Models of Chronic Myeloid Leukemia

Richard A. Van Etten, MD, PhD

Address
The Center for Blood Research and Department of Genetics, Harvard Medical School, Boston MA 02115, USA.
E-mail: vanetten@cbr.med.harvard.edu

Current Oncology Reports 2001, **3:**228–237
Current Science Inc. ISSN 1523-3790

Models of chronic myeloid leukemia (CML) have proven invaluable for furthering our understanding of the molecular pathophysiology of this disease. Xenotransplantation of primary human CML cells into immunodeficient mice allows investigation into the nature of the most primitive repopulating cells in this leukemia, but the system is limited by variability and difficulty with experimental manipulation. Accordingly, a large effort has been invested in developing models of CML through expression of the *BCR/ABL* oncogene in the hematopoietic system of laboratory mice. Despite numerous attempts, an accurate transgenic mouse model of CML has not been produced, possibly because of the toxicity of *BCR/ABL*. Conditional transgenic mice are a promising new approach to this problem. A more successful strategy is retroviral transduction of *BCR/ABL* into mouse bone marrow in vitro, followed by transplantation into syngeneic or immunodeficient recipient mice. Recipients of marrow transduced with p210 *BCR/ABL* develop a fatal myeloproliferative illness that closely resembles human CML. This model is being used to define the signaling pathways required for leukemogenesis by *BCR/ABL*, and for developing new therapeutic approaches.

Introduction

There are several reasons for developing animal models of human leukemia. The first is to assess the contribution of a genetic abnormality that is identified in a leukemia cell to the pathogenesis of the malignancy. The second is to investigate the pathophysiology of the leukemia in ways that would be difficult or impossible using primary human cells and cell lines. Finally, an accurate animal model of leukemia can serve as a platform for the development and evaluation of new treatments, including immune-based therapies.

Chronic myeloid or myelogenous leukemia (CML) is perhaps the best understood of all human malignancies. In part, this reflects identification of the Philadelphia chromosome over 40 years ago, and the subsequent isolation of the product of this translocation, the *BCR/ABL* oncogene. Fifteen years of intensive research demonstrated that the protein product of this gene, Bcr/Abl, was a dysregulated protein-tyrosine kinase that activated numerous intracellular signaling pathways and transformed fibroblasts and hematopoietic cells in culture. However, whether *BCR/ABL* was the principal cause of CML was in some doubt until the demonstration, a decade ago, that expression of this oncogene in mouse bone marrow induced a myeloproliferative illness closely resembling human CML [1,2]. Recently, the central role of Bcr/Abl in human CML was confirmed by the astounding clinical efficacy of an inhibitor of the Abl tyrosine kinase, STI571. Having established *BCR/ABL* as the cause of CML, current work on models of CML centers on studying the pathophysiology of the leukemia and testing novel therapeutic strategies.

This article discusses recent progress in the development and use of models of CML, focusing on three distinct experimental approaches: xenotransplantation of primary human CML cells into immunodeficient mice and *BCR/ABL* transgenic mice, and *BCR/ABL* retroviral bone marrow transduction/transplantation. With each method, emphasis is placed on what has been learned from the model, as well as the strengths and weaknesses of the system. The intention is to allow investigators considering the use of an animal model of CML to select the system that best fits their particular experimental requirements.

Xenotransplantation of Human CML Cells into Immunodeficient Mice

Engraftment of primary CML cells in SCID mice

Both normal and malignant human hematopoietic cells can engraft severe combined immunodeficient (SCID) and non-obese diabetic/LtSz/*scid*/*scid* (NOD/SCID) mice. Efficient engraftment of normal human myeloid cells in these mice requires concomitant administration of exogenous human cytokines, cotransplantation of marrow stroma or accessory cells, or use of large cell doses. Like most other established cell lines, cell lines derived from CML blast crisis patients efficiently engraft and disseminate in SCID mice [3,4]. In contrast, attempts to propagate primary cells from CML patients were generally successful when the patients were in blast crisis [3,5], but stable engraftment of primary chronic-phase cells was not observed in early attempts [3,6].

Success was achieved by injecting very large cell inoculums ($8–14 \times 10^7$ cells) from peripheral blood or marrow of chronic-phase CML patients into SCID mice [7], resulting in engraftment of recipient bone marrow with human

hematopoietic cells at levels from 1% to 10% within 30 to 60 days following injection. The degree of engraftment was cell dose-dependent, with very low engraftment observed at lower inoculums ($1–4 \times 10^7$ cells). Human myeloid ($CD13^+$), B-lymphoid ($CD19^+$), and progenitor ($CD34^+$) cells, as well as multilineage colony-forming cells were detected in recipient bone marrow, but there was no dissemination to peripheral blood, spleen, or other organs, nor did recipients develop any clinical illness. Surprisingly, treatment of recipients with exogenous human cytokines was not required for efficient engraftment, which may reflect aberrant production of cytokines by primitive CML progenitors [8], with autocrine and paracrine effects after transplantation. Surprisingly, only about one third of CFC were derived from Ph^+ progenitors even when peripheral blood was used as the graft, demonstrating that primitive normal cells are mobilized in chronic-phase CML along with their malignant counterparts, and may engraft SCID mice more efficiently.

Superiority of NOD/SCID mice as recipients

Recently, three different laboratories [9•,10•,11•] have demonstrated that NOD/SCID mice are superior to SCID mice for engraftment of primary cells from chronic-phase CML patients. When injected with similar numbers of cells from chronic-phase patients, NOD/SCID recipients reproducibly had higher levels of human hematopoietic engraftment than SCID recipients [10•]. Eighty-four percent of mice receiving more than 4×10^7 cells showed evidence of engraftment, with half of these demonstrating greater than 10% human cells in their bone marrow (BM), assessed by human-specific and *BCR/ABL*-specific fluorescence in situ hybridization (FISH) [9•]. Comparison of donor cells derived from CML patients in chronic, accelerated, and blastic phases demonstrated that the extent of engraftment of NOD/SCID recipients increased and time to engraftment decreased between the chronic phase and blast crisis [11•]. Exogenous cytokines were not required for engraftment [9•,10•], and both normal and Ph^+ engraftment was observed. The proportion of Ph^+ cells was between 23% and 64% in three highly engrafted mice in one study [9•], whereas another found that an average of 66% of total human BM cells and 71% of BM-derived human CFC were Ph^+ by southern blot or karyotype analysis, respectively [10•]. These findings contrast with those from SCID recipients, in which an average of 70% of human CFC were Ph-negative [7]. Interestingly, some NOD/SCID recipients demonstrated human cells in the spleen [9•,10•], with about two thirds of recipients with BM engraftment demonstrating detectable human cells in the spleen at variable (average, 16%) levels, accompanied by moderate splenomegaly in some cases [9•].

Applications of the xenotransplant model system

A major advantage of xenotransplantation is the ability to work directly with primary CML cells from patients. The NOD/SCID assay system should be valuable for elucidating the characteristics of the most primitive leukemia-initiating cell in CML. Cell fractionation studies demonstrated that the chronic-phase CML progenitor that engrafted marrow of NOD/SCID recipients was $CD34^+$ [9•,10•]. Interestingly, the NOD/SCID leukemia-initiating cell in Ph^+ ($P190^+$) acute B-lymphoblastic leukemia is also $CD34^+$ $CD38^-$ [12], but the relationship of this target cell to the engrafting cell in CML requires further study. Given the quantitative differences in engraftment of chronic, accelerated, and blast-phase cells [11•], xenotransplantation may yield useful prognostic information when performed serially with samples from the same CML patient. The assay will also be useful for investigating the cellular biology of CML. For example, a recent study identified a subpopulation of quiescent primitive leukemia cells in chronic-phase CML patients that was nonetheless capable of engraftment of NOD/SCID mice [13]. Finally, the assay should be of use in testing new treatments, such as gene therapy approaches.

Limitations of the xenotransplant model system

Despite many advantages, the current xenotransplantation model has several problems that will limit its usefulness unless they can be overcome. First, engraftment appears to be extremely variable. The extent of engraftment of individual mice transplanted with cells derived from the same patient may vary by over three orders of magnitude [10•], whereas the average overall engraftment observed between recipients of cells from clinically similar patients is also quite different [9•], although this variability may represent more advanced disease in some patients [11•]. Although the engraftment of normal human hematopoietic progenitors in NOD/SCID recipients is reproducible enough to allow statistically significant limiting dilution analysis, enabling estimation of the frequency of the repopulating cells [14], it is unclear whether similar quantitative analysis of engraftment will be possible with CML cells. Second, whether NOD/SCID recipients engrafted with chronic-phase CML cells accurately recapitulate the pathophysiology of human CML remains to be seen. Recipients do not appear to have circulating human myeloid cells or dissemination of human cells to the liver or other extramedullary sites. Although NOD/SCID recipients often exhibit human hematopoietic cells in the spleen with concomitant splenomegaly, most of these cells appear to be of T-cell origin and are Ph-negative [9•,10•]. Most significantly, recipients of CML chronic-phase cells do not appear to develop progressive clinical myeloproliferative disease or evolve to acute leukemia, although long-term studies of such recipients have not been reported. In part, these difficulties may reflect the same fundamental problem, that NOD/SCID mice may not be able to efficiently support long-term human normal or CML-derived myelopoiesis. It seems likely that factors other than host immune responses, such as the marrow microenvironment, may account for this. Lastly, the xenotransplant system is difficult to manipulate experimentally, for example,

in direct analysis of the role of Bcr/Abl and downstream pathways in the leukemic process. Although this can be attempted through use of chemical inhibitors and antisense treatments, it is not possible to express different forms of Bcr/Abl or introduce other modulatory genes or proteins in the system. Despite these limitations, there is certain to be great interest in this model system, and many of these issues should be addressed in the near future.

BCR/ABL Transgenic Mice

Conventional *BCR/ABL* transgenic mice

In conventional transgenic mice, a *BCR/ABL* transgene is expressed under control of a promoter/enhancer element, integrates at a single chromosomal location, is present in one to several copies in a tandem repeat, and is found in all cells of the animal, including the germline. The expression of the transgene in the mice is predominantly controlled by the particular promoter/enhancer element utilized in the DNA construct. Usually, the transgene can be passed from a founder mouse to offspring, inherited in a Mendelian fashion. The different *BCR/ABL* transgenic mice that have been generated to date are listed in Table 1.

The first *BCR/ABL* transgenic mice expressed a BCR/v-abl facsimile oncogene (similar to p210 *BCR/ABL*) under the immunoglobulin heavy-chain enhancer (Eμ) or the widely expressed promoter/enhancer from the long terminal repeat (LTR) of the murine myeloproliferative sarcoma virus (MPSV) [15]. There was a decreased yield of transgenic offspring from eggs injected with either transgene DNA, but a small number of transgenic founders of both types, and some progeny developed clonal T and B lymphomas. More consistent success was achieved with expression of *BCR/ABL* from a segment of the mouse metallothionein (δMT-1) promoter, which is inducible with heavy metals but constitutively and widely expressed in transgenic mice. A δMT-1/p190 *BCR/ABL* transgene efficiently generated founders that developed leukemia with high frequency between 4 to 24 weeks of age [16,17]. The malignant cells were positive for B220 (CD45R) and 6C3/BP1 antigens, suggestive of an immature B-lymphoid cell phenotype. When the MT-1 promoter was used to generate p210 *BCR/ABL* transgenic mice, founders and progeny developed predominantly T- and B-cell leukemia/lymphoma, with a penetrance of about 60% by the age of 44 weeks [18,19]. This disease pattern was significantly different from the δMT-1/p190 mice, which exhibit greater than 90% incidence of leukemia by 24 weeks and never develop T-cell disease [17,20]. These results suggest that p210 *BCR/ABL* has less potent and distinct lymphoid leukemogenic activity in transgenic mice when compared with p190.

These studies confirmed the leukemogenic activity of *BCR/ABL* in vivo, but none of the mice reproducibly developed myeloid leukemias or CML-like disease. The possible reasons for this are discussed herein. Recently, the p210 *BCR/ABL* gene has been expressed under control of the promoter of the *tec* gene, which encodes a cytoplasmic kinase preferentially expressed in the hematopoietic system [21•]. Five founders were obtained, two of which died of T-cell lymphoblastic leukemia between 3 and 4 months of age. One of these leukemic founders gave rise to a permanent line that showed no evidence of T-ALL but instead developed a slow myeloproliferative-like syndrome with 100% penetrance by 8 months of age. This syndrome was characterized by modestly increased peripheral blood leukocyte counts (9–15 × 10^3/μL) composed predominantly of mature neutrophils, moderate thrombocytosis (80–200 × 104/μL), and mild anemia (hemoglobin 8.0–14.5 g/dL). Some mice became moribund at the age of around 1 year and showed infiltration of spleen and mesenteric lymph nodes with neutrophils, marked thrombocytosis, and severe anemia. *BCR/ABL* transgene expression was detected in circulating neutrophils by reverse transcriptase-polymerase-chain reaction (RT-PCR). This novel transgenic model is intriguing, but further studies are necessary to determine whether it represents an accurate model of CML. In particular, it is not clear whether the disease process is progressive or fatal in all offspring or if the illness is transplantable, and the cause of death in some mice appeared to be severe anemia, which is not a prominent feature of chronic-phase CML in human patients.

Conditional *BCR/ABL* transgenic mice

Recently, attention has turned to the use of conditional transgenic systems as an approach to developing a model of CML, based on the assumption that suppressing expression of a *BCR/ABL* transgene until after birth would prevent toxicity that might interfere with transgene expression. One type of binary transgene system utilizes the tetracycline-regulated transcriptional activator tTA. tTA is a fusion of the *Escherichia coli* tetracycline operon repressor and the herpesvirus transactivator VP16, which binds to tet operator DNA sequences and activates transcription in the absence of tetracycline, but not in its presence. In a recent study [22•], four lines of "transresponder" transgenic mice with p210 *BCR/ABL* under control of the tet-response element (TRE) were generated, and transgenic offspring were born with the expected Mendelian frequency and developed normally, indicating that the TRE-p210 transgene was not toxic. Transresponder mice were then mated to "transactivator" mice with tTA under control of the mouse mammary tumor virus long terminal repeat (MMTV-LTR) promoter/enhancer, known to express tTA in epithelial cells and bone marrow. Females were maintained on tetracycline beginning 5 days prior to mating to suppress expression of the *BCR/ABL* transgene. Double transgenic mice were obtained at the predicted Mendelian frequency and remained healthy as long as tetracycline administration was continued. Withdrawal of tetracycline resulted in development of fatal B-lymphoid leukemia with 100% incidence in all four lines. Surprisingly, readministration of tetracycline resulted in rapid disappearance

Table 1. *BCR/ABL* transgenic mice

Transgene	Promoter	Disease	Comments	Study*
BCR/v-*abl* hybrid (p210-like)	$E_\mu V_H$, MPSV LTR	B, T-ALL	Transgene silenced but expressed in tumors	[15]
p190 *BCR/ABL*	Metallothionein (δMT-1)	B-ALL	Transgene expressed widely at low levels	[16,17]
p210 *BCR/ABL*	*BCR*	Embryonic lethal	Lack of vasculature in more mature transgenic embryos	[30]
p210 *BCR/ABL*	Metallothionein (MT-1, δMT-1)	B, T-ALL	Longer disease latency and reduced penetrance than δMT-1/p190	[18,19]
p190 *BCR/ABL*	*BCR* (knock-in)	B-ALL	Leukemias induced in chimeras via blastocyst injection	[23]
p210 *BCR/ABL*	*tec*	T-ALL, MPD†	Long latency MPD with thrombocytosis and anemia; accelerated T-ALL in $p53^{+/-}$ background	[21•,27]
p210 *BCR/ABL*	MMTV-LTR-tTA TRE/p210	B-ALL	Conditional induction and maintenance of leukemia	[22•]
p190 *BCR/ABL* (C-terminal deletion)	Metallothionein (δMT-1)	B-ALL	Decreased frequency of induction of B-ALL with p190 mutant lacking F-actin-binding domain	[28]

*Transgenic mice are listed chronologically by publication date but discussed in the text in slightly different order.
†MPD (myeloproliferative disease) with moderate leukocytosis (leukocytes 15–20 × $10^3/\mu L$) and excess neutrophils.
B-ALL—B-cell acute lymphoblastic leukemia; LTR—long terminal repeat; MMTV—mouse mammary tumor virus; MPSV—myeloproliferative sarcoma virus; T-ALL—T-cell acute lymphoblastic leukemia.

of leukemic cells from blood and involved lymph nodes, with the majority of malignant lymphoblasts undergoing apoptosis [22•]. These studies demonstrate that *BCR/ABL*-induced lymphoid leukemia is absolutely dependent on continued expression of the *BCR/ABL* oncogene, even at advanced stages of disease.

Embryonic stem-cell–based approaches to *BCR/ABL* expression in mice

A distinct approach to expression of *BCR/ABL* in mice is through the use of embryonic stem (ES) cells. The embryonic stem-cell system has principally been used to inactivate genes by homologous recombination ("knock-out"), but the potential also exists to introduce oncogenes, either as simple transgenes or by homologous recombination into the corresponding endogenous murine gene ("knock-in"). ES cells can also be differentiated into multiple hematopoietic lineages in culture, allowing the opportunity to determine if oncogene expression might perturb hematopoietic development in vitro. Employing the knock-in approach, a p190 *BCR/ABL* transgene was created by homologous recombination in ES cells using a construct with fusion of mouse bcr exon 1 sequence with human *BCR* exon 1/*ABL* cDNA, with the resulting chimeric DNA expressed from the endogenous mouse *bcr* promoter [23]. Of 40 chimeric mice generated by blastocyst injection with the targeted ES cell clone, 38 developed B-lymphoid leukemia of the pre-B type by the age of 4 months. Other studies have examined the effect of *BCR/ABL* expression on hematopoietic differentiation of ES cells in vitro. Conditional expression (via the tet-regulatable system) of p210 *BCR/ABL* in ES cells stimulated multipotential progenitor expansion and myeloid lineage commitment [24•], whereas constitutive *BCR/ABL* expression in ES cells decreased the efficiency of embryoid body (EB) formation but increased the generation of myeloid hematopoietic progenitors in vitro [25]. More work is necessary to determine if these novel approaches will yield useful models of *BCR/ABL*-induced leukemia.

Applications of *BCR/ABL* transgenic mice

A great deal has been learned from *BCR/ABL* transgenic mice. These mice confirm that *BCR/ABL* can induce leukemia in vivo. The δMT-1-*BCR/ABL* transgenic mice express low levels of *BCR/ABL* in many tissues [17], but only lymphoid leukemias develop, suggesting further that *BCR/ABL* is a leukemia-specific oncogene. With transgenic mice that can be maintained as a line, it is easy to generate more animals for study by breeding. This has facilitated studies of the karyotypic evolution of the B-lymphoid leukemia in p190 *BCR/ABL* transgenic mice [20]. The conditional *BCR/ABL* transgenic mice will be particularly valuable for studying the earliest events in development of lymphoid leukemia and the mechanism of the anti-apoptotic action of *BCR/ABL*. It is also simple to breed a *BCR/ABL* transgene into different genetic backgrounds to test the role of other genes in leukemogenesis. This has allowed the demonstration that lymphoid leukemogenesis by p190 *BCR/ABL*

does not require the normal *bcr* gene product [26], whereas the tec-p210 transgenic mice rapidly develop T lymphomas rather than myeloproliferative disease in a p53 heterozygous background [27]. Although it is difficult, transgenic mice can be used to test the requirement of different functional domains of Bcr/Abl for leukemogenesis, and a recent study demonstrated that the C-terminal actin-binding domain was required for efficient development of lymphoid leukemia in mice [28]. Finally, *BCR/ABL* transgenic mice can be used for testing new therapies for Ph-positive leukemia, such as farnesyltransferase inhibitors [29].

Limitations of *BCR/ABL* transgenic mice

Unfortunately, *BCR/ABL* transgenic mice have several disadvantages as a model system. The most significant drawback is that, despite over a decade of effort in generation of *BCR/ABL* transgenic mice by conventional techniques, there is no transgenic mouse model of CML (with the possible exception of the tec/p210 *BCR/ABL* mice, which require further validation). The reasons for this are unclear, but the most plausible explanation may be lack of sufficient expression of the *BCR/ABL* transgene in myeloid and early progenitor/stem cells, because retroviral expression of *BCR/ABL* in multipotent progenitor cells does induce CML-like disease in mice, as noted herein. Activated *ABL* genes are known to have cytotoxic effects, and the presence of the transgene in all tissues raises the possibility of toxicity during embryonic development. Toxicity was initially suggested by the inefficient generation of *BCR*/v-*abl* transgenic founders and the failure of this transgene to express prior to development of overt malignancy [15], whereas a subsequent study directly confirmed that a *BCR/ABL* transgene expressed from the *BCR* promoter caused embryonic lethality [30]. The δMT-1 promoter should be expressed in myeloid progenitors, but this promoter may be downregulated in these transgenic mice because of transgene toxicity, and the resulting low levels of *BCR/ABL* expression are below a threshold required for oncogenic transformation by tyrosine kinases. This might explain the very low levels of bone marrow expression in young δMT-1/*BCR/ABL* transgenic mice, but much greater expression in the lymphoid leukemia cells [17]. In support of this hypothesis, when efforts were made to direct p210 *BCR/ABL* expression to myeloid cells by use of the CD11b or cathepsin G promoters, which are known to direct high-level position-independent expression of reporter transgenes to myeloid cells, the p210 transgenic founders that were obtained neither expressed detectable levels of *BCR/ABL* in their myeloid cells nor developed disease (Dziennis S, Van Etten RA, Tenen DG, Unpublished work; Pandolfi PP, Personal communication). Alternatively, it is possible that the bone marrow expression of these transgenes is heterogeneous and restricted to lymphoid progenitors. In the conditional *BCR/ABL* transgenic mice, bone marrow fractionation demonstrated that the MMTV-LTR/tTA transgene was expressed in $B220^+$ cells but not in $CD11b^+$ or $Thy1^+$ cells, which could explain the induction of B-lymphoid leukemia rather than myeloid leukemia in the double-transgenic mice. The use of myeloid-specific or stem-cell–specific promoters such as MRP8 or Sca-1 to express the tTA transactivator may allow conditional *BCR/ABL* expression in these compartments and the development of myeloid leukemia. Even an accurate transgenic mouse model of CML would have some drawbacks. In contrast with human disease, where the Ph translocation occurs in a single stem or progenitor cell, *BCR/ABL* transgenic mice carry the oncogene in all hematopoietic cells, which may alter the pathophysiology of a resultant leukemia or its response to therapy. Transgenic mice also have the disadvantage that the study of different isoforms or mutants of a given oncogene requires the generation, characterization, and maintenance of additional transgenic lines, a time-consuming and costly effort.

BCR/ABL Retroviral Bone Marrow Transduction/Transplantation Models

BCR/ABL induces multiple hematopoietic malignancies in recipients of transduced marrow, including CML-like myeloproliferative disease

Currently, the most accurate and informative animal model of CML involves transfer of the *BCR/ABL* gene into primary murine bone marrow cells ex vivo, followed by transplantation of the genetically modified cells into syngeneic or immunodeficient recipient mice. The preferred method of gene transfer has been replication-defective ecotropic retroviral vectors, due to their unparalleled efficiency at stable transduction of hematopoietic progenitor and stem cells, and because the retroviral DNA integrates into the cell chromosomal DNA and serves as a unique clonal marker of that cell and its progeny. Published studies utilizing the retroviral bone marrow transduction/transplantation model of *BCR/ABL* leukemogenesis are summarized in Table 2.

After considerable effort, a major breakthrough was achieved in 1990 when two groups were able to induce CML-like myeloproliferative disease and other hematologic malignancies in recipients of p210 *BCR/ABL*-transduced bone marrow [1,2]. The CML-like leukemia was a fatal myeloproliferative disease arising 4 to 12 weeks following transplantation, characterized by peripheral blood leukocytosis with greatly increased neutrophils, and infiltration of spleen, liver, and lungs with maturing myeloid cells, leading to hepatosplenomegaly, and ultimately death from respiratory failure. Bone marrow of these mice exhibited myeloid hyperplasia with minimal fibrosis and less than 10% blasts. The myeloid cells express Bcr/Abl protein, contain increased levels of tyrosine phosphorylated proteins, and carry the retroviral provirus in chromosomal DNA, confirming that the disease was directly induced by *BCR/ABL*. In addition to the CML-like disease, other recipients developed distinct hematopoietic malignancies, including B-lymphoid leukemia (similar to that observed in *BCR/ABL* transgenic mice), tumors of monocyte–macrophage lineage, and occasionally erythroleukemia and T-lymphoid

Table 2. *BCR/ABL* retroviral bone marrow transduction/transplantation studies

Oncogene	Disease*	Comments	Study
p210	CML, B-ALL, macrophage disease	Original demonstration that p210 induced CML-like disease in mice	[1]
p210, *v-abl*	CML, B-ALL, macrophage disease	Retroviral stocks contained helper virus; *v-abl* later found not to induce CML-like disease [42•,47]	[2]
p210	B-ALL, macrophage disease, EL	Did not observe CML-like disease in C57Bl/6 and DBA mouse strains	[31]
p210	CML, mast-cell disease, B/T-ALL	Secondary transplantation of CML-like disease and clonally related acute myeloid and lymphoid leukemias	[32]
p190, p210	CML, B-ALL, macrophage disease	p190 induced leukemias of shorter latency than p210	[48]
p210	CML, B-ALL, macrophage disease, EL	BCR/ABL-induced leukemias influenced by mouse strain and transduction conditions	[37]
p210	CML, B/T-ALL, macrophage disease	Efficient secondary transplantation of CML-like disease; heterogeneity of target cells	[33]
p210	CML, AML	SCID mouse recipients; $p53^{+/+}$ donor marrow gave long latency MPD, whereas $p53^{-/-}$ donors gave AML-like disease	[49]
p210 ΔSH2	CML, AML	SCID recipients; p210 SH2 mutants induced less aggressive CML/AML-like disease	[44]
p210	CML, T-ALL	Efficient induction and secondary transplantation of CML-like disease	[34•]
p210	CML	Efficient induction/secondary transplantation of CML; elevated plasma IL-3/GM-CSF	[35•]
p190, p210, p230	CML, B-ALL, macrophage disease	Efficient/similar induction of CML by all three oncogenes; p190 more potent for induction of B-ALL	[36•]
p210 b3a3	CML	SH3-deleted p210 efficiently induces CML-like disease	[41]
p210 + ICSBP	CML	Coexpression of ICSBP delays p210-induced CML	[50]
p210 Y177F	B, T-ALL	Grb2 binding site required for induction of CML-like disease by p210	[42•]
p190, p210	CML, B-ALL	*Stat5a/b* genes not absolutely required for induction of CML-like disease by *BCR/ABL*	[45•]
p190 SH2 muts, p210 SH2 muts	CML, B-ALL	SH2 domain contributes to efficient induction of CML-like disease but not required for B-ALL	[38,51]
p210	CML	*Il3* and *Gmcsf* genes not required for induction of CML-like disease by p210	[46]

*Disease phenotypes are defined in the text.
AML—acute myeloid leukemia; B-ALL—B-cell acute lymphoblastic leukemia; CML—chronic myeloid leukemia; EL—erythroleukemia; GM-CSF—granulocyte-macrophage colony-stimulating factor; ICSBP—interferon consensus sequence binding protein; IL—interleukin; muts—mutations; SCID—severe combined immunodeficient; T-ALL—T-cell acute lymphoblastic leukemia.

leukemia [1,2,31]. The CML-like disease could be transferred to secondary recipients by transplantation of marrow, but with low efficiency [32,33]. Some secondary recipients developed acute myeloid and lymphoid leukemia arising from the same provirally marked clone as the CML-like disease [32], suggestive of disease progression to blast crisis.

These seminal studies established that *BCR/ABL* is the direct cause of CML, but the inefficiency of induction of CML-like disease made it difficult to use the model to study the pathophysiology of CML in mice. Using transient retroviral packaging systems to improve virus titer, several groups recently reported efficient induction of CML-like disease in mice by p210 *BCR/ABL* [34•,35•,36•], where 100% of recipients of p210-transduced marrow succumbed to fatal CML-like disease, identical to that described earlier, within 4 weeks after transplantation. The CML-like disease in these mice was polyclonal by provirus integration rather than the monoclonal pattern observed previously [1], which confirmed increased transduction efficiency, and suggested that *BCR/ABL* transduction alone was sufficient to induce the CML-like disease. However, because the number of transduced target cells received by each animal was not known, this could not be definitively established by these studies. The CML-like disease was efficiently transplanted from most, but not all, primary mice by transfer of bone marrow or spleen cells. Interestingly, only a small subset of the clones present in the primary mice contributed to day-12 spleen colonies [36•] and induced disease in secondary recipients [35•,36•], suggesting heterogeneity between individual clones contributing to the expansion of myeloid cells in the primary animal, with only a minor subset capable of self-renewal as assessed by secondary transplantation.

Advantages and limitations of the retroviral transduction/transplantation model

Expression of *BCR/ABL* in vivo by retroviral transduction has a number of distinct advantages over transgenic mice. In this system, it is relatively easy to test different forms and mutants of *BCR/ABL*, simply by making new retroviral stocks. It is also easy, at least in principle, to test leukemogenicity of *BCR/ABL* in different genetic backgrounds and in mice with targeted mutations in different genes by employing mutant mice as donors and/or recipients in the system. The retroviral transduction system has been extended to the study of other leukemia oncogenes, although not all putative oncogenes induce leukemia in this system. Using simultaneous transduction of bone marrow with two distinct retroviruses, it is possible to observe synergistic interactions between two genes in leukemogenesis, but the relatively low incidence of cotransduction of stem cells with two viruses makes it implausible that dominant negative effects can be detected in such experiments. An alternative means to efficiently coexpress two genes is the use of retroviral vectors with the internal ribosome entry site (IRES) derived from encephalomyocarditis virus, which allows expression of two cistrons from the same proviral mRNA transcript. Coexpression of *BCR/ABL* with enhanced green fluorescent protein (eGFP) facilitates rapid titering of retroviral stocks and has been used to directly identify *BCR/ABL*-expressing cells in vivo [34•,35•].However, the retroviral transduction/transplantation model has several disadvantages. The biggest drawback is that generation of diseased mice for study requires performing a new transduction/transplantation procedure, which is somewhat laborious. An additional concern is that *BCR/ABL* is expressed from the retroviral LTR in most of the vectors utilized, which obviously differs from the *BCR* promoter that expresses *BCR/ABL* in human CML. The LTR is probably a stronger promoter than *BCR*, possibly accounting for the short survival of mice with *BCR/ABL*-induced CML-like disease. As a consequence, the current system does not model the long latent period required for human CML patients to develop clinical symptoms following exposure to ionizing radiation. However, the short latency of murine CML-like disease may also reflect the requirement for stem cells to be cycling in order to be transduced by retroviral vectors, and the use of different vectors or transduction conditions may allow models of CML latency to be developed in mice [33].

Studying the pathophysiology of CML using the retroviral transduction/transplantation model

The ability to induce CML-like disease in 100% of recipients of *BCR/ABL*-transduced marrow has allowed the system used as an actual assay for the first time to investigate the pathogenesis and pathophysiology of CML. A very important question is why transduction of *BCR/ABL* can induce CML-like disease under some circumstances, but other recipients instead develop B-cell acute lymphoblastic leukemia (B-ALL) and other hematologic malignancies. The type of leukemia induced by *BCR/ABL* is influenced by the transduction conditions [37]. When the donors are pretreated with 5-fluorouracil (5-FU), all recipients of *BCR/ABL*-transduced marrow develop CML-like disease, but when the donors are not treated with 5-FU, recipients instead develop a mixture of CML-like disease, B-ALL, and monocyte–macrophage tumors [36•]. Omission of both 5-FU and myeloid cytokines from the transduction protocol results in development of exclusively B-ALL in recipients [38]. The most plausible explanation for this is that these diseases are the consequence of transduction of different target cells within the bone marrow. In mice with *BCR/ABL*-induced CML-like disease, the same spectrum of proviral clones is observed in neutrophils, macrophages, nucleated erythroid progenitors, B-lymphoid cells, and sometimes T-lymphoid cells, suggesting that the target cell for the CML-like disease is an early multipotential progenitor/stem cell [36•]. In contrast, in mice with *BCR/ABL*-induced B-lymphoid leukemia or macrophage disease, the provirus is found in the malignant B-lymphoid and monocyte–macrophage cells, respectively, but not in normal myeloid cells or in CFU-S12 derived from these mice [36•], suggesting that the target cells for these diseases are distinct progenitors with lineage-restricted differentiation potential. With the most efficient retroviral transduction, all recipients receive multiple *BCR/ABL*-transduced stem cells and rapidly develop CML-like disease [34,•35•,36•]. However, under less efficient or altered transduction conditions, some animals may receive no transduced stem cells and survive to develop B-lymphoid and monocyte–macrophage malignancies of longer latency that may require additional events in addition to *BCR/ABL*. The existence of multiple *BCR/ABL*-induced leukemias arising from different target cells makes the system quite complex, and great care is required to avoid incorrect conclusions. Proof of this model of multitarget leukemogenesis by *BCR/ABL* will require isolation of these distinct target cells from bone marrow, and is an important goal of current work in this system. Defining the nature of the cells that initiate these leukemias in mice is clinically relevant, because it may lead to methods for isolating normal stem cells from leukemogenic cells in patients undergoing autologous transplantation.

One of the first uses of the efficient mouse model of CML was a comparison of the leukemogenic activity of the three principal forms of *BCR/ABL*: p190, p210, and p230. These oncogenes arise from different breakpoints on chromosome 22, generating three distinct fusion proteins that contain the same portion of c-Abl with different amounts of Bcr polypeptide sequence at the N-terminus and exhibit different intrinsic tyrosine kinase activity [36•]. In humans, the three forms of *BCR/ABL* are predominantly associated with distinct forms of leukemia [39]. The p210 form is of course found in chronic-phase CML, p190 is commonly observed in Ph-positive ALL but very rarely detected in CML, and p230 has been detected in several patients with a clinically mild variant of CML, chronic neutrophilic leukemia. These

observations raise the question of whether different forms of *BCR/ABL* have intrinsically different leukemogenic activity in hematopoietic cells. The retroviral transduction/transplantation model offers an ideal system to compare the in vivo leukemogenic activity of these different *BCR/ABL* oncogenes after transduction of an identical spectrum of hematopoietic cells. All three forms of *BCR/ABL* were equally potent in induction of CML-like disease when marrow from donors treated with 5-FU was employed [36•]. There was no significant difference in survival, peripheral blood leukocyte counts, or spleen weights at death, suggesting that all three *BCR/ABL* oncogenes induced a similar proliferative stimulus to myeloid cells under these conditions. These results do not support the hypothesis that p230 induces a distinct and less aggressive form of CML, and suggest that the rarity of p190 in chronic-phase CML may reflect infrequent *BCR* intron 1 breakpoints during the genesis of the Ph chromosome in stem cells, rather than intrinsic differences in myeloid leukemogenicity between p190 and p210.

The retroviral transduction/transplantation CML model can also be used to identify and validate the signaling pathways activated by *BCR/ABL* that are critical to leukemogenesis. It is already clear that results of in vitro studies of *BCR/ABL* signaling and transformation frequently do not correlate with leukemogenesis. Despite cell culture studies that suggested a role for the SH3 domain of Bcr/Abl in leukemogenicity [40], SH3 can be deleted from p210 Bcr/Abl with no effect on induction of CML-like disease in vivo [41]. In contrast, the p210 Bcr/Abl Y177F point mutant that cannot bind the Grb2 adapter protein is profoundly defective for induction of CML-like disease in mice, with recipients instead developing B-lymphoid leukemia or a novel T-cell lymphoma [42•]. This result resolves a long-standing controversy over the importance of direct binding of Grb2 to Bcr/Abl for leukemogenesis [43], and validates the Grb2 pathway as an important target for rational drug design in CML. The SH2 domain is not required for induction of B-lymphoid leukemia by *BCR/ABL* [38], but it contributes to efficient induction of CML-like disease in mice [38,44]. Although the SH2 domain of Bcr/Abl was initially reported to be required for activation of the phosphatidylinositol 3-kinase (PI 3-K) pathway in hematopoietic cells [44], subsequent studies found no defect in PI 3-K activation in cells transduced with *BCR/ABL* SH2 mutants [38], and the mechanism of the modest defect in induction of CML-like disease by these mutants requires further study. The model system can also be employed to determine the role of other genes in leukemogenesis by *BCR/ABL*, by employing mice with naturally occurring or targeted mutations in these genes as donors and/or recipients. *BCR/ABL* induces constitutive activation of STAT5 in hematopoietic cells, but studies in *Stat5a/b*-deficient mice demonstrate that STAT5 is not absolutely required for induction of CML-like disease in mice [45•]. Recently, aberrant transcripts for interleukin 3 (IL-3) and granulocyte-colony stimulating factor (G-CSF) have been identified in primitive Ph-positive human CML progenitors that may account in part for the autonomous in vitro growth of these cells [8]. Interestingly, increased plasma levels of IL-3 [35•,36•] and granulocyte macrophage colony stimulating factor (GM-CSF) [35•] have been detected in plasma of mice with *BCR/ABL*-induced CML-like disease. The *Il3* and *Gmcsf* genes have been deleted in mice through homologous recombination, and neither gene is required in donor or recipient for efficient induction of CML-like disease by p210 *BCR/ABL* [46]. These examples illustrate how this CML model system can provide important new knowledge about the pathogenesis of CML that would be difficult or impossible to obtain from studies of human patients or primary human CML cells. However, continued correlative studies between human CML and the murine CML-like disease are necessary, and conclusions reached in the mouse model system require validation, if possible, in human CML patients and cells.

Conclusions

It is apparent that we have learned an enormous amount about chronic myeloid leukemia in the recent past, and one could argue fairly convincingly that it is the best understood of all human malignant disorders. In no small part, this is because of what has been learned from animal models of the disease. The power of animal model systems derives from their ability to accurately recapitulate the complex physiology of CML in vivo. This suggests that the most productive future applications of these model systems will be in areas related to therapy of CML that are controversial today, such as identifying signaling pathways whose inhibition might complement Abl kinase inhibitor therapy, and mechanisms of graft-versus-leukemia effects. Critical advances in these areas will be facilitated by the careful and creative application of the animal model systems described in this review.

Acknowledgments

This work was supported by National Institutes of Health grant CA57593. The author is a Scholar of the Leukemia and Lymphoma Society of America and the Carl and Margaret Walter Scholar in Blood Research at Harvard Medical School.

References and Recommended Reading

Papers of particular interest, published recently, have been highlighted as:

- • Of importance
- •• Of major importance

1. Daley GQ, Van Etten RA, Baltimore D: **Induction of chronic myelogenous leukemia in mice by the P210bcr/abl gene of the Philadelphia chromosome.** *Science* 1990, **247:**824–830.
2. Kelliher MA, McLaughlin J, Witte ON, Rosenberg N: **Induction of a chronic myelogenous leukemia-like syndrome in mice with v-abl and bcr/abl.** *Proc Natl Acad Sci U S A* 1990, **87:**6649–6653.
3. Sawyers CL, Gishizky ML, Quan S, *et al.*: **Propagation of human blastic myeloid leukemias in the SCID mouse.** *Blood* 1992, **79:**2089–2098.
4. Skorski T, Nieborowska-Skorska M, Calabretta B: **A model of Ph' positive chronic myeloid leukemia-blast crisis cell line growth in immunodeficient SCID mice.** *Folia Histochem Cytobiol* 1992, **30:**91–96.
5. 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.
6. 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.
7. 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.
8. 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.
9. • 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.

See annotation for reference [10•].

10. • 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.

This article is one of three describing efficient engraftment of primary human CML cells in NOD/SCID mice.

11. • 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.

See annotation for reference [10•].

12. 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.
13. Holyoake T, Jiang X, Eaves C, Eaves A: **Isolation of a highly quiescent subpopulation of primitive leukemic cells in chronic myeloid leukemia.** *Blood* 1999, **94:**2056–2064.
14. Bhatia M, Wang JCY, Kapp U, *et al.*: **Purification of primitive human hematopoietic cells capable of repopulating immune-deficient mice.** *Proc Natl Acad Sci U S A* 1997, **94:**5320–5325.
15. 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.
16. Heisterkamp N, Jenster G, ten Hoeve J, *et al.*: **Acute leukemia in bcr/abl transgenic mice.** *Nature* 1990, **344:**251–253.
17. Voncken JW, Griffiths S, Greaves MF, *et al.*: **Restricted oncogenicity of *BCR/ABL* p190 in transgenic mice.** *Cancer Research* 1992, **52:**4534–4539.
18. Voncken JW, Kaartinen V, Pattengale PK, *et al.*: ***BCR/ABL* P210 and P190 cause distinct leukemia in transgenic mice.** *Blood* 1995, **86:**4603–4611.
19. Honda H, Fujii T, Takatoku M, *et al.*: **Expression of p210bcr/abl by metallothionein promoter induced T-cell leukemia in transgenic mice.** *Blood* 1995, **10:**2853–2861.
20. Voncken JW, Morris C, Pattengale P, *et al.*: **Clonal development and karyotype evolution during leukemogenesis of *BCR/ABL* transgenic mice.** *Blood* 1992, **79:**1029–1036.
21. • Honda H, Oda H, Suzuki T, *et al.*: **Development of acute lymphoblastic leukemia and myeloproliferative disorder in transgenic mice expressing p210bcr/abl: a novel transgenic model for human Ph1-positive leukemias.** *Blood* 1998, **91:**2067–2075.

This article describes the only existing transgenic mouse model of CML, but the myeloproliferative disease in these animals has some distinct differences from human CML.

22. • Huettner CS, Zhang P, Van Etten RA, Tenen DG: **Reversibility of acute B-cell leukaemia induced by *BCR/ABL*1.** *Nat Genet* 2000, **24:**57–60.

The first report of conditional expression of *BCR/ABL* in mice using the tetracycline-regulated transactivator in a binary transgene system.

23. Castellanos A, Pintado B, Weruaga E, *et al.*: **A *BCR/ABL*p190 fusion gene made by homologous recombination causes B-cell acute lymphoblastic leukemias in chimeric mice with independence of the endogenous bcr product.** *Blood* 1997, **90:**2168–2174.
24. • 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.

The first report of effects of *BCR/ABL* expression on hematopoietic differentiation of embryonic stem cells.

25. Turhan AG, Bonnet ML, Le Pesteur D, *et al.*: **Generation of an embryonic stem (ES) cell model of chronic myelogenous leukemia (CML) [abstract].** *Blood* 1999, **94(suppl 1):**101a.
26. Voncken JW, Kaartinen V, Groffen J, Heisterkamp N: **Bcr/Abl associated leukemogenesis in bcr null mutant mice.** *Oncogene* 1998, **16:**2029–2032.
27. 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.
28. Heisterkamp N, Voncken JW, Senadheera D, *et al.*: **Reduced oncogenicity of p190 Bcr/Abl F-actin-binding domain mutants.** *Blood* 2000, **96:**2226–2232.
29. Heisterkamp N, Groffen J: **Efficacy of a farnesyltransferase inhibitor for therapy of mice with p190 *BCR/ABL*-induced leukemia.** *Blood* 2001, **96:**2226-2232.
30. Heisterkamp N, Jenster G, Kioussis D, *et al.*: **Human bcr-abl gene has a lethal effect on embryogenesis.** *Transgen Res* 1991, **1:**45–53.
31. Elefanty AG, Hariharan IK, Cory S: **bcr-abl, the hallmark of chronic myeloid leukemia in man, induces multiple hematopoietic neoplasms in mice.** *EMBO J* 1990, **9:**1069–1078.
32. Daley GQ, Van Etten RA, Baltimore D: **Blast crisis in a murine model of chronic myelogenous leukemia.** *Proc Natl Acad Sci U S A* 1991, **88:**11335–11338.
33. Gishizky MI, Johnson-White J, Witte ON: **Efficient transplantation of *BCR/ABL*-induced chronic myelogenous leukemia-like syndrome in mice.** *Proc Natl Acad Sci U S A* 1993, **90:**3755–3759.
34. • 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.

See annotation for reference [36•].

35. • 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.

See annotation for reference [36•].

36.• 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 syndrome in mice but have different lymphoid leukemogenic activity.** *J Exp Med* 1999, **189:**1399–1412.

These three articles describe efficient induction of CML-like leukemia in mice by *BCR/ABL*, allowing the model to be used as an assay for the study of leukemogenesis.

37. Elefanty AG, Cory S: **Hematologic disease induced in BALB/c mice by a bcr/abl retrovirus is influenced by infection conditions.** *Mol Cell Biol* 1992, **12:**1755–1763.

38. 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.

39. Melo JV: **The diversity of *BCR/ABL* fusion proteins and their relationship to leukemia phenotype.** *Blood* 1996, **88:**2375–2384.

40. Skorski T, Nieborowska-Skorska M, Wlodarski P, *et al.*: **The SH3 domain contributes to *BCR/ABL*-dependent leukemogenesis in vivo: role in adhesion, invasion, and homing.** *Blood* 1998, **91:**406–418.

41. Gross AW, Zhang X, Ren R: **Bcr-Abl with an SH3 deletion retains the ability to induce a myeloproliferative disease in mice, yet c-Abl activated by an SH3 deletion induces only lymphoid malignancy.** *Mol Cell Biol* 1999, **19:**6918–6928.

42.• 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.

This article employs the retroviral transduction/transplantation model to demonstrate a requirement for the Grb2 binding site on Bcr/Abl for induction of CML-like leukemia in mice.

43. 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.

44. 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.

45.• 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.

This study used mice with homozygous inactivation of the *Stat5a* and *Stat5b* genes to demonstrate that STAT5 is not absolutely required for leukemogenesis by *BCR/ABL* and *v-abl*.

46. 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.

47. Scott ML, Van Etten RA, Daley GQ, Baltimore D: **v-abl causes hematopoietic disease distinct from that caused by bcr-abl.** *Proc Natl Acad Sci U S A* 1991, **88:**6506–6510.

48. Kelliher M, Knott A, McLaughlin J, *et al.*: **Differences in oncogenic potency but not target cell specificity distinguish the two forms of the *BCR/ABL* oncogene.** *Mol Cell Biol* 1991, **11:**4710–4716.

49. Skorski T, Nieborowska-Skorska M, Wlodarski P, *et al.*: **Blastic transformation of p53-deficient bone marrow cells by p210bcr/abl tyrosine kinase.** *Proc Natl Acad Sci U S A* 1996, **93:**13137–13142.

50. Hao SX, Ren R: **Expression of interferon consensus sequence binding protein (ICSBP) is downregulated in Bcr-Abl-induced murine chronic myelogenous leukemia-like disease, and enforced coexpression of ICSBP inhibits Bcr-Abl induced myeloproliferative disorder.** *Mol Cell Biol* 2000, **20:**1149–1161.

51. 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