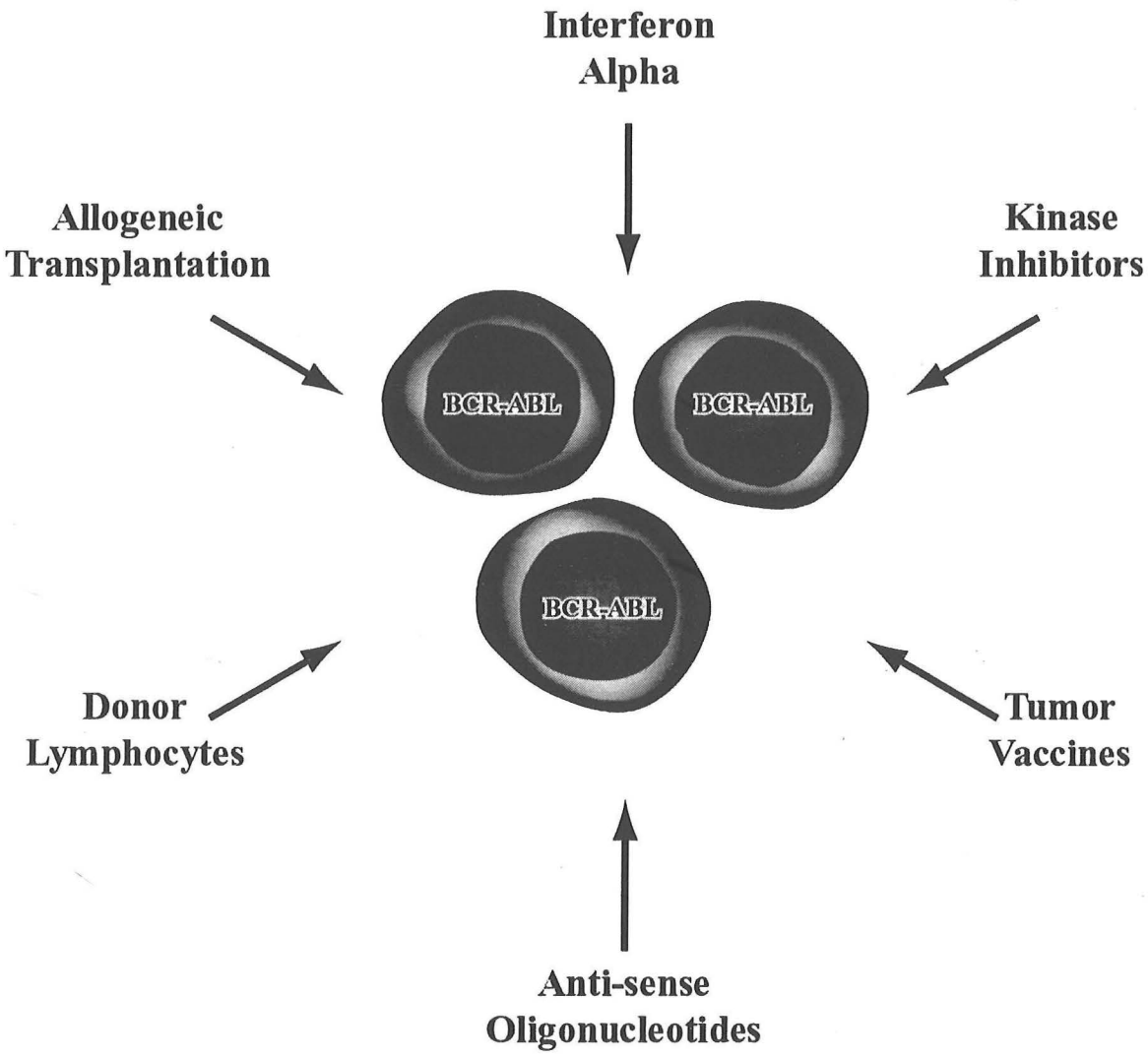
Chronic Myelogenous Leukemia: A Paradigm for Novel Molecular and Immunologic Approaches for Cancer Treatment



Internal Medicine Grand Rounds

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Epidemiology

CML accounts for 7% to 15% of all leukemias in adults, with approximately 1 to 1.5 cases per 100,000 population (16, 20). There is a male predominance, with a male to female ratio of 1.5 to 1. The incidence of CML has remained steady for the last 50 years. The median age at presentation is 50 to 60 years, but the disease occurs in all age groups (53). In early reports, more than 50% of patients were age 60 years and older, but this incidence has decreased in more recent studies to as low as 12% (77). This is likely due to the strict criteria of the Philadelphia chromosome positive disease and the exclusion of patients with other myeloproliferative disorders, Philadelphia chromosome (Ph)-negative CML and chronic myelomonocytic leukemia (CMML) (21, 28, 38).

Etiology

The underlying etiology of CML is unknown. There is little evidence for specific genetic predisposition for the development of CML. Children of parents with CML do not have a higher incidence of CML than in the general population. There is also no correlation in monozygotic twins, suggesting that CML is an acquired disorder. There may, however, be some correlation with the development of CML and the presence of the HLA antigens CW3 and CW4 (15). Survivors of the atomic disasters at Nagasaki and Hiroshima were reported to have a significantly higher incidence of CML, although this was not confirmed by cytogentic studies because the reports preceded the discovery of the Philadelphia chromosome. Thus many of these patients may have had CMML or other myelodysplastic syndromes (88). Therapeutic radiation has also been associated with increased risk of CML, as observed in some patients with ankylosing spondylitis given spinal radiation and women with uterine cervical cancer treated with radiation therapy (14, 17). Exposure to specific chemicals have not been associated with an increased risk for the development of CML.

Definitions of Accelerated and Blastic Phases of CML (78)

Accelerated phase CML
Multivariate analysis-derived criteria
Peripheral blasts 15% or more
Peripheral blasts plus promyelocytes 30% or more
Peripheral basophils 20% or more
Thrombocytopenia <100 X 10^9 /L unrelated to therapy
Cytogenetic clonal evolution
Other criteria used in common practice
Increasing drug dosage requirement
Splenomegaly unresponsive to therapy
Marrow reticulin or collagen fibrosis
Marrow or peripheral blasts ≥ 10%
Marrow of peripheral basophils ± eosinophils ≥ 10%
Triad of WBC>50 X 10^9 /L, hematocrit <25%, and platelets<100 X 10^9 /L not controlled with therapy
Persistent unexplained fever or bone pains
Blastic phase CML
30% or more blasts in the marrow or peripheral blood
Extramedullary disease with localized immature blasts

Definitions of CML Phases

Definitions of the different phases of CML is important in order to determine the appropriate therapeutic intervention for patients (77). CML usually has a biphasic, and sometimes triphasic, course. The disease presents in an indolent or chronic phase, which after 2 to 6 years of conventional therapy, evolves into an accelerated phase that lasts for less than 1 to 1.5 years. The accelerated phase is followed by the blast phase, which results in the patient's death within t. Twenty percent to 25% of patients die during the accelerated phase, and another 20% to 25% progress directly from chronic to blast phase without a discernible accelerated phase (97). Standard definitions of the accelerated and blastic phases of CML have been proposed (75, 77, 81).

Clinical Presentation

CML is frequently asymptomatic in the chronic phase of the disease. The incidence of asymptomatic cases has increased over the last decade from 15% to about 45% of all cases, due to diagnosis by routine blood counts (54, 62, 77). Patients with symptoms usually have a gradual onset of fatigue, anorexia, weight loss, increased sweating, left upper quadrant discomfort, and early satiety because of splenomegaly. The magnitude of splenomegaly correlates well with the total body granulocyte mass and the blood granulocyte count. The degree of splenomegaly may be an indication of the duration of the chronic phase of the disease with gross splenomegaly predicting a shorter time for the development of the blast phase. Splenomegaly was documented in approximately 70% of patients in older reports, but it has decreased to 50% in more recent studies. Hepatomegaly is less common (10% to 40% of patients). Lymphadenopathy is uncommon in chronic phase CML, and its appearance suggests either accelerated or blastic phase disease (73, 75). Rare patients with very high WBC counts may have manifestations of hyperviscosity, including priapism, tinnitus, stupor, visual changes from retinal hemorrhages, and cerebrovascular accidents (135, 137).

Presenting Features of Patients with Chronic Phase of CML (48)

Presenting Features	Patients at the University of Texas M.D. Anderson Cancer Center	Patients at Hammersmith Hospital (United Kingdom)
	%	
Age ≥ 60 y	15	0.2
Asymptomatic presentation	45	20
Hepatomegaly	9	2
Splenomegaly	48	76
Hemoglobin level < 120 g/L	45	62
Leukocyte count ≥ 100 cells x 10^9/L	52	72
Platelet count > 700 cells x 10^9/L	15	34
Peripheral blood blasts	52	NA
Peripheral basophils ≥ 7%	14	NA
Marrow blasts ≥ 5%	6	NA
Marrow basophils ≥ 3%	26	NA

The accelerated phase of CML is a somewhat ill defined transitional phase (73). It is occasionally asymptomatic and the diagnosis is made based on increased blasts in the peripheral blood and bone marrow. Some patients may have fever and night sweats, as well as progressive enlargement of the spleen (73). At least 20% of chronic phase patients develop a blast phase without evidence of an accelerated phase.

Poor Prognostic Factors in CML (78)

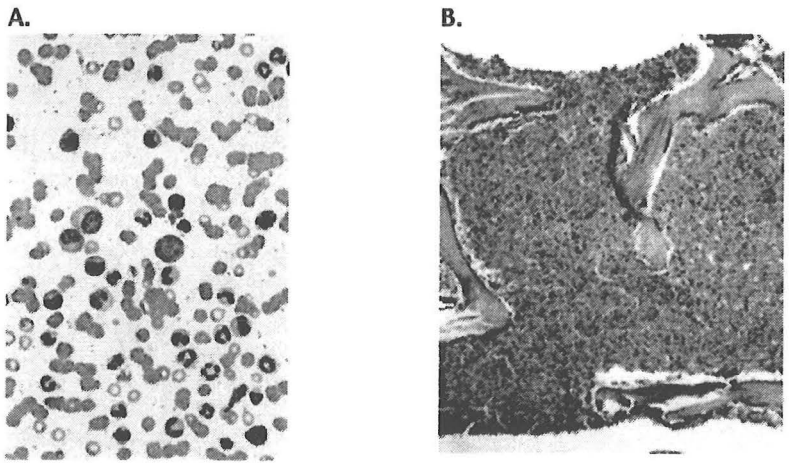
Clinical
Older age
Symptoms at diagnosis
Significant weight loss
Hepatomegaly
Splenomegaly
Poor performance
Black race
Laboratory
Anemia
Thrombocytosis, thrombocytopenia, megakaryocytopenia
Increased basts, or blasts + promyelocytes in blood or marrow
Increased basophils in blood or marrow
Collagen or reticulin fibrosis grade 3-4
Treatment-associated
Longer time to achieve hematologic remission with busulfan chemotherapy
Short remission duration
High total dose of busulfan or hydroxyurea therapy required in the first year to control the disease
Poor initial hematologic or cytogenetic response to interferon-alpha therepy

The blastic phase CML resembles acute leukemia (61, 75, 87, 152). Its diagnosis requires the presence of at least 30% of blasts in the bone marrow or peripheral blood. Patients in the blast phase are more likely to have symptoms, including weight loss, fever, night sweats, and bone pains (75). Symptoms of anemia, infectious complications, and bleeding are common and signs of CNS leukemia may be seen particularly with lymphoid blast transformation (30% incidence). In some patients the blastic phase is characterized by extramedullary deposits of leukemia called myeloblastomas or chloromas (72, 143). These usually appear in the CNS, lymph nodes, or bones, and occasionally they occur in the absence of blood or bone marrow evidence of blastic transformation (72, 75). Most of these patients develop hematologic manifestations within a few months (143). Patients in blastic phase usually die within 3 to 6 months. The major cell detected in the peripheral blood in the blastic phase is myeloid in approximately 50% of patients, lymphoid in 25% and undifferentiated in 25%. Patients with lymphoid blastic phase respond to therapy used to treat acute lymphoblastic leukemia (50% to 60% of the time). Although their median survival is better as compared with myeloid or undifferentiated cases (9 months versus 3 months) however, the prognosis for all patients with blastic phase CML is still very poor (37, 44).

Laboratory Features

The most common peripheral blood feature of CML is an elevated WBC count, usually above 25 x 10^9 /L, and frequently above 100 x 10^9 /L. (135). Some patients have wide cyclic variations in the WBC count of up to an order of magnitude in 50- to 70-day cycles (70). At diagnosis, circulating BFU-E and CFU-GM progenitor numbers in CML may be increased up to 180-fold and 9000-fold, respectively. Leukostasis is a particular problem in 60% of childhood cases, reflecting the very high WBC in children with Ph-positive CML. The platelet count is elevated in 30% to 50% of patients, and it may be greater than 1000 x 109 /L in some patients (98, 140). Although platelet function is frequently abnormal *in vitro* most frequently with a decreased secondary aggregation in response to epinephrine, this is not usually associated with bleeding. Most patients have mild anemia at diagnosis, but untreated patients may be severely anemic. Patients in chronic phase do not have an increased risk for infections, although *in vitro* neutrophil function abnormalities are common (29, 155). Marrow hyperplasia of myeloid cells in CML is caused by progenitor cell expansion, a slower cell cycle, prolonged maturation-division times, and delayed compartmental transit. The WBC differential usually shows granulocytes in all stages of maturation, from blasts to mature granulocytes, which look morphologically normal. Basophils are usually elevated, but only 10% to 15% of patients have at least 7% basophils in peripheral blood. A very high proportion of basophils in the peripheral blood (ie, at least 20%) is usually associated with accelerated phase disease (73). Eosinophils are also frequently elevated, although to a lesser degree.

Peripheral Blood and Bone Marrow in CML (129)



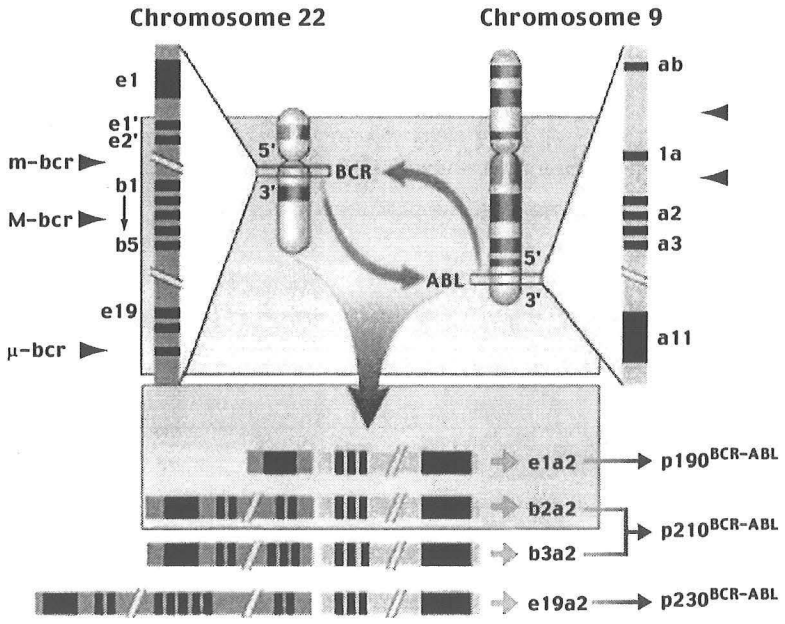
The bone marrow in the chronic phase of CML is hypercellular, with a cellularity of 75% to 90%, and very scarce fat (83). The myeloid to erythroid ratio is 10:1 to 30:1, rather than the normal 2:1 to 5:1. Bands plus segmented neutrophils, metamyelocytes, and the combined numbers of myeloblasts, promyelocytes, and myelocytes occur in equivalent proportions, demonstrating a marked shift toward myeloid immaturity. Megakaryocytic hyperplasia is common, and dysplastic changes variably affect all cell lines. About 30% of CML patients develop focal or diffuse increases in marrow reticulin fibers (reticulin fibrosis) early in the disease, and some 20% develop extensive new collagen formation (collagen fibrosis).

LAP activity is reduced in nearly all patients at diagnosis (125). Serum vitamin B12 levels are increased up to 10 times the normal levels in proportion to the amounts of transcobalamin I and III released during breakdown of CML granulocytes. Increased production of uric acid, with hyperuricemia and hyperuricosuria, is common in untreated CML. Serum levels of lactic dehydrogenase are also frequently elevated.

Molecular Analysis

The molecular diagnosis of CML is based on the detection of the Philadelphia (Ph) chromosome which is a translocation of chromosomes 9 and 22 (106, 126) t(9;22), two exellent reviews of this subject have recently been written (49, 129). This translocation is present in 95 percent of the patients with CML. Another 5 percent have complex or variant translocations. However, the result of these translocations is the fusion of the BCR (breakpoint cluster region) gene on chromosome 22 to the ABL (Ableson leukemia virus) gene on chromosome 9. This translocation is not limited to myeloid cells, but is also found in erythroid, megakaryocytic, and B lymphocytes. Thus CML is a stem-cell rather than a myeloid specific disease. During the development of blast crisis, a variety of additional chromosomal changes develop including duplication of the Ph chromosome and trisomy 8 (11). In addition, mutations or deletions of tumor-suppressor genes including p16 (132) and p53 (2) also occur with variable frequency late in the disease and likely contribute to the pathogenesis of blast crisis.

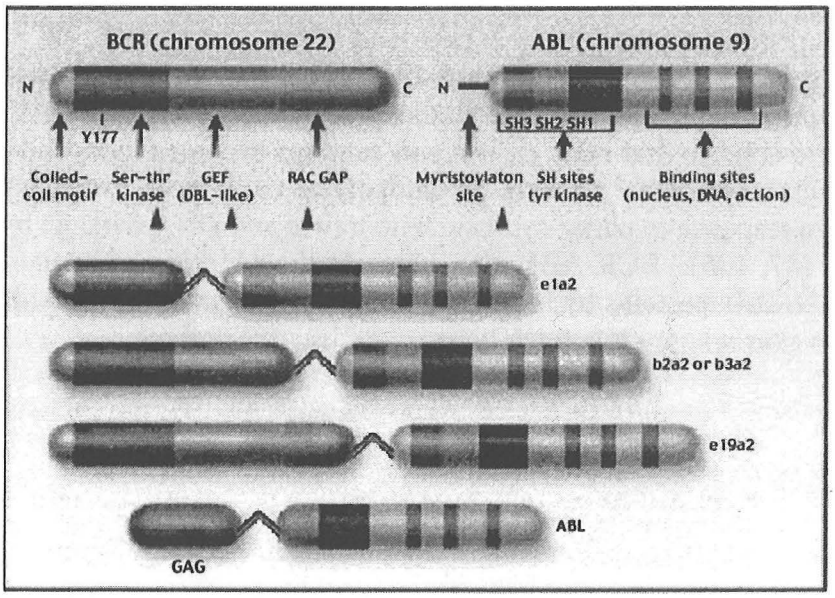
The Translocation of t(9;22)(q34;q11) in CML (49)



The result of the t(9;22) translocation is the generation of a 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 protein differs in the length of BCR sequence retained at the N terminus, but encodes the same portion of the ABL tyrosine kinase. Nearly all patients with

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. A 230-kd BCR-ABL fusion protein is found in a subgroup of patients with CML who present with a lower white-cell count than typical CML patients and in whom progression to blast crisis is slow (114). Laboratory studies of the biologic activity of these proteins 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 or 230 kd proteins Thus BCR-ABL fusion proteins of different sizes can be correlated with different biological activities with the magnitude of the tyrosine kinase signal likely correlating with the clinical outcome of the disease (95, 150).

Functional Domains of p160BCR, p145ABL, and p210BCR-ABL (49)



Highly sensitive and specific molecular BCR-ABL probes are useful for monitoring responses to therapy. Quantitative cytogenetic information can be obtained by fluorescence in situ hybridization (FISH) without the need to culture cells or analyze cells in metaphase (144). Polymerase-chain-reaction (PCR) testing of peripheral-blood RNA is highly sensitive resulting in the detection of 1 Ph-positive cell expressing the BCR-ABL fusion transcript present in 10^6 normal cells (30). Thus the response of CML to treatment can now be based on the hematologic, cytogenetic, and molecular criteria. 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.

Negative PCR results in patients treated by allogenic bone marrow transplantation clearly predict a favorable outcome (68, 118). However, the results of PCR assays can remain positive in interferon-treated patients who are in complete cytogentic remission and patients who have survived for several years after bone marrow transplantation, two subgroups with very favorable outcomes (66, 103). This is likely due to remaining small numbers of leukemic cells. Quantitative PCR assays are now being performed which

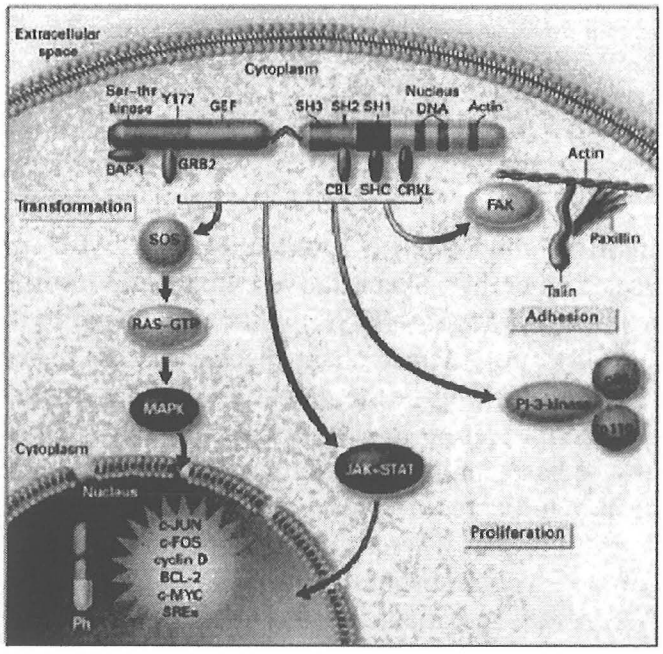
permit quantitation of the level of BCR-ABL messenger RNA transcripts. Using this assay, a progressive increase in BCR/ABL RNA levels in patients minimal residual disease after allogeneic transplantation appears to predict eventual relapse (30). It is likely that this quantitative PCR assay will become the standard in determining the clinical course of CML.

Mechanisms of BCR-ABL Leukemogenesis

The mechanism by which BCR-ABL results in leukemia has been studied in both mice models and by biochemical techniques. For example, transgenic mice containing the 190-kd BCR-ABL protein result in animals with acute leukemia at birth (64). These mice also contain secondary chromosomal abnormalities analogous to blast-crisis cells in humans (151). Retroviral-mediated transfer of the BCR-ABL gene into hematopoietic stem cells of normal mice results in the generation of acute and chronic myeloid leukemias depending on the genetic background of the mice (31, 46, 82).

The effects of overexpression of BCR-ABL on the growth and cellular transformation of hematopoietic cells has also been analyzed. BCR-ABL can transform hematopoietic cells so that their growth and survival becomes independent of cytokines (58, 100). Its expression protects hematopoietic cells from programmed cell death (apoptosis) in response to either cytokine withdrawal and DNA damage by chemotherapy or radiation (47, 105). BCR-ABL also increases the adhesion of hematopoietic cells to extracellular-matrix proteins by increasing the activity of integrin (6) which may localize these cells to sites where growth inhibitory cytokines are present.

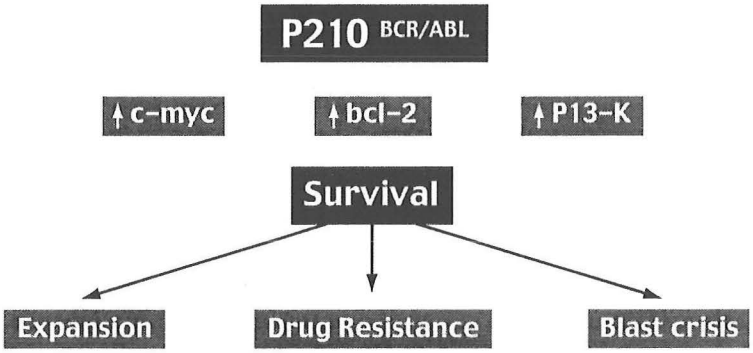
Signaling Pathways of p210BCR-ABL (49)



The BCR-ABL protein is a constitutively active tyrosine kinase which is present in the cytoplasm, whereas the wild-type ABL protein shuttles between the nucleus and cytoplasm (92, 146). The BCR-ABL protein can thus phosphorylate a number of

cytoplasmic proteins due to its increased tyrosine kinase activity, thereby activating multiple signal-transduction pathways that affect the growth and differentiation of cells. The substrates include CRKL (104, 110, 142), p62Dok (24, 154), paxillin (128), CBL (34) and RIN (1). These substrates are involved in activating a number of critical signalling pathways including RAS (96), RAF (113), phosphatidylinositol-3 kinase (133), JUN kinase (119), MYC (130), and STAT (23, 69, 131). Thus the BCR-ABL protein activates the same signaling cascades that are activated by cytokines and are involved in the control of growth and differentiation of normal hematopoietic cells. Since the BCR-ABL exhibits constitutive tyrosine kinase activity, cells with this translocation exhibit enhanced growth properties and become leukemic.

Role of $p210^{BCR-ABL}$ Protein in Leukemogenesis (148)



Cytotoxic therapy

As treatment for CML has improved, the goals of therapy have changed markedly. In more than 80% of chronic phase CML patients, hydroxyurea and other cytotoxic agents have the ability to control the signs and symptoms of CML caused by the myeloid hyperplasia, leukocytosis, and organomegaly (53, 63). However, these agents have little or no effect on progression of the disease into blast transformation. All patients receiving traditional cytotoxic therapy will eventually evolve into blast phase and succumb to their disease after a median survival of 3 to 6 years.

Criteria for Response to Therapy in CML (78)

RESPONSE	CATEGORY	CRITERIA
Hematologic remission	Complete	Normalization of WBC counts to <9 x 10⁹/L with normal differential
		Normalization of platelet counts to <450 x 10⁹/L
		Disappearance of all signs and symptoms of disease
	Partial	Normalization of WBC with persistent immature peripheral cells, or splenomegaly or thrombocytosis at <50% pretreatment level
Cytogenetic response	Complete	No evidence of Ph-positive cells
	Partial	1% to 34% of metaphases Ph-positive
	Minor	35% to 90% of metaphases Ph-positive
	None	All analyzable cells Ph-positive

Hydroxyurea is a cycle-specific inhibitor of DNA synthesis that has been used to treat CML since 1972 (127). Hydroxyurea gives a rapid but relatively transient control of the hematologic manifestations of CML and patients requires frequent follow-up. It is usually given at a dose of 20-30 mg/kg and in an attempt to keep the WBC at approximately 2 x 10^9. Hydroxyurea is very well tolerated by most patients and has very few side effects. Prolonged treatment with hydroxyurea causes red cell macrocytosis and megaloblastic changes in the marrow due to its effects on inhibiting DNA synthesis. Hydroxyurea and another agent busulfan can both control the hematologic manifestations of the disease in more than 80% of CML patients. A large randomized study of 458 patients prospectively compared these two agents in chronic phase CML (63). Patients randomized to hydroxyurea therapy had a significantly longer median survival (56 versus 44 months) than did the patients who received busulfan. The survival advantage conferred by hydroxyurea was evident in all prognostic subgroups. The median duration of chronic phase in the hydroxyurea cohort was significantly longer (47 versus 37 months), than in the busulfan cohort. However, no patients achieved a complete cytogenetic response to either agent. There were no serious adverse events with hydroxyurea, in contrast to serious adverse events including prolonged marrow aplasia or pulmonary toxicity in 6% of patients receiving busulfan. Therefore, hydroxyurea is clearly better in controlling CML giving less toxicity and a more prolonged survival as compared to busulfan. However, neither agent induces cytogenetic remission or significantly delays the time to the development of blast phase.

Chemotherapeutic Drugs Used to Treat the Chronic Phase of CML (129)

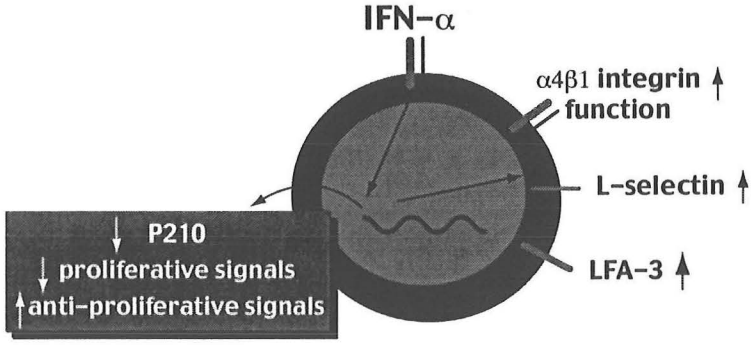
DRUG	DOSE	ADVERSE EFFECTS
Hydroxyurea	0.5–2.0 g/day orally	Cytopenias, rash, nausea
Busulfan	2.0–6.0 mg/day orally	Cytopenias, rash, bone marrow aplasia
Interferon alfa	5 million U/m²/day subcutaneously	Fever, myalgias, rash, depression, thrombocytopenia
Interferon alfa plus cytarabine	Interferon alfa, 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

Interferon-alpha

Interferon-alpha (IFNα) therapy prolongs survival and delays the progression to the blast phase in patients with CML when compared with therapy using either hydroxyurea or busulfan (3, 91, 112). The dose of IFNα used may be important for obtaining complete cytogenetic response. Patients receiving less than 5 MU/m^2 three times a week have less than 10% incidence of major cytogenetic remissions, as opposed to a 40% likelihood of a major cytogenetic response if patients 5 MU/m^2 daily. However,

IFNα toxicity increases with dose. Patients who develop serious toxicities must discontinue IFNα until they resolve, and therapy can then be reinstituted with a 50% dose reduction. Moderate chronic toxicities such as a WBC less than 2 x 10^9 or a platelet count less than 50 x 10^9 may be alleviated by a 25% reduction of the dose of IFNα.

Mechanisms Underlying Therapeutic Effects of Interferon-alpha (148)



Although major cytogenetic remissions induced by IFNα therapy are durable, it is uncertain how long they last after discontinuation of IFNα therapy. One approach is to continue IFNα therapy until a complete cytogenetic response is seen and PCR negativity is documented for 3 years. About 40% of such patients continue in complete remission at a median of 40 months off such therapy (39, 89).

Response to IFN-alpha by CML Phase (78)

PHASE	CHR (%)	CYTOGENETIC RESPONSE (%)	
		ANY	MAJOR
Early chronic	60-80	40-50	20-35
Late chronic	40-60	10-20	<10
Accelerated	20-30	<10	0
Blastic	<10-20	<10	0
CHR = Complete hematologic remission			

Initial studies of combining IFNα with cytotoxic agents were conducted to investigate whether patients who failed to achieve a cytogenetic remission to IFNα alone might do so with combined therapy. Furthermore, it was important to determine whether cytogenetic remission rates might be improved by combination therapy. Since ara-C selectively suppresses the growth of CML cells over that of normal hematopoietic cells *in*

vitro (134), combinations of IFNα and ara-C were investigated in patients with late chronic phase disease (55, 74).

A combination of daily IFN-α (5 MU/m2) and low-dose ara-C in different schedules (10 mg/day or 20 mg/m2 /day for 10 days) was well tolerated and associated with cytogenetic and clinical results similar to those seen in CML patients who receive therapy with IFN-α alone. However, lowering the dose of IFN-α in regimens with ara-C may be associated with a lower major cytogenetic response rate. A current International Oncology Study Group (IOSG) randomized study comparing IFN-α /HU with IFN-α /ara-C in early chronic phase CML is now underway to better evaluate the efficacy of ara-C in combination with IFN-α.

Cytogenetic and Hematologic Responses to Interferon-α Plus or Minus Cytarabine, Results of Three M.D. Anderson Cancer Center Phase II Studies (76)

Response	IFN-α +Daily low dose cytarabine [N=134]	IFN-α + Intermittent low dose cytarabine [N=45]	IFN-α alone [N=274]
Complete hematologic response	92	84	80
Cytogenetic response (overall)	74	73	58
CR	31 } 50%	20 } 38%	26 } 38%
PR	19	18	12
Minor response	24	33	20
Median follow-up (mo)	42	52	65

Allogeneic Transplantation

Allogeneic stem cell transplantation (alloSCT) is the only form of treatment for chronic myelogenous leukemia (CML) with a prospect of cure in the majority of patients. Several advances in the past two decades have made CML the most frequent indication for allogeneic stem cell transplantation (60, 124). Improved control of complications such as graft-versus-host disease (GVHD) has resulted from treatment with cyclosporin A and methotrexate (136) and by depletion of T-cells from the graft (59). Supportive treatment for prophylaxis of viral infections reduces the risk of complication from allografts further (102). The most important need to make more patients with CML eligible for transplantation is to further expand large registries of HLA-typed volunteer donors. In the last decade, the number of registered donors has increased worldwide from about 100,000 donors to more than 6 million. As a consequence the likelihood of finding a suitable donor has increased dramatically in the last decade (7) (National Marrow Donor Program Report 1998). Moreover, the methods for matching unrelated donors with patients has improved through the use of high resolution typing of DNA. Approximately 35% of Caucasian patients, 33% of American Indian/Alaskan, and 31% of Hispanic, but only 24% of Asian/Pacific and 22% of African/American proceed from search from a donor to transplantation. The median age of patients with CML is approximately 50 years so that increasing the age of patients has increased the proportion of patients grafted for the treatment of CML.

Results of Allogeneic Bone Marrow Transplantation in Patients with CML in Chronic Phase (129)

STUDY AND TYPE OF DONOR	No. OF PATIENTS	DURATION OF FOLLOW-UP	SURVIVAL	RELAPSE
		yr	percent	
HLA-matched related donor				
IBMTR	2231	3	57	13
EBMT	373	8	54	19
Clift and Anasetti	351	>10	70	20
HLA-matched unrelated donor				
NMD	779	3	40	5
IBMTR	331	3	38	NA
Hansen et al.	196	5	57	NA

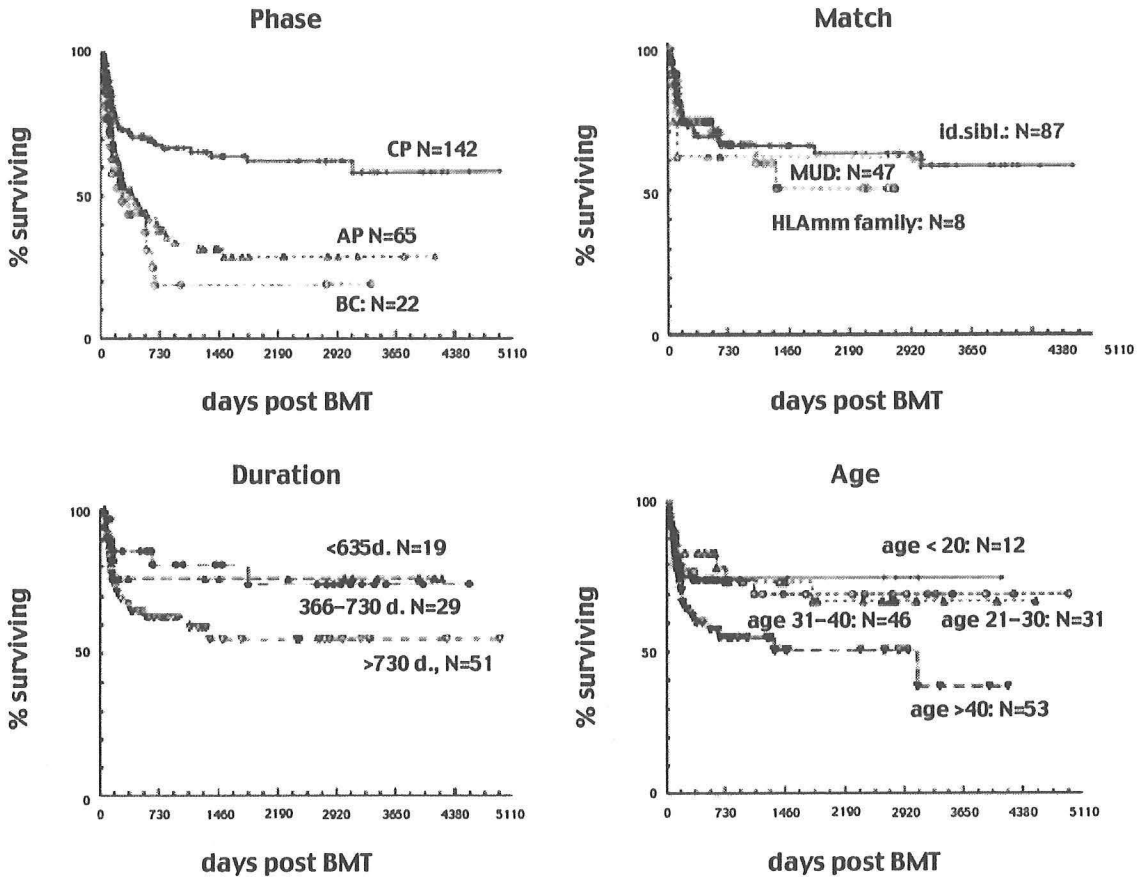
IBMTR denotes International Bone Marrow Transplant Registry, EBMT European Group for Blood and Marrow Transplantation, NMDP National Marrow Donor Program, and NA not available
This study was performed at the Fred Hutchinson Cancer Center in Seattle

The success of allogeneic stem cell and marrow transplantation for CML is dependent on the histocompatibility of the donor and host, the stage of the disease at the time of transplantation, the age and sex of the donor and host, and the time from diagnosis to transplantation. Adverse risk factors are patients with CML in the accelerated phase or blast crisis, less than a fully matched donor, age over 40 years, a female donor for a male patient, and transplantation more than a year from the diagnosis. Patients with 0 or 1 risk factors had a 5-year survival of 70-72%, while the survival of patients with 5 or 6 risk factors was only 18-22%. In transplantation from unrelated donors the age of the patient, matching of the HLA-DR locus, the time from diagnosis to transplant, obesity, and CMV status are risk factors.

Relapse and Survival of CML Patients Transplanted in First Chronic Phase (84)

Donor	N. Eval.	Relapse%	Survival%
Twin	49	51	86
HLA-identical sibling	4630	17	65
Unrelated	1234	18	46
HLA-identical, T-cell depleted	281	45	64

Survival of Patients Treated with Allogeneic Marrow Transplantation for CML (84)



Between 20 and 30% of patients with CML have an HLA-identical sibling as donor. Patients up to the age of 55 years are evaluated for transplantation, if the patient does not have additional serious diseases. About one half of the patients with CML treated with allogeneic transplants remain free of leukemia. However, relapses may occur late, more than 10 years after transplantation (45). The results of these unrelated donor transplants have improved considerably, due to better immunosuppressive agents and to better HLA-typing using high-resolution DNA probes. In several recent studies the results of unrelated transplants were not markedly worse than those of related donors. The most important factors for transplants using unrelated donor is that the disease is in the chronic phase and the age of the patient is less than 50 years of age (75% vs 45% chance of survival).

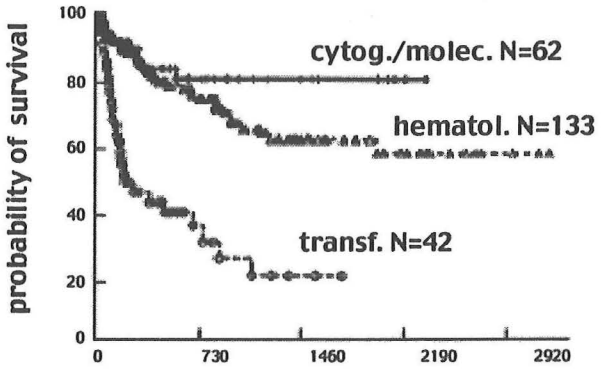
Donor Lymphocyte Transfusions

Treatment of patients with CML who relapse following an allogenic transplant sometimes respond to donor lymphocyte transfusions. Donor lymphocytes from HLA-identical sibling donors (85) can sometimes have marked effects on relapsed CML. Two large studies confirmed this experience with the transfusion of donor lymphocytes for the treatment of recurrent leukemia after allogeneic stem cell transplantation (27, 86). Best results were seen in patients with hematological relapse in chronic phase of the disease or

those who develop either a cytogenetic or molecular relapse. Intermediate results were seen in patients with accelerated or blast phase of CML. Graft versus host disease develops in about 52% of patients treated with donor lymphocyte transfusion and in 36% of the patients the graft-versus-host disease is severe enough to require treatment with immunosuppressive agents. Another complication of donor lymphocyte transplant is myelosuppression, which is transient in some patients but severe in up to 20% of patients. Factors that favorably influence the remission rate in patients with CML include patients with cytogenetic or hematological relapse rather than those I nthe blast phase, chronic phase of the disease at the time of transplantation, more than one year of remission after transplantation, the presence of donor-host chimerism, and the absence of chronic GVHD after transplantation. The potential role of combinations of IFNα and donor lymphocyte transfusions on patients with relapsed CML remains to be determined.

Survival after donor lymphocyte transfusion may be as good as that after transplantation. In certain settings, the survival probability for patients with a hematological relapse is 58% at 8 years and that for patients with a cytogenetic relapse is 80% at 6 years. Some patients who have a second relapse of CML will respond favorably to a second treatment with donor lymphocyte transfusion. However, after a single donor lymphocyte transfusion most patients become and remain negative for BCR/ABL by RTPCR (93).

Survival of patients treated with donor lymphocyte transfusion for recurrent CML after allogeneic transplantation (84)



The graft versus leukemia effect of donor lymphocyte transfusion is not well understood. Cells that may be involved in these effects include T-cells, natural killer cells (NK), macrophages and dendritic cells. The cells may recognize leukemia-specific antigens, histocompatibility antigens or other antigens present on leukemia cells only. The clinical response to donor lymphocyte transfusion requires several weeks to occur, while the cytogenetic and molecular responses may take several months. The median time to a cytogenetic and a molecular response is 4 to 6 months with late responses occurring even more than a year after transfusion (147). Responses to donor lymphocytes are seen in all groups with an allogeneic donor but are not seen in syngeneic

twins who have served as donors for transplants with CML. This finding supports the view that the graft versus leukemia effects is directed against minor histocompatibility antigens on leukemic cells. A remission has also been induced in a patient in which donor T-cells were ex vivo expanded and selected for reactivity to CML cells (50).

Autologous Transplantation

A number of agents are currently being explored to treat patients with CML in the accelerated or blast phase (76). High-dose chemotherapy when followed by infusion of purified stem-cells from CML patients should theoretically provide a means to perform autologous transplants with Ph-negative stem cells. Stem cells that are Ph-negative are harvested during the recovery phase after induction chemotherapy, and then are infused following high-dose chemotherapy where they successfully can engraft, to result in Ph-negative hematopoiesis (22). However, Ph-positive hematopoiesis inevitably recurs, usually within the first year after transplantation, with a return to the chronic phase of CML (99, 123). 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 (36). This result has provided a rationale to purge stem-cell preparations of residual CML cells with antisense messenger RNA directed against either BCR-ABL (33) or the MYB gene (52), perform *in vitro* culture conditions that select against Ph-positive cells (5), or physically separating Ph-negative stem cells from Ph-positive stem cells (149). The clinical feasibility and safety of each of these strategies have been demonstrated but their therapeutic value remains to be proved.

Autografting when it is combined with effective purging strategies, is unlikely to result in long-term remissions in most patients. This is due to the fact that a graft-versus-leukemia effect does not develop in these patients as compared to patients who receive an autologous transplant. For example, the relapse rate is two to three times as high in patients who receive bone marrow transplants from their identical twins – compared to patients who receive HLA-matched transplants from siblings who were not their identical twin (26, 67). Thus, it is likely that patients who receive autografts for CML will require post-transplantation therapy to remain in remission. For example, treatment with IFNα may be able to induce Ph-negative hematopoiesis in a subset of patients who receive an autologous transplants. This is based on the fact that IFNα can induce remissions in some patients who relapse after allogeneic bone marrow transplantation (4, 65).

Investigational Therapies

Homoharringtonine (HHT) is a plant alkaloid derived from the Cephalotaxus fortuneii tree. When HHT is used as a low-dose continuous infusion of 2.5 mg/m^2 daily for 14 days for induction, then for 7 days every month in patients with late chronic-phase CML, it can induce a complete hematologic response in two-thirds of patients (more than 50% of whom were resistance to IFN-α) and a cytogenetic response in one-third of these patients (half of which were major responses) (108). When HHT is given for 6 cycles as remission induction followed by IFNα maintenance to patients with early chronic-phase CML, the complete hematologic response rate is 92% and the cytogenetic response rate is 68% (109). Combinations to determine the efficacy of HHT and IFNα are now in progress (107).

Progression of CML is associated with hypermethylation of the Pa promoter region of the BCR-ABL gene (9, 71). 5-azycytidine and 5-aza-2'-deoxyazacytidine (decitabine) are cytidine analogues capable of inhibiting the DNA methyltransferase enzyme. Decitabine produces response rates of 25% in blast phase and 53% in accelerated phase disease (79). When decitabine is compared with intensive chemotherapy as initial therapy for CML blastic phase, it is associated with significantly better survival among patients 50 years or older. Investigations of decitabine in combination with busulfan and cyclophosphamide as part of a preparative regimen for allogeneic SCT and as salvage therapy with stem cell rescue after relapse from allogeneic transplantation are in progress (80).

A modified IFNα molecule can be covalently attached to polyethylene glycol. PEG interferon has a longer half-life than the parent compound and is given once weekly instead of daily. In a phase I study, Talpaz et al (139) treated 21 patients with CML in chronic phase with escalating doses of PEG interferon. In addition to a better side effect profile of PEG interferon, 50% of patients achieved a hematologic response, including 4 of 13 patients who had been resistant to IFN-α. Preliminary results with PEG interferon are promising since it appears to be easier to deliver (once weekly), less toxic, and possibly more effective than IFN-α.

Molecular Approaches for Treating CML

Early in the pathogenesis of CML, the only known genetic abnormality is the BCR-ABL gene itself. Due to its unique sequence structure, the BCR-ABL gene and its cognate mRNA and fusion protein are potentially ideal targets for disruption in an attempt to prevent expansion of the leukemic cells. Several strategies aimed at blocking BCR-ABL functions are currently being investigated. An alternative to inhibiting BCR-ABL itself is to target proteins which are directly or indirectly modulated by BCR-ABL in its various oncogenic pathways.

Attempts to design therapeutic tools for CML based on our current knowledge of the molecular and cell biology of the disease have concentrated on three main areas: (a) inhibition of gene expression at the translational level by 'antisense' strategies; (b) modulation of protein function by specific signal transduction inhibitors, and (c) stimulation of the immune system to recognize and destroy the leukemic cells.

Antisense Therapy Against BCR-ABL

The unique b2a2 or b3a2 junctional sequences of the BCR-ABL transcripts are potential targets for antisense approaches in CML. The first studies (32, 138, 153) provided encouraging results, reporting on suppression of colony formation by CML but not normal cells exposed *in vitro* to oligonucleotide decoys directed against either BCR-ABL junctional sequence. Nevertheless, other groups working on such systems have been unable to reproduce these results only with limited success. Attention then shifted to physiologically relevant proteins other than BCR-ABL that are involved in the pathogenesis of CML.

Targeting adaptor proteins required for BCR-ABL signal transduction is an alternative for antisense targeting. For example, Tari et al (101) used liposome-coated nuclease-resistant antisense oligonucleotide against the translation initiation sites of either CRKL or GRB2 adaptor proteins in cultures of two CML and one Ph+ ALL cell

lines. Downregulation of the respective protein expression was followed by a significant decrease in cell viability in the three BCR-ABL-positive lines, whereas ras induced proliferation of a control BCR-ABL-negative cell line was unaffected. Other candidate genes for antisense therapy are not directly linked to BCR-ABL but are expressed in early hematopoietic cells and seem to be more essential for growth of leukemic rather than normal hematopoietic cells. Examples of these are the KIT receptor (122) the VAV protein (94, 156) and MYB (18). MYB antisense oligonucleotide have been shown to preferentially inhibit the *in vitro* growth of CML, as compared to normal progenitors (19, 120) and to increase the survival of SCID mice transplanted with the K562 CML cell line (121).

Peptide Therapy to Generate Leukemia Specific CTLs

An exciting new approach to induce a CTL response is to pulse dendritic cells with exogenous 8-25 amino acid peptides derived from the b3a2 or b2a2 junctional regions of the BCR-ABL fusion protein. Four peptides spanning the b3a2 junction were found to bind with intermediate to high affinity to selected HLA class I molecules (13). One of these peptides (11 amino acids) was able to induce specific CTLs in two of three HLA3 donors against autologous and allogeneic HLA-matched peptide-pulsed mononuclear cells. A longer 25 amino acid peptide could stimulate an HLA class II-restricted T-cell proliferation in three out of seven donors with the HLA- DR11 haplotype (12). A recent study (141) succeeded in generating CD4 and CD4/CD8 T-cell clones by repetitive stimulation with a 17 amino acid peptide covering the b2a2 fusion region in an HLA-Dr51 normal individual.

A clinical trial was begun to determine the safety and immunogenicity of a multidose, multivalent b3a2 peptide vaccine in 12 patients with CML in the chronic phase of their disease. No significant adverse effects were seen. Three out of six patients treated at the two highest dose levels of vaccine, generated peptide-specific T cell proliferative responses ex vivo and/or delayed type hypersensitivity responses, lasting up to 5 months after vaccination. However, specific CTLs were not identified (116). The overall results suggest that a BCR-ABL derived peptide vaccine can be safely administered to CML patients and can elicit a specific immune response. It remains to be seen whether this type of vaccination will result in significant clinical benefit.

Tyrosine Kinase Inhibitors

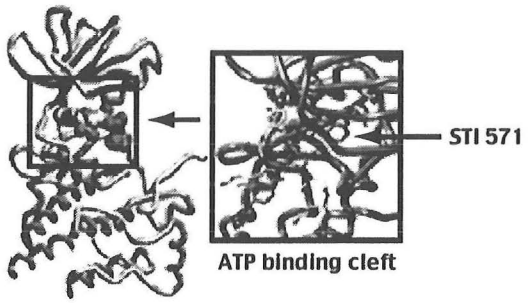
One of the tenets of rational drug design for cancer therapy is to define specific molecular abnormalities in tumors and then to use this data to develop specific inhibitors. In CML, we may be near the time when rational drug design to inhibit BCR-ABL function is a reality. The BCR-ABL fusion proteins are constitutively activated tyrosine kinases with increased protein tyrosine kinase activity as compared to the c-ABL tyrosine kinase (95, 100). Numerous studies have shown that tyrosine kinase activity is required for the transforming abilities of the BCR-ABL oncoprotein (95, 111). Because the BCR-ABL protein is a novel intracellular protein with elevated tyrosine kinase activity, an inhibitor of the BCR-ABL protein tyrosine kinase could be a potentially useful therapeutic agent for CML.

The crystal structure of several protein kinases has been determined therefore, it is now possible to rationally design compounds based on the structure of the ATP binding

site or active site of the enzyme. This in-formation, in combination with the knowledge of the structure of protein tyrosine kinase inhibitors, has allowed for the synthesis of inhibitors with increased potency and specificity. One such class of compounds is the 2-phenylaminopyrimidine derivatives. One compound in this class, CGP 57148, is a potent inhibitor of the ABL protein tyrosine kinase (43).

CGP 57148 or STI 571 inhibits the ABL tyrosine kinases at submicromolar concentrations *in vitro*. All ABL kinases, including p210BCR-ABL, p185BCR-ABL, v-ABL, and the c-ABL tyrosine kinase are inhibited by similar concentrations of CGP57148. Numerous tyrosine and serine/threonine protein kinases have been tested for inhibition by CGP 57148, and except for the platelet-derived growth receptor (PDGFR) and the c-Kit tyrosine kinases, no others are inhibited (25, 43).

Competition for the ATP Binding Site in BCR-ABL (41)



CGP 57148 or STI 571, at concentrations of 1 and 10 μM, kills or inhibits the proliferation of all BCR-ABL expressing cell lines tested to date (10, 25, 35, 43, 51). In contrast, a variety of immortalized or transformed cell lines that do not express BCR-ABL are not sensitive to CGP 57148. In colony-forming assays of CML bone marrow or peripheral blood samples, treatment with CGP 57148 decreases the number of colonies formed and may select for the growth of BCR-ABL-negative progenitor cells (35, 43). Minimal inhibition of the colony forming potential of normal bone marrow has been observed (35, 43). Thus, CGP 57148 appears to be selectively toxic to cells expressing the constitutively active BCR-ABL protein tyrosine kinase. Antitumor activity has been observed in syngeneic or nude mice injected with BCR-ABL-expressing cells followed by treatment with CGP 57148 (42, 43). CGP 57148 is highly bioavailable as an oral formulation and has minimal toxicity in rats and dogs.

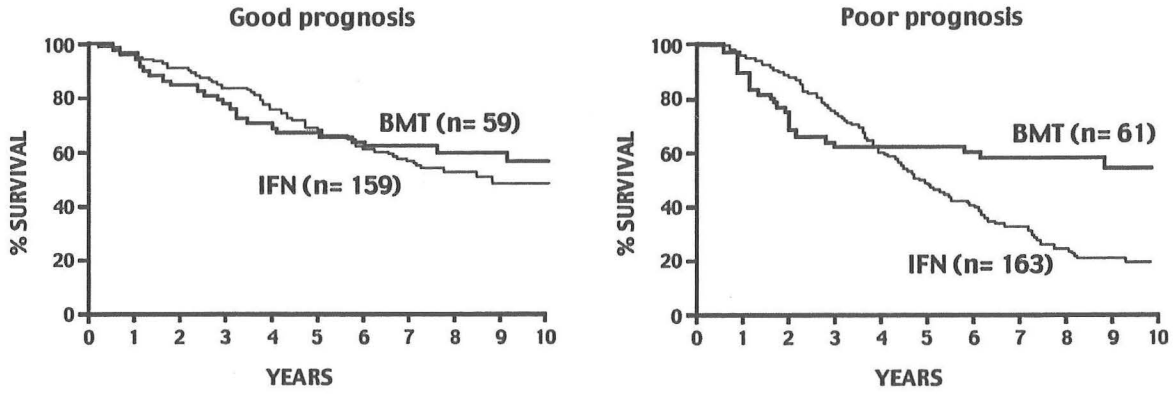
Based on the above data, an IND was obtained from the FDA and phase I trials in CML patients were begun in June 1998. The phase I study targeted CML patients who failed IFNα therapy. Over 40 patients have now been treated, and early results show that this drug is well tolerated with no significant side effects. Adequate bioavailabilty and pharmacokinetics have been observed with once daily administration. At the higher dose levels it has been possible to achieve levels *in vivo* that inhibit BCR-ABL kinase activity *in vitro*. Consistent with this finding, significant hematologic responses have been observed at the higher dose levels (42). Ph chromosome responses have not yet been observed; however, it is quite early and the patient population selected for these initial studies may have minimal Ph-negative hematopoiesis. Further studies of this agent either alone or in combination with other agents may provide a major breakthrough in the treatment of CML.

An alternative to direct inhibition of BCR-ABL is interference with proteins which are critical for BCR-ABL induced transformation. One of these proteins is GRB2, whose SH2 domain binds directly to BCR-ABL via the phosphorylated tyrosine 177 within the BCR portion of the chimera (115). This results in the formation of a BCR-ABL/GRB2/SOS complex which activates RAS GDP/GTP exchange (57). Several studies have provided compelling evidence for the role of GRB2 and RAS activation in this oncogenic process. Based on these observations, Gishizky and coworkers at Sugen Research initiated a screening program to identify small organic molecules that inhibit interaction between the SH2 domain of GRB2 and a tyrosine phosphorylated peptide found in BCR-ABL. One such compound was found to have GRB2-binding inhibitory capacity *in vitro* and in cells, and to reverse BCR-ABL-induced transformation of a murine cell line *in vitro*. This compound also inhibits the mitogenic responses induced by EGF and PDGF receptors, consistent with the participation of GRB2 in the signal transduction cascade of these two receptor tyrosine kinases. Provided that these molecular side effects do not adversely affect essential functions in normal cells, inhibitors of GRB2 may prove useful in the therapy for CML.

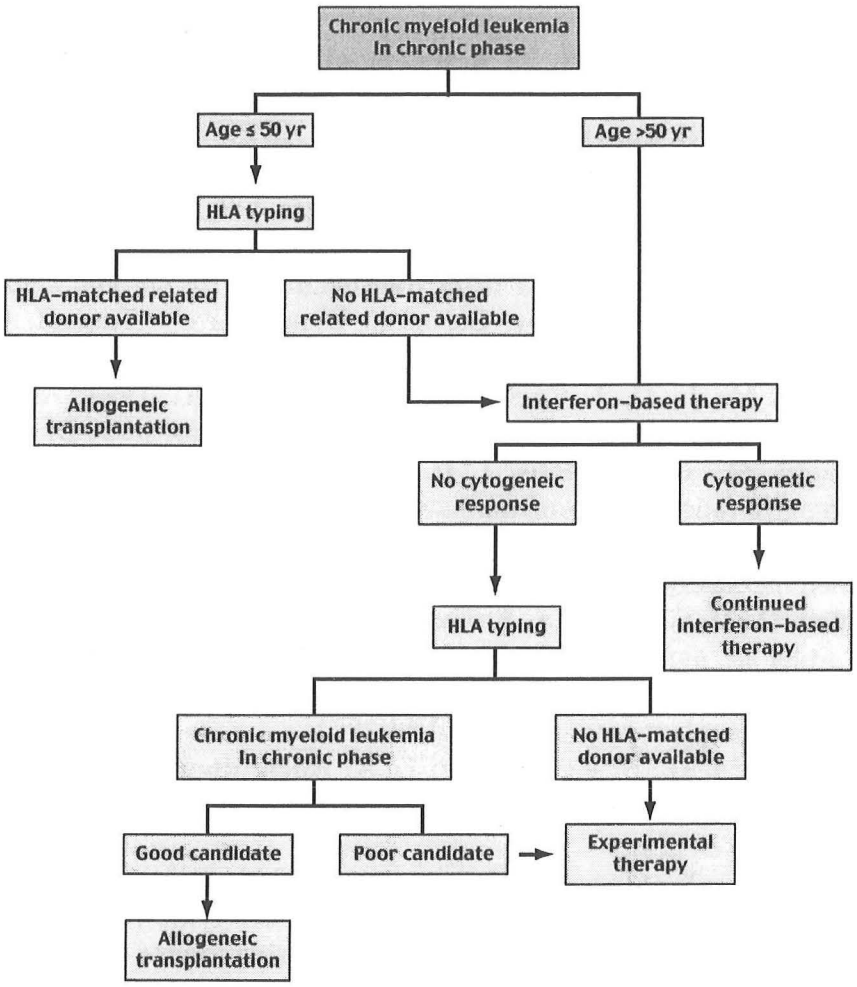
Clinical Decision Making: Transplantation versus Interferon-α Therapy

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.

Effect of Prognosis on the Survival of Patients with CML (145)



Aproach to the Treatment of Patients with Chronic Myeloid Leukemia in Chronic Phase (129)



One strategy supported by decision analysis (90) is to treat older patients or younger patients for whom no suitable donor of bone marrow is available with interferon alfa. 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 (8, 56, 157). Patients who relapse after allogeneic bone marrow transplantation can be treated successfully with infusion of donor lymphocytes, (40, 85, 117) IFNα, (4, 65) or a second allogeneic transplantation.

However, with the potential number of new agents and new strategies available to treat CML, it is likely that steady progress will be made in the treatment of this disease. It is likely that by combining immunologic approaches and drugs that target BCR-ABL itself and downstream signal transduction pathways that CML will finally become a true chronic but treatable disease.

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