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Perspectives in Cancer Research

Intensive Cytoreductive Regimen and Autologous Bone Marrow Transplantation in Leukemia. Present Status and the Future. A Review

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Abstract—*High-dose cytoreductive treatment followed by ABMT represents a new approach to the treatment of acute leukemia as an alternative when leukemic patients do not have HLA-identical donors. ABMT protocol seems to be a valuable treatment for AML if it is immediately employed after the remission obtained to consolidate the remission. For ALL adult patients, and so for poor prognosis ALL in children, intensive therapy with ABMT represents a new approach when conventional therapy has failed or failed to consolidate the remission. The results of ABMT in CML in the literature have been disappointing; the bone marrow could be collected during the course of the first chronic phase after hydroxyurea therapy or other treatment programs. In leukemia the ABMT approach will be more credible if the protocol includes* in vitro *immunologic or pharmacologic means to eliminate residual leukemic cells.*

INTRODUCTION

ALL EXPERIMENTAL and clinical data clearly favor high-dose therapy in acute leukemia [1]. However, there is the possibility of a resistant leukemic clone which would not be eliminated by the intensive treatment. The best results with high-dose therapy would be expected in cases where leukemic cells are very sensitive to therapy, but until recently the majority of patients have been treated at the end stage of the disease.

The hemopoietic toxicity of chemotherapy and total irradiation can be rescued by transplantation of bone marrow cells. These cells can be of allogenic or autologous origin.

Allogeneic bone marrow transplantation is perhaps the best treatment for patients with acute leukemia. Unfortunately less than 50% of leukemic patients have HLA-identical donors. As an alternative, intensive therapy followed by autologous bone marrow graft represents a new approach to the treatment of acute leukemia when conventional therapy has failed or to consolidate the obtained complete remission.

Autologous marrow transplantation represents a rescue for hemopoietic toxicity which is a common dose-limiting factor in cancer therapy. In this field in which the transplantation procedure is no longer a major problem, leukemic response, extramedullary toxicity and malignant cell contamination of the marrow cells are the limiting factors. The first studies done with autologous bone marrow transplantation demonstrated the feasibility of autotransplantation using frozen–thawed marrow cells.

Since the first publications on autologous bone marrow transplantation in the treatment of malignant disease numerous reports of autologous bone marrow rescues in patients with leukemia have been published.

This review will attempt to summarize all published works as well as our own experience in this field.

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TECHNICAL ASPECTS OF AUTOLOGOUS BONE MARROW TRANSPLANTATION

Autologous bone marrow collected from a leukemic patient and cryopreserved is by definition a malignant cell-contaminated bone marrow. Prior to marrow cell collection, the bone marrow 'normality' must be verified. It is generally defined with marrow smears and biopsy analysis. These cytological investigations detect malignant cells when their number is more than 10^9. Therefore they are not sufficient [2]. One can evaluate the type of CFU-GM growth in culture (especially ratio clusters/colonies). However, bone marrow culture alone is insufficient. Many attempts have been made to recognize, reduce and even eliminate malignant cells in leukemic bone marrow. Physical, pharmacological or immunological means proposed will be discussed further on.

The collection of stem cells must be scheduled at a time when the risk of malignant cell contamination is low. This period is assumed to occur when the marrow is cytologically free of leukemic cells.

Bone marrow processing

Marrow cells are collected under general anesthesia by aspiration from the iliac crests. The collected cells are suspended in anticoagulant tissue culture media [with heparin or acid-citrate dextrose (ACD-A)].

Table 1 summarizes our experience with the harvesting of 82 patients' bone marrow. The general tendency is to reduce the volume aspirated. With above 3 ml from the puncture site one observes a dilution with peripheral blood. If the marrow volume harvested is too large the marrow cell concentration step will be difficult and unsatisfactory. When the marrow volume aspirated is reduced, the total nucleated cell and CFU-GM counts remain satisfactory (Table 1).

Several methods have been proposed to concentrate the bone marrow before cryopreservation: the manual method, using a single centrifugation followed by a collecting of the buffy coat [Ehrsam, unpublished data]; the marrow can be processed with a Haemonetics semi-continuous flow cell separator [3, 4]. The aim of this concentration step is to reduce the volume and contamination by granulocytes, platelets and red cells without marked loss of stem cells; when it is necessary to remove malignant cells from the bone marrow collected by using pharmacological or immunological means, the purity of the marrow cell suspension becomes a fundamental parameter. We use a slightly modified method reported by Gilmore *et al.* [5]. It is an effective technique for the concentration of mononuclear cells from large volumes of bone marrow. The marrow mononuclear cells are isolated with the IBM 2991 blood cell processor. The bone marrow is layered on to Ficoll-Metrizoate and then the mononuclear cell layer is washed 3 times [Hervé *et al.*, unpublished data].

A sizeable reduction in volume represents a considerable saving not only in cryobiology material but also in storage space. Removal of granulocytes and platelets reduces all clumping after thawing.

Bone marrow preservation

Autologous bone marrow transplantation can be performed with either fresh or cryopreserved marrow:

Fresh marrow stem cells stored at 10°C have limited viability and must be reinfused within 3–4 days. This means of storage allows the evaluation of single agents in high doses but only conditionally of a short clearance of the anticancer agent used, such as melphalan. The possibility of short-term storage has been investigated in animals and in humans [6, 7].

It appears from our experience that the recovery time is slightly different from that obtained with frozen autologous bone marrow graft [8, Hervé *et al.*, in preparation].

Cryopreserved marrow stem cells allow more prolonged and intensive therapy.

The freezing procedures currently used are based on *in vitro* and *in vivo* studies performed over the last 25 yr since McGovern *et al.*'s first data [9]. After the bone marrow concentration step, several freezing methods could be proposed [10–14]. They all used the controlled-rate computerized freezer to protect marrow cells from warming up when the heat of fusion is released at the time of the change from the liquid to the solid phase.

Our method has already been described [11]. Briefly, dimethylsulfoxide (Me_2SO) in plasma

Table 1. Bone marrow aspiration: harvesting data

	Volume collected (ml)	Nucleated cell count ($\times 10^9$)	Total CFU-GM frozen ($\times 10^6$)
Adults ($n = 42$)	790 ± 234	13.92 ± 4.69	4.73 ± 2.81
Children ($n = 25$)	474 ± 181.54	11.46 ± 6.45	2.83 ± 1.80
Prior *in vitro* manipulations ($n = 15$)	373 ± 106.50	11.59 ± 4.51	5.61 ± 2.60

(2200 mosm) is added to the cell suspension. The final concentration of Me_2SO is 10%. The marrow suspension (100 ml) is then transferred into Teflon–Kapton freezing bags. The bags are cooled in two steps from 6 to −140°C at predetermined rates of 2 and 5°C/min in an electronic freezer equipped with a programmer. At the end of the procedure the bags are rapidly transferred to liquid nitrogen. This storage temperature is associated with long-term survival of stem cells [15].

Thawing of stem cells is done in a waterbath at 42°C. Thawed bone marrow can be reinfused with the cryoprotectant [10]. In accordance with Schaefer's studies we use a stepwise dilution method which can influence the osmotic stress [11, 13]. The Me_2SO is removed by centrifugation, the marrow cell pellet is resuspended in fresh frozen plasma + saline and infused without delay.

A correlation between the total CFU-GM infused and the time of granulocyte recovery had been reported by Spitzer *et al.* for autologous bone marrow grafts [16].

On the other hand, some data have shown that the time of hematological recovery did not correlate with the transplanted bone marrow dose in terms of the total nucleated cells and the CFU-GM/kg infused [12]. No correlation between the total number of infused nucleated cells and the autologous reconstitution time could be established in our experience (unpublished data). The rapidity of myeloid hemopoietic recovery is more proportional to the number of CFU-GM infused. Even this fact is not always obvious in autologous grafting and a minimal dose of stem cells is needed to achieve engraftment ($1–2 \times 10^4$ CFU-GM/kg). In reality the capability of the bone marrow collected for hemopoietic reconstitution is in close relation with the number of multipotential stem cells infused.

CLINICAL STUDIES AND RECENT TRIALS IN AUTOLOGOUS BONE MARROW TRANSPLANTATION (ABMT) IN LEUKEMIA

Acute leukemia (AL)

For patients with AL the incidence of complete remission (CR) has improved. However, the duration of CR in acute myeloid leukemia (AML) and in adult acute lymphoblastic leukemia (ALL) remains disappointingly short. It is well known that the treatment of AL during the first or the second remission with high-dose cyclophosphamide (Cy) and total-body irradiation (TBI) followed by allogeneic or syngeneic bone marrow transplantation has led to a number of long-term survivors.

If an HLA-identical donor is not available, autologous marrow cells might be used as an alternative therapy in AL. In this case the bone marrow has to be collected early in remission several months before relapse.

Case reports showed that high-dose cytoreductive regimens followed by ABMT can also induce CR in AL in relapse. This procedure was initially proposed at the time of relapse, but more recently several investigators have decided to test the efficacy of this intensive therapy when delivered soon after the induction of the first CR in leukemic patients with a high probability of relapse and no HLA-identical donor.

The main therapeutic protocols used prior to ABMT are summarized in Table 2. According to the authors, the conditioning regimen either did or did not include TBI.

Acute myeloid leukemia

The first clinical observations were published by Hellriegel [17] and Schaefer [13].

However, the largest series was reported by Dicke *et al.* [18, 19]. Fifteen patients with AML in relapse (13 of them in 1st relapse) were treated with high-dose piperazinedione (PPD) and TBI (Table 3).

For some patients the collected marrow was fractionated on a discontinuous bovine serum albumin gradient. The aim of this fractionation was to reduce residual leukemic cells. Unfortunately there was no evidence that patients who had received fractionated marrow cells had longer CR than those receiving unfractionated cells [19].

Gorin *et al.* [20] treated 4 patients with AML in relapse by combined chemotherapy (TACC regimen). In all the cases they obtained a 2nd CR.

We treated 7 patients with AML in 1st relapse (Tables 3 and 5). The conditioning regimen consisted either of combined chemotherapy, named 'TACC-modified' (see Table 2), for 5 patients, or high-dose cyclophosphamide and TBI for 2. All of the patients achieved CR but relapsed within 5–13 months [21].

The evaluation of single agents in high dose for acute leukemia therapy has been proposed. High-dose melphalan (140–200 mg/m^2) has been used in AML by Maraninchi and ourselves [8]. Six patients treated in relapse achieved CR (Table 3). Two of them relapsed 1 and 2 months later. Four patients remain in remission (8+, 9+, 13+, 14+ months). Among this group of patients, 3 have received 2 courses of HDM, with a space of 2 months between each treatment. The main side-effect with this agent has been mucositis.

The sensitivity of leukemic cells may be greater at the time of remission than at the time of relapse, when a greater level of resistance to therapy in the leukemic cells may occur.

Table 2. Main therapeutic protocols used prior autologous bone marrow transplantation in acute and chronic leukemia

Authors	Protocols	
	Chemotherapy	Radiotherapy
Bückner *et al.* (1978)	Cy	10 Gy
Dicke *et al.* (1979)	PPD	8.5–9.5 Gy
Goldman *et al.* (1981)	DNR, Ara-C, 6-TG, Cy, VP-16 ('RATE')	6–12 Gy
Gorin *et al.* (1979)	Cy, Ara-C, 6-TG, CCNU ('TACC')	
Kaizer *et al.* (1980; 1982)	ADM-Cy high-dose busulfan, Cy	8 Gy
Hervé *et al.* (1981)	Cy, Ara-C dose × 2, 6-TG dose × 2, CCNU dose × 2 ('TACC' modified)	
Zander *et al.* (1981)	Cy, BCNU, VP-16 ('CBV')	
Maraninchi *et al.* (1982)	high-dose melphalan	

Cy = cyclophosphamide; PPD = piperazinedione; DNR = daunorubicin; 6-TG = 6-thioguanine; Ara-C = cytosine arabinoside; ADM = adriamycin.

Table 3. High-dose therapy with autologous bone marrow transplantation in acute myeloblastic leukemia in relapse: clinical results

Authors	No. of patients	Clinical status: relapse	Therapy	No. of early deaths	Response	Remission duration (months)
Hellriegel *et al.* (1978)	1	1st	VCR, ADM, 6-TG MTX, Cy	0	CR	3
Schaefer *et al.* (1979)	1	1st	CY TBI	0	CR	4.8
Dicke *et al.* (1980)	15	1st and 2nd	PPD TBI	7	8 CR	$\bar{x}=4$ (2–14)
Gorin *et al.* (1981)	4	1st	TACC	0	4 CR	$\bar{x}=10.7+$ (3–32)
Hervé *et al.* (1982)	7	1st	TACC (5)	0	7 CR	$\bar{x}=8.9$ (5–13)
Maraninchi *et al.* (1982)	6	1st–3rd	high-dose melphalan	0	6 CR	$\bar{x}=8+$ (1.5–14)

The better clinical condition of the patients at the time of remission made them more able to tolerate the high dose therapy.

For these reasons several authors proposed the ABMT procedure soon after the CR was obtained.

We treated 8 patients in CR (7 in 1st remission and 1 in 2nd). All patients had received intensive induction and consolidation. Three patients could not be evaluated because of early death due to severe cardiac complications (cardiomyopathy) which occurred during conditioning treatment [22]. One of the evaluable patients relapsed on the 150th day but went into a 3rd remission after a high dose of melphalan (200 mg/m^2). This patient is well and has remained in 3rd CR for 9 months. The other four have remained in CR for 4–20 months. Our results are summarized in Tables 4 and 5, along with those of other investigators [22–24].

Present trends. The danger of grafting residual leukemic cells with the patient's marrow collected during CR remains the main argument against ABMT. A new approach is that of ABMT with *in vitro* elimination of residual malignant cells.

Using a rat model's AML, Sharkis *et al.* [25] appraised a metabolite of cyclophosphamide which showed high cytotoxic activity: the 4-hydroperoxycyclophosphamide (4-HC). The ability of 4-HC to clear a mixture of normal marrow cells and leukemic cells is dose-dependent.

Kaizer *et al.* [26] have designed a clinical trial using *in vitro* incubation of autologous bone marrow of 20 patients with AML in remission with 4-HC prior to cryopreservation. The initial concentration of 4-HC used was 40 μg/ml. At this concentration the growth of normal CFU-GM was inhibited by about 60–70%. Despite this inhibition they observed a prompt hematologic reconstitution. Today Kaizer *et al.* use higher doses of 4-HC (100–120 μg/ml). Despite the total inhibition of CFU-GM growth the hemopoietic recovery is only slightly delayed (personal communication). In terms of therapeutic outcome, 7 of the 20 patients remain in CR (2+–15+ months).

In our experience 4 patients have been grafted with bone marrow treated by 4-HC. It is too early

Table 4. High-dose therapy with autologous bone marrow transplantation in acute myeloblastic leukemia in remission: clinical results

Authors	No. of patients	Clinical status	Therapy	*In vitro* treatment of BM	No. of early deaths	Remission duration (months)
Fay *et al.* (1979)	4	CR	Cy + TBI	NO	0	not specified
	1	CR	BACT			2
Kaizer *et al.* (1982)	20	CR	Cy + TBI	4-HC	0	2+–15+ for 7 patients
Hervé *et al.* (1982)	7	1st CR	TACC	4-HC (5)	3	4+, 5+, 20+, 20+
	1	2nd CR		4-HC		5
Löwenberg *et al.* (1982)	2	1st CR	CY + TBI	NO	0	4, 12+

Table 5. High-dose therapy with autologous bone marrow transplantation in 12 patients with acute myeloblastic leukemia in relapse or in remission. Personal observations

7 patients grafted in 1st relapse: 7/7 CR
median CR duration: 9.9 months (5–14)
median survival: 14.4+ months (9–23.8)

5 patients grafted in remission:
1 relapse at 5 months: 3rd CR after high-dose melphalan = 9+
4CR: 4+, 5+, 20+, 20+ months

for evaluation of these cases, but the remission duration is of 4–9 months (unpublished).

Acute lymphoblastic leukemia (ALL)

In adult ALL patients the prognosis of this leukemia treated with conventional chemotherapy is poor.

Dicke *et al.* [27] treated 8 patients with ALL in 2nd or 3rd relapse using PPD and TBI as conditioning regimen followed by ABMT (Table 6). They obtained 4 CR with a median remission duration of 5 months. For ALL in relapse other reports with few patients have been published [21, 28–30].

The Houston group [28] proposed a program of two courses of high-dose chemotherapy (CBV), each followed by autologous bone marrow rescue in 14 ALL patients in 1st remission. After ABMT the patients did not have maintenance treatment.

We autografted a patient in 2nd remission after high-dose cyclophosphamide and TBI. This patient has remained in remission for 23 months and he does not receive maintenance treatment [21]. With the same conditioning regimen Porcellini *et al.* observed in 3 patients 2 relapses [31].

All these data are summarized in Table 7.

Present trends. Among various methods proposed to eradicate residual clonogenic leukemic cells in collected marrow, a possible approach involves the use of immunological methods.

Netzel *et al.* [32] used heteroantisera collected from a rabbit immunized with ALL leukemic cells (common ALL and T-ALL). After extensive absorption it appeared that the sera was cytotoxic for c-ALL cells and not for CFU-c. They treated 3 patients with c-ALL in 2nd and 3rd relapse. In 2 patients they obtained a CR. The remission duration was 6 and 4+ months [33]. Billing *et al.* [34] reported 3 c-ALL patients for which the marrow collected in remission was treated *in vitro* with c-ALL heteroantisera. When these patients relapsed the treated marrow was infused after intensive chemotherapy and TBI. Two patients showed a return to normal marrow function. One patient who died 4 months later showed no post-mortem evidence of leukemia.

Ritz *et al.* [35] used monoclonal antibody (named J_5) specific for the common ALL antigen (CALLA) and complement for *in vitro* treatment of marrow cells before freezing in 4 children with CALLA-positive ALL. Patients received ablative chemotherapy and TBI before reinfusion of autologous J_5-treated bone marrow. All the patients have had a complete hemopoietic reconstitution and 2 children remain in CR (7+ and 19+ months: Table 7).

Attempts have been made to treat the bone marrow of patients with T-ALL *in vitro* with the adenosine deaminase inhibitor, 2'-deoxycoformycin (dCF). *In vitro* studies showed a correlation between *in vitro* accumulation of deoxyadenosine and clinical response to dCF [36, 37]. So far no clinical studies using dCF-treated autologous marrow have been published.

Table 6. High-dose therapy with autologous bone marrow transplantation in acute lymphoblastic leukemia in relapse: clinical results

Authors	No. of patients	Clinical status relapse	Therapy	No. of early deaths	Response	Remission duration (months)
Dicke *et al.* (1979)	8	2nd or 3rd	PPD + TBI	1	4 CR 3NR	2, 2, 2, 14
Wells *et al.* (1979)	3	not specified	Cy + TBI (heterologous antisera)	2	1CR	4
Hervé *et al.* (1981)	1	2nd	TACC	0	NR	
Zander *et al.* (1981)	2	not specified	CBV	0	2 CR	6, 7
Netzel *et al.* (1981)	2	3rd	BCNU, Ara-C TBI (+heterologous antisera)	1	1 CR	not specified

Table 7. High-dose therapy with autologous bone marrow transplantation in acute lymphoblastic leukemia in remission: clinical results

Authors	No. of patients	Clinical status	Therapy	No. of early deaths	Remission duration (months)
Dicke *et al.* (1982)	14	CR	CBV (2 courses)	4	1, 2, 2 (after 1st course) 4, 10, 11, 28, 12+, 39+, 43+ (after 2nd course)
Hervé *et al.* (1981)	1	2nd CR	Cy, TBI	0	23+
Porcellini *et al.* (1982)	3	1st to 3rd CR	Cy, TBI	0	6, 3, 2.5+
Ritz *et al.* (1982)	4	2ndCR	Ara-C, Cy, TBI (+J5 monoclonal antibody *in vitro*)	1	19+, 17+ and 1 early relapse

Kaizer and ourselves have used 4-HC for bone marrow *in vitro* treatment in some cases of ALL patients. Today it is too early for evaluation.

Chronic myeloid leukemia (CML)

The treatment of patients in blast crisis has been attempted using high-dose radiochemotherapy followed by infusion of marrow stem cells collected early in the chronic phase or of peripheral blood stem cells obtained by cytapheresis at the time of diagnosis.

Goldman *et al.* [38] have shown that 3×10^6 CFU-c/kg can be collected from peripheral blood of CML patients.

This first report published by Bückner *et al.* [39] was about 7 CML patients grafted in blast crisis. All patients were treated with high-dose cyclophosphamide and TBI. Two patients failed to achieve marrow repopulation and 3 patients had partial marrow recovery. All died between 29 and 84 days. Two patients achieved complete marrow recovery and re-establishment of the chronic phase. One died 2 months later of fungal pneumonitis and the other relapsed within 4 months. The reason for this repopulation failure remains obscure but could be explained by technical problems such as freezing defect.

Goldman *et al.* [40, 41] treated 33 CML patients in blast crisis. Thirty-two patients were evaluable. Seventeen of them were splenectomized before autologous bone marrow graft. The median duration of the 2nd chronic phase was 13 weeks (range 2–125 weeks), and the median time from blast crisis to death was 23 weeks (range 2–129 weeks). Five patients remain alive in chronic phase. The introduction of TBI in the conditioning regimen did not improve the mean duration of the 2nd chronic phase.

For some patients the duration of the 2nd chronic phase was short (2 weeks). It is likely that the chronic phase was not obtained.

Körbling *et al.* have treated a CML patient in chronic phase with high-dose cyclophosphamide prior to blood stem cell harvesting. When the patient entered the blast crisis myeloablative treatment followed by ABMT was carried out. After restoration of hemopoiesis the patient's marrow cells were free of the Ph 1 chromosome, but the patient died of toxicity [42].

Gorin *et al.* reported a case of Ph 1 chromosome disappearance after ABMT for treatment of CML in blast crisis. These authors posed the hypothesis that either the Ph 1+ stem cells may have been selectively suppressed by chemotherapy before

marrow collection or that the Ph 1+ cell line might have been injured during cryopreservation [43].

We treated 1 CML patient in blast crisis with TACC modified regimen and ABMT. The 2nd chronic phase remains for more than 3 yr.

All these results have been tabulated in Table 8.

Present trends. In view of the poor results obtained, Goldman *et al.* proposed several courses of high-dose therapy followed by ABMT (unpublished data). It may therefore be preferable to further reduce the amount of chemotherapy given before ABMT and to schedule a second or subsequent procedure at a 3–6 months interval. However, non-hemopoietic toxic effects appear to limit a further increase in the number of ABMT.

It seems that in CML, stem cells should be collected from the bone marrow after initial therapy at a time when the proportion of Ph 1-positive cells is at a minimum.

In the same way as AML, collected CML marrow cells may be treated *in vitro* with cytotoxic drugs (such as analog of cyclophosphamide) before freezing.

Discussion and future trends

The feasibility of ABMT has been demonstrated [2, 12, 13, 20, 21, 27, 40, 44, 45].

Several factors determine the outcome of an ABMT protocol: the timing of the bone marrow collection; the quality of the patient's remission at the time of marrow collection; the efficiency of the storage procedure; the method(s) used to reduce or to eliminate residual leukemic cells; and the timing of the bone marrow reinfusion: end-stage acute leukemia in relapse or early in the course of the disease, during the CR.

Autologous bone marrow transplantation in first remission should be done in a defined group of patients with poor prognosis for remission duration. All the reports confirmed that the clinical conditions of the patients at the time of relapse are unfavorable in terms of resistance to the intensive therapy and of achieved remission duration [2, 17, 20, 21]; therefore ABMT should be included to support a high-dose therapy consolidation regimen ('supraconsolidation') during the course of CR. The bone marrow must be collected and stored for patients with AL as soon as a CR is obtained. The extramedullary toxicity of the conditioning regimen must be taken into account before entering a patient in CR into the ABMT procedure. We have had 3 patients treated during CR who died before marrow engraftment because of cardiac failure [22].

Intensive treatment early in remission is undertaken to reduce rapidly and maximally the number of leukemic cells.

After the recovery of hemopoiesis the patient can profit by a normal life without further chemotherapy. In terms of the quality of life, the ABMT procedure made during the 1st remission incontestably appears as the best proposal. However, a complete evaluation of the risks must be done and the patient informed before consenting to the procedure.

For the AML patients a comparative study of the antileukemic effect of different conditioning regimens, such as chemotherapy regimen alone vs TBI + cytoxan, must be evaluated. For poor prognosis ALL TBI + cyclophosphamide seems to be the best conditioning regimen.

One needs more patients grafted earlier in CR to compare this treatment modality in terms of survival with patient groups treated with conventional chemotherapy. It cannot be denied that long-term disease-free survival has been obtained with chemotherapy alone in adult myeloblastic as well as lymphoblastic disease.

For the CML in blast crisis treated by intensive therapy + ABMT the discussion remains open. However, the bone marrow could be collected during the course of the first chronic phase after hydroxyurea therapy or another treatment programme, aimed at reducing the abnormal clone bearing the Ph 1 chromosome.

In acute leukemia the intensive cytoreductive therapy with autologous stem cell reconstitution will be more satisfactory if the protocol include *in*

Table 8. High-dose therapy with autologous bone marrow transplantation in chronic myeloid leukemia in blast crisis: clinical results

Authors	No. of patients	Stem cell source	Therapy	Early death	Response (2nd chronic phase)	Follow-up (weeks)
Bückner *et al.* (1978)	7	marrow	Cy TBI	5	2	10, 17
Goldman *et al.* (1982)	33	blood	Cy TBI (12) DNR, 6-TG, VCR, Ara-C (16)	1	32	$\bar{x}$ = 13 (2–125)
Hervé *et al.* (1982)	1	marrow	TACC	0	1	164+
Körbling *et al.* (1981)	1	blood	busulfan-Cy	0	1	8
Gorin *et al.* (1982)	1	marrow	TACC	0	1	13

vitro immunologic or pharmacologic means to eliminate residual leukemic cells.

An innovative approach has been the use of antisera specific for various ALL antigens to try to remove leukemic cells from autologous marrow cells prior to cryopreservation [33–35].

The efficacy of monoclonal antibodies for the bone marrow *in vitro* treatment can be limited by some factors such as antigenic modulation on the cell surface and specificity of monoclonal antibodies that can react with hemopoietic cells [46].

One must direct all efforts toward this aim. We have reported the main works published in this field. Nevertheless, they are taken from preliminary studies which concern few patients, and it is too early for evaluation [24, 32–35].

CONCLUSION

Until recently the results of autografts in leukemia published in the literature have been disappointing in general and often very difficult to analyze. However, the interest of autologous stem cell grafting has been suggested by some privileged observations.

In the future incomplete and premature cases will hopefully be avoided in the publications. The major criticism that can be made is that in the past, early reports of interesting trials were never republished after a follow-up of 2 or 3 yr.

Intensive cytoreductive therapy seems a major part of leukemia. As potential benefits become better defined it will be possible to extend this new therapeutic protocol to more carefully selected patients.

Since the technical problems are well described, it will be possible to judge the results in a more homogeneous manner for a given chemotherapy in a given pathology.

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