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Pathogenesis and treatment of Ph⁺ leukemia: recent insights from mouse models

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Several methods to model human Ph⁺ leukemia in laboratory mice are available, including propagation of *BCR/ABL*-expressing cells in mice, xenotransplantation of primary Ph⁺ leukemia cells into immunodeficient mice, *BCR/ABL* transgenic mice, and *BCR/ABL* retroviral bone marrow transduction and transplantation. Recent studies in these different model systems have yielded important advances in our knowledge of the pathogenesis and therapy of human chronic myeloid leukemia and Ph⁺ B-lymphoblastic leukemia, and are the subject of this review. Curr Opin Hematol 2001, 8:224–230 © 2001 Lippincott Williams & Wilkins, Inc.

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Abbreviations

AGP	acid glycoprotein
B-ALL	acute B-lymphoblastic leukemia
CML	chronic myeloid leukemia
ES	embryonic stem
GM-CSF	granulocyte-macrophage colony-stimulating factor
IL-3	interleukin 3
LTC-IC	long-term culture-initiating cells
MMTV	mouse mammary tumor virus
MT	metallothionein
Ph⁺	Philadelphia-positive
PI 3-K	phosphatidylinositol 3-kinase
SCID	severe combined immunodeficiency disorder
SL-IC	SCID leukemia-initiating cells
SH2	Src homology 2
SRC	SCID-repopulating cell
STAT5	signal transducer and activator of transcription-5
TRE	tetracycline-responsive promoter element

Mouse models of human cancer are one of the most important areas of current molecular oncology research [1]. These models allow investigation of the molecular pathophysiology of these diseases in ways that would be difficult or impossible using primary human cancer cells and cell lines and provide a platform for the development and testing of new therapeutic approaches. Of all the models of human malignancy, murine models of the Philadelphia-positive (Ph⁺) leukemias are arguably the most accurate and faithful and the most advanced. In part, this is because the principal molecular abnormality of these leukemias, the *BCR/ABL* oncogene, was identified as the product of the t(9;22) Ph chromosome translocation more than 17 years ago, and many years of effort have been invested in expressing this gene in primary cells in mice. There are currently four main experimental approaches to modeling the 2 most common Ph⁺ leukemias, chronic myeloid leukemia (CML) and acute B-lymphoblastic leukemia (B-ALL), in mice: propagation of *BCR/ABL*-expressing cell lines in mice, xenotransplantation of primary Ph⁺ leukemia cells into immunodeficient mice, *BCR/ABL* transgenic mice, and *BCR/ABL* retroviral bone marrow transduction and transplantation (for more background on these models, see [2,3]). This article will focus on published advances using these four model systems during the past year.

Propagation of *BCR/ABL*-expressing cell lines in mice

One simple approach to modeling Ph⁺ leukemia *in vivo* is to propagate permanent cell lines expressing *BCR/ABL* in mice by parental inoculation. The cell lines used most often are mouse cytokine–dependent hematopoietic cell lines such as 32D or Ba/F3, which are transformed to cytokine independence by *BCR/ABL*. These cells can be grown in syngeneic hosts (C3H or Balb/c mice, respectively) without requiring irradiation of the recipient. Human Ph⁺ cell lines, derived from patients with B-ALL or blast crisis of CML, can also be grown in immunodeficient (most often SCID or nude/athymic) mice. In both cases, the *BCR/ABL*-expressing cells disseminate widely and cause death because of infiltration of marrow, spleen, liver, and other organs. This disease process does not accurately represent either Ph⁺ B-ALL or CML, so the value of this model system for studying leukemogenesis is limited. However, the system is quite useful for initial *in vivo* testing of new therapeutic approaches to Ph⁺ leukemia.

Several recent papers illustrate the usefulness of this model for drug testing. The Abl tyrosine kinase inhibitor STI-571 (Gleevec, formerly called CGP57148; Novartis Pharmaceuticals, East Hanover, NJ), given parenterally or orally, was able to eradicate human CML cell lines propagated in nude mice [4]. Pharmacokinetic data indicated that the effective half-life of STI-571 in mice (2–3 hours) was markedly shorter than in humans (16 hours), necessitating thrice-daily dosing. A subsequent study of mice with large burdens of Ph$^+$ leukemia cells found that most animals relapsed after an initial response to STI-571 [5•]. Although there was evidence of *in vivo* resistance to the inhibitor, cells isolated from relapsing mice were fully sensitive to STI-571 *in vitro*. Increased plasma levels of α1 acid glycoprotein (AGP) in these mice, produced from the liver as an acute-phase reactant, bound STI-571 and blocked the inhibition of Bcr/Abl kinase activity. The *in vivo* resistance could be partially overcome by co-administering erythromycin, which also binds AGP. The relevance of AGP to clinical resistance to STI-571 in patients remains to be established. Another study used *BCR/ABL*-transformed Ba/F3 cells propagated in Balb/c mice to assess the *in vivo* effectiveness of an orally administered farnesyltransferase inhibitor, SCH66336 [6•]. Oral SCH66336 administered twice daily for 1 month was able to eradicate the *BCR/ABL*-expressing cells and led to long-term survival. Biochemical studies indicated that other farnesylated proteins in addition to Ras were targets for the action of this drug, indicating that combinations of Abl kinase inhibitors and farnesyltransferase inhibitors may be advantageous for therapy of Ph$^+$ leukemias. The model system has also recently been used to demonstrate the potential of biologic interventions directed at *BCR/ABL*. Transduction of the Ph$^+$ cell line BV173 with retrovirus expressing a novel dimeric ribozyme directed against the *BCR/ABL* mRNA junction sequence abolished the leukemogenicity of these cells in nonobese diabetic (NOD)/SCID recipient mice [7], suggesting this strategy might be useful for *in vitro* purging of marrow autografts. Conditional expression of the normal *BCR* gene in the Ph$^+$ cell line K562 decreased the leukemogenicity of the cells in NOD/SCID mice [8], demonstrating that the normal Bcr protein can antagonize the actions of Bcr/Abl.

Xenotransplantation into NOD/SCID mice

Normal human bone marrow can engraft immunodeficient (SCID and NOD/SCID) mice, and this has allowed the definition of a CD34$^+$CD38$^-$ SCID-repopulating cell (SRC) that is more primitive than most long-term culture-initiating cells (LTC-IC) [9]. Primary leukemia blasts from human Ph$^+$ B-ALL and CML blast crisis patients are easily propagated in NOD/SCID mice [10,11], but cells from chronic-phase CML patients are more difficult to establish [10,12,13]. In 1998, three groups demonstrated efficient engraftment of immunodeficient NOD/SCID mice with large doses of primary

cells from chronic-phase CML patients [14–16]. Unfortunately, the extent of engraftment of individual mice transplanted with cells from the same patient may vary by more than three orders of magnitude [15], making quantitative studies impossible. Furthermore, the engrafted mice do not develop progressive or fatal myeloproliferative disease, suggesting that the murine hematopoietic microenvironment cannot support long-term *BCR/ABL*-induced myelopoiesis in cells of human origin.

In spite of these limitations, engraftment of NOD/SCID mice is useful because it is the only assay for the earliest malignant progenitors in patients with Ph$^+$ leukemia. A recent study used the NOD/SCID engraftment assay to demonstrate that a primitive quiescent population of progenitors from chronic-phase CML patients contained Ph$^+$ SRC [17]. A second paper examined the phenotype of primary cells from patients with Ph$^+$ B-ALL and the p190 isoform of *BCR/ABL* that were capable of engraftment of NOD/SCID mice [18]. The SCID leukemia-initiating cells (SL-IC) were primitive CD34$^+$CD38$^-$ cells that were more immature than most of the leukemic blasts, which were predominantly CD34$^+$CD38$^+$CD19$^+$. These results support previous observations that the leukemic clone in AML patients likewise has a hierarchical organization [19]. Although the authors suggested that the SL-IC in Ph$^+$ B-ALL might be hematopoietic stem cells, this cannot be definitively concluded from the cell surface markers used in their study. Evidence from the mouse retroviral bone marrow transduction/transplantation model system suggests that at least some non–stem cell progenitors can initiate B-ALL in mice after acquisition of the *BCR/ABL* oncogene [20•], (see "BCR/ABL retroviral bone marrow transduction/transplantation").

BCR/ABL transgenic mice

Reproducible expression of *BCR/ABL* in the hematopoietic system of transgenic mice has been hampered by the toxicity of this oncogene during mouse embryonic development, leading to down-regulation and silencing of the transgene [21] and even embryonic lethality [22]. The most successful *BCR/ABL* transgenic model system has used the metallothionein (MT) promoter to express either the p190 or p210 isoforms of *BCR/ABL* [23,24]. These mice express *BCR/ABL* in many tissues at very low levels [25], reproducibly develop B-ALL after several months' latency, and have proven valuable for investigating the pathophysiology of Ph$^+$ B-ALL and for testing treatments.

Expression of *BCR/ABL* in hematopoietic cell lines is associated with chromosomal instability [26] and increased frequency of point mutations [27], but it has been difficult to prove that these abnormalities are directly induced by Bcr/Abl rather than a secondary consequence of selection for increased growth in culture. A recent study in preleukemic MT-p190 *BCR/ABL* trans-

genic mice demonstrated an increased frequency of point mutations in a chromosomally integrated *lacI* reporter gene from both spleen and kidney, offering the first direct evidence that *BCR/ABL* expression causes a mutator phenotype in primary tissues [28•]. The precise mechanism of the increased mutation frequency induced by *BCR/ABL* is unknown. Previous studies in Ba/F3 cells demonstrated that *BCR/ABL* expression conferred resistance to apoptosis after ionizing radiation, accompanied by a prolonged G2/M arrest that was hypothesized to account for this genetic instability [29]. Surprisingly, primary cells from preleukemic p190 *BCR/ABL* transgenic mice showed no evidence of decreased apoptosis or abnormal checkpoint arrest after ionizing radiation [30]. Although the level of expression of p190 Bcr/Abl protein is low in the preleukemic mice, similar results were obtained with malignant lymphoblasts from leukemic mice, which express much higher levels of p190. These results suggest that the effects of *BCR/ABL* on cell cycle checkpoints in cultured cells may be irrelevant to the initiation and progression of leukemia *in vivo*, and further studies will be necessary to determine the mechanism of the increased mutation frequency induced by *BCR/ABL*.

The MT-*BCR/ABL* transgenic mice have also been used to study the signaling pathways important for leukemogenesis by *BCR/ABL*. One approach is to study leukemogenesis by different forms and mutants of *BCR/ABL* in this model. This is laborious, because testing each *BCR/ABL* mutant requires the generation, breeding, and characterization of multiple additional transgenic founder animals. In spite of this limitation, the approach has been used in the past year to demonstrate that both the coiled-coil oligomerization domain of Bcr [31] and the F-actin binding domain of Abl [32] are required for efficient development of B-ALL in *BCR/ABL* transgenic mice. A complementary strategy is breeding the MT-*BCR/ABL* transgene into different knockout and transgenic backgrounds to examine the effect on leukemogenesis. In this way, it has been shown that induction of B-ALL by *BCR/ABL* is independent of the normal *BCR* gene product [33], whereas overexpression of the CrkL adapter protein accelerates B-lymphoid leukemogenesis by *BCR/ABL* [34]. Finally, the MT-*BCR/ABL* mice have also been used to test the effectiveness of the farnesyl-transferase inhibitor SCH66336. Administration of this compound to early leukemic mice caused suppression of the leukemic phenotype and allowed disease-free survival in 80% of treated mice for the duration of the study [35]. These results suggest that inhibition of farnesyl-transferase may be of therapeutic benefit in Ph+ B-ALL, a disease where STI-571 is of only temporary benefit [36].

For unknown reasons, the MT-*BCR/ABL* transgenic mice do not develop myeloid leukemia or myeloprolif-

erative disease. The only *BCR/ABL* transgenic model of myeloproliferative disease is a line of mice expressing p210 *BCR/ABL* from the hematopoietic-specific *tec* promoter, which develop gradual increases in circulating neutrophils, thrombocytosis, anemia, and morbidity by 8 to 12 months of age [37]. Crossing the *tec*-p210 transgene into mice with heterozygous mutation of the *p53* tumor suppressor gene resulted in rapidly developing *BCR/ABL*-expressing T-lymphomas, most of which showed evidence of loss of the second *p53* allele [38]. This study provides convincing evidence of cooperation between *BCR/ABL* and loss of *p53*, but the lack of clear myeloproliferative disease in these mice prior to T-lymphoma development casts doubt on whether this represents blast crisis.

An exciting development in transgenic *BCR/ABL* models during the past year is the conditional expression of *BCR/ABL* in mice using the tetracycline-regulated system. In this system, two transgenic strains of mice are generated, one (transactivator) expressing the tetracycline-responsive transcriptional activator protein tTA, the other (transresponder) expressing *BCR/ABL* under control of a DNA response element with multiple tTA binding sites (Fig. 1A). The two strains are mated to generate double-transgenic mice, but the expression of the *BCR/ABL* transgene is suppressed during embryonic and postnatal development by dosing the drinking water of pregnant females and their offspring with tetracycline. Double-transgenic mice derived from four different p210 *BCR/ABL* transresponder lines remained healthy as long as tetracycline administration was continued, but developed fatal B-lymphoid leukemia with 100% incidence on withdrawal of the antibiotic [39•] (Fig. 1B). Surprisingly, retreatment of diseased mice with tetracycline led to the rapid death of the leukemic cells by apoptosis, demonstrating that *BCR/ABL*-induced B-ALL is absolutely dependent on continued expression of the oncogene, even at advanced stages of the leukemia (Fig. 1C). This model system should be useful for studying the earliest events in B-lymphoid leukemogenesis by *BCR/ABL* both *in vivo* and *in vitro*, and for analyzing the anti-apoptotic action of *BCR/ABL* in primary cells. The mice do not develop myeloid leukemia or CML because the tTA protein is not highly expressed in myeloid and stem cells [39•], but generation of different transactivator mice with myeloid-specific promoters might circumvent this problem. In related studies, another group expressed p210 *BCR/ABL* in totipotent embryonic stem (ES) cells using the tetracycline-regulated system, and demonstrated that *BCR/ABL* expression stimulated multipotential progenitor expansion and myeloid lineage commitment *in vitro* [40]. Generation of chimeric mice with these conditional *BCR/ABL* ES cells is the obvious next step, but has not been reported to date.

Figure 1. Conditional expression of *BCR/ABL* in transgenic mice
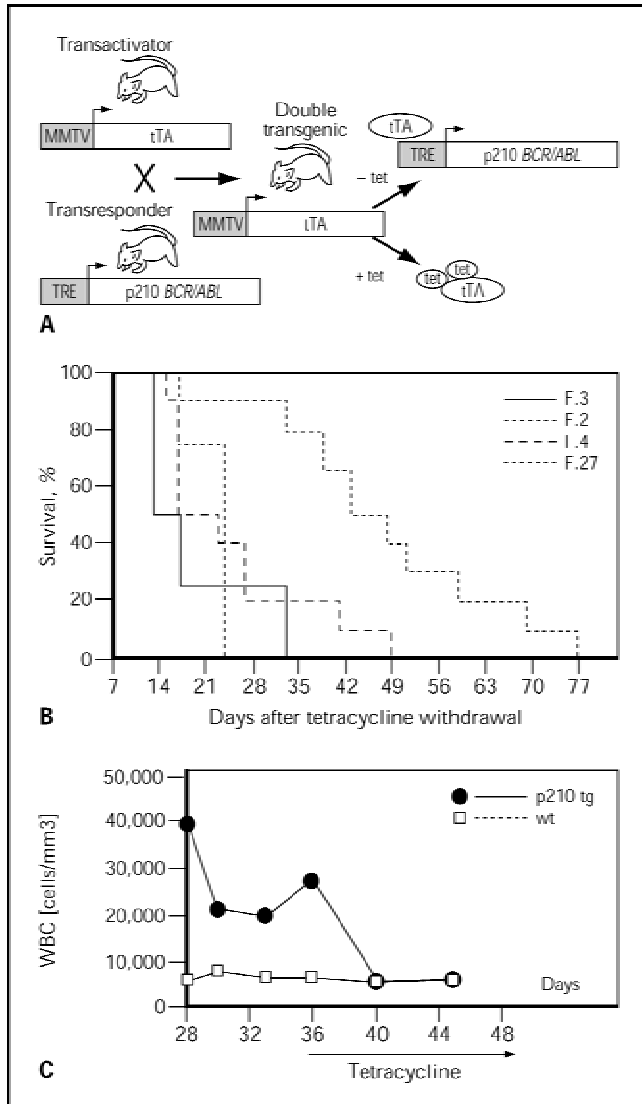
(A) Schematic representation of the double-transgene system for tetracycline-regulated expression of *BCR/ABL* in mice. The transactivator strain (top left) expresses the tTA protein from the mouse mammary tumor virus (MMTV) LTR, whereas the transresponder strain (bottom left) has a p210 *BCR/ABL* transgene under control of the tetracycline-responsive promoter element (TRE). Double transgenic mice (center) express tTA widely, but the *BCR/ABL* transgene is only expressed on removal of tetracycline (tet) (upper right). **(B)** Induction of *BCR/ABL* expression causes rapid development of B-ALL. Kaplan-Meier–style survival curve of double transgenic mice derived from four different TRE-p210 transresponder strains (F.2, F.3, F.4, and F.27) on removal of tetracycline (adapted from [39]). All mice developed pre-B acute lymphoblastic leukemia. **(C)** Reversion of leukemic mice on re-addition of tetracycline (adapted from [39]). Peripheral blood leukocyte count (WBC) of a leukemic mouse from line F.4. Tetracycline was withdrawn on day 1, frank leukemia developed by day 28 with an excess of blasts in peripheral blood (black circles), and the animal was again treated with tetracycline-supplemented water at day 36, with a precipitous decrease in WBC back to normal levels (illustrated by a wild-type littermate, open squares).

BCR/ABL retroviral bone marrow transduction/transplantation

The most accurate and quantitative mouse model of CML involves retroviral transduction of the *BCR/ABL* gene into primary murine bone marrow *ex vivo*, followed by transplantation of the cells into irradiated syngeneic recipient mice [41]. Two years ago, three groups reported efficient induction of CML-like myeloproliferative disease in recipients of *BCR/ABL*-transduced marrow, allowing the system to be used as an assay for the first time [42–44]. The retroviral marrow transduction/transplantation model has several distinct advantages over transgenic and knock-in strategies for modeling Ph+ leukemia in mice [45], including ease of testing different mutants of *BCR/ABL* and the ability to assess leukemogenicity in different bone marrow target cells.

This model system has been used recently to determine the role of particular signaling molecules in the pathogenesis of *BCR/ABL*-induced CML-like disease by using mice with naturally occurring or targeted mutations as donors, recipients, or both. *BCR/ABL* induces constitutive activation of the transcription factor signal transducer and activator of transcription-5 (STAT5) in hematopoietic cells, but some recipients of *BCR/ABL*-transduced marrow from mice with homozygous mutations in both *Stat 5a* and *Stat5b* genes still developed myeloproliferative disease [46], demonstrating that STAT5 is not absolutely required for the pathogenesis of *BCR/ABL*-induced CML-like disease. In contrast, a similar myeloproliferative illness induced by the *TEL/JAK2* fusion oncogene was absolutely dependent on STAT5 [47]. These studies emphasize that important pathophysiologic differences exist between phenotypically similar leukemias and suggest that molecularly targeted therapy of these diseases must be individualized. In another recent study, the role of cytokines in the pathogenesis of *BCR/ABL*-induced CML-like disease was investigated. Aberrant transcripts for interleukin 3 (IL-3) and granulocyte colony-stimulating factor (G-CSF) have been reported in primitive Ph+ progenitors from chronic-phase CML patients, raising the possibility of an autocrine growth mechanism in CML [48]. Although increased circulating IL-3 and granulocyte-macrophage colony-stimulating factor (GM-CSF) are detected in mice with *BCR/ABL*-induced CML-like leukemia [43], neither cytokine is required for induction of myeloproliferative disease in mice by *BCR/ABL* [49•]. These results argue that IL-3 does not play an essential role in human chronic-phase CML but do not exclude a role for cytokines in the long latent phase of CML, which is not modeled accurately in mice.

Another series of recent studies examined leukemogenesis by *BCR/ABL* mutants with alterations in well-defined structural domains of the polypeptide. Two critical domains in the Bcr portion of the fusion protein were found to be necessary for efficient induction of myeloproliferative disease by Bcr/Abl: the N-terminal coiled-coil oligomerization domain [50] and the direct binding site for the Grb2 adapter protein at Bcr tyrosine 177 [50,51]. Although the requirement for the oligomeriza-

Figure 2. Alternative signaling models to explain the leukemogenic defect of Bcr/Abl SH2 mutants
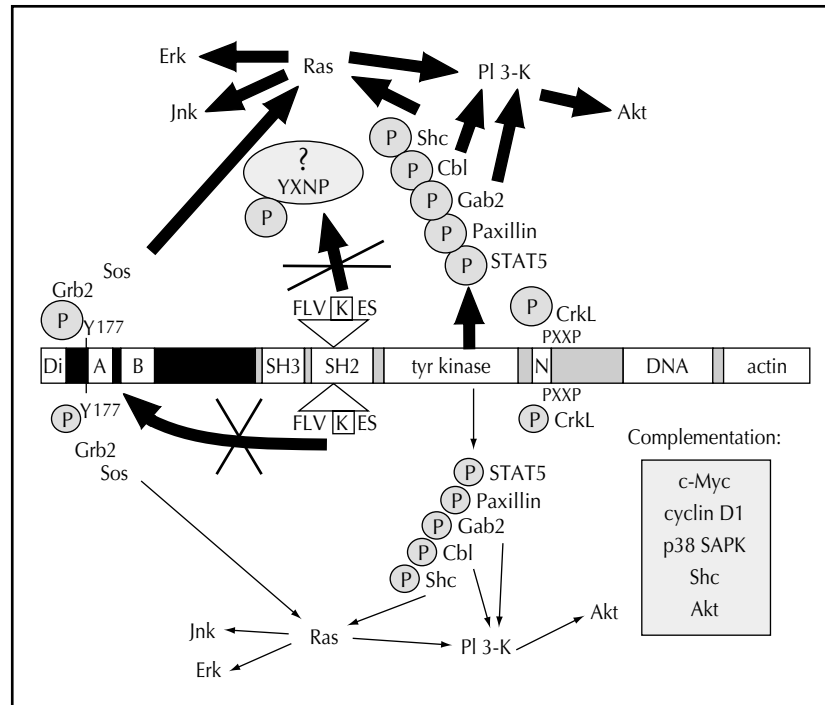
A schematic representation of the Bcr/Abl fusion protein is depicted in the middle, whereas several known Bcr/Abl substrates and downstream signaling pathways are shown above and below. Mutation of a critical arginine to lysine (K) in the highly conserved FLVRES motif of the Abl SH2 domain blocks binding of tyrosine-phosphorylated ligands to SH2 (represented by a hypothetical YXNP-containing signaling protein, top) but also decreases the intrinsic tyrosine kinase activity of the fusion protein (represented by the decreased thickness of the arrows and the smaller size of the phosphates (P), bottom). Globally decreased signaling in multiple Bcr/Abl-stimulated pathways (bottom model) is consistent with the lack of qualitative defects in signaling in malignant myeloid cells expressing the Bcr/Abl SH2 mutant and explains the complementation of this mutant on overexpression of many different downstream signaling proteins (box). Erk, extracellular signal-regulated kinase; Jnk, c-Jun N-terminal kinase; SH3, Src homology 3; Sos, son of sevenless; published with permission [20•].

tion domain was anticipated from *in vitro* studies [52], the necessity of direct Grb2 binding was unexpected [53] and validates this pathway as a target for rational drug design. The importance of direct binding of Grb2 is further emphasized by the fact that p160 v-Abl, the product of the transforming gene of Abelson murine leukemia virus, lacks Grb2 binding and is completely defective for induction of myeloproliferative disease in mice [51,54]. The effect of mutations in the Src homology 2 (SH2) domain of Bcr/Abl are worth emphasizing because they illustrate the complexity of this model system. The SH2 domain of Bcr/Abl was previously reported to be absolutely and specifically required for activation of phosphatidylinositol 3-kinase (PI 3-K) [55]. Recent studies demonstrated that the SH2 domain is required for efficient induction of CML-like disease by Bcr/Abl [20•,56] but not for B-lymphoid leukemogenesis [20•]. SH2 mutation decreased the intrinsic tyrosine kinase activity of Bcr/Abl, yet malignant lymphoblasts and myeloid cells from the diseased mice showed no impairment of PI 3-K activation [20•]. These results argue that the decreased induction of myeloproliferative disease by SH2-mutated Bcr/Abl is not the effect of an isolated defect in one specific signaling pathway, but is rather a consequence of slightly dampened signaling in many pathways (Fig. 2). As a corollary, maximal Bcr/Abl tyrosine kinase activity is required for induction of myeloproliferative disease but not for B-lymphoid leukemogenesis, which may in part explain the resistance of Ph+ B-ALL to kinase inhibitor therapy [36].

Conclusions

Human Ph+ leukemia is arguably the best understood of all human malignant disorders, in part because of what we have learned from mouse models of these diseases. These models are valuable because they accurately recapitulate the complex pathophysiology of the leukemias *in vivo*. We can expect the existing model systems to be improved, and they will continue to provide important new knowledge about the pathophysiology and therapy of the Ph+ leukemias that would impossible to derive from clinical studies. However, continued correlative studies between human and murine *BCR/ABL*-induced leukemias are essential, and conclusions reached in mouse model systems require validation, if possible, in human patients and cells.

Acknowledgments

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

*   Of special interest
•• Of outstanding interest

1   Klausner R: The Nation's Investment in Cancer Research: A budget proposal for fiscal year 2000: National Cancer Institute; 1998.

2   Van Etten RA: Animal models of Philadelphia-positive leukemia. In Chronic Myeloid Leukemia: Biology and Treatment. Edited by Carella AM, Daley GQ, Goldman JM, et al. London: Martin Dunitz; 2001:in press.

3   Van Etten RA: Models of chronic myeloid leukemia. Curr Oncol Rep 2001, 3:228–237.

4   le Coutre P, Mologni L, Cleris L, et al.: *In vivo* eradication of human BCR/ABL-positive leukemia cells with an ABL kinase inhibitor. J Natl Cancer Inst 1999, 91:163–168.

5   Gambacorti-Passerini C, Barni R, le Coutre P, et al.: Role of alpha1 acid
•   glycoprotein in the *in vivo* resistance of human BCR-ABL(+) leukemic cells to the Abl inhibitor STI571. J Natl Cancer Inst 2000, 92:1641–1650.
This study defines a novel *in vivo* mechanism of resistance to Abl kinase inhibitor therapy.

6   Peters DG, Hoover RR, Gerlach MJ, et al.: Activity of the farnesyl protein
•   transferase inhibitor SCH66336 against BCR/ABL-induced murine leukemia and primary cells from patients with chronic myeloid leukemia. Blood 2001, 97:1404–1412.
The authors offer a demonstration of the activity of a farnesyltransferase inhibitor in a cell line-based mouse model and against primary CML patient cells.

7   Tanabe T, Kuwabara T, Warashina M, et al.: Oncogene inactivation in a mouse model. Nature 2000, 406:473–474.

8   Lin F, Monaco G, Sun T, et al.: *BCR* gene expression blocks Bcr-Abl induced pathogenicity in a mouse model. Oncogene 2001, 20:1873–1881.

9   Larochelle A, Vormoor J, Hanenberg H, et al.: Identification of primitive human hematopoietic cells capable of repopulating NOD/SCID mouse bone marrow: implications for gene therapy. Nat Med 1996, 2:1329–1337.

10 Sawyers CL, Gishizky ML, Quan S, et al.: Propagation of human blastic myeloid leukemias in the SCID mouse. Blood 1992, 79:2089–2098.

11 Cesano A, Hoxie JA, Lange B, et al.: The severe combined immunodeficient (SCID) mouse as a model for human myeloid leukemias. Oncogene 1992, 7:827–836.

12 McGuirk J, Yan Y, Childs B, et al.: Differential growth patterns in SCID mice of patient-derived chronic myelogenous leukemias. Bone Marrow Transplant 1998, 22:367–374.

13 Sirard C, Lapidot T, Vormoor J, et al.: Normal and leukemic SCID-repopulating cells (SRC) coexist in the bone marrow and peripheral blood from CML patients in chronic phase, whereas leukemic SRC are detected in blast crisis. Blood 1996, 87:1539–1548.

14 Dazzi F, Capelli D, Hasserjian R, et al.: The kinetics and extent of engraftment of chronic myelogenous leukemia cells in non-obese diabetic/severe combined immunodeficiency mice reflect the phase of the donor's disease: An *in vivo* model of chronic myelogenous leukemia biology. Blood 1998, 92:1390–1396.

15 Wang JCY, Lapidot T, Cashman JD, et al.: High level engraftment of NOD/SCID mice by primitive normal and leukemic hematopoietic cells from patients with chronic myeloid leukemia in chronic phase. Blood 1998, 91:2406–2414.

16 Lewis ID, McDiarmid LA, Samels LM, et al.: Establishment of a reproducible model of chronic-phase chronic myeloid leukemia in NOD/SCID mice using blood-derived mononuclear or CD34+ cells. Blood 1998, 91:630–640.

17 Holyoake T, Jiang X, Eaves C, et al.: Isolation of a highly quiescent subpopulation of primitive leukemic cells in chronic myeloid leukemia. Blood 1999, 94:2056–2064.

18 Cobaleda C, Gutierrez-Cianca N, Perez-Losada J, et al.: A primitive hematopoietic cell is the target for the leukemic transformation in human Philadelphia-positive acute lymphoblastic leukemia. Blood 2000, 95:1007–1013.

19 Bonnet D, Dick JE: Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. Nat Med 1997, 3:730–737.

20 Roumiantsev S, de Aos I, Varticovski L, et al.: The Src homology 2 domain of
•   Bcr/Abl is required for efficient induction of chronic myeloid leukemia-like disease in mice but not for lymphoid leukemogenesis or activation of phosphatidylinositol 3-kinase. Blood 2001, 97:4–13.

This article presents a comprehensive assessment of the role of SH2 domain mutations on leukemogenesis and signaling by Bcr/Abl.

21 Hariharan IK, Harris AW, Crawford M, et al.: A *bcr-v-abl* oncogene induces lymphomas in transgenic mice. Mol Cell Biol 1989, 9:2798–2805.

22 Heisterkamp N, Jenster G, Kioussis D, et al.: Human *bcr-abl* gene has a lethal effect on embryogenesis. Transgenic Res 1991, 1:45–53.

23 Heisterkamp N, Jenster G, ten Hoeve J, et al.: Acute leukemia in bcr/abl transgenic mice. Nature 1990, 344:251–253.

24 Voncken JW, Kaartinen V, Pattengale PK, et al.: *BCR/ABL* P210 and P190 cause distinct leukemia in transgenic mice. Blood 1995, 86:4603–4611.

25 Voncken JW, Griffiths S, Greaves MF, et al.: Restricted oncogenicity of BCR/ABL p190 in transgenic mice. Cancer Res 1992, 52:4534–4539.

26 Laneuville P, Sun G, Timm M, et al.: Clonal evolution in a myeloid cell line transformed to interleukin-3 independent growth by retroviral transduction and expression of p210bcr/abl. Blood 1992, 80:1788–1797.

27 Canitrot Y, Lautier D, Laurent G, et al.: Mutator phenotype of BCR-ABL transfected Ba/F3 cell lines and its association with enhanced expression of DNA polymerase beta. Oncogene 1999, 18:2676–2680.

28 Salloukh HF, Laneuville P: Increase in mutant frequencies in mice expressing
•   the BCR-ABL activated tyrosine kinase. Leukemia 2000, 14:1401–1404.
This is the first demonstration of an increased mutation frequency in hematopoietic and nonhematopoietic tissues from *BCR/ABL* transgenic mice.

29 Bedi A, Barber JP, Bedi GC, et al.: BCR-ABL-mediated inhibition of apoptosis with delay of G2/M transition after DNA damage: A mechanism of resistance to multiple anticancer agents. Blood 1995, 86:1148–1158.

30 Salloukh HF, Vowles I, Heisterkamp N, et al.: Early events in leukemogenesis in P190Bcr-abl transgenic mice. Oncogene 2000, 19:4362–4374.

31 Heisterkamp N, Voncken JW, Senadheera D, et al.: The Bcr N-terminal oligomerization domain contributes to the full oncogenicity of P190 Bcr/Abl in transgenic mice. Int J Mol Med 2001, 7:351–357.

32 Heisterkamp N, Voncken JW, Senadheera D, et al.: Reduced oncogenicity of p190 Bcr/Abl F-actin-binding domain mutants. Blood 2000, 96:2226–2232.

33 Voncken JW, Kaartinen V, Groffen J, et al.: Bcr/Abl associated leukemogenesis in *bcr* null mutant mice. Oncogene 1998, 16:2029–2032.

34 Hemmeryckx B, van Wijk A, Reichert A, et al.: Crkl enhances leukemogenesis in *BCR/ABL* P190 transgenic mice. Cancer Res 2001, 61:1398–1405.

35 Reichert A, Heisterkamp N, Daley GQ, et al.: Treatment of Bcr/Abl-positive ALL in p190 transgenic mice with the farnesyltransferase inhibitor SCH66336. Blood 2001, 97:1399–1403.

36 Druker BJ, Sawyers CL, Kantarjian H, et al.: Activity of a specific inhibitor of the BCR-ABL tyrosine kinase in the blast crisis of chronic myeloid leukemia and acute lymphoblastic leukemia with the Philadelphia chromosome. N Engl J Med 2001, 344:1084–1086.

37 Honda H, Oda H, Suzuki T, et al.: Development of acute lymphoblastic leukemia and myeloproliferative disorder in transgenic mice expressing p210[bcr/abl]: A novel transgenic model for human Ph[1]-positive leukemias. Blood 1998, 91:2067–2075.

38 Honda H, Ushijima T, Wakazono K, et al.: Acquired loss of p53 induces blastic transformation in p210(bcr/abl)-expressing hematopoietic cells: a transgenic study for blast crisis of human CML. Blood 2000, 95:1144–1150.

39 Huettner CS, Zhang P, Van Etten RA, et al.: Reversibility of acute B-cell leu-
•   kaemia induced by *BCR-ABL1*. Nat Gen 2000, 24:57–60.
This is the first report of conditional expression of p210 *BCR/ABL* in mice using a binary tetracycline-regulated transgene system.

40 Era T, Witte ON: Regulated expression of p210 Bcr-Abl during embryonic stem cell differentiation stimulates multipotential progenitor expansion and myeloid cell fate. Proc Natl Acad Sci U S A 2000, 97:1737–1742.

41 Daley GQ, Van Etten RA, Baltimore D: Induction of chronic myelogenous leukemia in mice by the P210[bcr/abl] gene of the Philadelphia chromosome. Science 1990, 247:824–830.

42 Pear WS, Miller JP, Xu L, et al.: Efficient and rapid induction of a chronic myelogenous leukemia-like myeloproliferative disease in mice receiving P210 bcr/abl-transduced bone marrow. Blood 1998, 92:3780–3792.

43 Zhang X, Ren R: Bcr-Abl efficiently induces a myeloproliferative disease and production of excess interleukin-3 and granulocyte-macrophage colony-stimulating factor in mice: A novel model for chronic myelogenous leukemia. Blood 1998, 92:3829–3840.

44 Li S, Ilaria RL, Million RP, et al.: The P190, P210, and P230 forms of the *BCR/ABL* oncogene induce a similar chronic myeloid leukemia-like syn-

drome in mice but have different lymphoid leukemogenic activity. J Exp Med 1999, 189:1399–1412.

45   Van Etten RA: Retroviral transduction models of Ph+ leukemia: advantages and limitations for modeling human hematological malignancies in mice. Blood Cells Mol Dis 2001, 27:201–205.

46   Sexl V, Piekorz R, Moriggl R, et al.: Stat5a/b contribute to interleukin 7-induced B-cell precursor expansion, but *abl-* and *bcr/abl-*induced transformation are independent of STAT5. Blood 2000, 96:2277–2283.

47   Schwaller J, Parganas E, Wang D, et al.: STAT5 is essential for the myelo- and lymphoproliferative disease induced by TEL/JAK2. Mol Cell 2000, 6:693–704.

48   Jiang X, Lopez A, Holyoake T, et al.: Autocrine production and action of IL-3 and granuolcyte colony-stimulating factor in chronic myeloid leukemia. Proc Natl Acad Sci U S A 1999, 96:12804–12809.

49   Li S, Gillessen S, Tomasson MH, et al.: Interleukin-3 and granulocyte-
•   macrophage colony-stimulating factor are not required for induction of chronic myeloid leukemia-like myeloproliferative disease in mice by *BCR/ABL*. Blood 2001, 97:1442–1450.
This article provides an example of using mutant mice in the retroviral bone marrow transduction/transplantation system to determine the role of cytokines in the pathogenesis of *BCR/ABL*-induced CML-like leukemia.

50   Zhang X, Subrahmanyam R, Wong R, et al.: The NH_2-terminal coiled-coil domain and tyrosine 177 play important roles in induction of a myeloproliferative disease in mice by Bcr-Abl. Mol Cell Biol 2001, 21:840–853.

51   Million RP, Van Etten RA: The Grb2 binding site is required for induction of chronic myeloid leukemia-like disease in mice by the Bcr/Abl tyrosine kinase. Blood 2000, 96:664–670.

52   McWhirter JR, Galasso DL, Wang JYJ: A coiled-coil oligomerization domain of Bcr is essential for the transforming function of Bcr-Abl oncoproteins. Mol Cell Biol 1993, 13:7587–7595.

53   Goga A, McLaughlin J, Afar DE, et al.: Alternative signals to RAS for hematopoietic transformation by the BCR-ABL oncogene. Cell 1995, 82:981–988.

54   Gross AW, Ren R: Bcr-Abl has a greater intrinsic capacity than v-Abl to induce the neoplastic expansion of myeloid progenitor cells. Oncogene 2000, 19:6286–6296.

55   Skorski T, Bellacosa A, Nieborowska-Skorska M, et al.: Transformation of hematopoietic cells by BCR/ABL requires activation of a PI-3K/Akt-dependent pathway. EMBO J 1997, 16:6151–6161.

56   Zhang X, Wong R, Hao SX, et al.: The SH2 domain of Bcr-Abl is not required to induce a murine myeloproliferative disease; however, SH2 signaling influences disease latency and phenotype. Blood 2001, 97:277–287.