The molecular biology of chronic myeloid leukemia

Michael W. N. Deininger, John M. Goldman, and Junia V. Melo

Chronic myeloid leukemia (CML) is probably the most extensively studied human malignancy. The discovery of the Philadelphia (Ph) chromosome in 1960[1] as the first consistent chromosomal abnormality associated with a specific type of leukemia was a breakthrough in cancer biology. It took 13 years before it was appreciated that the Ph chromosome is the result of a t(9;22) reciprocal chromosomal translocation[2] and another 10 years before the translocation was shown to involve the *ABL* proto-oncogene normally on chromosome 9[3] and a previously unknown gene on chromosome 22, later termed *BCR* for breakpoint cluster region.[4] The deregulated Abl tyrosine kinase activity was then defined as the pathogenetic principle,[5] and the first animal models were developed.[6] The end of the millennium sees all this knowledge transferred from the bench to the bedside with the arrival of Abl-specific tyrosine kinase inhibitors that selectively inhibit the growth of *BCR-ABL*–positive cells in vitro[7,8] and in vivo.[9]

In this review we will try to summarize what is currently known about the molecular biology of CML. Because several aspects of CML pathogenesis may be attributable to the altered function of the 2 genes involved in the Ph translocation, we will also address the physiological roles of *BCR* and *ABL*. We concede that a review of this nature can never be totally comprehensive without losing clarity, and we therefore apologize to any authors whose work we have not cited.

The physiologic function of the translocation partners

The *ABL* gene is the human homologue of the v-*abl* oncogene carried by the Abelson murine leukemia virus (A-MuLV),[10] and it encodes a nonreceptor tyrosine kinase.[11] Human Abl is a ubiquitously expressed 145-kd protein with 2 isoforms arising from alternative splicing of the first exon.[11] Several structural domains can be defined within the protein (Figure 1). Three SRC homology domains (SH1-SH3) are located toward the NH_2 terminus. The SH1 domain carries the tyrosine kinase function, whereas the SH2 and SH3 domains allow for interaction with other proteins.[12] Proline-rich sequences in the center of the molecule can, in turn, interact with SH3 domains of other proteins, such as Crk.[13] Toward the 3′ end, nuclear localization signals[14] and the DNA-binding[15] and actin-binding motifs[16] are found.

Several fairly diverse functions have been attributed to Abl, and the emerging picture is complex. Thus, the normal Abl protein is involved in the regulation of the cell cycle,[17,18] in the cellular response to genotoxic stress,[19] and in the transmission of information about the cellular environment through integrin signaling.[20] (For a comprehensive review of Abl function, see Van Etten[21]). Overall, it appears that the Abl protein serves a complex role as a cellular module that integrates signals from various extracellular and intracellular sources and that influences decisions in regard to cell cycle and apoptosis. It must be stressed, however, that many of the data are based solely on in vitro studies in fibroblasts, not hematopoietic cells, and are still controversial. Unfortunately, the generation of *ABL* knockout mice failed to resolve most of the outstanding issues.[22,23]

The 160-kd Bcr protein, like Abl, is ubiquitously expressed.[11] Several structural motifs can be delineated (Figure 2). The first N-terminal exon encodes a serine–threonine kinase. The only substrates of this kinase identified so far are Bap-1, a member of the 14-3-3 family of proteins,[24] and possibly Bcr itself.[11] A coiled–coil domain at the N-terminus of Bcr allows dimer formation in vivo.[25] The center of the molecule contains a region with *dbl*-like and pleckstrin-homology (PH) domains that stimulate the exchange of guanidine triphosphate (GTP) for guanidine diphosphate (GDP) on Rho guanidine exchange factors,[26] which in turn may activate transcription factors such as NF-κB.[27] The C-terminus has GTPase activity for Rac,[28] a small GTPase of the Ras superfamily that regulates actin polymerization and the activity of an NADPH oxidase in phagocytic cells.[29] In addition, Bcr can be phosphorylated on several tyrosine residues,[30] especially tyrosine 177, which binds Grb-2, an important adapter molecule involved in the activation of the Ras pathway.[31] Interestingly, Abl has been shown to phosphorylate Bcr in COS1 cells, resulting in a reduction of Bcr kinase activity.[31,32] Although these data argue for a role of Bcr in signal transduction, their true biologic relevance remains to be determined. The fact that *BCR* knockout mice are viable and the fact that an increased oxidative burst in neutrophils is thus far the only recognized defect[33] probably reflect the redundancy of signaling pathways. If there is a role for Bcr in the pathogenesis of Ph-positive leukemias, it is not clearly discernible because the incidence and biology of $P190^{BCR\text{-}ABL}$-induced leukemia are the same in *BCR*−/− mice as they are in wild-type mice.[34]

Molecular anatomy of the *BCR-ABL* translocation

The breakpoints within the *ABL* gene at 9q34 can occur anywhere over a large (greater than 300 kb) area at its 5′ end, either upstream

From the Department of Hematology/Oncology, University of Leipzig, Germany; and the Department of Haematology, Imperial College School of Medicine, Hammersmith Hospital, London, United Kingdom.

Submitted November 16, 1999; accepted July 12, 2000.

Supported by grants from Leukaemia Research Fund (UK) and the Dr Ernst und Anita Bauer Stiftung (Germany).

Reprints: Michael W. N. Deininger, Department of Hematology/Oncology, University of Leipzig, Johannisallee 32, Leipzig 04103, Germany; e-mail: deim@medizin.uni-leipzig.de.

The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 U.S.C. section 1734.

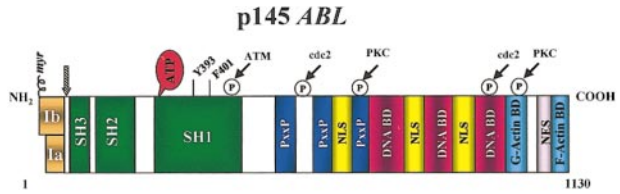

Figure 1. Structure of the Abl protein. Type Ia isoform is slightly shorter than type Ib, which contains a myristoylation (myr) site for attachment to the plasma membrane. Note the 3 SRC-homology (SH) domains situated toward the NH_2 terminus. Y393 is the major site of autophosphorylation within the kinase domain, and phenylalanine 401 (F401) is highly conserved in PTKs containing SH3 domains. The middle of each protein is dominated by proline-rich regions (PxxP) capable of binding to SH3 domains, and it harbors 1 of 3 nuclear localization signals (NLS). The carboxy terminus contains DNA as well as G- and F-actin–binding domains. Phosphorylation sites by Atm, cdc2, and PKC are shown. The arrowhead indicates the position of the breakpoint in the Bcr-Abl fusion protein.

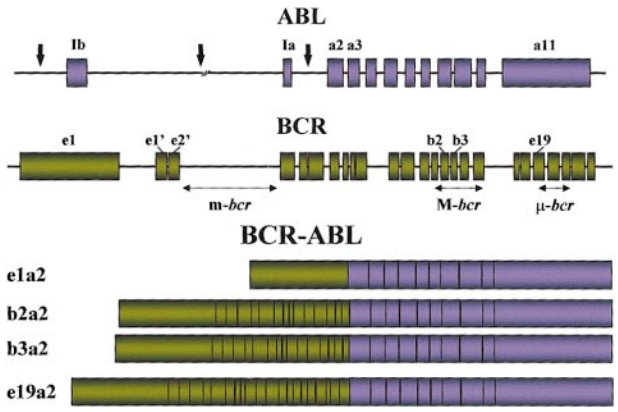

Figure 3. Locations of the breakpoints in the *ABL* and *BCR* genes and structure of the chimeric mRNAs derived from the various breaks.

of the first alternative exon Ib, downstream of the second alternative exon Ia, or, more frequently, between the two[35] (Figure 3). Regardless of the exact location of the breakpoint, splicing of the primary hybrid transcript yields an mRNA molecule in which *BCR* sequences are fused to *ABL* exon a2. In contrast to *ABL*, breakpoints within *BCR* localize to 1 of 3 so-called breakpoint cluster regions (*bcr*). In most patients with CML and in approximately one third of patients with Ph-positive acute lymphoblastic leukemia (ALL), the break occurs within a 5.8-kb area spanning *BCR* exons 12-16 (originally referred to as exons b1-b5), defined as the major breakpoint cluster region (M-*bcr*). Because of alternative splicing, fusion transcripts with either b2a2 or b3a2 junctions can be formed. A 210-kd chimeric protein ($P210^{BCR\text{-}ABL}$) is derived from this mRNA. In the remaining patients with ALL and rarely in patients with CML, characterized clinically by prominent monocytosis,[36,37] the breakpoints are further upstream in the 54.4-kb region between the alternative *BCR* exons e2′ and e2, termed the minor breakpoint cluster region (m-*bcr*). The resultant e1a2 mRNA is translated into a 190-kd protein ($P190^{BCR\text{-}ABL}$). Recently, a third breakpoint cluster region (μ-*bcr*) was identified downstream of exon 19, giving rise to a 230-kd fusion protein ($P230^{BCR\text{-}ABL}$) associated with the rare Ph-positive chronic neutrophilic leukemia,[38] though not in all cases.[39] If sensitive techniques such as nested reverse transcription–polymerase chain reaction are used, transcripts with the e1a2 fusion are detectable in many patients with classical $P210^{BCR\text{-}ABL}$ CML.[40] The low level of expression of these P190-type transcripts compared to P210 indicates that they are most likely the result of alternative splicing of the primary mRNA. Occasional cases with other junctions, such as b2a3, b3a3, e1a3, e6a2,[41] or e2a2,[42] have been reported in patients with ALL and CML. These "experiments of nature" provide important information as to the function of the various parts of *BCR* and *ABL* in the oncogenic fusion protein. Interestingly, *ABL* exon 1, even if retained in the genomic fusion, is never part of the chimeric mRNA. Thus, it must be spliced out during processing of the primary mRNA; the mechanism underlying this apparent peculiarity is unknown. Based on the observation that the Abl part in the chimeric protein is almost invariably constant while the Bcr portion varies greatly, one may deduce that Abl is likely to carry the transforming principle whereas the different sizes of the Bcr sequence may dictate the phenotype of the disease. In support of this notion, rare cases of ALL express a *TEL-ABL* fusion gene,[43,44] indicating that the *BCR* moiety can in principle be replaced by other sequences and still cause leukemia. Interestingly, a fusion between *TEL(ETV6)* and the *ABL*-related gene *ARG* has recently been described in a patient with AML.[45] Although all 3 major Bcr-Abl fusion proteins induce a CML-like disease in mice, they differ in their ability to induce lymphoid leukemia,[46] and, in contrast to P190 and P210, transformation to growth factor independence by $P230^{BCR\text{-}ABL}$ is incomplete,[47] which is consistent with the relatively benign clinical course of P230-positive chronic neutrophilic leukemia.[38]

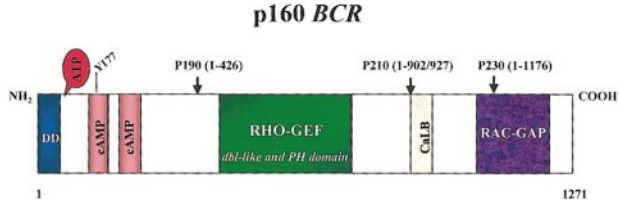

Figure 2. Structure of the Bcr protein. Note the dimerization domain (DD) and the 2 cyclic adenosine monophosphate kinase homologous domains at the N terminus. Y177 is the autophosphorylation site crucial for binding to Grb-2. The center of the molecule contains a region homologous to Rho guanidine nucleotide exchange factors (Rho-GEF) as well as dbl-like and pleckstrin homology (PH) domains. Toward the C-terminus a putative site for calcium-dependent lipid binding (CaLB) and a domain with activating function for Rac-GTPase (Rac-GAP) are found. Arrowheads indicate the position of the breakpoints in the *BCR-ABL* fusion proteins.

One of the most intriguing questions relates to the events responsible for the chromosomal translocation in the first place. From epidemiologic studies it is well known that exposure to ionizing radiation (IR) is a risk factor for CML.[48,49] Moreover, *BCR-ABL* fusion transcripts can be induced in hematopoietic cells by exposure to IR in vitro[50]; such IR-induced translocations may not be random events but may depend on the cellular background and on the particular genes involved. Two recent reports showed that the physical distance between the *BCR* and the *ABL* genes in human lymphocytes[51] and $CD34^+$ cells[52] is shorter than might be expected by chance; such physical proximity could favor translocation events involving the 2 genes. However, the presence of the *BCR-ABL* translocation in a hematopoietic cell is not in itself sufficient to cause leukemia because *BCR-ABL* fusion transcripts of M-*bcr* and m-*bcr* type are detectable at low frequency in the blood of many healthy individuals.[53,54] It is unclear why Ph-positive leukemia develops in a tiny minority of these persons. It may be that the translocation occurs in cells committed to terminal differentiation that are thus eliminated or that an immune response suppresses or eliminates Bcr-Abl–expressing cells. Indirect evidence that such a mechanism may be relevant comes from the observation that certain HLA types protect against CML.[55] Another possibility is that *BCR-ABL* is not the only genetic lesion required to induce chronic-phase CML. Indeed, a skewed pattern of G-6PD

isoenzymes has been detected in Ph-negative Epstein-Barr virus-transformed B-cell lines derived from patients with CML, suggesting that a Ph-negative pathologic state may precede the emergence of the Ph chromosome.[56]

Mechanisms of *BCR-ABL*–mediated malignant transformation

Essential features of the Bcr-Abl protein

Mutational analysis identified several features in the chimeric protein that are essential for cellular transformation (Figure 4). In Abl they include the SH1, SH2, and actin-binding domains (Figure 1), and in Bcr they include a coiled–coil motif contained in amino acids 1-63,[25] the tyrosine at position 177,[57] and phosphoserine–threonine-rich sequences between amino acids 192-242 and 298-413[58] (Figure 2). It is, however, important to note that essential features depend on the experimental system. For example, SH2 deletion mutants of Bcr-Abl are defective for fibroblast transformation,[59] but they retain the capacity to transform cell lines to factor independence and are leukemogenic in animals.[60]

Deregulation of the Abl tyrosine kinase

Abl tyrosine kinase activity is tightly regulated under physiologic conditions. The SH3 domain appears to play a critical role in this inhibitory process because its deletion[14] or positional alteration[61] activates the kinase; it is replaced by viral *gag* sequences in v-*abl*.[62] Both cis- and trans-acting mechanisms have been proposed to mediate the repression of the kinase. Several proteins have been identified that bind to the SH3 domain.[63-65] Abi-1 and Abi-2 (Abl interactor proteins 1 and 2) activate the inhibitory function of the SH3 domain; even more interesting, activated Abl proteins promote the proteasome-mediated degradation of Abi-1[66] and Abi-2. Another candidate inhibitor of Abl is Pag/Msp23. On exposure of cells to oxidative stress such as ionizing radiation, this small protein is oxidized and dissociates from Abl, whose kinase is in turn activated.[67] These results are in line with previous observations that highly purified Abl protein is kinase-active,[61] suggesting that its constitutive inhibition derives from a trans-acting mechanism. Alternatively, the SH3 domain may bind internally to the proline-rich region in the center of the Abl protein, causing a conformational change that inhibits interaction with substrates.[68] Furthermore, a mutation of Phe[401] to Val (within the kinase domain) leads to the transformation of rodent fibroblasts. Because this residue is highly conserved in tyrosine kinases with N-terminal SH3 domains, it may bind internally to the SH3 domain.[69] It is conceivable that the fusion of Bcr sequences 5′ of the Abl SH3 domain abrogates the physiologic suppression of the kinase. This might be the consequence of homodimer formation; indeed, the N-terminal dimerization domain is an essential feature of the Bcr-Abl protein but can be functionally replaced by other sequences that allow for dimer formation, such as the N-terminus of the *TEL* (*ETV-6*) transcription factor in the *TEL-ABL* fusion associated with the t(9;12).[43,70] It is possible that deregulated tyrosine kinase activity is a unifying feature of chronic myeloproliferative disorders. Several other reciprocal translocations have been cloned from patients with chronic *BCR-ABL*–negative myeloproliferative disorders. Remarkably, most of these turn out to involve tyrosine kinases such as fibroblast growth factor receptor 1[71] and platelet-derived growth factor β receptor (PDGFβR).[72]

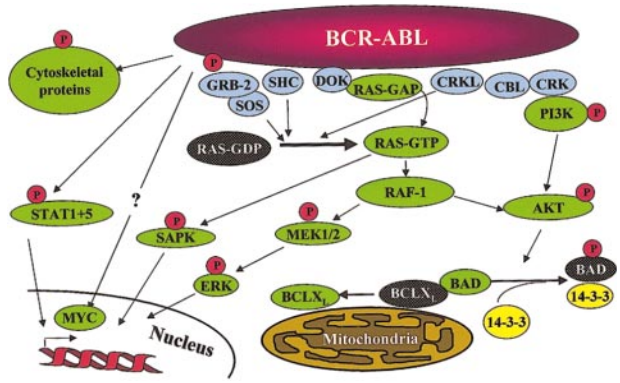

Figure 4. Signaling pathways activated in *BCR-ABL*–positive cells. Note that this is a simplified diagram and that many more associations between Bcr-Abl and signaling proteins have been reported.

Table 1. Substrates of BCR-ABL

Protein	Function	Reference
P62^{DOK}	Adapter	74
Crkl	Adapter	73
Crk	Adapter	13
Shc	Adapter	75
Talin	Cytoskeleton/cell membrane	76
Paxillin	Cytoskeleton/cell membrane	77
Fak	Cytoskeleton/cell membrane	78
Fes	Myeloid differentiation	79
Ras-GAP	Ras-GTPase	80
GAP-associated proteins	Ras activation?	214
PLCγ	Phospholipase	80
PI3 kinase (p85 subunit)	Serine kinase	127
Syp	Cytoplasmic phosphatase	83
Bap-1	14-3-3 protein	24
Cbl	Unknown	81
Vav	Hematopoietic differentiation	82

A host of substrates can be tyrosine phosphorylated by *Bcr-Abl* (Table 1). Most important, because of autophosphorylation, there is a marked increase of phosphotyrosine on *Bcr-Abl* itself, which creates binding sites for the SH2 domains of other proteins. Generally, substrates of *Bcr-Abl* can be grouped according to their physiologic role into adapter molecules (such as Crkl and p62^{DOK}), proteins associated with the organization of the cytoskeleton and the cell membrane (such as paxillin and talin), and proteins with catalytic function (such as the nonreceptor tyrosine kinase Fes or the phosphatase Syp). It is important to note that the choice of substrates depends on the cellular context. For example, Crkl is the major tyrosine-phosphorylated protein in CML neutrophils,[73] whereas phosphorylated p62^{DOK} is predominantly found in early progenitor cells.[74]

Tyrosine phosphatases counterbalance and regulate the effects of tyrosine kinases under physiologic conditions, keeping cellular phosphotyrosine levels low. Two tyrosine phosphatases, Syp[83] and PTP1B,[84] have been shown to form complexes with Bcr-Abl, and both appear to dephosphorylate Bcr-Abl. Interestingly, PTP1B levels increase in a kinase-dependent manner, suggesting that the cell attempts to limit the impact of Bcr-Abl tyrosine kinase activity. At least in fibroblasts, transformation by Bcr-Abl is impaired by the overexpression of PTP1B.[85] Interestingly, we recently observed the up-regulation of receptor protein tyrosine phosphatase κ (RPTP-κ) with the inhibition of Bcr-Abl in BV173 cells treated with the

tyrosine kinase inhibitor STI571,[86] which suggests that the opposite effect may also occur. Thus, though the pivotal role of Bcr-Abl tyrosine kinase activity is clearly established, much remains to be learned about the significance of tyrosine phosphatases in the transformation process.

Activated signaling pathways and biologic properties of BCR-ABL–positive cells

Three major mechanisms have been implicated in the malignant transformation by *Bcr-Abl*, namely altered adhesion to stroma cells and extracellular matrix,[87] constitutively active mitogenic signaling[88] and reduced apoptosis[89] (Figure 5). A fourth possible mechanism is the recently described proteasome-mediated degradation of Abl inhibitory proteins.[66]

Altered adhesion properties

CML progenitor cells exhibit decreased adhesion to bone marrow stroma cells and extracellular matrix.[87,90] In this scenario, adhesion to stroma negatively regulates cell proliferation, and CML cells escape this regulation by virtue of their perturbed adhesion properties. Interferon-α (IFN-α), an active therapeutic agent in CML, appears to reverse the adhesion defect.[91] Recent data suggest an important role for β-integrins in the interaction between stroma and progenitor cells. CML cells express an adhesion-inhibitory variant of β1 integrin that is not found in normal progenitors.[92] On binding to their receptors, integrins are capable of initiating normal signal transduction from outside to inside[93]; it is thus conceivable that the transfer of signals that normally inhibit proliferation is impaired in CML cells. Because Abl has been implicated in the intracellular transduction of such signals, this process may be further disturbed by the presence of a large pool of Bcr-Abl protein in the cytoplasm. Furthermore, Crkl, one of the most prominent tyrosine-phosphorylated proteins in Bcr-Abl–transformed cells,[73] is involved in the regulation of cellular motility[94] and in integrin-mediated cell adhesion[95] by association with other focal adhesion proteins such as paxillin, the focal adhesion kinase Fak, p130Cas,[96] and Hef1.[97] We recently demonstrated that Bcr-Abl tyrosine kinase upregulates the expression of α6 integrin mRNA,[86] which points to transcriptional activation as yet another possible mechanism by which Bcr-Abl may have an impact on integrin signaling. Thus, though there is sound evidence that Bcr-Abl influences integrin function, it is more difficult to determine the precise nature of the biologic consequences, and, at least in certain cellular systems, integrin function appears to be enhanced rather than reduced by Bcr-Abl.[98]

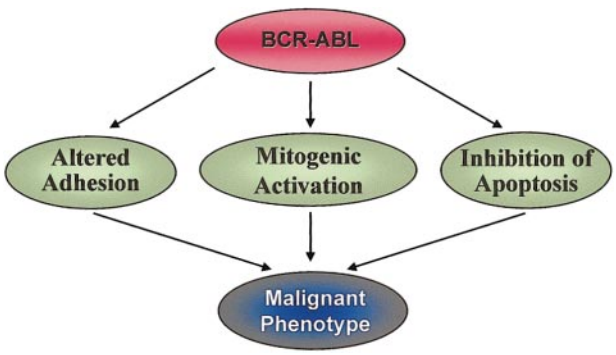

Figure 5. Mechanisms implicated in the pathogenesis of CML.

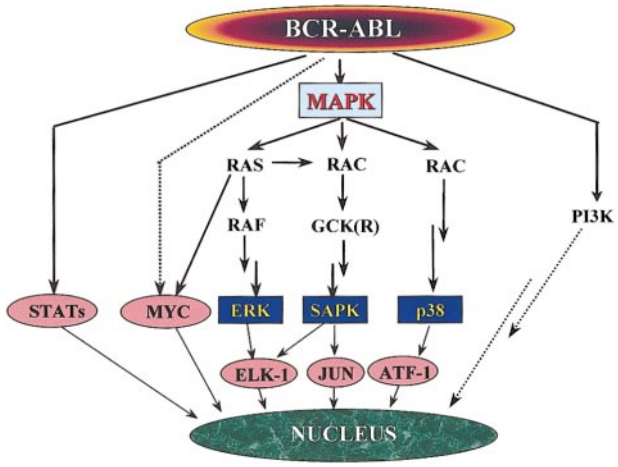

Figure 6. Signaling pathways with mitogenic potential in *BCR-ABL*–transformed cells. The activation of individual paths depends on the cell type, but the MAP kinase system appears to play a central role. Activation of p38 has been demonstrated only in v-*abl*–transformed cells, whereas data for *BCR-ABL*–expressing cells are missing.

Activation of mitogenic signaling

Ras and the MAP kinase pathways. Several links between Bcr-Abl and Ras have been defined. Autophosphorylation of tyrosine 177 provides a docking site for the adapter molecule Grb-2.[57] Grb-2, after binding to the Sos protein, stabilizes Ras in its active GTP-bound form. Two other adapter molecules, Shc and Crkl, can also activate Ras. Both are substrates of Bcr-Abl[73,99] and bind Bcr-Abl through their SH2 (Shc) or SH3 (Crkl) domains. The relevance of Ras activation by Crkl is, however, questionable because it appears to be restricted to fibroblasts.[100] Moreover, direct binding of Crkl to Bcr-Abl is not required for the transformation of myeloid cells.[101] Circumstantial evidence that Ras activation is important for the pathogenesis of Ph-positive leukemias comes from the observation that activating mutations are uncommon, even in the blastic phase of the disease,[102] unlike in most other tumors. This implies that the Ras pathway is constitutively active, and no further activating mutations are required. There is still dispute as to which mitogen-activated protein (MAP) kinase pathway is downstream of Ras in Ph-positive cells. Stimulation of cytokine receptors such as IL-3 leads to the activation of Ras and the subsequent recruitment of the serine–threonine kinase Raf to the cell membrane.[103] Raf initiates a signaling cascade through the serine–threonine kinases Mek1/Mek2 and Erk, which ultimately leads to the activation of gene transcription.[104] Although some data indicate that this pathway may be activated only in v-abl– but not in BCR-ABL–transformed cells,[105] this view has recently been challenged.[106] Moreover, activation of the Jnk/Sapk pathway by Bcr-Abl has been demonstrated and is required for malignant transformation[107]; thus, signaling from Ras may be relayed through the GTP–GDP exchange factor Rac[108] to Gckr (germinal center kinase related)[109] and further down to Jnk/Sapk (Figure 6). There is also some evidence that p38, the third pillar of the MAP kinase pathway, is also activated in BCR-ABL–transformed cells, and there are other pathways with mitogenic potential. In any case, the signal is eventually transduced to the transcriptional machinery of the cell.

It is also possible that Bcr-Abl uses growth factor pathways in a more direct way. For example, association with the βc subunit of the IL-3 receptor[110] and the Kit receptor[111] has been observed. Interestingly, the pattern of tyrosine-phosphorylated proteins seen

in normal progenitor cells after stimulation with Kit ligand is similar to the pattern seen in CML progenitor cells.[112] Dok-1 ($p62^{DOK}$), one of the most prominent phosphoproteins in this setting, forms complexes with Crkl, RasGAP, and Bcr-Abl. In fact, there may be a whole family of related proteins with similar functions—for example, the recently described Dok-2 ($p56^{DOK}2$).[113] Somewhat surprisingly, $p62^{DOK}$ is essential for transformation of Rat-1 fibroblasts but not for growth-factor independence of myeloid cells[114]; thus, its true role remains to be defined.

Jak-Stat pathway. The first evidence for involvement of the Jak-Stat pathway came from studies in v-abl–transformed B cells.[62] Constitutive phosphorylation of Stat transcription factors (Stat1 and Stat5) has since been reported in several BCR-ABL–positive cell lines[115] and in primary CML cells,[116] and Stat5 activation appears to contribute to malignant transformation.[117] Although Stat5 has pleiotropic physiologic functions,[118] its effect in BCR-ABL–transformed cells appears to be primarily anti-apoptotic and involves transcriptional activation of Bcl-xL.[119,120] In contrast to the activation of the Jak-Stat pathway by physiologic stimuli, Bcr-Abl may directly activate Stat1 and Stat5 without prior phosphorylation of Jak proteins. There seems to be specificity for Stat6 activation by $P190^{BCR\text{-}ABL}$ proteins as opposed to $P210^{BCR\text{-}ABL}$.[115] It is tempting to speculate that the predominantly lymphoblastic phenotype in these leukemias is related to this peculiarity.

The role of the Ras and Jak-Stat pathways in the cellular response to growth factors could explain the observation that *BCR-ABL* renders a number of growth factor–dependent cell lines factor independent.[105,121] In some experimental systems there is evidence for an autocrine loop dependent on the Bcr-Abl–induced secretion of growth factors,[122] and it was recently reported that Bcr-Abl induces an IL-3 and G-CSF autocrine loop in early progenitor cells.[123] Interestingly, Bcr-Abl tyrosine kinase activity may induce expression not only of cytokines but also of growth factor receptors such as the oncostatin M β receptor.[86] One should bear in mind, however, that during the chronic phase, CML progenitor cells are still dependent on external growth factors for their survival and proliferation,[124] though less than normal progenitors.[125] A recent study sheds fresh light on this issue. FDCPmix cells transduced with a temperature-sensitive mutant of *BCR-ABL* have a reduced requirement for growth factors at the kinase permissive temperature without differentiation block.[126] This situation resembles chronic-phase CML, in which the malignant clone has a subtle growth advantage while retaining almost normal differentiation capacity.

PI3 kinase pathway. PI3 kinase activity is required for the proliferation of *BCR-ABL*–positive cells.[127] Bcr-Abl forms multimeric complexes with PI3 kinase, Cbl, and the adapter molecules Crk and Crkl,[95] in which PI3 kinase is activated. The next relevant substrate in this cascade appears to be the serine–threonine kinase Akt.[128] This kinase had previously been implicated in anti-apoptotic signaling.[129] A recent report placed Akt in the downstream cascade of the IL-3 receptor and identified the pro-apoptotic protein Bad as a key substrate of Akt.[130] Phosphorylated Bad is inactive because it is no longer able to bind anti-apoptotic proteins such as Bcl_{XL} and it is trapped by cytoplasmic 14-3-3 proteins. Altogether this indicates that Bcr-Abl might be able to mimic the physiologic IL-3 survival signal in a PI3 kinase-dependent manner (see also below). Ship[131] and Ship-2,[132] 2 inositol phosphatases with somewhat different specificities, are activated in response to growth factor signals and by Bcr-Abl. Thus, Bcr-Abl appears to have a profound effect on phosphoinositol metabolism, which might again shift the balance to a pattern similar to physiologic growth factor stimulation.

Myc pathway. Overexpression of Myc has been demonstrated in many human malignancies. It is thought to act as a transcription factor, though its target genes are largely unknown. Activation of Myc by Bcr-Abl is dependent on the SH2 domain, and the overexpression of Myc partially rescues transformation-defective SH2 deletion mutants whereas the overexpression of a dominant-negative mutant suppresses transformation.[133] The pathway linking Myc to the SH2 domain of Bcr-Abl is still unknown. However, results obtained in v-abl–transformed cells suggest that the signal is transduced through Ras/Raf, cyclin-dependent kinases (cdks), and E2F transcription factors that ultimately activate the MYC promoter.[134] Similar results were reported for BCR-ABL–transformed murine myeloid cells.[135] How these findings relate to human Ph-positive cells is unknown. It seems likely that the effects of Myc in Ph-positive cells are probably not different from those in other tumors. Depending on the cellular context, Myc may constitute a proliferative or an apoptotic signal.[136,137] It is therefore likely that the apoptotic arm of its dual function is counterbalanced in CML cells by other mechanisms, such as the PI3 kinase pathway.

Inhibition of apoptosis

Expression of Bcr-Abl in factor-dependent murine[138] and human[122] cell lines prevents apoptosis after growth-factor withdrawal, an effect that is critically dependent on tyrosine kinase activity and that correlates with the activation of Ras.[88,139] Moreover, several studies showed that *BCR-ABL*–positive cell lines are resistant to apoptosis induced by DNA damage.[89,140] The underlying biologic mechanisms are still not well understood. Bcr-Abl may block the release of cytochrome C from the mitochondria and thus the activation of caspases.[141,142] This effect upstream of caspase activation might be mediated by the Bcl-2 family of proteins. Bcr-Abl has been shown to up-regulate Bcl-2 in a Ras-[143] or a PI3 kinase-dependent[128] manner in Baf/3 and 32D cells, respectively. Moreover, as mentioned previously, $Bclx_L$ is transcriptionally activated by Stat5 in *BCR-ABL*–positive cells.[119,120]

Another link between *BCR-ABL* and the inhibition of apoptosis might be the phosphorylation of the pro-apoptotic protein Bad. In addition to Akt, Raf-1, immediately downstream of Ras, phosphorylates Bad on 2 serine residues.[144,145] Two recent studies provided evidence that the survival signal provided by Bcr-Abl is at least partially mediated by Bad and requires targeting of Raf-1 to the mitochondria.[146,147] It is also possible that Bcr-Abl inhibits apoptosis by down-regulating interferon consensus sequence binding protein (ICSBP).[148,149] These data are interesting because ICSBP knockout mice develop a myeloproliferative syndrome,[150] and hematopoietic progenitor cells from ICSBP−/− mice show altered responses to cytokines.[151] The connection to interferon α, an active agent in the treatment of CML, is obvious.

It becomes clear that the multiple signals initiated by Bcr-Abl have proliferative and anti-apoptotic qualities that are frequently difficult to separate. Thus, Bcr-Abl may shift the balance toward the inhibition of apoptosis while simultaneously providing a proliferative stimulus. This is in line with the concept that a proliferative signal leads to apoptosis unless it is counterbalanced by an anti-apoptotic signal,[152] and Bcr-Abl fulfills both requirements at the same time. There is, however, controversy. One report found 32D cells transfected with *BCR-ABL* to be more sensitive to IR than the parental cells,[153] whereas 2 other studies failed to detect any difference between CML and normal primary progenitor cells with regard to their sensitivity to IR and growth factor withdrawal.[124,154] Furthermore, based on results obtained in transfected cell systems, it was suggested that Bcr-Abl inhibits apoptosis

mediated by the Fas receptor/Fas ligand system.[155] However, though there may be a role for this system in mediating the clinical response to interferon-α,[156] there is no indication that Fas-triggered apoptosis is defective in primary CML cells or in "natural" Ph-positive cell lines.[157] Moreover, Bcr-Abl accelerates C2 ceramide-induced apoptosis,[158] and it does not protect against natural killer cell-induced apoptosis.[159] These inconsistencies may reflect genuine differences between cell lines and primary cells. On the other hand, it is debatable whether complete growth-factor withdrawal and IR constitute stimuli that have much physiologic relevance. To allow for a representative comparison, it would be crucial to define the signals that induce apoptosis in vivo.

Degradation of inhibitory proteins.

The recent discovery that Bcr-Abl induces the proteasome-mediated degradation of Abi-1 and Abi-2,[66] 2 proteins with inhibitory function, may be the first indication of yet another way by which Bcr-Abl induces cellular transformation. Most compelling, the degradation of Abi-1 and Abi-2 is specific for Ph-positive acute leukemias and is not seen in Ph-negative samples of comparable phenotype. The overall significance of this observation remains to be seen, and one must bear in mind that the data refer to acute leukemias and not to chronic phase CML. It is nevertheless tempting to speculate that other proteins, whose level of expression is regulated through the proteasome pathway, may also be degraded. A good candidate would be the cell cycle inhibitor p27, but to our knowledge no data are available yet.

Experimental models of CML

Various experimental systems have been developed to study the pathophysiology of CML. All of them have their advantages and shortcomings, and it is probably fair to say that there is still no ideal in vitro or in vivo model that would cover all aspects of the human disease.

Cell lines

Fibroblasts. Fibroblast lines have been used extensively in CML research because they are easy to manipulate. Fibroblast transformation—that is, anchorage-independent growth in soft agar—is the standard in vitro test for tumorigenicity.[160] However, it became clear that the introduction of *BCR-ABL* into fibroblasts has diverse effects, depending on the type of fibroblast used. Thus, though $P210^{BCR\text{-}ABL}$ transforms Rat-1 fibroblasts,[161] there is no such effect in NIH3T3.[162] Moreover, transformation to serum-independent growth occurs only in few cells (permissive cells[163]), whereas most undergo growth arrest. These observations show that certain cellular requirements must be met if a cell is to be transformed by *BCR-ABL*. Interestingly, this is also the case for the various parts of the Bcr-Abl protein. Thus, a *BCR-ABL* mutant that lacks the SH2 domain retains the capacity to transform hematopoietic 32D cells to growth factor independence[60] but is defective for fibroblast transformation.[59] In addition, there are differences between hematopoietic cells and fibroblasts in terms of interactions with other proteins such as Crkl. The latter is functional in Ras activation and transformation in fibroblasts[100] but not in hematopoietic cells.[101] Thus, results obtained from studies in fibroblasts must be interpreted with great caution.

Hematopoietic cell lines. Until relatively recently, only a few *BCR-ABL*–positive lines derived from CML were available, but their number has grown considerably in the past few years.[164] They include cell lines with myeloid differentiation, such as the well-known K562, and lymphoid phenotype, such as BV173. The main drawback common to all these lines is the fact that they are derived from blast crisis and, thus, contain genetic lesions in addition to *BCR-ABL*. Consequently, they may reflect blast crisis fairly well but are insufficient models of chronic phase CML. Until now, no cell line from chronic phase CML has been established, just as no cell line could be derived from normal human bone marrow. Even attempts to immortalize Ph-positive B-cells from patients in the chronic phase of disease were not successful because these lines have a limited life span,[165] in contrast to their Ph-negative counterparts. One could therefore speculate that the very establishment of a line from a patient with CML would be diagnostic of advanced disease. In this context, it is surprising that most human CML lines remain dependent on Bcr-Abl tyrosine kinase activity for their proliferation and survival, as shown by their susceptibility to the effects of the Abl-specific tyrosine kinase inhibitor STI571.[8] However, the phenotype of these cell lines is that of an acute leukemia. Therefore great caution is warranted if experimental results are to be transferred to chronic phase CML. A striking example is the fact that inhibition of apoptosis by Bcr-Abl is easily demonstrable in cell lines[139] but not in primary cells.[124] It should also be noted that many of the lines used have undergone hundreds, if not thousands, of rounds of replication, and different laboratories frequently house lines that have little in common but their names and *BCR-ABL* positivity.

Transformation of factor-dependent cell lines to growth-factor independence is an important feature of Bcr-Abl, and, in fact, other oncoproteins that contain an activated tyrosine kinase.[43,166] Although it is usually difficult to obtain stable expression of *BCR-ABL* in previously immortalized cell lines, this is relatively easily achieved in factor-dependent lines, presumably because *BCR-ABL* expression is an advantage to the latter but useless or even detrimental to the former. Murine cell lines such as Baf/3 and 32D and human cell lines such as MO7 were used to study the effects of *BCR-ABL* by direct comparison between transduced and parental cells. A particular advantage of the murine lines is the fact that they are derived from normal nonmalignant hematopoietic cells. Unfortunately, this does not rule out the development of additional mutations[167] that confer a selective growth advantage. Furthermore, it is not clear how the transformation to complete factor independence relates to clinical CML in which the cells are still factor-dependent, though obviously less so than normal hematopoietic cells.[123] The subject of growth factor independence and *BCR-ABL* transformation has been reviewed recently.[168]

None of the cell lines mentioned above is capable of multilineage hematopoietic differentiation. Two strategies are promising in overcoming this restraint. A recent report[126] shows that murine FDCPmix cells, transduced with a temperature-sensitive mutant of *BCR-ABL*, become partially factor-independent at the permissive temperature, in analogy to chronic phase CML. They retain the capacity for terminal differentiation, similar to chronic phase CML cells. Another approach is the study of embryonic stem (ES) cells transduced with *BCR-ABL*. In one such experimental system, it was possible to reproduce one cardinal feature of the clinical disease in the model, namely the expansion of the myeloid compartment at the expense of the erythroid compartment.[169] Interestingly, the increase in total cell numbers in the *BCR-ABL*-transduced ES cells was found to result from increased proliferation though there was little effect on apoptosis, another finding in line with observations in primary Ph-positive cells.[124,154] In this system, a stromal cell layer is used on which the ES cells removed from leukemia-inhibitory factor

(LIF) differentiate into hemangioblasts and then into hematopoietic cells. This may explain why these results are not strictly comparable to those of another study, in which *BCR-ABL* resulted in the decreased formation of embryonal bodies along with increased output of all kinds of hematopoietic progenitors.[170] In yet another study, *BCR-ABL*–transformed ES cells transplanted into irradiated mice induced a leukemic syndrome with many features of CML.[171] If developed further, these models may well be able to retain the major advantage of cell lines—their ease of manipulation—while at the same time moving the in vitro system closer to the clinical disease.

Bearing all these caveats in mind, there is no doubt that the study of cell lines contributed significantly to our understanding of CML. Particularly, many of the proteins that interact with Bcr-Abl were identified in Ph-positive cell lines, where they are more abundantly expressed than in primary cells. Thus, though these lines are invaluable tools for screening, it is important to confirm the results in primary cells.

Primary cells. The study of patient material and its comparison with normal hematopoietic progenitor cells is certainly the gold standard of CML research, particularly for the chronic phase of the disease. Much of the data refer to operationally defined cellular properties of CML versus normal cells, such as clonogenicity or adherence to bone marrow stroma; to give a comprehensive account of the cellular biology of CML would require a review in its own right. We will therefore focus on some areas in which the study of primary CML cells has been particularly instrumental to the study of the molecular biology of the disease.

One of the main problems when studying primary cells is inherent in the very nature of chronic phase cells—they tend to mature when placed in culture. Thus, the window of time for in vitro studies is narrow, and expansion of very primitive cells, the least prevalent but most interesting population, is difficult and carries the risk for introducing nonphysiologic alterations.[172] Furthermore, there is considerable variation between patients that frequently results in an overlap rather than a clear distinction between normal cells and CML cells. Last, results are unreliable unless clearly defined cell populations such as $CD34^+$ cells are studied. To a large extent, these problems can be overcome by the introduction of retroviral *BCR-ABL* expression vectors to murine or human primary bone marrow cells (see "Animal models" below).

A striking example of how fruitful the comparison of primary cell populations can be is the study of tyrosine-phosphorylated proteins in $CD34^+$ cells.[112] This study led to the subsequent identification of $p62^{DOK}$[74;173] and SHIP2[132] as mediators of Bcr-Abl–induced transformation. Moreover, it produced the important notion that Bcr-Abl tyrosine kinase activity may have consequences similar to the activation of the KIT receptor.[112] Another example is the identification of CRKL as the major tyrosine-phosphorylated protein in CML neutrophils.[73]

The recent possibility of turning off the Bcr-Abl tyrosine kinase activity in cell lines and primary cells with STI571[7,8] provided the opportunity to study the effects of the *BCR-ABL* gene when expressed from its natural *BCR* promoter at "physiological" levels. This is certainly an advantage over transduced cell systems; the drawback, however, is that effects related to inhibition of the KIT and PDGFRβ kinases, and potentially other unidentified tyrosine kinases, cannot be ruled out. Furthermore, the Bcr-Abl protein, though kinase-inactive, is still present in the cells and may interfere with other proteins.

Animal models

Thus far, no animals other than mice have been used for the study of CML in vivo. Various approaches have been taken.

Engraftment of BCR-ABL–transformed cell lines in syngeneic mice. Murine factor-dependent cell lines such as 32D transduced with *BCR-ABL* give rise to an aggressive leukemia when transplanted into syngeneic recipients.[60,174] This is an excellent in vivo model to test the efficacy of new drugs, such as the tyrosine kinase inhibitor STI571, in vivo. Furthermore, the impact on leukemogenicity of modifications within the Bcr-Abl protein and modifications to the respective cell lines (such as the introduction of co-stimulatory molecules[174]) can be tested. The main drawback is that the disease is a form of acute leukemia and is thus far from chronic phase CML.

Engraftment of immunodeficient mice with human BCR-ABL–positive cells. Cell lines derived from human CML blast crisis are relatively easily propagated in severe combined immunodeficiency (SCID) mice.[175] The distribution of the leukemia cells is fairly similar to the human disease, that is, they home to bone marrow and peripheral blood before they metastasize to nonhematopoietic tissues. More recently, it was shown that SCID mice can be engrafted with chronic phase CML cells if the cell inoculum is large enough (in the range of 1×10^8 cells).[176] Up to 10% human cells were detectable in the recipient bone marrow and showed multilineage differentiation. Interestingly, most colonies were *BCR-ABL* negative and thus were derived from the patient's residual normal hematopoiesis. This is reminiscent of long-term bone marrow cultures of CML[177] and shows that host factors modify the disease to a great extent, a problem that will persist, even if higher percentages of engraftment can eventually be achieved. A step into this direction is the use of nonobese diabetes-SCID mice. In addition to the SCID defect in V(D)J recombination, these animals lack functional natural killer cells. Chronic phase CML cells and, even more so, cells from accelerated phase or blast crisis readily engraft in these mice, and there is a significant correlation between engraftment and disease state.[178] Interestingly, the cells were exclusively Ph-positive in most cases, in contrast to cells engrafted in SCID mice, as mentioned above. This may be attributed to technical reasons but may also reflect a genuine difference between the different strains of mice. We can anticipate that these murine models will be useful for studying certain aspects of CML, such as the response to novel forms of treatment. Their value in investigating the human disease will be limited because it is difficult to see how disease modification by host factors could ever be ruled out.

Transgenic mouse models. Attempts to use *BCR-ABL* transgenic mice as a CML model go back to the late 1980s, when a full-length cDNA of *BCR-ABL* was not yet available and an artificial construct of human BCR sequences fused to v-*abl* was used instead.[179] Since then, a number of studies have been published that clearly prove the oncogenic potential of *BCR-ABL*. Several different promoters were used to direct the expression to the desired target tissues. However, some problems were encountered. First, it became clear that Bcr-Abl has a toxic effect on embryogenesis,[180] perhaps the consequence of a cytostatic effect in nonhematopoietic tissues.[181] Recently, the expression of *BCR-ABL* from a tetracycline-repressible promoter effectively overcame this problem.[182] Most striking, the leukemia in these transgenic mice is completely reversible on re-addition of tetracycline. The second problem with transgenic mice is that the P210 *BCR-ABL* variant relevant to CML is difficult to study because it is less efficient in

inducing leukemia than P190, a finding that was again confirmed in a recent study.[47] Third and most important, the types of leukemia that developed in these mice were acute and of either B- or T-lymphoid phenotype, regardless of whether they arose in P190 or P210 transgenic animals. Thus, they resembled *BCR-ABL*–positive ALL but not chronic-phase CML[183,184]. In fact, myeloid leukemias developed rarely, if at all. A recent report[185] may represent a major advance in this respect. In this study, *BCR-ABL* was expressed from the Tec promoter, a cytoplasmic tyrosine kinase predominantly expressed in hematopoietic cells. Although the founder mice exhibited excessive proliferation of lymphoblasts, their progeny developed a CML-like disease, albeit after a relatively long latency period of approximately 10 months. Thus, it is likely that the problems of the transgenic models will eventually be resolved if the gene is targeted to the appropriate cell.

Transduction of murine bone marrow cells with BCR-ABL retroviruses. In 1990, several groups reported that a CML-like myeloproliferative syndrome could be induced when P*210BCR-ABL*–infected marrow was transplanted into syngeneic recipients.[6,186,187] Transplantation into secondary recipients frequently produced an identical disease while some mice developed acute leukemias of T- or B-cell phenotype, analogous to the development of lymphoid blast crisis in the clinical disease. Clonality was demonstrated in many cases. Roughly a quarter of the mice showed the myeloproliferative disease, whereas other recipients developed other distinct hematologic malignancies, such as macrophage tumors, B-ALL, T-ALL, and erythroleukemia. Most likely, these different diseases are the consequence of infection of different committed progenitor cells that, after transformation, give rise to the respective progeny. Not surprisingly, the infection conditions have a major impact on the disease phenotype.[188] Building on the foundations of this early work, major improvements to the transduction–transplantation system have been made in the past few years. High-titer *BCR-ABL* retroviral stocks can be produced rapidly by transient transfection of packaging lines; the culture conditions have been refined, and the murine stem cell virus LTR has been introduced that allows for more efficient expression of *BCR-ABL* in the desired target cell. Combining all 3 improvements, 2 recent studies[189,190] reported the induction of a transplantable CML-like disease in 100% of recipients. Pulmonary hemorrhage, a complication not found in human CML, was a frequent cause of death in both studies, demonstrating that these novel models, though a major step forward, may have their own distinct problems. Nevertheless, bone marrow transduction–transplantation most faithfully reproduces human CML, and further improvements are likely in the near future.

Transformation to blast crisis

Clinically, chronic-phase CML does not represent a major management problem because the elevated white blood cell count is readily controlled with cytotoxic agents in most patients, and neutrophil and platelet functions are largely normal. However, the disease progresses inexorably to acceleration and blast crisis, often within 5 years of diagnosis. The mechanisms underlying this evolution remain enigmatic. Deletion or inactivation of p16,[191] p53,[192] and the retinoblastoma gene product[193] have been reported but occur relatively rarely and, similar to the overexpression of *EVI-1*,[194] are not specific for blast crisis CML. This probably indicates that a wide variety of lesions, possibly multiple "cooperating" lesions, are required to induce the phenotype of blast crisis. Perhaps even more intriguing is why the cells acquire these additional lesions in the first place. A recent report shows that Bcr-Abl enhances the mutation rate in the Na-K-ATPase and in the HPRT genomic loci, both commonly used markers to measure mutation frequency. Along with this goes enhanced expression of DNA polymerase β, the mammalian DNA polymerase with the least fidelity.[195] $P210^{BCR-ABL}$, but not $P190^{BCR-ABL}$, phosphorylates and potentially interacts with xeroderma pigmentosum group B protein (XPB); as a result, the catalytic function of XPB may be reduced, and DNA repair may be impaired.[196] In a recent study $p210^{BCR-ABL}$ transgenic mice were cross-mated with p53 heterozygous mice. In the offspring, the remaining p53 was rapidly lost because of somatic mutation, and the mice developed a disease that resembled blast crisis.[197] Although this is still not a perfect model of human CML because the blasts are of T lineage, it strongly supports the concept of genomic instability in *BCR-ABL*–transformed cells. How Bcr-Abl leads to these phenomena is unclear, but they might form the basis of the presumed genomic instability of chronic phase CML. It is also possible that the alleged anti-apoptotic effect of Bcr-Abl favors inaccurate DNA repair where apoptosis would ensue in normal cells. In line with this concept, a prolonged G_2 arrest after IR has been observed in *BCR-ABL*–expressing cell lines exposed to DNA-damaging agents.[140] This arrest could allow for DNA (mis)repair, whereas in a normal cell the damage would induce apoptosis. Over time this could lead to the accumulation of mutations in *BCR-ABL*–positive cells that finally result in blastic transformation. There is no doubt that the excessive proliferation, with its high cell turnover, must be a risk factor per se for additional genetic lesions.

Molecular targets for therapy

Attempts at designing therapeutic tools for CML based on our current knowledge of the molecular and cell biology of the disease have concentrated on 3 main areas—the inhibition of gene expression at the translational level by "antisense" strategies, the stimulation of the immune system's capacity to recognize and destroy leukemic cells, and the modulation of protein function by specific signal transduction inhibitors. The antisense oligonucleotide[198,199] and ribozyme[200] approaches received much attention in the last decade but have in general failed to fulfill their theoretical promises. New modifications to the system, such as the use of *BCR-ABL* junction-specific catalytic subunits of RNase P,[201] may revitalize the field. The issue of immunologic stimulation, be it in the form of adoptive immunotherapy by donor lymphocyte infusions[202] or of *BCR-ABL* junction peptide vaccination,[203] is another avenue being extensively explored for the treatment of CML.

Perhaps the most exciting of the molecularly designed therapeutic approaches was brought about by the advent of signal transduction inhibitors (STI), which block or prevent a protein from exerting its role in the oncogenic pathway. Because the main transforming property of the Bcr-Abl protein is effected through its constitutive tyrosine kinase activity, direct inhibition of such activity seems to be the most logical means of silencing the oncoprotein. To this effect, several tyrosine kinase inhibitors have been evaluated for their potential to modify the phenotype of CML cells. The first to be tested were compounds isolated from natural sources, such as the iso-flavonoid genistein and the antibiotic herbimycin A.[204] Later, synthetic compounds were developed through a rational design of chemical structures capable of competing with the adenosine triphosphate (ATP) or the protein

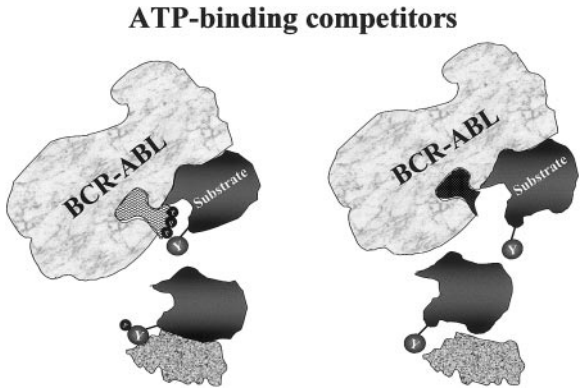

Figure 7. Mechanism of action of tyrosine kinase inhibitors. The drug competes with ATP for its specific binding site in the kinase domain. Thus, whereas the physiologic binding of ATP to its pocket allows Bcr-Abl to phosphorylate selected tyrosine residues on its substrates *(left diagram)*, a synthetic ATP mimic such as STI571 fits this pocket equally well but does not provide the essential phosphate group to be transferred to the substrate *(right diagram)*. The downstream chain of reactions is then halted because, with its tyrosines in the unphosphorylated form, this protein does not assume the necessary conformation to ensure association with its effector.

substrate for the binding site in the catalytic center of the kinase[205] (Figure 7).

The most promising of these compounds is the 2-phenylaminopyrimidine STI571 (formerly CGP57418B; Novartis Pharmaceutics, Basel, Switzerland), which specifically inhibits Abl tyrosine kinase at micromolar concentrations.[206] Inhibition of the Bcr-Abl kinase activity by this compound results in the transcriptional modulation of various genes involved in the control of the cell cycle, cell adhesion, and cytoskeleton organization, leading the Ph-positive cell to an apoptotic death.[86] Furthermore, STI571 selectively suppresses the growth of CML primary cells and cell lines in vitro[7,8] and in mice.[7,207] Its remarkable specificity and efficacy led to consideration of the drug for therapeutic use. Thus, in the spring of 1998, a phase 1 clinical trial was initiated in the United States in which patients with CML in chronic phase resistant to IFN-α were treated with STI571 in increasing doses. The drug showed little toxicity but proved to be highly effective. All patients treated with 300 mg/d or more entered a complete hematologic remission. Even more striking, many of the patients had cytogenetic responses.[9] This might mean that STI571 changes the natural course of the disease, though it is far too early to arrive at any definite conclusions. Altogether, the results were convincing enough to justify the initiation of phase 2 studies that included patients with acute Ph-positive leukemias (CML in blast crisis and Ph-positive ALL) and, at a later stage, a large cohort of interferon-intolerant or -resistant patients. These studies are ongoing. It turned out that STI571 is effective in many patients with acute Ph-positive leukemia, particularly of lymphoid phenotype. Although in many patients the remissions are not sustained, the advent of an effective oral medication with little toxicity represents a major step forward in this very poor risk group. Clearly, elucidation of the mechanisms underlying the resistance[208] will be of critical importance for the development of further treatment strategies, such as a combination of STI571 with conventional cytotoxic drugs or, perhaps, with other STIs (see below). In this context, the most interesting question is whether STI571 will be able to eradicate the malignant clone, at least in some patients with chronic-phase CML. From what we know about the disease, this seems unlikely—colony formation by CML progenitor cells is much reduced but not abrogated in the presence of STI571[7,8]—which might mean that a subset of these cells proliferates independently of Bcr-Abl tyrosine kinase activity and still relies on external growth factors. There is no doubt, however, that the clinical efficacy and low toxicity of STI571 sets a precedent for the further development of targeted forms of therapy in malignant disease.

An alternative or a supplement to direct inhibition of Bcr-Abl is interference with proteins that are critical for Bcr-Abl–induced transformation (Figure 4). One of these proteins is Grb2, whose SH2 domain binds directly to Bcr-Abl through the phosphorylated tyrosine 177 within the Bcr portion of the chimera[57] and is essential for activation of the Ras pathway (Figure 6).[209] Another good candidate is Ras itself, whose activity depends on its attachment to the cell membrane through a prenyl (usually a farnesyl) group. Thus, farnesyl transferase inhibitors (FTI) have been studied for their effect in inhibiting the proliferation of ALL[210] and juvenile myelomonocytic leukemia cells,[211] and they may be useful for the control of CML cells. Additional targets worth considering are represented by PI-3 and Src kinases, of which the available inhibitors have been shown to suppress colony formation of primary progenitors,[127] proliferation of *BCR-ABL*–transfected cell lines, or both.[212,213] It is envisaged that the progressive unraveling of which pathways are really essential for the development of the disease, coupled to rapid advances in biotechnology, will bring us the ideal combination of rationally designed drugs that can tip the balance toward the re-establishment of normal hematopoiesis in CML.

Conclusion

Though this be madness, yet there is method in it. (Shakespeare W., *Hamlet.* Act 2, scene 2.)

There are 2 ways to conclude this review after going through the vast amount of data presented. Surely one could argue that despite all these data, there is still no clear picture emerging and each piece of additional information adds only more confusion. Alternatively, what might help us against capitulation in the face of complexity is to try to simplify without oversimplification.

Can we build a model of CML that incorporates all the scientific data available but still retains clarity? In other words, could we explain how Bcr-Abl works in a few sentences to somebody who has never heard of it? Perhaps the most promising approach might be to try to link the biologic behavior of a CML cell to the underlying molecular events (Figure 5). Crucially, we should be able to picture this scenario relying on *BCR-ABL* alone because, at least until now, there is no unequivocal evidence that additional genetic lesions are present during chronic phase. We do not know how long it takes to move from the initial genetic event to fully established chronic-phase CML, but there is good reason to believe that the proliferative advantage of CML over normal cells is limited. Together with the largely normal differentiation capacity and function of CML blood cells, one feels that Ph-positive hematopoiesis cannot be so much different from normal hematopoiesis until the disease accelerates. Thus, Bcr-Abl is likely to hijack pathways that normally increase blood cell output in response to physiologic stimuli rather than to interrupt or replace them with pathways that are not normally used in hematopoietic cells. Indeed, there is plenty of experimental evidence to support this notion. Importantly, Bcr-Abl is capable of activating survival pathways along with proliferative stimuli without the need for a second cooperating genetic lesion; in this way, the apoptotic response that would otherwise follow an isolated proliferative stimulus is

avoided. The sustained dependence on growth factors is an indication that Bcr-Abl is not a complete substitute; rather, it tips the balance to provide a limited growth advantage in vivo. This growth advantage is also dependent on specific survival conditions: transient regeneration of Ph-negative hematopoiesis is often observed after autografting, even when the autograft seems to be comprised exclusively of Ph-positive stem cells, and long-term cultures initiated from patients with chronic-phase CML become dominated by *BCR-ABL*–negative cells after some time.[177] Thus, there appears to be a specific interaction (or noninteraction) of CML progenitor cells with their microenvironment that is crucial to maintain their proliferative advantage. Whether this interaction is stimulatory for CML over normal progenitor cells or inhibitory for normal over CML progenitor cells remains to be seen. Similarly, we can look at extramedullary hematopoiesis as a loss of function (ie, loss of the capacity to respond to negative signals) or a gain of function (ie, acquisition of a capacity to respond to positive signals that are not provided in the bone marrow) phenomenon. Much of the evidence implicates integrins in mediating these abnormal interactions, but other proteins may also play a role. Overall, it appears that the organization of cell membrane and cytoskeleton is more profoundly perturbed in CML progenitor cells than might be anticipated from the largely normal function of their progeny. Furthermore, Bcr-Abl may interfere with the "wiring" between integrin receptors on the cell surface and the nucleus and so disturb the communication of the cell with its environment. Another mechanism may also be important: Bcr-Abl appears to induce the degradation of certain inhibitory proteins. This might thwart cellular counter-reactions that would otherwise be activated, rather like cutting the telephone cable before the police can be called in.

Many questions remain unanswered. Why is there a predominantly myeloid expansion when all 3 lineages carry the translocation? What is the biologic basis for the extraordinary variability in the clinical course of a disease that appears to carry just a single genetic lesion? What is the molecular basis for the genomic instability that we see clinically as relentless progression to blast crisis?

Where do we go from here? The more we learn about the pathogenesis of CML, the more we realize its extraordinary complexity. Perhaps one should not be too surprised because it has become clear that cellular processes tend to rely on integrated networks rather than on straight unidirectional pathways. Only in this way can the cell achieve the flexibility required to respond to the various stimuli within a multicellular organism. Clearly, some components must be more important, and some less so, in the transformation network operated by Bcr-Abl. Absolutely essential features may be restricted to functional domains and to certain residues of the Bcr-Abl protein itself, and downstream effectors may be able to substitute for each other, at least to some extent. In this respect, the use of knockout mice that lack specific downstream molecules will allow one to define their precise relevance for Bcr-Abl–mediated cellular transformation. It may turn out that the combined elimination of several components abrogates transformation by Bcr-Abl, whereas each component individually is of limited significance. Chronic phase CML operates very much by exploiting physiologic pathways, perhaps by gently "coaxing" hematopoiesis toward the classical CML phenotype; nevertheless it prepares the ground for blast crisis. Thus, to understand CML, we must study its chronic phase. We must move away from artificial systems, such as transduced fibroblasts, and take on the demanding task of studying signal transduction in primary progenitor cells.

References

1. Nowell P, Hungerford D. A minute chromosome in human chronic granulocytic leukemia. Science. 1960;132:1497.
2. Rowley JD. A new consistent chromosomal abnormality in chronic myelogenous leukaemia identified by quinacrine fluorescence and Giemsa staining [letter]. Nature. 1973;243:290-293.
3. Bartram CR, de Klein A, Hagemeijer A, et al. Translocation of c-abl oncogene correlates with the presence of a Philadelphia chromosome in chronic myelocytic leukaemia. Nature. 1983;306: 277-280.
4. Groffen J, Stephenson JR, Heisterkamp N, de Klein A, Bartram CR, Grosveld G. Philadelphia chromosomal breakpoints are clustered within a limited region, bcr, on chromosome 22. Cell. 1984;36:93-99.
5. Lugo TG, Pendergast AM, Muller AJ, Witte ON. Tyrosine kinase activity and transformation potency of bcr-abl oncogene products. Science. 1990;247:1079-1082.
6. Daley GQ, Van Etten RA, Baltimore D. Induction of chronic myelogenous leukemia in mice by the P210bcr/abl gene of the Philadelphia chromosome. Science. 1990;247:824-830.
7. Druker BJ, Tamura S, Buchdunger E, et al. Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr-Abl positive cells. Nat Med. 1996;2:561-566.
8. Deininger M, Goldman JM, Lydon NB, Melo JV. The tyrosine kinase inhibitor CGP57148B selectively inhibits the growth of BCR-ABL positive cells. Blood. 1997;90:3691-3698.
9. Druker BJ, Talpaz M, Resta D, et al. Clinical efficacy and safety of an ABL-specific tyrosine kinase inhibitor as targeted therapy for chronic myeloid leukemia [abstract]. Blood. 1999:94:368.
10. Abelson HT, Rabstein LS. Lymphosarcoma: virus-induced thymic-independent disease in mice. Cancer Res. 1970;30:2213-2222.
11. Laneuville P. Abl tyrosine protein kinase. Semin Immunol. 1995;7:255-266.
12. Cohen GB, Ren R, Baltimore D. Modular binding domains in signal transduction proteins. Cell. 1995;80:237-248.
13. Feller SM, Knudsen B, Hanafusa H. c-Abl kinase regulates the protein binding activity of c-Crk. EMBO J. 1994;13:2341-2351.
14. Van Etten RA, Jackson P, Baltimore D. The mouse type IV c-abl gene product is a nuclear protein, and activation of transforming ability is associated with cytoplasmic localization. Cell. 1989;58:669-678.
15. Kipreos ET, Wang JY. Cell cycle-regulated binding of c-Abl tyrosine kinase to DNA. Science. 1992;256:382-385.
16. McWhirter JR, Wang JY. An actin-binding function contributes to transformation by the Bcr-Abl oncoprotein of Philadelphia chromosome-positive human leukemias. EMBO J. 1993;12:1533-1546.
17. Kipreos ET, Wang JY. Differential phosphorylation of c-Abl in cell cycle determined by cdc2 kinase and phosphatase activity. Science. 1990;248:217-220.
18. Sawyers CL, McLaughlin J, Goga A, Havlik M, Witte O. The nuclear tyrosine kinase c-Abl negatively regulates cell growth. Cell. 1994;77:121-131.
19. Yuan ZM, Shioya H, Ishiko T, et al. p73 is regulated by tyrosine kinase c-Abl in the apoptotic response to DNA damage. Nature. 1999;399:814-817.
20. Lewis JM, Schwartz MA. Integrins regulate the association and phosphorylation of paxillin by c-Abl. J Biol Chem. 1998;273:14225-14230.
21. Van Etten RA. Cycling, stressed-out and nervous: cellular functions of c-Abl. Trends Cell Biol. 1999; 9:179-186.
22. Tybulewicz VL, Crawford CE, Jackson PK, Bronson RT, Mulligan RC. Neonatal lethality and lymphopenia in mice with a homozygous disruption of the c-abl proto-oncogene. Cell. 1991;65:1153-1163.
23. Schwartzberg PL, Stall AM, Hardin JD, et al. Mice homozygous for the ablm1 mutation show poor viability and depletion of selected B and T cell populations. Cell. 1991;65:1165-1175.
24. Reuther GW, Fu H, Cripe LD, Collier RJ, Pendergast AM. Association of the protein kinases c-Bcr and Bcr-Abl with proteins of the 14-3-3 family. Science. 1994;266:129-133.
25. McWhirter JR, Galasso DL, Wang JY. A coiled-coil oligomerization domain of Bcr is essential for the transforming function of Bcr-Abl oncoproteins. Mol Cell Biol. 1993;13:7587-7595.
26. Denhardt DT. Signal-transducing protein phosphorylation cascades mediated by Ras/Rho proteins in the mammalian cell: the potential for multiplex signalling. Biochem J. 1996;318:729-747.
27. Montaner S, Perona R, Saniger L, Lacal JC. Multiple signaling pathways lead to the activation of the nuclear factor κB by the Rho family of GTPases. J Biol Chem. 1998;273:12779-12785.
28. Diekmann D, Brill S, Garrett MD, et al. Bcr encodes a GTPase-activating protein for p21rac. Nature. 1991;351:400-402.

29. Diekmann D, Nobes CD, Burbelo PD, Abo A, Hall A. Rac GTPase interacts with GAPs and target proteins through multiple effector sites. EMBO J. 1995;14:5297-5305.
30. Wu Y, Liu J, Arlinghaus RB. Requirement of two specific tyrosine residues for the catalytic activity of Bcr serine/threonine kinase. Oncogene. 1998; 16:141-146.
31. Ma G, Lu D, Wu Y, Liu J, Arlinghaus RB. Bcr phosphorylated on tyrosine 177 binds Grb2. Oncogene. 1997;14:2367-2372.
32. Liu J, Wu Y, Ma GZ, et al. Inhibition of Bcr serine kinase by tyrosine phosphorylation. Mol Cell Biol. 1996;16:998-1005.
33. Voncken JW, van Schaick H, Kaartinen V, et al. Increased neutrophil respiratory burst in bcr-null mutants. Cell. 1995;80:719-728.
34. Voncken JW, Kaartinen V, Groffen J, Heisterkamp N. Bcr/Abl associated leukemogenesis in bcr null mutant mice. Oncogene. 1998;16:2029-2032.
35. Melo JV. The diversity of BCR-ABL fusion proteins and their relationship to leukemia phenotype. Blood. 1996;88:2375-2384.
36. Melo JV, Myint H, Galton DA, Goldman JM. P190BCR-ABL chronic myeloid leukemia: the missing link with chronic myelomonocytic leukemia? Leukemia. 1994;8:208-211.
37. Ravandi F, Cortes J, Albitar M, et al. Chronic myelogenous leukaemia with p185(BCR/ABL) expression: characteristics and clinical significance. Br J Haematol. 1999;107:581-586.
38. Pane F, Frigeri F, Sindona M, et al. Neutrophilic-chronic myeloid leukemia: a distinct disease with a specific molecular marker (BCR/ABL with C3/A2 junction). Blood. 1996;88:2410-2414.
39. Wilson G, Frost L, Goodeve A, Vandenberghe E, Peake I, Reilly J. BCR-ABL transcript with an e19a2 (c3a2) junction in classical chronic myeloid leukemia. Blood. 1997;89:3064.
40. van Rhee F, Hochhaus A, Lin F, Melo JV, Goldman JM, Cross NC. p190 BCR-ABL mRNA is expressed at low levels in p210-positive chronic myeloid and acute lymphoblastic leukemias. Blood. 1996;87:5213-5217.
41. Melo JV. BCR-ABL gene variants. Baillieres.Clin-.Haematol. 1997;10:203-222.
42. Leibundgut EO, Jotterand M, Rigamonti V, et al. A novel BCR-ABL transcript e2a2 in a chronic myelogenous leukaemia patient with a duplicated Ph-chromosome and monosomy 7. Br J Haematol. 1999;106:1041-1044.
43. Golub TR, Goga A, Barker GF, et al. Oligomerization of the ABL tyrosine kinase by the Ets protein TEL in human leukemia. Mol Cell Biol. 1996;16: 4107-4116.
44. Papadopoulos P, Ridge SA, Boucher CA, Stocking C, Wiedemann LM. The novel activation of ABL by fusion to an ets-related gene, TEL. Cancer Res. 1995;55:34-38.
45. Cazzaniga G, Tosi S, Aloisi A, et al. The tyrosine kinase abl-related gene ARG is fused to ETV6 in an AML-M4Eo patient with a t(1;12)(q25;p13): molecular cloning of both reciprocal transcripts. Blood. 1999;94:4370-4373.
46. Li S, Ilaria RL Jr, Million RP, Daley GQ, Van Etten RA. The P190, P210, and P230 forms of the BCR/ABL oncogene induce a similar chronic myeloid leukemia-like syndrome in mice but have different lymphoid leukemogenic activity. J Exp Med. 1999;189:1399-1412.
47. Quackenbush RC, Reuther GW, Miller JP, Courtney KD, Pear WS, Pendergast AM. Analysis of the biologic properties of p230 Bcr-Abl reveals unique and overlapping properties with the oncogenic p185 and p210 Bcr-Abl tyrosine kinases. Blood. 2000;95:2913-2921.
48. Tanaka K, Takechi M, Hong J, et al. 9;22 translocation and bcr rearrangements in chronic myelocytic leukemia patients among atomic bomb survivors. J Radiat Res. 1989;30:352-358.
49. Corso A, Lazzarino M, Morra E, et al. Chronic myelogenous leukemia and exposure to ionizing radiation—a retrospective study of 443 patients. Ann Hematol. 1995;70:79-82.
50. Deininger MW, Bose S, Gora-Tybor J, Yan XH, Goldman JM, Melo JV. Selective induction of leukemia-associated fusion genes by high-dose ionizing radiation. Cancer Res. 1998;58:421-425.
51. Kozubek S, Lukasova E, Ryznar L, et al. Distribution of ABL and BCR genes in cell nuclei of normal and irradiated lymphocytes. Blood. 1997;89: 4537-4545.
52. Neves H, Ramos C, da Silva MG, Parreira A, Parreira L. The nuclear topography of ABL, BCR, PML, and RARα genes: evidence for gene proximity in specific phases of the cell cycle and stages of hematopoietic differentiation. Blood. 1999;93:1197-1207.
53. Bose S, Deininger M, Gora-Tybor J, Goldman JM, Melo JV. The presence of BCR-ABL fusion genes in leukocytes of normal individuals: implications for the assessment of minimal residual disease. Blood. 1998;92:3362-3367.
54. Biernaux C, Sels A, Huez G, Stryckmans P. Very low level of major BCR-ABL expression in blood of some healthy individuals. Bone Marrow Transplant. 1996;17(suppl 3):S45–S47.
55. Posthuma EF, Falkenburg JH, Apperley JF, et al. HLA-B8 and HLA-A3 coexpressed with HLA-B8 are associated with a reduced risk of the development of chronic myeloid leukemia: the Chronic Leukemia Working Party of the EBMT. Blood. 1999;93:3863-3865.
56. Fialkow PJ, Martin PJ, Najfeld V, Penfold GK, Jacobson RJ, Hansen JA. Evidence for a multistep pathogenesis of chronic myelogenous leukemia. Blood. 1981;58:158-163.
57. Pendergast AM, Quilliam LA, Cripe LD, et al. BCR-ABL-induced oncogenesis is mediated by direct interaction with the SH2 domain of the GRB-2 adaptor protein. Cell. 1993;75:175-185.
58. Pendergast AM, Muller AJ, Havlik MH, Maru Y, Witte ON. BCR sequences essential for transformation by the BCR-ABL oncogene bind to the ABL SH2 regulatory domain in a non-phosphotyrosine-dependent manner. Cell. 1991;66:161-171.
59. Afar DE, Goga A, McLaughlin J, Witte ON, Sawyers CL. Differential complementation of Bcr-Abl point mutants with c-Myc. Science. 1994;264: 424-426.
60. Ilaria RL Jr, Van Etten RA. The SH2 domain of P210BCR/ABL is not required for the transformation of hematopoietic factor-dependent cells. Blood. 1995;86:3897-3904.
61. Mayer BJ, Baltimore D. Mutagenic analysis of the roles of SH2 and SH3 domains in regulation of the Abl tyrosine kinase. Mol Cell Biol. 1994;14: 2883-2894.
62. Danial NN, Pernis A, Rothman PB. Jak-STAT signaling induced by the v-abl oncogene. Science. 1995;269:1875-1877.
63. Shi Y, Alin K, Goff SP. Abl-interactor-1, a novel SH3 protein binding to the carboxy-terminal portion of the Abl protein, suppresses v-abl transforming activity. Genes Dev. 1995;9:2583-2597.
64. Dai Z, Pendergast AM. Abi-2, a novel SH3-containing protein interacts with the c-Abl tyrosine kinase and modulates c-Abl transforming activity. Genes Dev. 1995;9:2569-2582.
65. Cicchetti P, Mayer BJ, Thiel G, Baltimore D. Identification of a protein that binds to the SH3 region of Abl and is similar to Bcr and GAP-rho. Science. 1992;257:803-806.
66. Dai Z, Quackenbush RC, Courtney KD, et al. Oncogenic Abl and Src tyrosine kinases elicit the ubiquitin-dependent degradation of target proteins through a Ras-independent pathway. Genes Dev. 1998;12:1415-1424.
67. Wen ST, Van ER. The PAG gene product, a stress-induced protein with antioxidant properties, is an Abl SH3-binding protein and a physiological inhibitor of c-Abl tyrosine kinase activity. Genes Dev. 1997;11:2456-2467.
68. Goga A, McLaughlin J, Pendergast AM, et al. Oncogenic activation of c-ABL by mutation within its last exon. Mol Cell Biol. 1993;13:4967-4975.
69. Jackson PK, Paskind M, Baltimore D. Mutation of a phenylalanine conserved in SH3-containing tyrosine kinases activates the transforming ability of c-Abl. Oncogene. 1993;8:1943-1956.
70. Janssen JW, Ridge SA, Papadopoulos P, et al. The fusion of TEL and ABL in human acute lymphoblastic leukaemia is a rare event. Br J Haematol. 1995;90:222-224.
71. Reiter A, Sohal J, Kulkarni S, et al. Consistent fusion of ZNF198 to the fibroblast growth factor receptor-1 in the 1(8;13)(p11;q12) myeloproliferative syndrome. Blood. 1998;92:1735-1742.
72. Golub TR, Barker GF, Lovett M, Gilliland DG. Fusion of PDGF receptor beta to a novel ets-like gene, tel, in chronic myelomonocytic leukemia with t(5;12) chromosomal translocation. Cell. 1994;77:307-316.
73. Oda T, Heaney C, Hagopian JR, Okuda K, Griffin JD, Druker BJ. Crkl is the major tyrosine-phosphorylated protein in neutrophils from patients with chronic myelogenous leukemia. J Biol Chem. 1994;269:22925-22928.
74. Carpino N, Wisniewski D, Strife A, et al. p62(dok): a constitutively tyrosine-phosphorylated, GAP-associated protein in chronic myelogenous leukemia progenitor cells. Cell. 1997;88:197-204.
75. Matsuguchi T, Salgia R, Hallek M, et al. Shc phosphorylation in myeloid cells is regulated by granulocyte macrophage colony-stimulating factor, interleukin-3, and steel factor and is constitutively increased by p210BCR/ABL. J Biol Chem. 1994;269:5016-5021.
76. Salgia R, Brunkhorst B, Pisick E, et al. Increased tyrosine phosphorylation of focal adhesion proteins in myeloid cell lines expressing p210BCR/ABL. Oncogene. 1995;11:1149-1155.
77. Salgia R, Li JL, Lo SH, et al. Molecular cloning of human paxillin, a focal adhesion protein phosphorylated by P210BCR/ABL. J Biol Chem. 1995; 270:5039-5047.
78. Gotoh A, Miyazawa K, Ohyashiki K, et al. Tyrosine phosphorylation and activation of focal adhesion kinase (p125FAK) by BCR-ABL oncoprotein. Exp Hematol. 1995;23:1153-1159.
79. Ernst TJ, Slattery KE, Griffin JD. p210Bcr/Abl and p160v-Abl induce an increase in the tyrosine phosphorylation of p93c-Fes. J Biol Chem. 1994; 269:5764-5769.
80. Gotoh A, Miyazawa K, Ohyashiki K, Toyama K. Potential molecules implicated in downstream signaling pathways of p185BCR-ABL in Ph-positive ALL involve GTPase-activating protein, phospholipase C-gamma 1, and phosphatidylinositol 3'-kinase. Leukemia. 1994;8:115-120.
81. Andoniou CE, Thien CB, Langdon WY. Tumour induction by activated abl involves tyrosine phosphorylation of the product of the cbl oncogene. EMBO J. 1994;13:4515-4523.
82. Matsuguchi T, Inhorn RC, Carlesso N, Xu G, Druker B, Griffin JD. Tyrosine phosphorylation of p95Vav in myeloid cells is regulated by GM-CSF, IL-3 and steel factor and is constitutively increased by p210BCR/ABL. EMBO J. 1995;14: 257-265.
83. Tauchi T, Feng GS, Shen R, et al. SH2-containing phosphotyrosine phosphatase Syp is a target of p210 bcr-abl tyrosine kinase. J Biol Chem. 1994; 269:15381-15387.
84. LaMontagne KR Jr, Flint AJ, Franza BR Jr, Pandergast AM, Tonks NK. Protein tyrosine phosphatase 1B antagonizes signaling by oncoprotein tyrosine kinase p210 bcr-abl in vivo. Mol Cell Biol. 1998;18:2965-2975.
85. LaMontagne KR Jr, Hannon G, Tonks NK. Protein tyrosine phosphatase PTP1B suppresses p210

bcr-abl-induced transformation of rat-1 fibroblasts and promotes differentiation of K562 cells. Proc Natl Acad Sci U S A. 1998;95:14094-14099.

86. Deininger MW, Vieira S, Mendiola R, Schultheis B, Goldman JM, Melo JV. BCR-ABL tyrosine kinase activity regulates the expression of multiple genes implicated in the pathogenesis of chronic myeloid leukemia. Cancer Res. 2000;60:2049-2055.
87. Gordon MY, Dowding CR, Riley GP, Goldman JM, Greaves MF. Altered adhesive interactions with marrow stroma of haematopoietic progenitor cells in chronic myeloid leukaemia. Nature. 1987;328:342-344.
88. Puil L, Liu J, Gish G, et al. Bcr-Abl oncoproteins bind directly to activators of the Ras signalling pathway. EMBO J. 1994;13:764-773.
89. Bedi A, Zehnbauer BA, Barber JP, Sharkis SJ, Jones RJ. Inhibition of apoptosis by BCR-ABL in chronic myeloid leukemia. Blood. 1994;83:2038-2044.
90. Verfaillie CM, Hurley R, Lundell BI, Zhao C, Bhatia R. Integrin-mediated regulation of hematopoiesis: do BCR/ABL-induced defects in integrin function underlie the abnormal circulation and proliferation of CML progenitors? Acta Haematol. 1997;97:40-52.
91. Bhatia R, Wayner EA, McGlave PB, Verfaillie CM. Interferon-alpha restores normal adhesion of chronic myelogenous leukemia hematopoietic progenitors to bone marrow stroma by correcting impaired beta 1 integrin receptor function. J Clin Invest. 1994;94:384-391.
92. Zhao RC, Tarone G, Verfaillie CM. Presence of the adhesion inhibitory β1B integrin isoform on CML but not normal progenitors is at least in part responsible for the decreased CML progenitor adhesion [abstract]. Blood. 1997;90:393a.
93. Lewis JM, Baskaran R, Taagepera S, Schwartz MA, Wang JY. Integrin regulation of c-Abl tyrosine kinase activity and cytoplasmic-nuclear transport. Proc Natl Acad Sci U S A. 1996;93:15174-15179.
94. Uemura N, Griffin JD. The adapter protein Crkl links Cbl to C3G after integrin ligation and enhances cell migration. J Biol Chem. 1999;274:37525-37532.
95. Sattler M, Salgia R, Okuda K, et al. The proto-oncogene product p120CBL and the adaptor proteins CRKL and c-CRK link c-ABL, p190BCR/ABL and p210BCR/ABL to the phosphatidylinositol-3' kinase pathway. Oncogene. 1996;12:839-846.
96. Salgia R, Pisick E, Sattler M, et al. p130CAS forms a signaling complex with the adapter protein CRKL in hematopoietic cells transformed by the BCR/ABL oncogene. J Biol Chem. 1996;271:25198-25203.
97. Sattler M, Salgia R, Shrikhande G, et al. Differential signaling after β1 integrin ligation is mediated through binding of CRKL to p120(CBL) and p110(HEF1). J Biol Chem. 1997;272:14320-14326.
98. Bazzoni G, Carlesso N, Griffin JD, Hemler ME. Bcr/Abl expression stimulates integrin function in hematopoietic cell lines. J Clin Invest. 1996;98:521-528.
99. Pelicci G, Lanfrancone L, Salcini AE, et al. Constitutive phosphorylation of Shc proteins in human tumors. Oncogene. 1995;11:899-907.
100. Senechal K, Halpern J, Sawyers CL. The CRKL adaptor protein transforms fibroblasts and functions in transformation by the BCR-ABL oncogene. J Biol Chem. 1996;271:23255-23261.
101. Heaney C, Kolibaba K, Bhat A, et al. Direct binding of CRKL to BCR-ABL is not required for BCR-ABL transformation. Blood. 1997;89:297-306.
102. Watzinger F, Gaiger A, Karlic H, Becher R, Pillwein K, Lion T. Absence of N-ras mutations in myeloid and lymphoid blast crisis of chronic myeloid leukemia. Cancer Res. 1994;54:3934-3938.
103. Marais R, Light Y, Paterson HF, Marshall CJ. Ras recruits Raf-1 to the plasma membrane for activation by tyrosine phosphorylation. EMBO J. 1995;14:3136-3145.
104. Cahill MA, Janknecht R, Nordheim A. Signalling pathways: jack of all cascades. Curr Biol. 1996;6:16-19.
105. Kabarowski JH, Allen PB, Wiedemann LM. A temperature sensitive p210 BCR-ABL mutant defines the primary consequences of BCR-ABL tyrosine kinase expression in growth factor dependent cells. EMBO J. 1994;13:5887-5895.
106. Cortez D, Reuther GW, Pendergast AM. The BCR-ABL tyrosine kinase activates mitotic signaling pathways and stimulates G1-to-S phase transition in hematopoietic cells. Oncogene. 1997;15:2333-2342.
107. Raitano AB, Halpern JR, Hambuch TM, Sawyers CL. The Bcr-Abl leukemia oncogene activates Jun kinase and requires Jun for transformation. Proc Natl Acad Sci U S A. 1995;92:11746-11750.
108. Skorski T, Wlodarski P, Daheron L, et al. BCR/ABL-mediated leukemogenesis requires the activity of the small GTP-binding protein Rac. Proc Natl Acad Sci U S A. 1998;95:11858-11862.
109. Shi C-S, Tuscano JM, Witte O, Kehrl JH. GCKR links the BCR-ABL oncogene and RAS to the stress-activated protein kinase pathway. Blood. 1999;93:1338-1345.
110. Wilson Rawls J, Xie S, Liu J, Laneuville P, Arlinghaus RB. P210 Bcr-Abl interacts with the interleukin 3 receptor beta(c) subunit and constitutively induces its tyrosine phosphorylation. Cancer Res. 1996;56:3426-3430.
111. Hallek M, Danhauser Riedl S, Herbst R, et al. Interaction of the receptor tyrosine kinase p145c-kit with the p210bcr/abl kinase in myeloid cells. Br J Haematol. 1996;94:5-16.
112. Wisniewski D, Strife A, Berman E, Clarkson B. c-kit ligand stimulates tyrosine phosphorylation of a similar pattern of phosphotyrosyl proteins in primary primitive normal hematopoietic progenitors that are constitutively phosphorylated in comparable primitive progenitors in chronic phase chronic myelogenous leukemia. Leukemia. 1996;10:229-237.
113. Di Cristofano A, Carpino N, Dunant N, et al. Molecular cloning and characterization of p56dok-2 defines a new family of RasGAP-binding proteins. J Biol Chem. 1998;273:4827-4830.
114. Bhat A, Johnson KJ, Oda T, Corbin AS, Druker BJ. Interactions of p62(dok) with p210(bcr-abl) and Bcr-Abl-associated proteins. J Biol Chem. 1998;273:32360-32368.
115. Ilaria RL Jr, Van Etten RA. P210 and P190(BCR/ABL) induce the tyrosine phosphorylation and DNA binding activity of multiple specific STAT family members. J Biol Chem. 1996;271:31704-31710.
116. Chai SK, Nichols GL, Rothman P. Constitutive activation of JAKs and STATs in BCR-Abl-expressing cell lines and peripheral blood cells derived from leukemic patients. J Immunol. 1997;159:4720-4728.
117. de Groot RP, Raaijmakers JA, Lammers JW, Jove R, Koenderman L. STAT5 activation by BCR-Abl contributes to transformation of K562 leukemia cells. Blood. 1999;94:1108-1112.
118. Nosaka T, Kawashima T, Misawa K, Ikuta K, Mui AL, Kitamura T. STAT5 as a molecular regulator of proliferation, differentiation and apoptosis in hematopoietic cells. EMBO J. 1999;18:4754-4765.
119. Horita M, Andreu EJ, Benito A, et al. Blockade of the Bcr-Abl kinase activity induces apoptosis of chronic myelogenous leukemia cells by suppressing signal transducer and activator of transcription 5-dependent expression of Bcl-xL. J Exp Med. 2000;191:977-984.
120. Sillaber C, Gesbert F, Frank DA, Sattler M, Griffin JD. STAT5 activation contributes to growth and viability in Bcr/Abl-transformed cells. Blood. 2000;95:2118-2125.
121. Daley GQ, Baltimore D. Transformation of an interleukin 3-dependent hematopoietic cell line by the chronic myelogenous leukemia-specific P210bcr/abl protein. Proc Natl Acad Sci U S A. 1988;85:9312-9316.
122. Sirard C, Laneuville P, Dick JE. Expression of bcr-abl abrogates factor-dependent growth of human hematopoietic M07E cells by an autocrine mechanism. Blood. 1994;83:1575-1585.
123. Jiang X, Lopez A, Holyoake T, Eaves A, Eaves C. Autocrine production and action of IL-3 and granulocyte colony-stimulating factor in chronic myeloid leukemia. Proc Natl Acad Sci U S A. 1999;96:12804-12809.
124. Amos TA, Lewis JL, Grand FH, Gooding RP, Goldman JM, Gordon MY. Apoptosis in chronic myeloid leukaemia: normal responses by progenitor cells to growth factor deprivation, X-irradiation and glucocorticoids. Br J Haematol. 1995;91:387-393.
125. Jonuleit T, Peschel C, Schwab R, et al. Bcr-Abl kinase promotes cell cycle entry of primary myeloid CML cells in the absence of growth factors. Br J Haematol. 1998;100:295-303.
126. Pierce A, Owen-Lynch PJ, Spooncer E, Dexter TM, Whetton AD. p210 Bcr-Abl expression in a primitive multipotent haematopoietic cell line models the development of chronic myeloid leukaemia. Oncogene. 1998;17:667-672.
127. Skorski T, Kanakaraj P, Nieborowska Skorska M, et al. Phosphatidylinositol-3 kinase activity is regulated by BCR/ABL and is required for the growth of Philadelphia chromosome-positive cells. Blood. 1995;86:726-736.
128. Skorski T, Bellacosa A, Nieborowska-Skorska M, et al. Transformation of hematopoietic cells by BCR/ABL requires activation of a PI-3k/Akt-dependent pathway. EMBO J. 1997;16:6151-6161.
129. Franke TF, Kaplan DR, Cantley LC. PI3K: downstream AKTion blocks apoptosis. Cell. 1997;88:435-437.
130. del Peso L, Gonzalez-Garcia M, Page C, Herrera R, Nunez G. Interleukin-3-induced phosphorylation of bad through the protein kinase akt. Science. 1998;278:687-689.
131. Lioubin MN, Algate PA, Tsai S, Carlberg K, Aebersold A, Rohrschneider LR. p150Ship, a signal transduction molecule with inositol polyphosphate-5-phosphatase activity. Genes Dev. 1996;10:1084-1095.
132. Wisniewski D, Strife A, Swendeman S, et al. A novel SH2-containing phosphatidylinositol 3,4,5-trisphosphate 5-phosphatase (SHIP2) is constitutively tyrosine phosphorylated and associated with src homologous and collagen gene (SHC) in chronic myelogenous leukemia progenitor cells. Blood. 1999;93:2707-2720.
133. Sawyers CL, Callahan W, Witte ON. Dominant negative MYC blocks transformation by ABL oncogenes. Cell. 1992;70:901-910.
134. Zou X, Rudchenko S, Wong K, Calame K. Induction of c-myc transcription by the v-Abl tyrosine kinase requires Ras, Raf1, and cyclin-dependent kinases. Genes Dev. 1997;11:654-662.
135. Stewart MJ, Litz Jackson S, Burgess GS, Williamson EA, Leibowitz DS, Boswell HS. Role for E2F1 in p210 BCR-ABL downstream regulation of c-myc transcription initiation: studies in murine myeloid cells. Leukemia. 1995;9:1499-1507.
136. Bissonnette RP, Echeverri F, Mahboubi A, Green DR. Apoptotic cell death induced by c-myc is inhibited by bcl-2. Nature. 1992;359:552-554.
137. Evan GI, Wyllie AH, Gilbert CS, et al. Induction of apoptosis in fibroblasts by c-myc protein. Cell. 1992;69:119-128.
138. Daley GQ, Baltimore D. Transformation of an interleukin 3-dependent hematopoietic cell line by the chronic myelogenous leukemia-specific $P210^{bcr/abl}$ protein. Proc Natl Acad Sci U S A. 1992;85:9312-9316.
139. Cortez D, Kadlec L, Pendergast AM. Structural

and signaling requirements for BCR-ABL-mediated transformation and inhibition of apoptosis. Mol Cell Biol. 1995;15:5531-5541.

140. Bedi A, Barber JP, Bedi GC, et al. BCR-ABL-mediated inhibition of apoptosis with delay of G2/M transition after DNA damage: a mechanism of resistance to multiple anticancer agents. Blood. 1995;86:1148-1158.
141. Dubrez L, Eymin B, Sordet O, Droin N, Turhan AG, Solary E. BCR-ABL delays apoptosis upstream of procaspase-3 activation. Blood. 1998; 91:2415-2422.
142. Amarante Mendes GP, Naekyung Kim C, Liu L, et al. Bcr-Abl exerts its antiapoptotic effect against diverse apoptotic stimuli through blockage of mitochondrial release of cytochrome C and activation of caspase-3. Blood. 1998;91:1700-1705.
143. Sanchez Garcia I, Martin Zanca D. Regulation of Bcl-2 gene expression by BCR-ABL is mediated by Ras. J Mol Biol. 1997;267:225-228.
144. Wang HG, Rapp UR, Reed JC. Bcl-2 targets the protein kinase Raf-1 to mitochondria. Cell. 1996; 87:629-638.
145. Zha J, Harada H, Yang E, Jockel J, Korsmeyer SJ. Serine phosphorylation of death agonist BAD in response to survival factor results in binding to 14-3-3 not BCL-X(L). Cell. 1996;87:619-628.
146. Neshat MS, Raitano AB, Wang HG, Reed JC, Sawyers CL. The survival function of the Bcr-Abl oncogene is mediated by Bad-dependent and -independent pathways: roles for phosphatidylinositol 3-kinase and Raf. Mol Cell Biol. 2000;20: 1179-1186.
147. Majewski M, Nieborowska-Skorska M, Salomoni P, et al. Activation of mitochondrial Raf-1 is involved in the antiapoptotic effects of Akt. Cancer Res. 1999;59:2815-2819.
148. Gabriele L, Phung J, Fukumoto J, et al. Regulation of apoptosis in myeloid cells by interferon consensus sequence-binding protein. J Exp Med. 1999;190:411-421.
149. Hao SX, Ren R. Expression of interferon consensus sequence binding protein (ICSBP) is downregulated in Bcr-Abl-induced murine chronic myelogenous leukemia-like disease, and forced coexpression of ICSBP inhibits Bcr-Abl-induced myeloproliferative disorder. Mol Cell Biol. 2000; 20:1149-1161.
150. Holtschke T, Lohler J, Kanno Y, et al. Immunodeficiency and chronic myelogenous leukemia-like syndrome in mice with a targeted mutation of the ICSBP gene. Cell. 1996;87:307-317.
151. Scheller M, Foerster J, Heyworth CM, et al. Altered development and cytokine responses of myeloid progenitors in the absence of transcription factor, interferon consensus sequence binding protein. Blood. 1999;94:3764-3771.
152. Evan GI, Brown L, Whyte M, Harrington E. Apoptosis and the cell cycle. Curr Opin Cell Biol. 1995;7:825-834.
153. Santucci MA, Anklesaria P, Laneuville P, et al. Expression of p210 bcr/abl increases hematopoietic progenitor cell radiosensitivity. Int J Radiat Oncol Biol Phys. 1993;26:831-836.
154. Albrecht T, Schwab R, Henkes M, Peschel C, Huber C, Aulitzky WE. Primary proliferating immature myeloid cells from CML patients are not resistant to induction of apoptosis by DNA damage and growth factor withdrawal. Br J Haematol. 1996;95:501-507.
155. McGahon AJ, Nishioka WK, Martin SJ, Mahboubi A, Cotter TG, Green DR. Regulation of the Fas apoptotic cell death pathway by Abl. J Biol Chem. 1995;270:22625-22631.
156. Selleri C, Maciejewski J, Pane F, et al. Fas-mediated modulation of Bcr/Abl in chronic myelogenous leukemia results in differential effects on apoptosis. Blood. 1998;92:981-989.
157. Gora-Tybor J, Deininger M, Goldman JM, Melo JV. The susceptibility of Philadelpha chromosome-positive cells to FAS-mediated apoptosis is not linked to the tyrosine kinase activity of BCR-ABL. Br J Haematol. 1998;103:716-720.
158. Maguer Satta V, Burl S, Liu L, et al. BCR-ABL accelerates C2-ceramide-induced apoptosis. Oncogene. 1997;16:237-248.
159. Roger R, Issaad C, Pallardy M, et al. BCR-ABL does not prevent apoptotic death induced by human natural killer or lymphokine-activated killer cells. Blood. 1996;87:1113-1122.
160. Tordaro GJ, Green H. An assay for cellular transformation by SV40. Virology. 1964;23:117-119.
161. Lugo TG, Witte ON. The BCR-ABL oncogene transforms Rat-1 cells and cooperates with v-myc. Mol Cell Biol. 1989;9:1263-1270.
162. Daley GQ, McLaughlin J, Witte ON, Baltimore D. The CML-specific P210 bcr/abl protein, unlike v-abl, does not transform NIH/3T3 fibroblasts. Science. 1987;237:532-535.
163. Renshaw MW, Kipreos ET, Albrecht MR, Wang JY. Oncogenic v-Abl tyrosine kinase can inhibit or stimulate growth, depending on the cell context. EMBO J. 1992;11:3941-3951.
164. Drexler HG, MacLeod RA, Uphoff CC. Leukemia cell lines: in vitro models for the study of Philadelphia chromosome-positive leukemia. Leuk Res. 1999;23:207-215.
165. Spencer A, Yan XH, Chase A, Goldman JM, Melo JV. BCR-ABL-positive lymphoblastoid cells display limited proliferative capacity under in vitro culture conditions. Br J Haematol. 1996;94:654-658.
166. Carroll M, Tomasson MH, Barker GF, Golub TR, Gilliland DG. The TEL/platelet-derived growth factor beta receptor (PDGF beta R) fusion in chronic myelomonocytic leukemia is a transforming protein that self-associates and activates PDGF beta R kinase-dependent signaling pathways. Proc Natl Acad Sci U S A. 1996;93:14845-14850.
167. Klucher KM, Lopez DV, Daley GQ. Secondary mutation maintains the transformed state in BaF3 cells with inducible BCR/ABL expression. Blood. 1998;91:3927-3934.
168. Ghaffari S, Daley GQ, Lodish HF. Growth factor independence and BCR/ABL transformation: promise and pitfalls of murine model systems and assays. Leukemia. 1999;13:1200-1206.
169. Era T, Witte ON. Regulated expression of P210 Bcr-Abl during embryonic stem cell differentiation stimulates multipotential progenitor expansion and myeloid cell fate. Proc Natl Acad Sci U S A. 2000;97:1737-1742.
170. Turhan AG, Bonnet ML, Le Pesteur D, Mitjavila M, Vainschenker W, Sainteny F. Generation of an embryonic stem cell (ES) model of chronic myelogenous leukemia (CML) [abstract]. Blood. 1999;94:101a.
171. Peters DG, Perlingeiro RC, Klucher KM, et al. Generation of a hematopoietic stem cell line from ES cells using the oncogene BCR/ABL [abstract]. Blood. 1999;94:252a.
172. Garin MI, Apperley JF, Melo JV. *Ex vivo* expansion and characterisation of CD34+ cells derived from chronic myeloid leukaemia bone marrow and peripheral blood, and from normal bone marrow and mobilised peripheral blood. Eur J Haematol. 2000;64:85-92.
173. Kashige N, Carpino N, Kobayashi R. Tyrosine phosphorylation of p62dok by p210bcr-abl inhibits RasGAP activity. Proc Natl Acad Sci U S A. 2000;97:2093-2098.
174. Matulonis UA, Dosiou C, Lamont C, et al. Role of B7–1 in mediating an immune response to myeloid leukemia cells. Blood. 1995;85:2507-2515.
175. Sawyers CL, Gishizky ML, Quan S, Golde DW, Witte ON. Propagation of human blastic myeloid leukemias in the SCID mouse. Blood. 1992;79: 2089-2098.
176. Sirard C, Lapidot T, Vormoor J, et al. Normal and leukemic SCID-repopulating cells (SRC) coexist in the bone marrow and peripheral blood from CML patients in chronic phase, whereas leukemic SRC are detected in blast crisis. Blood. 1996;87: 1539-1548.
177. Coulombel L, Kalousek DK, Eaves CJ, Gupta CM, Eaves AC. Long-term marrow culture reveals chromosomally normal hematopoietic progenitor cells in patients with Philadelphia chromosome-positive chronic myelogenous leukemia. N Engl J Med. 1983;308:1493-1498.
178. Dazzi F, Capelli D, Hasserjian R, et al. The kinetics and extent of engraftment of chronic myelogenous leukemia cells in non-obese diabetic/severe combined immunodeficiency mice reflect the phase of the donor's disease: an in vivo model of chronic myelogenous leukemia biology. Blood. 1998;92:1390-1396.
179. Hariharan IK, Harris AW, Crawford M, et al. A bcr-v-abl oncogene induces lymphomas in transgenic mice. Mol Cell Biol. 1989;9:2798-2805.
180. Heisterkamp N, Jenster G, Kioussis D, Pattengale PK, Groffen J. Human bcr-abl gene has a lethal effect on embryogenesis. Transgenic Res. 1991;1:45-53.
181. Wen ST, Jackson PK, Van Etten RA. The cytostatic function of c-Abl is controlled by multiple nuclear localization signals and requires the p53 and Rb tumor suppressor gene products. EMBO J. 1996;15:1583-1595.
182. Huettner CS, Zhang P, Van Etten RA, Tenen DG. Reversibility of acute B-cell leukaemia induced by BCR-ABL1. Nat Genet. 2000;24:57-60.
183. Heisterkamp N, Jenster G, ten Hoeve J, Zovich D, Pattengale PK, Groffen J. Acute leukaemia in bcr/abl transgenic mice. Nature. 1990;344:251-253.
184. Voncken JW, Kaartinen V, Pattengale PK, Germeraad WT, Groffen J, Heisterkamp N. BCR/ABL P210 and P190 cause distinct leukemia in transgenic mice. Blood. 1995;86:4603-4611.
185. Honda H, Oda H, Suzuki T, et al. Development of acute lymphoblastic leukemia and myeloproliferative disorder in transgenic mice expressing p210 bcr/abl: a novel transgenic model for human Ph1-positive leukemias. Blood. 1998;91:2067-2075.
186. Elefanty AG, Hariharan IK, Cory S. bcr-abl, the hallmark of chronic myeloid leukaemia in man, induces multiple haemopoietic neoplasms in mice. EMBO J. 1990;9:1069-1078.
187. Kelliher MA, McLaughlin J, Witte ON, Rosenberg N. Induction of a chronic myelogenous leukemia-like syndrome in mice with v-abl and BCR/ABL. Proc Natl Acad Sci U S A. 1990;87:6649-6653.
188. Elefanty AG, Cory S. Hematologic disease induced in BALB/c mice by a bcr-abl retrovirus is influenced by the infection conditions. Mol Cell Biol. 1992;12:1755-1763.
189. Pear WS, Miller JP, Xu L, et al. Efficient and rapid induction of a chronic myelogenous leukemia-like myeloproliferative disease in mice receiving P210 bcr/abl-transduced bone marrow. Blood. 1998;92: 3780-3792.
190. Zhang X, Ren R. Bcr-Abl efficiently induces a myeloproliferative disease and production of excess interleukin-3 and granulocyte-macrophage colony-stimulating factor in mice: a novel model for chronic myelogenous leukemia. Blood. 1998; 92:3829-3840.
191. Sill H, Goldman JM, Cross NC. Homozygous deletions of the p16 tumor-suppressor gene are associated with lymphoid transformation of chronic myeloid leukemia. Blood. 1995;85:2013-2016.
192. Feinstein E, Cimino G, Gale RP, et al. p53 in chronic myelogenous leukemia in acute phase. Proc Natl Acad Sci U S A. 1991;88:6293-6297.
193. Towatari M, Adachi K, Kato H, Saito H. Absence of the human retinoblastoma gene product in the megakaryoblastic crisis of chronic myelogenous leukemia. Blood. 1991;78:2178-2181.
194. Ogawa S, Mitani K, Kurokawa M, et al. Abnormal

expression of Evi-1 gene in human leukemias. Hum Cell. 1996;9:323-332.

195. Canitrot Y, Lautier D, Laurent G, et al. Mutator phenotype of BCR-ABL transfected Ba/F3 cell lines and its association with enhanced expression of DNA polymerase beta. Oncogene. 1999; 18:2676-2680.
196. Takedam N, Shibuya M, Maru Y. The BCR-ABL oncoprotein potentially interacts with the xeroderma pigmentosum group B protein. Proc Natl Acad Sci U S A. 1999;96:203-207.
197. Honda H, Ushijima T, Wakazono K, et al. Acquired loss of p53 induces blastic transformation in p210(bcr/abl)-expressing hematopoietic cells: a transgenic study for blast crisis of human CML. Blood. 2000;95:1144-1150.
198. O'Brien SG, Smetsers TF. BCR-ABL as a target for antisense intervention. In: Stein CA, Krieg AM, eds. Applied antisense oligonucleotide technology. New York: Wiley-Liss; 1997:207-230.
199. Gewirtz AM, Sokol DL, Ratajczak MZ. Nucleic acid therapeutics: state of the art and future prospects. Blood. 1998;92:712-736.
200. James HA, Gibson I. The therapeutic potential of ribozymes. Blood. 1998;91:371-382.
201. Cobaleda C, Sanchez-Garcia I. In vivo inhibition by a site-specific catalytic RNA subunit of RNase P designed against the BCR-ABL oncogenic products: a novel approach for cancer treatment. Blood. 2000;95:731-737.
202. Dazzi F, Szydlo RM, Goldman JM. Donor lymphocyte infusions for relapse of chronic myeloid leukemia after allogeneic stem cell transplant: where we now stand. Exp Hematol. 1999;27:1477-1486.
203. Pinilla-Ibarz J, Cathcart K, Korontsvit T, et al. Vaccination of patients with chronic myelogenous leukemia with bcr-abl oncogene breakpoint fusion peptides generates specific immune responses. Blood. 2000;95:1781-1787.
204. Boutin JA. Tyrosine protein kinase inhibition and cancer. Int J Biochem. 1994;26:1203-1226.
205. Levitzki A, Gazit A. Tyrosine kinase inhibition: an approach to drug development. Science. 1995; 267:1782-1788.
206. Buchdunger E, Zimmermann J, Mett H, et al. Selective inhibition of the platelet-derived growth factor signal transduction pathway by a protein-tyrosine kinase inhibitor of the 2-phenylaminopyrimidine class. Proc Natl Acad Sci U S A. 1995; 92:2558-2562.
207. LeCoutre P, Mologni L, Cleris L, et al. In vivo eradication of human BCR/ABL-positive leukemia cells with an ABL kinase inhibitor. J Natl Cancer Inst. 1999;91:163-168.
208. Mahon FX, Deininger MW, Schultheis B, et al. Selection and characterization of *BCR-ABL* positive cell lines with differential sensitivity to the tyrosine kinase inhibitor STI571: diverse mechanisms of resistance. Blood. 2000;96:1070-1079.
209. Gishizky ML, Cortez D, Pendergast AM. Mutant forms of growth factor-binding protein-2 reverse BCR-ABL-induced transformation. Proc Natl Acad Sci U S A. 1995;92:10889-10893.
210. Perrey DA, Scannell MP, Narla RK, Navara C, Uckun FM. RAS endoprotease inhibitors are potent cytotoxic agents against acute lymphoblastic leukemia. Blood [abstract]. 1998;92:599.
211. Emanuel PD, Snyder RC, Wiley T, Gopurala B, Castleberry RP. Inhibition of juvenile myelomonocytic leukemia cell growth in vitro by farnesyl transferase inhibitors. Blood. 2000;95:639-645.
212. Gaston I, Stenberg PE, Bhat A, Druker BJ. Abl kinase but not PI3-kinase links to the cytoskeletal defects in Bcr-Abl transformed cells. Exp Hematol. 2000;28:77-86.
213. Warmuth M, Bergmann M, Priess A, Hauslmann K, Emmerich B, Hallek M. The Src family kinase Hck interacts with Bcr-Abl by a kinase-independent mechanism and phosphorylates the Grb2-binding site of Bcr. J Biol Chem. 1997;272:33260-33270.
214. Druker B, Okuda K, Matulonis U, Salgia R, Roberts T, Griffin JD. Tyrosine phosphorylation of rasGAP and associated proteins in chronic myelogenous leukemia cell lines. Blood. 1992;79:2215-2220.