DNA constructs encoding BCR/ABL P210 have been introduced into the mouse germ line using microinjection of one-cell fertilized eggs. Kinetics of BCR/ABL P210 expression in transgenic mice were very similar to those of BCR/ABL P190 constructs in transgenic mice. mRNA transcripts were detectable early in embryonic development and also in hematopoietic tissue of adult animals. Expression of BCR/ABL in peripheral blood preceded development of overt disease. P210 founder and progeny transgenic animals, when becoming ill, developed leukemia of B, T-lymphoid, or myeloid origin after a relatively long latency period. In contrast, P190-transgenic mice exclusively developed leukemia of B-cell origin, with a relatively short period of latency. The observed dissimilarities are most likely due to intrinsically different properties of the P190 and P210 oncoproteins and may also involve sequences that control transgene expression. The delayed progression of BCR/ABL P210-associated disease in the transgenic mice is consistent with the apparent indolence of human chronic myeloid leukemia during the chronic phase. We conclude that, in transgenic models, comparable expression of BCR/ABL P210 and BCR/ABL P190 results in clinically distinct conditions.

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ABL P210 was constructed from three segments: MT-I promoter (a 200-bp SstI from the previously described cosmid cl(8)der, a BCWABL DNA fragment containing the human BCR gene promoter. The latter fragment was derived by ligating a 5’ MT-BCRIABL P210 construct (Fig 1) lacking the glucocorticoid-responsive element and is identical to that used before to generate AMT-BCWABL P190 transgenic animals.29 The SMT-BCR/ABL P210 construct was generated by ligating a 5’ Cla I-Sal I fragment containing the truncated MT-I promoter (a 200-bp Sst I-Bgl I fragment) and BCR gene exon 1 until the Sal I site to a large Sal I-Cla I fragment encompassing the remainder of BCR/ABL. The latter fragment was derived from the previously described cosmid cl(8)der, a BCR/ABL DNA construct containing the human BCR gene promoter.30 The Sal I-Cla I fragments were ligated into vector pHEP and packaged in cDNA sequences were used to generate these constructs. Thin lines indicate intron sequences. Hatched boxes represent BCR gene exons. Black boxes represent ABL gene exons. The small black boxes 5’ to BCR exon 1 represent murine MT-1 promoter sequences.

controlled by the human BCR promoter failed because of deleterious effects of the construct on embryogenesis; no live offspring were obtained.31 We present here the generation of mouse lines transgenic for MT-BCR/ABL P210 constructs and describe the development of hematopoietic neoplasia caused by the P210 transgene.

MATERIALS AND METHODS

Transgenes and mice. The MT promoter in the SMT-BCR/ABL P210 construct (Fig 1) lacks the glucocorticoid-responsive element and is identical to that used before to generate AMT-BCR/ABL P190 transgenic animals.29 The SMT-BCR/ABL P210 construct was generated by ligating a 5’ Cla I-Sal I fragment containing the truncated MT-I promoter (a 200-bp Sst I-Bgl I fragment) and BCR gene exon 1 until the Sal I site to a large Sal I-Cla I fragment encompassing the remainder of BCR/ABL. The latter fragment was derived from the previously described cosmid cl(8)der, a BCR/ABL DNA construct containing the human BCR gene promoter.30 The Sal I-Cla I fragments were ligated into vector pHEP and packaged in cDNA sequences. The rest of ABL includes introns and is a genomic sequence. The BCR/ABL P210 was constructed from three segments: (1) the complete murine MT-I promoter fused to BCR exon 1 plus a segment of 3’ intron; (2) the intron immediately preceding BCR gene exon 2 and additional BCR gene exons up to and including Mbc exon 2 and Mbc exon 3 with flanking introns; and (3) ABL exon 2 with 5’ and 3’ introns and ABL exons 3 through 11 as cDNA.

Transgenic δMT-BCR/ABL P190 animals have been described in detail elsewhere.29-30 Mice transgenic for the MT-BCR/ABL P210 constructs were generated as described.28 Founder animals were the offspring of matings between C57BL/CBA F1 animals. Transgenic progeny was the result of matings between transgenics and C57BL/CBA F1 mice. Progeny were identified by eight-digit numbers: the first four digits are those of the founder of that specific line. Histology. Pathologic analysis was performed, as described previously,31 on autopsy material obtained at killing from mice that were generally terminally ill. Routine histology examinations included bone marrow, lymph nodes, liver, kidney, spleen, and thymus. Tissue sections were fixed in 10% formalin, 90% B5. All MT-BCR/ABL P210 males displayed fertility problems; severely reduced testis size and aspermia were frequently encountered. For this reason, only a limited amount of offspring was obtained from MT-BCR/ABL founders. The δMT-BCR/ABL P210 and P190 animals apparently had normal fertility.

Periodic monitoring of peripheral blood for signs of disease. White blood cell (WBC) counts and BCR/ABL expression assays were performed regularly on peripheral blood from transgenic and control mice. Briefly, approximately 70 µL of blood was obtained via retro-orbital sinus bleeding. WBC counts were performed manually, as were differential WBC counts. Peripheral blood films were stained with Wright-Giemsa and evaluated histologically. Evidence of myeloid involvement (differential counts) in the period that preceded the development of overt leukemia in BCR/ABL P210 transgenic animals was not found. Fifty microliters of heparinized blood was used for RNA isolation. Red blood cells were lysed in 200,000 nucleated cells.32 To verify that a certain RNA sample was a limited amount of offspring was obtained from MT-BCR/ABL founders. The δMT-BCR/ABL P210 and P190 animals apparently had normal fertility.

Composition of hematopoietic tumors and peripheral blood samples. Composition of hematopoietic tissues was determined by double-color flow cytometry on a FACScan (Becton Dickinson, Lincoln Park, NJ). Briefly, tissues were disaggregated and washed twice in cold PBS. Approximately 1 × 10^6 WBCs were stained with phycoerythrin (PE)- and/or fluorescein isothiocyanate (FITC)-conjugated antibodies for 20 to 30 minutes at 4°C in the dark. For each experiment, RNA was isolated essentially as described.34 The RNA pellet was suspended in 40 µL of solution D; RNA was isolated essentially as described.34 In some experiments, an oligonucleotide probe (S'-ACTCC-TATGTGGG-TG ACGAGG-3') contained within the amplified region was used for hybridization probe.

Southern analysis. Tissue DNAs were isolated as described.35 DNAs digested with EcoR1, HindIII, and BamHI were run on 0.7% gels, blotted to nitrocellulose, and hybridized to a 1.2-kb Msp I
EcoRI, a 0.7-kb Pst I T-cell receptor-β (TCR-β), or a 2.8-kb BamHI C5 fragment respectively. Posthybridization washings were at 0.1X SSC at 65°C.

RNA isolation and expression in other tissues. RNAs were isolated as described using guanidine-isothiocyanate. To test for early expression, timed matings between transgenic males and non-transgenic C57BlxCBA F1 females were set up and embryos isolated between days 13 and 15 of gestation. Three fetuses were pooled and homogenized immediately in solution D and stored at -80°C. Bone marrow, spleen, or tumor RNA was isolated after homogenizing part of the tissue in l/sD. One to three micrograms of total RNA was used, per RT-PCR reaction. RT-PCR products were separated on 1% agarose gels, blotted to Nytran (Schleicher and Schuell, Keene, NH) and hybridized as indicated above.

Immunoblotting. Tissues were minced and homogenized in a Potter homogenizer in 2X sodium dodecyl sulfate (SDS) sample buffer and analyzed by Western blotting, as described. The chimeric Bcr/Abl P190 and P210 oncoproteins were detected with the mouse monoclonal antibody Ab-3 (anti-c-Abl; Oncogene Science, Inc, Uniondale, NY).

RESULTS

Transgenes and transgenic animals. To circumvent the problem of embryonic lethality observed with the human BCR promoter, the mouse metallothionein-1 promoter was chosen as an alternative regulatory sequence for BCR/ABL P210 transgenes. Analysis of a mouse model for human ALL in which this MT promoter was used to control P190 expression (the δMT-BCR/ABL P190 construct; Fig 1) showed that transgenic animals developed pre-B-cell leukemia without prior exposure to heavy metals. BCR/ABL mRNA could be detected, by RT-PCR, as early as the 1 cell stage embryo and was found in all tissues examined at later stages. This low level of transgene expression is apparently enough to predispose for cancer development. Subsequently, two BCR/ABL P210 DNA constructs were made (Fig 1), both controlled by the mouse MT-1 promoter. They differ in the length of the MT promoter segment included and in their intron/exon structure; the δMT-BCR/ABL P210 and δMT-BCR/ABL P190 constructs share identical MT promoter sequences (Fig 1). In transfected mouse NIH-3T3 cells, expression of both BCR/ABL P210 constructs resulted in the production of 210-kD proteins, which were active in autophosphorylation assays in vitro (data not shown).

Transgenic founder and progeny animals were generated with both BCR/ABL P210 constructs (Table 1) and hematologic tumors were obtained. Animals containing a double δMT-BCR/ABL P210 transgene were also generated by breeding (ie, P210/P210). However, the transgene copy number had no influence on the kinetics and/or type of disease that developed (data not shown).

Disease. Unlike mice transgenic for BCR/ABL P190, which developed pre-B-cell acute lymphoblastic leukemia, BCR/ABL P210 animals developed B- as well as T-cell leukemias with approximately the same frequency; no difference was found in type of disease between the two BCR/ABL P210 constructs (Table 1). Tumor phenotype was confirmed by flow cytometric analysis (Fig 2) and Ig heavy and light chain and TCR-β rearrangement (data not shown). The incidence of hematologic disease in δMT-BCR/ABL P210 mice was 58% and in MT-BCR/ABL P210 was 53%; in comparison, δMT-BCR/ABL P190 had a disease incidence of nearly 100% (Fig 3; see also Groffen et al). The experimental end point was arbitrarily chosen at 44 weeks; some P210-transgenic animals died of BCR/ABL related disease after more than 1 year.

In contrast to studies by others that reported the development of T-cell neoplasias upon injection of either BCR/ABL P190 or P210 retroviral vectors directly into the thymus, we have found no evidence that T-lymphoid cells are targets for BCR/ABL P190. Subsequent to the first reported study, among 14 newly generated founders and more than 200 transgenic offspring, none has shown any leukemia other than of the B-lineage.

Neoplasias in BCR/ABL P210 animals were of both B- and T-cell type and, less frequently, of myeloid origin. B-lymphoid leukemias or lymphomas were invariably accompanied by splenomegaly and involvement of lymph nodes, but never involvement of the thymus. The majority of T-cell lymphoid leukemias involved the thymus, frequently lymph nodes and spleen; WBC counts were often extremely high. Mature lymphocytic leukemia (B or T) was further characterized by absence of mitotic figures in the peripheral blood. In both B- and T-lymphoid tumors, a coexisting but separate and presumably non-neoplastic prominent hematopoiesis (erythropoiesis and megakaryocytosis) was frequently observed in the spleen (data not shown). Because clonality (Ig heavy or light chain and/or TCR rearrangements) and/or Bcr/Abl protein levels of isolated megakaryocyte populations were not evaluated, we cannot exclude the possibility that these phenomena related to direct oncogenic action of BCR/ABL. Macrophage tumors were not found in either BCR/ABL P190 or BCR/ABL P210 animals: primary granulocytic disease was not very prevalent. All leukemias were rapid in onset; no evidence for chronic disease was found in the peripheral blood.

In a few cases, the myeloid compartment was involved at terminal stages of disease. Circulating mature neutrophil counts were increased in 1 case (1994/2663), and a relatively high percentage of cells in the bone marrow compartment of another terminally sick animal (1994/2685) stained positive for both B220 and GR-1 (Fig 2B). Myeloblastic leukemia with a sarcomatous component in animal 2663 involved the red pulp in the spleen, lymph nodes, liver, and kidney (Table 1). In contrast to neoplastic lymphoblasts, the myeloblasts in animal 2663 showed a conspicuous rim of pinkish cytoplasm and appeared cohesive, particularly in the non-lymphoid red-pulp area of the spleen. Because mouse 2685 showed a mild lymphoid proliferative disorder at an earlier stage, the myeloid involvement could have been secondary to lymphoid tumorigenesis (ie, a deregulated cytokine production), as has been suggested before. Alternatively, BCR/ABL P210 expression could disturb differentiation or proliferation of relatively rare myeloid-lymphoid precursors. Taking these findings together, we conclude from our data that P190 and P210 cause clinically distinct disorders in a transgenic setting.

Kinetics of transgene expression. To investigate whether differences in type of disease were associated with different levels of BCR/ABL expression, total protein was isolated
Table 1. Leukemogenesis in BCR/ABL P210-Transgenic Animals

<table>
<thead>
<tr>
<th>Animal</th>
<th>Age* (wk)</th>
<th>Tissue Involvement</th>
<th>WBC† (×10⁹/mL)</th>
<th>Diagnosis§</th>
</tr>
</thead>
<tbody>
<tr>
<td>MT-BCR/ABL P210</td>
<td></td>
<td>BM     SPL   THY   LN</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1063</td>
<td>11</td>
<td>+      +      -    +</td>
<td>110</td>
<td>5-lineage lymphoblastic leukemia</td>
</tr>
<tr>
<td>1063-1164</td>
<td>13</td>
<td>+      +      -    +</td>
<td>53</td>
<td>Small B-lymphocytic leukemia</td>
</tr>
<tr>
<td>1102-1684</td>
<td>17</td>
<td>+      +      +      +</td>
<td>376</td>
<td>Small T-lymphocytic leukemia/lymphoma</td>
</tr>
<tr>
<td>1702-1691</td>
<td>15</td>
<td>+      +      +      +</td>
<td>219</td>
<td>Small T-lymphocytic leukemia/lymphoma</td>
</tr>
<tr>
<td>2160-2302</td>
<td>20</td>
<td>+      +      -    +</td>
<td>ND</td>
<td>B-lineage lymphocytic lymphoma</td>
</tr>
<tr>
<td>2179</td>
<td>15</td>
<td>+      +      +      +</td>
<td>ND</td>
<td>T-lineage leukemia/lymphoma</td>
</tr>
<tr>
<td>6MT-BCR/ABL P210</td>
<td></td>
<td>BM     SPL   THY   LN</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1994-2619</td>
<td>18</td>
<td>+      +      +    +</td>
<td>ND</td>
<td>T-lymphoblastic leukemia</td>
</tr>
<tr>
<td>1994-2661</td>
<td>44</td>
<td>+      +      +    +</td>
<td>126</td>
<td>T-lymphocytic leukemia/lymphoma</td>
</tr>
<tr>
<td>1994-2663</td>
<td>54</td>
<td>+      +      -    +</td>
<td>98</td>
<td>Myeloblastic leukemia†</td>
</tr>
<tr>
<td>1994-2683</td>
<td>22</td>
<td>+      +      +    +</td>
<td>1</td>
<td>B-lineage lymphoblastic lymphoma</td>
</tr>
<tr>
<td>1994-2685</td>
<td>21</td>
<td>+      +      -    +</td>
<td>1</td>
<td>B-lymphocytic leukemia/lymphoma</td>
</tr>
<tr>
<td>1994-2710</td>
<td>14</td>
<td>+      +      +    +</td>
<td>2</td>
<td>B-lymphoblastic lymphoma</td>
</tr>
<tr>
<td>1994-2720</td>
<td>19</td>
<td>+      +      +    +</td>
<td>128</td>
<td>Small T-lymphoblastic leukemia/lymphoma</td>
</tr>
<tr>
<td>1994-2725</td>
<td>11</td>
<td>+      +      +    +</td>
<td>84</td>
<td>T-lymphoblastic leukemia/lymphoma</td>
</tr>
<tr>
<td>1994-2744</td>
<td>18</td>
<td>+      +      +    +</td>
<td>ND</td>
<td>T-lineage leukemia/lymphoma</td>
</tr>
<tr>
<td>1994-2748</td>
<td>25</td>
<td>+      +      +    +</td>
<td>19</td>
<td>T-lymphoblastic leukemia</td>
</tr>
<tr>
<td>1994-2762</td>
<td>12</td>
<td>+      +      +    +</td>
<td>29</td>
<td>Small B-lymphocytic leukemia/lymphoma</td>
</tr>
<tr>
<td>1994-2777</td>
<td>9</td>
<td>+      +      +    +</td>
<td>56</td>
<td>T-lymphocytic leukemia/lymphoma</td>
</tr>
<tr>
<td>1994-2779</td>
<td>47</td>
<td>+      +      +    +</td>
<td>32</td>
<td>T-lymphoblastic lymphoma</td>
</tr>
<tr>
<td>1994-2781</td>
<td>28</td>
<td>+      +      +    +</td>
<td>196</td>
<td>B-lineage lymphoblastic leukemia</td>
</tr>
<tr>
<td>1994-2784</td>
<td>28</td>
<td>+      +      +    +</td>
<td>ND</td>
<td>T-lymphoblastic lymphoma</td>
</tr>
</tbody>
</table>

Abbreviation: ND, not done.

* Age at death; autopsies were performed on terminally ill animals or animals were followed up until they died.
† Tissue involvement was established by gross pathologic and histologic examination.
‡ WBC count was either determined at or a few days before death.
§ Phenotype of lymphoid tumors was assessed by flow cytometry and Ig and TCR gene rearrangement (see the Materials and Methods).
¶ These animals displayed granulocytic involvement in the peripheral blood at terminal stages of disease.
†† Diagnosis of myeloblastic leukemia in animal 2663 was based on pattern, general morphology, and anatomic sites involved (see also the Results).

from different end-stage tumors. However, lymphomas of all three transgenic animal lines expressed comparably high levels of the chimeric Bcr/Ab1 oncoproteins (Fig 4).

We examined transgene expression using RT-PCR early in development and in bone marrow. Both BCR/ABL P210 transgenes were expressed early in utero in nonhematopoietic tissues (testis and brain; data not shown) and in the bone marrow and spleen of different transgenic animal lines (Table 2) without prior exposure to zinc or cadmium. As was the case in the 6MT-BCR/ABL P190 animal model, the appearance of BCR/ABL P210 expressing cells in the peripheral blood preceded the development of overt leukemia.29 Transgene expression in three transgenic mouse lines (founders animals 1102, 1989, and 1994) was investigated in more detail. Transgene expression in the bone marrow of nonleukemic animals was low (on average, 50 to >100× lower than in K562; data not shown); there was no apparent correlation between relative transgene expression levels and the development of disease among the different lines. Not all transgenic animals within the lines developed detectable peripheral transgene expression (Table 2). Moreover, appearance of transgene expression in circulating blood cells did not necessarily precede immediate development of disease. Some animals for which transgene expression in circulating nucleated cells had been confirmed between 10 and 16 weeks of age remained disease free for a long period, with some remaining disease free past 16 months of age. These observations were valid for both BCR/ABL P210 constructs. In P190 animals, a close correlation exists between transgene expression and development of disease.29 The difference between P190 and P210 transgenic lines is also reflected in the different mortality curves (Fig 3). The large differences in latency time among transgenic animals indicates that BCR/ABL P210 expression is not the sole cause of leukemia but rather predisposes for the cancer. In agreement with current concepts, additional (epigenetic) mutations, directly or indirectly resulting from BCR/ABL expression, most likely contribute to malignant tumor evolution.

DISCUSSION

Transgenic mouse models allow direct comparison of P210- and P190-associated disease. In recent years, attempts to generate a reliable animal model for the study of CML have been thwarted by lack of reliability and reproducibility.24 The outcome of bone marrow transplantation experiments using retroviral BCR/ABL P210 constructs was shown to depend largely on infection conditions, the retroviral and internal regulatory sequences used, and also on genetic background of the irradiated recipient mice; in essence, the out-
come of the disease was thought to depend on which hematopoietic progenitor cell was targeted by retroviral vectors.\textsuperscript{25,26} Myeloproliferative disorders could be obtained by manipulating these experimental conditions. In this fashion, \textit{BCR/ABL} P190 and P210 were shown to cause similar disease.\textsuperscript{24} Even with \textit{v-abl}, which, under natural conditions, exclusively induces pre-B-cell leukemia in mice, myeloproliferative illness could be produced.\textsuperscript{25}

In \textit{BCR/ABL} transgenic animals, every cell, including hematopoietic stem cells, will contain a \textit{BCR/ABL} transgene. Although the MT promoter used here does not target transgene expression exclusively to hematopoietic cells, it does allow expression in these cell types. However, leukemogenesis in our \textit{BCR/ABL} transgenic mice is independent of influences of retroviral sequences or other experimental factors, such as infection and bone marrow culture conditions. Hence, a transgenic mouse model is expected to more accurately reflect the leukemogenic properties of \textit{BCR/ABL}.

\textbf{Influence of Bcr protein moiety and BCR gene promoter on leukemogenesis.} The P210 constructs and P190 construct were designed to resemble the genomic \textit{BCR/ABL} gene structures, with the exception of the MT promoter, which was used to circumvent embryonic lethality observed when the \textit{BCR} promoter was used.\textsuperscript{31} With the exception of additional \textit{BCR} coding sequences in the \textit{BCR/ABL} P210 constructs, the \textit{ABL} and promoter sequences were identical in these transgenes. One additional difference should be noted: introns are positioned differently among the constructs. However, both \textit{BCR/ABL} P190- and P210-mediated leukemogenesis clearly differ, despite the similarities in transgene design. An independent study using an \textit{MT-BCR/ABL} P210 transgenic construct supports the distinct oncogenicity of \textit{BCR/ABL} P190 and P210.\textsuperscript{43} However, in this study, mice exclusively developed T-cell leukemias, which may be related to transgene design including lack of introns and integration effects in the single line studied.

The longer latency time of P210-associated leukemogene-
sis, independent of whether a B- or T-lineage or myeloid leukemia develops, would seem to indicate intrinsic variation in biochemical properties between the two distinct oncoproteins. It has been reported that P190 Bcr/Abl has a higher autophosphorylation activity than P210 in vitro, a property that correlated with its higher transforming ability in cultured fibroblast cell lines. Such differences in tyrosine kinase activity might, for example, have a differential effect on the differentiation of certain hematopoietic lineages and may help explain the altered disease-spectrum associated with P210 as compared with P190.

The combined findings in our transgenic mice show that, as in human leukemia, the additional Bcr domains included in the P210 oncoprotein modulate the outcome of the disease. However, the mechanisms that govern these distinct leukemic processes are not understood. As indicated above, the Bcr domains modify the tyrosine kinase activity of the Ab1 moiety and also F-actin binding activities of c-Abl. In addition, the Dbl-homology and pleckstrin-homology domains only present in P210 may allow protein-protein interactions with other cellular factors that cannot occur with P190.

Apart from its structural role in the Bcr/Abl oncprotein, there appears to be a second discrete mechanism through which BCR may contribute to leukemogenesis. We have recently shown that the effect of bcr gene ablation specifically affects hematopoietic cells in mice affected by BCR/ABL in humans and mice. It seems reasonable to conclude from our data that a correlation exists between the cell type affected in Ph+ leukemia and Bcr function. The BCR promoter controls expression of both the remaining nonrearranged BCR allele and the BCR/ABL oncogene in human

![Table 2. Transgene Expression in Bone Marrow of Different BCR/ABL P210 Lines](image)

<table>
<thead>
<tr>
<th>Animal of Line*</th>
<th>TG1 xpr</th>
<th>Disease in Mouse Line: Founder or Offspring</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \delta MT-BCR/ABL ) P210</td>
<td></td>
<td></td>
</tr>
<tr>
<td>168</td>
<td>ND</td>
<td>NO</td>
</tr>
<tr>
<td>170</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>171</td>
<td>ND</td>
<td>NO</td>
</tr>
<tr>
<td>1989</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td>1994</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2039</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2043</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>MT-BCR/ABL P210</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1063</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>1102</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>1105</td>
<td>ND</td>
<td>NO</td>
</tr>
<tr>
<td>2148</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>2160</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2179</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Of animal line 170, the founder (170) and at least two of his offspring were evaluated for transgene expression; founder and 2 offspring of the 1989 line were found positive for low transgene expression in bone marrow; of line 1994, at least 20 F1 and F2 animals have been monitored periodically for transgene expression and development of disease (see the Materials and Methods). Founders and at least two offspring of lines 2039 and 2043 were evaluated for transgene expression in bone marrow; only the 2043 line had detectable BCR/ABL P210 mRNA. Animal lines 1063, 1102, 2148, 2160, and 2179 all showed equal levels of transgene expression in both spleen and bone marrow on average somewhat higher than the \( \delta MT-BCR-\) P210 lines. Offspring of lines 1063 (n = 1), 1102 (n = 8), and 2148 (n = 3) were evaluated for transgene expression and found to be positive. Of line 1102, at least 7 offspring were monitored periodically for transgene expression and development of disease (see the Materials and Methods). Transgene integrity in the different transgenic lines was assessed by restriction endonuclease digestion and Southern analysis.

Abbreviations: ND, not determined; NO, no offspring sired by this founder.

* Animal lines are represented by founder identification numbers.

† Transgene expression in bone marrow was evaluated in at least one transgenic animal per line by RT-PCR.
leukemia. It is very well possible that regulatory sequences that normally govern BCR expression have substantial influence in directing BCR/ABL-mediated leukemogenesis.

The prominent myeloid development in BCR/ABL P210-mediated human CML may be related to the presence of factors in myeloid lineages that regulate BCR expression through specific elements in the BCR promoter. These factors may be less prevalent in human T- and B-cell lineages and preferentially direct BCR-promoted malignant transformation in granulocytes.

All experimentally derived BCR/ABL lymphoid tumors were generated with regulatory sequences other than the BCR promoter. To date, all animals models for human Ph+ leukemia, whether generated through retroviral or transgenic technologies (Heisterkamp et al., Honda et al., Hariharan et al., and this study), develop predominantly lymphoid malignancy of the B and T type. Although hematologic tumors in Ph+ ALL are principally of the pre-B phenotype, B-lymphoid involvement occurs in about 25% of cases of human CML in blast crisis and T-cell involvement is rarely seen in humans. It is tempting to speculate that regulatory sequences within the BCR transcriptional unit are important for the type of disease found in humans and mice. This concept contrasts with the postulate that (in transplantation experiments using BCR/ABL-expressing, retrovirus-infected bone marrow) neither BCR sequences nor myeloid cell promoter elements are essential for myeloid leukemogenic transformation. Similarly, in a comparable experimental setting, the mere introduction of an activated abl gene into a certain target cell was proposed to control the selection of particular hematopoietic cell types and not the BCR sequences that distinguish BCR/ABL P190 and BCR/ABL P210. The findings presented here do not support these conclusions.

Ph+ myeloid leukemia in human and mouse. BCR/ABL expression in human chronic myeloid leukemia results in myeloid expansion, which may increase the chance for additional mutagenic events, that ultimately accompany malignant transition to fatal blast crisis. We found evidence of myeloid disease at terminal stages of several BCR/ABL P210-transgenic animals, although the frequency was relatively low. Because no obvious evidence was found for the development of myeloid proliferative disorders secondary to other primary malignancies, we assume that the observed myeloid involvement was neoplastic. The mixed lymphoid-myeloid leukemia, which was found in 1 case, seems to indicate that BCR/ABL P210 expression, in contrast to BCR/ABL P190, can disturb differentiation or proliferation of relatively rare myeloid-lymphoid precursors in the mouse. Such rare stem cell origins in Ph+ leukemia are occasionally encountered, also, switching within myeloid and pre-B lymphoid tumor lineages has been reported, showing a close developmental link between these hematopoietic lineages.

The absence of a chronic phase and the low frequency of myeloid disease in the present study could be a direct result of the restricted size of the cell population subject to secondary mutational events in a small animal like the mouse. A similar scenario was suggested to explain the absence of retinoblastoma in animals heterozygous for the tumor-suppressor retinoblastoma susceptibility gene (Rb)-mutant animals. Alternatively, the mouse may be more prone to develop lymphoid neoplasias than spontaneous myeloid disorders. This finding, combined with the observation that BCR/ABL P190 targets cells of both myeloid and lymphoid origin in a transgenic setting, may explain the natural predilection for BCR/ABL-mediated lymphoid leukemia.

In humans, about 50% of cases of Ph+ ALL carry the BCR/ABL P190 translocation, with the other 50% carrying the P210 translocation. Consequently, the issue of whether Ph+ ALL is actually CML in blastic phase has been controversial for some time. However, clinical and molecular studies have recently established that Ph+ ALL and acute-phase CML are indeed two distinct diseases. All results of the present study support this concept. In transgenic mice, BCR/ABL P210 clearly causes a distinct disease from BCR/ABL P190. This is caused by inherently distinct properties of P190 and P210, possibly including the intrinsically different kinase activities of the ABL moiety in the hybrid oncoproteins and/or cellular interactions of the DbI and pleckstrin homology domains or as of yet to be identified domains in Bcr. In addition, regulatory sequences upstream from or within the BCR gene are likely to contribute to the occurrence of myeloid leukemia in humans and presumably in mice. These ideas will be the subject of future investigations in our laboratory.

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BCR/ABL P210 and P190 cause distinct leukemia in transgenic mice

JW Voncken, V Kaartinen, PK Pattengale, WT Germeraad, J Groffen and N Heisterkamp