kinase inhibitors impair cellular transformation by BCR-ABL in cultured cells [54]. Stimulatory role of SRC kinases in transformation of lymphoid cells by BCR-ABL is demonstrated clearly using mice deficient for three SRC kinases LYN, HCK and FGR, and these three SRC kinases are shown to play a specific role in the induction of B-ALL not CML [11]. This lineage-specific function of SRC kinases indicates that SRC kinases are good therapeutic targets for B-ALL. The role of Lyn in BCR-ABL-induced lymphoid leukaemia is also supported by a study using lymphoid blastic cells from CML patients [55]. Lyn overexpression or inhibition of Lyn by the Src kinase inhibitor PP2 leads to an increase or decrease of Bcl-2 expression in LAMA84 and K562 cells [56], suggesting Bcl-2 may be a downstream signalling molecule of Lyn in BCR-ABL-positive cells. Because BCR-ABL activates SRC kinases, it is reasonable to think that inhibition of BCR-ABL kinase activity by imatinib would shut down SRC kinases

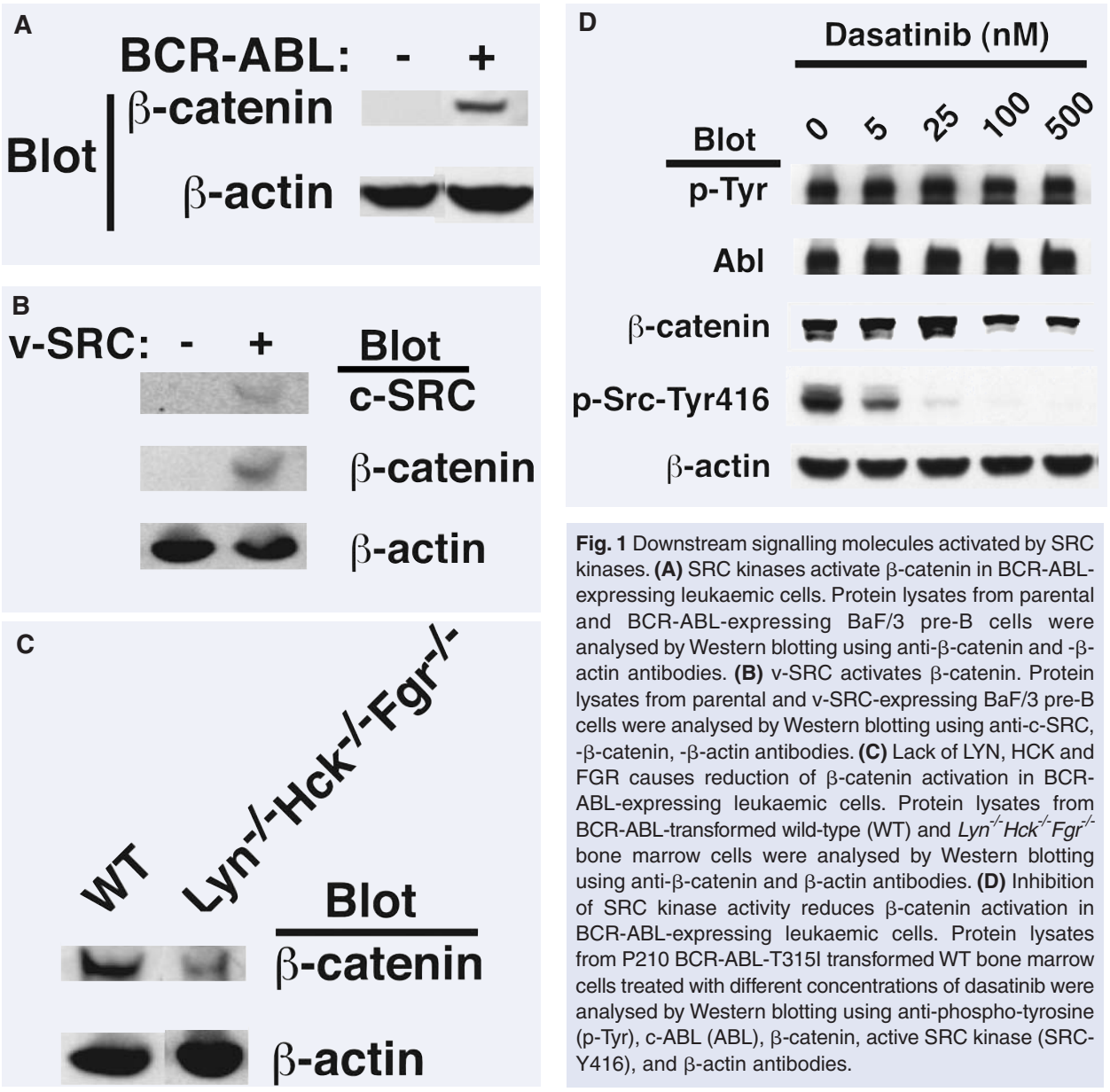

Fig. 1 Downstream signalling molecules activated by SRC kinases. **(A)** SRC kinases activate β-catenin in BCR-ABL-expressing leukaemic cells. Protein lysates from parental and BCR-ABL-expressing BaF/3 pre-B cells were analysed by Western blotting using anti-β-catenin and -β-actin antibodies. **(B)** v-SRC activates β-catenin. Protein lysates from parental and v-SRC-expressing BaF/3 pre-B cells were analysed by Western blotting using anti-c-SRC, -β-catenin, -β-actin antibodies. **(C)** Lack of LYN, HCK and FGR causes reduction of β-catenin activation in BCR-ABL-expressing leukaemic cells. Protein lysates from BCR-ABL-transformed wild-type (WT) and *Lyn*$^{-/-}$*Hck*$^{-/-}$*Fgr*$^{-/-}$ bone marrow cells were analysed by Western blotting using anti-β-catenin and β-actin antibodies. **(D)** Inhibition of SRC kinase activity reduces β-catenin activation in BCR-ABL-expressing leukaemic cells. Protein lysates from P210 BCR-ABL-T315I transformed WT bone marrow cells treated with different concentrations of dasatinib were analysed by Western blotting using anti-phospho-tyrosine (p-Tyr), c-ABL (ABL), β-catenin, active SRC kinase (SRC-Y416), and β-actin antibodies.

that are downstream of BCR-ABL. The striking finding is that in B-lymphoid cells imatinib markedly inhibited BCR-ABL kinase activity but did not result in a decrease in SRC activation, indicating that while imatinib was very effective in inhibiting BCR-ABL phosphorylation, it was unable to affect BCR-ABL-stimulated phosphorylation of SRC kinases [19]. These observations indicate that activation of SRC kinases by BCR-ABL is independent upon its kinase activity, suggesting the necessity of targeting both BCR-ABL and SRC kinases in treating B-ALL. The inability of imatinib to inactivate SRC kinases may explain the relatively poor activity of this drug against Ph$^+$ B-ALL and acute lymphoid leukaemia.

Role of SRC kinases in the development of B-ALL

As mentioned above, the critical role of SRC kinases in the development of BCR-ABL-induced is first

demonstrated using the SRC-deficient mice [11]. Are SRC kinases good therapeutic targets for B-ALL? This question was answered by treating B-ALL mice with the dual SRC/ABL inhibitor dasatinib. The P210 form of BCR-ABL-T315I mutant is resistant to inhibition by both imatinib [4, 54, 57] and dasatinib [13]. Treatment of mice with B-ALL induced by BCR-ABL-T315I with imatinib or dasatinib showed that imatinib had no therapeutic effect, whereas dasatinib significantly prolonged survival of the mice [19], indicating that targeting SRC kinases alone prolongs survival of B-ALL mice. However, targeting SRC kinases alone did not cure the disease, which may be due to the incomplete inhibition of SRC kinase activity *in vivo*. Testing a stronger SRC inhibitor would help to reveal the full potential of SRC kinases as therapeutic targets for B-ALL induced by BCR-ABL.

The role of SRC kinases in B-ALL development is further supported by comparing growth potential of BCR-ABL-transduced wild-type (WT) and *Lyn*$^{-/-}$*Hck*$^{-/-}$*Fgr*$^{-/-}$ bone marrow cells [19]. Levels of BCR-ABL-expressing B220^{+} B-lymphoid leukaemic cells were significantly lower in mice receiving BCR-ABL transduced *Lyn*$^{-/-}$*Hck*$^{-/-}$*Fgr*$^{-/-}$ bone marrow cells than in those receiving BCR-ABL transduced WT bone marrow cells. Strikingly, B-lymphoid leukaemic cells in some mice receiving the transduced *Lyn*$^{-/-}$*Hck*$^{-/-}$*Fgr*$^{-/-}$ bone marrow cells almost disappeared 5 weeks following B-ALL induction, demonstrating more definitively a critical role of SRC kinases in B-ALL development.

Inhibition BCR-ABL kinase activity and SRC kinase leads to long-term survival of B-ALL mice

Imatinib only has a weak therapeutic effect on B–ALL [19], and this is likely due to the fact that SRC kinases are still active when BCR-ABL phosphorylation is inhibited by imatinib [19]. If so, shutting down simultaneously both SRC kinases and BCR-ABL kinase activity with dasatinib should provide much more dramatic therapeutic effect on B-ALL. Indeed, dasatinib maintained long-term survival of the mice with B-ALL induced by P190 or P210 form of BCR-ABL. The weak therapeutic effect of imatinib is not attributed to an inability to inhibit BCR-ABL kinase activity *in vivo*, as imatinib significantly inhibited BCR-ABL phosphorylation, to similar extend compared to dasatinib, in B-lymphoid leukaemic cells from the treated B-ALL mice. These results support the critical role of SRC kinases in B-ALL development.

Role of SRC kinases in progression of CML to lymphoid blast crisis

Additional genetic alterations, such as mutations in the tumour suppressor genes INK4^{a}, pRB and p53 are associated with the transition from CML chronic phase to acute (blastic) phase [23–25]. A recent study showed that Arf gene loss enhances oncogenicity of and limits imatinib response to BCR-ABL-induced B-ALL in mice [58]. Chronic phase CML responds to imatinib treatment, but imatinib becomes much less effective after the disease advances to blastic phase. In mice, serial transplantation of CML bone marrow cells to recipient mice leads to developing acute lymphoid leukaemia [59]. When mice were transplanted with BCR-ABL transduced bone marrow cells from either WT or *Lyn*$^{-/-}$*Hck*$^{-/-}$*Fgr*$^{-/-}$ mice to induce CML, followed by subsequently transferring bone marrow CML cells into lethally irradiated syngeneic recipient mice, mice receiving WT CML bone marrow cells developed B-ALL, whereas none of the mice receiving *Lyn*$^{-/-}$*Hck*$^{-/-}$*Fgr*$^{-/-}$ CML BM cells developed this disease. This result indicates that CML transition to lymphoid blast phase requires SRC kinases. Inhibition of SRC kinases by dasatinib may reflect therapeutic effect of this drug on B-ALL patients [60, 61]. It is important to mention that targeting SRC kinases are less effective in treating B-ALL when tumour suppressor gene function is defective, as dasatinib-treated recipients of BCR-AB-transduced bone marrow cells from p53-deficient mice survived longer than those treated with imatinib, but eventually died [19]. These results suggest that loss of tumour suppressor function impedes effectiveness of dasatinib or likely other kinase inhibitors in treating BCR-ABL-induced B-ALL, and identification of mutations in these tumour suppressor genes in patients is useful in guiding drug therapy of Ph^{+} leukaemia.

Comments and conclusions

It is still an open question whether BCR-ABL kinase inhibitors cure Ph^{+} leukaemia. Studies in mice show

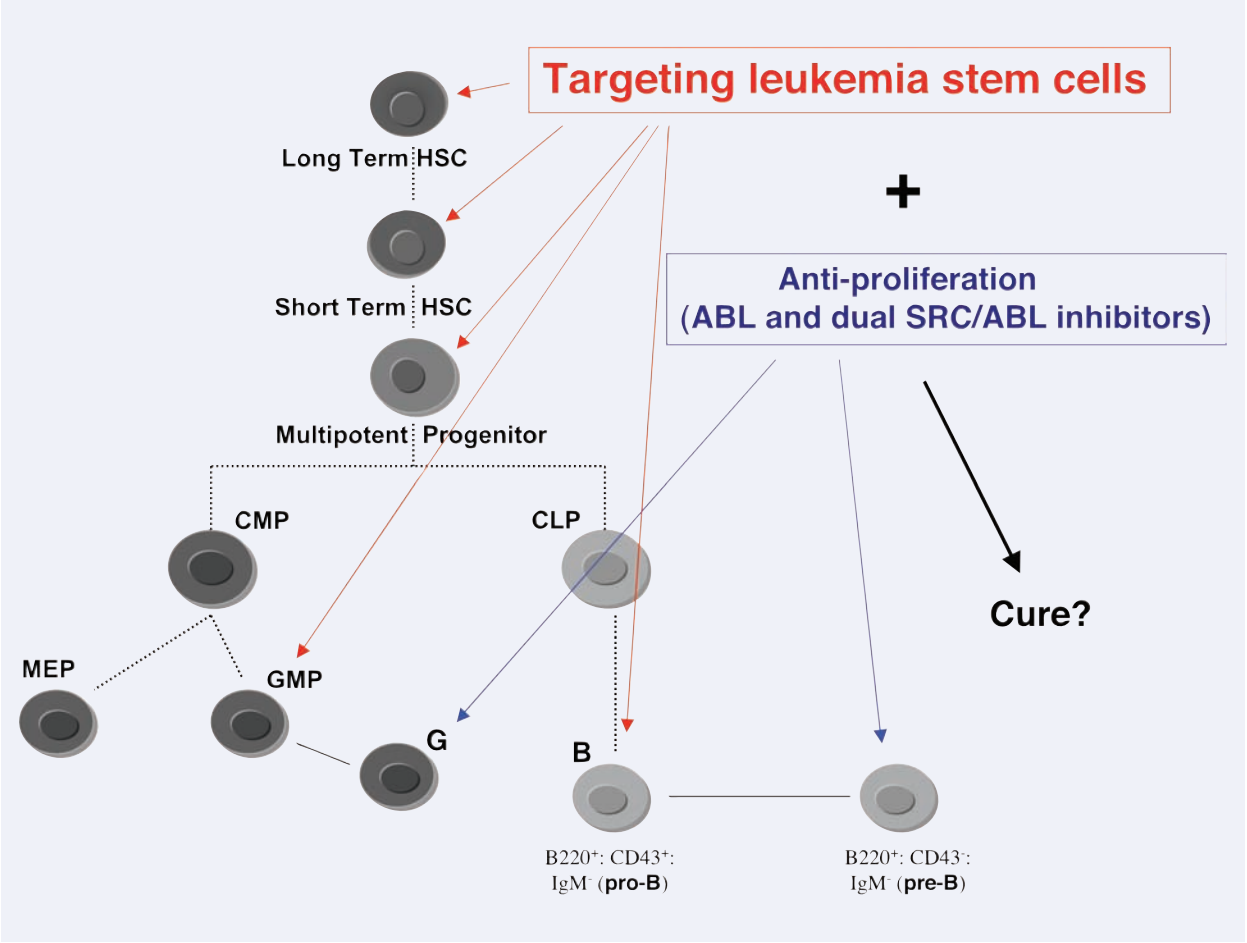

Fig. 2 Simultaneous targeting of both leukaemic stem cells and highly proliferative leukaemic cells may lead to cure of Ph^+ leukaemia. Treatment with BCR-ABL kinase inhibitors does not cure CML and B-ALL induced by BCR-ABL in mice, this is likely due to the inability of these inhibitors to kill leukaemic stem cells. Therefore, combination of anti-stem cell agents and BCR-ABL kinase inhibitors would be a promising therapeutic strategy for Ph^+ leukaemia.

that sole inhibition of BCR-ABL kinase activity by imatinib has an effect in treating Ph^+ B-ALL and CML but is not sufficient to achieve complete control of the diseases. One of the reasons is because inhibition of BCR-ABL kinase activity by imatinib does not inactivate some BCR-ABL activated signalling pathways such as SRC kinases, which are essential to B-ALL development. Sustained activation of these pathways would allow leukaemic cells to survive treatment with drugs that inhibit only BCR-ABL kinase activity, allowing accumulation of BCR-ABL mutations associated with drug resistance. Thus, simultaneous targeting of these BCR-ABL kinase activity-independent pathways and BCR-ABL kinase activity would provide a significantly improved therapeutic strategy for Ph^+ leukaemia. This strategy is against the idea that complete and sole inhibition of BCR-ABL kinase activity would completely inhibit BCR-ABL functions. On the other hand, BCR-ABL-activated SRC kinases alone may not be efficient in transformation of B-lymphoid cells, however, they are sufficient to maintain survival and stimulate proliferation of the leukaemic cells under treatment of BCR-ABL kinase inhibitors. Although the next generation of BCR-ABL kinase inhibitors aims at increasing drug potency or overriding imatinib resistance, BCR-ABL kinase activity-independent pathways must be targeted to achieve a durable therapeutic effect in patients with Ph^+ acute lymphoid leukaemia. Effectiveness of dasatinib in preventing or delaying transition of CML chronic

phase to acute lymphoid leukaemia and in treating acute lymphoid leukaemia with compromised tumour suppressor function provides a rationale for the early and continuous use of dasatinib in chronic phase CML patients. Comparing to patients with compromised tumour suppressor functions, the early use of dasatinib would be more effective in preventing transition of patients with chronic phase CML to lymphoid blast crisis and for management of patients with advanced lymphoid leukaemia. This idea is indirectly supported by the clinical observation that dasatinib is effective in treating Ph^+ B-ALL patients [60]. Our thoughts are that, if the BCR-ABL-T315I mutation that does not respond to dasatinib treatment is absent from the leukaemic cell population, dasatinib treatment may lead to long-term remission of B-ALL.

Most challenging issue in therapy of Ph^+ leukaemia deals with leukaemic stem cells. Although dasatinib could help achieve long-term control of B-ALL in mice, curative drug therapy of this disease would require targeting quiescent leukaemic stem cells [62] in addition to BCR-ABL kinase activity and SRC-dependent pathways. Identification of CML and B-ALL stem cells in mice [19] is significant, as it provides a model system for studying the biology of leukaemic stem cells. Identification of pro-B leukaemic cells as stem cells for B-ALL is important, as it indicates that pro-B progenitors could acquire self-renewal capacity to become the major source of highly proliferating B-lymphoid leukaemic cells in B-ALL mice. Therefore, complete inhibition of growth of this leukaemic population could achieve long-term survival of B-ALL mice. This also promotes the effort in testing whether other progenitor lineages could also acquire stem-like properties. It will be important to assess whether the stem cells identified in leukaemic mice can be similarly found in Ph^+ leukaemia patients. Finally, insensitivity of leukaemic stem cells in mice to inhibition by both imatinib and dasatinib [19] prompts us to identify unknown pathways in leukaemic stem cells for developing curative therapies for Ph^+ leukaemia (Fig. 2). It is important to mention that in human CML patients the ineffectiveness of kinase inhibitors to completely eradicate leukaemic cells could also be due to the pre-existing BCR-ABL kinase domain mutations, as shown by the elegant work from Ottmann's group [63]. It will be critical to investigate whether these mutations exist in leukaemic stem cells of CML patients before they are treated with kinase inhibitors. On the other hand, there are other kinase inhibitors that inhibit multiple kinases in cancer cells. For example, the Aurora kinase VX-680 (MK-0457) suppresses tumour cell growth and also inhibits BCR-ABL kinase including imatinib-resistant mutant BCR-ABL [64–69]. It is worth testing whether this kind of inhibitors would have an inhibitory effect on leukaemic stem cells.

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