

# ADVANCES IN BIOLOGY AND TREATMENT OF

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Chronic myeloid leukaemia (CML) (also chronic myelogenous leukaemia, chronic granulocytic leukaemia) is a clonal disease that results from an acquired genetic change in a pluripotential haemopoietic stem cell (reviewed in refs 1-3). This altered stem cell proliferates and generates a population of differentiated cells that gradually replaces normal haemopoiesis and leads to a greatly expanded total myeloid mass. The disease was clearly recognized as distinct from other types of leukaemia with the advent of panoptic stains for blood films at the end of the last century. One important landmark in the study of CML was the discovery of the Philadelphia (Ph) chromosome in 1960; the next was the characterization in the last decade of the BCR-ABL chimeric gene. Until the 1980s CML was assumed to be incurable and was treated palliatively, first with radiotherapy and more recently with alkylating agents, notably busulphan. It has become apparent in the last ten years that CML can be cured by bone marrow transplantation (BMT), but the proportion of patients eligible for BMT is still relatively small.

## **Classification**

The majority of patients with CML have a relatively homogeneous disease characterized at diagnosis by splenomegaly, leucocytosis and the presence of a Ph chromosome in all leukaemic cells. A minority of patients have a less typical disease that may be classified as atypical CML, chronic myelomonocytic leukaemia or chronic neutrophilic leukaemia. Children may have a disease referred to as juvenile chronic myeloid leukaemia. In none of these variants is there a Ph chromosome.

## **Epidemiology, Aetiology and Natural History**

The incidence of CML appears to be constant worldwide. It occurs in about 1.0-1.5 per 100,000 of the population in all countries where statistics are adequate. CML is rare below the age of 20 years but occurs in all decades; the median age of onset is 40-50 years. The incidence is slightly higher in males than in females.

The risk of developing CML is slightly but significantly increased by exposure to high doses of irradiation, as occurred in survivors of the atomic bombs exploded in Japan in 1945 and in patients irradiated for ankylosing spondylitis, but in general almost all cases must be regarded as 'sporadic' and no predisposing factors are identifiable. In particular, there is no familial predisposition and no association with HLA genotypes has been recognized. No contributory infectious agent has been incriminated.

CML is a biphasic or triphasic disease that is usually diagnosed in the initial 'chronic' or stable phase. There has been much debate about the duration of disease before the diagnosis is established, a question that is essentially unanswerable. If it is assumed that the disease starts with a 'transformation event' occurring in a single stem cell, it could be 5 to 10 years before the disease becomes clinically manifest. This

estimate depends on the assumption that the leucocyte doubling time in the pre-diagnosis phase is not fundamentally different from the doubling time after diagnosis (which may not be the case) and on the observation that the latent interval between exposure to irradiation from atomic bombs and earliest identifiable increased incidence of CML was about 7 years. One recent study concluded that a routine blood count might have identified CML on average 6 months before it was actually diagnosed in individual patients.

Patients are usually in the chronic or stable phase when CML is diagnosed. This chronic phase lasts typically 2 to 6 years but it may on occasion last more than 10 or even 15 years. Very rare spontaneous remissions have been described. In about one-half of cases, the chronic phase transforms unpredictably and abruptly to a more aggressive phase, which used to be referred to as blast crisis and is now usually described as acute or blastic transformation. In the other half of cases, the disease evolves somewhat more gradually through an intermediate phase described as 'accelerated' disease, which may last for months or occasionally years before frank transformation. Occasional patients have a disease that progresses gradually to a myelofibrotic or osteomyelosclerotic picture characterised by extensive marrow fibrosis and sometimes gross overgrowth of bony trabeculae; the clinical problems are then due to failure of haemopoiesis rather than to blast cell proliferation. The duration of survival after onset of transformation is usually 2-6 months; the median survival from diagnosis is 4-5 years.

Many attempts have been made to sub-classify or stage CML at diagnosis in a manner that would permit some prediction of the duration of chronic phase in individual patients. The most commonly quoted classification, devised by Sokal and colleagues in 1984, is based on a formula that takes account of the patient's age, blast cell count, spleen size and platelet count at diagnosis but is still too inaccurate to be clinically useful. At present the best prognostic indicator seems to be the response to initial treatment with interferon- $\alpha$  (IFN $\alpha$ ). Those who achieve haematological control live longer than those who do not, and the longest survival is seen in those patients who convert to Ph-negative haemopoiesis.

## **Cytogenetics**

The Philadelphia (Ph) chromosome is an acquired cytogenetic abnormality that characterises all leukaemic cells in CML. It is formed as a result of a reciprocal translocation of chromosomal material between the long arms of one no. 22 chromosome and one no. 9 chromosome, an event referred to as t(9;22)(q34;q11). In CML patients, the Ph chromosome is present in all myeloid cell lineages, in some B cells and in a very small proportion of T cells. Some but not all patients acquire additional clonal cytogenetic abnormalities during the course of the chronic phase. There is suspicion that some such changes might be caused in part by administration of alkylating agents, but they undoubtedly can occur spontaneously. The observation of non-random changes, typically +8, +Ph, iso-17q or +19, usually means that such new clones will expand and that blastic transformation will manifest itself within weeks or months. Eighty percent of patients in overt blastic transformation have clonal cytogenetic changes in addition to the Ph translocation.

## **Molecular Biology**

It was shown in the early 1980s that the ABL protooncogene was located normally on chromosome 9 and was translocated to chromosome 22 in CML patients. In 1984 Groffen and colleagues showed that the precise position of the genomic breakpoint on chromosome 22 in different CML patients was clustered in a relatively small 5.8 kb region to which they gave the name 'breakpoint cluster region' (bcr) or major breakpoint cluster region (M-bcr). Later it became clear that this region formed the central part of a relatively large gene now known as the BCR gene, whose normal function is unknown. The translocation results in juxtaposition of 5' sequences from the BCR gene with 3' ABL sequences derived from chromosome 9. Thus the Ph chromosome carries a chimeric gene, designated BCR-ABL, that is transcribed as an 8.5 kb mRNA and encodes a protein of molecular weight 210 kd that has greater tyrosine kinase activity than the normal ABL gene product. The p210 BCR-ABL must play a pivotal role in the pathogenesis of CML (reviewed in refs 3-5).

The mechanism by which the BCR-ABL gene alters stem cell kinetics remains obscure. Some evidence suggests that the BCR-ABL gene, but not the BCR gene, can form a complex with the GRB-2/SOS molecular complex that plays a key role in the intracellular second messenger system downstream of RAS. Alternatively, BCR-ABL may complex with precursors that activate MYC. An activated ABL opposes cellular apoptosis, and the BCR-ABL gene might act by impeding 'programmed cell death' in target stem cells.

The molecular basis of disease progression is still obscure, but it seems reasonable to infer that one or more additional genetic events - probably a sequence - occur in the Ph-positive clone. When the critical combination of additional events is achieved, transformation ensues. About 20% of patients with CML in myeloid transformation have point mutations or deletions in the coding sequence of the tumour suppressor gene p53, a gene implicated in progression of a variety of solid tumours, notably colonic carcinoma. Deletions in the tumour suppressor gene p16 are found in about 50% of patients with lymphoid transformations. The retinoblastoma (RB) gene is deleted in rare cases of CML in megakaryoblastic transformation, and changes in EVI-1 and MYC are described. Molecular changes underlying the non-random cytogenetic changes described above have not been identified.

## **Management of CML**

### ***Chronic Phase***

The management of newly diagnosed CML patients has changed very greatly in the last ten or so years (reviewed in refs 6 & 7). In the 1970s it was conventional for the physician to start treatment soon after diagnosis with busulphan and then to await further developments. Today, in most but not all countries, the patient is informed of the diagnosis and given some information about prognosis at the time of diagnosis. The various options for treatment are discussed at this stage. For younger patients the question of BMT should be addressed as soon as possible after diagnosis. The patient, all siblings

and other family members should be HLA typed. The issue of gonadal function is important. Treatment with busulphan should not be initiated unless the patient is willing to accept permanent sterility. Conversely, a patient who might be a candidate for BMT should be offered the possibility of semen or embryo cryopreservation.

There is no immediate urgency to start treatment in asymptomatic patients with leucocyte counts below  $100 \times 10^9/l$ . Most patients will, however, prefer to be treated once the diagnosis is confirmed. If the possibility of treatment by BMT is excluded or uncertain, treatment should be initiated with IFNa (see refs 8-10) or hydroxyurea at standard dosage. Busulphan should be reserved for special indications.

IFNa is a member of a large family of glycoproteins of biological origin with antiviral and antiproliferative properties. Studies in the early 1980s using material purified from human cell lines showed that it was active in reducing the leucocyte count and reversing all features of CML in 70-80% of CML patients. Of particular interest was the observation that 5 to 15% of patients sustained major reduction in the percentage of Ph-positive marrow metaphases with restoration of Ph-negative (putatively normal) haemopoiesis. This effect is very rarely achieved with standard cytotoxic drugs. It raised the important question of whether these 'cytogenetic responders' would have their life prolonged by treatment with IFNa, and prospectively randomized controlled studies were initiated in many European countries and in North America. Results of some of these studies have now been reported. It appears that treatment with IFNa has two important effects: (1) it identifies on the basis of speed of haematological response and degree of cytogenetic response subgroups of patients who will survive longer than others; and (2) it probably prolongs survival by perhaps 1 or 2 years in the majority of patients. Patients who obtain complete cytogenetic response have an extremely low risk of disease transformation, and their median survival may exceed 10 years. For the present it seems reasonable to conclude that IFNa should be offered to all newly diagnosed patients who are not candidates for allogeneic BMT and that treatment should be continued in haematological responders for as long as the drug is tolerated. IFNa is now available in various recombinant DNA preparations. It must be administered by subcutaneous injection. It may be started at low dosage, e.g., 3 mega units daily, with gradual increases or at high dosage, e.g., 5 mega units/m<sup>2</sup> daily with dose reduction if necessary. There is some evidence that the greatest chance of cytogenetic response is achieved with the higher dose levels. The drug is not, however, without side-effects. Almost all patients experience fevers, shivers, muscle aches and general 'flu-like' features on starting the drug; these last usually one to two weeks but may be alleviated by paracetamol. They recur when dosage is increased. A significant minority of patients cannot tolerate the drug on account of lethargy, malaise, anorexia, weight loss, depression and other affective disorders or alopecia. Autoimmune syndromes, such as thyrotoxicosis, may also occur. The drug is very much more expensive than hydroxyurea.

A number of efforts have been made to increase the proportion of patients who achieve Ph-negativity by combining IFNa with other drugs. The combination of IFNa with hydroxyurea (see below) has been tested in a number of centres. With this combination it is relatively easy to maintain the leucocyte count in the normal range, but the probability of achieving Ph-negativity is not increased. In contrast, the combination of IFNa with cytarabine at low dosage has approximately doubled the proportion of the

patients who achieve Ph-negativity. The toxicity, especially prolonged mucositis, of this combination has been considerable and it is not yet clear whether patients treated in this way achieve clinical benefit. Further controlled studies are warranted.

Younger patients with HLA-identical sibling donors should be offered the opportunity of treatment by allogeneic BMT (see refs 11,12). Most specialist centres exclude from consideration patients over the age of 50 or 55 years. In general, patients are 'conditioned' for transplant with cyclophosphamide at high dosage followed by total body irradiation or with the combination of busulphan and cyclophosphamide at high dosage. If all goes well, reasonable marrow function is achieved in three to four weeks after the infusion of donor marrow and the patient leaves the hospital. The possible major complications include graft-versus-host disease, reactivation of infection with cytomegalovirus or other viruses, idiopathic pneumonitis and veno-occlusive disease of the liver. For patients with CML treated by BMT with marrow from HLA-identical siblings, the overall leukaemia-free survival at 5 years is now 60-70%. There is a 20% chance of transplant-related mortality and a 15% chance of relapse. Patients surviving without haematological evidence of disease can be monitored by serial cytogenetic studies and by use of the much more sensitive reverse transcription polymerase chain reaction (RT-PCR), which can detect very low numbers of BCR-ABL transcripts in the blood or marrow. These studies suggest (but do not prove) that the majority of long-term survivors have been cured of CML.

This qualified success with BMT using matched siblings has led to increasing use of 'matched' unrelated donors for BMT for patients with CML. At present serologically matched unrelated donors can be identified for about 50% of Caucasian patients and for lower percentages of patients of other ethnic origins. The results of transplants using such unrelated donors are currently somewhat less good than results of using HLA-identical siblings, but an appreciable proportion of patients can probably be cured.

Patients who relapse after allogeneic BMT may be treated with IFNa or hydroxyurea. Second transplants are successful in some cases. Of great interest is the observation that transfusion of lymphoid cells from the original transplant donor without use of cytotoxic drugs may induce remissions in 70-80% of patients.(13,14) The incidence of complications, especially graft-versus-host disease, may be reduced if low numbers of donor lymphoid cells are administered at spaced intervals.

Because only a minority of patients are eligible for allogeneic BMT, much interest has focused recently on the possibility that life may be prolonged and some cures effected by autografting CML patients still in chronic phase. It is possible that the pool of leukaemic stem cells can be substantially reduced by an autograft procedure, and autografting may confer a short-term proliferative advantage on Ph-negative (presumably normal) stem cells (see refs 15,16). In practice, some patients have achieved temporary Ph-negative haemopoiesis after autografting. The possibility that more durable Ph-negativity may be achieved by autografting with marrow or blood stem cells treated in vitro to reduce the proportion of Ph-positive stem cells is currently being studied in various centres.

### *Advanced Phase Disease*

Patients in blastic transformation may be treated with combinations of cytotoxic drugs in the hope of prolonging life, but cure can no longer be a realistic objective. Conversely, it is not unreasonable to use a relatively innocuous drug such as hydroxyurea at higher dosage to restrain blast cell numbers and maintain the patient at home for as long as possible. If the patient has a myeloid transformation, he or she can be treated with drugs appropriate to the induction of remission in AML, namely daunorubicin, cytosine arabinoside with or without 6-thioguanine or etoposide. The blast cell numbers will be reduced substantially in most cases, but their numbers usually increase again within 3 to 6 weeks. Perhaps 20% of patients are restored to a situation resembling chronic phase disease and this benefit may last for 3-6 months.

Patients in lymphoid transformation may be treated with a little more optimism with drugs applicable to the management of adult acute lymphoblastic leukaemia (e.g., prednisolone, vincristine and daunorubicin, with or without L-asparaginase). More than 50% of patients will be restored to 'second' chronic phase, at which point this status can be maintained with daily 6-mercaptopurine and weekly methotrexate. Patients who achieve second chronic phase should have neuroprophylaxis with intrathecal methotrexate weekly for six consecutive weeks, but the administration of cranial irradiation is probably not indicated. Some patients treated for lymphoid transformation of CML may sustain long periods of apparent 'remission'.

Allogeneic BMT using HLA-matched sibling donors can be performed in accelerated phase; the probability of leukaemia-free survival at 5 years is 30-50%. BMT performed in overt blastic transformation is nearly always unsuccessful. The mortality resulting from graft-versus-host disease is extremely high and the probability of relapse in those who survive the transplant procedure is considerable. The probability of survival at 5 years is consequently 0-10%.

## References

1. Gale RP, Goldman JM, Grosveld G, Goldman JM. Chronic myelogenous leukemia: biology and therapy. (Meeting Report) *Leukemia* 1993, 7: 653-658.
2. Kantarjian HM, O'Brien S, Anderlini P, Talpaz M. Review: Treatment of chronic myeloid leukemia: current status and investigational options. *Blood* 1996, 87: 3069-3081.
3. Enright H, McGlave PB. Chronic myelogenous leukemia. *Current Opinion in Hematology* 1995, 2: 293-299.
4. Kurzrock R, Gutterman JU, Talpaz M. The molecular genetics of Philadelphia chromosome-positive leukemias. *N Engl J Med* 1988, 319: 990-998.
5. Bergamaschi G, Rosti V. Pathogenesis of chronic myelogenous leukemia. *Haematologica* 1994, 79: 1-3.
6. Goldman JM. Options for the management of chronic myeloid leukemia - 1990. *Leukemia and Lymphoma* 1990, 3: 159-164.
7. Goldman JM. Management of chronic myeloid leukaemia. *Blood Reviews* 1994, 8: 21-29.

8. Talpaz M, Kantarjian H, Kurzrock R, Trujillo JM, Gutterman JU. Interferon alpha produces sustained cytogenetic responses in chronic myelogenous leukemia Philadelphia chromosome-positive patients. *Ann Intern Med* 1991, 114: 532-538.
9. 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: 820-825.
10. Allan NC, Richards SM, Shepherd PCA. UK Medical Research Council randomised multicentre trial of interferon-a for chronic myeloid leukaemia: improved survival irrespective of cytogenetic response. *Lancet* 1995, 345: 1392-1397.
11. Thomas ED, Clift RA. Indications for marrow transplantation in chronic myelogenous leukemia. *Blood* 1989, 73: 861-864.
12. Clift RA, Appelbaum FR, Thomas ED. Editorial: treatment of chronic myeloid leukemia by marrow transplantation. *Blood* 1993, 82: 1954-1956.
13. Kolb HJ, Schattenberg A, Goldman JM et al. Graft-versus-leukemia effect of donor lymphocyte transfusion in marrow grafted patients. *Blood* 1995, 86: 2041-205.
14. Mackinnon S, Papadopoulos EP, Carabasi MH et al. Adoptive immunotherapy evaluating escalating doses of donor leukocytes for relapse of chronic myeloid leukemia following bone marrow transplantation: separation of graft-versus-leukemia responses from graft-versus-host disease. *Blood* 1995, 86: 1261-1267.
15. O'Brien SG, Goldman JM. Current approaches to hematopoietic stem cell purging in chronic myeloid leukemia. *J Clin Oncol* 1995, 13: 541-546.
16. Lemoli RM. Characterization and selection of benign stem cells in chronic myeloid leukemia. *Haematologica* 1993, 78: 393-400.