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NEW INSIGHTS IN BIOLOGY AND CURRENT THERAPEUTIC OPTIONS FOR PATIENTS WITH CHRONIC MYELOGENOUS LEUKEMIA*

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ABSTRACT

Background and Objective. From the discovery of the Ph-chromosome, there has been an extraordinary progress in our understanding of chronic myeloid leukemia (CML). During the last three decades, new findings arising from dissection of the genetic abnormalities at a molecular level have received the most attention, but there have also been important new observations arising from studies of the biologic behaviour of normal and leukemic stem cells and, more recently, from clinical investigations. In this review we first report the most important observations relevant to understanding the oncogenic potential of the BCR-ABL chimeric gene, and the behaviour and the relationships of normal and leukemic stem cells. From a clinical point of view, allogeneic stem cell transplantation is the only procedure able to cure CML. The main issues are: who can receive this procedure, and when and how it can be given. The situation is more complex in unrelated transplants. In patients without HLA compatible donors, many large trials in different countries have demonstrated that interferon alpha therapy is indicated and effective in the majority of patients. On the other hand, autologous stem cell transplantation is still an experimental procedure. These aspects will be analyzed in detail and, at the end, a therapeutic algorithm of a possible approach to the patients with untreated CML is provided.

Evidence and Information Sources. The method used for preparing this review was an informal consensus development. All the authors of the present review have been working in the field of chronic myeloid luekemia, and have contributed original papers in peer-reviewed journals. In addition, the material examined in the present review includes articles and abstracts published in journals covered by the Science Citation Index® and Medline®.

State of Art and Perspectives. The oncogenic potential of BCR-ABL has been demonstrated in a number of in vitro and in vivo model systems. Current research efforts are focused on defining the mechanism by which BCR-ABL transforms primary hematopoietic cells. The fact that BCR-ABL contains tyrosine residues, an SH2 domain, an SH3 domain, and proline-rich sequences raises the possibility of multiple protein-protein interactions. Indeed, BCR-ABL is reported to bind and/or phosphorylate more than 20 proteins. The insights into the signal transduction pathways activated by BCR-ABL will hopefully provide a new basis for the treatment of CML patients. Clinical evidence of the existence of a transplantable CML stem cell population has recently been extended to xenogeneic recipients of transplanted CML cells and by retroviral marking to autograft recipients. The potential of using immunodeficient mice as recipients of CML stem cells to create an in vivo model of chronic phase CML should be invaluable for testing novel therapies designed to eliminate residual disease in the patient. Current therapeutic options include conventional chemotherapy, IFN-a and allogeneic stem cell transplantation as established procedures, and autografting as an experimental procedure.While IFN-a as a first line therapy does not seem to jeopardize further treatments, autografting, according to the Genoa approach or other procedures, i.e. Ph-positive cells collected at diagnosis without mobilization therapy, raises the question of an ideal sequential strategy in the management of CML patients. There seems to be a general agreement that a patient less than 50 years old, with an HLA identical sibling, should receive an allogeneic stem cell transplant. This approach should be offered also to younger patients (≤ 40 years) who are able to find an unrelated matched donor. Since it seems that the normal hematopoietic reservoir declines with time, it may be desiderable to mobilize and collect peripheral stem cells in order to store Ph-negative progenitors as soon after diagnosis as possible when the WBC count has been controlled by hydroxyurea while searching for a MUD is proceeding. Then six-eight months should be allowed for a MUD search. If the donor is not found, the patient may undergo autografting with the previously stored Ph-negative progenitors followed by IFN-a therapy. However, at this moment, this is an experimental procedure and must be employed only in selected centers.

Key words: chronic myelogenous leukemia, oncogenes, stem cells, transplantation, interferon, chemotherapy

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Chronic myeloid leukemia (CML) is a stem cell disorder which progresses from a "benign" chronic phase to a refractory acute leukemia. In more than 90% of patients it is associated with the Philadelphia (Ph) chromosomal t(9;22) translocation, which results in the juxtaposition of BCR and ABL genes to form a BCR-ABL chimeric gene. From the discovery of the Ph-chromosome, there has been extraordinary progress in our understanding of CML. During the last three decades, new findings arising from dissection of genetic abnormalities at the molecular level have received the most attention, but there have also been important new observations arising from studies of the biologic behavior of normal and leukemic stem cells and, more recently, from clinical investigations. As a result of this new information, CML is probably now as well understood as any human neoplasm.

In this review, we first report the most important observations at different levels of investigation relevant to understanding the oncogenic potential of BCR-ABL. Subsequently, the biologic section will focus on the assays currently used to elucidate the behavior and the relationships of normal and leukemic stem cells.

From a clinical standpoint, allogeneic stem cell transplantation is the only procedure able to cure CML. The main issues are: who can receive this procedure, and when and how it can be given?

The situation is more complex in unrelated transplants but the data now available seem to demonstrate a better prognosis for younger patients if they are transplanted early during the disease.

In patients without HLA-compatible donors, many large trials in different countries have demonstrated that interferon-α therapy is indicated and effective in the majority of patients. On the other hand, autologous stem cell transplantation has been attempted in selected patients with CML since 1972 although no patient has been cured. The obstacles to more widespread use of autografting are the problems of providing grafts predominantly, if not completely, free of leukemic cells and of minimizing the toxicity of myeloablative therapies.

In this review all these aspects will be analyzed in detail and, at the end, a therapeutic algorithm of a possible approach to patients with untreated CML is provided.

Molecular and biological basis of CML

Molecular biology

Chronic myeloid leukemia is probably the best characterized form of human leukemia. It was the first hematological malignancy to be associated with a specific chromosomal translocation, t(9;22)(q34;q11), which is generally regarded as the hallmark of CML. In the classical t(9;22) translocation, a single breaks occur within the BCR gene on chromosome 22, and within the ABL gene on chromosome 9 and a reciprocal exchange of the telomeric ends of these two chromosomes results in a shortened 22q- or Philadelphia (Ph) chromosome containing the BCR-ABL fusion gene and a 9q+ derivative harboring the reciprocal ABL-BCR gene. This t(9;22) is found in approximately 85% of all patients diagnosed as CML. In another 5% the Ph chromosome resulting from complex translocations. These usually involve three or more chromosomes, but always include 9 and 22. In the remaining 10% of CML cases, a Ph chromosome cannot be identified by conventional cytogenetics. Within this group, more than 50% have a BCR-ABL gene which can be detected by fluorescence *in situ* hybridization (FISH) on interphase nuclei, or other methods [such as Southern blotting demonstrating a BCR gene rearrangement, or reverse transcription/polymerase chain reaction (RT/PCR) amplification of BCR-ABL mRNA transcripts]. Thus less than 5% of all patients clinically diagnosed as CML are found to be both Ph-negative and BCR-ABL negative.[1,2] The molecular mechanisms of the disease in these rare latter patients have been widely investigated but remain unknown.[3-5]

Several lines of investigation have disclosed that the essential and pathologically important outcome of the t(9;22) is the creation of a BCR-ABL gene, which encodes a fusion protein with elevated tyrosine kinase activity and is now regarded as central to the mechanism that underlies the chronic phase of CML (see below). Although the reciprocal fusion gene ABL-BCR is transcriptionally active in approximately 60% of CML patients, its functional role, if any, remains unknown.[6,7]

In the formation of BCR-ABL, the breakpoint in the ABL gene can occur anywhere within a > 300kb segment at the 5' end of the gene, either upstream from the first alternative exon Ib, between exons Ib and Ia, or downstream from exon Ia.[6] In the vast majority of CML patients and in about one-third of ALLs the breakpoint in the BCR gene is found within a 5.8 kb region known as the major breakpoint cluster region (M-bcr), spanning 5 exons historically named b1 to b5, now known to be exons 12 to 16 of the BCR gene. Regardless of the position of the ABL breakpoint, processing of the primary BCR-ABL transcript usually results in a hybrid BCR-ABL mRNA molecule with a b3a2 and/or a b2a2 junction encoding a p210 BCR-ABL fusion protein. There is apparently no significant difference in the evolution of the disease or in response to treatment between patients with a 5' or a 3' M-bcr breakpoint, except for a slight predominance of b3a2-expressing cases among those with increased platelet counts.[8-14]

In two-thirds of the patients categorized clinically as ALL and in rare cases of CML and AML patients, the breakpoint in BCR falls further upstream, in the

long (54.4 kb) intron[15] between the two alternative exons e2' and e2, known as the minor bcr (m-bcr). In these circumstances, exons e1' and e2' are removed by splicing. The hybrid BCR-ABL transcript, containing an e1a2 junction, is translated into a smaller 185-190 kDa BCR-ABL fusion protein (p190 BCR-ABL). In the majority of these very rare CML cases with the p190-type of BCR-ABL gene, the disease tends to have a prominent monocytic component, resembling chronic myelomonocytic leukemia (CMML).[16]

Recently, a third breakpoint cluster region was identified and named m-bcr.[17] The break occurs in the 3' region of the BCR gene, and the larger BCR-ABL hybrid gene is transcribed into a chimeric BCR-ABL mRNA with an e19a2 (originally described as c3a2) junction.[18] The translation product, p230 BCR-ABL, carries 180 additional amino acids encoded by 540 bp of *extra* BCR sequences as compared with the classical p210 BCR-ABL. Like the p210 and p190 proteins, p230 BCR-ABL also has tyrosine kinase activity.[19] CML resulting from a p230 BCR-ABL gene is extremely rare, and has been associated with either the chronic neutrophilic leukemia (CNL) variant[17] and/or with marked thrombocytosis.[20]

Exceptional CML cases have been described with BCR breakpoints outside the three defined cluster regions, or with unusual breakpoints in ABL resulting in BCR-ABL transcripts with b2a3 or b3a3 junctions, or with aberrant fusion transcripts containing variable lengths of intronic sequence inserts.[21] The identification of these cases has two implications. The first is that the BCR-ABL protein translated from these unusual transcripts, in spite of lacking a variable number of amino acids encoded by the missing exons, is still oncogenic, since the patients still have CML. The second important point is that these BCR-ABL transcripts may escape detection if the primers used for RT/PCR amplification are not appropriate, in which case an apparently paradoxical pattern of a Ph-positive karyotype without detectable BCR-ABL message may occur.

Oncogenic potential of BCR-ABL

The oncogenic potential of BCR-ABL has been demonstrated in a number of *in vitro* and *in vivo* model systems. Current research efforts are focused on defining the mechanism by which BCR-ABL transforms primary hematopoietic cells. The fact that BCR-ABL contains tyrosine residues, an SH2 domain, an SH3 domain, and proline-rich sequences raises the possibility of multiple protein-protein interactions. Indeed BCR-ABL is reported to bind and/or phosphorylate more than 20 proteins (Table 1). Many of these can be directly linked to signal transduction pathways based on defined roles in other systems, but others have no known function. Here we review current views of the mechanism of BCR-ABL transformation with emphasis on substrates related to the Ras pathway.

Table 1. Proteins implicated in BCR-ABL signal transduction through tyrosine phosphorylation and/or complex formation.

Ras pathway	*Definition*	*Refs*
Grb-2	SH2 and SH3 domain-containing adapter protein	28,47
CRKL	SH2 and SH3 domain-containing adapter protein	29,32,48
Shc	SH2 and SH3 domain-containing adapter protein	47,49
Ras-GAP	Ras GTPase activating protein	50,51
mSOS	Guanine nucleotide releasing protein	52
p62-Dok	Ras-GAP associated protein	53,54
p190	Ras-GAP associated protein	25,51
Signal transducers	*Definition*	*Refs.*
Syp	Protein tyrosine phosphatase	55
PLC-γ	Phospholipase C-γ	51
P13K p85 subunit	Phosphatidylinositol 3' kinase regulatory subunit	51,56
Vav	Hematopoietic cell-restricted SH2 and SH3 domain-containing protein	57
Fes	Hematopoietic cell-restricted tyrosine kinase	58
FAK	Focal adhesion kinase	59
STAT1/STAT5	Signal transducer and activator of transcription	60,61
Structural proteins	*Definition*	*Refs*
F-actin	Cytoplasmic actin protein	62
Paxillin	Focal adhesion complex protein	32,63
Vinculin	Focal adhesion complex protein	64
Talin	Focal adhesion complex protein	64
Tensin	Focal adhesion complex protein	64
Others		
Bcr	Serine/threonine kinase and Rac-GTPase	65,66
Bap-1	Bcr-associated protein-1	67
Cbl	Cytoplasmic protein	68
Abi-1/Abi-2	Abl interactor proteins 1 and 2	69,70

Activation of Ras is a hallmark of signal transduction by receptor tyrosine kinase (RTKs).[22,23] Like RTKs, BCR-ABL also activates Ras.[24,25] Ras is required for BCR-ABL function since a dominant negative mutant of Ras (Asn 17) blocks BCR-ABL transformation[26] and anti-apoptosis[27] activity. The mechanism for Ras activation involves at least three distinct adaptor proteins: Grb-2, SHC and CRKL. Each forms complexes with BCR-ABL and has the

potential to link BCR-ABL to Ras through recruitment of guanine nucleotide exchange factors. Mutations in BCR-ABL which impair its binding to either Grb2[28] or CRKL[29] show partial loss of function in fibroblast transformation models. Furthermore, dominant negative mutants of Grb-2 reverse the BCR-ABL transformed phenotype.[30] CRKL and Grb2 appear to have non-overlapping functions since deletion of both binding sites in BCR-ABL cripples the protein more severely than single-site mutations.[29] CRKL fulfills criteria for a relevant physiological substrate since it is among the most prominent phosphoproteins in clinical CML cells[31] and it is sufficient to recapitulate much of the activity of BCR-ABL. CRKL becomes hyperphosphorylated when overexpressed, activates Ras-dependent signaling pathways and transforms fibroblasts, all of which occur with BCR-ABL expression.[29] CRKL may also meditate other BCR-ABL functions. Through binding to paxillin, CRKL can bring BCR-ABL into contact with focal adhesion complexes.[32] This may provide insight into the cellular adhesive defects of CML cells.

With RTKs a primary signaling event following Ras activation is activation of mitogen-activated protein kinase (MAPK) signaling pathways.[22,33] Three MAPK cascades have been extensively characterized: the extracellularly regulated kinase (ERK), the stress-activated protein kinase (SAPK) or Jun kinase (JNK) pathway, and the p38 kinase pathway.[34-38] An endpoint for each of these pathways is the phosphorylation and activation of transcription factors. BCR-ABL and v-ABL activate the SAPK pathway in fibroblasts and hematopoietic cells.[39] Other constitutively active Abl alleles such as DSH3 c-ABL also activate SAPK 1.[40] Activation of SAPK by BCR-ABL is physiologically relevant since dominant negative Jun, which inhibits the endpoint of the SAPK pathway, inhibits BCR-ABL transformation. Similar results are obtained when a protein that specifically inhibits JNK but not ERK or p38 is expressed in target cells for BCR-ABL transformation.[41] BCR-ABL also activates Jun-dependent promoters in a Ras-, Mekk- and SAPK-dependent manner.[39] While the effects of BCR-ABL on the JNK pathway seem clear, studies of the ERK pathway show that BCR-ABL functions differently from most RTKs. Although BCR-ABL activates c-Raf,[42,43] the signal is not propagated through the entire pathway to ERK.[39,44] This result argues for a Raf-dependent, ERK-independent signaling pathway in BCR-ABL transformation. One candidate for this pathway is the Raf-dependent phosphorylation of Bad, a protein whose pro-apoptotic activity is impaired by an interleukin-3 (IL-3) signal.[45,46] Insights into the signal transduction pathways activated by BCR-ABL will provide a new basis for the treatment of CML patients.

Assays for normal and leukemic stem cells: what can they tell us?

Historically, quantitative assays for primitive human hematopoietic cells have mirrored those previously developed and validated for murine cells. Initially, these were focused on stimulating the formation of colonies of recognizable blood cells of a particular lineage (or multiple lineages) in growth factor-supplemented semisolid culture media. Thus in order for a cell to be identified as a progenitor using this approach, that cell and its progeny have to be able to execute a certain minimum number of cell divisions and differentiate to maturity in semisolid medium in response to a particular soluble growth factor, or combination of soluble growth factors. In fact, most, if not all, intermediate stages of hematopoietic cell development appear to possess all of these properties, providing they are exposed to adequate concentrations of cytokines to which they and their progeny are responsive.

More recently, we and others have focused on the use of the long-term culture (LTC) system to devise quantitative assays for cells that give rise to CFC for extended periods (≥ 4 weeks) under these conditions.[71-73] Interestingly, such cells, which are referred to as LTC-initiating cells (LTC-IC), when isolated from normal human marrow have been found to be unable to proliferate in semisolid media in spite of their ability to proliferate and differentiate in liquid media containing the same growth factors.[74] Thus, the cells in normal marrow that are identified as LTC-IC and CFC are more readily discriminated by differential sensitivity to changes in the physical chemistry of their microenvironment than by changes in growth factor responsiveness. On the other hand, studies of growth factor-stimulated marrow cells have suggested that the ability of a primitive hematopoietic cell to be detected as an LTC-IC is not mutually exclusive of its ability to be detected as a CFC.[75]

Comparison in the mouse of the frequency and properties of LTC-IC and cells with long-term *in vivo* lympho-myeloid reconstituting potential have indicated a close relationship between the cells detected by these two assays.[76,77] The latter can be quantitated by limiting dilution analysis and are referred to as *competitive repopulating units* (CRU). Recognition of the ability of human hematopoietic cells to home into the bone marrow of intravenously injected immunodeficient mice and initiate human multilineage hematopoiesis there, particularly in myeloablated recipients,[78,79] has now led to the development of an analogous limiting dilution assay for quantitating transplantable human CRU (Conneally, unpublished observations). As previously demonstrated in transplanted fetal sheep,[80] human cord blood cells, either expressing CD38 or not, have been shown to have transplantable hematopoietic reconstituting potential. However, the majority

(80%) are $CD38^-$ as is also the case for cord blood LTC-IC. The availability of a procedure for quantitating human CRU with lympho-myeloid reconstituting ability represents a crucial first step towards the further characterization of this cell, investigation of its relationship to human cells detectable as LTC-IC, and the elucidation of mechanisms that maintain their defining properties through successive cell divisions.

Properties of leukemic (CML) cells and leukemic cell populations quantitated using assays developed for normal cells

A cardinal feature of the chronic phase of CML is the continuation of essentially normal differentiation processes. This feature, together with the presumed origin of the clone in a stem cell with lympho-myeloid developmental potential, is believed to explain the generation in patients of clonal RBCs, platelets and lymphocytes as well as granulocytes and monocytes that are indistinguishable from their normal counterparts.[81] The minimal impact of BCR-ABL gene expression on hematopoietic cell differentiation has also made it possible to use the same functional assays developed for quantitating normal CFC and LTC-IC to discern a similar progenitor hierarchy within the $BCR\text{-}ABL^+/Ph^+$ clone.[82]

Analysis of the frequency and absolute numbers of these early progenitor types in a large number of individual chronic phase patients has revealed an interesting pattern of deregulation with evidence of the following common features, in spite of wide patient-to-patient variability. At the level of the most primitive (LTC-IC) compartment(s), residual normal cells commonly outnumber the expanding population of $BCR\text{-}ABL^+/Ph^+$ cells.[82-84] Nevertheless, the use of measurements of even minimally detectable leukemic LTC-IC in some patients' marrow samples ($\geq$1 LTC-IC/2×10^7 marrow cells) together with estimates of the total number of cells in the marrow (~10^{12}) indicates that populations of $\geq$ 50,000 leukemic LTC-IC are not uncommon. Whether this represents an abnormally amplified population (considering its origin from a single stem cell) is not known, since values for the distribution of LTC-IC numbers among the clones that are active in normal individuals are not available.

The phenotype, cycling control and self-renewal behavior of leukemic LTC-IC have also been compared with normal controls. A greater proportion of leukemic LTC-IC have been found to express readily detectable levels of HLA-DR than is typical for the LTC-IC that are present in normal marrow[85,86] but other markers of primitive hematopoietic cells (e.g., absence of detectable CD38, CD45, RA and CD71) also appear to be characteristic of $BCR\text{-}ABL^+/Ph^+$ LTC-IC,[87] in spite of the abnormally high numbers of these leukemic progenitors that are cycling. The self-renewal behavior of these cells, as inferred from studies demonstrating their rapid disappearance in LTC in the absence of added growth factors[84] or in serum-free medium in the presence of added growth factors[88] appears, however, to be rather defective. This could explain the slow rate of growth of the initial chronic phase clone *in vivo,*[89] due to the competing effects of an increased turnover rate but a reduced probability of self-renewal.[82] This latter finding also opens the possibility of new purging strategies based on the incubation of purified *stem cell candidates* under conditions that may amplify co-existing normal stem cells.[74]

Abnormalities in cell cycle control of leukemic CFC in patients with CML are well established.[90,91] The fact that these changes in the rate of turnover of the leukemic CFC compartment are accompained by a significant and lineage-wide increase in their numbers suggests a causal relationship. However, decreased sensitivity of $BCR\text{-}ABL^+/Ph^+$ progenitors to apoptosis in the absence of exogenously provided growth factors, as has been shown by several groups,[92-94] may also be a contributing mechanism.

Future directions

Valuable as they have been, surrogate assays for CML stem cells are limited in many respects. Clinical evidence of the existence of a transplantable CML stem cell population has been in the literature for many years[95,96] and has recently been extended to xenogeneic recipients of transplanted CML cells[97] and by retroviral marking to autograft recipients.[98] The potential of using immunodeficient mice as recipients of CML stem cells to create an *in vivo* model of chronic phase CML should be invaluable for testing novel therapies designed to eliminate residual disease in the patient. In addition, this last approach may result in more relevant assays for quantitating and characterizing the leukemic cells that maintain the developing chronic phase clone. It is hoped such efforts will lead to more effective and broadly applicable treatments as well as a better understanding of how the BCR-ABL gene product perturbs normal hematopoietic stem cell behavior.

Current therapeutic options

Conventional chemotherapy

Few patients die in the chronic phase of CML because it is relatively easy to control the clinical manifestations of the disease. Busulfan has been considered the drug of choice for palliation during the chronic phase for about 40 years. All studies have shown its efficacy and reliability since its introduction in 1953.[99] The only true alternative was hydroxyurea (HU) which was introduced 10 years

later.[100] Most other cytostatic drugs had shown to be either inferior to busulfan and HU or to lack any clear advantage.

Long survival times of CML patients with busulfan-induced mosaicisms, i.e. reduction of Ph-positive cells and the simultaneous presence of normal Ph-negative cells, led to trials to reduce or eliminate the Ph-positive cell clones by intensive combination chemotherapy.[101-104] The remarkable feature of these studies was the observation of reductions of the Ph-positive cells in up to 70% of the cases studied and, in some rare instances, of complete cytogenetic remissions. Intensive therapy of the chronic phase has been attempted with drugs effective in the treatment of acute leukemias, with the goal of ablating the Ph-bearing cells.[105-107] In at least six studies comprising about 200 patients, more than 20 complete cytogenetic remissions were observed.[108] The duration of cytogenetic improvement after combination chemotherapy was relatively brief, lasting only 6 to 8 months. Although there was no special maintenance therapy, the survival of these patients, as a group, was longer.

These trials were uncontrolled and the patient numbers small; a significant advantage over conventional therapy (mostly busulfan or hydroxyurea) was demonstrated in only one study.[109] Despite these interesting studies, hydroxyurea became increasingly popular because of its rapid action, low level of adverse effects and an increased survival versus patients treated with busulfan.[100,110] Based on these reports and similar unpublished experiences in other centers, the German CML study group in 1983 started a randomized trial comparing hydroxyurea vs. busulfan in order to evaluate the duration of the chronic phase and survival. Three hundred and seventy-one patients entered the study and a significant advantage for the hydroxyurea-treated patients was found.[111] The median survival in the busulfan group was 45 months and in the hydroxyurea group 58 months (p=0.008). In conclusion, hydroxyurea has replaced busulfan in the management of the chronic phase and remains the best therapy for CML patients at diagnosis.

Interferon-α therapy

The first reports on the efficacy of natural interferon-α (IFN-α) in CML date back to 1983 and were published by Talpaz *et al*. who demonstrated be hematologic remissions in five of seven untreated or minimally pretreated chronic phase CML patients.[112] Subsequently, Talpaz, reported that 73% of patients achieved hematological remissions and 19% complete cytogenetic remissions in a trial of 96 untreated CML patients.[113] Cytogenetic remissions were durable and long-lasting in the majority of patients. These results were confirmed by several groups with IFN-α alone or in combination.[114-128] Several randomized studies were started after these first reports in order to confirm the usefulness of IFN-α vs conventional chemotherapy.

Randomized IFN-α studies: results

Germany: 513 Ph-positive patients were randomized (133 for IFN-α, 186 for busulfan, 194 for hydroxyurea).[129] The median survival was 66 months for IFN-α, 56 for hydroxyurea and 45 for busulfan. IFN-α treated patients had a significant survival advantage over busulfan-treated patients (p=0.008), but not over hydroxyurea-treated patients (p=0.44). These results were recognized in all risk groups[130] as defined by Sokal's risk grouping.[131] The rates of patients reaching complete or partial hematologic remissions were 83% in IFN-α treated patients and 90% both in the hydroxyurea and busulfan groups. In the IFN-α arm, the time of any hematological response was approximately 2.5 months, to complete hematological remission approximately 6.5 months. Complete hematological remissions with IFN-α showed a significant survival advantage over partial or non-responders (p=0.007). Of special interest was the evolution of the disease in patients after IFN-α had been discontinued. The survival of the 65 patients who had discontinued IFN-α for various reasons when still in the chronic phase was significantly inferior to that of the 61 who had continued IFN (p=0.007).

Italy. The Italian Cooperative Group for Chronic Myeloid Leukemia compared recombinant IFN-α with conventional chemotherapy (hydroxyurea or busulfan) in a trial designed to have a power of 80% to detect a difference of 20% in a median survival between the one receiving IFN-α and the group given conventional chemotherapy. Between 1986 and 1988, 322 patients with previously untreated and minimally treated Ph-positive CML were randomly assigned to IFN-α (218 patients) or chemotherapy (104 patients, mostly hydroxyurea). Analysis was performed as of April 1993.[123] The rate of karyotypic responses (defined as >33% of Ph-negative metaphases) was 30% in the IFN-α group and 5% in the chemotherapy arm (p<0.001). The time for progression to accelerated and blastic phase was longer in the IFN-α group than in the conventional chemotherapy (median > 72 vs 45 months, p<0.001); as for survival, median survival was > 72 vs 52 months and six-year survival was 50% vs. 29% (p=0.002). Survival was longer in the patients with karyotypic responses and the Cox multivariate model showed that karyotypic response was more strongly related to survival than Sokal's risk groups (p=<0.001 vs. p=0.002). The cost of IFN-α treatment was 200 times that of conventional therapy. As of May 1997, the updated figures of that study are as follows: 228/322 patients have died; ninety-four patients are alive with a minimum follow-up of 90 and a maximum of 120 months (median 104). In the IFN-α arm, 56

patients are alive, 41% with a cytogenetic response (14% complete, 16% major and 11% minor). In the chemotherapy arm, 16 patients are alive (16%) but only 2 of them have shown a minor cytogenetic response. Overall, the median survival is 76 months (IFN-α) vs 52 months (chemotherapy) (p=0.002) and the risk of progression is lower for IFN-α (p=0.0005) (unpublished data).

United Kingdom. 587 CML patients (IFN-α: 293 patients; no IFN-α therapy: 294 patients)were reported to have a median survival time of 63 months in the IFN-α group and 43 months in the 266 patients treated with chemotherapy.[124] This difference was significant (p=0.0009). This study also found a significant survival advantage for IFN-α treated cytogenetic non-responders.

France. The results of a randomized multicentric French trial comparing IFN-α alone (324 pts) versus IFN-α + low-dose ARA-C (322 pts) were recently reported by Guilhot.[132] He has provided the following results: the rate of complete HR at 6 months was 54% vs 67%, respectively; major and complete cytogenetic responses were 22% (55/249 pts) and 39% (96/248 pts), respectively. Survival at 3 years was 76% and 88%. The survival was superior in those patients achieving major and complete cytogenetic remission (p=0.0001). However, 75 and 71 patients, respectively, discontinued IFN-α.

Japan. A Japanese study compared the influence of IFN-α (n=80) and busulfan (n=79) on the duration of the chronic phase, on survival, and on hematological and cytogenetic response in Ph-positive CML.[133] The predicted 5 year survival was 54% in the IFN-α group and 32% in the busulfan group (p=0.029). Seven patients (8.8%) in the IFN-α arm and 2 (2.5%) in the busulfan arm reached a complete cytogenetic remission. Cytogenetic IFN-α responders had no significant survival advantage over non-responders, but a trend was recognized (p=0.1065). Table 2 lists all the details of these studies.

Can we consider interferon first-line therapy for CML?

Defining any treatment procedure as first line is a matter of compromise and requires first that the treatment satisfies the patient, second that it can be administered, and third that it is more cost-effective than other treatments. Moreover, a fourth important feature of a first-line treatment should be that it does not prevent the patient from receiving other important treatments, if the first-line choice fails. In fact, it should not be overlooked that the disease is chronic and that treatment should aim at prolonging survival, so that more than one treatment may contribute substantially to overall survival.[134,135]

Based on these considerations, the first-line treatment for CML is: conventional chemotherapy for all elderly patients (e.g. more than 69 years old), IFN-α for all the *older adults* (55 to 69 years old) and for non low-risk adults without a matched family donor, and IFN-α also for low-risk *younger adults* (19 to 39 years old) without a matched family donor. When an HLA-identical sibling is available, allogeneic bone marrow transplantation is first line in all children (less than 19 years old), in all younger adults and in non low-risk adults. When only a *well-matched* unrelated donor is available, allogeneic bone marrow transplantation is first line in all children and non low-risk younger adults. Precise age boundaries are obviously controversial and open to criticism, and any final decision would also depend on the general health of patient as well as on his/her wishes. This is especially important for allogeneic bone marrow transplantation, which can either cure or kill a patient.

The basis for these options, that are summarized in Table 3, are the data that have been reported so far for IFN-α and allogeneic bone marrow transplantation.[113,123,124,128,129,136-139] These do not take into account experimental or non evidence-based treatments that are still investigational and cannot be considered for first-line treatment outside a con-

Table 2. Randomized studies with IFN-α in CML: results of treatment in the IFN-α arm.

Author (ref)	Pt. no.	Risk profile (Sokal) Low n (%)	Intermediate n (%)	High n (%)	HR (%)	Cytogenetic response Any (%)	Major (%)	Complete (%)	Median survival (months)
Kantarijan (128)	274	124(52)	59 (25)	54 (23)	87	56	38	26	89
Alimena (116)	35	16 (46)	12 (34)	7 (20)	68	55	12		
Ozer (119)	107						29	13	66
Mahon (120)	81	39 (48)	32 (40)	10 (12)	98		44	38	
Tura (123)	218	94 (43)	72 (33)	52 (24)	62	55	19	8	72
Hehlmann (129)	133	36 (27)	47 (35)	50 (38)	83	18	10	7	66
Allan (124)	267	67 (25)	89 (33)	111 (42)	86	22	11	6	63
Ohnishi (133)	80	29 (37)	26 (33)	23 (30)	78	44	7	9	65

HR = hematological remission complete and partial.

Table 3. Main options for first-line treatment according to age and risk. The risk is defined according to Sokal *et al*.[131]

Age and risk	*HLA-identical sibling* *Available*	*Non available*
Children (≤ 18 y), any risk	BMT	BMT/UD
Young adults (19-39 y)		
low risk	BMT	IFN-α
non-low risk	BMT	BMT/UD
Adults (40-55 y)		
low risk	IFN-α	IFN-α
non-low risk	BMT	IFN-α
Old adults (56-69 y)		
any risk	IFN-α	
Elderly (≥ 70 y)		
any risk	CHT	

BMT= allogeneic bone marrow transplantation from an HLA-identical sib donor. BMT/UD= allogeneic bone marrow transplantation from an unrelated donor. CHT= conventional chemotherapy.

trolled study.[140] The arguments for a decision in favor of IFN-α in several age and risk groups are not only that the response to IFN-α usually results in a long survival with a median that is not yet reached after 10 years, but also that IFN-α does not kill patients, that the response to IFN-α can be easily predicted within 6 months in 75% of patients and within 1 year in almost all cases, and that neither that period of time nor IFN-α treatment itself will adversely affect the outcome of subsequent evidence-based treatments, with special reference to allogeneic bone marrow transplantation, which can be offered to all patients who fail to respond to IFN-α.[140-143] It is likely that patients who would fail experimental treatment intensifications, including autologous bone marrow transplantation, would have a much lower chance of surviving a subsequent procedure of allogeneic bone marrow transplantation.

Summary and perspectives

Complete cytogenetic remissions can be achieved with IFN-α in up to 39% of cases in phase II studies with selected patient populations, and in a somewhat lower proportion of patients in randomized studies which have to adhere to the *intention-to-treat* principle. The majority of these remissions are relatively stable and long-lasting (more than 1 year and up to 6 years and longer). The median time to complete cytogenetic remission ranges between 12 and 17 months, but complete remissions may occur 36 months after start of IFN-α therapy and even later. Due to the inherent limitations of cytogenetic analyses (bone marrow puncture and analysis of a sufficiently large number of mitoses required), the determination of the exact time when complete cytogenetic remission occurs may be difficult. Likewise, the detection of transient cytogenetic remissions may depend to a considerable extent on the frequency of cytogenetic analyses. With regard to these limitations, hematologic remission probably is the most practicable prognostic and follow-up parameter. However, in some important studies (Italian, Houston) cytogenetic remission was shown to be of major relevance. It has to be defined whether the survival advantage, due to a delay in blast transformation, seen in major and complete cytogenetic remissions is related to the achievement of CR once in the course of the disease, or whetever the length of that CR has a major importance.

Toxicity appears to limit the use of IFN-α in a proportion of patients. The contention that IFN-α is less well tolerated in patients older than 60 years was not confirmed in the German randomized study, in which the mean age of patients who continued or discontinued IFN-α was virtually identical (47 years). If the full IFN-α dosage cannot be resumed, hydroxyurea is added with the aim of keeping the leukocyte counts at 2.0-4.0×10^9/L. In comparing median survival times between studies, it should be kept in mind that the impact of risk profile (Sokal) on survival overrides that of drug therapy by a factor of about 2.[144]

The mechanism of the life-prolonging effect of IFN-α in CML is unclear. Possible ways include non-specific inhibition of proliferation of the leukemic cell clone as well as modulation of cytokine actions and of the immune surveillance system. The different therapeutic effects of cytostatics and of IFN-α suggest that, at least in part, different modes of action may be responsible for their effects on CML.

Allogeneic hemopoietic stem cell transplantation for CML

It has become clear in the last fifteen years that selected patients with chronic myeloid leukemia can be cured by allogeneic bone marrow transplantation. A number of unresolved issues remain, most of which are interdependent. These include the selection of eligible patients, the choice of an optimal donor, the timing of the transplant procedure within chronic phase, the technology of the actual transplant, the best way to monitor individual patients and the best approach to management of relapse. Some of the issues are addressed below.

Patient eligibility

There is general agreement that the risk of transplant-related mortality (TRM) increases in parallel with the age of the patient but no agreement as to the maximal age for transplant using a genetically HLA-identical sibling (sibling donor transplant) or a phenotypically-matched family member or volun-

teer unrelated donor (alternative donor transplant). Most transplant centers are reluctant to offer sibling donor transplants to patients over 50 years or alternative donor transplants to patients over 45 years. Some specialist centers use higher age limits.

Choice of donors

Currently, the optimal donor is an HLA-identical sibling but it is at least theoretically possible that a *well-matched* unrelated donor could be a better choice. Registries established in more than 20 countries worldwide now contain details of about 4 million people who have volunteered to donate bone marrow for suitable patients. HLA-typing techniques have advanced greatly in the last five years so that polymorphic class II genes can now routinely be typed by molecular methods and comparable molecular techniques should soon be generally available for typing class I genes. Cross-matching techniques such as measurement of cytotoxic and helper T-cell precursor frequencies in the blood of prospective donors have some value in donor identification. It appears also that the use of younger donors may be associated with lower incidence of transplant-related mortality than with older donors. The role of stem cells collected from the umbilical cord of neonates is still uncertain.

Timing of the transplant procedure

It is clearly established that the risk of relapse and of TRM are both substantially higher if the transplant is performed in advanced phase disease rather than in chronic phase. For patients in chronic phase it is now generally accepted that TRM is lower and leukemia-free survival (LFS) correspondingly higher if the transplant is performed within the first 12 months after diagnosis.[145] It should be noted, however, that this conclusion is based on analysis of survival in patients treated before transplant with busulfan or hydroxyurea; it is not certain that the same adverse effect of delay to transplant would be seen in patients treated pre-transplant with interferon-α. Whatever the case, the fact that the onset of transformation cannot reliably be predicted in any given patient is a strong argument in favor of proceeding to transplant as rapidly as possible in any eligible patient.

Details of the transplant procedure

The use of cyclophosphamide and total body irradiation (TBI) remains the gold standard but the combination of busulfan and cyclophosphamide may be equally effective and easier to administer. TBI is usually given in fractions over 3 to 6 days but in practice fractionated TBI may offer no advantage over TBI administered as a single dose. Most centers use a combination of cyclosporin A and methotrexate for prevention of GVHD, although this is associated with a higher risk of relapse than the use of cyclosporin A alone. A minority of centers use one method or another of T-cell depletion of donor marrow; all such methods are associated with an increased probability of relapse.

Results of transplant

In general recent analyses show that the projected probabilities of survival, leukemia-free survival and relapse at 5 years for patients allografted with stem cells from sibling donors are 50-70%, 30-60% and 15-30% respectively. The age of the patient is

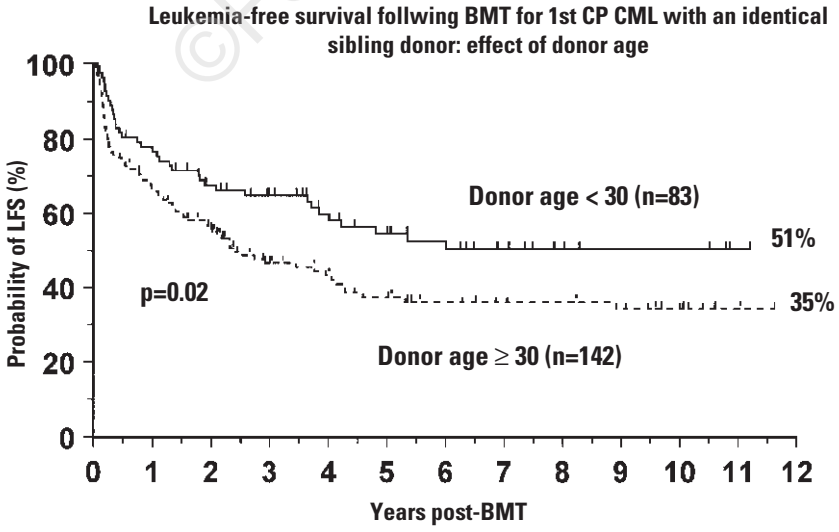

Figure 1. Probability of leukemia-free survival by patient age at time of transplant for 225 patients allografted for CML in chronic phase with marrow stem cells from HLA-identical sibling donors. (Hammersmith Hospital, London, May 1997).

an important variable (Figure 1). Comparable figures for patients allografted in chronic phase with stem cells from alternative donors are 40-60%, 30-50% and 5-25%. The major causes of transplant failure are GVHD and relapse. Deaths from infection and pneumonitis are now relatively rare.

Monitoring individual patients

The majority of patients who relapse do so within 3 years post-transplant. The first evidence of relapse is identifiable at the molecular level, followed after some months by cytogenetic relapse and thereafter by hematologic relapse. The finding of BCR-ABL transcripts in the blood or marrow in the first 6 months post-transplant has little prognostic significance,[146] but their persistence or appearance at 9 months or later is ominous. Thereafter, a rising level of BCR-ABL transcripts defines molecular relapse and means that the patient is likely to proceed to cytogenetic and eventually to hematologic relapse. Thus a reasonable formula would be to measure transcript levels at 2- or 3-month intervals starting 6 months post-transplant and continuing for at least three years.

Treatment of relapse

Relapse, however defined, may be treated by a second transplant, IFN-α or donor lymphocyte transfusions (DLT). This last approach can restore remission in 70-80% of cases[147] but can also cause marrow aplasia or GVHD. Because the response rate may be higher and the incidence of complications may be lower in those treated in molecular or cytogenetic relapse compared with those treated in hematologic relapse,[148] a reasonable approach would be to initiate treatment with DLT for any patient in established molecular relapse. Recent evidence suggests that administration of DLT in an escalating dosage schedule may be a way of inducing a graft-versus-leukemia effect with minimal risk of associated GVHD.

Patients who achieve cytogenetic responses almost all proceed to PCR negativity and this is usually sustained indefinitely. Thus a portion of the patients who respond to DLT may eventually prove to be cured despite their earlier relapse. This means that estimation of leukemia-free survival by conventional techniques may fail to recognize a subset of patients who have relapsed but have subsequently been treated with success. One formula for taking into account these patients is to add them to the conventionally defined leukemia-free survivors in a new Kaplan-Meier curve designated *current leukemia-free survival*.[149]

Alternative donor transplants for chronic myeloid leukemia

For many patients, alternative donor transplant is a real consideration. In general the results using unrelated donors are inferior to those using siblings. The reasons for decreased disease-free survival (DFS) and increased TRM are undoubtedly multifactorial. They include increases in the incidences of acute and chronic GVHD, of graft failure and of life-threatening infections, all of which presumably reflect the presence of varying degrees of HLA-disparity and a tendency to delay the procedure so that the interval from diagnosis to transplant is greater than in an equivalent cohort of patients transplanted from sibling donors.

Attempts to modify the risks of graft failure and GVHD by using intensified conditioning regimens and more rigorous GVHD prophylaxis have, in turn, resulted in increases in infectious complications, pneumonitis and disease recurrence. These modifications are necessary to compensate for the presence of HLA-disparity between the recipient and donor. Theoretically therefore, improvements in outcome would seem to depend on improved donor selection.

Selection of unrelated donors previously relied upon serological identification of HLA-A, B and DR alleles. Sequencing of the HLA-genes has now revealed a greater degree of polymorphism at these loci than that detected by serology. As a consequence many unrelated pairs matched serologically have subsequently been shown to have multiple undisclosed mismatches. The influence of matching for Class I HLA-alleles has long been recognized. The cytotoxic T-cell precursor (CTLp) frequency assay reflects differences at the Class I loci and at least two groups have identified the presence of high frequency CTLp to be a useful prognostic indicator of outcome after unrelated donor transplants.[150,151] Donor-recipient pairs with a high frequency of CTLp are more likely to develop severe acute GVHD and have a decreased DFS compared to those pairs with a low frequency of CTLp. More recently, high frequency CTLp has been shown to be closely correlated with mismatching at the HLA-C locus,[152] and it is likely that HLA-C matching will now assume greater importance in donor selection.

In a recent analysis of 320 patients transplanted for CML in first chronic phase from unrelated donors and reported to the *Chronic Leukemia Registry of the European Group for Blood and Marrow Transplantation* (EBMT), matching for HLA-DRB1 was the most important factor influencing DFS.[153] The 211 patients in the *matched* group had a DFS at 2 years of 41% compared to 17% for 109 patients who were *mismatched* at this locus. This confirmed data previously reported by the *Seattle Transplant Team* derived from 364 patients transplanted for a variety of hematological malignancies.[154]

The dilemma now facing physicians is that the use of sophisticated techniques for HLA-matching is likely to render the identification of a fully matched unrelated donor much more difficult. The

goal must now be to identify acceptable degrees of *mismatch* but with the enormous heterogeneity of the HLA locus, even this may be a major challenge.

Variables known to influence the outcome of HLA-identical sibling transplants also have a prognostic role in unrelated donor grafts. Older age, advanced phase disease, prolonged interval from diagnosis to transplant and T-cell depletion all adversely affect DFS. Whereas age and disease status cannot be influenced by the physician, the timing of the transplant and the method of GVHD prophylaxis are amenable to modification. For many patients it is difficult to justify an unrelated transplant before an adequate trial of interferon-α, but the latter approach may delay the transplant beyond the first year of diagnosis. The nature of GVHD prophylaxis is also a complex problem. In HLA-identical sibling transplants for CML, T-cell depletion is associated with a reduction in the incidence and severity of GVHD but at the expense of an higher incidence of graft failure and disease recurrence. These effects were confirmed in recipients of unrelated donor transplants in the EBMT study mentioned above, with 2 year disease-free survival and relapse incidences of 41% and 5% in patients receiving cyclosporine and methotrexate (n=202) vs 28% and 29% (n=58) for those who were T-cell depleted *in vivo* (n=71). In contrast, in a series of 48 consecutive patients with CML who received T-cell depleted unrelated marrow, the 2 year probability of relapse was low at 8.8%, suggesting an apparent preservation of graft versus leukemia activity.[155]

The *Seattle Transplant Team* who continue to use conventional cyclosporine and methotrexate as GVHD prophylaxis report a 3 year overall survival of 60% and a relapse incidence of less than 10% for patients transplanted in first chronic phase from unrelated donors.[156] The extent to which the use of DLT may compensate for the increased relapse rate is as yet unclear. At the Hammersmith Hospital we have successfully restored 12 of 24 recipients of unrelated marrow to complete cytogenetic remission using donor lymphocytes. A re-definition of the terminology relating to disease free survivors may now be appropriate.[149]

Several groups have reported an increase in the incidence of late infections in recipients of unrelated marrow compared to those receiving sibling cells. Again the causes are likely to be multifactorial. T-cell depletion delays T-cell re-population as do increasing degrees of HLA-disparity and the increased incidence of GVHD in these patients necessitates the use of long-term immunosuppressive agents. We and others have identified recipient CMV seropositivity prior to transplant as an adverse prognostic indicator, although improved methods of CMV detection may now permit effective pre-emptive therapy in this group.

A knowledge of the prognostic indicators allows us to identify a good risk group for unrelated transplantation. In our hospital 10-year survival and DFS following unrelated transplant for CML in first chronic phase were 50% and 40%, respectively, for patients who were less than 40 years at transplant, CMV seronegative and HLA-matched (by the best available method at the time of transplant) and 25% and 19% for all other patients (Figures 1 and 2). The intelligent and compassionate use of this knowledge should enable better selection of patients and their donors and begin to solve the dilemma related to the timing of the transplant.

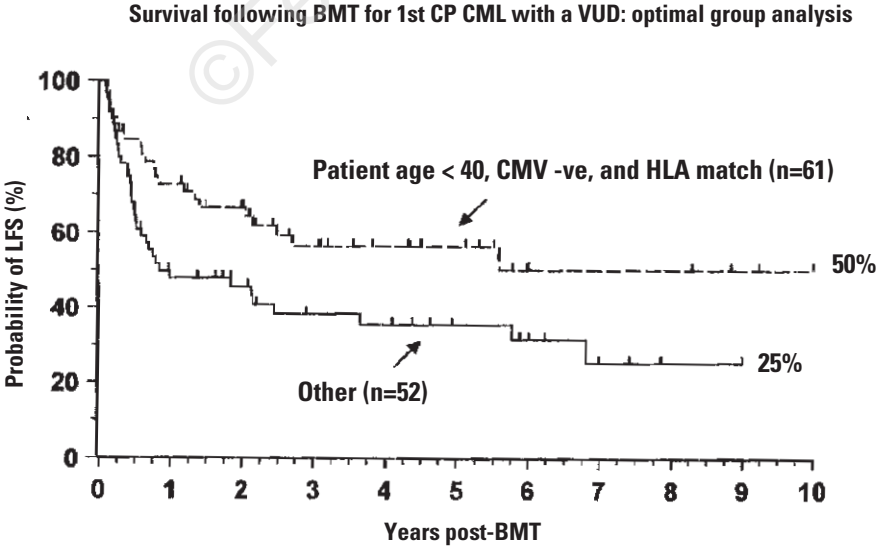

Figure 2. Survival following BMT for CML in 1st chronic phase from a volunteer unrelated donor. A comparison of a good risk group defined by age < 40 years at transplant, CMV seronegativity and HLA-match, vs. all other patients.

Alternative sources of stem cells, i.e. cord blood and family mismatched donors, are now available for patients lacking HLA-identical sibling or adult unrelated donors. Data relating to cord blood transplants for CML are limited to case reports. More useful information should be generated from a Eurocord pilot study restricting cord blood transplant for CML to a uniform protocol of conditioning, GVHD prophylaxis and minimum cell dose. Data from murine transplants has long since demonstrated that engraftment across HLA-barriers can be achieved by increasing the inoculum of infused stem cells. The resulting increased incidence of GVHD can be overcome by rigorous T-cell depletion. This approach has been unsuccessful in humans due to the unacceptably high incidence of graft failure. Recently, however, this obstacle was overcome in acute leukemia by the use of T-cell-depleted bone marrow- and blood-derived stem cells.[157] This exciting approach has great potential and results in CML are eagerly awaited.

Autografting for chronic myeloid leukemia: does it make sense?

In patients lacking matched related or unrelated donors, autologous stem cell transplantation (ASCT) has been attempted since 1972 in order to restore chronic phase in patients who have evolved to blast crisis.[158] Subsequently, ASCT was performed in chronic phase patients using unmodified stem cells. In this situation, cytogenetic response was achieved in about 40% of patients and the results of retrospective analyses of registries suggest that it could prolong survival. This needs to be demonstrated prospectively. If so, further studies

Table 4. Summary of results of ASCT for 497 in chronic phase.

No. pts	*Source of stem cells**	*Cytogenetic conversion°*	*Outcome#*	*Refs.*
34	Unmanipulated BSC	18/32 pts.	31/34 A/W (median follow-up of 12 months)	158
21	Unmanipulated BSC	11/17 pts.	5-year survival of 56%	159
23	Unmanipulated BSC	14/23 pts.	3-year survival of 66.8±23%	160
22	Unmanipulated BSC or marrow=10 Mobilized BSC=9 Purged marrow=3	5/22 pts.	Median survival of 34 months	161
5	Mafosfamide-treated marrow	5/5 pts.	2/5 A/W	166
16	Cultured marrow	7/11 pts.	12/16 A/W	167
6	IFN-γ treated marrow	5/6 pts.	5/6 A/W	168
23	Mobilized BSC	18/22 pts. treated in early chronic phase achieved a CR	12/12 A/W $2^{+}/47^{+}$ months after autografting	174

**BSC: Blood stem cells; °More than 50% Ph-negative cells in most cases.; #A/W: alive and well (in chronic phase). CR: complete response*

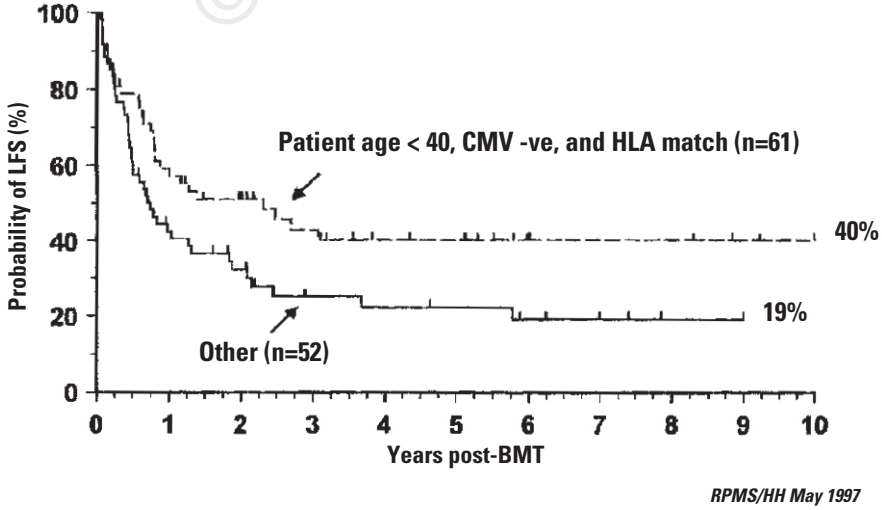

Figure 3. Disease-free survival following BMT for CML in 1st chronic phase from a volunteer unrelated donor. A comparison of a good risk group defined by age < 40 years at transplant, CMV seronegativity and HLA-match, vs. all other patients.

multicentric studies correspond to those of unicentric studies. In the report by Mc Glave *et al*.,[163] 142 patients transplanted during chronic phase in eight major transplant centers were analyzed; the four-year survival was around 60%. The source of stem cells (blood versus marrow) and the use of *ex vivo* cell treatment did not influence survival. In a retrospective analysis of registry of the *European Group for Blood and Marrow Transplantation* (EBMT), 174 patients who underwent autologous blood (66%) or marrow (34%) stem cell transplantation during chronic phase after different conditioning regimens were evaluated.[164] Most of these patients were treated with IFN-α after transplantation. The actuarial survival at five years was 68.4±11% and was significantly higher for younger patients and, more importantly, for those who achieved hematological or cytogenetic responses following autografting. In this series of patients, the survival was not influenced by the source of stem cells (marrow versus peripheral blood) and autografting was able to restore IFN-α sensitivity in some patients. Thus, ASCT performed during chronic phase has an acceptable toxicity (as the transplant-related mortality does not exceed 5%) and produces a five-year survival from transplantation around 50%-70%.

What source of stem cells?

Whatever the source of stem cells (blood or marrow), the main question concerns purging. It is most unlikely that ASCT would cure CML if Ph-positive stem cells were reinfused into the patients. Moreover, Deisseroth *et al*. using gene marking techniques have reported that transplanted leukemic progenitor cells could contribute to relapse after ASCT.[165] Thus many attempts have been made to eradicate Ph-positive leukemic cells from the graft.

Ex vivo purging. These techniques include the use of hyperthermia, cyclophosphamide derivatives (hydroperoxycyclophosphamide and mafosfamide-AZTA-Z), interferons and/or interleukin-2 and, more recently, ribozymes, antisense oligonucleotides or tyrosine kinase inhibitors.[166] Some of the *in vitro* results are encouraging but their possible advantage in terms of prolongation of survival over unpurged ASCT, has not yet been demonstrated (Table 4). They also have some drawbacks, such as delayed engraftment.[167-169] *In vitro* experiments published by the Vancouver group and the selection of $CD34^+/DR^-$ cells by Minneapolis, represent the most interesting new perspectives in this field.[86,168] The initial idea of exploiting Ph-negative cells in CML gained strength and enthusiasm. It was shown in a series of elegant experiments that Ph-positive cell numbers decline when put in culture, whereas Ph-negative cells not previously identifiable emerged in those cultures and showed better survival.[82-85] The basic mechanism of this behavior is still unclear; however, it is important to point out that some of the emerging Ph-negative cells show characteristics of very primitive hematopoietic cells (LTC-IC). As a result of these findings, the Vancouver group devised a trial consisting of a 10-day culture of CML bone marrow and subsequent infusion into a conditioned patient previously selected on the basis of the ability of his bone marrow to produce *in vitro* an adequate number of normal LTC-ICs. Only 30% of newly diagnosed patients were suitable to this procedure. The results show that patients initially regenerated with Ph-negative cells and this was maintained, in some patients, up to two years or more before Ph-positive cells made their reappearance.[168] What could the basic mechanisms of this temporary advantage be? Recent fascinating molecular studies have produced evidence that pluripotent hemopoietic murine cells infected with a retrovirus containing the BCR/ABL sequence can reproduce a disease very similar to CML and have shed some light on the perspective of autografting in CML. Transfer of these leukemic cells into a syngeneic recipient resulted in the reconstitution of normal hematopoiesis in short term. Subsequently, some mice developed either chronic or blast phase leukemia in cells derived from the leukemic clone. However, in some mice no evidence of regrowth of leukemic cells was observed.[170] This suggests that the underlying mechanism of partial success of the autografting procedure relies on the presence of Ph-negative cells and their expansion, made possible by reshuffling and the new dynamics between normal and leukemic cells.

Other groups have focused their attention on the separation of Ph-positive from Ph-negative progenitors on the basis of their phenotype. Some authors have suggested that $CD34^+/DR^-$[86,171] but not $CD34^+/38^-$[171] may select for Ph-negative progenitor cells; however, other reports have argued against this sharp distinction.[172] Nevertheless, it has been recently reported that, in patients in early chronic phase, $CD34^+/DR^-$ cells are BCR-ABL mRNA negative in 80% of patients. Large-scale selection with a high-speed FACS, starting from a marrow harvest of 2-2.5 liters results in $1\text{-}3\times10^5$/kg $CD34^+/DR^-$ cells. The frequency of CFC and LTC-IC ranged from 2.6-8.6% and 0.187-0.233%, respectively. Both $CD34^+/DR^-$ and secondary CFC (from LTC-IC) were BCR-ABL mRNA negative. Therefore this large-scale clinical grade selection of $CD34^+/DR^-$ cells allows a highly purified autograft and represents a promising step forward toward further gene manipulation developments.

In vivo purging. It has been clearly demonstrated that most CML patients harbor some Ph-negative cells and these cells could be collected even in advanced stage, during regeneration after chemotherapy and G-CSF.[173] However, only when the disease was approached during early chronic phase in

patients not pretreated with IFN-α did the collection of Ph-negative progenitors became satisfactory. Thirty-three patients with CML entered the Genoa protocol, all within 12 months from diagnosis. All patients completed the mobilization protocol and from most of them, especially those in the first few weeks after diagnosis, a high number of Ph-negative progenitors (CFC and LTC-IC) could be collected.[174] These values were not far from those obtained by the same team when mobilization of normal donors for allografting was performed. This indicates that the hematopoietic reservoir is still well preserved at least early after diagnosis. Furthermore, the number of Ph-negative progenitors is by far superior to what can be achieved by mobilizing patients later on in the course of their disease. A successful collection was obtained in 75% of patients; more precisely, the collection contained ≤ 35% Ph-positive cells and the number of CFC and $CD34^+$ cells was superior to 2×10^4 and 2×10^6 per kg, respectively.

To date, twenty-two (66%) of the 33 untreated patients have been autografted and 90% of the autografted patients achieved major or complete cytogenetic bone marrow remission after engraftment. Low-dose IL-2 and IFN-α were given after autografting. Sixteen patients maintain major or complete cytogenetic remission at 1 year after autografting. Median follow-up from autografting was 18 months (range, 3-58). No patient experienced late graft failure or required a second transplant. One patient evolved to blast transformation at 6 months post-autografting and died of leukemia a few months later.

Taken together, the results suggest that this approach, when applied in early chronic phase, is able to restore and maintain a major or complete cytogenetic response in 50% of the initial population. This percentage seems to be superior to that obtained with interferon therapy; however, such a small series cannot be compared with the results of large-scale trials. Therefore, at the moment, any survival projection is premature. Pilot studies followed by randomized trials are needed to evaluate these two approaches. Such trials are now in progress. Autografting in blastic phase CML is associated with prohibitive toxicity in the absence of any advantages in terms of survival.[175] The use of antisense oligonucleotides for *in vivo* purging in autografting programs is not yet feasible.[176]

Conclusions

While IFN-α as first-line therapy does not seem to jeopardize further treatments, autografting accord-

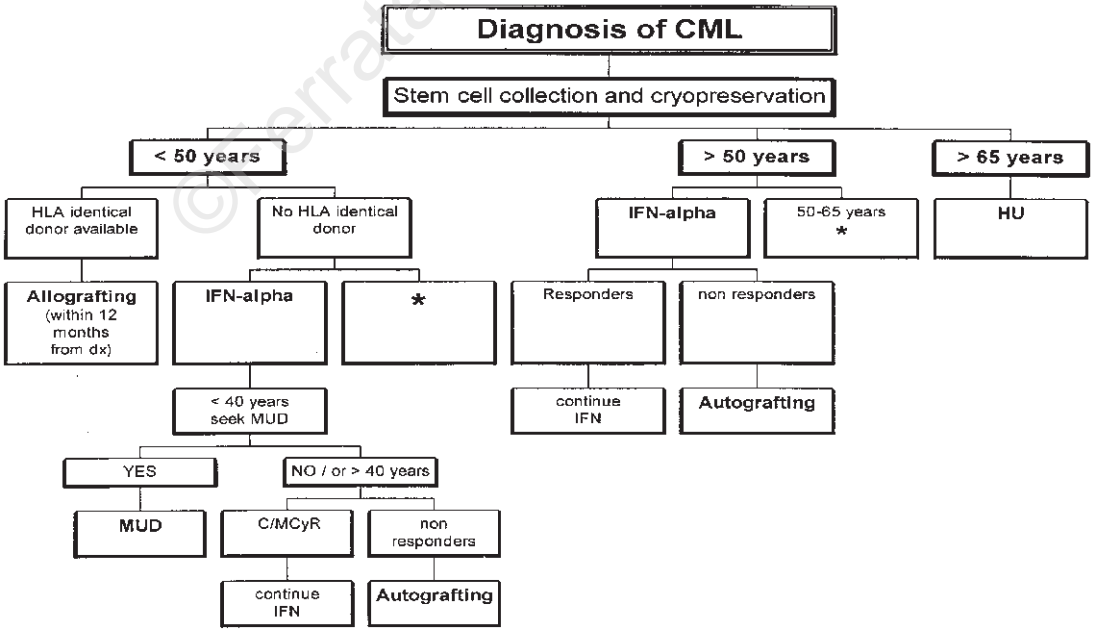

Figure 4. Therapeutic algorhithm for CML.
***Autografting with unmodified peripheral stem cells collected at diagnosis or with BSC mobilized with chemotherapy + G-CSF.**

ing to the Genoa approach or other procedures, i.e. Ph-positive cells collected at diagnosis without mobilization therapy, raises the question of an ideal sequential strategy in the management of CML patients. There seems to be general agreement that a patient less than 50 years old with an HLA-identical sibling should receive an allogeneic stem cell transplant (Figure 4). This approach should also be offered to younger patients (≤40 years) who are able to find a matched unrelated donor (MUD). Since it seems that the normal hematopoietic reservoir declines with time, it may be desirable to mobilize and collect peripheral stem cells in order to store Ph-negative progenitors as soon after diagnosis as possible when the WBC count is being controlled by hydroxyurea while the search for a MUD proceeds. Six-eight months should be allowed for a MUD search. If a donor is not found, the patient could undergo autografting with the previously stored Ph-negative progenitors followed by IFN-α therapy. However, at present, this is an experimental procedure and must be employed only in selected centers.

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