Aberrant Cytokine Signaling in Leukemia

Richard A. Van Etten
Molecular Oncology Research Institute and Division of Hematology/Oncology, Tufts-New England Medical Center, Boston, MA 02111, USA, Tel 617-636-6449, Fax 617-636-5935
Richard A. Van Etten: rvanetten@tufts-nemc.org

Abstract
Abnormalities of cytokine and growth factor signaling pathways are characteristic of all forms of leukemia: lymphoid and myeloid, acute and chronic. In normal hematopoietic cells, cytokines provide the stimulus for proliferation, survival, self-renewal, differentiation, and functional activation. In leukemic cells, these pathways are usurped to subserve critical parts of the malignant program. In this review, our current knowledge of leukemic cell cytokine signaling will be summarized, and some speculations on the significance and implications of these insights will be advanced. A better understanding of aberrant cytokine signaling in leukemia should provide additional targets for the rational therapy of these diseases.

Keywords
Tyrosine kinase; kinase inhibitor; leukemia stem cell

Introduction
Leukemia is a clonal malignancy of lymphoid or myeloid cells that can be further subdivided into acute and chronic forms. The chronic leukemias and myeloproliferative diseases (MPDs) are characterized by overproduction of relatively mature differentiated cells, for example neutrophils in chronic myeloid leukemia (CML), or B-lymphocytes in chronic lymphocytic leukemia (CLL) (Van Etten & Shannon, 2004). By contrast, in the acute lymphoid and myeloid leukemias there is a profound block in hematopoietic differentiation, with overproduction of immature blasts. Leukemic cells, like other cancers, must acquire a set of aberrant properties during their genesis (Hanahan & Weinberg, 2000). Among these are the ability to survive and proliferate, to overcome negative growth signals, and to spread to different tissues within and outside the hematolymphoid system. In addition, it is now recognized that leukemias (and perhaps all cancers) are organized as a hierarchy, and that a small less differentiated population of leukemia-initiating cells or leukemia “stem” cells with the aberrant capacity to self-renew is responsible for maintenance of the disease (Wang & Dick, 2005). In normal hematopoiesis, cytokine and growth factors are essential for these various functions, and act by binding to their cell-surface receptors and triggering complex cascades of intracellular signaling (see sections on “Cytokine Receptors and Hematopoietic Development” and “Hematopoietic Cytokine Receptor Signaling” in this issue). The acute lymphoid and myeloid leukemias require additional mutations to block the process of differentiation (Gilliland & Tallman, 2002),
and, in many instances, this is a consequence of aberrant or mutant transcription factor expression (see sections on “Hox Genes in Hematopoiesis and Leukemogenesis”, and on “Myeloid Transformation” and “Lymphoid Transformation”, in this issue). This article will focus on how aberrant cytokine and growth factor signaling contributes to the pathogenesis of the leukemias, with emphasis on possible therapeutic implications.

**Mechanisms of cytokine and growth factor receptor tyrosine kinase activation**

Several cytokine and growth factor receptors involved in human leukemia have intracellular domains with tyrosine kinase (TK) catalytic subunits and multiple tyrosyl phosphorylation sites. These include c-FMS (the receptor for CSF-1/macrophage colony-stimulating factor), Fms-like tyrosine kinase 3 (FLT3), c-KIT, platelet-derived growth factor receptors (PDGFR) α and β, anaplastic lymphoma kinase (ALK), neurotrophin-3 receptor kinase (NTRK3), and fibroblast growth factor receptors (FGFR)-1 and -3. In addition to receptor TKs, there are several non-receptor TKs that have been implicated in the pathogenesis of leukemia. The most famous and intensively studied of these is c-ABL, which is activated by several growth factors (Pendergast, 2002) and in the DNA damage response (Van Etten, 1999). Receptors for other cytokines (such as the interleukins, interferons, and many colony-stimulating factors) lack intrinsic TK activity, and downstream signaling is dependent on recruitment and activation of a non-receptor TK of the Janus kinase (JAK) family. Immunoreceptor-associated TKs of the Syk/ZAP-70 family are also involved in distinct forms of human leukemia.

Dysregulated TK signaling in leukemic cells can result from several mechanisms. In normal cells in the absence of ligand, cytokine receptor TKs are unphosphorylated, monomeric, and the conformation of their kinase domains is inactive. In some cytokine receptor TKs, the cytoplasmic juxtamembrane region contributes to inhibition by direct interaction with the kinase domain (Griffith et al., 2004). Binding of ligand to the extracellular domain results in receptor oligomerization, disruption of the autoinhibitory juxtamembrane interaction, and intermolecular autophosphorylation, which increases the catalytic activity of the enzyme and generates binding sites for signaling proteins, recruiting them to the membrane and activating multiple signaling pathways (Schlessinger, 2000). Non-receptor TKs are maintained in an inactive state by cellular inhibitor proteins and lipids and through intramolecular autoinhibition (Van Etten, 2003), and become active during signaling through dissociation of inhibitors, recruitment to transmembrane receptors, and trans-phosphorylation by other kinases. In leukemia, a common mechanism of TK dysregulation is the fusion of a receptor or non-receptor TK with a partner protein, usually resulting from a balanced chromosomal translocation. Many of the partner proteins contain a domain that mediates constitutive oligomerization of the TK in the absence of cytokine binding or physiologic activating signals, thereby promoting autophosphorylation and activation. With some receptor TKs, lack of the juxtamembrane inhibitory domain in the fusion protein contributes to activation. A second major cause of TK deregulation is mutations that disrupt autoregulation of the kinase. A final mechanism of cytokine receptor TK deregulation is increased expression of the receptor TK, its cytokine ligand, or both.
Dysregulated receptor and non-receptor TKs in human leukemia

Cytokine receptor TKs and associated non-receptor TKs are dysregulated via these different mechanisms in a wide variety of specific leukemias (Table 1). BCR-ABL is non-receptor fusion TK that is the product of the Philadelphia chromosome translocation in CML, where a tetramerization domain in BCR overcomes ABL autoinhibition by oligomerization and autophosphorylation (Smith et al., 2003). ABL is also activated by fusion to NUP214 in about 6% of T-cell acute lymphoblastic leukemias (T-ALL) (Graux et al., 2004), to EML1 in infrequent cases of T-ALL (De Keersmaecker et al., 2005), and to the transcription factor ETV6/TEL in rare cases of atypical CML and acute leukemia (Golub et al., 1996). In TEL-ABL, an oligomerization domain in TEL is required for leukemogenesis in a mouse model (Million et al., 2002). PDGFRα is activated by cryptic interstitial chromosome 4 deletions that generate a FIP1L1-PDGFRα fusion TK in some patients with chronic eosinophilic leukemia (Cools et al., 2003) or systemic mastocytosis (Pardanani et al., 2003). FIP1L1 appears to activate PDGFRα by disruption of the juxtamembrane regulatory motif rather than through oligomerization (Stover et al., 2006). In rare CEL variants, PDGFRα is fused to other partners including BCR (Baxter et al., 2002) and CDK5RAP2 (Walz et al., 2006). The closely related receptor PDGFRβ is dysregulated in some patients with proliferative variants of chronic myelomonocytic leukemia (CMML) via balanced chromosomal translocations that lead to fusion with one of many different partner proteins (Jones & Cross, 2004), including TEL (Golub et al., 1994). c-KIT is activated by point mutations, most commonly involving D816 in the activation loop of the KIT kinase domain, in many cases of systemic mastocytosis or mast cell leukemia, and less frequently in AML. In addition, the normal c-KIT receptor is expressed on most AML blasts and may be overexpressed and activated in some patients. FLT3 expressed on blasts in most cases of AML, and is dysregulated by internal tandem duplications within the juxtamembrane domain (Nakao et al., 1996) in 25–30% of patients and by point mutations at position D835 within the kinase domain in another 5–7% of patients (Yamamoto et al., 2001). In some AML patients with overexpression of unmutated FLT3, there is evidence that an autocrine FLT3 loop promotes the survival and growth of the AML blasts in vitro (Estey et al., 2003; Zheng et al., 2004). In the 8p11 myeloproliferative syndrome (EMS), characterized by MPD and non-Hodgkin’s lymphoma where both malignancies share chromosomal translocations involving 8p11, FGFR1 is fused with one of several partners (Macdonald et al., 2002), including the transcription factor ZNF198 (Xiao et al., 1998). A related receptor TK, FGFR3, is mutated and overexpressed in multiple myeloma with t(4;14) (Chesi et al., 1997). Translocations involving the ALK gene are pathognomonic of anaplastic large cell lymphoma and generate fusions of the ALK, an orphan receptor TK normally expressed in neuronal cells, with several partners (Pulford et al., 2004). Fusions of the non-receptor TK JAK2 with TEL or BCR have been found in cases of acute leukemia and atypical CML (Lacroix et al., 1997). Finally, an activating point mutation (V617F) in the pseudokinase domain of JAK2 is found in the majority of polycythemia vera patients and some cases of essential thrombocytosis and idiopathic myelofibrosis (Baxter et al., 2005; James et al., 2005). The role of JAK kinases in leukemia will be discussed in greater detail below, in the section on JAK-STAT signaling.
Pathogenic and therapeutic implications of dysregulated TK signaling in leukemia

For many of these dysregulated cytokine receptor and non-receptor TKs, there is persuasive evidence from mouse models that expression of the specific TK in the hematopoietic system can recapitulate most of the important clinical features of the human leukemia (Table 1), directly implicating these TKs in the pathogenesis of the disease. These model systems include retroviral transduction of the relevant TK gene into hematopoietic progenitors followed by transplantation, or the generation of transgenic mice (Van Etten, 2001). For dysregulated TKs found in the MPDs, the cardinal features of the corresponding disease are often induced rapidly and in polyclonal fashion using retroviral transduction, suggesting that the TK is both necessary and sufficient to cause the disease. Examples include CML-like disease induced by BCR-ABL (Daley et al., 1990), EMS-like MPD and lymphoma induced by ZNF198-FGFR1 (Chen et al., 2004; Roumiantsev et al., 2004), and polycythemia induced by JAK2 V617F (James et al., 2005; Zaleskas et al., 2006). For dysregulated TKs found in acute leukemias, such as mutant FLT3, expression of the kinase alone typically generates an MPD-like syndrome in mice (Kelly et al., 2002b), whereas induction of an acute leukemia phenotype requires co-expression of a mutant transcription factor such as PML-RAR (Kelly et al., 2002a). This confirms the hypothesis that multiple genetic lesions are required for the pathogenesis of acute leukemia (Gilliland & Tallman, 2002). As a corollary, this also suggests that dysregulated TK signaling acts predominantly as a cytokine surrogate in leukemogenesis, providing proliferation and survival functions, without a predominant effect on differentiation.

The primary role of these dysregulated TKs in leukemogenesis predicts that drugs targeting the catalytic activity of these enzymes should have therapeutic efficacy in leukemia (Deininger et al., 2005), and this has been confirmed in spectacular fashion in a subset of these leukemias, most notably in CML. Imatinib mesylate (Gleevec®), a 2-phenylaminopyrimidine compound that is a specific inhibitor of the ABL, ARG, c-KIT, and PDGFR TKs, induces complete hematologic and cytogenetic remissions in most patients with chronic phase CML (O’Brien et al., 2003) but is less effective in the accelerated and blast crisis phases of the disease (Druker et al., 2001). As predicted by the spectrum of its TK inhibitory activity, imatinib also induces dramatic clinical and molecular responses in CMML patients with dysregulated PDGFRβ (Apperley et al., 2002) and in CEL patients with PDGFRα fusions (Klion et al., 2003). The non-receptor SH2-containing TK Syk is fused to TEL in some patients with myelodysplastic syndrome, and small molecule Syk kinase inhibitors impair proliferation and survival of some precursor B-lymphoid leukemias and lymphomas (Gururajan et al., 2007; Wossning et al., 2006). Several inhibitors of FLT3 kinase activity in clinical development (Wadleigh et al., 2005) can inhibit growth and induce apoptosis in hematopoietic cell lines expressing activated FLT3, and have therapeutic efficacy in murine models of FLT3-induced leukemia (Weisberg et al., 2002). In phase II clinical trials, the drugs are well-tolerated and reduce circulating and bone marrow blasts in 20–50% of patients with relapsed or refractory AML (Fiedler et al., 2003; Smith et al., 2004; Stone et al., 2005). Whether FLT3 inhibitors will benefit AML patients whose blasts overexpress the normal FLT3 receptor is unclear.
The outstanding response of chronic phase CML and other MPDs to kinase inhibitor therapy suggest that the deregulated TK is the sole or predominant genetic abnormality in the malignant cells, which is supported by the mouse model studies cited above. In contrast, the multiple genetic abnormalities found in AML may explain the less profound activity of FLT3 inhibitors in AML. In some AML patients, FLT3 mutations appear to be acquired late in the course of the disease, and therefore may not be central to the pathogenesis of the leukemia (Kottaridis et al., 2002). The extreme sensitivity of some leukemias to TK inhibition may reflect their absolute dependence on the targeted TK signaling pathway for survival. This phenomenon has been termed “oncogene addiction” (Weinstein, 2002), and is best illustrated by the fact that imatinib suppresses Ph+ bone marrow myeloid colony formation without affecting colonies from normal progenitors (Druker et al., 1996).

Cytokine receptors signal primarily through associated TK activity, resulting in tyrosyl phosphorylation of multiple substrates. One category of substrate is specific tyrosine residues on the receptor itself or on scaffolding-adapter proteins such as Gab2, which provide binding sites for SH2-containing signaling proteins. These signaling proteins link to a wide array of downstream signaling pathways, including the signal transducer and activator of transcription (STAT) pathway, the Ras/Raf/MEK/mitogen-activated protein kinase (MAPK) pathway, and the phosphatidylinositol 3-kinase (PI 3-kinase)/Akt/mammalian target of rapamycin (mTOR) pathway. The importance of understanding these downstream pathways is that they may offer additional rational targets for therapy of leukemia. It is important to realize that phenotypically similar leukemias may rely on different signaling pathways for their pathogenesis (Van Etten, 2004), which is of obvious therapeutic relevance. Furthermore, tyrosine kinase inhibitor therapy is limited by the development of resistance, which is due in large part to mutations in the target TK that render the kinase insensitive to the drug. By analogy to HIV therapy, simultaneous treatment with drugs targeting the relevant TK and critical downstream signaling pathways may overcome or prevent resistance to kinase inhibitor therapy in leukemia (Van Etten, 2004). The roles of these various downstream pathways in leukemogenesis will be discussed in more detail below.

**JAK-STAT signaling in leukemia**

Members of the Janus kinase (JAK) family of non-receptor TKs are constitutively associated with cytokine receptors, including receptors for interferons, interleukins 2 through 7, and the hematopoietic growth factors GM-CSF, G-CSF, erythropoietin (Epo), and thrombopoietin (Tpo). Upon cytokine binding and receptor dimerization, JAK kinases cross-phosphorylate one another, become activated, and subsequently phosphorylate multiple substrates. A major substrate of the JAKs is the STAT family of transcription factors, which form homo- and heterodimers through their SH2 domains following phosphorylation, translocate to the nucleus, and activate transcription of specific target genes.

JAK2 is required for signaling from the Epo receptor and other type I cytokine receptors (Parganas et al., 1998). In 2005, a somatic mutation in the JAK2 tyrosine kinase was identified in many non-CML MPD patients. Studies of erythroid progenitors from polycythemia vera (PV) patients demonstrated that Epo-independent erythroid maturation
was impaired by a JAK2 inhibitor (Ugo et al., 2004) and by siRNA knockdown of JAK2 (James et al., 2005). This prompted sequencing of the JAK2 gene, which identified a G to A point mutation, resulting in substitution of phenylalanine for valine at amino acid 617 (V617F), in the JAK2 pseudokinase domain in the majority of PV patients (James et al., 2005). The same mutation was independently identified through sequencing of TK genes in MPD patients (Baxter et al., 2005; Levine et al., 2005), and by investigation of loss of heterozygosity involving the JAK2 gene on chromosome 9p (Kralovics et al., 2005). The JAK2 V617F mutation is found in nearly every patient with PV and is present in homozygous form through mitotic recombination in up to 30% of patients. The mutation is also found in 40–60% of patients with essential thrombocythemia (ET) and chronic idiopathic myelofibrosis (CIMF), but is rarely found outside MPD (Jones et al., 2005; Scott et al., 2005; Steensma et al., 2005). Recently, mutations in exon 12 of JAK2, located just proximal to the pseudokinase domain, were described in patients with PV and idiopathic erythrocytosis who lacked the V617F mutation (Scott et al., 2007). Together, this suggests that mutant JAK2 is found in virtually every patient with PV.

The JAK2 V617F mutant has constitutive kinase activity in vivo in the absence of Epo stimulation, and can transform cytokine dependent cell lines that express a type I cytokine receptor (Epo receptor, G-CSF receptor, or MPL, the receptor for Tpo) to become independent of cytokine for survival and growth (James et al., 2005; Lu et al., 2005). The requirement for cytokine receptor expression suggests that mutant JAK2 still requires association with a receptor in order to signal. In the retroviral transduction mouse model, JAK2 V617F reproduces the entire clinical spectrum of PV, including splenomegaly, moderate leukocytosis, polycythemia due to overproduction of erythrocytes, increased red cell mass, low plasma Epo levels, and presence of Epo-independent erythroid progenitors in marrow and spleen (Lacout et al., 2006; Wernig et al., 2006; Zaleskas et al., 2006). This suggests that mutant JAK2 is the direct cause of PV, and the erythrocytosis and reticulocytosis responded to a small molecule JAK2 inhibitor, confirming that JAK2 is a target for therapy of PV (Zaleskas et al., 2006).

There was no effect of JAK2 V617F on platelet number in mice (Lacout et al., 2006; Wernig et al., 2006; Zaleskas et al., 2006), despite a profound abnormality of platelet function, manifested as prolonged tail bleeding time (Zaleskas et al., 2006). This suggests that the mutant JAK2 is insufficient to cause thrombocytosis, and that additional mutations may be required for an ET phenotype. Several lines of evidence also suggest that one or more mutations may precede JAK2 V617F in the non-CML MPDs, such as clonal hematopoiesis in these patients that is not explained by the allele frequency of a JAK2 mutation (Kralovics et al., 2006). Sequencing of candidate signaling molecules (type I cytokine receptors, other JAKs, STATs) in MPD patients lacking JAK2 V617F revealed activating mutations at position W515 in MPL in a small subset of CIMF (5%) and ET (1%) patients (Pardanani et al., 2006; Pikman et al., 2006). The mutant MPL receptor induced MPD, thrombocytosis, and myelofibrosis in the retroviral transduction mouse model, confirming that it is likely the direct cause of ET or CIMF in those patients who express this mutant. Further studies are necessary to define the pathogenesis of those ET and CIMF cases that lack JAK2 or MPL mutations.
Dysregulated JAK-STAT signaling is also found in CML. Activation of STAT5 in BCR-ABL-expressing cell lines and primary leukemia cells was reported a decade ago (Carlesso et al., 1996; Ilaria & Van Etten, 1996), but the role of STAT5 in the pathogenesis of CML has been controversial. BCR-ABL may activate STAT5 through direct phosphorylation, or the activation could be indirect, via phosphorylation by JAK2 (Samanta et al., 2006) or by Src family kinases (Nieborowska-Skorska et al., 1999), both of which are activated by BCR-ABL. In the mouse retroviral transduction model of CML, initial studies using donor mice with targeted mutations in Stat5a and Stat5b suggested that STAT5 was not absolutely required for induction of CML-like leukemia by BCR-ABL (Sexl et al., 2000). However, the Stat5a/b mutations employed in these studies were hypomorphic rather than true null alleles (Bunting et al., 2002). In more recent studies, induction of murine CML-like MPD was attenuated in donor hematopoietic cells with a single null mutation in Stat5a (Ye et al., 2006), while fetal liver hematopoietic progenitors from mice with the entire Stat5ab locus deleted (Cui et al., 2004) were incapable of generating leukemia in recipient mice following retroviral transduction with BCR-ABL (Hoelbl et al., 2006). Together, these studies suggest that STAT5 signaling does indeed contribute to BCR-ABL leukemogenesis, consistent with a recent report that siRNA against STAT5 in human CML patient samples impaired Ph+ myeloid colony formation (Scherr et al., 2006).

Finally, there is considerable evidence of activation JAK-STAT signaling in the acute leukemias. Recent studies have identified activating mutations in JAK3 in several acute megakaryocytic leukemia cell lines and patient samples (Walters et al., 2006; see section on Erythroid and Megakaryocytic Transformation). Activated STAT proteins are also found in blasts from patients with AML and ALL (Gouilleux-Gruart et al., 1996; Weber-Nordt et al., 1996; Xia et al., 1998). The spectrum of STAT activation varies, but activation of STAT3 and STAT5 is particularly common in AML (Benekli et al., 2002; Steensma et al., 2006). In some cases, a dysregulated TK such as mutant FLT3 is responsible for STAT phosphorylation, but in other cases, the identity of the kinase responsible for STAT activation is not clear. In contrast to the situation in the MPDs, whether STAT activation contributes to pathogenesis of acute leukemia has generally not been defined, but as additional specific inhibitors of potential STAT kinases (FLT3, JAKs, Src kinases) become available, this question can be addressed pharmacologically.

**Ras/Raf/MEK/MAPK signaling in leukemia**

The Ras-MAPK signaling pathway is activated by a diverse range of cytokines, growth factors, and activated TKs. In the leukemias, activation of this pathway is commonly mediated by dysregulated TK signaling, coupling to the adapter protein growth factor receptor bound-2 (Grb2), which in turn binds activators of Ras, including the guanine nucleotide exchange factor son-of-sevenless (Sos). In some leukemias, Ras isoforms (most commonly N- or K-Ras) are constitutively activated by mutations at codon 12 or 61, leading to impaired GTPase activity and prolonged signaling (Shannon, 1995). By contrast, activating mutations in the downstream cascade of serine-threonine kinases (Raf/MEK/MAPK) are uncommon in leukemia (Lee et al., 2004). Biochemical evidence of activation of the Ras-MAPK pathway is frequently present in both the MPDs and in the acute leukemias, but the pathogenic and therapeutic significance of this signaling pathway in
leukemogenesis is less clear. The activity of specific inhibitors of MEK against different leukemia patient samples and cell lines suggests that the MAPK pathway alone does not, in general, contribute substantially to leukemic cell survival or proliferation (Lunghi et al., 2003), but simultaneous targeting of MEK/MAPK and other pathways (such as Bcl2 or a TK) may have synergistic effects (Milella et al., 2002; Wu et al., 2004; Yu et al., 2002). Inhibiting cell cycle kinases (such as Cdk4 or Cdk6) that are downstream of the MAPK pathway may be a more productive therapeutic approach in AML (Wang et al., 2007).

In contrast to acute leukemia, there is a myeloproliferative-like syndrome, juvenile myelomonocytic leukemia (JMML), where Ras-MAPK signaling plays a central pathogenetic role (Van Etten & Shannon, 2004). JMML is a rare fatal myeloid disorder of infancy characterized by overproduction of maturing myelomonocytic cells. A hint to the pathogenesis of JMML came from the observation that the disorder is frequent in children with neurofibromatosis or Noonan syndrome (characterized by cardiopulmonary and truncal defects). The NFI tumor suppressor gene encodes a GTPase activating protein that negatively regulates Ras signaling, and the normal NFI allele is frequently deleted in JMML cells from children with neurofibromatosis-1 (Side et al., 1997). Germline missense mutations in PTPN11, which encodes the SHP-2 tyrosine phosphatase, are found in about half of Noonan syndrome patients and nearly all of those with JMML, while somatic PTPN11 mutations are detected in about 35% of sporadic JMML (Tartaglia et al., 2001). These mutations dysregulate SHP-2 phosphatase activity by destabilizing an auto-inhibitory interaction, and increase signaling to Ras and other downstream effectors (Tartaglia et al., 2004). Overall, about 85% of JMML patient samples have mutations in KRAS2, NRAS, NFI, or PTPN11 that are largely mutually exclusive of one another, arguing strongly that hyperactive Ras is central to the pathogenesis of this disease. This hypothesis is further supported by the fact that mice with haploinsufficiency for NFI, or gain-of-function mutations in Kras or Ptpn11, all develop myeloid disorders resembling JMML or CMML (Araki et al., 2004; Braun et al., 2004; Chan et al., 2004; Le et al., 2004). The central role of Ras in JMML would predict that Ras inhibitors, such as farnesyltransferase inhibitors (FTI), might have therapeutic efficacy in this disease (Emanuel et al., 2000). However, the clinical responses of JMML patients to FTIs have been somewhat disappointing, possibly due to alternative prenylation pathways for Ras.

**PI 3-kinase/PTEN/Akt/mTOR signaling in leukemia**

The other major pathway downstream of cytokine receptors is the PI3 kinase signaling pathway. Type IA PI 3-kinase is a heterodimeric enzyme with a regulatory 85 kDa regulatory subunit containing an SH2 domain, and a 110 kDa catalytic subunit. Activation of PI 3-kinase by binding of the p85 subunit to a tyrosyl phosphorylated ligand stimulates the lipid kinase activity of the p110 subunit, resulting in phosphorylation of the membrane lipid phosphatidylinositol (4, 5)-bisphosphate at the 3’ position to generate phosphatidylinositol (3, 4, 5)-trisphosphate (PIP3). PI 3-kinase is antagonized by several lipid phosphatases that dephosphorylate phosphoinositides, including SHIP and PTEN. PIP3 serves as a membrane binding site for proteins containing plextrin homology domains, including Akt/protein kinase B. Akt is activated through PIP3 binding and by phosphorylation by phosphoinositide-dependent kinase-1 (PDK1). Subsequently, PDK1 and Akt phosphorylate
a wide variety of substrates, including the proapoptotic protein BAD, the Cdk inhibitor p27, Forkhead transcription factors, glycogen synthase kinase-3, mTOR, and the p70 and p90 ribosomal subunit kinases. These substrates in turn mediate a wide variety of cellular responses to cytokines, including proliferation, resistance to apoptosis, and altered metabolism.

In leukemia cells, activation of the PI 3-kinase/Akt pathway is widespread, based on biochemical measurements (Martelli et al., 2006). Studies with PI 3-kinase inhibitors suggest that this pathway is involved in mediating survival responses in AML (Sujobert et al., 2005; Xu et al., 2003), possibly through phosphorylation of BAD (Zhao et al., 2004) and other substrates (Grandage et al., 2005). The downstream mTOR pathway has also emerged as a promising therapeutic target in leukemia (Giles & Albitar, 2005; Xu et al., 2005), and several mTOR inhibitors, including rapamycin/sirolimus and RAD001/everolimus, are in clinical trials for leukemia. The PI 3-kinase pathway is activated by dysregulated TKs and by Ras, but the mode of PI 3-kinase activation in leukemias not associated with mutant TKs or Ras is not clear.

In CML cells, the mechanism of activation of the PI 3-kinase pathway is particularly instructive. BCR-ABL contains a phosphotyrosine residue, pTyr177, that forms a direct binding site for the SH2 domain of Grb2 (Pendergast et al., 1993; Puil et al., 1994). It was initially presumed that this allowed coupling of BCR-ABL signaling to the Ras pathway through Sos, but it is now apparent that a different Grb2 effector protein is primarily involved. Gab2 is a complex scaffolding/adapter protein that is highly tyrosyl-phosphorylated in CML cells and serves as a binding protein and activator of the p85 subunit of PI 3-kinase and of SHP2 (Gu et al., 1998). Gab2 is bound by Grb2 and recruited to BCR-ABL via pTyr177, and this is required for maximal tyrosyl phosphorylation of Gab2 by BCR-ABL. A BCR-ABL Y177F mutant is profoundly defective for induction of CML-like MPD in the retroviral mouse model system (Million & Van Etten, 2000; Zhang et al., 2001), and cell lines and primary bone marrow progenitors expressing BCR-ABL Y177F had decreased phosphorylation of Gab2 and decreased activation of PI 3-kinase, Akt, and ERK (Sattler et al., 2002). BCR-ABL-induced PI 3-kinase/Akt and Erk/MAPK activation were also impaired in primary myeloid and lymphoid cells from mice with a homozygous null mutation in the Gab2 gene. Bone marrow myeloid progenitors from Gab2−/− mice were absolutely resistant to transformation by BCR-ABL, while lymphoid transformation was diminished as a consequence of markedly increased apoptosis (Sattler et al., 2002). These results identify Gab2 as a critical determinant of transformation and leukemogenesis by BCR-ABL, through signaling to the PI 3-kinase and SHP2 pathways. Mice lacking Gab2 have impaired mast cell allergic responses but normal hematopoiesis (Gu et al., 2001), suggesting that the Gab2 pathway might be an excellent target for leukemia-selective therapy.

**Leukemia stem cell signaling pathways**

Leukemia stem cells (LSC) are defined functionally by their ability to transfer the malignancy by xenotransplantation into immunodeficient mice. In AML, only leukemic blasts with the immature cell surface phenotype CD34+CD38−, comprising a minority of the
total population, are capable of transferring leukemia to SCID mice (Lapidot et al., 1994). LSC have also been defined in acute lymphoid leukemia (Castor et al., 2005; Cobaleda et al., 2000). LSC are postulated to be responsible for the relapse of disease following a remission induced by cytotoxic or targeted therapy. As a corollary, eradication of LSC may be necessary for the permanent cure of leukemia.

Self-renewal, a central feature of both normal HSC and LSC, is defined by the ability to reconstitute normal hematopoiesis or leukemia, respectively, following serial transplantation. The signaling pathways controlling self-renewal of normal and leukemic stem cells have been defined primarily by studies in mice. Overexpression of the Hox genes *HoxB4* or *HoxA9* in bone marrow causes expansion of HSCs in vitro and in vivo (Antonchuk et al., 2002; Thorsteinsdottir et al., 2002) (see the section on “Hox Genes in Hematopoiesis and Leukemogenesis”). Treatment of HSC with hedgehog ligands induces their proliferation and expansion through a pathway involving bone morphogenetic protein-4 (Bhardwaj et al., 2001; Trowbridge et al., 2006). Wnt ligands bind to their cell-surface receptors (frizzled), leading to stabilization of β-catenin, which translocates to the nucleus and activates specific gene transcription. Overexpression of a stabilized β-catenin mutant in HSCs induces their expansion, while expression of the β-catenin antagonist axin impairs HSC proliferation and repopulating function (Reya et al., 2003). Expression of the Notch receptor ligand Jagged-1 in stromal cells triggers proteolysis and membrane release of the intracellular domain of Notch (ICN) in hematopoietic cells, which then translocates to the nucleus and activates gene transcription in concert with several co-factors, leading to expansion of self-renewing HSC (Karanu et al., 2000). Retroviral expression of a constitutively active ICN protein in hematopoietic progenitors generates cytokine-dependent cell lines with multilineage repopulating potential in vivo (Varnum-Finney et al., 2000), further supporting a role for notch signaling in HSC self-renewal. The polycomb group protein Bmi-1 is also required for self-renewal of normal HSC through loss-of-function (Park et al., 2003) and overexpression studies (Lessard & Sauvageau, 2003), acting through repression of the cell cycle and apoptosis regulators p16 and ARF.

In acute leukemia stem cells, the cytokine signaling pathways governing self-renewal are less well-defined, but probably overlap considerably with those in normal HSC. NF-κB is constitutively active in most AML stem cells but not in normal, unstimulated hematopoietic progenitors (Guzman et al., 2001), although the signaling pathway governing NF-κB activation are unknown. Inhibition of NF-κB activation by treatment with a proteasome inhibitor (Guzman et al., 2001; Guzman et al., 2002) or an inhibitor of IkB kinase induces selective apoptosis in AML stem cells (Guzman et al., 2005). In mouse retroviral models, leukemogenic transcription factors such as MLL-ENL (Cozzio et al., 2003), MLL-AF9 (Krivtsov et al., 2006), and MOZ-TIF2 (Huntly et al., 2004) can induce self-renewal in committed myeloid cells such as common myeloid progenitors (CMP) and granulocyte-macrophage progenitors (GMP) that ordinarily lack this capacity (see section on “The Cellular Basis of Hematopoiesis” in this issue). Transcriptional profiling of purified LSCs induced by MLL-AF9 demonstrated re-activation of a set of genes expressed at high levels in HSC, including multiple *HoxA* cluster genes and the transcription factor genes *Meis1* and *Mef2c* (Krivtsov et al., 2006; Somervaille & Cleary, 2006), and shRNA knockdown of...
Mef2c impaired the repopulating ability of MLL-AF9<sup>+</sup> progenitors (Krivtsov et al., 2006). As with normal HSC, Bmi-1 expression may be required within the LSC for self-renewal of AMLs induced by these dysregulated transcription factors (Lessard & Sauvageau, 2003).

In CML, the LSC appear to be very similar if not identical to normal HSC, with multilineage differentiation capacity in patients (Haferlach et al., 1997) and multilineage repopulating activity in NOD-SCID mice (Eisterer et al., 2005; Wang et al., 1998). In mouse models, whereas transgenic expression of BCR-ABL in CMP causes a CML-like phenotype (Jaiswal et al., 2003), retroviral expression of BCR-ABL in purified CMP or GMP does not produce MPD in recipient mice (Huntly et al., 2004). This suggests that dysregulated tyrosine kinases such as those found in the MPDs do not activate self-renewal pathways in committed progenitors. As a corollary, mutations that dysregulate TKs in the MPDs may be found exclusively in self-renewing HSC, because they cannot be sustained if they occur in committed progenitors that lack self-renewal. When CML progresses from chronic phase to myeloid blast crisis, a condition resembling AML, the Wnt signaling pathway may play an important role. Relative to normal or chronic phase CML marrow, the phenotypic GMP compartment in patients with CML myeloid blast crisis was expanded and aberrantly expressed transcriptionally active nuclear β-catenin (Jamieson et al., 2004). Leukemic GMP from CML blast crisis patients also exhibited self-renewal in a replating assay that was reduced by enforced expression of axin, an inhibitor of β-catenin signaling (Jamieson et al., 2004). These observations suggest that activation of β-catenin in CML GMP may confer self-renewal potential and contribute to disease progression.

**Summary and future directions**

Aberrant cytokine and growth factor signaling is a prominent feature of all leukemias, and contributes to proliferation, survival, self-renewal, and resistance to chemotherapy. Understanding of these signaling pathways is a prerequisite to the development and rational use of molecularly targeted therapies in leukemia. Our knowledge has already yielded some impressive therapeutic agents, including drugs targeting tyrosine kinases, serine-threonine kinases, Ras and other small G proteins, lipid kinases, and some transcription factors. It is likely that important differences in signaling exist between different classes of leukemia, and possibly even between phenotypically similar variants within the same class of leukemia. This, in turn, implies that signaling networks in leukemic cells should be defined on a case-by-case basis, with an emphasis on accurate and faithful mouse models and on analysis of primary human leukemia samples. Particular attention should be paid to characterization of the leukemic stem cells, which may be the ultimate targets of curative therapy. New systems biology approaches, such as interrogation of intracellular phosphorylation networks by flow cytometry (Irish et al., 2004) and analysis of gene expression profiles in response to drug treatment (Lamb et al., 2006) may offer fresh insights into this complex field.

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### Table 1

**Dysregulated TKs in leukemia**

| Kinase | Activating mechanism(s)                                                                 | Leukemia                                           | Kinase inhibitor(s) |
|--------|----------------------------------------------------------------------------------------|----------------------------------------------------|---------------------|
| ABL    | Fusion to BCR, Fusion to TEL, Fusion to NUP214 or EML1                                  | CML, AML, ALL, atypical CML, T-ALL                 | Imatinib, Dasatinib, Nilotinib |
| ALK    | Fusion to NPM                                                                           | Anaplastic large cell lymphoma                      | (None available)    |
| ARG    | Fusion to TEL                                                                           | AML                                               | Imatinib            |
| FGFR1  | Fusion to multiple partners, including ZNF198                                           | 8p11 myeloproliferative syndrome (EMS)             | (None available)    |
| FGFR3  | Overexpression from t(4;14)                                                             | Myeloma                                           | (None available)    |
| FMS    | Kinase domain mutations                                                                  | MDS, AML                                          | (None available)    |
| FLT3   | ITD mutations, Kinase domain mutations                                                  | AML                                               | Lestaurntinb, PKC-412|
| KIT    | Overexpression, Kinase domain mutations                                                 | AML, Systemic mast cell disease                    | (None available)    |
| JAK2   | Fusion to TEL or BCR Pseudokinase domain mutations (V617F)                             | Acute leukemia, atypical CML, PV, ET, CIMF         | Lestaurntinb        |
| JAK3   | Point mutations                                                                        | M7 AML                                            | (None available)    |
| NTRK3  | Fusion to TEL                                                                           | AML                                               | (None available)    |
| PDGFRα | Fusion to FIP1L1, Fusion to BCR, Fusion to CDK5RAP2                                     | CEL, systemic mast cell disease                    | Imatinib            |
| PDGFRβ | Fusion to TEL or other partners                                                         | CMML                                              | Imatinib            |
| Syk    | Fusion to TEL                                                                           | MDS                                               | Imatinib (?)        |

Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CEL, chronic eosinophilic leukemia; CIMF, chronic idiopathic myelofibrosis; CML, chronic myeloid leukemia; CMML, chronic myelomonocytic leukemia; ET, essential thrombocythemia; PV, polycythemia vera; MDS, myelodysplastic syndrome; T-ALL, T-cell acute lymphoblastic leukemia