BCL6: A Novel Target for Therapy of Ph+ B Cell Acute Lymphoblastic Leukemia

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BCL6 is a zinc-finger transcriptional repressor known for its oncogenic role in B cell lymphoma. In a recent issue of *Nature*, Duy et al. describe a novel role for BCL6 at the center of a transcriptional network in Ph+ acute lymphoblastic leukemia cells that modulates their leukemogenicity and response to kinase inhibitors.

The Philadelphia chromosome-positive (Ph^+) leukemias, chronic myeloid leukemia (CML), and Ph^+ B cell acute lymphoblastic leukemia (B-ALL) are prevalent blood cancers that comprise about a quarter of all leukemias in adults. The product of the Ph chromosome, BCR-ABL1, is a dysregulated protein-tyrosine kinase that is the direct cause of both forms of leukemia, as ectopic expression of BCR-ABL1 in the relevant bone marrow target cells leads to phenotypically identical neoplasms in mice (Hu et al., 2006). In CML patients in the chronic phase of their disease, treatment with any one of the three BCR-ABL1 tyrosine kinase inhibitors (TKIs) approved by the United States Food and Drug Administration (imatinib, dasatinib, or nilotinib) is remarkably effective, with the great majority of patients achieving sustained cytogenetic remissions. Unfortunately, the outcome of TKI therapy in Ph^+ B-ALL is not nearly as favorable, with most patients relapsing quickly after transient decreases in the burden of their leukemia (Druker et al., 2001). Although TKIs are being incorporated into chemotherapy regimens for treatment of Ph^+ B-ALL, the best chance for long-term survival for such patients is to undergo allogeneic hematopoietic stem cell transplantation, a risky and costly procedure.

The mechanism of selective killing of BCR-ABL1-expressing leukemic cells by ABL1-specific TKIs is not completely understood, but upregulation of proapoptotic pathways may contribute, as leukemias induced by BCR-ABL1 in bone marrow cells from mice lacking p53 (Wendel et al., 2006) or Bim (Kuroda et al., 2006) are relatively resistant to imatinib. The reasons for relapse of Ph^+ B-ALL following TKI therapy are also murky. While some recurrences can be ascribed to amplification of the *BCR-ABL1* fusion gene or to BCR-ABL1 kinase domain mutations that render the enzyme resistant to the TKI (Gorre et al., 2001), the majority of treatment failures are blamed on the inability of the drug to eliminate the so-called leukemia stem cells, defined rigorously as clonogenic leukemic cells capable of recapitulating the disease upon transplantation. Studies in syngeneic mouse models (Hu et al., 2006) and from xenotransplantation of human B-ALL into immunodeficient mouse recipients (Castor et al., 2005) indicate that these leukemia-initiating cells have the phenotype of early B-lymphoid progenitors. Hence, there is currently great interest in identifying molecular strategies to accentuate the killing of leukemic stem cells by TKIs in patients with Ph^+ B-ALL, with the aim of eliminating residual disease and increasing the chance of cure.

In a study published in a recent issue of *Nature*, Markus Müschen and colleagues, via an elegant and sophisticated set of experiments, implicate BCL6 as a key element of a transcriptional network in BCR-ABL1-expressing B-ALL cells that controls the cell cycle and apoptotic responses to TKI treatment (Duy et al., 2011). Microarray analysis of changes in gene expression triggered by TKI treatment of B-ALL cells expressing BCR-ABL1 or several other dysregulated TKs identified *BCL6* as the most highly TKI-induced gene. Biochemical studies demonstrated that *BCL6* expression is normally inhibited in BCR-ABL1-expressing B-ALL cells through direct repression by activated STAT5 and via AKT-mediated inhibition of the *BCL6* activator FoxO4. The transcriptional targets of BCL6 in TKI-treated Ph^+ B-ALL cells, identified through ChIP on chip and comparative gene expression analyses, include p53, ARF, and the cell cycle regulators p21 and p27. While BCR-ABL1 could readily immortalize B-lymphoid progenitors from $Bcl6^{+/+}$ and $Bcl6^{-/-}$ mice in vitro, the BCR-ABL1-transformed $Bcl6^{-/-}$ B-lymphoblasts were profoundly impaired in their ability to induce fatal B-ALL in immunodeficient mice and were unable to establish leukemia in secondary transplant recipients, suggestive of a defect in the self-renewal of $Bcl6^{-/-}$ leukemic stem cells. When challenged with imatinib, $Bcl6^{-/-}$ BCR-ABL1$^+$ B-ALL cells were nearly 10-fold more sensitive than their $Bcl6^{+/+}$ counterparts and exhibited an altered cellular response to BCR-ABL1 inhibition with increased p53 expression and apoptosis, whereas $Bcl6^{+/+}$ lymphoblasts had a more restrained induction of p53 and exited the cell cycle to become quiescent. These findings were reproduced in primary human Ph^+ B-ALL samples transduced with a dominant-negative *BCL6* mutant. Together, these results suggest a model wherein tonic repression of BCL6 increases the leukemogenicity of BCR-ABL1-transformed B-lymphoid progenitors, whereas induction of BCL6 tends to counteract TKI-induced apoptosis through repression of p53 and ARF (Figure 1).

How might these new findings be translated to the clinic? In drug development, targeting transcription factors (at least those that lack natural ligands such as nuclear hormone receptors) has proven notoriously difficult. In this work, the authors turned to a novel peptidomimetic inhibitor of BCL6, RI-BPI, which binds to BCL6 and blocks its ability to recruit corepressor proteins. RI-BPI increased the sensitivity of BCR-ABL1$^+$ B-ALL cells to imatinib to a similar extent as BCL6

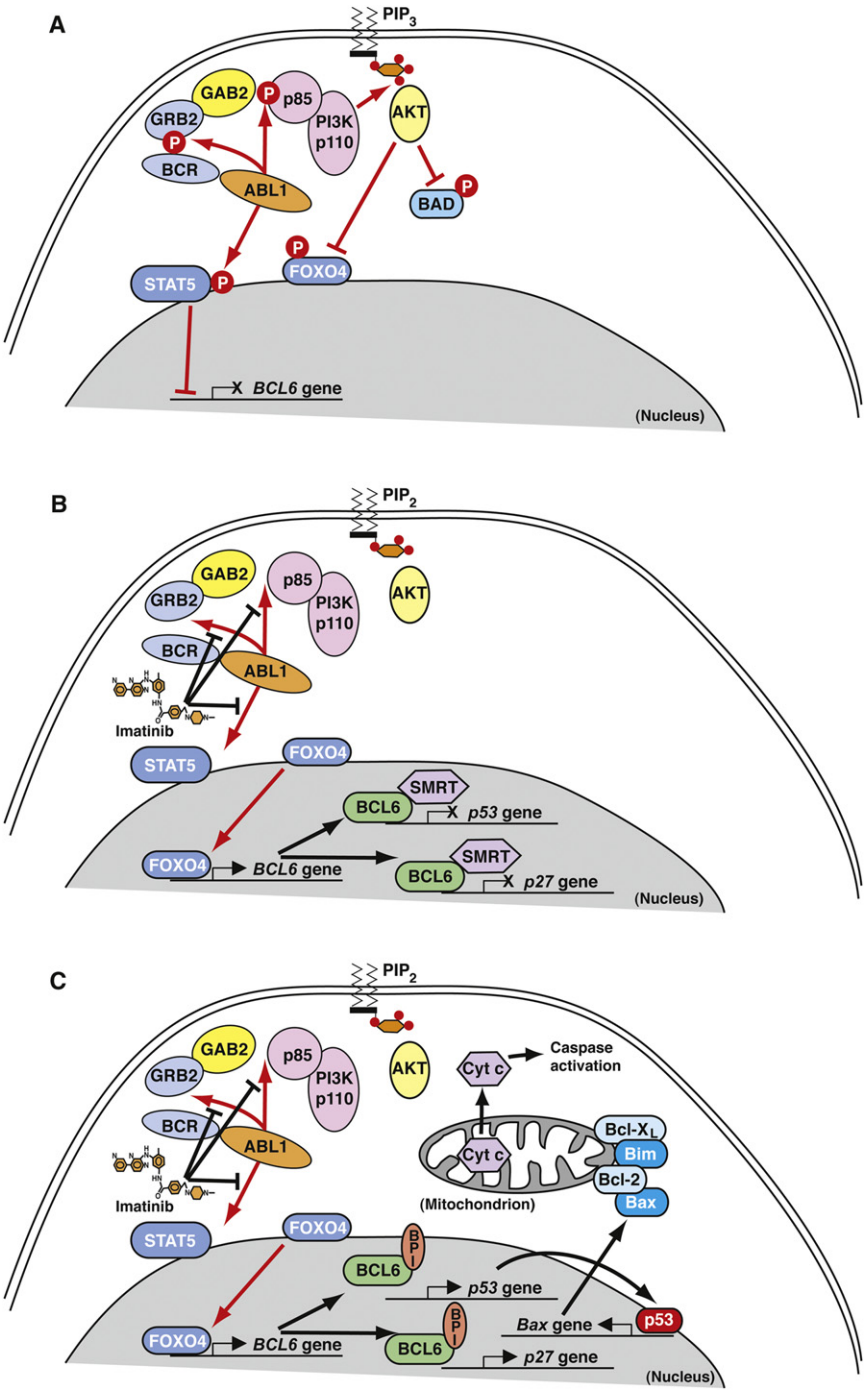

Figure 1. BCL6 Attenuates the Response to BCR-ABL1 Kinase Inhibition in Ph$^+$ B-ALL Cells
(A) In untreated Ph$^+$ lymphoid leukemia cells, *BCL6* gene expression is inhibited by direct repression via activated STAT5 and through inhibition of the *BCL6* activator FOXO4 via AKT.
(B) Upon inhibition of BCR-ABL1 kinase activity by imatinib treatment, BCR-ABL1 signaling is reversed, but induction of BCL6 expression through activated FOXO4 leads to repression of p53 and p27 genes, blunting the apoptotic response.
(C) When the BCL6 inhibitor RI-BPI is added to imatinib treatment, BCL6 target genes including *p53* are induced, accentuating the antileukemic effect of TKIs.

deficiency, an effect that was partially dependent on p53. Together with imatinib, RI-BPI effectively suppressed the emergence of resistant Ph$^+$ B-ALL in vitro, while the combination of nilotinib (a more potent analog of imatinib) and RI-BPI was well-tolerated and cured the majority of mice bearing Ph$^+$ B-ALL xenografts.

These exciting findings suggest that simultaneously targeting BCR-ABL1 and BCL6 is a promising strategy for preventing TKI resistance and relapse in patients with Ph$^+$ B-ALL (Figure 1). Several aspects of the study will require clarification. The experiments do not clearly distinguish between the effects of BCL6 loss or inhibition on the bulk of BCR-ABL1$^+$ B-ALL cells versus the more primitive and refractory leukemic stem cells. Moreover, loss of ARF is a frequent event in the progression of BCR-ABL1-transformed B-lymphoid progenitors (Williams et al., 2006) and in human Ph$^+$ B-ALL; it is unclear how this might affect the response to RI-BPI. The role of BCL6 at the interface between BCR-ABL1 signaling and response to TKIs argues that the use of other signal transduction inhibitor drugs in Ph$^+$ leukemia should be approached with caution, as inhibitors of the phosphatidylinositol 3-kinase/AKT pathway might induce BCL6 and diminish the effectiveness of TKI therapy. Finally, the development of clinical TKI resistance based on acquired ABL1 kinase domain mutations, particularly the troublesome gatekeeper mutation T315I, would be expected to thwart the efficacy of dual nilotinib/RI-BPI treatment. In this regard, testing RI-BPI in combination with third-generation BCR-ABL1 TKIs capable of inhibiting the T315I mutant (Chan et al., 2011; O'Hare et al., 2009) is warranted.

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Comprehensive Identification of Somatic Mutations in Chronic Lymphocytic Leukemia

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Massively parallel sequencing enables the sequencing of whole genomes, exomes, and transcriptomes from many tumor samples. Thus, it now is possible to comprehensively identify somatic mutations, including single base changes, deletions, insertions, and genomic rearrangements. Early results for hematopoietic tumors show great promise, but many questions remain to be answered.

Improved methods for sequencing the human genome at an increasingly lower cost are now being applied to all kinds of tumors (Lander, 2011). A recent paper in *Nature* (Puente et al., 2011) offers a glimpse of the power of integrating genomic technologies, including global sequence analysis, in B cell chronic lymphocytic leukemia (CLL). Genomic analysis started from an in-depth analysis of four patients representing the two major molecular subtypes of CLL: two with and two without somatic hypermutation of the immunoglobulin heavy chain variable region (IGHV). Paired advanced tumor and normal blood cells isolated before and after treatment, respectively, were studied using multiple independent technologies: whole genome sequencing (WGS); mate pair sequencing of 2.5 kb DNA fragments for efficient detection of DNA rearrangements; and chip analyses for single nucleotide polymorphisms (SNPs), DNA copy number, and RNA expression. This allowed the authors to determine that the sequencing identified 99.4% of the heterozygous SNPs. Importantly, for a subset of the putative somatic mutations, they were able to validate 96% by Sanger sequencing. They found approximately 1000 somatic substitutions per CLL genome. The pattern of base changes and dinucleotide context differed for the IGHV-mutated and IGHV-unmutated tumors. The authors suggested that the higher frequency of A > T and C > G transversions in the IGHV-mutated cases was consistent with their introduction by the error prone-polymerase η during somatic hypermutation in immunoglobulin genes. Altogether, they identified changes in the protein-coding region of 45 genes in the four tumors, including 41 nonsynonymous single base substitutions and 5 insertions/deletions (indels). Typically, CLL have only a limited number of genomic rearrangements. The comprehensive mate pair analyses enabled the authors to identify and characterize ten large genomic alterations, six of which—including large 13q14 deletions in three tumors—have been reported previously in CLL.

Focusing on the 26 mutated, expressed genes, Puente et al. (2011) then extended these findings to a cohort of 169 CLL patients. Using a clever pooled-sequencing strategy, they determined that other CLL tumors had mutations, suggesting identification of four driver genes: *NOTCH1,* 12.2%; *MYD88,* 2.9%; XPO, 2.4%; and *KLHL6*, 1.8%. The nonsynonymous to synonymous mutation ratio in the remaining 22 expressed genes and the 19 unexpressed genes were 2.83 and 2.71, respectively, consistent with the lack of selection expected if most are passenger genes (Chapman et al., 2011).

Of the NOTCH1 mutations, which occurred in 20% of IGHV-unmutated and 7% of IGHV-mutated CLL, 29/31 were the P2515Rfs*4. This mutation previously had been identified in lymphoid malignancies, including T cell acute lymphoblastic leukemia and B-CLL. The P2515Rfs*4 mutation and the other two NOTCH 1 mutations all generate premature stop codons predicted to result in truncated proteins lacking the destabilizing PEST domain. The authors confirmed that leukemias carrying the *NOTCH1 P2515Rfs*4* mutation expressed higher levels of truncated NOTCH1, together with higher levels of NOTCH1 target genes. In addition, the NOTCH1 mutation is correlated with a more advanced clinical stage at diagnosis, a shorter survival, and an increased frequency of transformation into diffuse large B cell lymphoma (DLBCL). All of the MYD88 mutations,