REVIEW ARTICLE

MECHANISMS OF DISEASE

Chronic Myeloid Leukemia — Advances in Biology and New Approaches to Treatment

John M. Goldman, F.R.C.P., and Junia V. Melo, M.D., Ph.D.

From the Department of Haematology, Faculty of Medicine, Imperial College London, London. Address reprint requests to Dr. Goldman at Hammersmith Hospital, Imperial College London, Du Cane Rd., London W12 0NN, United Kingdom, or at jgoldman@imperial.ac.uk.

N Engl J Med 2003;349:1451-64.

CHRONIC MYELOID LEUKEMIA (CML) WAS PROBABLY THE FIRST FORM OF leukemia to be recognized as a distinct entity. In 1845, two patients were described as having massive splenomegaly associated with leukocytosis,[1] which seemed to be a novel entity not explained by the other causes of splenomegaly, such as tuberculosis, that were already widely accepted in the 1840s. The first important clue to its pathogenesis came only very much later, when in 1960 newly developed techniques for studying human cells in mitosis allowed Nowell and Hungerford to detect a consistent chromosomal abnormality,[2] later termed the Philadelphia (Ph[1], or just Ph) chromosome and identified as 22q–, in persons with this disease. In 1973, Rowley observed that the Ph chromosome resulted from a reciprocal translocation that also involved chromosome 9; the abnormality is now designated t(9;22)(q34;q11).[3] In the 1980s, the Ph chromosome was shown to carry a unique fusion gene, termed *BCR-ABL*,[4] the generation of which is now believed to be the principal cause of the chronic phase of CML.

Until the 1980s, CML was regarded as incurable and thus inexorably fatal. We know now that selected patients can be treated, and in many cases cured, by allogeneic stem-cell transplantation. However, efforts to extend this form of treatment to all patients with CML have been thwarted by the lack of suitable donors and the increased incidence of potentially lethal graft-versus-host disease (GVHD) in older recipients. The recent introduction into clinical practice of a tyrosine kinase inhibitor that specifically blocks the enzymatic action of the BCR-ABL fusion protein promises to be a major contribution to the management of CML and may also prove to be the lead agent that ushers in an era of success with molecularly targeted therapy for other leukemias, lymphomas, and cancers.

The evolving story of CML and modern views on its biology and treatment have been reviewed in the *Journal* and elsewhere in recent years.[5-8] Here, we briefly summarize recent advances in knowledge related to the pathogenesis of CML and indicate where these may have important therapeutic implications.

CYTOKINETICS

It is generally believed that CML develops when a single, pluripotential, hematopoietic stem cell acquires a Ph chromosome carrying the *BCR-ABL* fusion gene, which confers on its progeny a proliferative advantage over normal hematopoietic elements and thus allows the Ph-positive clone gradually to displace residual normal hematopoiesis.[9,10] The evidence for this hypothesis derives in part from the consistency of the molecular abnormality in any given patient, but the mechanism by which the molecular and cytogenetic changes occur remains enigmatic. Similarly, the molecular basis of the apparent proliferative advantage is not well defined, but it may relate in part to constitutive expression by leukemic progenitors of growth-stimulating factors, notably interleukin-3 and granulocyte colony-stimulating factor.[11,12] Moreover, CML cells seem to survive longer than their normal counterparts, as a result of a defective apoptotic response to

stimuli that would otherwise lead to physiologic cell death.[13]

Whatever the primary mechanism of the disease, it is clear that the myeloid mass is greatly increased in patients with newly diagnosed disease, owing to an expansion of mature elements, as well as to increased numbers of progenitor cells and putative stem cells.[10] The latter probably include a population of leukemic cells that are "deeply quiescent" and that may thus be relatively resistant to standard chemotherapy.[14] Until the 1980s, there was uncertainty as to whether substantial numbers of normal stem cells were still present in the bone marrow (or elsewhere) in patients with a new diagnosis of CML. However, several findings — the demonstration of the presence of Ph-negative progenitors in myeloid-cell cultures,[15] the observation that Ph-negative progenitor cells can be identified in the blood after high-dose chemotherapy,[16] and the ability of interferon alfa to induce Ph-negativity in the marrow — all constitute persuasive circumstantial evidence that the Ph-positive clone displaces normal hematopoiesis but does not destroy residual normal stem cells. The final proof comes from the observation that imatinib mesylate, a new, ABL-specific tyrosine kinase inhibitor (formerly called STI571 and marketed under the name Gleevec in the United States and Glivec in Europe), can induce complete or nearly complete cytogenetic remissions in up to 80 percent of patients.[17,18]

It is reasonably certain that the majority of patients who survive allogeneic stem-cell transplantation and who appear to be free of disease five years after the procedure can be regarded as cured,[19] but the definition and mechanism of "cure" remain controversial. In other words, cure might theoretically require the total eradication of all leukemia cells from a patient's body, but alternatively, an operational definition of cure might apply if low numbers of leukemia cells persisted but were unable to reestablish clinical disease. Such symbiosis of low numbers of leukemia cells with normal cells could, for example, be due to the continuing action of a graft-versus-leukemia effect after allogeneic stem-cell transplantation.[20]

Currently, the reverse-transcriptase polymerase chain reaction (RT-PCR) is the most sensitive method for detecting low numbers of *BCR-ABL* transcripts in a patient after apparently successful stem-cell transplantation.[21] In practice, the results may be difficult to interpret because even if the test is negative, there may still be a million or more residual Ph-positive cells in the body; in other cases, the test may be persistently positive at a low level for many years. Moreover, it has been suggested that some residual Ph-positive cells may be "transcriptionally silent" and thus not detectable by conventional RT-PCR techniques,[22] although this idea has been disputed.[23,24] Finally, interpretation of RT-PCR results might theoretically be confounded by the fact that *BCR-ABL* transcripts can be detected at a very low level in the blood of many normal persons, the majority of whom will never have CML.[25,26]

CYTOGENETICS

The mechanism by which the Ph chromosome is first formed and the time required for progression to overt disease are unknown. Radiation may play a role in some cases, since persons exposed to high-dose irradiation have a significantly increased risk of leukemia,[27] and high-dose irradiation of myeloid cell lines in vitro induces the expression of *BCR-ABL* transcripts indistinguishable from those that characterize CML.[28] It has also been proposed that the close proximity of the *BCR* and *ABL* genes in hematopoietic cells in interphase may favor translocations between the two genes.[29] Very recently, a 76-kb duplicon (a two-copy DNA repeat sequence) was identified on chromosome 9 near the *ABL* gene and on chromosome 22 near the *BCR* gene; it may be implicated in the translocation, but the mechanism is purely speculative.[30]

The *BCR-ABL* gene on the Ph chromosome is, of course, expressed in all patients with CML, but the *ABL-BCR* gene on the 9q+ derivative is expressed in only 70 percent of cases.[31] Of great interest are the recent observations that about 20 percent of patients with CML have deletions of chromosomal material of varying size on the derivative 9q+ and that patients who have such deletions have significantly shorter survival than those who do not.[32,33] These deletions presumably occur at the same time as the formation of the Ph chromosome, and their recognition will probably prove important in predicting survival for individual patients. Nonexpression of the *ABL-BCR* gene, which is always included in the deleted region, does not by itself have the same ominous prognostic implication.[34] Thus, the identification of the precise gene or genes on 9q+, the deletion of which adversely influences prognosis in CML, would be an important achievement.

It is generally believed that the Ph-positive clone has an increased susceptibility to additional molec-

ular changes that underlie disease progression. If so, the nonrandom cytogenetic changes identified in 60 to 80 percent of patients with disease in blastic transformation[35] — notably +8, +Ph, +19, and i(17)q — should provide some clues as to the activation of oncogenes (other than *BCR-ABL*) or the deletion of tumor-suppressor genes in the transformation from chronic to advanced phase disease. However, in only a minority of cases has the transformation actually been linked to mutations, deletions, or altered expression of known genes, notably *p53*,[36] *p16*,[37] *Rb*,[38] and *EVI-1*.[39] In general, no specific pattern can be discerned. All in all, it seems likely that a variety of molecular mechanisms, rather than a single gene defect, underlies the arrest of maturation that occurs in a subclone of Ph-positive cells and manifests itself in the blastic phase of the disease. The advent of powerful microarray techniques that permit comparisons of gene-expression profiles should help to identify the genes involved in this transformation.

Although the majority of patients with a leukemia that appears on morphologic grounds to be CML prove to have a Ph chromosome and a classic *BCR-ABL* fusion gene, some do not. About one third of the patients with CML who appear to have normal karyotypes actually have a cytogenetically occult *BCR-ABL* gene, usually located on a normal-appearing chromosome 22 but very occasionally on chromosome 9.[40] In the remaining patients, in whom the disease is described as Ph-negative, *BCR-ABL*–negative, the leukemia has no known molecular basis. There is also a very small group of patients with a form of chronic leukemia superficially resembling classic CML who have consistent cytogenetic aberrations (in most cases, translocations) other than the Ph chromosome (Table 1). The commonest non-Ph translocations are t(5;12)(q33;p13)[41] and t(8;13) (p11;q12).[42] Both translocations generate fusion genes, one component of which is a gene that encodes a receptor tyrosine kinase — namely, platelet-derived growth factor receptor β in t(5;12) and fibroblast growth factor receptor 1 in t(8;13). Functional studies performed with these fusion genes suggest that they cause a chronic myeloid leukemia by signaling pathways that closely parallel the pathways activated by the BCR-ABL oncoprotein.[43-45]

MOLECULAR EVENTS

The idea that CML, like other cancers, may be the result of a multistep pathogenetic process was first broached more than 20 years ago,[46] but there is still very little evidence of any acquired molecular abnormalities preceding the t(9;22) translocation. Instead, it seems more likely that the generation of a classic *BCR-ABL* fusion gene in a specific type of cell (namely, a pluripotential hematopoietic stem cell), possibly under conditions of reduced immunologic surveillance, is sufficient to initiate the expansion of a hematopoietic clone that leads to CML. The proposition that the acquisition of a *BCR-ABL* fusion gene is the first step in the genesis of CML is supported by murine models in which a CML-like disease has been produced by transfecting stem cells with a *BCR-ABL* gene[47-49]; however, once established, the tempo or aggressiveness of the chronic phase disease varies from patient to patient and thus must be influenced by other factors.

The classic *BCR-ABL* gene of CML results from the fusion of parts of two normal genes: the *ABL*

Table 1. Cytogenetic Abnormalities Leading to the Expression of Deregulated Tyrosine Kinases in Chronic Myeloproliferative Disorders.*

Cytogenetic Abnormality	Tyrosine Kinase Fusion Protein	Chronic Myeloproliferative Disorder
t(9;22)(q34;q11)	BCR-ABL	CML or acute lymphoblastic leukemia
t(8;22)(p11;q11)	BCR-FGFR1	BCR-ABL–negative CML
t(4;22)(q12;q11)	BCR-PDGFRA	Atypical CML
t(8;13)(p11;q12)	ZNF198-FGFR1	8p Myeloproliferative syndrome
t(6;8)(q27;p11)	FOP-FGFR1	8p Myeloproliferative syndrome
t(8;9)(p12;q33)	CEP110-FGFR1	8p Myeloproliferative syndrome
t(8;19)(p12;q13)	HERV-K-FGFR1	8p Myeloproliferative syndrome
t(5;12)(q33;p13)	TEL-PDGFRB	Chronic myelomonocytic leukemia or atypical CML
t(5;7)(q33;q11)	HIP1-PDGFRB	Chronic myelomonocytic leukemia or atypical CML
t(5;17)(q33;p13)	RAB5-PDGFRB	Chronic myelomonocytic leukemia or atypical CML
t(5;10)(q33;q21)	H4-PDGFRB	Chronic myelomonocytic leukemia or atypical CML
t(9;12)(q34;p13)	TEL-ABL	Atypical CML or BCR-ABL–negative CML
t(9;12)(p24;p13)	TEL-JAK2	Atypical CML or BCR-ABL–negative CML
t(9;22)(p24;q11)	BCR-JAK2	Atypical CML or BCR-ABL–negative CML
del(4)(q12)	FIP1L1-PDGFRA	Hypereosinophilic syndrome

* CML denotes chronic myeloid leukemia, FGFR1 fibroblast growth factor receptor 1, PDGFRA platelet-derived growth factor receptor α, ZNF198 zinc finger protein 198, FOP FGFR1 oncogene partner, CEP110 centrosome-associated protein 110, HERV-K human endogenous retrovirus protein (K family), TEL translocation E26 transforming-specific leukemia protein, PDGFRB platelet-derived growth factor receptor β, HIP1 Huntingtin interacting protein 1, RAB5 rabaptin-5, H4 histone-4, JAK2 Janus kinase 2, and FIP1L1 FIP1-like 1.

gene on chromosome 9 and the *BCR* gene on chromosome 22. Both genes are ubiquitously expressed in normal tissues, but their precise functions are not well defined. In the translocation that forms the fusion gene, a break occurs in *ABL* somewhere upstream of exon a2, and simultaneously a break occurs in the major breakpoint cluster region of the *BCR* gene. As a result, a 5′ portion of *BCR* and a 3′ portion of *ABL* are juxtaposed on a shortened chromosome 22 (the derivative 22q−, or Ph, chromosome) (Fig. 1). The messenger RNA (mRNA) molecules transcribed from this hybrid gene usually contain one of two *BCR-ABL* junctions, designated e13a2 (formerly b2a2) and e14a2 (or b3a2). There is no evidence that the type of junction has any prognostic significance. Both *BCR-ABL* mRNA molecules are translated into an 210-kD oncoprotein, usually referred to as $p210^{BCR\text{-}ABL}$. Other variant breakpoints and fusions can give rise to full-length, functionally oncogenic BCR-ABL proteins, notably $p190^{BCR\text{-}ABL}$ (associated with an e1a2 mRNA junction) and $p230^{BCR\text{-}ABL}$ (associated with an e19a2 mRNA junction),[50] but they are rather rare in classic CML.[51,52]

The leukemogenic potential of $p210^{BCR\text{-}ABL}$ resides in the fact that the normally regulated tyrosine kinase activity of the ABL protein is constitutively activated by the juxtaposition of alien BCR sequences (Fig. 2). BCR acts by promoting dimerization of the oncoprotein, such that the two adjacent BCR-ABL molecules phosphorylate each other on tyrosine residues in their kinase-activation loops.[56,57] The uncontrolled kinase activity of BCR-ABL then usurps the physiologic functions of the normal ABL enzyme by interacting with a variety of effector pro-

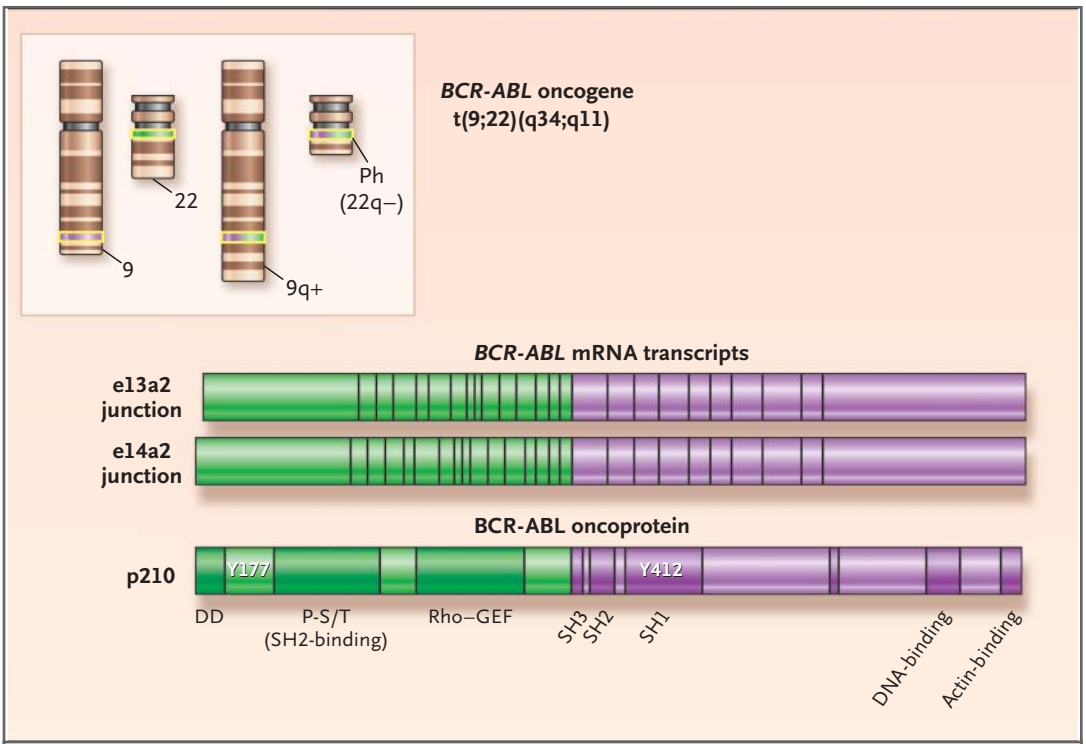

Figure 1. The t(9;22) Translocation and Its Products: the *BCR-ABL* Oncogene on the Ph Chromosome and the Reciprocal *ABL-BCR* on the Derivative 9q+ Chromosome.

In classic CML, *BCR-ABL* is transcribed into messenger RNA (mRNA) molecules with e13a2 or e14a2 junctions, which are then translated into the $p210^{BCR\text{-}ABL}$ oncoprotein. This oncoprotein is a hybrid containing functional domains from the N-terminal end of BCR (dimerization [DD], SRC-homology 2 [SH2]–binding, and the Rho GTP–GDP exchange-factor [GEF] domains) and the C-terminal end of ABL. (Only SRC-homology regions 2, 3, and 1 [SH2, SH3, and SH1, respectively], and the DNA- and actin-binding domains are shown.) Tyrosine 177 (Y177) in the BCR portion of the fusion gene and tyrosine 412 (Y412) in the ABL portion are important for the docking of adapter proteins and for BCR-ABL autophosphorylation, respectively. P-S/T denotes phosphoserine and phosphothreonine.

teins, the net result of which is deregulated cellular proliferation, decreased adherence of leukemia cells to the bone marrow stroma, and a reduced apoptotic response to mutagenic stimuli. Unfortunately, the relative contributions of these effects to the phenotype of chronic-phase CML is still poorly understood.[8]

The structure of the BCR-ABL protein and the biochemical pathways in which it is involved have been extensively studied.[5,8] Knowledge of the roles of several functional domains derived from the parental BCR and ABL proteins allows one to test for certain properties of the fusion product (Fig. 1 and 2). Thus, the tyrosine kinase encoded by the SRC-homology 1 (SH1) domain of the ABL component of BCR-ABL is undoubtedly the most crucial for oncogenic transformation. Other important motifs in the ABL portion are the protein-interaction SRC-homology 2 (SH2) and the C-terminal actin-binding domains. On the BCR moiety, the coiled-coil motif encoded by the first BCR exon is responsible for dimerization of the oncoprotein; a tyrosine at position 177 is crucial for the binding of adaptor proteins such as growth factor receptor–bound protein 2; and N-terminal phosphoserine and phosphothreonine residues are required for interaction

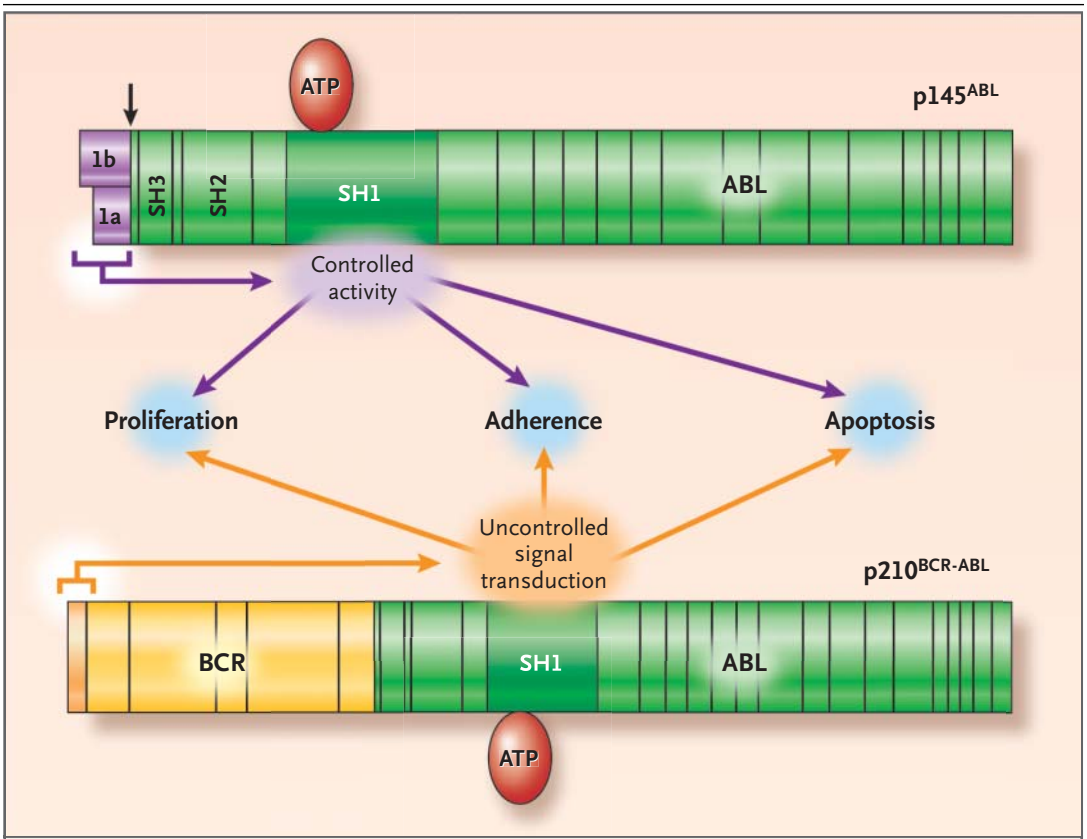

Figure 2. Physiologic Regulation by the Normal ABL Protein and Deregulation by BCR-ABL of Key Cellular Processes Such as Proliferation, Adherence, and Apoptosis.

The enzymatic (tyrosine kinase) activity of the normal ABL protein ($p145^{ABL}$), encoded by its SRC-homology 1 (SH1) domain, is kept under tight control, probably by the intramolecular binding of an N-terminal cap region encompassed by the first exon (1b or 1a) and the first part of exon a2.[53] In the BCR-ABL fusion protein ($p210^{BCR-ABL}$), lack of the ABL cap region and a dimerization domain encoded by the first exon of BCR are responsible for constitutive activation of the ABL SH1 domain, resulting in uncontrolled signal transduction and an abnormal cellular phenotype. The various functional domains of the ABL protein include the SRC-homology 3 and 2 regulatory domains (SH3 and SH2, respectively), the SH1 domain with its ATP-binding site, the nuclear-localization signal motif, the nuclear-export signal motif, the DNA-binding domain, and the G-actin and F-actin DNA-binding domains. The last two are important for the control of cytoskeletal organization, cell adherence, cell motility, and integrin receptor–mediated signal transduction.[54,55]

with SH2-containing proteins, including ABL itself. Numerous substrates have been found to bind to BCR-ABL and to be tyrosine-phosphorylated by it (Table 2 and Fig. 3), and the list of substrates keeps increasing. Associations with tyrosine phosphatases such as protein tyrosine phosphatase B1 (PTPB1) and Syp have also been reported, but these phosphatases may in fact limit the kinase activity of BCR-ABL, an effect that would oppose other BCR-ABL leukemogenic forces. However, most interactions and activation processes have been studied only in cell lines in vitro and under conditions of forced (ectopic) overexpression. In most cases, therefore, their very existence in primary leukemic cells and their relevance to the CML phenotype in vivo remain uncertain.

Table 2. BCR-ABL Substrates.

Protein	Function	Source
Growth factor receptor–bound protein 2 (GRB-2)	Adapter	Li et al.[59]
DOK	Adapter	Yamanashi and Baltimore[60]
CRK-like protein (CRKL)	Adapter	Oda et al.[61]
CRK	Adapter	Sattler et al.[62]
SRC-homology–containing protein (SHC)	Adapter	Tauchi et al.[63]
Talin	Cytoskeleton and cell membrane	Salgia et al.[64]
Paxillin	Cytoskeleton and cell membrane	Salgia et al.[65]
Focal adhesion kinase (FAK)	Cytoskeleton and cell membrane	Gotoh et al.[66]
FES	Myeloid differentiation	Ernst et al.[67]
RAS GTPase-activating protein (GAP)	RAS GTPase	Gotoh et al.[68]
Phospholipase C-γ (PLC-γ)	Phospholipase	Gotoh et al.[68]
Germinal-center kinase-related protein (GCKR)	Serine–threonine kinase	Shi et al.[69]
Phosphatidylinositol 3-kinase (p85 subunit)	Serine kinase	Skorski et al.[70]
ABL-interacting proteins 1 and 2	Tumor suppression	Dai et al.[71]
Syp	Cytoplasmic phosphatase	Tauchi et al.[72]
SH2-containing inositol 5' phosphatase 1 (SHIP-1), SHIP-2	Cytoplasmic phosphatase	Wisniewski et al.[73]
BAP-1	14-3-3 protein	Reuther et al.[74]
Casitas-B-lineage protein (CBL)	Unknown	Sattler et al.,[62] Bhat et al.[75]
VAV	Hematopoietic differentiation	Matsuguchi et al.[76]

One of the most striking differences between the normal ABL protein and BCR-ABL is in their contrasting subcellular locations. The ABL protein is found in both the nucleus and the cytoplasm and can shuttle between these two compartments under the influence of its nuclear-localization signal and nuclear-export signal domains, whereas BCR-ABL is exclusively cytoplasmic. Nuclear ABL is an essentially proapoptotic protein, playing a key part in the cellular response to genotoxic stress. BCR-ABL, in contrast, is largely antiapoptotic and, although it retains the ABL nuclear-localization and nuclear-export sequences, seems unable to enter the nucleus. The main reason the BCR-ABL protein is retained in the cytoplasm is its constitutively activated tyrosine kinase.[78] When the kinase is inhibited in vitro with imatinib and its nuclear export simultaneously blocked with leptomycin B, the oncoprotein enters the nucleus and is trapped there. Interestingly, when imatinib is removed and the now nuclear BCR-ABL is allowed to reactivate its tyrosine kinase, it is converted from an antiapoptotic to a proapoptotic protein and induces, rather than prevents, cell death. It has recently been shown that BCR-ABL also translocates to the nucleus in leukemia cells subjected to genotoxic stress and there slows DNA repair by interaction with effectors of the ataxia telangiectasia–related (ATR) protein, a phenomenon that may underlie the genomic instability of the CML clone.[79]

In the late 1980s, accumulating data on the mechanisms of BCR-ABL function set the scene for the design of molecularly targeted therapy. Since the tyrosine kinase is the effector part of the oncoprotein, it was obviously the most attractive target for inhibition. The aim was to design a small chemical compound that could compete with ATP for its binding site in the kinase domain. Whereas the normal binding of ATP allows BCR-ABL to phosphorylate selected tyrosine residues on its substrates, an ATP mimic occupying the binding pocket would not provide any phosphate group for transfer to the substrate. With its tyrosine residues in the unphosphorylated form, the substrate protein would not then undergo the required conformational change to allow it to associate with its downstream effector. The entire chain of downstream reactions would then be impeded, interrupting transmission of the oncogenic signal to the nucleus.

During the 1990s, a number of tyrosine kinase inhibitors were purified from natural substances (e.g., herbimycin A and genistein) or designed and synthesized in the laboratory (e.g., tyrphostins).[80-82] However, by far the most successful inhibitor was imatinib, a small, 2-phenylaminopyrimidine molecule that, at micromolar concentrations, inhibits the kinase activity of all proteins that contain ABL, ABL-related gene (ARG) protein, or platelet-derived growth factor receptor, as well as the KIT receptor.[83-85] This compound inhibits cellular growth and induces apoptosis in CML, both in vitro and in vivo.[86-88]

The importance of imatinib goes beyond its undoubted therapeutic value in CML. Because it was rationally designed to carry out a predetermined function and proved so successful, other candidate agents for targeted therapy in CML have been rapidly emerging; among them are adaphostin (an ABL tyrphostin), geldanamycin (an inhibitor of the BCR-ABL chaperone heat-shock protein 90), and the farnesyl transferase inhibitors (which prevent activation of RAS and other farnesylated oncoproteins). Each of these could prove clinically valuable if combined with imatinib, even after resistance to imatinib as a single agent has developed.[89-91] Moreover, the combined use of imatinib and leptomycin B in a regimen similar to the experimental one developed by Vigneri and Wang[78] may prove valuable for eliminating residual leukemia cells before

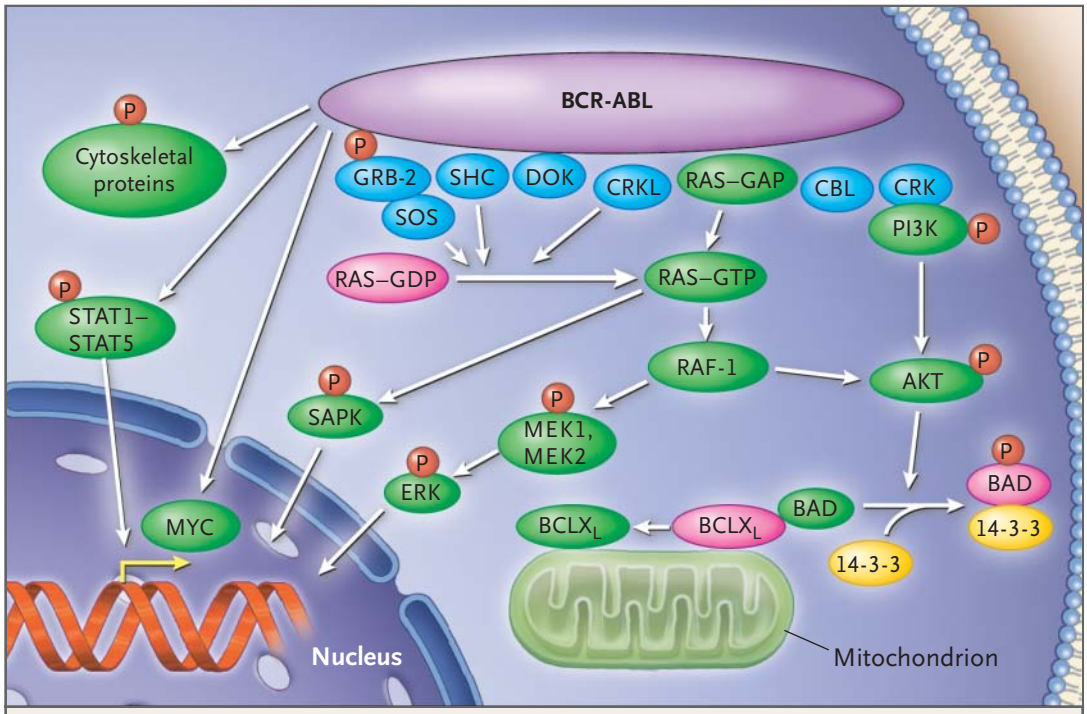

Figure 3. Signal-Transduction Pathways Affected by BCR-ABL.

The cellular effects of BCR-ABL are exerted through interactions with various proteins that transduce the oncogenic signals responsible for the activation or repression of gene transcription, of mitochondrial processing of apoptotic responses, of cytoskeletal organization, and of the degradation of inhibitory proteins.[77] The key pathways implicated so far are those involving RAS, mitogen-activated protein (MAP) kinases, signal transducers and activators of transcription (STAT), phosphatidylinositol 3-kinase (PI3K), and MYC. Most of the interactions are mediated through tyrosine phosphorylation and require the binding of BCR-ABL to adapter proteins such as growth factor receptor–bound protein 2 (GRB-2), DOK, CRK, CRK-like protein (CRKL), SRC-homology–containing protein (SHC), and casitas-B-lineage lymphoma protein (CBL). As we start to dissect these various interactions, we can now design drugs aimed at disrupting specific branches of these pathways, in an attempt either to kill the CML cell or to cause its phenotype to revert to normal. It is obvious that the best target is BCR-ABL proper, since this is the only protein that is exclusive to the leukemic clone. The second-best approach is to target key downstream effectors of BCR-ABL; however, this approach might, in principle, adversely affect normal hematopoiesis as well. P denotes phosphate.

autografting, a setting in which imatinib alone is not very effective.[92] A family of SRC tyrosine kinase inhibitors (in particular, PD166326, PD173955, and PD180970) was also recently reported to be active against BCR-ABL and to inhibit the in vitro growth of primary CML progenitor cells.[93]

Because of the excitement they have generated, the synthetic signal-transduction inhibitors may, for the present, overshadow other potential antileukemia agents, but the development of drug resistance — already documented for imatinib[94-99] — should be a reminder of the possible value of combining different molecular approaches. Although traditional oligodeoxynucleotide or ribozyme antisense molecules against BCR-ABL mRNA failed to fulfill their initial promise for the treatment of CML, better sequence design and chemical techniques have recently provided new ideas for the success of this approach, among them a dimeric ribozyme or "maxizyme"[100] and RNA-interference strategies.[101] Equally interesting is the possibility of expressing a single-chain antibody, or "intrabody," against ABL (or possibly BCR-ABL) by retroviral-mediated gene transfer into CML cells.[102] Finally, attempts at immunization with molecularly engineered BCR-ABL fusion peptides or plasmid DNA should be considered a possible adjunct to debulking regimens with signal transduction inhibitors.

GRAFT-VERSUS-LEUKEMIA EFFECT

The notion that the eradication of leukemia by allogeneic stem-cell transplantation does not depend entirely on the chemotherapy and radiotherapy given during the conditioning phase that precedes stem-cell infusion, but that it relies also on an ill-defined graft-versus-leukemia effect, has been generally accepted for some years.[20,103] The concept that this graft-versus-leukemia effect may be mediated by allogeneic T cells derives support from four somewhat discrete lines of evidence — namely, the observations that the incidence of relapse in leukemia is inversely related to the incidence of GVHD, that relapse is more common after the transplantation of hematopoietic stem cells from syngeneic rather than allogeneic sibling donors, that depletion of T cells from the donor inoculum greatly increases the risk of relapse, and that the infusion of lymphocytes collected from the original donor can restore complete remission in a patient who has had a relapse after allografting, especially in the case of CML. These observations led logically to a search for the target antigen or antigens recognized by the allogeneic T cells.

Almost all the peptide sequences that make up the BCR-ABL protein are also present in the normal ABL or BCR proteins, but the junctional codons and their corresponding amino acids are unique and thus specific to leukemia. There is recent evidence that such leukemia-specific oligopeptides may be presented on the leukemia-cell surface in conjunction with HLA class II molecules.[104] Alternatively, the target antigens for the graft-versus-leukemia effect could be lineage-specific rather than leukemia-specific. Cytotoxic T lymphocytes that recognize the PR-1 component of proteinase 3 or the Wilms' tumor 1 antigen, both of which are overexpressed in leukemia cells, kill CML cells in vitro but spare control cells from normal persons.[105,106]

Attempts are now being made to immunize patients against their own leukemia. A group at Memorial Sloan-Kettering Cancer Center, in New York, showed that patients with the e14a2 junction may generate cytotoxic T lymphocytes in response to immunization with an e14a2-derived junctional oligopeptide presented in conjunction with HLA molecules and a suitable adjuvant agent.[107] Cytotoxic T lymphocytes directed against known antigens could prove valuable in treating minimal residual disease but may not be so effective in dealing with large quantities of leukemia.

TREATMENT OPTIONS

ALTERNATIVES TO TRANSPLANTATION

The options for treating patients with newly diagnosed CML have changed fundamentally since the introduction of imatinib. Until recently, interferon alfa was generally accepted as the best single agent for treating CML in the chronic phase in patients who were not eligible for allogeneic stem-cell transplantation. The drug may cause a wide range of side effects, especially in older persons, but it induces complete or nearly complete cytogenetic responses in 10 to 30 percent of patients and probably prolongs survival to a greater extent than hydroxyurea.[108] A French multicenter study showed that survival among patients treated with the combination of interferon alfa and cytarabine was superior to that among patients treated with interferon alfa alone,[109] but that potentially important observation was not confirmed in a more recent study.[110]

Imatinib was first used in 1998 to treat patients

who were judged to have disease refractory to treatment with interferon alfa or who could not tolerate interferon alfa.[84] Imatinib induced complete hematologic responses in more than 95 percent of patients with CML resistant to interferon alfa but still in the chronic phase, and it induced major cytogenetic responses in 40 to 50 percent.[17] Preliminary analyses of the results of treatment in relatively large numbers of patients treated in six countries have shown that event-free survival and overall survival are better than might be expected with alternative therapies.[111] One recent study suggests that patients who achieve good cytogenetic responses live longer than historical control patients.[112] Among patients treated in the accelerated phase of the disease, overall survival and event-free survival appear to be superior to those in historical controls.[113] In contrast, the majority of patients with disease in blastic transformation have a response to imatinib, but these responses are short-lived, and no definite survival benefit can be discerned.[114,115] The drug can cause nausea, headache, rashes, fluid retention, clinically significant cytopenia, and other side effects, but these problems are generally manageable and seem to be appreciably less troublesome than those associated with interferon alfa.[84]

Although data on cytogenetic responses as a surrogate marker of survival suggest that imatinib prolongs the survival of patients treated in the chronic phase of disease, this conclusion must for the present be regarded as unproved. For this reason, the Food and Drug Administration recommended to the manufacturers of the drug that they undertake a multicenter study in which imatinib is compared prospectively with the combination of interferon alfa and cytarabine in previously untreated patients with CML. The study was undertaken, and patients were recruited in 2001. According to a recent interim assessment of the results, the rate of complete cytogenetic response in the imatinib group is 74 percent,[18] and some of those patients have achieved a molecular remission (the absence of detectable BCR-ABL transcripts in the blood). As a consequence, the study has been complicated by the desire of some patients in the interferon alfa–cytarabine group to cross over to the imatinib group. It is hoped, nonetheless, that the trial will show before too long whether treatment with imatinib prolongs the survival of patients with newly diagnosed CML.

Although resistance to imatinib as a single agent seems to be rare in patients treated in the chronic phase of disease, resistance does eventually develop in the majority of patients treated in the advanced phase. The mechanism of this acquired resistance is of great interest. Studies in vitro of resistant cell lines have shown amplification of the *BCR-ABL* gene in association with overexpression of the oncoprotein.[94] Moreover, in the clinic, a proportion of patients whose disease has become resistant to imatinib have acquired point mutations in the ABL kinase domain that lead to specific amino acid substitutions that could, theoretically, interfere with the binding of imatinib in the kinase pocket[97,98,116-122] (Table 3). Presumably, these mutations do not prevent the kinase from accepting ATP and thus phosphorylating the substrates that generate the CML phenotype. In a substantial proportion of patients, however, resistance to imatinib is probably not due to BCR-ABL overexpression or mutations in the ABL kinase domain, particularly because some of the point mutations detected do not result in loss of sensitivity to imatinib.[123] In these cases, the resistant phenotype is probably due to the capacity of CML cells to recruit signal-transduction pathways that bypass the block or, as recently demonstrated by in vitro mutagenesis, to point mutations outside the kinase domain.[124]

STEM-CELL TRANSPLANTATION

It is now generally accepted that allogeneic hematopoietic stem-cell transplantation can cure CML in selected patients,[19,125-127] but stem-cell transplantation[19,127] is still associated with an appreciable risk of complications and death, due principally to GVHD and opportunistic infections. The decision about whether to offer transplantation to a given patient is aided to some degree by knowledge that the factors that influence the risk of transplantation-related death are now reasonably well defined and include the patient's age, the phase of disease, the duration of disease, the degree of donor–recipient histocompatibility, and the donor's sex.[128,129] Thus, for example, a young patient with newly diagnosed CML in the chronic phase who has an HLA-identical sibling donor can expect to fare much better after transplantation than an older patient with accelerated-phase disease who has a less well matched, unrelated donor.

The recognition that the graft-versus-leukemia effect[20] plays a major part in the eradication of CML after allografting led to the concept that the toxic effects associated with allografting could be substantially reduced by lowering the overall intensity of the pretransplantation conditioning regimen. This

Table 3. *BCR–ABL* Point Mutations Associated with Imatinib Resistance in Patients with Chronic Myeloid Leukemia and Philadelphia Chromosome–Positive Acute Lymphoblastic Leukemia.

Nucleotide Change*	Amino Acid Change†	Proposed Mechanism of Resistance	Cases‡	Source
			no. detected/no. tested	
A1094G	M244V (M263V)	Impairs conformational change (?)	1/32 1/66	Shah et al.[119] Hochhaus et al.[120]
G1113A	G250E (G269E)	Impairs conformational change	2/28 2/32	Branford et al.[121] Shah et al.[119]
A1119G	Q252R (Q271R)	Impairs conformational change	1/32	Shah et al.[119]
G1120C/T	Q252H (Q271H)	Impairs conformational change	6/32 1/66	Shah et al.[119] Hochhaus et al.[120]
T1121C	Y253H (Y272H)	Impairs conformational change	2/8 1/28 2/32 4/66	von Bubnoff et al.[116] Branford et al.[121] Shah et al.[119] Hochhaus et al.[120]
A1122T	Y253F (Y272F)	Impairs conformational change	3/32 1/66	Shah et al.[119] Hochhaus et al.[120]
G1127A	E255K (E274K)	Impairs conformational change	1/12 2/8 4/28 6/9 10/32 3/66	Barthe et al.[98] von Bubnoff et al.[116] Branford et al.[121] Hofmann et al.[122] Shah et al.[119] Hochhaus et al.[120]
A1128T	E255V (E274V)	Impairs conformational change	1/8 1/66	von Bubnoff et al.[116] Hochhaus et al.[120]
T1495C	F311L (F330L)	Unknown	1/24	Roche-Lestienne et al.[118]
C1308T	T315I (T334I)	Affects imatinib binding	2/8 10/32 3/28 1/9 3/24 6/66	von Bubnoff et al.[116] Shah et al.[119] Branford et al.[121] Hofmann et al.[122] Roche-Lestienne et al.[118] Hochhaus et al.[120]
C1315G	F317L (F336L)	Affects imatinib binding	1/28 3/32	Branford et al.[121] Shah et al.[119]
T1392C	M343T (M362T)	Unknown	1/32	Shah et al.[119]
T1416C	M351T (M370T)	Impairs conformational change	2/28 10/32 1/24 4/66	Branford et al.[121] Shah et al.[119] Roche-Lestienne et al.[118] Hochhaus et al.[120]
A1428G	E355G (E374G)	Impairs conformational change	1/32 1/66	Shah et al.[119] Hochhaus et al.[120]
T1439G	F359V (F378V)	Affects imatinib binding (?)	2/32	Shah et al.[119]
G1499A	V379I (V398I)	Impairs conformational change (?)	1/32	Shah et al.[119]
T1508C	F382L (F401L)	Unknown	1/32	Shah et al.[119]
T1523A	L387M (L406M)	Impairs conformational change (?)	1/32	Shah et al.[119]
A1551G	H396R (H415R)	Impairs conformational change (?)	3/32 1/66	Shah et al.[119] Hochhaus et al.[120]

* The positions are those of the GenBank sequence (accession number M14752).

† The positions are those of the GenBank sequence (accession number AAB60394); those in parentheses are the corresponding positions for ABL type 1b (where the N-terminal domain is 19 amino acids longer). Amino acids are denoted with the single-letter code.

‡ Patients in whom resistance developed or those who never had a response to imatinib were tested. The table collates data from seven independent series of patients who were screened for DNA mutations in the tyrosine kinase–encoding region of *BCR-ABL*. A total of 114 mutational events were detected in a total of 179 patients (a few of whom had more than 1 mutation in the resistant clone). This indicates that point mutations in the tyrosine kinase domain account for approximately 60 percent of patients in whom no response or loss of response to imatinib is observed in the clinic.

approach involves substantial reductions in the dose of cytotoxic drugs or radiation used in conditioning, with emphasis instead on the use of immunosuppressive agents. At the same time, the number of hematopoietic stem cells and lymphocytes transfused to the patient is maximized to ensure engraftment and an optimal graft-versus-leukemia effect. A wide variety of regimens have been devised for such transplantations, which have been called "nonmyeloablative," "reduced-intensity conditioning transplantation," and "mini-transplantation."[130-132] By reducing the intensity of conditioning in this way, it may be possible to offer transplantation to patients who would not otherwise be eligible because of older age or the presence of concomitant disease. Some patients who have received this type of treatment have had durable cytogenetic and molecular remissions,[132] but long-term results are still difficult to assess.

STRATEGIES FOR DECISION MAKING

The questions of whether one should recommend allogeneic stem-cell transplantation to patients with CML in the chronic phase and, if so, to which patients remain challenging.[133] One possible approach is to balance the perceived benefits and risks of transplantation against the likelihood of long-term survival if the best available nontransplantation therapy is used. The results of nontransplantation therapy in a given patient may be predicted in very general terms by reference to scoring systems devised by Sokal and colleagues[134] and subsequently updated by Hasford and colleagues.[135] Because neither the results of transplantation nor the long-term outcome of treatment with imatinib or imatinib-containing combinations can be accurately predicted, one possibility would be to offer patients with newly diagnosed CML a trial of treatment with imatinib and then to offer transplantation to those in whom a complete or nearly complete cytogenetic response has not been achieved by six to nine months, provided that the estimated risk of transplantation-related death is reasonably low (e.g., less than 20 percent); other patients could be offered alternative nontransplantation therapy.[133] The best approach to managing CML in young patients who have a suitable transplant donor may be clearer in one or two years, when we have gained more experience in the use of imatinib.

FUTURE PROSPECTS

Although much has been achieved, many important issues pertaining to the biology and treatment of CML remain unresolved. To mention just a few, we know little of the mechanisms that cause the chromosomal rearrangement. We still need to clarify how deregulation of signal transduction by the BCR-ABL oncoprotein leads to the proliferative advantage of the Ph-positive clone. We need a much better understanding of the molecular basis of disease progression. Of tremendous value would be certain identification of the target antigens in the graft-versus-leukemia effect. In therapeutic terms, we need to define as rapidly as possible the true clinical potential of imatinib and to ascertain whether combining this agent with other signal-transduction inhibitors, other cytotoxic drugs, or differentiating agents can improve its efficacy. We need to know whether immunizing patients with CML can prolong their survival or contribute to the eradication of disease. It seems that at least some of these problems will be solved within the next five years.

We are indebted to our clinical and nonclinical colleagues who contributed many of the ideas and the bulk of the data included in this review, especially colleagues at Hammersmith Hospital, including Professors Jane Apperley and Myrtle Gordon and Drs. David Barnes, Francesco Dazzi, Edward Kanfer, Eduardo Olavarria, David Marin, Amin Rahemtulla, and Alex Tipping.

REFERENCES

1. Geary CG. The story of chronic myeloid leukaemia. Br J Haematol 2000;110:2-11.
2. Nowell PC, Hungerford DA. A minute chromosome in human chronic granulocytic leukemia. Science 1960;132:1497.
3. Rowley JD. A new consistent chromosomal abnormality in chronic myelogenous leukaemia identified by quinacrine fluorescence and Giemsa staining. Nature 1973;243:290-3.
4. Shtivelman E, Lifshitz B, Gale RP, Canaani E. Fused transcript of abl and bcr genes in chronic myelogenous leukaemia. Nature 1985;315:550-4.
5. Sawyers CL. Chronic myeloid leukemia. N Engl J Med 1999;340:1330-40.
6. Verfaillie CM. Chronic myelogenous leukemia: from pathogenesis to therapy. J Hematother 1999;8:3-13.
7. Faderl S, Talpaz M, Estrov Z, O'Brien S, Kurzrock R, Kantarjian HM. The biology of chronic myeloid leukemia. N Engl J Med 1999;341:164-72.
8. Deininger MW, Goldman JM, Melo JV. The molecular biology of chronic myeloid leukemia. Blood 2000;96:3343-56.
9. Eaves CJ, Eaves AC. Stem cell kinetics. Baillieres Clin Haematol 1997;10:233-57.
10. *Idem.* Progenitor cell dynamics. In: Carella AM, Daley GQ, Eaves CJ, Goldman JM, Hehlmann R, eds. Chronic myeloid leukaemia: biology and treatment. London: Martin Dunitz, 2001:73-100.
11. 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;96:12804-9.
12. Holyoake TL, Jiang X, Jorgensen HG, et al. Primitive quiescent leukemic cells from patients with chronic myeloid leukemia spontaneously initiate factor-independent growth

in vitro in association with up-regulation of expression of interleukin-3. Blood 2001;97:720-8.
13. Bedi A, Zehnbauer BA, Barber JP, Sharkis SJ, Jones RJ. Inhibition of apoptosis by BCR-ABL in chronic myeloid leukemia. Blood 1994;83:2038-44.
14. Graham SM, Jorgensen HG, Allan E, et al. Primitive, quiescent, Philadelphia-positive stem cells from patients with chronic myeloid leukemia are insensitive to STI571 in vitro. Blood 2002;99:319-25.
15. 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;308:1493-8.
16. Carella AM, Podesta M, Frassoni F, et al. Collection of 'normal' blood repopulating cells during early hemopoietic recovery after intensive conventional chemotherapy in chronic myelogenous leukemia. Bone Marrow Transplant 1993;12:267-71.
17. Druker BJ, Talpaz M, Resta DJ, et al. Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. N Engl J Med 2001;344:1031-7.
18. O'Brien SG, Guilhot F, Larson RA, et al. Imatinib compared with interferon and low-dose cytarabine for newly diagnosed chronic-phase chronic myeloid leukemia. N Engl J Med 2003;348:994-1004.
19. van Rhee F, Szydlo RM, Hermans J, et al. Long-term results after allogeneic bone marrow transplantation for chronic myelogenous leukemia in chronic phase: a report from the Chronic Leukemia Working Party of the European Group for Blood and Marrow Transplantation. Bone Marrow Transplant 1997;20:553-60.
20. Barrett AJ, Malkovska V. Graft-versus-leukaemia: understanding and using the alloimmune response to treat haematological malignancies. Br J Haematol 1996;93:754-61.
21. Cross NCP. Assessing residual disease. In: Goldman JM, ed. Baillieres Clin Haematol 1997;10:389-403.
22. Chomel JC, Brizard F, Veinstein A, et al. Persistence of BCR-ABL genomic rearrangement in chronic myeloid leukemia patients in complete and sustained cytogenetic remission after interferon-alpha therapy or allogeneic bone marrow transplantation. Blood 2000;95:404-8.
23. Chase A, Parker S, Kaeda J, Sivalingam R, Cross NC, Goldman JM. Absence of host-derived cells in the blood of patients in remission after allografting for chronic myeloid leukemia. Blood 2000;96:777-8.
24. Deininger M, Lehmann T, Krahl R, Hennig E, Muller C, Niederwieser D. No evidence for persistence of BCR-ABL-positive cells in patients in molecular remission after conventional allogenic transplantation for chronic myeloid leukemia. Blood 2000;96:779-80.
25. Biernaux C, Loos M, Sels A, Huez G, Stryckmans P. Detection of major bcr-abl gene expression at a very low level in blood cells of some healthy individuals. Blood 1995;86:3118-22.
26. Bose S, Deininger M, Gora-Tybor J, Goldman JM, Melo JV. The presence of typical and atypical BCR-ABL fusion genes in leukocytes of normal individuals: biologic significance and implications for the assessment of minimal residual disease. Blood 1998;92:3362-7.
27. Preston DL, Kusumi S, Tomonaga M, et al. Cancer incidence in atomic bomb survivors. III. Leukemia, lymphoma and multiple myeloma, 1950-1987. Radiat Res 1994;137:Suppl:S68-S97. [Erratum, Radiat Res 1994;139:129.]
28. 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;58:421-5.
29. Neves H, Ramos C, da Silva MG, Parreira A, Parreira L. The nuclear topography of ABL, BCR, PML, and RARalpha genes: evidence for gene proximity in specific phases of the cell cycle and stages of hematopoietic differentiation. Blood 1999;93:1197-207.
30. Saglio G, Storlazzi CT, Giugliano E, et al. A 76-kb duplicon maps close to the BCR gene on chromosome 22 and the ABL gene on chromosome 9: possible involvement in the genesis of the Philadelphia chromosome translocation. Proc Natl Acad Sci U S A 2002;99:9882-7.
31. Melo JV, Gordon DE, Cross NC, Goldman JM. The ABL-BCR fusion gene is expressed in chronic myeloid leukemia. Blood 1993;81:158-65.
32. Sinclair PB, Nacheva EP, Leversha M, et al. Large deletions at the t(9;22) breakpoint are common and may identify a poor-prognosis subgroup of patients with chronic myeloid leukemia. Blood 2000;95:738-43.
33. Huntly BJ, Reid AG, Bench AJ, et al. Deletions of the derivative chromosome 9 occur at the time of the Philadelphia translocation and provide a powerful and independent prognostic indicator in chronic myeloid leukemia. Blood 2001;98:1732-8.
34. de la Fuente J, Merx K, Steer J, et al. *ABL-BCR* expression does not correlate with deletions on the derivative chromosome 9 or survival in chronic myeloid leukemia. Blood 2001;98:2879-80.
35. Johansson B, Fioretos T, Mitelman F. Cytogenetic and molecular genetic evolution of chronic myeloid leukemia. Acta Haematol 2002;107:76-94.
36. Feinstein E, Cimino G, Gale RP, et al. p53 In chronic myelogenous leukemia in acute phase. Proc Natl Acad Sci U S A 1991;88:6293-7.
37. 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;85:2013-6.
38. 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;78:2178-81.
39. Ogawa S, Mitani K, Kurokawa M, et al. Abnormal expression of Evi-1 gene in human leukemias. Hum Cell 1996;9:323-32.
40. Cortes JE, Talpaz M, Beran M, et al. Philadelphia chromosome-negative chronic myelogenous leukemia with rearrangement of the breakpoint cluster region: long-term follow-up results. Cancer 1995;75:464-70.
41. 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;77:307-16.
42. Reiter A, Sohal J, Kulkarni S, et al. Consistent fusion of ZNF198 to the fibroblast growth factor receptor-1 in the t(8;13)(p11;q12) myeloproliferative syndrome. Blood 1998;92:1735-42.
43. Wilbanks AM, Mahajan S, Frank DA, Druker BJ, Gilliland DG, Carroll M. TEL/PDGFbetaR fusion protein activates STAT1 and STAT5: a common mechanism for transformation by tyrosine kinase fusion proteins. Exp Hematol 2000;28:584-93.
44. Smedley D, Demiroglu A, Abdul-Rauf M, et al. ZNF198-FGFR1 transforms Ba/F3 cells to growth factor independence and results in high level tyrosine phosphorylation of STATS 1 and 5. Neoplasia 1999;1:349-55.
45. Steer EJ, Cross NC. Myeloproliferative disorders with translocations of chromosome 5q31-35: role of the platelet-derived growth factor receptor beta. Acta Haematol 2002;107:113-22.
46. Fialkow PJ, Martin PJ, Najfeld V, Penfold GK, Jacobson RJ, Hansen JA. Evidence for a multistep pathogenesis of chronic myelogenous leukemia. Blood 1981;58:158-63.
47. 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;247:824-30.
48. 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;92:3780-92.
49. 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;92:3829-40.
50. 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;88:2410-4. [Erratum, Blood 1997;89:4244.]
51. Melo JV, Myint H, Galton DA, Goldman JM. P190BCR-ABL chronic myeloid leukaemia: the missing link with chronic myelo-

monocytic leukaemia? Leukemia 1994;8:208-11.
52. Melo JV. The diversity of BCR-ABL fusion proteins and their relationship to leukemia phenotype. Blood 1996;88:2375-84.
53. Pluk H, Dorey K, Superti-Furga G. Autoinhibition of c-Abl. Cell 2002;108:247-59.
54. 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;93:15174-9.
55. Salgia R, Li JL, Ewaniuk DS, et al. BCR/ABL induces multiple abnormalities of cytoskeletal function. J Clin Invest 1997;100:46-57.
56. 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;13:7587-95.
57. Smith KM, Yacobi R, Van Etten RA. Autoinhibition of Bcr-Abl through its SH3 domain. Mol Cell 2003;12:27-37.
58. Barnes DJ, Melo JV. Cytogenetic and molecular genetic aspects of chronic myeloid leukaemia. Acta Haematol 2002;108:180-202.
59. Li S, Couvillon AD, Brasher BB, Van Etten RA. Tyrosine phosphorylation of Grb2 by Bcr/Abl and epidermal growth factor receptor: a novel regulatory mechanism for tyrosine kinase signaling. EMBO J 2001;20:6793-804.
60. Yamanashi Y, Baltimore D. Identification of the Abl- and rasGAP-associated 62 kDa protein as a docking protein, Dok. Cell 1997;88:205-11.
61. 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;269:22925-8.
62. 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;12:839-46.
63. Tauchi T, Boswell HS, Leibowitz D, Broxmeyer HE. Coupling between p210bcr-abl and Shc and Grb2 adaptor proteins in hematopoietic cells permits growth factor receptor-independent link to ras activation pathway. J Exp Med 1994;179:167-75.
64. Salgia R, Brunkhorst B, Pisick E, et al. Increased tyrosine phosphorylation of focal adhesion proteins in myeloid cell lines expressing p210BCR/ABL. Oncogene 1995;11:1149-55.
65. 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;270:5039-47.
66. Gotoh A, Miyazawa K, Ohyashiki K, et al. Tyrosine phosphorylation and activation of focal adhesion kinase (p125FAK) by BCR-ABL oncoprotein. Exp Hematol 1995;23:1153-9.
67. 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;269:5764-9.
68. Gotoh A, Miyazawa K, Ohyashiki K, Toyama K. Potential molecules implicated in downstream signaling pathways of p185BCR-ABL in Ph+ ALL involve GTPase-activating protein, phospholipase C-gamma 1, and phosphatidylinositol 3′-kinase. Leukemia 1994;8:115-20.
69. Shi CS, Tuscano JM, Witte ON, Kehrl JH. GCKR links the Bcr-Abl oncogene and Ras to the stress-activated protein kinase pathway. Blood 1999;93:1338-45.
70. 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;86:726-36.
71. 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;12:1415-24.
72. Tauchi T, Feng GS, Shen R, et al. SH2-containing phosphotyrosine phosphatase Syp is a target of p210bcr-abl tyrosine kinase. J Biol Chem 1994;269:15381-7.
73. 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;93:2707-20.
74. 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;266:129-33.
75. Bhat A, Kolibaba K, Oda T, Ohno-Jones S, Heaney C, Druker BJ. Interactions of CBL with BCR-ABL and CRKL in BCR-ABL-transformed myeloid cells. J Biol Chem 1997;272:16170-5.
76. 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;14:257-65.
77. Dai Z, Kerzic P, Schroeder WG, McNiece IK. Deletion of the Src homology 3 domain and C-terminal proline-rich sequences in Bcr-Abl prevents Abl interactor 2 degradation and spontaneous cell migration and impairs leukemogenesis. J Biol Chem 2001;276:28954-60.
78. Vigneri P, Wang JY. Induction of apoptosis in chronic myelogenous leukemia cells through nuclear entrapment of BCR-ABL tyrosine kinase. Nat Med 2001;7:228-34.
79. Dierov JK, Dierova R, Carroll M. BCR/ABL translocates to the nucleus after DNA damage and interacts with the ataxia-telangiectasia mutant (ATM) protein to modify DNA repair. Blood 2002;100:204a. abstract.
80. Levitzki A, Gazit A. Tyrosine kinase inhibition: an approach to drug development. Science 1995;267:1782-8.
81. Druker BJ, Lydon NB. Lessons learned from the development of an Abl tyrosine kinase inhibitor for chronic myelogenous leukemia. J Clin Invest 2000;105:3-7.
82. Traxler P, Bold G, Buchdunger E, et al. Tyrosine kinase inhibitors: from rational design to clinical trials. Med Res Rev 2001;21:499-512.
83. Buchdunger E, Zimmermann J, Mett H, et al. Inhibition of the Abl protein-tyrosine kinase in vitro and in vivo by a 2-phenylaminopyrimidine derivative. Cancer Res 1996;56:100-4.
84. Savage DG, Antman KH. Imatinib mesylate — a new oral targeted therapy. N Engl J Med 2002;346:683-93.
85. Okuda K, Weisberg E, Gilliland DG, Griffin JD. ARG tyrosine kinase activity is inhibited by STI571. Blood 2001;97:2440-8.
86. 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;2:561-6.
87. Deininger MWN, Goldman JM, Lydon NB, Melo JV. The tyrosine kinase inhibitor CGP57148B selectively inhibits the growth of BCR-ABL-positive cells. Blood 1997;90:3691-8.
88. le Coutre 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;91:163-8.
89. Mow BM, Chandra J, Svingen PA, et al. Effects of the Bcr/abl kinase inhibitors STI571 and adaphostin (NSC 680410) on chronic myelogenous leukemia cells in vitro. Blood 2002;99:664-71.
90. Gorre ME, Ellwood-Yen K, Chiosis G, Rosen N, Sawyers CL. BCR-ABL point mutants isolated from patients with imatinib mesylate-resistant chronic myeloid leukemia remain sensitive to inhibitors of the BCR-ABL chaperone heat shock protein 90. Blood 2002;100:3041-4.
91. Hoover RR, Mahon FX, Melo JV, Daley GQ. Overcoming STI571 resistance with the farnesyl transferase inhibitor SCH66336. Blood 2002;100:1068-71.
92. Schultheis B, Yong ASM, Goldman JM, Melo JV. The combined use of STI571 and leptomycin-B (LMB) for *in vitro* purging of leukemic cells in autografts for CML. Blood 2001;98:618a. abstract.
93. Wisniewski D, Lambek CL, Liu C, et al. Characterization of potent inhibitors of the Bcr-Abl and the c-kit receptor tyrosine kinases. Cancer Res 2002;62:4244-55.
94. 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;96:1070-9.
95. le Coutre P, Tassi E, Varella-Garcia M,

et al. Induction of resistance to the Abelson inhibitor STI571 in human leukemic cells through gene amplification. Blood 2000;95:1758-6.
96. Weisberg E, Griffin JD. Mechanism of resistance to the ABL tyrosine kinase inhibitor STI571 in BCR/ABL-transformed hematopoietic cell lines. Blood 2000;95:3498-505.
97. Gorre ME, Mohammed M, Ellwood K, et al. Clinical resistance to STI-571 cancer therapy caused by BCR-ABL gene mutation or amplification. Science 2001;293:876-80.
98. Barthe C, Cony-Makhoul P, Melo JV, Mahon JR. Roots of clinical resistance to STI-571 cancer therapy. Science 2001;293:2163.
99. Hochhaus A, Kreil S, Corbin A, et al. Roots of clinical resistance to STI-571 cancer therapy. Science 2001;293:2163.
100. Tanabe T, Kuwabara T, Warashina M, Tani K, Taira K, Asano S. Oncogene inactivation in a mouse model. Nature 2000;406:473-4.
101. Wilda M, Fuchs U, Wossmann W, Borkhardt A. Killing of leukemic cells with a BCR/ABL fusion gene by RNA interference (RNAi). Oncogene 2002;21:5716-24.
102. Xu D, Song J, Li D, Verfaillie CM, Zhao RCH. Anti-ABL tyrosine kinase intrabody promotes apoptosis in K562 cells. Blood 2001;98:146a. abstract.
103. Barrett AJ. Allogeneic stem cell transplantation for chronic myeloid leukemia. Semin Hematol 2003;40:59-71.
104. Clark RE, Dodi IA, Hill SC, et al. Direct evidence that leukemic cells present HLA-associated immunogenic peptides derived from the BCR-ABL b3a2 fusion protein. Blood 2001;98:2887-93.
105. Molldrem JJ, Lee PP, Wang C, et al. Evidence that specific T lymphocytes may participate in the elimination of chronic myelogenous leukemia. Nat Med 2000;6:1018-23.
106. Gao L, Bellantuono I, Elsasser A, et al. Selective elimination of leukemic CD34(+) progenitor cells by cytotoxic T lymphocytes specific for WT1. Blood 2000;95:2198-203.
107. 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;95:1781-7.
108. Chronic Myeloid Leukemia Trialists' Collaborative Group. Interferon alfa versus chemotherapy for chronic myeloid leukemia: a meta-analysis of seven randomized trials. J Natl Cancer Inst 1997;89:1616-20.
109. Guilhot F, Chastang C, Michallet M, et al. Interferon alfa-2b combined with cytarabine versus interferon alone in chronic myelogenous leukemia. N Engl J Med 1997;337:223-9.
110. Baccarani M, Rosti G, de Vivo A, et al. A randomized study of interferon-alpha versus interferon-alpha and low-dose arabinosyl cytosine in chronic myeloid leukemia. Blood 2002;99:1527-35.
111. Kantarjian H, Sawyers C, Hochhaus A, et al. Hematologic and cytogenetic responses to imatinib mesylate in chronic myelogenous leukemia. N Engl J Med 2002;346:645-52. [Erratum, N Engl J Med 2002;346:1923.]
112. Marin D, Marktel S, Szydlo R, et al. Survival of patients with chronic-phase chronic myeloid leukaemia on imatinib after failure on interferon alfa. Lancet 2003;362:617-9.
113. Talpaz M, Silver RT, Druker BJ, et al. Imatinib induces durable hematologic and cytogenetic responses in patients with accelerated phase chronic myeloid leukemia: results of a phase 2 study. Blood 2002;99:1928-37.
114. Druker BJ, Sawyers CL, Kantarjian H, et al. Activity of a specific inhibitor of the BCR-ABL tyrosine kinase in the blast crisis of chronic myeloid leukemia and acute lymphoblastic leukemia with the Philadelphia chromosome. N Engl J Med 2001;344:1038-42. [Erratum, N Engl J Med 2001;345:232.]
115. Wadhwa J, Szydlo RM, Apperley JF, et al. Factors affecting duration of survival after onset of blastic transformation of chronic myeloid leukemia. Blood 2002;99:2304-9.
116. von Bubnoff N, Schneller F, Peschel C, Duyster J. BCR-ABL gene mutations in relation to clinical resistance of Philadelphia-chromosome-positive leukaemia to STI571: a prospective study. Lancet 2002;359:487-91.
117. Branford S, Rudzki Z, Walsh S, et al. High frequency of point mutations clustered within the adenosine triphosphate-binding region of BCR/ABL in patients with chronic myeloid leukemia or Ph-positive acute lymphoblastic leukemia who develop imatinib (STI571) resistance. Blood 2002;99:3472-5.
118. Roche-Lestienne C, Soenen-Cornu V, Grardel-Duflos N, et al. Several types of mutations of the Abl gene can be found in chronic myeloid leukemia patients resistant to STI571, and they can pre-exist to the onset of treatment. Blood 2002;100:1014-8.
119. Shah NP, Nicoll JM, Nagar B, et al. Multiple BCR-ABL kinase domain mutations confer polyclonal resistance to the tyrosine kinase inhibitor imatinib (STI571) in chronic phase and blast crisis chronic myeloid leukemia. Cancer Cell 2002;2:117-25.
120. Hochhaus A, Kreil S, Corbin AS, et al. Molecular and chromosomal mechanisms of resistance to imatinib (STI571) therapy. Leukemia 2002;16:2190-6.
121. Branford S, Rudzki Z, Walsh S, et al. Detection of BCR-ABL mutations in patients with CML treated with imatinib is virtually always accompanied by clinical resistance, and mutations in the ATP phosphate-binding loop (P-loop) are associated with a poor prognosis. Blood 2003;102:276-83.
122. Hofmann WK, Jones LC, Lemp NA, et al. Ph(+) acute lymphoblastic leukemia resistant to the tyrosine kinase inhibitor STI571 has a unique BCR-ABL gene mutation. Blood 2002;99:1860-2.
123. Corbin AS, La Rosee P, Stoffregen EP, Druker BJ, Deininger MW. Several Bcr-Abl kinase domain mutants associated with imatinib mesylate resistance remain sensitive to imatinib. Blood 2003;101:4611-4.
124. Azam M, Latek RR, Daley GQ. Mechanisms of autoinhibition and STI-571/imatinib resistance revealed by mutagenesis of BCR-ABL. Cell 2003;112:831-43.
125. Thomas ED, Clift RA, Fefer A, et al. Marrow transplantation for the treatment of chronic myelogenous leukemia. Ann Intern Med 1986;104:155-63.
126. Goldman JM, Szydlo R, Horowitz MM, et al. Choice of pretransplant treatment and timing of transplants for chronic myelogenous leukemia in chronic phase. Blood 1993;82:2235-8.
127. Gale RP, Hehlmann R, Zhang MJ, et al. Survival with bone marrow transplantation versus hydroxyurea or interferon for chronic myelogenous leukemia. Blood 1998;91:1810-9.
128. Gratwohl A, Hermans J, Goldman JM, et al. Risk assessment for patients with chronic myeloid leukaemia before allogeneic blood or marrow transplantation. Lancet 1998;352:1087-92.
129. Passweg JR, Walker I, Sobocinski KA, et al. Validation of the EBMT risk score for recipients of allogeneic stem cell transplants for CML. Bone Marrow Transplant 2002;27:Suppl 2:S33. abstract.
130. Giralt S, Thall PF, Khouri I, et al. Melphalan and purine analog-containing preparative regimens: reduced-intensity conditioning for patients with hematologic malignancies undergoing allogeneic progenitor cell transplantation. Blood 2001;97:631-7.
131. Or R, Shapira MY, Resnick I, et al. Nonmyeloablative allogeneic stem cell transplantation for the treatment of chronic myeloid leukemia in first chronic phase. Blood 2003;101:441-5.
132. Uzunel M, Mattsson J, Brune M, Johansson JE, Aschan J, Ringden O. Kinetics of minimal residual disease and chimerism in patients with chronic myeloid leukemia after nonmyeloablative conditioning and allogeneic stem cell transplantation. Blood 2003;101:469-72.
133. Goldman JM, Druker BJ. Chronic myeloid leukemia: current treatment options. Blood 2001;98:2039-42.
134. Sokal JE, Cox EB, Baccarani M, et al. Prognostic discrimination in "good-risk" chronic granulocytic leukemia. Blood 1984;63:789-99.
135. Hasford J, Pfirrmann M, Hehlmann R, et al. A new prognostic score for survival of patients wtih chronic myeloid leukemia treated with interferon alfa. J Natl Cancer Inst 1998;90:850-8.