Review Articles

Medical Progress

Chronic Myeloid Leukemia

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In the past decade clinical and laboratory studies have led to important new insights into the biology of chronic myeloid leukemia (CML). Basic science has defined the molecular pathogenesis of CML as unregulated signal transduction by a tyrosine kinase. Clinical science has demonstrated that it is curable through immune-mediated elimination of leukemia cells by allogeneic T lymphocytes.

CLINICAL FEATURES

CML is a malignant clonal disorder of hematopoietic stem cells that results in increases in not only myeloid cells but also erythroid cells and platelets in peripheral blood and marked myeloid hyperplasia in the bone marrow (Fig. 1). The median age at presentation is 53 years, but all age groups, including children, are affected. Most patients also have thrombocytosis, which is consistent with the presence of a defect in a pluripotent hematopoietic stem cell. The typical symptoms at presentation are fatigue, anorexia, and weight loss, but about 40 percent of patients are asymptomatic, and in these patients, the diagnosis is based solely on an abnormal blood count (Table 1). The most common abnormality on physical examination is splenomegaly, which is present in up to half of patients. The natural history of CML is progression from a benign chronic phase to a rapidly fatal blast crisis within three to five years. The blast crisis is often preceded by an accelerated phase in which increasing doses of hydroxyurea or busulfan are required to lower the neutrophil count. In contrast to the maturation of CML cells during the chronic phase, during a blast crisis cells fail to mature and resemble the myeloblasts or lymphoblasts found in patients with acute leukemias.

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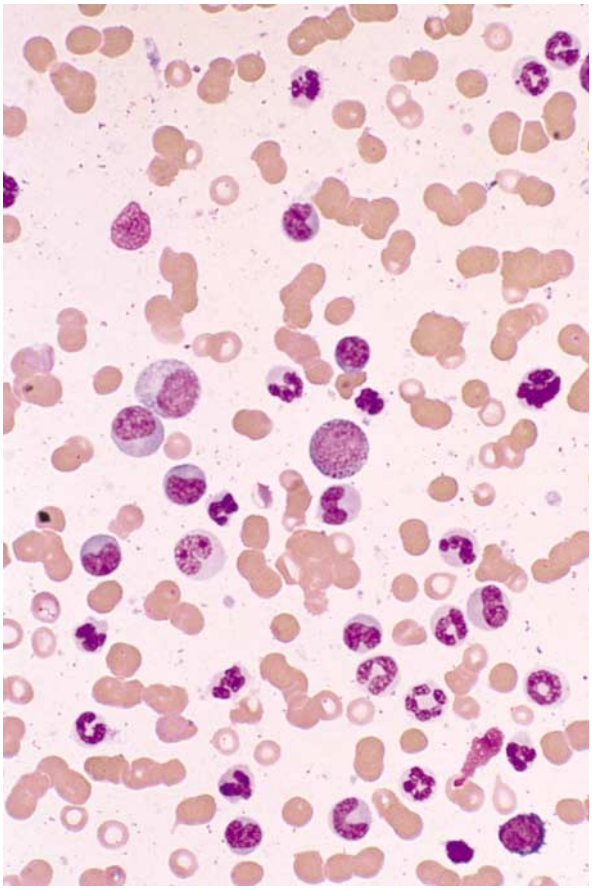
MOLECULAR PATHOPHYSIOLOGY

The diagnosis of CML is usually based on detection of the Philadelphia (Ph) chromosome. This abnormality, first described as a shortened chromosome 22 in 1960[1] and then as a t(9;22) translocation in 1973,[2] is present in 95 percent of patients. Another 5 percent have complex or variant translocations involving additional chromosomes that have the same end result, which is fusion of the *BCR* (breakpoint cluster region) gene on chromosome 22 to the *ABL* (Ableson leukemia virus) gene on chromosome 9. The Ph chromosome is found in cells from the myeloid, erythroid, megakaryocytic, and B lymphoid lineages, indicating that CML is a stem-cell disease. During the evolution to a blast crisis, a range of nonrandom, secondary chromosomal changes occur, including duplication of the Ph chromosome and trisomy 8.[3] Mutations or deletions of tumor-suppressor genes such as *p16*[4] and *p53* occur with variable frequency and presumably contribute to the malignant phenotype.[5]

The molecular consequence of the t(9;22) translocation is the creation of the fusion protein BCR–ABL, which is a constitutively active cytoplasmic tyrosine kinase. Depending on the site of the breakpoint in the *BCR* gene, the fusion protein can vary in size from 185 kd to 230 kd. Each fusion gene encodes the same portion of the ABL tyrosine kinase but differs in the length of BCR sequence retained at the N terminal (Fig. 2). Nearly all patients with typical chronic-phase CML express a 210-kd BCR–ABL protein, whereas patients with Ph-positive acute lymphoblastic leukemia express either a 210-kd or a 190-kd BCR–ABL protein (the latter is sometimes referred to as 185/190 kd). Recently, a larger, 230-kd BCR–ABL fusion protein was found in a subgroup of patients with CML who presented with a lower white-cell count than is usual for the disease and in whom progression to blast crisis was slow.[6] The fact that fusion proteins of different sizes can be correlated with different outcomes has led to laboratory studies of the biologic activity of the proteins. The results indicate that the 190-kd BCR–ABL protein has greater activity as a tyrosine kinase and is a more potent oncogene than the 210-kd protein, suggesting that the magnitude of the tyrosine kinase signal affects the expression of the disease.[7,8]

The cloning of the Ph translocation has led to the development of highly sensitive and specific molecular probes that are valuable tools for monitoring responses to therapy. Quantitative cytogenetic information can be obtained by fluorescence in situ hybridization without the need to culture cells or



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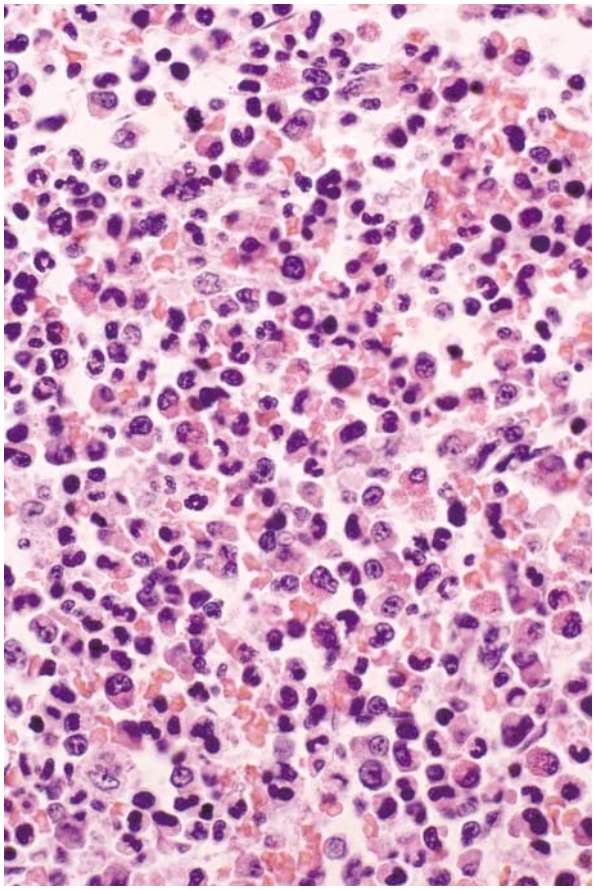

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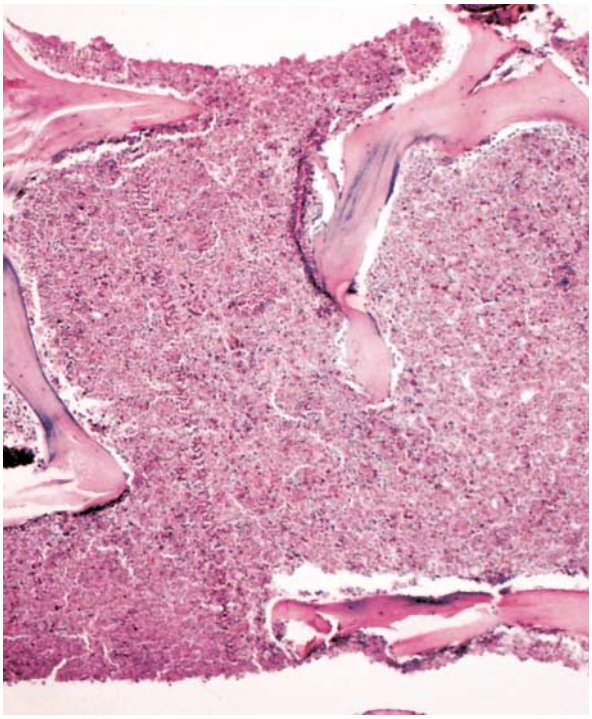

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Figure 1. Photomicrographs of a Peripheral-Blood Sample and Bone Marrow Samples from a Patient with Chronic Myeloid Leukemia.

Panel A shows a peripheral-blood smear with numerous myeloid cells, including myelocytes and a basophil (center) (Wright's stain, ×40). In Panel B, a bone marrow specimen obtained with a trephine shows marked hypercellularity and almost no fat (hematoxylin and eosin, ×40). In Panel C, marked myeloid hyperplasia is evident in the bone marrow specimen at a higher magnification (hematoxylin and eosin, ×160).

TABLE 1. CHARACTERISTICS OF PATIENTS WITH CHRONIC MYELOID LEUKEMIA AT PRESENTATION.

Clinical findings*
Fatigue, anorexia, weight loss
Splenomegaly
Hepatomegaly
Peripheral-blood findings
Elevated white-cell count (usually greater than 25,000/mm^3)
Elevated platelet count in 30 to 50 percent of cases
Basophilia
Reduced leukocyte alkaline phosphatase activity
All stages of granulocyte differentiation visible on peripheral smear
Bone marrow findings
Hypercellularity, reduced fat content
Increased ratio of myeloid cells to erythroid cells
Increased numbers of megakaryocytes
Blasts and promyelocytes constitute less than 10 percent of all cells

*Approximately 40 percent of patients are asymptomatic.

analyze cells in metaphase.[9] Polymerase-chain-reaction (PCR) testing of peripheral-blood RNA is highly sensitive: it can detect 1 Ph-positive cell expressing the BCR–ABL fusion transcript in 10^5 to 10^6 normal cells.[10] One consequence of these newer diagnostic tests has been the reclassification of the response to treatment on the basis of hematologic, cytogenetic, and molecular remissions. A hematologic remission indicates a return of peripheral-blood cell counts and bone marrow morphology to normal, whereas cytogenetic and molecular remissions indicate the disappearance of the Ph chromosome or the *BCR–ABL* gene, respectively. The favorable prognostic value of hematologic and cytogenetic remissions is clear, on the basis of data on survival among patients treated with interferon alfa,[11] but the clinical value of molecular testing remains to be defined. Negative PCR results in patients treated by allogeneic bone marrow transplantation clearly predict a favorable outcome,[12,13] but positive results are difficult to interpret, because of the extreme sensitivity of the PCR assay. For example, the results of PCR assays can remain positive in interferon-treated patients who are in complete cytogenetic remission and patients who have survived for several years after bone marrow transplantation, two subgroups with very favorable outcomes,[14,15] presumably because small numbers of leukemic cells remain. Newly developed quantitative PCR assays allow monitoring of the level of BCR–ABL messenger RNA transcripts over time. On the basis of these assays, a progressive increase in minimal residual disease after allogeneic transplantation appears to predict eventual relapse.[10]

MECHANISM OF LEUKEMOGENESIS

Laboratory studies of *BCR–ABL* have established that it is an oncogene that induces leukemias in animals. Transgenic expression of the 190-kd BCR–ABL protein in mice causes acute leukemia at birth, suggesting that it confers a potent oncogenic signal in hematopoietic cells.[16] Karyotypic studies of the leukemia cells from these mice indicate secondary chromosomal abnormalities analogous to blast-crisis cells in humans.[17] Another model of CML involves

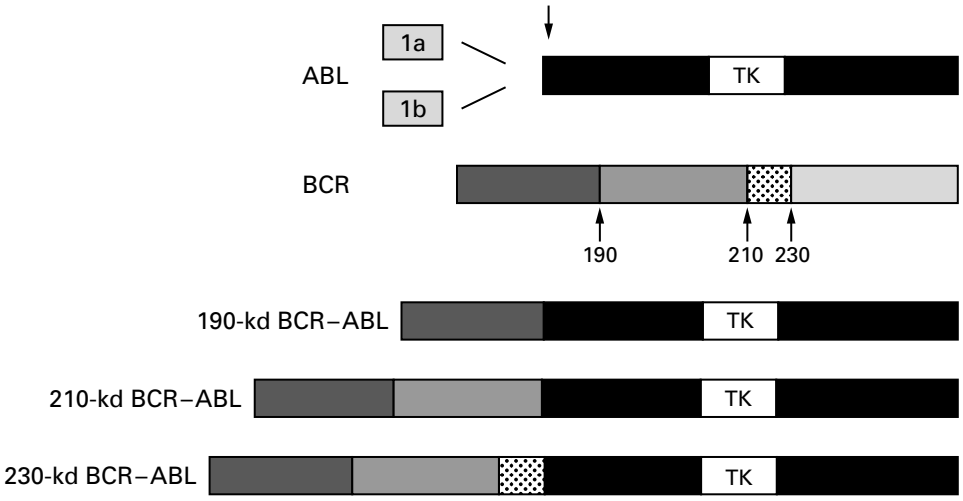

Figure 2. Structure of BCR–ABL Fusion Proteins.

The structure of the wild-type ABL and BCR proteins is shown, with the site of the break points in each marked by the arrows. The sizes of the fusion proteins differ depending on the amount of the BCR sequence that is retained. The length of the ABL sequence is the same in all cases. ABL has two alternative first exons (1a and 1b). TK denotes the tyrosine kinase domain.

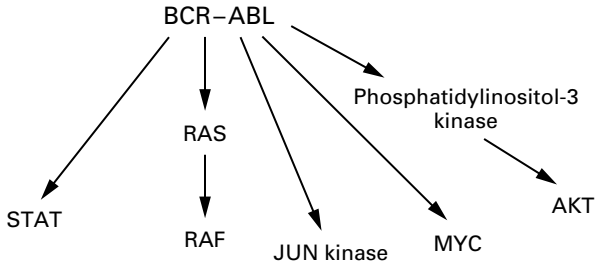

Figure 3. Signal Transduction by the BCR–ABL Protein.
Constitutive activation of its tyrosine kinase domain causes the BCR–ABL protein to activate a number of cytoplasmic and nuclear signal-transduction pathways that affect the growth and survival of hematopoietic cells.

the transfer of the *BCR–ABL* gene into hematopoietic stem cells of normal mice by infection with a retrovirus. In these animals a range of acute and chronic myeloid leukemias develops that varies in strains with different genetic backgrounds.[18-20] These findings suggest that the pathogenesis of CML is a multistep process.

Most laboratory studies of the function of the BCR–ABL protein have focused on determining the effects of overexpression on growth and cellular transformation. The protein can transform hematopoietic cells so that their growth and survival in vitro become independent of cytokines.[21,22] It can also protect hematopoietic cells from programmed cell death (apoptosis) in response to cytokine withdrawal and DNA damage by chemotherapy or radiation.[23,24] However, in primary CML cells, the effects of the BCR–ABL protein are less dramatic, particularly with regard to apoptosis.[25,26] It also increases adhesion of hematopoietic cells to extracellular-matrix proteins by increasing the activity of integrin.[27] One mechanism for this effect may involve the BCR–ABL substrate CRKL, which induces adhesion when it is phosphorylated, by allowing the assembly of focal adhesion complexes.[28] Curiously, primary CML cells adhere poorly to bone marrow stroma in vitro.[29,30] This result appears to contrast with data from cells engineered to express the BCR–ABL protein and will require further study. Nevertheless, the adhesion defect offers another mechanism for leukemogenesis, since CML cells may escape negative regulatory influences that stromal cells normally exert on hematopoietic cells through contact between stromal cells and stem cells.

Biochemical studies of leukemogenesis indicate that the BCR–ABL protein is a constitutively active tyrosine kinase confined to the cytoplasm, whereas wild-type ABL shuttles between the nucleus and cytoplasm.[31,32] As a consequence of increased tyrosine kinase activity, the BCR–ABL protein can phosphorylate several substrates, thereby activating multiple signal-transduction cascades affecting the growth and differentiation of cells. The substrates include CRKL,[33-35] p62Dok,[36,37] paxillin,[38] CBL,[39] and RIN,[40] which activate pathways involving RAS,[41] RAF,[42] phosphatidylinositol-3 kinase,[43] JUN kinase,[44] MYC,[45] and STAT[46-48] (Fig. 3). The ways in which these pathways become activated are not well defined, but an emerging theme is that the BCR–ABL protein activates the same signaling cascades activated by cytokines that control the growth and differentiation of normal hematopoietic cells. Since the BCR–ABL signal is constitutive, these cells escape constraints on normal growth and become leukemic.

A major goal of current research is to define the specific signal-transduction events that contribute to leukemogenesis so that rational therapeutic interventions can be designed. One clear theme is that loss of tyrosine kinase activity through either mutation or the use of pharmacologic inhibitors blocks the leukemogenic activity of the BCR–ABL protein.[49] In addition, inhibition of the RAS,[50] RAF, phosphatidylinositol-3 kinase,[43] AKT,[51] JUN kinase,[44,52] and MYC[45,53] pathways prevents the transformation of cells in which the BCR–ABL tyrosine kinase is constitutively activated. Although the signaling pathways are complex, it is possible that BCR–ABL function could be blocked by the inhibition of single pathways.

TREATMENT OPTIONS

During the chronic phase of CML, cytoreductive therapy is required in most patients to avoid thrombotic complications that can result from high circulating levels of neutrophils. Fortunately, CML cells are sensitive to several oral chemotherapeutic drugs. Ninety percent of patients who are treated with hydroxyurea or busulfan have hematologic remissions.[54] Hydroxyurea is preferred to busulfan because the median duration of the chronic phase and median survival were significantly better in a comparative trial of long-term therapy to maintain the neutrophil count in the normal range (Table 2).[54] Hydroxyurea is advantageous primarily because of its favorable toxicity profile rather than because it has a specific effect on CML cells. Treatment with either drug results in a negligible rate of cytogenetic response and has no effect on the rate of progression to blast crisis; therefore, these treatments must be considered palliative.

Allogeneic Bone Marrow Transplantation

CML is a disease of hematopoietic stem cells. High-dose chemotherapy that destroys the leukemic cells also destroys normal bone marrow and therefore must be followed by allogeneic bone marrow or stem-cell transplantation. Decades of follow-up data from multiple centers have confirmed that high-dose

TABLE 2. CHEMOTHERAPEUTIC DRUGS USED TO TREAT THE CHRONIC PHASE OF CHRONIC MYELOID LEUKEMIA.

DRUG	DOSE*	ADVERSE EFFECTS†
Hydroxyurea	0.5–2.0 g/day orally	Cytopenias, rash, nausea
Busulfan	2.0–6.0 mg/day orally	Cytopenias, rash, bone marrow aplasia
Interferon alfa	5 million U/m²/day subcutaneously	Fever, myalgias, rash, depression, thrombocytopenia
Interferon alfa plus cytarabine	Interferon alfa, 5 million U/m²/day subcutaneously, plus cytarabine, 20 mg/m²/day for 10 days each month	Fever, myalgias, rash, depression, thrombocytopenia, nausea, vomiting, diarrhea, mucositis, weight loss

*Doses are modified on an individual basis according to changes in the patient's peripheral-blood counts.

†Data are from randomized clinical trials.[54-56]

TABLE 3. RESULTS OF ALLOGENEIC BONE MARROW TRANSPLANTATION IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA IN CHRONIC PHASE.*

STUDY AND TYPE OF DONOR	NO. OF PATIENTS	DURATION OF FOLLOW-UP	SURVIVAL	RELAPSE
		yr	percent	
HLA-matched related donor				
IBMTR[57]	2231	3	57	13
EBMT[58]	373	8	54	19
Clift and Anasetti[59]†	351	>10	70	20
HLA-matched unrelated donor				
NMDP[60,61]	779	3	40	5
IBMTR[57]	331	3	38	NA
Hansen et al.[62]†	196	5	57	NA

*IBMTR denotes International Bone Marrow Transplant Registry, EBMT European Group for Blood and Marrow Transplantation, NMDP National Marrow Donor Program, and NA not available.

†This study was performed at the Fred Hutchinson Cancer Center in Seattle.

chemotherapy with busulfan and cyclophosphamide or combined chemotherapy with cyclophosphamide and fractionated total-body irradiation followed by allogeneic bone marrow transplantation is curative therapy in patients with CML in chronic phase. The International Bone Marrow Transplant Registry[57] and the European Group for Blood and Marrow Transplantation[58] have recently reported survival rates of 50 to 60 percent among patients with CML in chronic phase who received chemotherapy alone or radiotherapy plus chemotherapy followed by transplantation of marrow cells from HLA-matched sibling donors (Table 3). The largest single-institution experience is that of the Fred Hutchinson Cancer Center in Seattle, which reports a survival rate of 70 percent at 10 years.[59] The success of allogeneic transplantation is age-dependent, being significantly lower in patients over the age of 40 years, primarily because of higher treatment-related mortality. Although age is clearly an important prognostic variable, transplantation decisions must be considered individually in the light of other variables that influence outcome, such as disease stage and the level of donor–recipient HLA matching.

Because of the risk of transplantation-related mortality, it may be tempting to delay the procedure in patients with HLA-matched related donors until the disease progresses. This approach is inappropriate for two reasons. First, among patients with CML, those who undergo transplantation when the disease is in the chronic phase have a higher likelihood of survival than do those who undergo transplantation during the accelerated phase or blast crisis.[57,59] Second, patients who undergo transplantation within the first year after diagnosis have a higher likelihood of survival than do those who undergo transplantation later, even if it is before progression to the accelerated phase.[57,63] These findings indicate that CML cells are capable of becoming resistant to high-dose chemotherapy and radiotherapy. Given that CML progresses steadily to a more malignant phenotype and that it is not possible to define the point in the chronic phase at which the likelihood of survival tends to decrease, allogeneic transplantation should be considered at the time of diagnosis in eligible patients with HLA-matched sibling donors.

Unfortunately, only 15 to 20 percent of patients with CML are candidates for allogeneic transplantation from HLA-matched related donors because of age limitations and the low probability of having an HLA-matched sibling donor. This number can be increased to 30 percent through the use of HLA-matched unrelated donors identified by bone marrow–donor registries.[64] However, the survival rate among recipients of transplants from unrelated donors identified by the National Marrow Donor Program is substantially lower than that among recipients of transplants from related donors (Table 3).[60,61] Recent results from a Seattle study are better, particularly in patients who were 50 years of age or younger who received a transplant matched for HLA-A, B, and DRB1 by molecular studies.[62] Molecular typing allows a distinction to be made between subtypes of serologically related but immunologically distinct HLA antigens. Although this strategy appears to improve survival, it will decrease the number of patients with CML for whom an appropriate donor can be found.

The Role of the Graft-versus-Leukemia Effect in Curing CML

A major lesson from clinical research on transplantation in patients with CML is the role of the immune system in achieving a cure. The first hint of

this concept was a correlation between graft-versus-host disease, a potentially fatal complication of allogeneic transplantation, and the long-term success of transplantation, as measured by leukemia-free survival.[65] The importance of this phenomenon, termed the graft-versus-leukemia effect, became evident when transplantations were performed with bone marrow depleted of T lymphocytes to ameliorate graft-versus-host disease. This approach successfully reduced mortality from graft-versus-host disease, but the relapse rate of CML approached 60 percent.[66] These results implicated T lymphocytes in the donor marrow as a critical ingredient in the success of allogeneic transplantation in patients with CML. Formal proof of this hypothesis has come with the demonstration that infusions of donor lymphocytes are sufficient to induce complete remissions in the absence of any conditioning chemotherapy or radiotherapy in patients who relapse after allogeneic bone marrow transplantation.[67-69]

Current efforts are focused on making allogeneic transplantation safer and more available by reducing the risk of graft-versus-host disease without sacrificing the curative graft-versus-leukemia phenomenon. One approach is to remove T cells from the donor bone marrow and then reinfuse them at a later date. Threshold doses of T cells have been defined that can induce the graft-versus-leukemia effect but not graft-versus-host disease when they are infused after engraftment.[70] The most likely explanation for the reduction in graft-versus-host disease is the delivery of T cells at a time when target tissues such as the gut and liver have recovered from damage induced by chemotherapy or radiation. A future challenge is to test this concept more widely and extend its utility to transplantation involving unrelated donors, after which the risk of graft-versus-host disease is higher.

A major research question in transplantation biology is whether graft-versus-host disease and the graft-versus-leukemia effect can be separated at the cellular level, in order to obtain the clinical benefits of the graft-versus-leukemia effect while avoiding the complications induced by graft-versus-host disease. In this regard, efforts are under way to isolate from donor T cells leukemia-specific cytotoxic T-cell clones whose numbers might be expanded in vitro. CML cells express the tumor-specific *BCR–ABL* fusion gene, and therefore it might be possible to produce tumor-specific immunity to peptides spanning the *BCR–ABL* junction. These peptides induce T-cell immune responses,[71,72] but it is not clear whether these T-cells specifically recognize and kill autologous CML cells.

Interferon Alfa

Because the majority of patients with CML are not candidates for allogeneic transplantation, alternative therapies have been studied extensively. One such therapy is interferon alfa (Table 2). It can induce hematologic and cytogenetic remissions in patients with CML in chronic phase.[73] In several clinical trials, the cytogenetic-response rate and the survival rate were higher in patients who received long-term treatment with subcutaneous interferon alfa at a dose of 5 million U per square meter of body-surface area per day than in patients who received other drugs[54,55,74-76] (Table 4). Therefore, interferon alfa is now first-line therapy in patients with CML who are not eligible for allogeneic bone marrow transplantation.

A complete cytogenetic response or a partial cytogenetic response to treatment with interferon alfa, defined as a reduction in the percentage of Ph-positive cells in metaphase to less than 34 percent, occurs in 20 to 30 percent of patients. Minor responses (defined as the presence of less than 67 percent Ph-positive cells in metaphase) occur in an additional 10 percent. Patients with complete cytogenetic responses gain the most clinical benefit from treatment with interferon alfa,[11] but those with lesser responses also benefit, as compared with patients treated with hydroxyurea.[75] Only 5 to 10 percent of patients have sustained, complete disappearance of

TABLE 4. RESULTS OF CLINICAL TRIALS OF INTERFERON ALFA AND OTHER DRUGS IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA.

STUDY AND TREATMENT	CYTOGENETIC-RESPONSE RATE*	5-YR SURVIVAL
	percent	
Italian Cooperative Study Group on Chronic Myeloid Leukemia[74]		
Hydroxyurea	1	29†
Interferon alfa	19	50†
Hehlmann et al.[54]		
Hydroxyurea	<1	44
Busulfan	<1	32
Interferon alfa	9.6	5.9
Allan et al.[75]		
Hydroxyurea or busulfan	—	34
Interferon alfa	11	52
Ohnishi et al.[55]		
Busulfan	5	32
Interferon alfa	16.3	54
Guilhot et al.[56]		
Interferon alfa	24	79‡
Interferon alfa plus cytarabine	41	86‡
Chronic Myeloid Leukemia Trialists' Collaborative Group[76]§		
Chemotherapy		42
Interferon alfa		57

*Complete responses (defined as the absence of Ph-positive cells) and partial responses (defined as the presence of <34 percent Ph-positive cells in metaphase) are included.

†This is the rate at six years.

‡This is the rate at three years.

§This study was a meta-analysis.

Ph-positive cells, and they survive the longest. In efforts to improve the response rate, current trials are focused on the use of combination therapy. The most promising results have been obtained with a combination of cytarabine and interferon alfa.[56] Since cytarabine has substantial gastrointestinal and hematologic toxicity, the superiority of this combination needs to be confirmed before it becomes standard therapy.

Little is known about the mechanism of action of interferon alfa in CML. It has immune modulatory effects on tumor cells, such as increased expression of HLA class I antigens,[77] but it is not clear whether these have a role in CML. In a recent study, interferon alfa in combination with granulocyte–macrophage colony-stimulating factor stimulated an increase in the number of antigen-presenting dendritic cells in vitro.[78] Dendritic cells from patients with CML may selectively stimulate killing of autologous Ph-positive cells but not Ph-negative cells by T cells,[79] and therefore the anti-CML activity of interferon alfa may be mediated through the activation of dendritic cells.

Clinical Decision Making: Transplantation versus Interferon Alfa Therapy

Over the past 10 years the survival of patients with CML has improved as a consequence of early diagnosis through routine blood counts and treatment with transplantation or interferon alfa. In view of the improved cytogenetic-response rates in patients treated with a combination of interferon alfa and cytarabine, physicians counseling patients with CML who are eligible for allogeneic bone marrow transplantation may face a difficult decision. Although curative, allogeneic bone marrow transplantation is associated with substantial mortality and potentially disabling morbidity among those who survive for long periods. Treatment with interferon alfa is safer, but the percentage of patients who have a complete cytogenetic remission is low and the durability of the survival benefit has not been defined in large numbers of patients.

One strategy supported by decision analysis[80] is to treat older patients or younger patients for whom no suitable donor of bone marrow is available with interferon alfa (Fig. 4). In patients who have a cytogenetic response within one year, treatment with interferon alfa is continued indefinitely; the others undergo transplantation. With improvements in HLA-matching procedures and pretransplantation risk assessment, this algorithm will require modification. An implicit assumption of this approach is that the success of allogeneic bone marrow transplantation is not affected by prior treatment with interferon alfa, but there have been conflicting reports on this topic and the issue remains unsettled.[81-83] Patients who relapse after allogeneic bone marrow transplantation can be treated successfully with infusion of donor lymphocytes,[67-69] interferon alfa,[84,85] or a second allogeneic transplantation.

NOVEL TREATMENTS

Autografts

Studies of combination chemotherapy with cytarabine and anthracyclines, similar to that used in the treatment of acute myeloid leukemia, have demonstrated transient cytogenetic remissions but minimal long-term clinical benefit in patients with CML in chronic phase.[86,87] Currently, there is renewed interest in combining high-dose chemotherapy with stem-cell purification so that autologous transplants with Ph-negative stem cells can be used. Stem cells that are Ph-negative are harvested during the recovery phase after induction chemotherapy, and they successfully engraft, resulting in Ph-negative hematopoiesis.[88] However, Ph-positive hematopoiesis inevitably recurs, usually within the first year after transplantation, with a return to the chronic phase of CML.[89,90] This recurrence probably results from the failure to remove all cells that are positive for BCR–ABL during the enrichment process. This hypothesis has been confirmed in retrovirus-marking trials, which demonstrate that virus-marked CML cells contribute to relapse.[91] This result has provided a rationale to purge stem-cell preparations of residual CML cells with antisense messenger RNA directed against the *BCR–ABL*[92] or the *MYB*[93] gene, in in vitro culture conditions that select against Ph-positive cells,[94] or by physically separating Ph-negative stem cells from Ph-positive stem cells.[95] The clinical feasibility and safety of these strategies have been demonstrated, but their therapeutic value remains to be proved.

Autografting alone, however, even when it is combined with effective purging strategies, is unlikely to result in long-term remissions in most patients, presumably because the graft-versus-leukemia effect or graft-versus-host disease does not develop in these patients. The reason for this effect is explained by studies in twins. The relapse rate was two to three times as high in patients who received bone marrow transplants from their identical twins — an approach theoretically equivalent to the use of autografts purged of Ph-positive stem cells — as in comparable patients who received HLA-matched transplants from siblings who were not their identical twin.[57,59] On the basis of this experience, it seems likely that patients who receive autografts will require post-transplantation therapy to remain in remission. Treatment with interferon alfa offers some promise because it can restore Ph-negative hematopoiesis in some patients who relapse after allogeneic bone marrow transplantation.[84,85] An alternative approach is treatment with interleukin-2, which is active against CML cells and is useful as post-remission therapy for acute myeloid leukemia.[96]

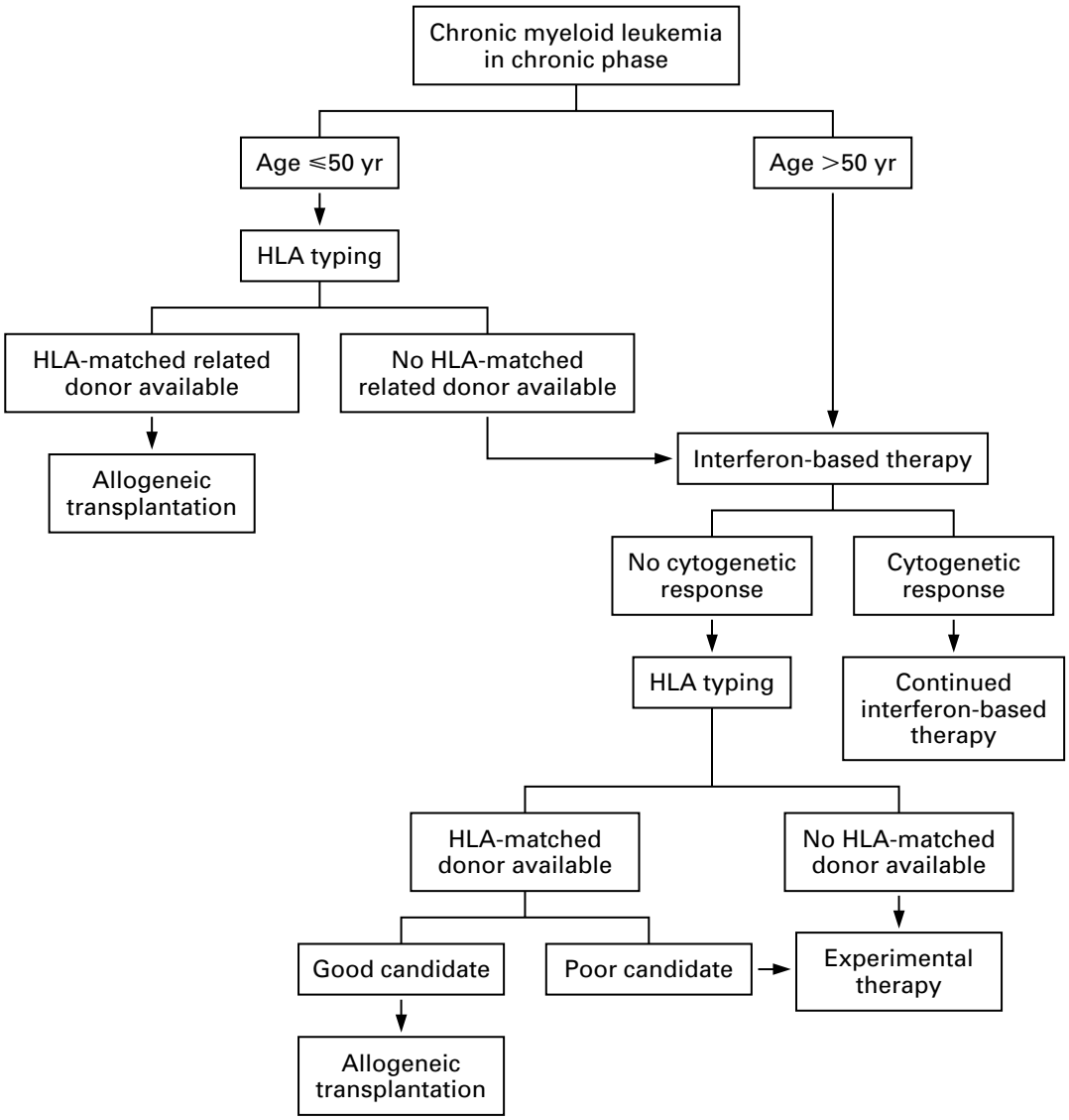

Figure 4. Approach to the Treatment of Patients with Chronic Myeloid Leukemia in Chronic Phase.

Molecular Therapy

Recent success in defining the molecular basis of many types of cancer has shifted the search for treatments toward identifying compounds that specifically impair proteins involved in signal transduction by cancer cells. Knowledge about the pathways critical for the leukemogenic activity of the BCR–ABL protein provides a number of potential targets for drug therapy. One compound that can selectively inhibit the tyrosine kinase activity of BCR–ABL has shown promise in preclinical studies in vitro and in animals.[49] Another promising target is the RAS pathway, which is required for the anti-apoptotic as well as the transforming activity of the BCR–ABL protein.[50,97] A series of potential anti-RAS drugs called farnesyltransferase inhibitors, which block a lipid modification required for RAS to function as a signaling molecule,[98] are currently under study in clinical trials in patients with other cancers.

CONCLUSIONS

Allogeneic marrow transplantation is firmly established as the treatment of choice for patients with CML, but only a small percentage of patients benefit from this curative procedure, because of limitations in the availability of donors and advanced age of patients. Treatment with interferon alfa, alone or perhaps in combination with other drugs, is suitable

for most patients but fails to induce long-term cytogenetic remissions in most patients. Laboratory and clinical research has defined the critical roles of the BCR–ABL fusion protein in the pathogenesis of CML and the immune system in curing patients by means of transplantation. On the basis of these insights, new treatment strategies are currently under investigation that involve pharmacologic inhibitors of the BCR–ABL signal-transduction pathway and peptides or cell-based immunotherapy to activate a leukemia-specific immune response.

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REFERENCES

1. Nowell PC, Hungerford DA. A minute chromosome in human chronic granulocytic leukemia. Science 1960;132:1497. abstract.
2. Rowley JD. A new consistent chromosomal abnormality in chronic myelogenous leukaemia identified by quinacrine fluorescence and Giemsa staining. Nature 1973;243:290-3.
3. Bernstein R. Cytogenetics of chronic myelogenous leukemia. Semin Hematol 1988;25:20-34.
4. 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.
5. Ahuja H, Bar-Eli M, Arlin Z, et al. The spectrum of molecular alterations in the evolution of chronic myelocytic leukemia. J Clin Invest 1991; 87:2042-7.
6. Pane F, Frigeri F, Sindona M, et al. Neutrophilic-chronic myeloid leukemia: a distinct disease with a specific molecular marker. Blood 1996;88: 2410-4. [Erratum, Blood 1997;89:4244.]
7. Lugo TG, Pendergast AM, Muller AJ, Witte ON. Tyrosine kinase activity and transformation potency of bcr-abl oncogene products. Science 1990;247:1079-82.
8. 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;86:4603-11.
9. Tkachuk DC, Westbrook CA, Andreeff M, et al. Detection of bcr-abl fusion in chronic myelogenous leukemia by in situ hybridization. Science 1990;250:559-62.
10. Cross NC, Feng L, Chase A, Bungey J, Hughes TP, Goldman JM. Competitive polymerase chain reaction to estimate the number of BCR-ABL transcripts of chronic myeloid leukemia patients after bone marrow transplantation. Blood 1993;82:1929-36.
11. Kantarjian HM, Smith TL, O'Brien S, Beran M, Pierce S, Talpaz M. Prolonged survival in chronic myelogenous leukemia after cytogenetic response to interferon-α therapy. Ann Intern Med 1995;122:254-61.
12. Hughes TP, Morgan GJ, Martiat P, Goldman JM. Detection of residual leukemia after bone marrow transplant for chronic myeloid leukemia: role of polymerase chain reaction in predicting relapse. Blood 1991;77:874-8.
13. Radich JP, Gehly G, Gooley T, et al. Polymerase chain reaction detection of the BCR-ABL fusion transcript after allogeneic marrow transplantation for chronic myeloid leukemia: results and implications in 346 patients. Blood 1995;85:2632-8.
14. Hochhaus A, Lin F, Reiter A, et al. Variable numbers of BCR-ABL transcripts persist in CML patients who achieve complete cytogenetic remission with interferon-alpha. Br J Haematol 1995;91:126-31.
15. Miyamura K, Tahara T, Tanimoto M, et al. Long persistent bcr-abl positive transcript detected by polymerase chain reaction after marrow transplant for chronic myelogenous leukemia without clinical relapse: a study of 64 patients. Blood 1993;81:1089-93.
16. Heisterkamp N, Jenster G, ten Hoeve J, Zovich D, Pattengale PK, Groffen J. Acute leukaemia in bcr/abl transgenic mice. Nature 1990;344: 251-3.
17. Voncken JW, Morris C, Pattengale P, et al. Clonal development and karyotype evolution during leukemogenesis of BCR/ABL transgenic mice. Blood 1992;79:1029-36.
18. 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.
19. 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;87:6649-53. [Erratum, Proc Natl Acad Sci U S A 1990;87:9072.]
20. Elefanty AG, Hariharan IK, Cory S. bcr-abl, The hallmark of chronic myeloid leukaemia in man, induces multiple haematopoietic neoplasms in mice. EMBO J 1990;9:1069-78.
21. McLaughlin J, Chianese E, Witte O. In vitro transformation of immature hematopoietic cells by the P210 BCR/ABL oncogene product of the Philadelphia chromosome. Proc Natl Acad Sci U S A 1987;84:6558-62.
22. Gishizky ML, Witte ON. Initiation of deregulated growth of multipotent progenitor cells by bcr-abl in vitro. Science 1992;256:836-9.
23. Nishii K, Kabarowski JH, Gibbons DL, et al. ts BCR-ABL kinase activation confers increased resistance to genotoxic damage via cell cycle block. Oncogene 1996;13:2225-34.
24. Evans CA, Owen-Lynch PJ, Whetton AD, Dive C. Activation of the Abelson tyrosine kinase activity is associated with suppression of apoptosis in hemopoietic cells. Cancer Res 1993;53:1735-8.
25. 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;91:387-93.
26. 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;95:501-7.
27. Bazzoni G, Carlesso N, Griffin JD, Hemler ME. Bcr/Abl expression stimulates integrin function in hematopoietic cell lines. J Clin Invest 1996; 98:521-8.
28. Senechal K, Heaney C, Druker B, Sawyers CL. Structural requirements for function of the Crkl adapter protein in fibroblasts and hematopoietic cells. Mol Cell Biol 1998;18:5082-90.
29. 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;328:342-4.
30. Bhatia R, McCarthy JB, Verfaillie CM. Interferon-alpha restores normal beta 1 integrin-mediated inhibition of hematopoietic progenitor proliferation by the marrow microenvironment in chronic myelogenous leukemia. Blood 1996;87:3883-91.
31. 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;58:669-78.
32. Lewis JM, Baskaran R, Taagepera S, Schwartz MA, Wang JYJ. Integrin regulation of c-Abl tyrosine kinase activity and cytoplasmic-nuclear transport. Proc Natl Acad Sci U S A 1996;93:15174-9.
33. Nichols GL, Raines MA, Vera JC, Lacomis L, Tempst P, Golde DW. Identification of CRKL as the constitutively phosphorylated 39-kD tyrosine phosphoprotein in chronic myelogenous leukemia cells. Blood 1994; 84:2912-8.
34. ten Hoeve J, Arlinghaus RB, Guo JQ, Heisterkamp N, Groffen J. Tyrosine phosphorylation of CRKL in Philadelphia$^+$ leukemia. Blood 1994; 94:1731-6.
35. 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.
36. 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.
37. Yamanashi Y, Baltimore D. Identification of the Abl- and rasGAP-associated 62 kDa protein as a docking protein, Dok. Cell 1997;88:205-11.
38. Salgia R, Uemura N, Okuda K, et al. CRKL links p210BCR/ABL with paxillin in chronic myelogenous leukemia cells. J Biol Chem 1995; 270:29145-50.
39. de Jong R, ten Hoeve J, Heisterkamp N, Groffen J. Crkl is complexed with tyrosine-phosphorylated Cbl in Ph-positive leukemia. J Biol Chem 1995;270:21468-71.
40. Afar DE, Han L, McLaughlin J, et al. Regulation of the oncogenic activity of BCR-ABL by a tightly bound substrate protein RIN1. Immunity 1997;6:773-82.
41. Mandanas RA, Leibowitz DS, Gharehbaghi K, et al. Role of p21 RAS in p210 bcr-abl transformation of murine myeloid cells. Blood 1993;82: 1838-47.
42. Okuda K, Matulonis U, Salgia R, Kanakura Y, Druker B, Griffin JD. Factor independence of human myeloid leukemia cell lines is associated with increased phosphorylation of the proto-oncogene Raf-1. Exp Hematol 1994;22:1111-7.
43. 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.
44. 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;92:11746-50.
45. Sawyers CL, Callahan W, Witte ON. Dominant negative MYC blocks transformation by ABL oncogenes. Cell 1992;70:901-10.
46. Shuai K, Halpern J, ten Hoeve J, Rao X, Sawyers CL. Constitutive activation of STAT5 by the BCR-ABL oncogene in chronic myelogenous leukemia. Oncogene 1996;13:247-54.
47. Carlesso N, Frank DA, Griffin JD. Tyrosyl phosphorylation and DNA binding activity of signal transducers and activators of transcription (STAT) proteins in hematopoietic cell lines transformed by Bcr/Abl. J Exp Med 1996;183:811-20.
48. 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;271:31704-10.
49. 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.
50. Sawyers CL, McLaughlin J, Witte ON. Genetic requirement for Ras in the transformation of fibroblasts and hematopoietic cells by the Bcr-Abl oncogene. J Exp Med 1995;181:307-13.
51. 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;16:6151-61.
52. Dickens M, Rogers JS, Cavanagh J, et al. A cytoplasmic inhibitor of the JNK signal transduction pathway. Science 1997;277:693-6.
53. Afar DEH, Goga A, McLaughlin J, Witte ON, Sawyers CL. Differential complementation of Bcr-Abl point mutants with c-Myc. Science 1994; 264:424-6.
54. Hehlmann R, Heimpel H, Hasford J, et al. Randomized comparison of interferon-α with busulfan and hydroxyurea in chronic myelogenous leukemia. Blood 1994;84:4064-77.
55. Ohnishi K, Ohno R, Tomonaga M, et al. A randomized trial comparing interferon-α with busulfan for newly diagnosed chronic myelogenous leukemia in chronic phase. Blood 1995;86:906-16.
56. Guilhot F, Chastang C, Michallet M, et al. Interferon alpha-2b combined with cytarabine versus interferon alone in chronic myelogenous leukemia. N Engl J Med 1997;337:223-9.
57. Horowitz MM, Rowlings PA, Passweg JR. Allogeneic bone marrow transplantation for CML: a report from the International Bone Marrow Transplant Registry. Bone Marrow Transplant 1996;17:Suppl 3:S5-S6.
58. 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.
59. Clift RA, Anasetti C. Allografting for chronic myeloid leukaemia. Baillieres Clin Haematol 1997;10:319-36.
60. McGlave P, Bartsch G, Anasetti C, et al. Unrelated donor marrow transplantation therapy for chronic myelogenous leukemia: initial experience of the National Marrow Donor Program. Blood 1993;81:543-50.
61. McGlave P, Kollman C, Shu XO, et al. The first 1000 unrelated donor transplants for CML: lessons from the National Marrow Donor Program (NMDP) experience. Blood 1996;88:Suppl 1:483a. abstract.
62. Hansen JA, Gooley TA, Martin PJ, et al. Bone marrow transplants from unrelated donors for patients with chronic myeloid leukemia. N Engl J Med 1998;338:962-8.
63. Thomas ED, Clift RA, Fefer A, et al. Marrow transplantation for the treatment of chronic myelogenous leukemia. Ann Intern Med 1986;104: 155-63.
64. O'Brien SG. Autografting for chronic myeloid leukaemia. Baillieres Clin Haematol 1997;10:369-88.
65. Weiden PL, Sullivan KM, Flournoy N, Storb R, Thomas ED, Seattle Marrow Transplant Team. Antileukemic effect of chronic graft-versus-host disease: contribution to improved survival after allogeneic marrow transplantation. N Engl J Med 1981;304:1529-33.
66. Goldman JM, Gale RP, Horowitz MM, et al. Bone marrow transplantation for chronic myelogenous leukemia in chronic phase: increased risk for relapse associated with T-cell depletion. Ann Intern Med 1988;108: 806-14.
67. Kolb HJ, Mittermuller J, Clemm C, et al. Donor leukocyte transfusions for treatment of recurrent chronic myelogenous leukemia in marrow transplant patients. Blood 1990;76:2462-5.
68. Drobyski WR, Keever CA, Roth MS, et al. Salvage immunotherapy using donor leukocyte infusions as treatment for relapsed chronic myelogenous leukemia after allogeneic bone marrow transplantation: efficacy and toxicity of a defined T-cell dose. Blood 1993;82:2310-8.
69. Porter DL, Roth MS, McGarigle C, Ferrara JLM, Antin JH. Induction of graft-versus-host disease as immunotherapy for relapsed chronic myeloid leukemia. N Engl J Med 1994;330:100-6.
70. Mackinnon S, Papadopoulos EB, Carabasi MH, et al. Adoptive immunotherapy evaluating escalating doses of donor leukocytes for relapse of chronic myeloid leukemia after bone marrow transplantation: separation of graft-versus-leukemia responses from graft-versus-host disease. Blood 1995;86:1261-8.
71. Bocchia M, Korontsvit T, Xu Q, et al. Specific human cellular immunity to bcr-abl oncogene-derived peptides. Blood 1996;87:3587-92.
72. Nieda M, Nicol A, Kikuchi A, et al. Dendritic cells stimulate the expansion of bcr-abl specific $CD8^+$ T cells with cytotoxic activity against leukemic cells from patients with chronic myeloid leukemia. Blood 1998;91: 977-83.
73. Talpaz M, McCredie KB, Mavligit GM, Gutterman JU. Leukocyte interferon-induced myeloid cytoreduction in chronic myelogenous leukemia. Blood 1983;62:689-92.
74. The Italian Cooperative Study Group on Chronic Myeloid Leukemia. Interferon alfa-2a as compared with conventional chemotherapy for the treatment of chronic myeloid leukemia. N Engl J Med 1994;330:820-5.
75. Allan NC, Richards SM, Shepherd PC. UK Medical Research Council randomised, multicentre trial of interferon-alpha n1 for chronic myeloid leukaemia: improved survival irrespective of cytogenetic response. Lancet 1995;345:1392-7.
76. The Chronic Myeloid Leukemia Trialists' Collaborative Group. Interferon alpha versus chemotherapy for chronic myeloid leukemia: a meta-analysis of seven randomized trials. J Natl Cancer Inst 1997;89: 1616-20.
77. Friedman RL, Stark GR. alpha-Interferon-induced transcription of HLA and metallothionein genes containing homologous upstream sequences. Nature 1985;314:637-9.
78. Lardon F, Snoeck HW, Berneman ZN, et al. Generation of dendritic cells from bone marrow progenitors using GM-CSF, TNF-alpha, and additional cytokines: antagonistic effects of IL-4 and IFN-gamma and selective involvement of TNF-alpha receptor-1. Immunology 1997;91:553-9.
79. Choudhury A, Gajewski JL, Liang JC, et al. Use of leukemic dendritic cells for the generation of antileukemic cellular cytotoxicity against Philadelphia chromosome-positive chronic myelogenous leukemia. Blood 1997; 89:1133-42.
80. Lee SJ, Kuntz KM, Horowitz MM, et al. Unrelated donor bone marrow transplantation for chronic myelogenous leukemia: a decision analysis. Ann Intern Med 1997;127:1080-8.
81. Beelen DW, Graeven U, Elmaagacli AH, et al. Prolonged administration of interferon-alpha in patients with chronic-phase Philadelphia chromosome-positive chronic myelogenous leukemia before allogeneic bone marrow transplantation may adversely affect transplant outcome. Blood 1995;85:2981-90.
82. Giralt SA, Kantarjian HM, Talpaz M, et al. Effect of prior interferon alpha therapy on the outcome of allogeneic bone marrow transplantation for chronic myelogenous leukemia. J Clin Oncol 1993;11:1055-61.
83. Zuffa E, Bandini G, Bonini A, et al. Prior treatment with alpha-interferon does not adversely affect the outcome of allogeneic BMT in chronic phase chronic myeloid leukemia. Haematologica 1998;83:231-6.
84. Higano CS, Chielens D, Raskind W, et al. Use of alpha-2a-interferon to treat cytogenetic relapse of chronic myeloid leukemia after marrow transplantation. Blood 1997;90:2549-54.
85. Arcese W, Goldman JM, D'Arcangelo E, et al. Outcome for patients who relapse after allogeneic bone marrow transplantation for chronic myeloid leukemia. Blood 1993;82:3211-9.
86. Sharp JC, Joyner MV, Wayne AW, et al. Karyotypic conversion in Ph1-positive chronic myeloid leukaemia with combination chemotherapy. Lancet 1979;1:1370-2.
87. Smalley RV, Vogel J, Huguley CM Jr, Miller D. Chronic granulocytic leukemia: cytogenetic conversion of the bone marrow with cycle-specific chemotherapy. Blood 1977;50:107-13.
88. Carella AM, Chimirri F, Podesta M, et al. High-dose chemo-radiotherapy followed by autologous Philadelphia chromosome-negative blood progenitor cell transplantation in patients with chronic myelogenous leukemia. Bone Marrow Transplant 1996;17:201-5.
89. McGlave PB, De Fabritiis P, Deisseroth A, et al. Autologous transplants for chronic myelogenous leukaemia: results from eight transplant groups. Lancet 1994;343:1486-8.
90. Reiffers J, Goldman J, Meloni G, Cahn JY, Gratwohl A. Autologous stem cell transplantation in chronic myelogenous leukemia: a retrospective analysis of the European Group for Bone Marrow Transplantation. Bone Marrow Transplant 1994;14:407-10.
91. Deisseroth AB, Zu Z, Claxton D, et al. Genetic marking shows that Ph+ cells present in autologous transplants of chronic myelogenous leukemia (CML) contribute to relapse after autologous bone marrow in CML. Blood 1994;83:3068-76.
92. de Fabritiis P, Amadori S, Petti MC, et al. In vitro purging with BCR-

ABL antisense oligodeoxynucleotides does not prevent haematologic reconstitution after autologous bone marrow transplantation. Leukemia 1995;9:662-4.
93. Gewirtz AM. Treatment of chronic myelogenous leukemia (CML) with c-myb antisense oligodeoxynucleotides. Bone Marrow Transplant 1994;14:Suppl 3:S57-S61.
94. Barnett MJ, Eaves CJ, Phillips GL, et al. Autografting with cultured marrow in chronic myeloid leukemia: results of a pilot study. Blood 1994; 84:724-32.
95. Verfaillie CM, Bhatia R, Miller W, et al. BCR/ABL-negative primitive progenitors suitable for transplantation can be selected from the marrow of most early-chronic phase but not accelerated-phase chronic myelogenous leukemia patients. Blood 1996;87:4770-9.
96. Toren A, Ackerstein A, Slavin S, Nagler A. Role of interleukin-2 in human hematological malignancies. Med Oncol 1995;12:177-86.
97. Cortez D, Stoica G, Pierce JH, Pendergast AM. The BCR-ABL tyrosine kinase inhibits apoptosis by activating a Ras-dependent signaling pathway. Oncogene 1996;13:2589-94.
98. Gibbs JB, Oliff A, Kohl NE. Farnesyltransferase inhibitors: Ras research yields a potential cancer therapeutic. Cell 1994;77:175-8.