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MECHANISMS OF DISEASE

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THE MOLECULAR GENETICS OF PHILADELPHIA CHROMOSOME–POSITIVE LEUKEMIAS

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ACTIVATION of cellular oncogenes by chromosomal translocation has been strongly implicated in the pathogenesis of several cancers. One of the most striking examples of this phenomenon occurs in leukemias involving the Philadelphia (Ph^1) chromosome. The Ph^1 chromosome is a shortened chromosome 22 that arises from a reciprocal translocation, t(9;22)(q34;q11).[1,2] This cytogenetic aberration occurs in the majority of patients with chronic myelogenous leukemia (CML)[3] and in a much smaller proportion of patients with acute lymphoblastic leukemia (ALL) or acute myelogenous leukemia (AML).[4,5] The Ph^1 translocation results in transposition of rhe cellular *abl* (c-abl) gene from its usual residence on chromosome 9 to chromosome 22.[6,7] The enzymatic properties of this gene's protein product suggest that it could be involved in the control of normal cell growth. The transposition appears to activate the tumorigenic potential of c-abl. Interestingly, despite the presence of a cytogenetically identical t(9;22) anomaly in CML, ALL, and AML, the c-*abl* gene can interact with different, albeit nearby, chromosome 22 DNA sequences in these diseases.[8-14] Thus, chromosomal translocation may serve as a mechanism to disrupt the functions of normal genes and hence to convert proto-oncogenes to oncogenes. Furthermore, subtle genomic differences may be critical determinants of cancer phenotype. Here, we review the concepts that have emerged from studies of the molecular basis of Ph^1-positive neoplasms.

BACKGROUND

Chronic Myelogenous Leukemia

CML is a clonal cancer arising from neoplastic transformation of the hematopoietic stem cell.[15] The hallmark of CML, found in at least 90 to 95 percent of cases, is a truncated chromosome 22 — the Ph^1 chromosome.[1,2] This karyotypic anomaly is generally detected in 100 percent of bone marrow metaphases observed in patients with CML who are positive for Ph^1. The classic Ph^1 chromosome results from a translocation between chromosomes 9 and 22, t(9;22)(q34;q11).[2] In addition, some patients may have complex translocations involving three, four. or five chromosomes (including chromosomes 9 and 22). On the basis of Cytogenetic findings, a small proportion of patients were previously thought to have involvement of chromosome 22 and a chromosome other than 9 (variant translocations)[16]; however, recent molecular studies have established that chromosome 9 is universally affected by the Ph^1 translocation.[6]

Clinically, CML is characterized by a triphasic course, which exemplifies the malignant evolution believed to occur in most cancers but is less well defined in other types of tumors. The disease begins with a chronic phase marked bv a greatly increased pool of committed myeloid progenitor cells. Terminal differentiation of cells is maintained, resulting in profoundly elevated counts of circulating mature granulocytes. It is not known why the myeloid compartment is preferentially affected in the chronic phase, since CML is a stem-cell disorder. The chronic phase can be man-

Glossary:

3'	The end of a gene at which transcription ceases
5'	The end of a gene at which transcription begins
Exon	A segment of a gene that is represented in the mRNA product
Intron	A segment of a gene that is not represented in the mRNA product because it has been removed through the splicing together of sequences (exons) on either side of it
Kilobase (kb)	1000 base pairs of DNA or 1000 bases of RNA
N-terminal	The end of a polypeptide that has an amino acid with a free amino group; proteins are synthesized starting from the N-terminal amino acid
Oncogene	A gene that causes cancer; oncogenes are altered versions of ordinarily benign genes present in normal cells
Proto-oncogene	A normal gene with the potential to become activated and hence a cancer gene (oncogene)
Splice	The removal of introns and joining of exons in RNA: introns are spliced out, whereas exons are spliced together
Transcription	Synthesis of RNA on a DNA template
Transformation	The conversion of cells in culture to a state of unrestrained growth similar to that of neoplasia
Translation	Synthesis of protein on the mRNA template

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aged by a variety of well-tolerated treatments, and patients generally have a nearly normal quality of life. Unfortunately, after several weeks to many years (median interval, 3.5 years),[17,18] a state of myeloproliferative acceleration develops in which the myeloid cell progressively loses its capacity for terminal differentiation, and thrombocytosis, basophilia, and additional clonal cytogenetic abnormalities often appear. These changes herald the terminal, blast-crisis stage, during which immature blast cells rapidly proliferate and the patient inevitably dies. The phenotype of the blasts at the time of transformation may reflect any of the lineages of the hematopoietic stem cell involved in CML: myeloid (65 percent of cases). lymphoid (30 percent of cases), and erythroid, megakaryocytic, or undifferentiated (<5 percent of cases). Currently, the variables dictating the length of time to blast crisis are unknown.

Ph¹-Positive Acute Leukemia

The Ph¹ chromosome has also been observed in approximately 20 percent of adults with ALL, 2 percent of adults with AML, and 5 percent of children with ALL.[3-5,19] The presence of the Ph' chromosome in a patient with acute leukemia carries a poor prognosis. At the cytogenetic level, this chromosomal anomaly is identical to the Ph¹ translocation of CML. It has been questioned whether Ph¹-positive acute leukemia is a true de novo acute leukemia or, alternatively, a blast-crisis stage of CML that has remained asymptomatic and thus escaped detection during the chronic stage.[20] Evidence supporting a single disease state for Ph¹-positive acute leukemia and CML includes their similar clinical and laboratory manifestations,[''] and well-documented reports of transition from acute leukemia to chronic-phase CML after chemotherapy in some patients with Phi-positive ALL or, rarely, AML.[20] Even so, it seems likely that at least some patients with ALL or AML who have the Ph' anomaly have a bona fide acute presentation.

Molecular Biology of the Ph¹ Translocation

The *abl* Gene

The c-*abl* proto-oncogene resides on the long arm of chromosome 9 (band q34).[7] This DNA region is perturbed by the t(9;22) anomaly associated with CML and acute leukemia. Interestingly, the c-*abl* gene is highly conserved in evolution and is present not only in humans and other vertebrates but even in Drosophila *melanogaster*.[22] This remarkable phylogenetic conservation suggests that the normal c-abl gene has an important role in human ontogeny.

The emergence of molecular technology has allowed extensive characterization of this gene in humans. Cloning of c-*abl* has revealed that it spans at least 230 kb (kilobases), contains at least 11 exons, and is oriented with its 5' end toward the centromere[23-25] (Fig. I). Two alternative first exons exist (exons 1a and 1b). Exon 1a is 19 kb proximal to exon 2; exon 1b is a formidable ≥200 kb proximal to exon 2.

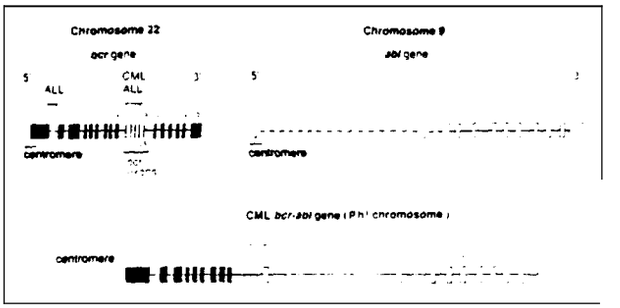


Figure 1. Normal c-*abl* and *bcr* Genes on Chromosomes 9 and 22, Respectively, and the Chimeric *bcr–abl* Gene Associated with CML.

The boxes represent exons (DNA sequences that are included in the mRNA); the number above each box denotes the exon number. The exons are separated by stretches of DNA that are not included in the mRNA product (introns). The relative sizes of the exons and introns and the number of exons may not be exactly as shown. Transcription starts at the 5' end of the genes. The dashed line in the c-*abl* gene represents the DNA segment in which breakpoints occur. The symbol, ↔, above the *bcr* gene. denotes the sections of this gene in which breaks occur in CML or acute leukemia, as designated.

In CML, the breakpoints on chromosome 22 are restricted to a 5.8-kb region, bcr. which is in the midportion of the *bcr* gene. The breakpoints on chromosome 9 span a large 5' region (at least 200 kb) starting proximal to c-abl exon 1b and extending to c-*abl* exon 2. A reciprocal exchange of DNA results: distal *bcr* sequences are moved from chromosome 22 to 9, and distal c-*abl* sequences from chromosome 9 to 22. Consequently. proximal (5') *bcr* sequences are juxtaposed to distal (3') c-abl sequences, and a hybrid *bcr–abl* gene is formed on chromosome 22. Since the exact location of the breakpoint varies among patients, certain exons, denoted by ±, may or may not be found on the Ph' chromosome of individual patients.

In contrast to patients with CML. patients with Ph¹-positive ALL have breakpoints that can occur either within the 5.8-kb bcr or far proximal, within the first intron of the *bcr* gene. (Although not shown in the figure, breaks immediately proximal to the 5.8-kb bcr have been found in a small number of patients with ALL.[11,13,48]) Like patients with ALL, Ph¹-positive patients with AML may be divided into two subgroups, and have chromosome 22 rearrangement either inside or outside the 5.8-kb bcr[11,14]; because the translocation in the latter subgroup has not been cloned, it is not known for certain if this abnormality also occurs within the first intron of the *bcr* gene.

As a result of this genomic configuration, two major c-abf messages are transcribed: 6-kb and 7-kb messenger RNAs (mRNA) (Table I). The 6-kb transcript consists of exons 1a through 11; the 7-kb transcript begins with exon 1b, skips the impressive 200-kb distance to exon 2 (omitting exon 1a), and joins to exons 2 through 1I. An analogous situation occurs in mice, which have four types of c-*abl* message with distinct first exons.[26] Therefore, all the c-*abl* messages share a set of 3' exons, starting from c-*abl* exon 2. These observations indicate that the splice acceptor site associated with exon 2 is unusual in that it can accept multiple exon donor sites, can ignore nearby exons in favor of those farther away, and can jump very long molecular distances (≥ 200 kb). The property of c-abl exon 2 that allows this facile interchange of attaching *abl* DNA segments is pivotal to the tumorigenicity of this

Table 1. Molecular Consequences of the t(9;22)(q34;q11) (Philadelphia) Translocation.

CHROMOSOME	TRANSCRIPTION PRODUCT	TRANSLATION PRODUCT
Normal 9	6- and 7-kb c-*abl* mRNA	145-kd c-ab61 protein ($p145^{c\text{-}abl}$)
Normal 22	4.5- and 6.7-kb *bcr* mRNA	160-kd *bcr* protein ($p160^{bcr}$)
9q+	No message found	No message found
22q−	CML: 8.5-kb *bcr–abl* mRNA	$p210^{bcr\text{-}abl}$
	ALL: 7.0-kb *bcr–abl* mRNA	$p190^{c\text{-}abl}$
	or	or
	8.5-kb *bcr–abl* mRNA	$p210^{bcr\text{-}abl}$
	AML: not yet reported	$p190^{c\text{-}abl}$*

*Protein-expression analysis has been reported in only one patient.[14]

gene, for it also allows c-ab1 to be fused with non-*abl* sequences and thus become activated.

To date, only one normal human c-*abl* protein has been identified. This protein has a molecular mass of 145 kd and is termed $p145^{c\text{-}abl}$.[27] The c-*abl* polypeptide belongs to a family of tyrosine protein kinases. Protein kinases (phosphokinases) are enzymes that catalyze the transfer of the terminal phosphate of adenosine triphosphate to the amino acid residue of the substrate protein. These enzymes may also autophosphorylate. Phosphorylation of proteins is an important cellular mechanism for regulating their functions. Protein kinases usually add phosphate to the amino acids serine or threonine, which have a hydroxyl group to which the phosphate can be linked. Another amino acid that has a hydroxyl group, and thus can be phosphorylated, is tyrosine. This latter amino acid is phosphorylated infrequently; indeed, phosphotyrosine constitutes less than 1 percent of cellular phosphoamino acids. Proteins with tyrosine phosphokinase activity are of intense interest since this enzymatic property is associated with several growth factor receptors, such as epidermal growth factor, platelet-derived growth factor, and colony-stimulating factor receptor, and is closely linked with the tumorigenic potential of some oncogenes.[28] Although amino acid sequence analysis suggests that $p145^{c\text{-}abl}$ is not a receptor for extracellular factors, the close relation between c-*abl* and other tyrosine phosphokinase enzymes indicates that this protein may be involved in control of normal cell growth.

Murine Leukemia

Murine leukemia serves as a model for elucidating the mechanisms responsible for converting c-*abl* from a proto-oncogene to an oncogene, and thus for understanding the events in human Ph^1-positive leukemogenesis.

The *abl* gene was initially discovered through analysis of the Abelson murine leukemia virus.[29] It is now established that the c-abl gene represents the progenitor of the viral *abl* (v-abf) transforming gene within the genome of Abelson murine leukemia virus.[29] This viral genome was formed by a process of recombination between a murine retrovirus and part of the c-*abl* DNA of animal cells. Thus, v-*abl* originates from c-abl. In other words, at some point in evolution, a murine retrovirus incorporated some of the normal c-*abl* DNA of its vertebrate host into its own genome. The protein produced by Abelson murine leukemia virus is formed by N-terminal deletion of part of the c-abl polypeptide and substitution of a *gag* (core) viral moiety. Hence, the v-*abl* protein is a fusion gag–ab1 product (reviewed in references 30 and 31). This alteration in c-abl, which occurred after its integration into the virus genome, activated its tumorigenic potential. The gag–ab1 or v-ab1 protein confers on the Abelson murine leukemia virus the capacity to transform a broad range of hematopoietic cells in vitro.[30,31] In vivo, v-ab1 causes primarily B-cell leukemia in susceptible murine hosts infected with Abelson leukemia virus. The v-*abl* (gag–abl) protein has a much more elevated tyrosine phosphokinase activity than its normal c-*abl* counterpart; this enhanced enzymatic function is probably a key element in its oncogenicity, as attested to by experiments showing that loss of the phosphokinase activity of the v-*abl* protein results in abolishment of its potential for transformation.[32]

A critical concept derived from the murine model of virally induced leukemia is that molecular alteration of normal cellular genes can activate their tumorigenic properties. The corollary of this concept is that similar molecular changes, produced by mechanisms unrelated to viral infection, may be operative in some leukemias. It follows that chromosomal aberrations, such as the Ph^1 translocation, may distort the structure and properties of the normal c-*abl* gene and hence be implicated in the deranged cell growth associated with Ph^1-positive leukemias in humans.

The *bcr* Gene

In 1984 Groffen et al.[8] reported that the breakpoints on chromosome 22 in patients with Ph^1-positive CML were clustered within a 5.8-kb DNA segment designated as the breakpoint cluster region (bcr). In 1985 Heisterkamp et al.[9] demonstrated that the 5.8-kb bcr is actually part of a gene that spans at least 90 kb. This gene was named the brr gene, but has also been called the *phl* gene in some reports.

Over the past few years, molecular studies have yielded prodigious amounts of information about the bcr gene. In fact, the entire coding region for this gene has been cloned,[9,33,34] and its central portion has been shown to be occupied by the 5.8-kb bcr (Fig. 1). The *bcr* gene expresses a 4.5-kb and a 6.7-kb mRNA[24] (Table 1). These transcripts are ubiquitous; indeed, they are detected in fibroblasts, diverse myeloid and lymphoid cell lines, and cells of epithelial origin.[33] The translation product of the bcr gene is a 160-kd protein ($p160^{bcr}$).[35] Only limited data concerning this protein are available: $p160^{bcr}$ does not appear to be a membrane receptor[33] and it can be phosphorylated on serine or threonine,[35] but whether the phosphokinase enzymatic activity associated with this protein is intrinsic to this molecule or is only coprecipitated

with it is unclear. Unfortunately, the role of the *bcr* product in normal hematopoietic processes has not been defined.

Chronic-Phase CYL

The crucial molecular events in Ph¹-positive CML include disruptions of both the c-*abl* gene on chromosome 9 and the bcr gene on chromosome 22. The breakage on chromosome 22 has been pinpointed to the small, 5.8-kb bcr segment in the central portion of the bcr gene, in over 200 published cases of Ph¹-positive CML (Table 2). About 98 percent of patients with CML who have the Ph¹ chromosome have been found to have bcr rearrangement, suggestinq that this molecular change is a sensitive test for Ph¹-positive CML. Importantly, an accurate search for bcr rearrangement requires the use of probes spanning 5' in addition to 3' bcr segments; otherwise, because deletions of bcr sequences (especially in the 3' end) occur in 10 to 20 percent of patients, a single 3' probe will give false negative results for bcr rearrangement in the small group of patients in whom the deletion corresponds to the DNA segment recognized by the probe.[39,50]

The precise point of breakage within bcr is unique in most patients with CML but generally occurs between bcr exons 2 and 3 or exons 3 and 4 (Fig. 1). As a result of the reciprocal exchange of cytogenetic information between chromosomes 9 and 22, all 3' *bcr*-gene exons distal to the point of breakage within bcr are physically removed and relocated to chromosome 9. Proximal 5' bcr-gene exons (including bcr exons 1 and 2, with or without exon 3) remain on chromosome 22. At the same time, the c-*abl* proto-oncogene is transferred from its normal residence at chromosome 9 band q34 to chromosome 22 band q11. In contrast to the small DNA segment involved in rearrangement on chromosome 22, the chromosome 9 breakpoints can span an extended region of ≥200 kb at the 5' end of the c-*abl* gene.[23] However c-*abl* exons 2 through 11 are always included in the segment transposed to chromosome 22, and exons 1a and 1b can be included as well. The end result is that proximal bcr-gene sequences are juxtaposed to c-*abl* sequences in a head-to-tail fashion and thus form a chimeric *bcr–abl* gene on chromosome 22 (Fig. 1).[24] This aberrant genomic configuration occurs even in patients with variant Ph¹ translocations. Therefore, at the molecular level, chromosome 9 is always involved in CML, even if this is not apparent on cytogenetic analysis.

During the process of mRNA formation, the splice acceptor site associated with c-*abl* exon 2 can skip splice donor sites in c-ab1exons 1a and 1b, to fuse with the splice donor sites of the juxtaposed bcr exons.[25] The result is that c-*abl* exons 1a and 1b are not included in the *bcr–abl* mRNA (Fig. 2, Panel a), even though they may be part of the *bcr–abl* gene on chromosome 22 (Fig. 1). Therefore, the aberrant 8.5-kb *bcr–abl* transcript consists of proximal bcr exons (ending in bcr exon 2 or 3) fused to c-*abl* exons 2 through 11 (Fig. 2, Panel a).

Table 2. Summary of Reported Cases of Philadelphia Chromosome–Positive Leukemia Studied for Rearrangement within *the* 5.8-kb bcr Region of Chromosome 22.

Diagnosis	No. of Patients Examined	No. with Rearrangement (%)	References
CML	253	247 (98)	8, 36–42
ALL — adult	46	24 (52)	11–13. 38. 42–49
AML — adult	2	1 (50)	11, 14
ALL — children	9	1 (11)	10–12, 38. 44. 45

The translation product is a chimeric protein of 210 kd — $p210^{bcr-abl}$.[51,52] At least two molecular variants of the *bcr–abl* protein (those with and those without the amino acid segment translated from bcr exon 3) are generated as a consequence of the variability among patients in the exact localization of bcr breakpoint ~$p210^{bcr-abl}$ (regardless of the presence of bcr exon 3) has a much higher tyrosine kinase enzymatic activity than its normal counterpart, $p145^{c-abl}$, in vitro[51-53]; $p210^{bcr-abl}$ and $p145^{c-abl}$ also have dissimilar patterns of autophosphorylation and may interact

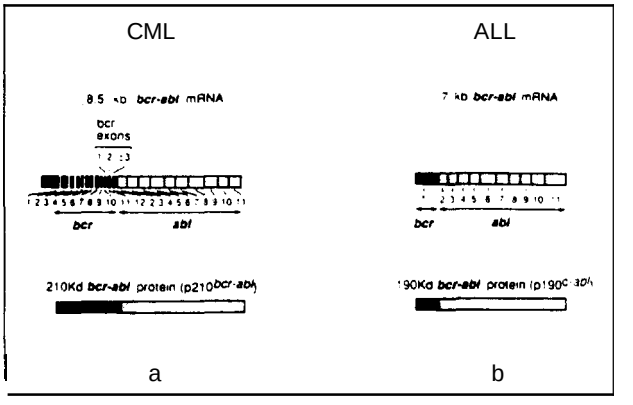


Figure 2. mRNA and Protein Produced as a Result of the Ph¹ Translocation in Cases of CML and Cases of ALL and AML with bcr Rearrangement (Panel a) and in Cases of ALL without bcr Rearrangement (Panel b).

In Panel a the chimeric *bcr–abl* gene (*see* Fig. 1) is transcribed into a hybrid 8.5-kb *bcr–abl* mRNA. The splice acceptor site associated with c-*abl* exon 2 can skip donor sites in c-abl exons 1a and 1b, to fuse with bcr exon 2 or 3. Therefore, c-*abl* exons 1a and 1b are not included in the *bcr–abl* mRNA even though they may be part of the *bcr–abl* gene on chromosome 22q–. The translation product is a 210-kd protein ($p210^{bcr-abl}$), which differs from the normal 145-kd c-*abl* protein ($p145^{c-abl}$) because of the N-terminal substitution of *bcr* sequences.

In Panel b, because the chromosome 22 breakpoint occurs within the first intron of the *bcr* gene (Fig. 1), the first exon of the *bcr* gene is juxtaposed to the translocated portion of the c-*abl* gene. This form of the *bcr–abl* gene is expressed as a 7.0-kb mRNA, which consists of the sequences encoded by bcr exon 1 fused to those encoded by c-*abl* exons 2 through 11. The translation product is a 190-kd protein, termed $P190^{c-abl}$, which differs from both the normal 145-kd c-*abl* protein and the CML-related 210-kd *bcr–abl* protein. pIVV— has also been detected in a case of Ph¹-positive, bcr rearrangement–negative AML.[14]

differently with substrates.[27] Therefore, usurpation of the N-terminal of c-abf by the bcr moiety through chromosomal translocation dysregulates the function of a previously normal gene.

Bcr rearrangement and a *bcr–abl* message are detected in virtually all patients with CML but not in patients with hematologic neoplasms other than Ph^1-positive leukemia.[54] Therefore, p210$^{bcr-abl}$ may represent the first genuine cancer-specific marker! Furthermore, both the alteration in the tyrosine phosphokinase enzymatic activity of this molecule and recent evidence that the *bcr–abl* product can transform hematopoietic cells in vitro[55] constitute powerful support for the belief that p210$^{bcr-abl}$, unlike other tumor markers, such as carcinoembryonic antigen, plays a direct and crucial part in the malignant process.

Ph^1-Negative CYL

A small percentage (≤5 percent) of patients with a presumptive diagnosis of CML do not have the Ph^1 chromosome. Cytogenetic analysis reveals a normal karyotype in about two thirds of patients with Ph^1-negative CML; a variety of cytogenetic anomalies, most commonly trisomy of chromosome 8, are observed in the other third. A minority of patients have a break at 9q34 and reciprocal translocation to another site within the karyotype. Initial studies reported that Ph^1-negative patients with CML had distinct clinical features? short survival, a poor response to therapy, absence of basophilia, and thrombocytopenia. Recently, it has become clear that the disorder of many of these patients can be reclassified on morphologic grounds as one of the myelodysplastic syndromes.[57] However, in view of the bimodal distribution of survival of patients with Ph^1-negative CML,[56] it appears that among them are a subgroup whose clinical features and outcome are remarkably similar to those of patients with Ph^1-positive CML.

Over the past three years, several investigators[58-64] have reported that some patients with Phi-negative CML have molecular features indistinguishable from those of patients with Ph^1-positive CML (Table 2): rearrangement in the 5.8-kb bcr region, juxtaposition of c-*abl* and the *5′* portion of the bcr gene, and expression of the aberrant 8.5-kb *bcr–abl* mRNA and the 210-kd bcr–abf protein. In situ hybridization studies have suggested that, in patients with Ph^1-negative CML and bcr rearrangement, interstitial insertion of the c-*abl* gene next to bcr on chromosome 22 occurs without reciprocal translocation of genomic material to chromosome 9.[60,62] Thus, the mechanism for juxtaposing bcr and c-abf is distinct from that operating in Ph^1-positive CML, but the resultant molecular fingerprint is identical.

The hematologic indexes, clinical course, response to treatment (chemotherapy or interferon alfa), and survival of the majority of patients negative for Ph^1 and positive for bcr rearrangement are much like those of patients with Ph^1-positive disease.[64] On the basis of these results, it appears that regardless of their status for the Ph' chromosome, all patients with leukemia who have bcr rearrangement and expression of p210$^{bcr-abl}$ have a single disease — CML. Importantly, the presence of bcr rearrangement in Ph^1-negative patients with CML whose cytogenetic profile is normal may be the only indication of malignant disease when these patients enter hematologic remission.[64] Therefore, molecular studies may provide a critical adjunct to cytogenetic and morphologic diagnostic workup, as well as follow-up, in Ph^1-negative patients with CML.

The question remains whether all Ph^1-negative patients whose morphologic assessment appears to indicate true CML will have bcr rearrangement. Although intuitively we have felt that this should be so, we have encountered four patients in whom the disease assessed at diagnosis was clinically and morphologically indistinguishable from CML but in whom no bcr rearrangement could be detected despite an extensive molecular analysis[58] (and Kunrock R, et al.: unpublished data). The clinical outcome of these patients has been rapid progression[58] or a long chronic phase (Kunrock R: unpublished data), a heterogeneity compatible with Ph^1-positive CML. Since the mRNA and proteins of these patients were not available for study, the possibility remains that a bcr–abf product was expressed, albeit through a unique and masked mechanism for genomic reorganization of chromosomes 22 and 9. Alternatively, it may be that completely novel molecular events participated in leukemogenesis in these patients. In this regard, it is of interest that Bartram has described a patient with CML in whom an aberrant bcr mRNA was expressed, but no *bcr–abl* message.[61] If distinct genetic events can result in identical disease patterns, the discovery of these events would enhance our understanding of the biology of the leukemic state.

Ph^1-Positive Acute Leukemia

Interestingly, some patients with ALL have a t(9;22) translocation that is cytogenetically indistinguishable from that found in patients with CML, yet do not have bcr rearrangement[10-13,43-49] (Table 1). Molecular analysis has demonstrated that, in these patients with ALL, the c-*abl* gene is transferred to chromosome 22 but the break on chromosome 22q11 occurs at a more proximal position than in patients with CML — the first intron of the bcr gene (Fig. 1).[47,65] Consequently, the first exon of the *bcr* gene is juxtaposed to the translocated portion of the c-*abl* gene. Hence, only a portion of the bcr-gene segment involved in CML participates in the molecular interactions in some cases of Ph^1-positive ALL.

Our work, as well as that of other groups, has revealed a novel c-abl protein — p190^{c-abl} — in Ph^1-positive, bcr rearrangement–negative patients with

ALL; $p210^{bcr-abl}$ is not expressed.[43-45] The ALL-derived protein is a chimera composed of the translation product of the first exon of the bcr gene joined to the c-abl gene product (starting with c-*abl* exon 2) (Fig. 2, Panel b).[46] Therefore, $p190^{c-abl}$ differs from $p210^{bcr-abl}$ by virtue of the absence of some bcr-gene sequences in the N-terminal of the former protein. $p190^{c-abl}$ is highly active as a tyrosine phosphokinase enzyme,[43-46] an observation consistent with the direct involvement of this aberrant protein in the development of leukemia.

It may be that $p190^{c-abl}$ has a unique role in the genesis of Ph^1-positive acute lymphoid leukemia. However, we have recently demonstrated that the Ph^1 translocation in AML can also be associated with a 22q11 break outside the 5.8-kb bcr locus and with the production of $p190^{c-abl}$ without an accompanying $p210^{bcr-abl}$.[14] Analysis of additional cases is needed to support the generality of the above phenomenon in Ph^1-positive AML. Nevertheless, this finding suggests the possibility that $p190^{c-abl}$ is not specifically linked with lymphoid morphology.

A review of the literature (Table 2) reveals that bcr rearrangement occurs in virtually all patients with Ph^1-positive CML (including >30 patients in blast crisis[39]), but in only approximately 50 percent of adults with Ph^1-positive ALL or AML and 10 percent of children with Ph^1-positive ALL. Regardless of the presence of acute or chronic Ph^1-positive disease, most patients with bcr rearrangement have expression of $p210^{bcr-abl}$, and those without bcr rearrangement have $p190^{c-abl}$. The two molecular subgroups of Ph^1-positive acute leukemia (patients with $p190^{c-abl}$ and those with $p210^{bcr-abl}$) appear to have similar clinical and morphologic features[10-13,43-49]; however, their genetic distinctions may have biologic relevance. The presence of $p210^{bcr-abl}$ makes Ph^1-positive acute leukemia virtually indistinguishable from blast crisis in CML[21] and suggests a single disease state in which the acute leukemia represents the blast-transformation phase of subclinical CML. Conversely, Ph^1-positive ALL and AML with expression of $p190^{c-abl}$ may reflect true de novo acute leukemia.

Although a wealth of evidence implicates the truncation and activation of c-abf in the development of Ph^1-positive leukemia, it is not known if the generation of $p190^{c-abl}$ as opposed to that of $p210^{bcr-abl}$ has a primary role in determining the phenotype of the resultant hematologic neoplasm. It is possible that the distinct molecular breakpoints associated with the Ph^1 translocation in CML, ALL, and AML are preferentially generated or maintained in certain hematopoietic lineages, and that the leukemic phenotype is determined by the target cell affected by the t(9;22) anomaly, regardless of whether $p190^{c-abl}$ or $p210^{bcr-abl}$ is expressed. Alternatively, modifications of the N-terminal of c-abf that result in the formation of $p190^{c-abl}$ rather than $p210^{bcr-abl}$ may be the critical pathogenetic factor in acute as distinct from chronic Ph^1-positive leukemias.

Blast Transformation in CML

A fascinating enigma of the malignant evolution of CML is the extraordinary range of intervals to blast crisis. Most patients enter this end stage in about four years; however, blast crisis can occur within several weeks of diagnosis, and a case of an ongoing chronic phase lasting at least 27 years has been reported.[17,18] It is not known whether disease progression represents a stochastic process or is predetermined, and the molecular events linked to the inexorable malignant evolution of CML from a phenotype of chronic leukemia to one of acute leukemia remain largely unexplored.

Although almost all patients with CML have bcr rearrangement, they vary in the precise localization of the breakpoint within the 5.8-kb bcr.[8] Therefore, several investigators have searched for a correlation between the exact molecular site of the bcr break and the clinical course of CML.[37,39,66] The results of these studies are conflicting but currently do not provide firm evidence for such a correlation. Furthermore, comparison of the point of breakage within the 5.8-kb bcr in the chronic and blast-crisis phases of individual patients reveals that, in the majority of cases, no change in bcr rearrangement occurs with disease progression.[38,39]

Since the Ph^1 chromosome is found in the early chronic phase, it seems likely that bcr rearrangement lays the groundwork, but is not sufficient, for the development of the blastic phase; superimposed, secondary genetic driving forces are required. Additional cytogenetic aberrations occur in over 80 percent of patients with CML approaching blast crisis[67] and commonly include trisomy of chromosome 8, a second Ph^1 chromosome, and isochromosome 17q (i(17q)). These karyotypic abnormalities may provide clues in the search for the genetic perturbations of blast transformation.

Because an additional chromosome 8 is frequently acquired during the evolution of leukemia, several investigators have studied the c-myc proto-oncogene (located[68] at chromosome position 8q34) in different stages of CML. Both c-*myc* gene amplification[69] and elevated expression of c-*myc* mRNA[70] have been observed in advanced CML. However, it has not been established if these findings represent pathogenetic events or are merely epiphenomena.

The emergence of an additional Ph^1 chromosome during clonal evolution raises the issue of the contribution of secondary changes in the c-*abl* gene to blast metamorphosis. Furthermore, all the sequences needed to code for the $p190^{c-abl}$ associated with acute leukemia are included in the bcr–abf gene of CML. If this gene were expressed as $p190^{c-abl}$ in addition to $p210^{bcr-abl}$, instead of $p210^{bcr-abl}$ exclusively, would the phenotype change? An alternative notion is that a

quantitative change in *abl* expression is relevant to disease progression. Although an increase in *bcr–abl* expression during the blast crisis as compared with the chronic phase of the disease has been reported by some authors,[71] such a change has been disputed by others.[72] In addition, Bartram and colleagues[73] have documented deletion of *bcr–abl* sequences in cells from a patient with CML who entered blast crisis, indicating that $p210^{bcr-abl}$ may be of subordinate importance in sustaining the leukemic state once CML has evolved beyond the chronic phase.

Overall, a paucity of studies makes the construction of a unified molecular theory of the pathogenesis of blast crisis impossible. Furthermore, the clinical heterogeneity of blast crisis and the multiplicity of associated secondary cytogenetic aberrations suggest that more than one molecular pathway may lead to this terminal event.

Conclusions and Future Directions

Over the past few years, it has become apparent that the basic defects responsible for many cancers fall within the purview of molecular biology. The discovery of consistent chromosomal aberrations in certain cancers has provided a starting point for molecular studies. However, on cytogenetic analysis, several tumors may have indistinguishable anomalies, whereas the higher resolving power of molecular techniques will reveal the involvement of discrete segments of DNA (Fig. 3).

The Ph^1 translocation (t(9;22)(q34;q11)) is found in about 90, 20, and 2 percent of adults with CML, ALL, and AML, respectively, as well as in 5 percent of children with ALL.[3-5,19,67] Ph^1-positive leukemia represents a nosologic continuum, in which CML evolves to a clinical state strongly resembling Ph^1-positive AML or ALL; conversely, Ph^1-positive AML or ALL may revert to CML after chemotherapy.[20] Yet, the distinct clinical presentation of acute leukemia as opposed to that of CML leaves unresolved the question whether these cancers represent one disease or two. These are not moot points, since the relation between the chronic-phase and the acute-phase Ph^1-positive phenotypes may be relevant to our understanding of the events that lead to blast crisis in CML.

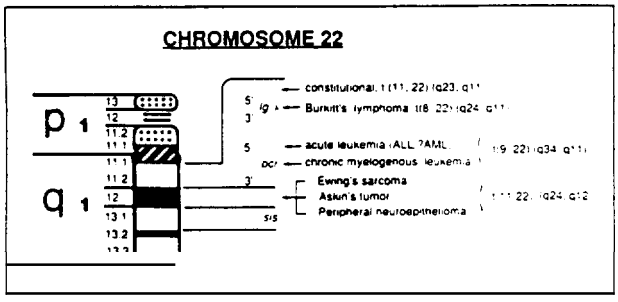


Figure 3. Breakpoints on Chromosome 22 in Band q11 and Nearby Regions, in Various Neoplasms.

The breakpoints are localized relative to the *sis* gene, the immunoglobulin lambda light-chain gene cluster, and the *bcr* gene. Molecular genetic techniques have demonstrated that the breakpoint at 22q11 in Burkitt's lymphoma and Ph^1-positive leukemia occurs within different *genes:* the t(8;22) break in Burkitt's lymphoma is within the immunoglobulin lambda light-chain gene, and the *break* in Ph^1-positive CML and ALL is within distinct segments of the *bcr* gene. The constitutional *break* at 22q11 is not associated with malignancy; it has been localized proximal to the constant region of the immunoglobulin lambda light-chain gene cluster.[74]

Recently, a series of incisive studies has resulted in important advances toward the elucidation of the basic genetic abnormalities associated with the various categories of Ph^1-positive leukemogenesis. It is now known that CML is characterized by a consistent set of molecular perturbations: a break within a 5.8-kb region — bcr — that lies within the *bcr* gene at chromosome 22q11; transfer of the *c-abl* proto-oncogene from chromosome 9q34 to 22q11, in juxtaposition to 5′ bcr sequences; and expression of a novel chimeric 210-kd *bcr–abl* protein ($p210^{bcr-abl}$) (Fig. 1 and 2a). By contrast, Ph^1-positive acute leukemia takes two molecular forms (Table 2, Fig. 1 and 2b). Molecular abnormalities identical to those found in CML are present in half the patients and may represent the end stage of subclinical CML; in addition, transfer of the c-*abl* gene from 9q34 to 22q11, albeit to a more proximal region within the bcr gene than in CML, occurs in the other half of patients, with the resultant expression of a unique aberrant 190-kd *bcr-abl* protein ($p190^{c-abl}$). These patients probably have a true acute leukemia, and most cases of Ph^1-positive childhood acute leukemia occur in this group. A comparison of normal and leukemia-associated molecular events is summarized in Table 1. The mechanisms responsible for this genomic diversity are probably related to the striking promiscuity of the second c-*abl* exon, which accommodates multiple exon donor sites and skips nearby sites in favor of those farther away. The similar but not identical molecular events involved in Ph^1-positive acute and chronic leukemia parallel the clinical phenotypic manifestations of these neoplasms, which appear to be related but not the same. Thus, subtle subchromosomal variations in genetic interaction may be key factors in heterogeneity of disease expression.

The formation of $p210^{bcr-abl}$ and $p190^{c-abl}$ is highly specific to Ph^1-positive leukemia. Furthermore, these proteins have a pronounced tyrosine phosphokinase enzymatic activity,[43-46,51-53] a property strongly related to the ability of some oncogenes and growth factor receptors to exert their effects. Therefore, $p210^{bcr-abl}$ and $p190^{c-abl}$ represent unique, cancer-specific markers that are probably directly involved in the malignant process. The use of these markers for diagnostic purposes has led to the identification of a subgroup of Ph^1-negative patients with CML who have bcr rearrangement, and expression of a *bcr–abl* message,

and a *clinical* outcome similar to that of Phi-positive patients with CML. The possibility of exploiting $p210^{bcr-abl}$ and $p190^{c-abl}$ for therapeutic purposes is yet to be explored.

There still remains a dearth of data on several important issues surrounding the molecular events in Ph^1-positive leukemia. For instance, recent studies have detected several candidate substrates for the *abl* phosphotyrosine kinase,[54,75] and one report has suggested that these substrates function as DNA-binding proteins that regulate gene expression.[75] However, the definitive identification and characterization of these target proteins, which are essential to understanding how *abl* mediates changes in normal and neoplastic growth or differentiation, have not been accomplished. The molecular events leading to blast crisis are also largely unknown. Since bcr rearrangement is present in the early, chronic phase, it seems likely that superimposed, secondary genetic perturbations are required for blastic conversion. The patterns of clonal cytogenetic evolution commonly seen in patients with CML (trisomy *8*, a second Ph^1 chromosome, and i(17q)) may provide clues to the chromosomal localization of these genetic changes. Whether or not additional qualitative or quantitative changes in c-*abl* itself occur and participate in malignant evolution remains speculative. These questions are especially pertinent, because the chronic phase of CML can be easily controlled by a variety of nontoxic therapeutic regimens whereas blast crisis is inevitably fatal. Identification of the secondary genetic changes contributing to the evolution of disease may provide an important first step toward preventing these changes and thus impeding disease progression.

Finally, the unraveling of the molecular abnormalities linked to the Ph^1 translocation may serve as a paradigm for the discovery of oncogene aberrations in other cancers. An increasing number of nonrandom chromosomal anomalies have been identified in cancer patients. Characterization of the genes affected by these exchanges in cytogenetic material will be pivotal to understanding the complex regulatory circuits involved in tumorigenesis.

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