PROCEEDINGS ARTICLE

Where are we going with CML research?

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The introduction of Abl tyrosine kinase inhibitors (TKI; that is, imatinib, dasatinib and nilotinib) as front-line therapy completely changed the course of chronic myelogenous leukemia (CML) to the point that most of the TKI-responsive newly diagnosed CML patients can be considered 'clinically' cured and their progression into blast crisis (BC) a rare event. However, a therapy for those patients who transform is still lacking, and TKIs do not eradicate CML at the stem cell level, therefore leaving a reservoir of cancer stem cells in a dormant stage. Thus, it is not surprising that the focus of CML research has shifted significantly toward the dissection of the mechanisms regulating the survival and self-renewal of TKI-resistant Philadelphia-positive leukemic chronic phase and BC stem cells, with the ultimate goal of developing small molecules capable of selectively killing leukemic but not normal hematopoietic stem cells, thereby achieving a 'biological' cure for this disease.

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The Philadelphia-positive (Ph⁺) chronic myelogenous leukemia (CML) is a biphasic hematopoietic stem cell (HSC)-derived, but progenitor-driven, myeloproliferative disorder that, if left untreated, progresses from a therapeutically manageable chronic phase (CML-CP) to an untreatable blastic phase (CML-BP).^{1,2} CML is characterized by the presence of the t(9;22) BCR-ABL1 oncogene, whose expression and constitutive tyrosine kinase activity in hematopoietic progenitors is determinant for disease emergence, maintenance, progression and response to tyrosine kinase inhibitor (TKI) monotherapy.^{1,2} Most CML patients with wild-type or mutated BCR-ABL1 achieve complete cytogenetic response and major or complete molecular remission (CMR) with low risk of relapse or progression to blastic phase when treated with first- or second-generation TKIs (imatinib, nilotinib and dasatinib);^{3,4} a few patients in CMR (undetectable BCR-ABL1 transcripts) for more than 2 years remain disease free after imatinib discontinuation.⁵ However, disease relapse is usually observed following cessation of TKIs,⁵ and the persistence of cells from the original BCR-ABL1⁺ leukemic clone in imatinib-treated patients but not in post-stem cell transplantation CML patients in CMR⁶ are both suggestive of the presence of nonproliferating Ph⁺ HSCs that demonstrate innate resistance to TKI treatment.^{7–10} Similarly, CML patients undergoing blastic transformation show an initial response to TKIs, but almost all of them relapse within 2 years.¹ This is commonly ascribed to the heterogeneous nature of the disease.¹¹ In fact, a variety of epigenetic and genetic changes that often disrupt tumor suppressor genes and genes regulating cell differentiation are detectable in almost all CML-BP patients, but only in a minority of CML-CP patients.^{11,12} At present, the molecular mechanisms underlying disease progression are still uncertain, but most likely involve activation of oncogenic factors and/or inactivation of tumor suppressors. A plausible assumption is that BC is a multistep, time-dependent process initiated by both BCR-ABL1-dependent and -independent mechanisms leading to enhanced DNA damage and aberrant DNA repair in CML-CP that, if facilitated by increased dosage of BCR-ABL1 activity, leads to the develop-

ment of CML-BP cells with HSC-like properties.¹ Indeed, published^{1,13–15} and unpublished (presented at the 2010–2011 *Annual Meetings of The American Society of Hematology*) work from different groups revealed that both BCR-ABL kinase-dependent and -independent mechanisms are responsible for the acquisition of β -catenin- and/or Hedgehog-regulated self-renewal, enhanced survival and arrested differentiations of leukemic granulocyte-macrophage progenitors (GMPs) that act as leukemia-initiating cells in CML-BP. Several lines of evidence suggest that stemness of Ph⁺ HSCs might be sustained through a BCR-ABL1 kinase-independent mechanism,¹⁶ which can only be partly explained by either altered drug uptake/efflux or BCR-ABL1 mutations;^{7,17,18} however, what is controlling leukemic stemness and whether BCR-ABL1 expression *per se* is required for survival and self-renewal of Ph⁺ quiescent HSCs (CFSE^{MAX}/CD34⁺/CD38[−]), and CML-BP GMPs still remain to be elucidated.

Although there are obvious benefits to exploring therapies that prevent disease progression into CML-BP, the current low incidence of blastic transformation together with the possibility to overcome the inherent TKI resistance of Ph⁺ quiescent HSCs^{7,19} has shifted the focus of CML research to the dissection of the mechanisms regulating the survival and self-renewal of TKI-resistant quiescent Ph⁺ HSCs and GMPs with the ultimate goal of developing small molecules capable, at the same time, of eradicating CML-CP at the stem cell level and, perhaps, successfully treating CML-BP patients.

Several strategies to target CML HSCs and CML-BP GMPs with acquired stemness behavior have shown promising results in preclinical studies with the following: (i) pharmacologic protein phosphatase 2A (PP2A) activators;²⁰ (ii) JAK2,^{21–23} HDAC,²⁴ Smo1,²⁵ β -catenin, BCL2/MCL1,^{26–28} autophagy and proteasome inhibitor,^{29,30} and (iii) natural killer cells or IL-1RAP antibodies to target leukemic cells.^{31,32} These studies have also provided insight into the mechanisms by which LSCs are capable of self-renewal and survival. In some cases, targeting such pathways does not induce killing but renders leukemic HSCs sensitive to

TKIs;^{24,29,30,33–38} however, it is still questionable whether the therapeutic window of some of these compounds is wide enough not to halt normal hematopoiesis or exhaust the reservoir of normal HSCs.

We have evidence that drugs such as FTY720 might represent a novel and powerful class of anti-leukemic agents. FTY720 (Fingolimod; Gilenia) is an orally administered sphingosine analog used in patients with relapsing multiple sclerosis for its immunosuppressive activity,^{39,40} which depends on impaired lymphocyte egress into the peripheral blood.⁴¹ To act as an immunosuppressor, FTY720 undergoes sphingosine kinase 2 (SPHK2) phosphorylation and internalization upon interaction with the sphingosine-1-phosphate receptor 1 (S1PR1).⁴¹ FTY720 is also a potent activator of PP2A,^{42,43} a tumor suppressor found inactivated in solid tumors and hematological malignancies including CML (CP and BP).^{15,43} However, the anticancer activity of FTY720 does not require SPHK2 phosphorylation or S1PR1 interaction,^{42,44} but depends, at least in Ph⁺ leukemia, on its ability to restore PP2A function that, in turn, promotes BCR-ABL1 inactivation/proteasome degradation and inhibition of key proliferation/survival factors such as Jak2, Akt and ERK1/2.^{15,42,45} In fact, FTY720 markedly suppresses cell proliferation and induces apoptosis of CD34⁺ progenitors from TKI-sensitive and -resistant Ph⁺ leukemia patients but not of BM progenitors from healthy individuals, which already present highly active PP2A.^{15,42} Accordingly, long-term FTY720 treatment of leukemic animals extensively prolongs survival and restores normal myelopoiesis without exerting any toxic effects in hematopoietic and non-hematopoietic organs,⁴² although it reversibly reduces B and T lymphocytes.^{43,46} The ability of FTY720 to act as an anti-leukemic drug seems to depend on its ability to interfere with the activity of the PP2A inhibitor SET, which was previously described as the cause of PP2A inhibition in Ph leukemia. Interestingly, recent work from the Druker's laboratory also showed that peptides (for example, OP449) that bind SET are capable of inducing apoptosis of CML progenitors through a mechanism that depends on reactivation of PP2A.⁴⁷

We have evidence (Neviani *et al*, manuscript submitted) that BCR-ABL1 expression but not the activity in Ph⁺ quiescent HSCs is important for recruitment and activation of Jak2, which induces SET-mediated PP2A inactivation and enhances β -catenin activity. Restoration of PP2A activity by FTY720 and three FTY720 non-immunosuppressive derivatives markedly impairs *in vitro* and *in vivo* survival and self-renewal of CML but not of normal quiescent HSCs. Mechanistically, FTY720 disrupts the SET-PP2A interaction, thereby allowing PP2A activation that inhibits the BCR-ABL1-recruited Jak2 and impairs β -catenin-dependent survival of quiescent Ph⁺ HSCs through GSK3 β activation. Importantly, FTY720 and its non-immunosuppressive analogs were capable of killing mouse BCR-ABL transgenic and CML patient leukemic HSCs in serial transplantation assays with syngeneic and xenograft models of CML.²⁰

Although the discovery of the pan-BCR-ABL1 inhibitor ponatinib^{48–50} might overcome TKI resistance owing to imatinib- and dasatinib-resistant BCR-ABL mutations, the complexity of CML-BP physiopathology, together with the failure of TKI-based therapies as therapeutic agents in CML-BP and their inability to eradicate CML at the stem cell level, fully justify the ongoing CML research. Furthermore, the fast pace at which these studies are proceeding makes safe to conclude that we might be very close to the identification of the mechanism controlling TKI resistance and survival of LSCs. Importantly, it is becoming clear that the key for a successful CML eradication therapy rests on understanding the bidirectional network of signals between leukemic HSC/progenitors and BM microenvironment rather than those operating in a cell-autonomous manner. In fact, it appears that variations in oxygen levels in the BM niche might also control quiescence and TKI resistance of primitive Ph⁺ HSCs.^{51,52} The understanding of

the molecular event governing LSC behavior will not only allow the 'biological' cure of CML but, perhaps, it may also represent an amenable treatment for patients with CML-BP and other acute leukemia originating at HSC level.

CONFLICT OF INTEREST

DP received consulting fees from Pfizer, grant support from the NIH and Karyopharm Therapeutics and has a patent pending for PP2A-activating drugs for CML.

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