PROCEEDINGS ARTICLE

Stem cell persistence in chronic myeloid leukemia

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Tyrosine kinase inhibitors (TKIs) of BCR-ABL have turned chronic myeloid leukemia (CML) from a deadly disease into a chronic ailment. Unfortunately, evidence is accumulating that TKIs are not curative, since CML stem cells are not addicted to BCR-ABL, and persist despite TKI therapy. On closer view this is not surprising, as it reflects fundamental principles of CML pathogenesis. Strategies to eradicate CML stem cells will most likely be based on synthetic lethality through parallel inhibition of BCR-ABL and other critical pathways.

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Imatinib, nilotinib and dasatinib, small-molecule inhibitors of the BCR-ABL tyrosine kinase, have revolutionized the treatment of chronic myeloid leukemia (CML).¹ In 2011, the overwhelming majority of patients diagnosed and treated in the chronic phase (CML-CP) can expect long-term survival with good quality of life. In fact, life expectancy of patients with CML-CP who have maintained a complete cytogenetic response on imatinib for 2 years is identical to that of an aged-matched control.² Unfortunately, recurrence of active leukemia is common if treatment is discontinued, even in patients whose disease has decreased to very low or undetectable levels as assessed by reverse transcription-PCR. This situation is commonly referred to as disease persistence and is different from resistance, which implies loss of response or the failure to attain a meaningful response in the first place.³ Although small studies have suggested that imatinib may be safely discontinued in some patients with undetectable BCR-ABL transcripts,⁴ current recommendation is that outside of a clinical trial treatment with tyrosine kinase inhibitors (TKIs) should continue indefinitely. In view of the increasing prevalence of CML, this places a considerable strain on health-care resources.

Why are TKIs so effective at reducing disease burden in CML but so ineffective at eradicating the disease? In the following, we will try to shed some conceptual light on this question, which has captured the center of the current scientific interest in CML.

ORGANIZATIONAL PRINCIPLES IN CML HEMATOPOIESIS AND THEIR IMPACT ON TKI RESPONSE

It is generally accepted that the initial Philadelphia translocation occurs in a multipotent hematopoietic stem cell, based on the observation that BCR-ABL is detectable in all hematopoietic lineages.⁵ This notion is further supported by murine models, which demonstrate that expression of BCR-ABL does not confer self-renewal capacity to committed hematopoietic progenitor cells, suggesting that the induction of CML, at least in the CP, requires the transformation of a cell that is intrinsically able to self-renew.⁶ Thus CML-CP largely recapitulates the hierarchical structure of normal hematopoiesis. A fundamental change is thought to occur at the time of transformation to blastic phase (CML-BP), when additional mutations endow granulocyte–macrophage progenitor cells with self-renewal capacity, possibly through activation of nuclear β -catenin.⁷ An important observa-

tion from a therapeutic perspective is that the primitive hematopoietic cell compartment of CML-CP is frequently dominated by Ph-negative cells, particularly at the time of diagnosis. To reconcile this with the vast expansion of myeloid cells that characterizes the clinical disease, one must postulate that the initial BCR-ABL-driven proliferative push targets almost exclusively the myeloid progenitor cell compartment, whose ordered differentiation is largely preserved.⁸ We speculate that these key biological features of CML are reflected in clinical responses to TKIs. Thus, as the expansion of the progenitor cells is solely dependent on BCR-ABL, it comes as no surprise that inhibition of BCR-ABL leads to a rapid contraction of this cell compartment. The clinical manifestations of this are rapid hematologic and deep cytogenetic responses to imatinib and even more so to the highly potent second-line TKIs dasatinib and nilotinib. Furthermore, the relatively small numbers of CML leukemic stem cell (LSC) suggest that BCR-ABL has limited control over the size of this compartment. In this frame of thinking inhibition of BCR-ABL may have only minor effects upon the size of the LSC pool, evident as persistence of minimal residual leukemia even after years of TKI therapy. On the other hand, the relatively small number of LSCs also limits the risk of additional mutations, consistent with the durability of TKI responses in CML-CP. This effect may be further enhanced by genomic stabilization, achieved as a result of BCR-ABL inhibition.⁹ Finally, acquisition of self-renewal by progenitor cells at the time of blastic transformation undermines the hierarchical organization of CML-CP; unsurprisingly responses to TKI therapy are much less profound and not usually durable.⁷

TARGETING CML LSCS

The study of disease persistence is difficult due to the lack of specific markers for identification and enrichment of CML LIC. Most data are based on *in vitro* studies of 'primitive' lineage-negative (Lin[−]) CD34⁺38[−] CML progenitor cells isolated from newly diagnosed CML patients, a population that was previously shown to contain cells capable of long-term engraftment in immunodeficient mice.¹⁰ Unfortunately, the Lin[−]34⁺38[−] phenotype does not distinguish between normal and leukemic cells, and the majority of these cells are not stem cells. It is important to be aware of these technical limitations when interpreting data

derived from *in vitro* studies of Lin⁻34⁺38⁻ cells isolated from newly diagnosed patients. Recent reports on CML LIC-specific markers, such as interleukin-1 receptor accessory protein, are promising but will require independent confirmation.¹¹

From a therapeutic perspective, the key question is whether persistence is a BCR-ABL-dependent or a BCR-ABL-independent phenomenon. If persistence is due to incomplete inhibition of BCR-ABL, then efforts need to be directed toward optimizing TKIs to achieve target inhibition in the relevant cell population; if not, additional or alternative approaches will be needed to target residual leukemia cells. Recent reports from several groups including ours strongly suggest that primitive CML cells are not oncogene addicted and do not require BCR-ABL kinase activity for their survival.^{12,13} This suggests that eradication of all CML LSCs by BCR-ABL TKIs may be biologically impossible. Moreover, evidence is accumulating that the signals affording survival of BCR-ABL LSCs despite BCR-ABL inhibition are extrinsic, derived from their microenvironment.¹⁴

It is obvious that strategies to target these residual leukemia cells should ideally be based on a precise mechanistic understanding of disease persistence. Conceptually, one can approach this by comparing a normal hematopoietic stem cell with a CML LSC in which BCR-ABL kinase activity is blocked by a TKI.

Several scenarios are possible:

- (1) Hypomorph: A loss-of-function situation could result if chemically silenced BCR-ABL kinase exhibited a dominant-negative effect. Such cells may still survive despite BCR-ABL inhibition, but may have become dependent on another pathway that could be targeted.
- (2) Hypermorph: A gain-of-function situation could be maintained despite BCR-ABL inhibition because of preservation of kinase-independent BCR-ABL activities.¹⁵ Inhibiting these activities may allow eliminating CML LSCs.
- (3) Neutral: Inactive BCR-ABL could be neutral, producing a cell that is biologically indistinguishable from a normal cell. In this case, elimination of residual leukemia may not be possible.

In the first two scenarios, combination of TKIs with inhibitors of other pathways may generate a situation of synthetic lethality. In fact, most studies combining TKIs with inhibition of other pathways are implicitly based on this paradigm. Multiple combination partners have been assessed in preclinical studies, and some are being evaluated in clinical trials (Table 1). Major categories include developmental pathways (Wnt/ β -catenin; Hedgehog), canonical signaling pathways (PP2A, BCL6) and nonspecific mechanisms, such as autophagy and chromatin remodeling (histone deacetylase). As a comprehensive review of all these approaches is beyond the scope of this brief paper, suffice it to say that thus far none of the strategies has emerged as clearly superior to the others.

Table 1. Pathways under investigation for targeting CML stem cells

Pathway	Clinical trials	Reference
Wnt/ β -catenin	—	Zhao <i>et al.</i> ¹⁸
Hedgehog	+	Dierks <i>et al.</i> ¹⁹ Zhao <i>et al.</i> ²⁰
Alox5	+	Chen <i>et al.</i> ²¹
BCL6	—	Hurtz <i>et al.</i> ²²
TGF β	—	Naka <i>et al.</i> ²³
PP2A	—	Neviani <i>et al.</i> ²⁴
Autophagy	+	Bellodi <i>et al.</i> ²⁵
HDAC	+	Chu <i>et al.</i> ²⁶
Proteasome	—	Heaney <i>et al.</i> ²⁷

Abbreviation: HDAC, histone deacetylase.

DO WE NEED TO KILL ALL LEUKEMIC CELLS TO CURE CML?

When is a patient with CML cured from her or his disease? A dogmatic definition would be to define cure as the complete annihilation of the leukemic cell clone. Unfortunately, although clean, this definition is impractical as it cannot be verified because of the limited sensitivity of assays to detect residual disease. In addition, BCR-ABL transcripts are detectable in many healthy individuals, blurring the difference between very low levels of residual leukemia and physiological 'noise'.¹⁶ More pragmatically one could define cure as a risk of developing clinical CML that is not different from the risk of the general population, even if residual disease remains detectable. This could be accomplished if TKI-induced reduction of the leukemia clone below a certain threshold allowed for effective control of the residual population by other mechanisms, such as the immune system. This might explain why some patients have maintained PCR negativity for BCR-ABL for several years after discontinuation of imatinib.⁴ Alternatively, a certain number of CML LSCs may be required to prevent the leukemic clone from extinction by random attrition, for example, through symmetrical cell divisions that generate two differentiating daughter cells.¹⁷ Whatever the correct explanation, these data raise hopes that TKIs may be able to cure some patients with CML. Clearly, longer follow-up will be required.

CONFLICT OF INTEREST

The author declares no conflict of interest.

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