THE PHILADELPHIA (Ph) chromosome was the first chromosomal abnormality associated with a specific malignant disease in humans, namely chronic myeloid leukemia (CML). Later it was identified as one partner in a reciprocal translocation between chromosomes 9 and 22, referred to as t(9;22)(q34;q11). We know now that this translocation results in the formation of two hybrid genes, BCR-ABL on the Ph chromosome and ABL-BCR on 9q+. The BCR-ABL gene encodes a fusion protein with elevated tyrosine kinase activity which is regarded as central to the mechanism that underlies the chronic phase of CML. The role, if any, of the reciprocal fusion gene, ABL-BCR, remains unknown.

More than 95% of patients with clinically 'acceptable' CML have a BCR-ABL gene in their leukemia cells, so the t(9;22) can generally be regarded as the hallmark of CML. However, it is not exclusive to CML because it is found in 10% to 20% of adults and in 2% to 5% of children with acute lymphoblastic leukemia (ALL), as well as in occasional bone fide cases of acute myeloid leukemia (AML), lymphoma, and myeloma. In the current issue of Blood, Pane et al add to this list a new disease entity, namely Ph+ chronic neutrophilic leukemia (CNL), with a novel position for the breakpoint in the BCR gene. They bring into focus the fascinating suggestion that the precise location of the breakpoint in BCR (and in ABL), and thus the composition of the fusion BCR-ABL protein, may determine the disease phenotype.

STRUCTURE OF THE VARIOUS BCR-ABL FUSION GENES AND THEIR TRANSCRIPTS

The breakpoint in the ABL gene can occur anywhere within a >300-kb segment at the 5' end of the gene, either upstream of the first alternative exon Ib, between exons Ib and Ia, or downstream of exon Ia (Fig 1). In the vast majority of CML patients and in about one third of ALLs the breakpoint in the BCR gene is found within a 5.8-kb region known as the major breakpoint cluster region (M-bcr), spanning 5 exons historically named b1 to b5, now known to be exons 12 to 16 of the BCR gene. Regardless of the position of the ABL breakpoint, processing of the primary BCR-ABL transcript usually results in hybrid BCR-ABL mRNA molecules with a b3a2 and/or a b2a2 junction encoding a p210BCR-ABL fusion protein. In two thirds of ALLs and in very rare cases of CML and AML patients (see below), the breakpoint in BCR falls further upstream, in the long (54.4 kb) intron between the two alternative exons e2' and e2, known as the minor bcr (m-bcr). In these circumstances, exons e1' and e2' are removed by splicing, and the hybrid BCR-ABL transcript contains an e1a2 junction, and is translated into a smaller 190-kD BCR-ABL fusion protein (p190BCR-ABL).

In 1990, Saglio et al described the first cases of CML with a 3' BCR breakpoint, between exons e19 and e20 (originally named exons c3 and c4). The resulting mRNA transcript in these two cases contained an e19a2 BCR-ABL fusion, coding for a large p230BCR-ABL product. A similar patient was reported last year from whom a Ph+ cell line expressing the p230BCR-ABL fusion protein was derived at the time of terminal myeloid blast crisis. The report by Pane et al describes 3 BCR-ABL+ CNL patients, all of whom had a BCR breakpoint located in this 3' end of the gene; the investigators suggest the notation μ-bcr for this region. Of considerable interest is the investigators' comment that the original two patients reported in 1990 might be, in retrospect, better reclassified as CNL rather than as classical CML. A patient of Wada et al was described as atypical Ph+ CML. Does this preferential, if not exclusive, association mean that the inclusion of "additional" BCR sequences in the BCR-ABL fusion gene enables more of the leukemic granulocytes to proceed to complete maturity? Are there other precedents for similar associations between particular types of BCR-ABL+ leukemias and specific BCR-ABL gene/protein structures? What could be the molecular and cellular mechanisms that dictate the final disease phenotype in the t(9;22)-derived malignancies?

Table 1 lists the types of malignant diseases reported so far in connection with the t(9;22)(q34;q11), and the various BCR breakpoints and BCR-ABL fusion transcripts charac-
Fig 1. Schematic representation of the ABL and the BCR genes disrupted in the t(9;22)(q34;q11). Note the 8604Met gene, which is located immediately upstream of the ABL locus, and whose function is unknown. Exons are represented by boxes and introns by connecting horizontal lines. Breakpoints in ABL, illustrated as vertical arrows, almost invariably occur either upstream of exon Ib, between Ib and Ia, or between Ia and a2. The BCR gene contains 25 exons, including two putative alternative first (e1') and second (e2') exons. The breakpoints in BCR usually occur within one of three breakpoint cluster regions (bcr), the location and probable extents of which are shown by the three double-headed horizontal arrows. In exceptional cases the BCR breakpoints fall between m-bcr and M-bcr, within the region indicated by the double-headed dashed-line arrow. The lower half of the figure shows the structure of the various BCR-ABL mRNA transcripts which are formed in accordance with the position of the breakpoint in BCR. Breaks in m-bcr give origin to BCR-ABL mRNA molecules with an ela2 junction. The breaks in M-bcr occur either between exons b2 (e13) and b3 (e14) or between b3 and b4 (e15), generating fusion transcripts with a b2a2 or a b3a2 junction, respectively. Breakpoints in μ-bcr, the most 3' cluster region, result in BCR-ABL transcripts with an e19a2 junction (originally described as c3a2).

Table 1. Types of Ph+ Leukemias Associated With Specific BCR Breakpoint Cluster Region (bcr) Rearrangements and Corresponding BCR-ABL Fusion Transcripts

<table>
<thead>
<tr>
<th>Disease (references)</th>
<th>M-bcr BCR-ABL Fusion</th>
<th>m-bcr BCR-ABL Fusion</th>
<th>μ-bcr BCR-ABL Fusion</th>
</tr>
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<tbody>
<tr>
<td>CML</td>
<td>b3a2</td>
<td>e1a2</td>
<td>e19a2</td>
</tr>
<tr>
<td>(8, 23, 25, 26, 36, 74)</td>
<td>b3a2</td>
<td>e1a2</td>
<td>e19a2</td>
</tr>
<tr>
<td>(9, 11, 27, 28, 70, 72, 73)</td>
<td>b3a2</td>
<td>e1a2</td>
<td>e19a2</td>
</tr>
<tr>
<td>ALL</td>
<td>b3a2</td>
<td>e1a2</td>
<td>e19a2</td>
</tr>
<tr>
<td>AML (12, 16, 27)</td>
<td>b3a2</td>
<td>e1a2</td>
<td>e19a2</td>
</tr>
<tr>
<td>ET (60-62)</td>
<td>b3a2</td>
<td>e1a2</td>
<td>e19a2</td>
</tr>
<tr>
<td>MM (20)</td>
<td>e1a2</td>
<td>e1a2</td>
<td>e1a2</td>
</tr>
<tr>
<td>B lymphoma (17)</td>
<td>e1a2</td>
<td>e1a2</td>
<td>e1a2</td>
</tr>
</tbody>
</table>

Abbreviation: MM, multiple myeloma.

Variability between BCR breakpoint cluster regions. In adults with Ph+ ALL the leukemias with M-bcr breakpoints (p210 ALLs) do not differ in any significant biologic or clinical respect from those with m-bcr breakpoints, ie, p190 ALLs. The situation may be somewhat different in childhood Ph+ ALL, where p210-type disease represents a small minority of cases: in a single series of 28 children studied by molecular methods, 24 had e1a2 BCR-ABL transcripts, ie, p190 ALL, and only 4 had an M-bcr breakpoint with p210-type BCR-ABL transcripts. It is noteworthy that these 4 children all had white blood cell (WBC) counts >250 × 10^9/L at diagnosis, in contrast to only 2 of the 24 patients with p190 ALL, suggesting that in the pediatric group the presence of p210 BCR-ABL is more frequently associated with the hyperleukocytotic ALL. This interpretation should nevertheless be viewed with caution, because some of these childhood p210 ALLs may represent lymphoid blast crisis of CML following a clinically silent chronic phase.

However, it is among leukemias that affect predominantly the myeloid lineage that the impact of the amount of BCR sequences in the BCR-ABL gene is seen most clearly. The
classical Ph+ CML phenotype, almost invariably derived from a p210 type of BCR-ABL fusion protein (M-bcr breakpoint), is characterized by a neoplastic expansion of the granulocytic and megakaryocytic lineages. Although the erythroid, the monocytic, and at least part of the B- and T-lymphoid lineages have the t(9;22),32,34 there is scant evidence that these cells are functionally compromised in CML. The presence of p210BCR-ABL in CML granulocytic progenitors causes, by some still unknown mechanism, a relatively "moderate" degree of disruption in their differentiation and maturation pathways,35 allowing a small proportion of cells to reach the terminal stages of mature neutrophils, eosinophils and basophils, but apparently arresting the majority at the intermediate stages of myelocytes and metamyelocytes. Pane et al36 show now that, in contrast to p210, the larger p230BCR-ABL protein seems to disrupt the normal process of granulocytic differentiation much less, leading to a disorder characterized by exuberant production of mature neutrophils (CNL), and an apparently indolent or "benign" clinical course. Another good example of the way in which the quantity of BCR sequences influences the phenotype of the leukemia is the rare group of CML patients with m-bcr breakpoints and exclusive production of a p190BCR-ABL fusion protein.36 The common characteristic of these cases is a prominent absolute and relative monocytosis, with a low neutrophil-to-monocyte ratio, features that are more frequently associated with chronic myelomonocytic leukemia (CMML) than with CML. Conversely, these patients have a variable degree of basophilia, a relatively high proportion of circulating immature granulocytes, and a low neutrophil alkaline phosphatase (NAP) score, so that on balance they seem to have a disease with features intermediate between CML and CMML.36 It appears that the presence of p190BCR-ABL in a committed early myeloid cell results in a myeloproliferative defect that includes the monocytic lineage, whereas p210BCR-ABL in the same type of progenitor restricts the excessive proliferation to the granulocytic pathway. This hypothesis is supported by the fact that among the rare patients with Ph+ AML who were studied by molecular techniques, all those with m-bcr breakpoints also had a myelomonocytic-phenotype (French-American-British [FAB] M4 or M5a).12-16 Conversely, m-bcr rearrangement leading to p190BCR-ABL has not been reported in any Ph+ AML with phenotypes other than M4.

Taken together, the available data so far suggest that one can distinguish at least three distinct clonal/hematological entities among the chronic (p210 CML, p190 CML, and p230 CML), and two among the acute (p210 AML and p190 AML) Ph+ myeloid leukemias, depending on the type of BCR-ABL fusion protein produced by different BCR breakpoints.

For the sake of completeness one should include in this category some sporadic Ph+ CML and ALL patients characterized by BCR breakpoints outside the three identified cluster regions (Table 2). The most recent report37 in fact describes a case of Ph- CML, although the BCR-ABL translocation could be recognized by fluorescence in situ hybridization (FISH) of metaphase chromosomes. This patient had an unusual BCR-ABL transcript with an e6a2 junction, resulting from a breakpoint in BCR intron 6, and encoding a BCR-ABL hybrid protein of approximately 195 kD. Negrini et al38 and Saglio et al39 reported one and three cases, respectively, in whom the breakpoints were mapped by restriction sites as occurring 2 to 12 kb upstream of the first BgII site in M-bcr. With the recent availability of the complete sequence of the BCR locus34 it is possible to define more precisely the position of these breakpoints, which are all found to be located in intron 8, or between introns 8 and 10. In the single case among those four tested for BCR-ABL expression39 no RT/PCR product was detected. Although the investigators used three pairs of primers covering the possible junctions of BCR-ABL transcripts derived from breakpoints in any of the three defined bcrs, they might still have missed a more likely RNA fusion for this breakpoint, such as e6a2. (For a discussion on potential in-frame BCR-ABL fusions see reference 37). Analysis of the Southern hybridization results40 from case no. 6 (Table 2) suggests that the disruption of the BCR gene probably occurred in intron 10. The breakpoint region in the last patient of this group41 (Table 2) can be defined less precisely because it was mapped with a cDNA probe spanning the 3′ mid-portion of exon 2 up to the middle of exon 6. The break in this case could have occurred anywhere along introns 2 to 5, and in these circumstances it is surprising that the chimeric BCR-ABL mRNA transcript had the same size as that of classical p210 CMLs.41 Based on the possible in-frame BCR-ABL fusions,37 the most likely BCR-ABL transcript junction from this breakpoint would be e1a2, which would give rise to a 7-kb mRNA species, as seen in p190BCR-ABL leukemias.

Could the genomic rearrangement observed in these cases be part of yet another rare BCR breakpoint cluster region? And if that proved to be the case, are there any common or particular features of the disease in these cases? The patient with the e6a2 BCR-ABL transcript37 had an unusual hematologic picture, in so far as the disease started with a phase of cycling leukocytosis not requiring treatment over 20 months, after which an extramedullary myeloblastic infiltration occurred. A patient of Bartram et al41 was maintained in chronic phase for 9 years with busulfan, then developed a lymphoid blast crisis but showed a remarkably good response to combination chemotherapy. A patient of Negrini et al42 was reported 3.5 years after diagnosis, during which time he had remained in chronic phase controlled by hydroxyurea alone. The three cases studied by Saglio et al43 had clinical features and prognosis that did not appear any worse compared with those generally seen in CML. Negrini et al38 made the interesting observation that both their patient and all the patients with breakpoints outside M-bcr reported by Saglio et al and by two other groups42,44 came from Northern Italy, from an area where occupational exposure to pesticides is very common. (The patient of Negrini et al was a farmer with a 20-year unprotected exposure to organophosphates and carbamates.) Although it is tempting to speculate on a possible link between exposure to some classes of pesticides and particular breakpoints in BCR, it is obvious that well-controlled combined epidemiologic and molecular studies would be needed to substantiate any such association.
Variability within M-bcr. Does the position of the breakpoint within the M-bcr region (5' M-bcr v 3' M-bcr), or the type of fusion transcript (b2a2 v b3a2) influence the disease phenotype? This debate has now been going on for several years, with some evidence in favor8-52 and some against45-51 a possible link between M-bcr breakpoint location and disease features. Like so many other long-standing arguments in biology, the truth may actually lie somewhere between the two extreme views.

Much of the discrepancy or discordance between the various studies could be due to qualitative and/or quantitative differences in patient or sample selection. A survey of the literature shows that the majority of the reports that suggest that CML patients with 5' M-bcr breakpoints and/or b2a2 fusion transcripts do better (longer chronic phase, blast crisis in patients with 3' M-bcr breakpoint),45-51 when the total number of patients analyzed was increased by small groups of patients.45-51 More recent prospective studies of large series failed to confirm any significant correlation between M-bcr breakpoint location and disease outcome.45-52 A similar 'normalization' seems to occur with reference to the postulated higher frequency of lymphoid blast crisis in patients with 3' M-bcr breakpoints,55,56 when the total number of patients analyzed was increased by combining data sets from three comparable series54 the apparently significant difference observed in the previous studies could not be confirmed.

On the other hand, another type of association does seem to be supported at least partly by published studies, and that is the link between the type of BCR-ABL fusion transcript and the platelet counts. The overall results from a recent prospective CML trial in the United Kingdom also failed to detect a correlation between either genomic or RNA findings and platelet numbers.8 However, when the UK series was divided into two groups according to the WBC counts at diagnosis, significantly higher platelet counts were observed in patients with b3a2 transcripts who had <100 × 10⁹/L. Although the number of patients in each subgroup was small, and therefore prone to statistical error, the data suggest that there may be a subpopulation of p210 CML patients in whom the b3a2 transcript is associated with significant excess thrombopoiesis.8 It is interesting to note incidentally that of a total of 10 patients with Ph+ essential thrombocythemia (ET) reported in three separate molecular studies,60-62 nine expressed b3a2 and only one expressed b2a2 mRNA fusion transcripts. Because these patients with a so-called Ph+ ET have several features resembling CML, but a typically moderate leukocytosis (WBC = 11 to 22 × 10⁹/L in the eight cases from references 61 and 62), they may be regarded as a special form of CML characterized by a preferential involvement of the megakaryocytic lineage.

Finally, it is worth considering the rare cases of Ph+ childhood CML. This form of CML, which differs from the Ph+ juvenile type, exhibits the same clinical, morphologic, and cytogenetic features as CML in adults. Also similar to the adult classical CML profile is the fact that Ph+ CML in children is apparently always of the p210 type, with no significant difference in the distribution of 5' and 3' M-bcr breakpoints.64,65 Nevertheless, the populations in the two age groups seem to differ in relation to the type of mRNA fusion transcripts: the two-third preponderance of b3a2 plus dual b3a2/b2a2 expressing cases observed in adults66 is reversed in Ph+ childhood CML, where 10 of 12 patients analyzed by reverse transcriptase-polymerase chain reaction (RT-PCR) in a single series expressed only b2a2 BCR-ABL.67 Since the number of children studied by molecular methods is obvi-

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Reference</th>
<th>Diagnosis</th>
<th>Region</th>
<th>Delimiters (nt. no.)* (restriction site)</th>
<th>BCR-ABL Transcript (method of detection)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>37</td>
<td>CML</td>
<td>Intron 6</td>
<td>106451-107181 (Bcll-BglII)</td>
<td>e6a2 (RT/PCR)</td>
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<tr>
<td>2</td>
<td>38</td>
<td>CML</td>
<td>Intron 8 &lt;&lt; ?10</td>
<td>112077 &lt;&lt; ?119935 (BamHI-Bcll)</td>
<td>Not detected (RT-PCR)</td>
</tr>
<tr>
<td>3</td>
<td>39</td>
<td>CML</td>
<td>Intron 8</td>
<td>108778-110790 (EcoRI-Bcll)</td>
<td>Not tested</td>
</tr>
<tr>
<td>4</td>
<td>39</td>
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<td>Intron 8</td>
<td>110790-112077 (Bcll-BamHI)</td>
<td>Not tested</td>
</tr>
<tr>
<td>5</td>
<td>39</td>
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<td>Intron 8 &lt;&lt; ?10</td>
<td>117214-119935 (KpnI-BamHI)</td>
<td>Not tested</td>
</tr>
<tr>
<td>6</td>
<td>40</td>
<td>ALL</td>
<td>Intron 10</td>
<td>119935-121621 (Bcll-BglII)</td>
<td>Not tested</td>
</tr>
<tr>
<td>7</td>
<td>41</td>
<td>CML</td>
<td>Intron 2 &lt;&lt; 5</td>
<td>87913-105648 (PstI-BamHI)</td>
<td>8 kb (Northern)</td>
</tr>
</tbody>
</table>

(7) The question marks before some intron and nucleotide numbers are due to a disagreement between the location of the Bcll site reported by Saglio et al and the actual Bcll sites found between introns 8 and 10.45

* Nucleotide numbers (nt. no.) are according to the complete BCR gene sequence, GenBank Accession No. U07000.
ously small, any statistical and possibly functional significance of these differences is so far uncertain.

**Unusual breakpoints in ABL.** Although several different junctional configurations can be identified in the various leukemia-associated BCR-ABL transcripts (eg, b2a2, b3a2, e1a2, e19a2, e6a2), these are usually characteristically produced by a variation in the position of the breakpoint in the BCR gene. Therefore, this seemingly consistent BCR-a2 junction structure led to the belief that the sequence encoded by exon a2 must be essential for ensuring the functional properties of the BCR-ABL protein. However, in 1990 Soekarman et al. reported two cases of Ph+ ALL where the BCR sequences in the hybrid transcripts were fused to ABL exon a3 rather than a2. In one patient the BCR-ABL mRNA had a b2a3 junction, and in the other an e1a3 junction; in both cases the breakpoint in ABL was located between exons a2 and a3. Interestingly, the BCR-ABL fusion proteins produced in the cells from the b2a3 and the e1a3 patients had approximately the same molecular weight as those from classical p210BCR-ABL and p190BCR-ABL leukemias, respectively, and also exhibited similar tyrosine kinase activities. Five other cases of Ph+ leukemias with BCR-a3 fusion transcripts have since been reported, comprising 3 additional ALLs and 2 CMLs. Among the 7 reported patients, 2 had mRNA transcripts with e1a3, 3 with b2a3, and 2 with b3a3 junctions. Whereas the ABL breakpoint fell between exons a2 and a3 in 3 of 5 cases tested, in the remaining 2 (both b3a3) no rearrangement was detected in this intron, suggesting that deletion of exon a2 from the chimeric transcript in the latter patients was caused by splicing of BCR exon b3 to ABL exon a3. All in all, regardless of the molecular mechanism of the BCR-a3 hybrid messages, the few patients described so far seem to be clinically indistinguishable from other Ph+ CML and ALL patients. Iwata et al. did speculate that the low WBC counts observed in 3 of the ALL individuals might be a characteristic of this molecular variant of Ph+ ALL, but more cases must be studied to clarify the issue.

**Alternative splicing of the BCR-ABL message.** This last category of variability is typically exemplified by the simultaneous presence in cells from CML and ALL patients of both p210-type (ie, b3a2 and/or b2a2) and p190-type (e1a2) BCR-ABL transcripts. Until very recently, identification of what seemed to be sporadic cases with this sort of dual mRNA expression led to various speculations on its possible meaning. The favored explanation was that the appearance of e1a2 transcripts in p210-expressing CML patients heralded the emergence of acute transformation or blast crisis. Two recent studies have independently shown that coexpression of p210 and p190-type BCR-ABL mRNA molecules can in fact be detected by sensitive RT-PCR assays in the vast majority (>90%) of CML and ALL patients with M-bcr breakpoints. In CML, the phenomenon was observed in both chronic phase and blast crisis samples, and even in a large proportion of patients receiving interferon-α treatment. Quantification of both types of BCR-ABL transcripts by different methods produced discordant results between the two studies: whereas van Rhee et al. found that the level of e1a2 mRNA molecules was, on average, 3 orders of magnitude lower than that of b3a2 or b2a2 transcripts, Saglio et al. reported it to be as high as 20% to 30% of the total BCR-ABL transcripts. This coexpression of the two mRNA forms of BCR-ABL in Ph+ leukemias with M-bcr breakpoints is probably due to alternative splicing or to mis- }

**STRUCTURE OF THE BCR-ABL FUSION PROTEIN AND DISEASE PHENOTYPE**

Although the position of the breakpoints in individual regions of the BCR and ABL genes seems to be associated with particular clinical and/or morphologic forms of Ph+ CML or ALL, the key determinant of this effect must be the structure/composition of the actual BCR-ABL fusion proteins produced by these different genomic rearrangements. Over the past 10 years, an enormous amount of effort has been devoted to the study of the normal ABL and BCR proteins. The fine dissection of their individual domains, as well as the study of their interactions with other cellular proteins, are beginning to reveal some clues as to their mechanism of action in both normal (ABL and BCR) and leukemic (BCR-ABL) pathways. What are these clues, and how do they explain the translation of molecular into cellular and, ultimately, clinical events?

Figure 2 is a schematic representation of the normal ABL, the normal BCR, and some forms of BCR-ABL proteins associated with Ph+ leukemias. As illustrated in this figure and discussed by Pane et al., the BCR-ABL rearrangement derived from BCR breakpoints in M-bcr, m-bcr, and µ-bcr will differ considerably in the number and integrity of the BCR protein domains included or excluded in the final BCR-ABL product. The smallest, p190BCR-ABL (m-bcr), contains only the coiled-coil motif from the dimerization domain, the GRB2 binding site at Tyr177, and the phospho-serine/threonine-rich SH2 binding domains encoded by BCR exon e1, all of which are essential and sufficient for activation of the ABL tyrosine kinase. p190BCR-ABL is predominantly associated with ALL, and this may indicate a preferential functional involvement of this form of hybrid product in metabolic pathways of the lymphoid lineage progenitors. In other words, the lack of BCR domains encoded by sequences downstream of exon e1 in the hybrid BCR-ABL protein may be irrelevant to the mechanism by which signal transduction is deregulated by p190BCR-ABL in lymphoid precursors, but may be more restrictive or inefficient in CML progenitors. The rarity with which p190BCR-ABL leads to CML and AML, and the fact that in both types of Ph+ myeloid leukemia there
is a marked monocytic involvement support the idea that a BCR-ABL protein with only a small extent of BCR sequences interferes with a specific chain of protein interactions that is activated preferentially during lymphoid and monocytic differentiation.

The commonest p230BCR-ABL protein (M-bcr breakpoints) contains, in addition to the aforementioned amino-terminal domains, the dbl-like domain of BCR that encodes a GDP-GTP exchange factor for the ras-like polypeptide CDC42Hs. A BCR-ABL protein containing these additional domains seems to affect the signal transduction pathways common to both ALL (lymphoid-committed) and CML (pluripotential stem cell) precursors, endowing either type of cell with a growth advantage. As mentioned above, the leukemic clones of p190 ALL and p210 ALL seem to be indistinguishable in their clinical phenotype, at least in the adult form of the disease, suggesting that deregulation of either type of metabolic pathway in lymphoid progenitors has the same cellular and clinical consequences. This is somewhat in contrast with in vitro and animal studies which suggested that p190BCR-ABL has a greater capacity to transform immature B-lymphoid lineage precursors than p210BCR-ABL. In CML, it is possible that inclusion of the dbl-like domain in the BCR-ABL fusion protein blocks the biochemical regulation of normal granulocytic differentiation with variable efficiency, since a spectrum of maturation arrest stages can be produced in cells derived from the same leukemic stem cell clone. Alternatively, this apparently quantitative effect may be unrelated to the presence of the dbl-like domain, but rather the consequence of exclusion from p190BCR-ABL of a specific BCR carboxy (C)-terminal domain (see below).

The largest of the BCR-ABL proteins, p230BCR-ABL (μ-bcr breakpoint), includes over 90% of the BCR amino acids, lacking only the C-terminal two thirds of the GAP domain. This domain is responsible for the GTPase activating protein (GAP) function of the normal BCR protein for p210, a small GTP-binding protein that is involved in the induction of plasma membrane ruffling and stimulation of NADPH neutrophil oxidase. The amino(N)-terminal GAP amino acid residues retained in p230BCR-ABL are essential for the GAP activity of BCR, and it is thus possible that p230BCR-ABL, unlike p210BCR-ABL, has an intact GAP function. Thus, it is tempting to speculate that the reason why p230BCR-ABL cells are capable of proceeding normally through the stages of granulocytic differentiation is because both copies of their BCR gene (ie, the normal and the translocated p230BCR-ABL alleles) encode proteins that have a normal, ‘physiological’ GAP function for rac, a protein which displays relative myeloid specificity. Conversely, p210BCR-ABL and p190BCR-ABL CML cells have only one BCR gene (the normal copy) with an intact GAP domain located in its original, normal position. The GAP domain from the disrupted BCR gene is translocated to chromosome 9 and, depending on the breakpoint in the latter, the 3’ BCR sequences are juxtaposed to 5’ ABL sequences (reciprocal ABL-BCR gene). If the location of the BCR GAP domain is responsible for impairing granulocytic maturation in p210 and p190 CML, this could be due partly to a dosage effect, consequent to the functional haploidy for the normal GAP domain in these CML cells. The lack of a detectable phenotype in mutant BCR (+/-) heterozygous mice argues against a gene dosage effect of monoallelic loss of BCR, although the situation is not entirely analogous to that of human BCR-ABL+ leukemias. Alternatively or additionally, a positional effect of the BCR GAP domain could result from expression in both p190 and p210 CML of an altered GAP-containing protein, the production and cellular localization of which are dictated by the promoter and 5’ coding sequences of a different gene. This gene could be either ABL in cases where an ABL-BCR reciprocal gene is formed, or even possibly the 8604Met gene, mapped immediately upstream of the ABL locus (Fig 2), in patients where the chromosome 9 breakpoint is found in this region. It must be
emphasized that this model is of necessity speculative at this stage, because it is not clear whether the level of normal p160BCR production in CML cells differs from that of normal leukocytes, nor whether ABL-BCR (or 8604Met-BCR) functional proteins are synthesized in Ph+ leukemia patients.

Very little can be said about the possible associations between other types of BCR-ABL rearrangements reviewed here and disease phenotype. These cases, involving breakpoints outside the three recognized cluster regions of the BCR gene or in atypical locations in ABL, are rare and some of them were described before the advent of sensitive molecular techniques. However, it is worth remembering that the BCR-ABL protein translated from unusual transcripts (eg, e6a2, b2a3, b3a3, e1a3), despite lacking a number of amino acids coded for by the ‘missing’ exons, is still oncogenic, because the patients do have CML or ALL. Therefore, cases such as these can be seen as nature’s ‘deletion mutants,’ and their detailed molecular characterization can provide invaluable clues to our understanding of the mechanisms of BCR-ABL leukemogenesis. Thus, it is clear that BCR sequences between amino acid residues 819 and 927 (Fig 2) must be nonessential to the CML-inducing potential of BCR-ABL, since the whole or part of this region is missing from the fusion protein in the e6a237 and possibly some of the other patients described in Table 2, as well as from a Ph+ CML patient studied by Selleri et al85 who had an interstitial 3-exon skipping (BCR e9, e10, and e11) in a 'p210-type' BCR-ABL mRNA. Similarly, the absence of 58 amino acid residues encoded by exon a2, which is part of the ABL SH3 domain, seems to be irrelevant in the context of the BCR-ABL fusion protein, probably because, as argued by Sockerman et al,70 the inhibiting function of the SH3 domain is likely to have been superseded by the juxtaposition of the 5' BCR sequences.

It is fascinating to witness the progress in our present knowledge of the molecular basis of the t(9;22)-derived leukemias since the translocation was first described barely 20 years ago.2 Although there are still several fundamental gaps to be filled, hopefully within the next few years, we are now in a position where we could draw a tentative map linking the DNA, RNA, and protein defects to the specific cells affected and to the clinical features in the patient. Further carefully conducted molecular studies coupled with a keen clinical insight as demonstrated by Pane et al83 in their report should help us to unravel some of the links still missing in the map.

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