# 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

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**Summary**

The product of the Philadelphia chromosome (Ph) translocation, the *BCR/ABL* oncogene, exists in three principal forms (P190, P210, and P230 *BCR/ABL*) that are found in distinct forms of Ph-positive leukemia, suggesting the three proteins have different leukemogenic activity. We have directly compared the tyrosine kinase activity, in vitro transformation properties, and in vivo leukemogenic activity of the P190, P210, and P230 forms of *BCR/ABL*. P230 exhibited lower intrinsic tyrosine kinase activity than P210 and P190. Although all three oncogenes transformed both myeloid (32D cl3) and lymphoid (Ba/F3) interleukin (IL)-3–dependent cell lines to become independent of IL-3 for survival and growth, their ability to stimulate proliferation of Ba/F3 lymphoid cells differed and correlated directly with tyrosine kinase activity. In a murine bone marrow transduction/transplantation model, the three forms of *BCR/ABL* were equally potent in the induction of a chronic myeloid leukemia (CML)–like myeloproliferative syndrome in recipient mice when 5-fluorouracil (5-FU)–treated donors were used. Analysis of proviral integration showed the CML-like disease to be polyclonal and to involve multiple myeloid and B lymphoid lineages, implicating a primitive multipotential target cell. Secondary transplantation revealed that only certain minor clones gave rise to day 12 spleen colonies and induced disease in secondary recipients, suggesting heterogeneity among the target cell population. In contrast, when marrow from non–5-FU–treated donors was used, a mixture of CML-like disease, B lymphoid acute leukemia, and macrophage tumors was observed in recipients. P190 *BCR/ABL* induced lymphoid leukemia with shorter latency than P210 or P230. The lymphoid leukemias and macrophage tumors had provirus integration patterns that were oligo- or monoclonal and limited to the tumor cells, suggesting a lineage-restricted target cell with a requirement for additional events in addition to *BCR/ABL* transduction for full malignant transformation. These results do not support the hypothesis that P230 *BCR/ABL* induces a distinct and less aggressive form of CML in humans, and suggest that the rarity of P190 *BCR/ABL* in human 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.

Key words: Abelson virus • retroviral vector • chronic myelogenous leukemia • mouse cancer model

The *BCR/ABL* fusion oncogene, the product of the t(9;22) Philadelphia chromosome (Ph),[1] exists in three principal forms (P190, P210, and P230) that arise from distinct breakpoints in the *BCR* gene on chromosome 22, resulting in translocation of *BCR* exon 1 (1), exons 1–12/13 (2), or exons 1–19 (3), respectively, to the c-*ABL* gene on chromosome 9. These different oncogenes give rise to three distinct fusion proteins of molecular mass 190, 210, and 230 kD, which contain the same portion of the c-Abl tyrosine kinase in the COOH terminus but include different amounts of Bcr sequence at the $NH_2$ terminus.

[1]*Abbreviations used in this paper:* ALL, acute B lymphoid leukemia; CML, chronic myeloid leukemia; 5-FU, 5-fluorouracil; GST, glutathione *S*-transferase; MSCV, murine stem cell virus; Ph, Philadelphia (chromosome); STAT, signal transducer and activator of transcription.

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In humans, there is evidence that the three different forms of *BCR/ABL* are associated with distinct forms of leukemia (for reviews, see references 4 and 5). The P210 form of *BCR/ABL* is found in hematopoietic cells of patients with chronic myeloid leukemia (CML) in stable phase, and in acute lymphoid and myeloid leukemias (6–8), although some patients with acute leukemia and P210 are likely to be cases of CML diagnosed in blast crisis. In contrast, the P190 form of *BCR/ABL* is commonly found in Ph-positive acute B lymphoid leukemia (ALL [9]) and occasionally in acute myeloid leukemia (10), but is rarely if ever observed in CML (11–13). Recently, several patients were described who had a Ph-positive myeloproliferative syndrome resembling CML but with very mild clinical symptoms, including a lower peripheral blood white cell count consisting principally of neutrophils, less anemia, less severe splenomegaly, and a delayed or absent transformation to blast crisis (14). These patients were found to have the *BCR* exon 19/*ABL* exon 2 (e19/a2) junction characteristic of P230 *BCR/ABL*. It was proposed that patients with the e19/a2 fusion comprise a distinct clinical entity called neutrophilic CML, with a much more benign clinical course than that associated with the traditional b2/a2 or b3/a2 P210 *BCR/ABL* fusion. However, several subsequent reports described patients with the e19/a2 fusion and typical CML (15–17). These observations raise the question of whether different forms of Bcr/Abl protein have intrinsically different leukemogenic activity in hematopoietic cells.

Consideration of the primary structures of P190, P210, and P230 indicates that P210 and P230 contain several potential functional motifs encoded by the *BCR* portion of the fusion gene (Cdc24/Dbl and plekstrin homology domains and a portion of a RacGAP catalytic domain, respectively) that might plausibly alter the oncogenic activity of these larger proteins relative to P190. Indeed, characterization of the tyrosine kinase and in vitro transformation activities of P190 and P210 Bcr/Abl has suggested that these proteins have distinct transforming properties. In an immune complex kinase assay, P190 has higher tyrosine kinase than P210, with P190 and P210 activity both elevated relative to c-Abl (18, 19). P190 is more efficient than P210 in transformation of Rat-1 fibroblasts, as assessed by colony formation in soft agar (18). Both P210 and P190 *BCR/ABL* can stimulate the growth of primary B cells in long-term in vitro cultures, eventually leading to oligoclonal populations of pre-B cells that are independent of stroma and leukemogenic in syngeneic mice (20). P190 is more potent than P210 in this growth-stimulatory assay, with P190-transduced cultures reaching high cell density and stromal independence more frequently and sooner than with P210 (21). The mechanism of increased growth stimulation of B lymphoid cells by P190 is not known. Most studies of P190 and P210 have found similar sets of tyrosine-phosphorylated proteins in cells transformed by the two oncogenes (22). A notable exception is signal transducer and activator of transcription STAT6, a transcription factor normally activated by IL-4 and implicated in lymphoid proliferative responses (23) that is preferentially tyrosine phosphorylated and activated by P190 but not P210 Bcr/Abl (24).

The only model system where Bcr/Abl induces a myeloproliferative syndrome closely resembling human CML is retroviral transduction of mouse bone marrow in vitro, followed by transplantation of transduced cells into irradiated syngeneic recipients (for a review, see reference 25). In this assay, the P210 form of *BCR/ABL* induced a fatal CML-like syndrome with massive elevation of neutrophils and maturing myeloid cells in peripheral blood, marrow, spleen, and liver (26–29). In addition to the CML-like syndrome, other transplanted mice developed leukemias of B lymphoid, monocyte/macrophage, T lymphoid, or erythroid lineages (26, 30). Because these mice develop leukemias that closely resemble several human Ph-positive leukemias, the bone marrow transduction/transplant model is perhaps the best system for directly comparing the leukemogenic activity of different forms of *BCR/ABL*. However, as originally described, the system was hampered by difficulty in generating high-titer replication-defective *BCR/ABL* retrovirus stocks, leading to a low efficiency of generation of diseased animals. Under the best circumstances, the CML-like syndrome was observed in only ~25% of transplant recipients, and some transplanted mice did not develop any malignancies (26). Despite these difficulties, P190 was observed to induce a similar CML-like syndrome in mice at low frequency (31). In experiments which used replication-competent Moloney murine leukemia helper virus to increase transduction efficiency, P190 was also observed to induce myeloid leukemias with excess neutrophils, suggestive of CML (32). In addition, the latency of P190-induced myeloid and lymphoid leukemias after transplant was shorter than for P210, suggesting that P190 was more potent in the induction of both types of leukemia.

With modifications to the original bone marrow transduction protocol, we and others (33) have recently been able to obtain 100% incidence of the CML-like syndrome in mice transplanted with P210 *BCR/ABL*-transduced marrow, allowing this method to be used as an assay for the first time. Here, we report a direct comparison of the tyrosine kinase activity, in vitro transformation properties, and in vivo leukemogenic activity of the P190, P210, and P230 forms of *BCR/ABL*.

## Materials and Methods

*DNA Constructs.* A cDNA encoding the P230 form of *BCR/ABL* was generated by a PCR strategy. A 5′ primer containing the AatII site at nucleotide 3184 in the *BCR* cDNA open reading frame and the adjacent e19/a2 *BCR/ABL* junction sequence and a 3′ primer containing the KpnI site at nucleotide 744 of the human c-*ABL* type Ia cDNA were used to generate a 307-bp PCR product from AatII to KpnI, spanning the P230 *BCR/ABL* junction. The product was subcloned and completely sequenced to verify the correct reading frame and lack of other mutations, then used to generate a complete P230 cDNA. The P190, P210, and P230 *BCR/ABL* cDNAs were introduced as EcoRI fragments into the unique EcoRI site of the murine stem cell virus (MSCV)-based retroviral vector, MSCVneoEB (34).

*In Vitro Kinase Assay.* Immunoprecipitation of Abl proteins from transfected 293 cells or transduced Ba/F3 cells and immune

complex kinase assay was as described previously (19, 35), except that a glutathione *S*-transferase (GST)-c-$Crk_{120–225}$ fusion protein was used as an exogenous substrate. To normalize for the amount of Abl proteins, cells were labeled for 12 h with [$^{35}$S]l-methionine before harvesting. The amount of $^{32}$P incorporation was quantitated by PhosphorImager analysis (STORM 850; Molecular Dynamics), whereas $^{35}$S incorporation was quantitated by digital camera and analysis by NIH Image v1.59 software. Relative protein levels, which were within 15% of one another, were corrected for methionine content and used to normalize $^{32}$P incorporation to calculate relative kinase activity.

*Generation of Retrovirus Stocks.* All DNAs were purified by two rounds of buoyant density centrifugation in CsCl. 10 μg of retroviral vector DNA and 5 μg of MCV-ecopac, an ecotropic single-genome packaging construct (36), were transfected per 6-cm dish of 293T cl. 17 cells (37), as described (36). Medium was changed at 24 h, and virus supernatant was harvested at 48 h after transfection. Supernatant was passed through a 0.45-μ filter, aliquoted, and frozen at −80°C. An aliquot was thawed, the virus titer for neomycin resistance was determined by transduction of NIH 3T3 cells, and a screen for the presence of replication-competent helper virus was carried out with a *lacZ*-3T3 indicator cell line. All viruses had titers of 3.5–4.0 × $10^6$ neomycin-resistant CFU/ml, passed the correct proviral structure by Southern blot analysis, and were free of detectable helper virus activity.

*Transformation of Cytokine-dependent Hematopoietic Cell Lines.* 32D cl3 cells and Ba/F3 cells were grown, transduced, and selected for G418 resistance and IL-3 independence as described previously (19). At 96 h after transduction, viable cells were counted and plated at 4 × $10^4$ cells in 1.0 ml RPMI medium without IL-3 and neomycin in triplicate wells of a 24-well plate. Cells were incubated at 37°C, and the viable cell count was determined daily for 3 d.

*Bone Marrow Transduction and Transplantation.* All animal studies were approved by the Animal Use and Care Committees of the Center for Blood Research and Harvard Medical School. Balb/c mice (The Jackson Laboratory or Taconic Farms) from 6 to 12 wk of age were used in all experiments. In some experiments, male donor mice were primed by intravenous injection with 5-fluorouracil (5-FU; 200 mg/kg) 4 d before harvest. Male donors were killed by $CO_2$ asphyxiation, femur and tibia were collected, and bone marrow was harvested by flushing with syringe and 26-gauge needle. Cells were counted and plated without removal of erythrocytes at 2 × $10^7$ cells per 10-cm plate in prestimulation medium (38) of DME, 15% (vol/vol) inactivated FCS, 5% (vol/vol) WEHI-3B conditioned medium, penicillin/streptomycin, 1.0 μg/ml ciprofloxacin, 200 μM l-glutamine, 6 ng/ml recombinant murine IL-3 (Genzyme), 10 ng/ml recombinant murine IL-6 (Genzyme), and 50–100 ng/ml recombinant murine stem cell factor (SCF; PeproTech). With non–5-FU–treated marrow, 10 ng/ml recombinant murine IL-7 (Genzyme) was also included. After prestimulation for 24 h at 37°C, viable cells were counted and transduced with retroviral stocks in the same medium containing 50% retroviral supernatant, 10 mM Hepes, pH 7.4, and 2 μg/ml polybrene. To increase transduction efficiency (39), virus and cells were cosedimented at 1,000 *g* for 90 min in a Sorvall RT-6000 centrifuge. Medium was changed after a 2–4-h adsorption period. At 48 h, a second round of transduction and cosedimentation was performed, and the cells were collected 2 h later, washed once in HBSS, and counted. Recipient female mice were prepared by two doses of 450-cGy gamma irradiation separated by 3 h. Transduced marrow cells were transplanted by injection of 0.2–0.5 × $10^6$ cells (5-FU–treated marrow) or 1.0 × $10^6$ cells (non–5-FU–treated marrow) in 0.4–0.5 ml HBSS into the lateral tail vein. After transplant, recipients were housed in microisolator cages supplied with acidified (pH 2.0) water.

*Analysis of Diseased Mice.* After transplant, recipient mice were evaluated daily for signs of morbidity, weight loss, failure to thrive, and splenomegaly. Premorbid animals were killed by $CO_2$ asphyxiation, peripheral blood was obtained from the retroorbital venous plexus, and hematopoietic tissues were removed. Depending on the individual animal, hematopoietic tissues and cells were used for several applications, including histopathology, in vitro culture, FACS® analysis, secondary transplantation, genomic DNA preparation, protein lysate preparation, or lineage analysis (see below). The clinical features and histopathology of *BCR/ABL*-induced CML-like disease, B lymphoid leukemia, and macrophage tumors were very similar to those observed previously (26).

*Lineage Analysis.* Peripheral blood samples generally served as a nearly pure source of neutrophils and their precursors; on occasion, neutrophils were purified further by positive selection for Ly-6G (Gr-1) antigen expression by immunomagnetic beads (MicroMACS®; Miltenyi Biotec). Macrophages were isolated from peritoneal washes, bone marrow, liver, or spleen by adherence and often subsequent culture on bacterial petri dishes in the presence of L929-conditioned medium as a source of CSF-1. Erythroid cells were purified from spleen by sedimentation through Ficoll-hypaque or by positive selection with TER119 mAb and immunomagnetic beads. B lymphoid cells were purified from pooled lymph nodes or spleen by positive selection with anti-B220 mAb, and T cells were purified by isolation of thymocytes or by positive selection of spleen or peripheral blood with anti–Thy-1.2 mAb. In all cases, the purity of the population was assessed by Wright-Giemsa staining of a cytospin specimen, and only those samples with at least 80% purity as judged from cell morphology were used for Southern blot analysis. Genomic DNA was prepared from each population, digested with BglII, and hybridized with a radioactive probe from the proviral neomycin resistance gene to determine the number of distinct proviral integrations in each sample. Subsequently, the blots were stripped and reprobed with a radioactive probe from the human c-*ABL* gene that detects a common 2.2-kb fragment from all proviruses, allowing determination of the total proviral content of each hematopoietic lineage. To control for differences in DNA loading, the intensity of hybridization of a fragment from the endogenous mouse c-*abl* gene was used as an internal control. Intensity of hybridization was determined by PhosphorImager analysis (Molecular Dynamics), and the ratio of intensity of hybridization of the *BCR/ABL* fragment to murine c-*abl* fragment was compared with that of genomic DNA standards containing a single proviral copy, with the results expressed as proviral copy number per diploid genome. A proviral copy number of 0.2 or less is consistent with the absence of provirus from a particular hematopoietic lineage.

*Secondary Transplants and Day 12 Spleen Colony Assay.* For adoptive transfer of the CML-like syndrome, 1–2 × $10^6$ bone marrow and/or splenocytes from primary animals were injected intravenously into sublethally irradiated (450 cGy) female Balb/c recipient mice. For isolation of day 12 spleen colonies, pairs of lethally irradiated (900 cGy) Balb/c mice were injected intravenously with 3 × $10^3$, 1 × $10^4$, and 3 × $10^4$ nucleated marrow cells from primary animals. Recipients were killed 12 d later, spleens were isolated, and macroscopic colonies were dissected out with the aid of a stereoscopic microscope. For those colonies not visibly red in appearance, cytospin preparations were examined by Wright-Giemsa staining to ensure the mixed myeloid origin of the colony. Genomic DNA was then prepared and analyzed as described above.

*Western Blot Analysis.* Protein lysates were prepared from peripheral blood myeloid cells by resuspension of $10^7$ cells in 75 μl RIPA buffer with immediate addition of an equal volume of 95°C 2× sample buffer and boiling for 10 min. Amounts of protein were standardized by SDS-PAGE and Coomassie blue staining. From other hematopoietic tissues and cultures, cell lysates in RIPA were quantitated by Bio-Rad protein assay. SDS-PAGE and Western blotting with anti-Abl (8E9; PharMingen) and antiphosphotyrosine (4G10; Upstate Biotechnology, Inc.) antibodies were performed as described (19).

## Results

*P230 Has Lower In Vitro Kinase Activity Than P210 and P190.* Previous studies indicated that P190 Bcr/Abl had intrinsically higher tyrosine kinase activity, measured as autophosphorylation or phosphorylation of an exogenous substrate, than did P210, while both were higher than c-Abl (18, 19). In a direct comparison after immunoprecipitation from transfected 293 cells, we again confirmed this and found a significantly lower kinase activity for P230 Bcr/Abl (Fig. 1). Corrected for levels of Abl protein, P190 had 7-fold increased phosphorylation of a GST-Crk substrate relative to c-Abl, whereas P210 and P230 had 5.4- and 3.7-fold increased activity, respectively, with similar results for autophosphorylation. There was no difference in the kinase activity of c-Abl and the oncogenic SH3-deleted c-Abl protein, as observed previously (40). Similar results were obtained when the Bcr/Abl proteins were immunoprecipitated from stably transformed Ba/F3 cells (see below; data not shown).

*The Three Forms of BCR/ABL Induce Different Proliferative Responses in Cultured Lymphoid Cells.* To compare the transforming ability of the three *BCR/ABL* oncogenes in cultured hematopoietic cells, we introduced them by retroviral transduction into IL-3–dependent myeloid (32D cl3) and lymphoid (Ba/F3) cell lines. P210 *BCR/ABL* has been previously demonstrated to transform each cell line to become independent of IL-3 for survival and growth (41, 42) by a mechanism that does not involve autocrine production of growth factors. To avoid any differences due to prolonged culture in the presence of *BCR/ABL*, populations of transduced cells were selected in neomycin and immediately deprived of IL-3, with measurement of cell survival and proliferation within 96 h of transduction. With both cell types, populations of cells that were independent of IL-3 for survival and growth were selected with equal efficiency after transduction with all three forms of *BCR/ABL*, but not with the parental MSCVneoEB virus, demonstrating that P190, P210, and P230 are all capable of transforming myeloid and lymphoid cells to cytokine independence (Fig. 2). There was no difference in the growth rate of 32D cells transduced with any of the three forms of *BCR/ABL* in the absence of IL-3 or of vector-transduced cells in the presence of IL-3 (Fig. 2 A). In contrast, *BCR/ABL*-transduced Ba/F3 cells in the absence of IL-3 proliferated more slowly than vector-transduced cells in the presence of IL-3, with P190-transduced cells showing the highest proliferation rate, followed by P210 and then P230 (Fig. 2 B). The level of

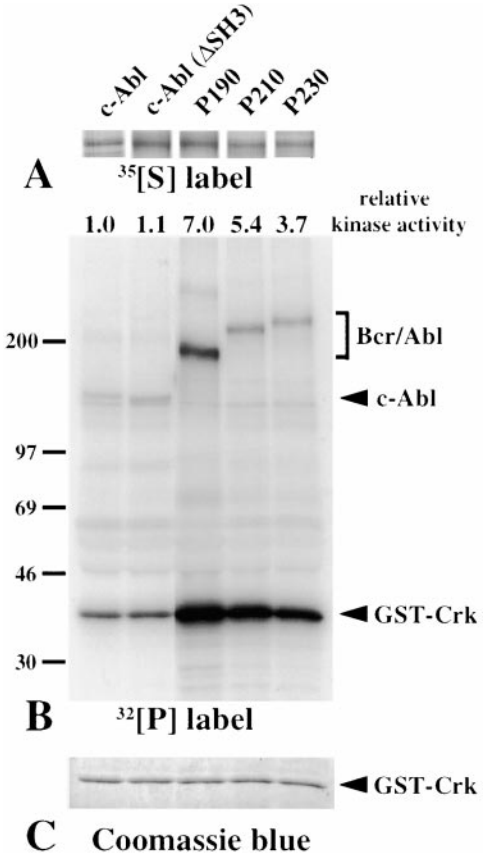


**Figure 1.** In vitro kinase activity of Bcr/Abl proteins. The indicated Abl proteins were expressed by transient transfection of 293 cells, immunoprecipitated, and incubated with [γ-$^{32}$P]ATP and GST-Crk substrate as described in Materials and Methods. (A) $^{35}$S incorporation into immunoprecipitated Abl proteins. (B) $^{32}$P label. The positions of c-Abl and Bcr/Abl proteins and the GST-Crk substrate are indicated. (C) Coomassie blue stain indicating equal amounts of GST-Crk substrate in each reaction. The transkinase activity of each Abl protein, relative to c-Abl, is given at the top.

expression of the three Bcr/Abl proteins and the spectrum of tyrosine phosphorylated proteins were similar within each of the two cell types (data not shown). These results suggest that the three forms of *BCR/ABL* induce an identical proliferative response in myeloid factor-dependent cells, but deliver a submaximal growth stimulus in lymphoid cells that parallels their intrinsic tyrosine kinase activity.

*All Three Forms of BCR/ABL Induce an Identical CML-like Syndrome in Mice.* To compare directly the ability of the three forms of *BCR/ABL* to induce a myeloproliferative syndrome in mice, we used the bone marrow transduction/transplantation model system (26), with several modifications to the original protocol designed principally to increase transduction efficiency (see Materials and Methods). With the modified protocol, we observed that all mice transplanted with bone marrow transduced with each of the three forms of *BCR/ABL* developed a fatal CML-like myeloproliferative syndrome within 4 wk after transplantation when marrow from 5-FU–treated donors was used (Fig. 3). The disease was characterized principally by massive expansion of maturing myeloid cells in bone marrow,

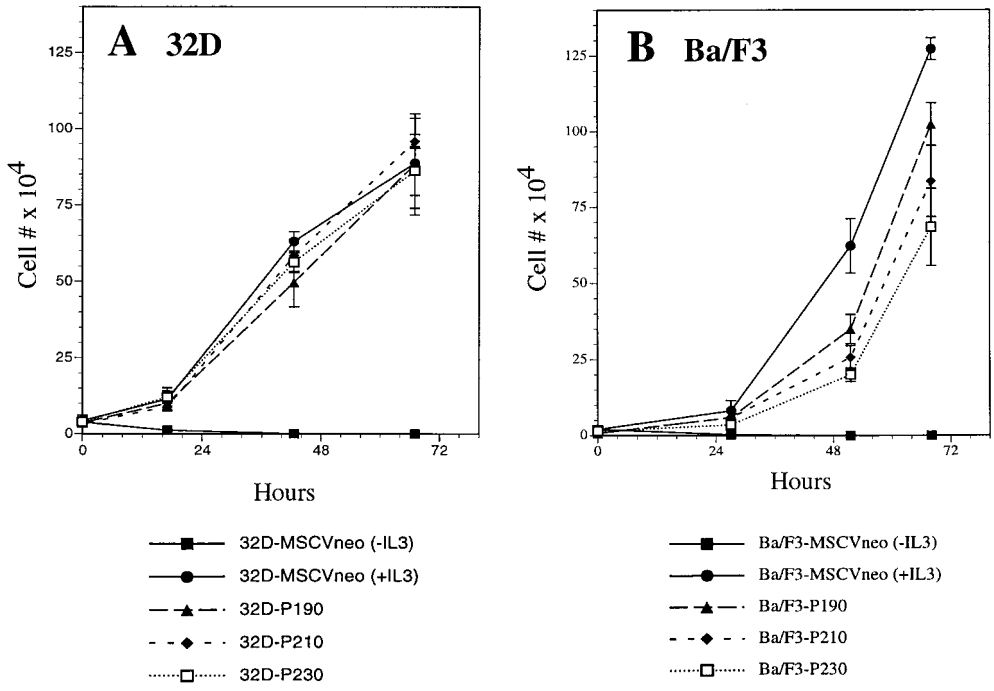


**Figure 2.** Proliferation of factor-dependent hematopoietic cells transformed by the three *BCR/ABL* oncogenes. (A) 32D cl3 myeloid cells. (B) Ba/F3 lymphoid cells. In each case, MSCVneo vector–transduced cells were grown in either the presence or absence of IL-3. The difference between the day 2 mean cell number for Ba/F3-MSCVneo (+IL-3) and Ba/F3-P190, and between Ba/F3-P190 and Ba/F3-P210 or Ba/F3-P230 was significant ($P = 0.05$, *t* test). The difference between the day 2 mean cell number between Ba/F3-P210 and Ba/F3-P230 was not significant.

spleen, liver, and peripheral blood. The peripheral blood leukocyte count at death was 2–4 $\times$ $10^5/\mu$l, composed predominantly of mature neutrophils, along with metamyelocytes, myelocytes, and promyelocytes, indicative of a shift towards less differentiated myeloid cells. The spleen and liver were greatly enlarged and disrupted by large numbers of maturing myeloid cells from the neutrophil lineage, along with significant erythropoiesis and increased megakaryocytes. Nearly all mice with the CML-like syndrome also had small to medium-sized collections of macrophages centered on portal areas in the liver. In addition, all animals had focal consolidation of the lungs with maturing myeloid cells and extensive intraparenchymal hemorrhage, which may have been the ultimate cause of death of these animals. Bone marrow showed increased cellularity with maturation and an increased myeloid to erythroid ratio, while lymph nodes and thymus were normal. In most cases, the CML-like syndrome could be efficiently transferred to secondary recipients by injection of bone marrow (data not shown), but as previously reported (43), for some primary animals the disease was not readily transplanted.

There was no significant difference in the histopathology, average survival, peripheral blood leukocyte count, or spleen weight of the disease induced by the three forms of *BCR/ABL* (Fig. 3 insert, and data not shown). Because the spleen is the main reservoir of myeloid cells in these diseased mice, this implies that mice transplanted with P190-, P210-, and P230-transduced marrow have identical total myeloid cell burdens at death, indicating that the proliferative stimulus induced in myeloid cells by the three *BCR/*

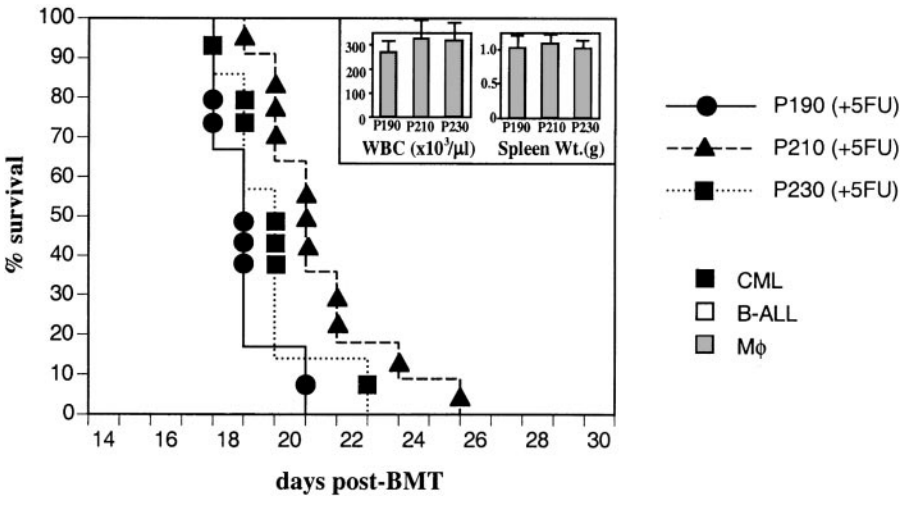


**Figure 3.** The three forms of *BCR/ABL* induce an identical CML-like disease in mice. Survival curve for recipients of *BCR/ABL*-transduced marrow from 5-FU–treated donors. The individual mice in each arm are indicated by the symbols. All animals developed the same CML-like syndrome (filled symbols). Similar survival curves were observed in three independent transplant experiments (data not shown). (Insert) Similar myeloid cell levels in mice with CML-like disease induced by the three forms of *BCR/ABL*. Average $\pm$ SE (bars) of peripheral blood leukocyte count ($\times 10^3/\mu$l, left) and spleen weight (in grams, right) for the three different *BCR/ABL* genotypes. The difference in mean survival of P190-transduced versus P210-transduced mice was significant at $P = 0.1$ but not at $P = 0.05$ (*t* test); none of the other pairwise differences in survival, leukocyte count, or spleen weight were significant ($P = 0.1$). BMT, bone marrow transplantation.

*ABL* oncogenes is very similar. Although human P190$^+$ CMLs may be characterized by monocytosis (13), the percentage of monocytes in peripheral blood of mice with CML-like disease was <10% and did not differ significantly between *BCR/ABL* genotypes (data not shown).

*The CML-like Syndrome Is Polyclonal and Involves Several Hematopoietic Lineages, Suggesting a Primitive Multipotential Target Cell.* Mice with the CML-like syndrome were analyzed for the presence of Bcr/Abl protein by Western blot and for the *BCR/ABL* provirus by Southern blot of genomic DNA. Bcr/Abl protein was detected in peripheral blood leukocytes (principally neutrophils and their precursors; Fig. 4 A) and in peritoneal macrophages (Fig. 4 B), confirming the expression of the *BCR/ABL* provirus in two distinct myeloid lineages. To identify the *BCR/ABL* provirus, genomic DNA from total spleen, liver, peripheral blood, or bone marrow was digested with XbaI (which cuts once in each proviral LTR) or BglII (which cuts once in proviral DNA sequences 3′ of the *BCR/ABL* cDNA insert) and probed with a radioactive fragment from the neomycin resistance gene. The XbaI digest indicated that these tissues contained the *BCR/ABL* provirus at about one proviral copy per cell (Fig. 5), confirming that the increased myeloid cells in these animals were a primary part of the malignant process and not a secondary or reactive phenomenon (30, 44). The BglII digest, which yields a distinct hybridizing fragment for each unique proviral integration site, demonstrated that multiple independent clones contributed to the myeloid cell expansion in these mice (Fig. 5), with 4–12 clones (average 9) observed in recipients of P190-, P210-, and P230-transduced marrow. Interestingly, the relative abundance of some clones differed between DNA samples from the same animal, suggesting a variable contribution of individual clones to myelopoiesis in different tissues. The presence of polyclonal disease suggests that the presence of *BCR/ABL* alone is sufficient to induce the CML-like syndrome.

To determine which hematopoietic lineages were involved in the malignant process, we performed lineage analysis by purifying different hematopoietic cell populations and determining the *BCR/ABL* proviral status by Southern blot. With all three oncogenes, mice with the CML-like syndrome carried the *BCR/ABL* provirus at or above single-copy levels in neutrophils, macrophages, erythroid cells, and splenic B lymphoid cells, and in some cases peripheral node lymphocytes and thymocytes (Fig. 6, A–C). Most of the independent proviral clones present were found in each lineage. These data indicate transduction of a bone marrow target cell capable of differentiation via multiple myeloid and lymphoid pathways, suggesting that the *BCR/ABL* target cell for the CML-like syndrome is a multipotential progenitor cell.

To further define the nature of this target cell, we performed secondary transplant experiments to isolate day 12 spleen colonies derived from selected primary mice with the CML-like syndrome. Such colonies are clonal, of mixed myeloid origin, have limited self-renewal potential, and are de-

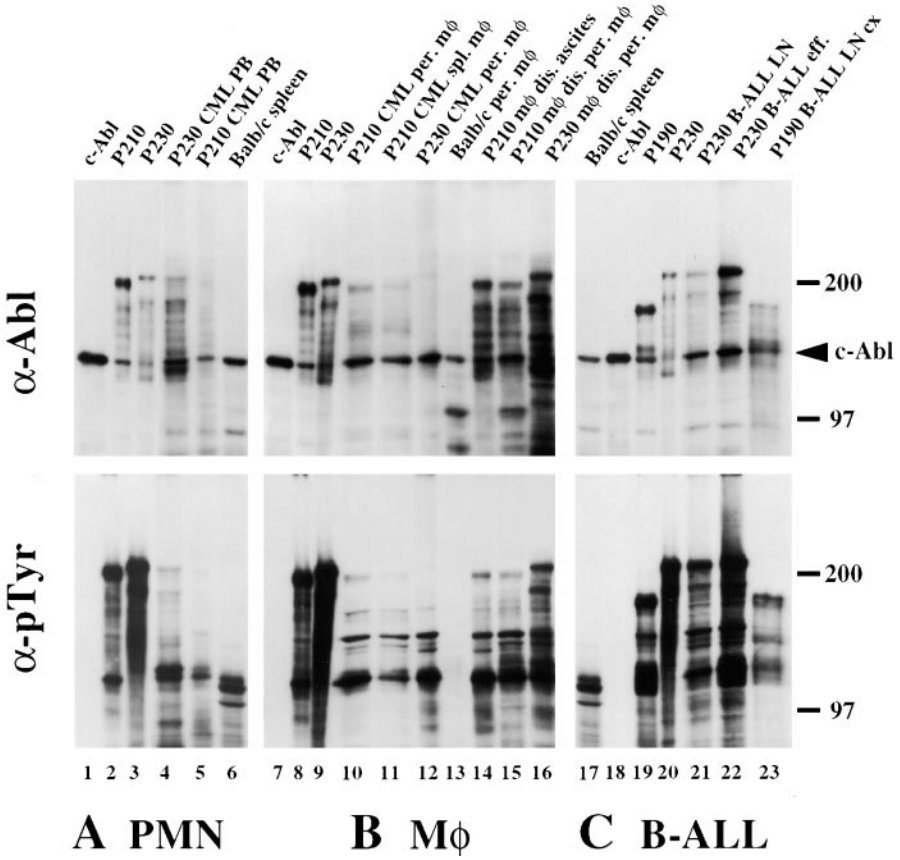


**Figure 4.** Detection of Bcr/Abl protein in primary hematopoietic cells from mice with *BCR/ABL*-induced leukemias. Extracts from cell lines expressing c-Abl or P190, P210, or P230 Bcr/Abl are included in each panel as size markers (lanes 1–3, 7–9, and 18–20). Control extracts from splenocytes or thioglycollate-induced peritoneal macrophages from untransduced Balb/c mice are included to demonstrate specificity of anti-Abl staining (lanes 6, 13, and 17). Top panels are anti-Abl blots; bottom panels are the same membranes blotted with antiphosphotyrosine (pTyr) antibody. (A) Peripheral blood (predominantly neutrophils [PMN]) from two mice with P230- (lane 4) and P210-induced (lane 5) CML-like disease. (B) Primary macrophages (mϕ) from mice with P210- (lanes 10 and 11) and P230-induced (lane 12) CML-like disease, and from mice with P210- (lanes 14 and 15) and P230-induced (lane 16) primary macrophage disease. (C) B lymphoid tumor cells (B-ALL) from lymph node (LN, lane 21) or pleural effusion (eff., lane 22) of two mice with P230-induced disease, and from cultured lymph node cells (LNCX, lane 23) from a mouse with P190-induced disease. Molecular weight markers are on the right.

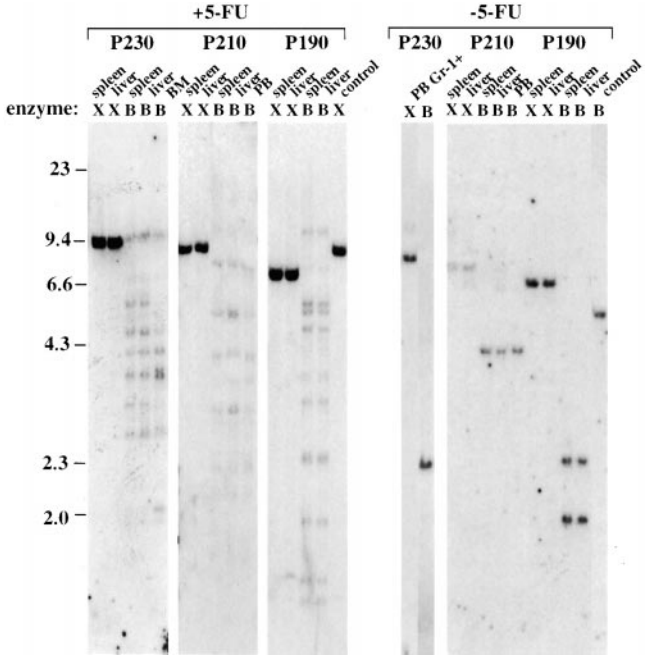


**Figure 5.** Polyclonal disease in mice with the CML-like syndrome. Southern blot of genomic DNA from six mice with CML-like disease, three receiving transduced marrow from 5-FU–treated donors (+5-FU, left) and three recipients of marrow from non–5-FU–treated donors (−5-FU, right). Genomic DNA from spleen, liver, peripheral blood (PB), or bone marrow (BM) was digested with the indicated restriction enzyme, transferred to nylon membranes, and hybridized with a radioactive probe to the proviral neomycin resistance gene, as described in Materials and Methods. In one case, Gr-$1^+$ peripheral blood neutrophils were isolated from a P230-transduced animal with mixed CML and ALL. The XbaI digest (X) yields a single full-length proviral species from each individual clone, and indicates presence of the provirus at about single-copy levels in myeloid cells of all four mice. The BglII digest (B) yields a distinct sized species for each individual proviral integration. Control DNA samples from a P210 *BCR/ABL*-transformed B cell line containing a single provirus are used to indicate one proviral copy per diploid genome.

rived from a cell (CFU-$S_{12}$) with characteristics of an early multipotential progenitor with some stem cell–like properties (45). It was previously shown (26) that an animal with the CML-like syndrome carried the retroviral provirus in the majority of secondary day 12 spleen colonies, suggesting that the target cell for the CML-like syndrome was an early progenitor that could generate CFU-$S_{12}$. In this study, we also detected the *BCR/ABL* provirus in the majority of day 12 spleen colonies in secondary transplants from mice with the CML-like syndrome (Fig. 6, A, B, and D). Collectively, in 10 primary animals with the CML-like syndrome, 64% (28 out of 44) of day 12 spleen colonies from secondary transplants carried the *BCR/ABL* provirus (Fig. 6, and data not shown). Interestingly, of the many distinct proviral clones present in the bone marrow of the primary animals, a small subset of clones was detected frequently or exclusively in secondary day 12 spleen colonies. For example, two minor clones were found in four out of seven colonies derived from an animal with P190-induced CML-like disease (Fig. 6 A), whereas a single minor clone generated seven out of eight colonies from a mouse with P210-induced disease (Fig. 6 D).

We observed a similar phenomenon in secondary recipients that developed the CML-like syndrome after transplantation of larger numbers of marrow cells. Instead of developing polyclonal disease with the same spectrum of proviral clones as the primary animal, myeloid cells from secondary mice with CML-like disease typically contained only a single minor clone from the donor (Fig. 6, C and D). In one animal, the same clone that contributed exclusively to day 12 spleen colonies was also recovered from secondary mice with CML-like disease (Fig. 6 D). These findings suggest that the target cell for induction of the CML-like syndrome is heterogeneous, with all clones exhibiting multilineage repopulating ability but only some clones capable of efficient generation of CFU-$S_{12}$ and secondary disease.

*P190 BCR/ABL Induces More Aggressive B Lymphoid Leukemia Than P210 or P230 When Non–5-FU–treated Marrow Is Used for Transduction.* Because animals with the CML-like syndrome die within 4 wk of transplantation, it is not possible to assess the incidence or severity of *BCR/ABL*-induced hematologic malignancies of longer latency or duration in this system. Multipotential myeloid target cells for *BCR/ABL* are enriched for by 5-FU treatment (46), whereas the target cell for Abelson virus–induced pre-B leukemia is fairly abundant in normal marrow from Balb/c mice (47). Therefore, we reasoned that the use of bone marrow from donors that had not been pretreated with 5-FU might allow us to observe the characteristics of other leukemias induced by *BCR/ABL*, including lymphoid leukemia.

Indeed, when marrow from non–5-FU–treated donors was used for retroviral transduction, we observed three distinct hematopoietic neoplasms with each of the three forms of *BCR/ABL* (Fig. 7). We observed the CML-like syndrome in about half of transplant recipients. The survival of mice with the CML-like syndrome induced by transduction of non–5-FU marrow (21–49 d) was somewhat longer than for recipients of 5-FU–treated marrow. Otherwise, the syndrome appeared pathologically identical to that observed with 5-FU–treated marrow, with increased neutrophil counts, hepatosplenomegaly, and pulmonary hemorrhage. Analysis of proviral integration in myeloid cells showed that significantly fewer (one to three) independent proviral clones contributed to the CML-like disease in these mice, compared with recipients of transduced marrow from 5-FU–treated donors (Fig. 5). Lineage analysis of selected mice demonstrated the same proviral clone(s) in neutrophils, macrophages, erythroid cells, and B lymphoid cells (data not shown), indicating the target cell for the CML-like disease in normal marrow, like that from 5-FU–treated marrow, had multilineage differentiation potential.

The other animals developed acute leukemia of B lymphoid type, or tumors of monocyte/macrophages. Animals with B lymphoid leukemia exhibited modest splenomegaly (0.2–0.4 g) and lymphadenopathy with infiltration with lymphoblasts, and a bloody pleural effusion, containing high levels of malignant lymphoid cells, that appeared to be the cause of death. The lymphoblasts expressed high levels of Bcr/Abl protein (Fig. 4 C), were negative for myeloid and T lymphoid cell surface markers, but positive for CD45R (B220),

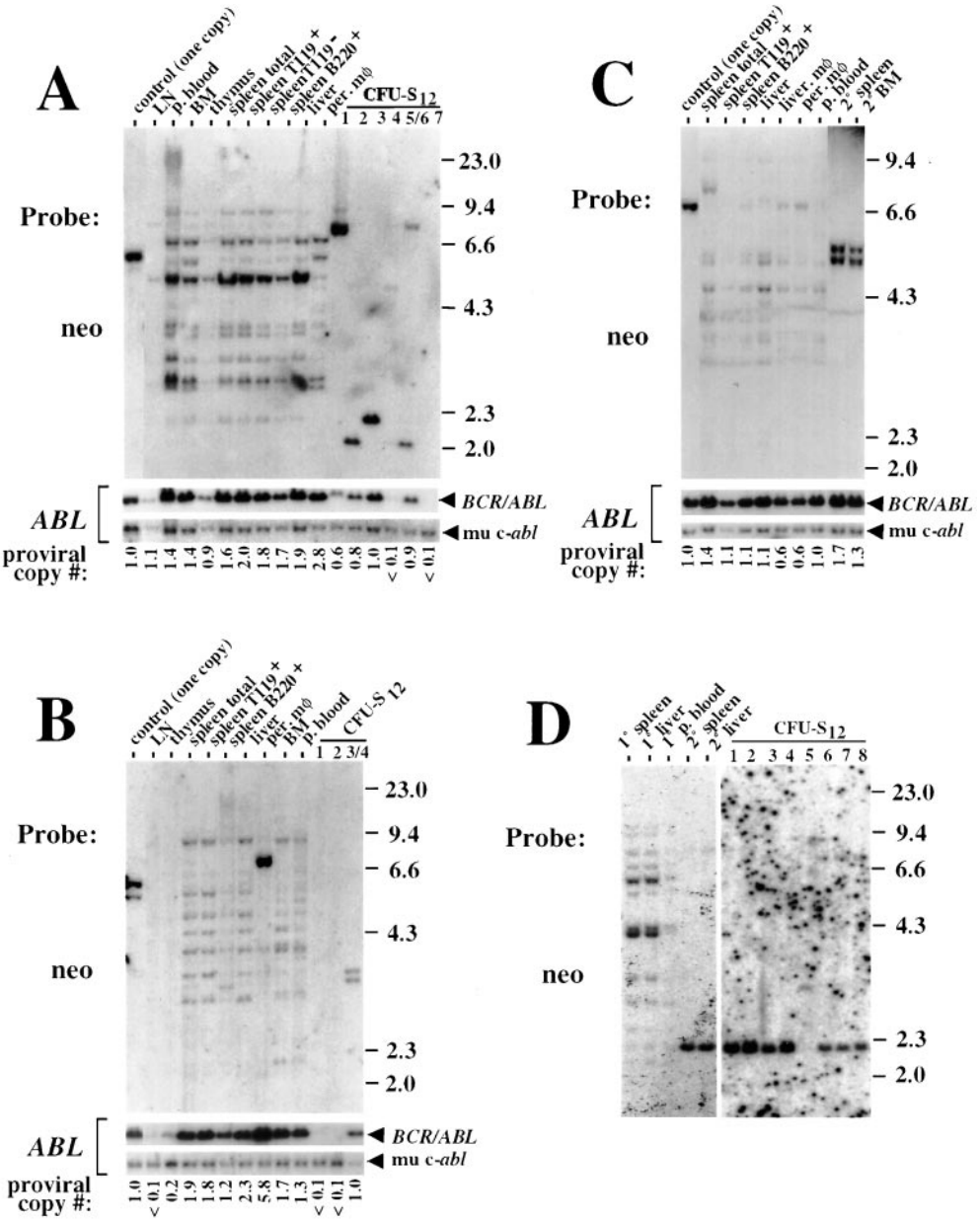


**Figure 6.** The target cell for the CML-like disease has multilineage repopulating ability but is heterogeneous for self-renewal as assessed by secondary transplantation. (A) Lineage analysis of a primary animal with P190-induced CML-like disease. In the top panels, DNAs from the indicated hematopoietic tissues and lineages were digested with BglII and hybridized with a radioactive probe from the neomycin resistance gene. The blot was then stripped and reprobed with a radioactive probe from the human c-*ABL* gene (bottom panels), allowing determination of the proviral copy number in each sample (see Materials and Methods). LN, pooled peripheral lymph nodes; p. blood, peripheral blood; BM, bone marrow; spleen $T119^+$, $T119^-$, and $B220^+$, splenocytes purified using mAbs against TER119 and B220; per. mϕ, purified peritoneal macrophages. Genomic DNA from a control cell line containing a single copy of the *BCR/ABL* provirus is on the left; DNAs from seven day 12 spleen colonies derived from bone marrow from this animal are on the right; one sample contained two distinct proviral integrants and likely represents a mixture of two individual colonies ($CFU\text{-}S_{12}$ 5/6). Provirus was detected at 1.4–2.0 copies per cell in several primary tissues, indicating that one or more target cells had been transduced with multiple proviruses. Purified $TER119^+$ and $B220^+$ splenocytes contained the *BCR/ABL* provirus at levels equal to or higher than total spleen, indicating repopulation of erythroid and B lymphoid lineages with provirus-positive cells. (B) Lineage analysis of a primary animal with P230-induced CML-like disease. Nomenclature as in A. In this animal, provirus is absent from lymph node and thymus, but present in $B220^+$ splenocytes. Peritoneal macrophages from this animal were cultured for 1 wk in the presence of CSF-1 to increase cell number, resulting in selection for a clone containing an amplified, rearranged provirus (data not shown); because of this frequent tendency, short-term adherence was used subsequently for purification of macrophages. (C) Lineage analysis of a primary animal with P210-induced CML-like disease, and of a secondary marrow transplant recipient from this mouse that also developed CML-like disease. Nomenclature as in A. At the right, spleen and bone marrow from the secondary (2°) animal carry two proviruses each present at single-copy levels, indicating a doubly transduced target cell. (D) Analysis of secondary transplants from an animal with P210-induced CML-like disease. A single minor clone from the 13 clones present in the primary mouse (1°) was selectively recovered from a secondary animal (2°) with CML-like disease and from 7 out of 8 day 12 spleen colonies from this primary animal.

CD43, 6C3/BP-1, and CD24 (data not shown), indicating an immature B cell phenotype. The lymphoid leukemias were efficiently transplanted to secondary recipients, with animals receiving $3 \times 10^6$ tumor cells from lymph node or pleural effusion succumbing to an identical disease within 4–5 wk. Animals with the monocyte/macrophage tumors presented with enlargement of the liver and infiltration of periportal areas with tumors of cells with large vacuolated cytoplasm; similar tumors were often found in the spleen and mesentery, and most animals had a prominent ascites composed exclusively of macrophages. The tumor cells expressed high levels of Bcr/Abl protein (Fig. 4 B) and were positive for the cell surface markers Mac-1 and F4/80 but negative for lymphoid markers (data not shown). Some mice exhibited characteristics of two diseases simultaneously (Fig. 7); because of the distinct and very uniform features of the three

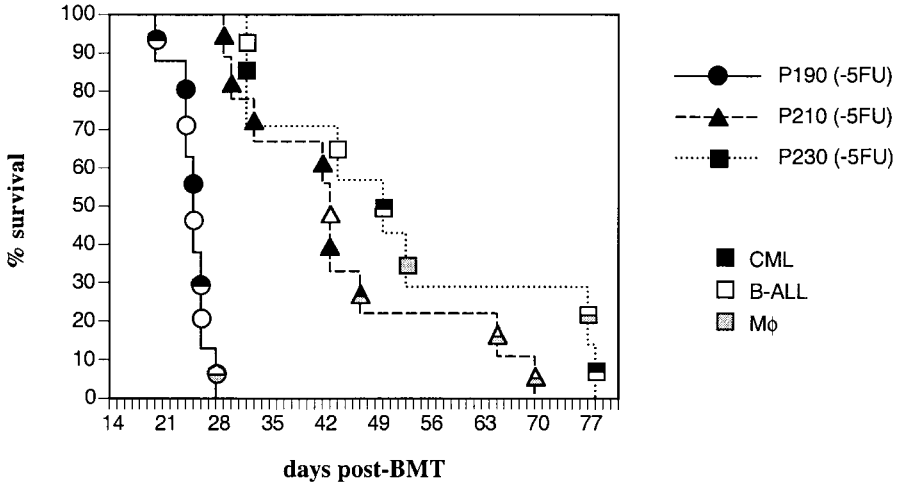


**Figure 7.** Survival curve for recipients of *BCR/ABL*-transduced marrow from non–5-FU–treated donors. The individual mice in each arm are indicated by the symbols. Filled symbols, CML-like disease; open symbols, B lymphoid leukemia; gray symbols, macrophage disease. Animals diagnosed with two disease processes simultaneously based on histopathological and molecular analysis are indicated by two-color symbols. The difference in survival between recipients of P190-transduced marrow and either P210- or P230-transduced marrow was highly significant ($P < 0.001$, Cox test), whereas there was no significant difference between P210 and P230. BMT, bone marrow transplantation.

malignancies, it was relatively easy to recognize these animals based upon clinical features and histopathology.

The survival curve of mice transplanted with P190-transduced marrow differed significantly from that of P210- or P230-transduced recipients (50% death in 25 d vs. 43 and 50 d, respectively; Fig. 7). Principally, this was because P190 induced B lymphoid leukemia in the majority (6/8) of recipients, which rapidly lead to the death of the host. In contrast, recipients of P210- or P230-transduced marrow that developed B lymphoid leukemia (3/9 and 5/7 mice, respectively) succumbed to these diseases much later after transplantation. The longer latency associated with P210 and P230 appeared to reflect a longer time required for establishment of the disease rather than a more aggressive leukemia, because the survival of secondary recipients transplanted with $3 \times 10^6$ malignant lymph node cells from either P190- or P210-induced leukemia was similar, ~4 wk (data not shown). These data confirm earlier observations on the relative potency of P190 and P210 *BCR/ABL* for in vitro lymphoid transformation (21), and suggest that P190 induces lymphoid leukemia with shorter latency and perhaps greater efficiency than either P210 or P230.

*The Lymphoid Leukemias and Macrophage Tumors Are Oligo- or Monoclonal and Lineage Restricted.* To further characterize the lymphoid leukemias and macrophage tumors, we analyzed genomic DNA from the primary tumors (lymph node or pleural effusion cells for the lymphoid leukemias, and liver, ascites, or purified macrophages for the macrophage tumors). In addition, we performed lineage analysis and isolated secondary day 12 spleen colonies from selected mice. In contrast to the polyclonal nature of the CML-like syndrome, we generally observed only one or two independent proviral integrations in the lymphoid leukemias and macrophage tumors (Fig. 8, A–C). In some mice with lymphoid leukemia, different clones predominated in different anatomical sites such as lymph nodes and the pleural effusion. The presence of oligo- or monoclonal disease suggests that the presence of *BCR/ABL* alone is insufficient to induce these diseases, and that secondary genetic or epigenetic events are required.

In contrast to the CML-like syndrome, where provirus-positive cells were observed in multiple myeloid and lymphoid lineages in addition to the neutrophils, the provirus in the B lymphoid leukemias and macrophage tumors was confined to the tumor cells, and not present in cells of other hematopoietic lineages (Fig. 8, B and C). In animals with the macrophage disease, provirus was absent from the neutrophils, a closely related myeloid lineage. In those animals diagnosed with two diseases based on clinicopathological criteria, two different proviral integrants were usually observed, restricted to the individual tumors (26; Fig. 8 B, and data not shown). In addition, provirus was uniformly absent from day 12 spleen colonies derived from bone marrow of these mice; collectively, a total of 47 colonies derived from 3 primary animals with lymphoid leukemia and 3 primary animals with macrophage disease lacked the retroviral provirus (Fig. 8, B and C, and data not shown). These results suggest that the target cells for these diseases are progenitor cells whose differentiation capacity is lineage restricted and cannot give rise to CFU-$S_{12}$.

## Discussion

In human Ph-positive leukemia, the clear association of different forms of the *BCR/ABL* oncogene with distinct types of leukemia begs a biological explanation. This is most evident for P190 *BCR/ABL*, which is only rarely if ever observed in CML. Recently, it has been suggested that the P230 form of *BCR/ABL* identifies a group of patients with a distinctly benign form of CML, neutrophilic CML (14). There are two models that might explain such a phenotypic correlation with different *BCR/ABL* genotypes. It is possible that the three forms of *BCR/ABL* have different intrinsic leukemogenic activities when expressed in a hematopoietic progenitor cell that has acquired a t(9; 22) translocation. Alternatively, the three oncogenes might have identical leukemogenic properties, but their expression might be largely restricted to different hematopoietic lineages because a particular chromosome 22 breakpoint is favored in a given lineage during the formation of the Ph chromosome. For example, in this model, the *BCR* intron 1 breakpoint might be frequent in B lymphoid progenitor

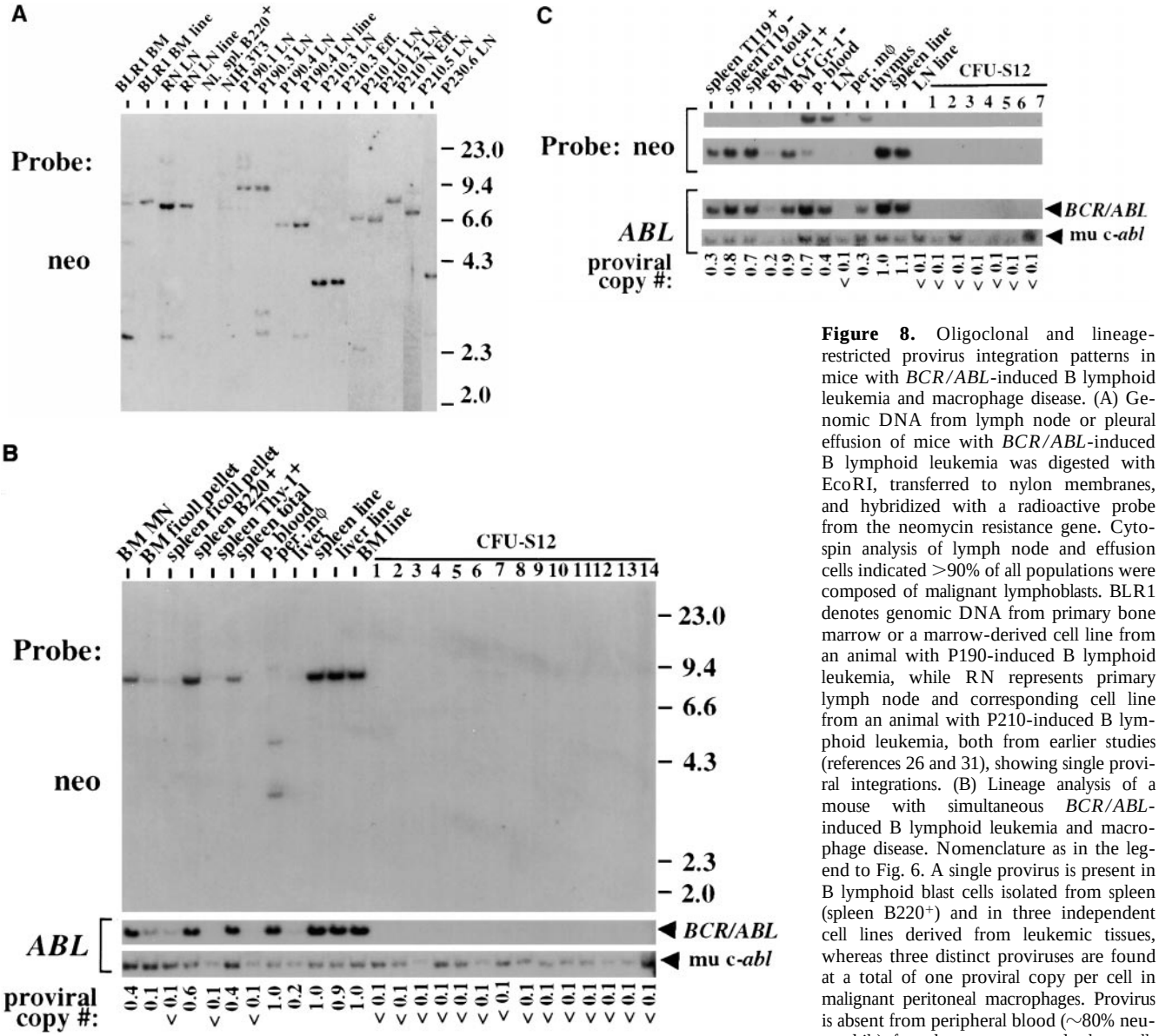


**Figure 8.** Oligoclonal and lineage-restricted provirus integration patterns in mice with *BCR/ABL*-induced B lymphoid leukemia and macrophage disease. (A) Genomic DNA from lymph node or pleural effusion of mice with *BCR/ABL*-induced B lymphoid leukemia was digested with EcoRI, transferred to nylon membranes, and hybridized with a radioactive probe from the neomycin resistance gene. Cytospin analysis of lymph node and effusion cells indicated >90% of all populations were composed of malignant lymphoblasts. BLR1 denotes genomic DNA from primary bone marrow or a marrow-derived cell line from an animal with P190-induced B lymphoid leukemia, while RN represents primary lymph node and corresponding cell line from an animal with P210-induced B lymphoid leukemia, both from earlier studies (references 26 and 31), showing single proviral integrations. (B) Lineage analysis of a mouse with simultaneous *BCR/ABL*-induced B lymphoid leukemia and macrophage disease. Nomenclature as in the legend to Fig. 6. A single provirus is present in B lymphoid blast cells isolated from spleen (spleen $B220^+$) and in three independent cell lines derived from leukemic tissues, whereas three distinct proviruses are found at a total of one proviral copy per cell in malignant peritoneal macrophages. Provirus is absent from peripheral blood (~80% neutrophils), from bone marrow and spleen cells that sedimented through Ficoll-Hypaque (ficoll pellet; entirely neutrophils and immature erythroid cells by cytospin analysis), and from splenic T lymphocytes (spleen Thy-$1^+$). Provirus was also absent from 14 day 12 spleen colonies derived from this mouse. (C) Lineage analysis of a mouse with *BCR/ABL*-induced B lymphoid leukemia. This animal demonstrated two distinct proviral integrations in tumor cells, one predominant in lymph node and the other in spleen and marrow, with both clones contributing to peripheral blood. Provirus is absent from peritoneal macrophages, bone marrow neutrophils, and decreased in thymocytes and $TER119^+$ splenocytes, both of which were contaminated with blasts at ~25%.

cells but uncommon in hematopoietic stem cells, explaining the rarity of P190 *BCR/ABL* in CML.

Several studies of the cell of origin of the Ph chromosome translocation appear to support the latter model. In CML patients, the Ph chromosome is present in myeloid, erythroid, megakaryocytic, and B lymphoid cells, confirming that the t(9;22) translocation took place in a very early multipotential progenitor or stem cell (48). In Ph-positive ALL, about half of adult patients have traditional *BCR* intron 13 or 14 breakpoints on chromosome 22 generating the P210 form of *BCR/ABL*. Some of these patients have persistence of the Ph chromosome in remission and carry the Ph chromosome in myeloid cells and myeloid colonies grown in vitro, suggesting they represent cases of CML presenting in blast crisis after an unrecognized chronic phase (6–8, 49). In contrast, the majority of ALL patients with the *BCR* intron 1 breakpoint characteristic of P190 *BCR/ABL* do not exhibit the additional cytogenetic abnormalities typical of CML blast crisis, lack the Ph chromosome in myeloid cells, and become Ph-negative during clinical remissions, suggesting they represent transformation of a cell type that is more limited in its differentiation potential (50). However, some patients with P190-associated ALL show persistence of the Ph chromosome in remission and in myeloid cells, suggesting a multipotential target cell (51–53). Detailed studies of the nature of the cell of origin in patients

with neutrophilic CML have not been reported. Therefore, the available clinical and molecular studies of human Ph-positive leukemia patients do not provide a biological distinction between the above two models.

The purpose of this study was to characterize the kinase activity and in vitro transformation properties of the newly described P230 Bcr/Abl protein, and to compare the in vivo leukemogenic activity of the three principal forms of Bcr/Abl to distinguish between these alternative models. We found elevated kinase activity for P230 Bcr/Abl relative to c-Abl, although the activity of P230 was reproducibly lower than that of P210. While all three forms of *BCR/ABL* transformed IL-3–dependent myeloid and lymphoid cell lines to IL-3 independence, they were all less effective than IL-3 at promoting the growth of Ba/F3 lymphoid cells, with the rates of proliferation directly correlating with the intrinsic tyrosine kinase activity of Bcr/Abl. The reason for this difference is not known, but it is plausible that increased tyrosine phosphorylation of critical substrates such as STAT6 by P190 might contribute to proliferation.

To test the hypothesis that the three forms of *BCR/ABL* have different leukemogenic properties in vivo, we used the bone marrow transduction/transplantation model system. This assay allowed us to directly determine whether P190 is able to cause a CML-like syndrome and whether P230 is less potent than P210 upon transduction of an identical spectrum of primary hematopoietic cells. When the three forms of *BCR/ABL* were transduced into marrow from donors treated with 5-FU, they induced an identical fatal CML-like disease in recipients transplanted with the transduced cells, with no appreciable differences in histopathology, hematologic parameters, or disease latency. These observations argue that the three forms of *BCR/ABL* induce an identical proliferative stimulus to myeloid cells in our experimental model. Human patients with neutrophilic CML are described as having lower peripheral blood leukocyte counts, less splenomegaly, and requiring little or no myelosuppressive therapy (14), features which imply less myeloid cell proliferation and expansion relative to traditional CML. However, our results do not support the hypothesis that P230 generates a weaker proliferative stimulus in myeloid cells than P210, and suggest that the mild clinical symptoms observed in some patients with P230 *BCR/ABL* are due to other variables, such as genetic differences between individuals or a selection bias (15–17). In recipients of transduced marrow from non–5-FU–treated donors, there is a suggestion of increased latency of P210- and P230-induced CML-like disease compared with P190, which leaves open the possibility of differences in *BCR/ABL*-induced CML-like disease under conditions where small numbers of proviral clones and perhaps lower Bcr/Abl expression exist.

Similarly, because P190 *BCR/ABL* can induce a CML-like syndrome in mice which is identical to that induced by P210, it is difficult to argue that the rarity of P190 in human CML reflects different leukemogenic activity of P190 in myeloid cells. Rather, our results suggest that the restriction of P190 *BCR/ABL* to human acute leukemias may be due to a relative lack of *BCR* first intron breakpoints during the generation of the Ph chromosome in stem cells. Alternatively, patients with the P190 form of *BCR/ABL* may develop the Ph translocation in a stem cell but manifest only a brief chronic phase. This is supported by a recent study of human P190-positive ALL, where the Ph chromosome was detected by interphase fluorescence in situ hybridization in granulocytes of five out of five patients, suggesting multilineage involvement (53).

Although there was no difference between the three *BCR/ABL* oncogenes in induction of a CML-like disease in recipients of transduced marrow from 5-FU–treated donors, there was a highly significant difference in survival of recipients of P190-transduced marrow compared with recipients of P210- and P230-transduced marrow when donors were not pretreated with 5-FU. Under these conditions, rather than succumbing to CML-like disease, most recipients of P190-transduced marrow developed B lymphoid leukemia. Although several recipients of P210- and P230-transduced marrow also developed B lymphoid leukemia, the disease developed significantly later with P210 and P230. These results demonstrate that P190 *BCR/ABL* induces lymphoid leukemia in vivo with shorter latency than P210 or P230, and are in agreement with previous studies which found P190 to be more potent than P210 at transformation of B lymphoid cells in vitro (21) and induction of lymphoid leukemia after marrow transduction (32). Our data further suggest that P230 and P210 have similar lymphoid leukemogenic activity. The mechanism of the increased lymphoid leukemogenic activity of P190 relative to P210 and P230 is not known. One possibility is that the increased intrinsic tyrosine kinase activity of P190 allows elevated tyrosine phosphorylation of a substrate critical to proliferation or transformation of lymphoid cells. One candidate for such a key substrate is STAT6, whose DNA-binding activity is activated via tyrosine phosphorylation by P190 but not P210 (24). In support of this, we have observed that bone marrow from *stat6*$^{-/-}$ mice (23) exhibits significant resistance to transformation by P190 *BCR/ABL* in vitro when compared with *stat6*$^{+/+}$ marrow (our unpublished observations). An alternative possibility is that the presence of distinct functional motifs in the Bcr portion of P210 and P230 *BCR/ABL* impairs lymphoid transformation by these oncogenes. The only known domain common to P210 and P230 but lacking in P190 is a homology to Cdc24Hs/Dbl, which is a member of a class of guanine nucleotide exchange factors for small G proteins of the Rac/Cdc42 family. Our system allows us to readily test the importance of this domain in lymphoid leukemogenesis.

What accounts for the very different spectrum of leukemia observed when non–5-FU–treated marrow is used? Data in this report argue that the CML-like disease, B lymphoid leukemia, and macrophage disease are the consequence of *BCR/ABL* transduction of distinct bone marrow target cells, and the relative abundance and/or infectability of these target cells is influenced by 5-FU treatment of donors. In mice with the CML-like syndrome, multiple myeloid and lymphoid lineages carry the same spectrum of proviral clones, demonstrating that the target cell is a pro-

genitor with both lymphoid and myeloerythroid differentiation potential. In addition, we found the *BCR/ABL* provirus in the majority of secondary day 12 spleen colonies derived from primary mice with the CML-like syndrome, demonstrating that some of these target cells can generate CFU-$S_{12}$. In contrast, the bone marrow target cells for induction of *BCR/ABL*-induced B lymphoid leukemia and macrophage disease have restricted in vivo differentiation potential, so that provirus-positive cells do not contribute to other hematopoietic lineages; further, the provirus is never found in day 12 spleen colonies derived by secondary transplantation of marrow from these animals. These observations suggest the *BCR/ABL* B lymphoid leukemia target cell is a committed B lymphoid progenitor similar or identical to the Abelson virus target cell, which is known to be an immature Thy-$1^{lo}B220^{+}$ committed lymphoid progenitor (54) that is distinct from CFU-$S_{12}$ (55).

Analysis of proviral integration patterns suggests other pathogenetic differences between *BCR/ABL*-induced CML-like disease and B lymphoid leukemia. The CML-like disease observed in recipients of transduced marrow from 5-FU–treated donors is polyclonal, whereas the B lymphoid leukemias are mono- or oligoclonal. The short latency of the CML-like disease together with the polyclonal nature suggest that *BCR/ABL* alone is capable of inducing CML-like disease, whereas the longer latency and monoclonal nature of the B lymphoid leukemias imply that expression of *BCR/ABL* alone may be insufficient for malignant transformation of primary B lymphocytes and that additional events are required, a concept supported by many previous observations (20, 56–59). However, proof of the sufficiency of *BCR/ABL* for induction of the CML-like disease will require limiting dilution analysis and knowledge of the efficiency of infection of the CML target cell.

Interestingly, we and others (29) have found evidence for heterogeneity among the presumptive target cells for the murine CML-like syndrome. Of the many clones contributing to the expansion of myeloid cells in several primary CML animals, only one or two relatively minor clones were observed to contribute to day 12 spleen colonies and to efficiently transfer the CML-like disease in secondary transplants. These data imply two distinct target cells for the murine CML-like syndrome, both with the capacity for multilineage differentiation in the primary animal, but with different ability for repopulation of secondary recipients. These properties are reminiscent of the hierarchy of murine hematopoietic stem cells, where a distinct population of progenitor cells enriched for CFU-$S_{12}$ has transient multilineage repopulating and self-renewal potential, and a more differentiated subset lacks secondary transplantability (60). An alternative possibility is that all the CML target cells are equivalent immediately after infection, but additional genetic or epigenetic abnormalities confer transplantability on a small subset of transduced progenitors. Distinguishing between these models will require isolation of the various target cells from whole marrow.

We found small to medium-sized accumulations of macrophages in the livers of nearly every animal with the CML-like syndrome. Previous studies have shown that some animals with extensive *BCR/ABL*-induced macrophage tumors exhibit elevated serum GM-CSF and/or G-CSF levels, and a secondary elevation in peripheral blood neutrophils that lack the retroviral provirus and are therefore not a direct part of a malignant process (26, 30, 44). In our study, immunoassay of plasma from several mice with primary or secondary CML-like disease revealed normal levels of GM-CSF but increased levels of IL-3 (data not shown). The biological significance of the elevation in plasma IL-3 in these animals is not clear and is under investigation. However, in contrast to mice with primary macrophage tumors, the neutrophils in all animals with the CML-like disease contain the retroviral provirus and express Bcr/Abl protein, demonstrating that this disease represents a bona fide myeloproliferative process. The lineage analysis suggests that the macrophage collections in mice with the CML-like syndrome represent the product of differentiation of the myeloid clones, as previously suggested (32).

Our original goal in expressing *BCR/ABL* in the murine hematopoietic system by retroviral transduction (26) was to determine whether *BCR/ABL* was the direct cause of CML, a point which has been established beyond reasonable doubt (26–29). However, our initial model system was difficult to use for comparative studies because of the low efficiency and variability of induction of the CML-like disease. In this report, the mouse bone marrow transduction/transplantation system has been improved, and we have used this system for a direct comparison of the leukemogenic properties of the three principal forms of *BCR/ABL*. We found no significant difference in the ability of the three oncogenes to induce a CML-like myeloproliferative syndrome, but observed that P190 *BCR/ABL* had increased potency for induction of B lymphoid leukemia. This model system should be very useful for studying the molecular pathophysiology of the Ph-positive leukemias, such as testing the requirement for various functional domains of Bcr/Abl, determining signaling pathways relevant to leukemogenesis, and investigating the physiological effects of *BCR/ABL* expression in primary hematopoietic cells.

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*Note added in proof.* Pear et al. (Pear, W.S., J.P. Miller, L. Xu, J.C. Pui, B. Soffer, R.C. Quackenbush, A.M. Pendergast, R. Bronson, J.C. Aster, M.L. Scott, and D. Baltimore. 1998. *Blood*. 92:3780–3792) and Zhang and Ren (Zhang, X., and R. Ren. 1998. *Blood*. 92:3829–3840) have also recently reported efficient induction of CML-like disease in mice by *BCR/ABL*.

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