

Low Transplant Mortality in Allogeneic Bone Marrow Transplantation for Acute Myeloid Leukemia: A Randomized Study of Low-Dose Cyclosporin Versus Low-Dose Cyclosporin and Low-Dose Methotrexate

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Sixty patients undergoing allogeneic bone marrow transplant for acute myeloid leukemia (AML) in first remission (CR1; $n = 49$) or more advanced phase ($n = 11$) were entered in a prospective trial of graft-versus-host disease (GvHD) prophylaxis: low-dose cyclosporin A (IdCSA; 1 mg/kg/d from day -1 to $+20$ day; $n = 28$) or IdCSA plus low-dose methotrexate (IdMTX; 10 mg/m² for day $+1$, 8 mg/m² for days $+3$, $+6$, and $+11$; $n = 32$). Primary end points were acute GvHD (aGvHD) and transplant-related mortality (TRM); secondary end points were relapse and survival. The conditioning regimen consisted of cyclophosphamide (120 mg/kg) and fractionated total body irradiation (3.3 Gy/d for 3 consecutive days). The actuarial risk of developing aGvHD grade II-III was 61% for IdCSA alone and 34% for IdCSA + IdMTX ($P = .02$). The actuarial risk of TRM at 1 year was 11% versus 13%, respectively, and older patients (≥ 29 years) had higher

TRM than younger patients (22% v 5%, $P = .01$). The age effect was significant in the IdCSA group ($P = .04$) but not in the IdCSA + IdMTX group ($P = .1$). The median follow-up is 4.4 years, with an overall actuarial survival of 78% for CR1 patients and 36% for patients with advanced disease. For patients in CR1 the outcome of the two regimens was as follows: survival 77% versus 80% ($P = .6$), relapse 20% versus 9% ($P = .1$), and TRM 13% versus 17% ($P = .6$). This study suggests that TRM can be reduced in AML patients undergoing allogeneic marrow transplants with a mild conditioning regimen and low-dose immunosuppression, and this translates in a 78% 5-year survival for CR1 patients. Beyond CR1 the major obstacle remains leukemia relapse, which is not prevented by low-dose in vivo immunosuppression.

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ALLOGENEIC BONE MARROW transplantation (BMT) is a well-established therapy for patients with acute myeloid leukemia (AML), and has been proven to be at least as effective or superior to chemotherapy and autologous transplants in first remission (CR1) patients.¹ The success of allogeneic BMT has been limited by transplant-related mortality (TRM). Graft-versus-host disease (GvHD) alone or in combination with infections or interstitial pneumonia accounts for a substantial proportion of failures after allogeneic BMT.² Between 20% and 50% of patients administered grafts from HLA identical siblings develop significant acute GvHD (aGvHD). HLA disparity, older age, donor-recipient sex mismatch, and posttransplantation immunosuppressive regimen are all predictors of aGvHD.³ In addition, the intensity of the conditioning regimen should be taken into account. Protocols including high-dose chemotherapy⁴ or high-dose total body irradiation (TBI)⁵ are associated with a high incidence of GvHD and TRM. At a given intensity of pretransplant conditioning, the combination of cyclosporin (CSA) and methotrexate (MTX) seems to be superior to either regimen alone in preventing aGvHD.⁶

We have previously described a fractionated TBI (fTBI) regimen consisting of three daily fractions of 3.3 Gy each that is associated with a low risk of interstitial pneumonia.⁷ We have subsequently shown that small variations within that schedule had a significant impact on leukemia relapse,⁸ and we have since then corrected the dose according to thermoluminescent dosimeter readings to deliver the nominal dose.

With this relatively mild conditioning regimen we then investigated a possible effect of reducing in vivo immunosuppression. Patients were randomized to receive high-dose CSA (5 mg/kg/d) or low-dose CSA (IdCSA, 1 mg/kg/d), from day -1 to day $+20$.⁹ Although the two groups differed significantly in CSA dose and CSA serum levels only between day 0 and day 10, the IdCSA regimen (1 mg/kg) significantly reduced leukemia relapse and resulted in improved survival.⁹ An update of that study with median follow-up of 8.8 years (range, 7 to 10) still shows a survival advantage for IdCSA in young patients

(<35 years, $P = .03$), but not in older patients ($P = .7$) (unpublished data).

In the attempt of reducing GvHD, especially in older patients, we have therefore compared the best arm of that study (CSA, 1 mg/kg) with the combination of IdCSA and low-dose methotrexate (IdMTX). We are now reporting the results of this second randomized trial.

MATERIALS AND METHODS

Study design. Patients aged 16 to 50 years with AML were eligible. Patients were randomized to receive IdCSA (1 mg/kg body weight) administered in continuous intravenous infusion, from day -1 to day $+20$, or IdCSA with IdMTX (10 mg/m² on day $+1$, and 8 mg/m² on days $+3$, $+6$, and $+11$). The dose of MTX could be further reduced or omitted by the attending physician in case of severe mucositis or febrile neutropenia. Patients were stratified at randomization for age (<30 or ≥ 30) and phase of the disease (CR1 or >CR1). On day $+21$, after BMT patients were placed on oral CSA (10 mg/kg/d), the dose was adjusted according to creatinine levels and CSA serum levels. The dose of CSA was reduced in case of creatinine levels greater than 1.7 mg% and CSA levels greater than 700 ng/mL. CSA was tapered at 1 year after BMT and discontinued in the absence of chronic GvHD. If aGvHD devel-

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oped, patients were treated with 6methylprednisolone in doses ranging between 2 and 10 mg/kg/d.

Aim of the study. The aim of the study was to test whether the association of MTX with IdCSA would reduce aGvHD as compared with IdCSA alone. With a baseline incidence of 61% in our previous study⁹ and with an expected reduction of 35%, we expected to enroll 36 patients in each arm to achieve a significant difference in aGvHD, with a significance level of 0.05 and a power of 0.80.

End points. Primary end points were aGvHD and TRM; secondary end points were relapse and survival.

Patients. Sixty patients with AML underwent BMT from HLA identical siblings at our institute between 1990 and 1994 after giving informed consent. Forty-nine patients were in CR1, eight in first untreated relapse, two in second remission, and one in second relapse. Clinical details of patients are outlined in Table 1. The two groups were comparable for age, sex, French-American-British (FAB) classification, and time between diagnosis and transplant (Table 1).

Transplantation. Conditioning regimen was cyclophosphamide (60 mg/kg on days -7 and -6; total, 120 mg/kg), followed by TBI; (3.3 Gy/d on days -3, -2, and -1 delivered at a dose rate of 12 cGy/min from a linear accelerator with lung shielding to 9.9 Gy). The received dose of TBI was monitored with thermoluminescent dosimeters as described⁸ and adjusted to deliver the prescribed dose of 3.3 Gy/d. All patients received ciprofloxacin from day -7 until the onset of fever or resolution of neutropenia, and trimethoprim-sulfamethoxazole was then administered from day +30 until day +365 to prevent *Pneumocystis carinii* infections. High-dose immunoglobulins (400 mg/kg/wk) were administered until day +100. All patients received acyclovir (500 mg/m² intravenously every 8 hours from day -1 to day +30, 800 mg orally every 6 hours from day +31 to day +100, followed by 800 mg orally every 12 hours from day +101 to day +365). Bone marrow cells were slowly infused unmanipulated after the last dose of TBI. The day of infusion was designed as day 0. The median number of infused cells was 4.5×10^8 /kg/body weight (range, 1.3 to 11; $3.4 \nu 4.5$ for the two

groups, respectively; $P = .09$). Bone marrow was obtained under general anesthesia (20 to 25 mL/kg of donor weight), aiming at a cell dose greater than 4×10^8 /kg. Cytomegalovirus (CMV) infections were monitored with antigenemia¹⁰ and treated with ganciclovir or foscarnet,¹¹ and more recently, with combination of the two.¹²

Engraftment and chimerism. Engraftment was defined as the first of 3 consecutive days with absolute number of neutrophils (PMN) greater than 0.5×10^9 /L. Chimerism for DNA polymorphism using microsatellites (HUMF13A1, chromosome 6; HUMTH01, chromosome 11; HUMvWA31, chromosome 12; HUMFES/FPS, chromosome 15; and D19S253, chromosome 19) was assessed.¹³

aGvHD. aGvHD was scored according to current criteria.¹⁴

Statistical analysis. Kaplan-Meier curves were used for survival, relapse, and TRM incidence as also the incidence of aGvHD grade II-IV.¹⁵ The Mann-Whitney test was used to compare means for continuous variables between groups. Fisher's exact test was used for 2×2 tables. Cox regression analysis was used to define predictors on survival and incidence of chronic GvHD (cGvHD).¹⁶

RESULTS

Patient compliance. The median administered dose of CSA between day -1 and day +20 was 1.03 mg/kg (range, 0.9 to 2.5) for 28 patients in the IdCSA arm and 1.01 mg/kg (range, 0.9 to 1.4) for 32 patients in the IdCSA + IdMTX arm. For the latter, the median administered dose of MTX on days +1, +3, +6, and +11 was 10 mg/m² (range, 8.77 to 11.43), 7.38 mg/m² (2.94 to 8.75), 7.14 mg/m² (5.26 to 8.75), and 6.67 mg/m² (2.94 to 8.75), respectively. On days +1, +3, +6, and +11 MTX was administered to 32 patients (100%), 30 (94%), 21 (66%), and 15 (47%), respectively. The median cumulative dose of MTX was 24 mg/m² (range, 10 to 35).

Engraftment. All patients achieved engraftment as proven by sex markers or DNA polymorphism. Patients administered MTX achieved a neutrophil count of greater than or equal to 0.5×10^9 /L on day 16 as compared with day 12 for CSA alone ($P < .0001$). There was also a delay in platelet engraftment of 2 days (14 ν 16, $P = .08$). Median platelet counts on days 0-20, 21-50, 51-100, and greater than 100 were 31 versus 25×10^9 /L, 88 versus 82×10^9 /L, 100 versus 96×10^9 /L, and 189 versus 191×10^9 /L for IdCSA versus IdCSA + IdMTX, respectively (statistically not different).

aGvHD. No patient developed aGvHD grade IV. aGvHD was scored as grade 0-I in 11 versus 21 patients, and grade II-III in 17 versus 11 patients for the two groups, respectively ($P = .03$, Fisher's exact test). The median day to develop aGvHD was 14 (range, 7 to 40) versus 17 (range, 7 to 57) for two groups ($P = .1$). The overall incidence was 47% for the whole group. The actuarial probability of developing aGvHD grade II-III was 61% versus 34%, respectively ($P = .02$; Fig 1). Patients receiving IdCSA alone had a 2.3-fold higher risk to develop aGvHD grade II-III than those receiving IdCSA + IdMTX, and this was also true for patients in CR1. In patients 30 years or older, the incidence of aGvHD grade 0-I, II, and III was 27%, 55%, 18% versus 75%, 25%, and 0%, respectively, for the IdCSA versus IdCSA + IdMTX group ($P = .03$). We could not find a correlation between the dose of administered MTX and aGvHD.

cGvHD. cGvHD was scored in 54 of 60 patients (26 ν 28 from each group, respectively) alive 100 days posttransplant. This was scored as absent or limited in 15 versus 19 patients and

Table 1. Clinical Characteristics of Patients

	IdCSA	IdCSA + IdMTX	P
No. of patients	28	32	
Age (y)			
Median (range)	29 (14-43)	30 (11-43)	NS*
Recipient gender			
Male/female	13/15	18/14	NS†
Donor age (y)			
Median (range)	27 (15-47)	30 (9-51)	NS*
Donor gender			
Male/female	16/12	14/18	NS*
Disease status			
CR1	23	26	NS†
>CR1	5	6	NS†
FAB classification			
M1-M2	13	14	
M3	7	6	
M4-M5	7	11	
M6	0	1	
M7	1	0	
Days from diagnosis to HSCT			
Median (range)	183 (108-356)	173 (58-542)	NS*
No. of infused cells ($\times 10^8$ /kg)			
Median (range)	3.4 (1.5-10)	4.5 (1.3-11)	.09*

Abbreviations: HSCT, hematopoietic stem cell transplants; NS, not significant.

*Mann-Whitney.

†Fisher's exact test.

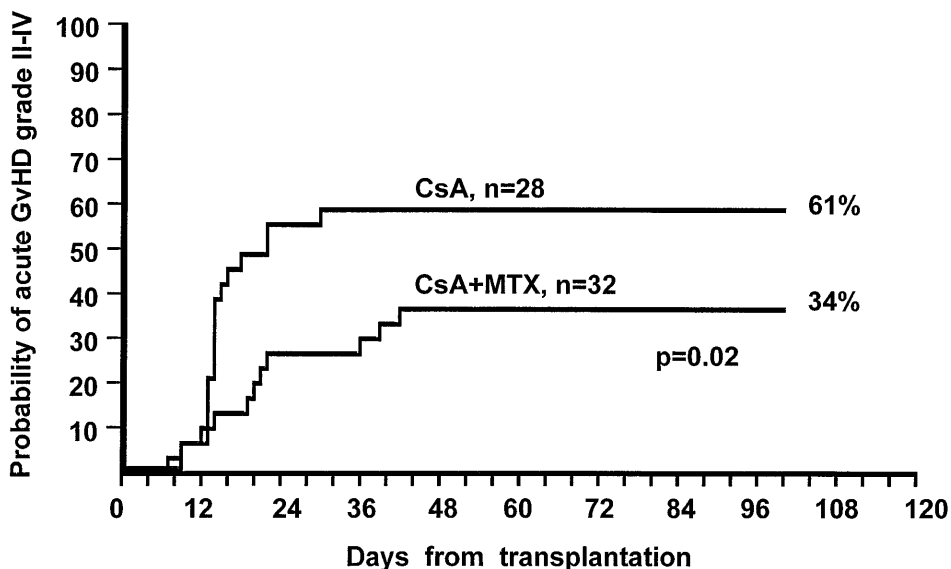


Fig 1. Actuarial probability of developing aGvHD grade II-IV, showing a significant difference between the two groups: CSA + MTX, 34% versus CSA alone, 61% ($P = .02$).

as extensive in 11 versus 9 patients for the two groups, respectively ($P = .1$).

Relapse rate. At the time of analysis 12 patients had relapsed and 9 of them (6 v 3 for the two groups, respectively) had died. The overall probability of relapse was 22% for all patients and 14% for CR1 patients. Among 28 patients in the IdCSA group, 8 relapsed at a median interval of 266 days (range, 64 to 1,175) versus 4 of 32 patients in the IdCSA + IdMTX group, who relapsed at a median interval of 115 days (range, 80 to 954; $P = .6$). The actuarial relapse rate is 32% versus 14% for the two groups, respectively ($P = .1$). The results were not significantly different when stratified by median age (29 years, 33% v 19%, $P = .07$), grade of aGvHD (grade 0-I, 27% v 11%, $P = 0.3$; grade II-IV, 35% v 19%, $P = .36$), or phase of the disease (CR1, 20% v 9%, $P = .3$; Fig 2). In multivariate analysis CR1 ($P = .008$) and cGvHD ($P = .01$) were favorable predictors of persisting remission.

TRM. The actuarial TRM at 5 years is 14% for all patients, and 15% for CR1 patients. The probability of TRM is 11% versus 17% in the two groups, respectively ($P = .6$), and 13% versus 17% for CR1 patients (Fig 3). The major nonrelapse causes of death were GvHD (1 v 1 deaths), infections (1 v 2 deaths), and IP (1 v 0 deaths) for the two groups, respectively.

In multivariate COX analysis on TRM the only predictor was patient's age (RR 1.2, $P = .01$), whereas donor age, gender, disease phase, aGvHD, interval diagnosis-BMT, FAB subtype, and GvHD prophylaxis had no significant impact. The actuarial TRM of patients under the age of 29 (median) was 5% versus 22% for older patients ($P = .01$).

Survival. Forty-three of sixty patients are currently alive with a median follow-up of 4.4 years (range, 0.5 to 7). The 5-year actuarial survival is 71% for whole group and 78% for patients in CR1 (Fig 3). Nineteen of twenty-eight patients in the IdCSA group are alive, at a median interval of 2,119 days

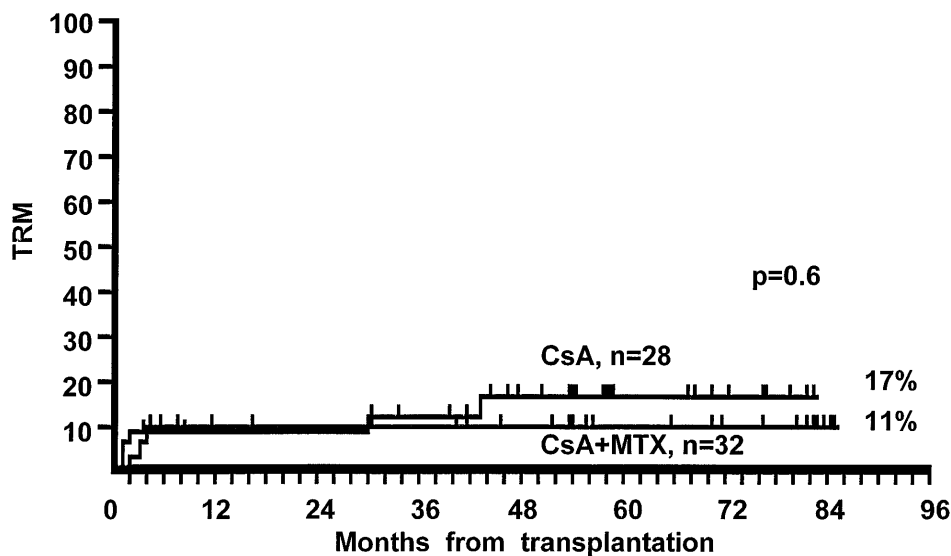


Fig 2. Actuarial risk of TRM. There was no significant difference between patients receiving CSA + MTX or CSA alone.

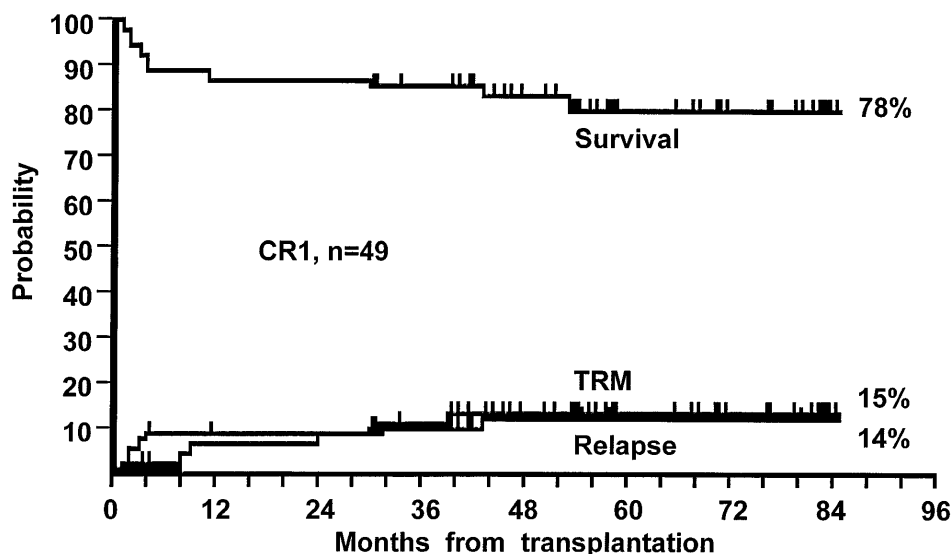


Fig 3. Overall outcome for patients in CR1. The actuarial survival rate for the two groups was 78%, relapse rate was 14%, and TRM was 15%.

(range, 904 to 2,534) versus 24 of 32 patients in the IdCSA + IdMTX group, at a median interval of 1,728 days (910 to 2,476). The 5-year actuarial survival is 67% versus 74% for the two groups, respectively ($P = .1$). There was no difference between groups when we stratified patients according to recipient age (≤ 29 or >29 , 81% v 73%, $P = .5$ and 59% v 68%, $P = .5$), phase of disease (CR1 or $>$ CR1, 77% v 80%, $P = .8$ and 20% v 50%, $P = .5$), or time between diagnosis to BMT (<180 or ≥ 180 days, 92% v 78%, $P = .34$ and 46% v 69%, $P = .4$). In multivariate COX analysis there was no variable which predicted failure in survival analysis.

Table 2. Results

	IdCSA	IdCSA + IdMTX	<i>P</i>
PMN $> 0.5 \times 10^9/L$	12 (11-20)	16 (12-21)	$<.0001^*$
aGvHD			
Grade 0-I	11	21	
Grade II-III	17	11	.037†
Median day (range)	14 (7-40)	17 (7-57)	.1*
cGvHD			
Absent	3	7	
Limited	12	12	
Extensive	11	9	.2†
Causes of death			
Relapse	6	3	
GvHD	1	1	
Infections (+IP)	2	2	
Heart failure	0	1	
Multiorgan failure	0	1	
Survival			
Alive (%)	19 (68)	24 (74)	
Median day (range)	2,119 (904-2,539)	1,728 (910-2,476)	.16*
Relapse			
Relapsed (%)	8 (29)	4 (13)	
Median day (range)	266 (64-1,175)	115 (80-954)	.6*
TRM	11%	17%	.6‡

Abbreviation: IP, interstitial pneumonia.

*Mann-Whitney.

†Fisher's exact test.

‡Log rank test.

DISCUSSION

In the present study we have shown that IdCSA combined with IdMTX reduces the risk of aGvHD when compared with IdCSA alone, although this does not have a significant impact on survival; for patients in CR1, TRM was 12%, relapse rate was 14%, and survival rate was 78%; and for patients with advanced disease, alternative strategies to control leukemia should be devised.

Concerning the first point, better prevention of aGvHD is one reason for improved outcome of allogeneic BMT, and in particular, the widely used combination of CSA and full-dose MTX.^{6,17} In the present study, despite a significant reduction of the dose of CSA and also of MTX, no patient had grade IV and only two patients experienced grade III GvHD, possibly also a result of a mild conditioning regimen. There was a reduction of GvHD in patients administered CSA + MTX as compared with CSA alone, especially in patients over 30 years of age. Many centers use CSA at the dose of 2.5, 3, or 5 mg/kg, but to our knowledge there is no trial suggesting this is the best dose, and it may actually be a high dose in terms of toxicity and immunosuppression.¹⁸ The dose of 1 mg/kg of CSA was derived from a randomized study which suggested that 1 mg/kg/d in the first 10 days post-BMT produced less multiorgan toxicity, and a lower rate of relapse when compared with 5 mg/kg.⁹ The median administered dose of MTX was 24 mg/m², approximately 50% less than the full dose of the original protocol.¹⁹ All patients randomized to the MTX arm received MTX (10 mg/m²) on day +1; some patients did not receive their MTX on days +3, +6, or +11 because of clinical conditions such as severe mucositis and or febrile neutropenia. Within the MTX arm we could not find a correlation between the number of doses of MTX and aGvHD, nor between the total dose of MTX administered in the first 11 days and aGvHD. Nevertheless, patients in the CSA + MTX arm had a significant reduction of aGvHD as compared with controls receiving CSA alone, suggesting a primary role for the MTX dose on day +1. The encouraging control of aGvHD did not eliminate the effect of patient age: TMR was 5% in younger patients (≤ 29 years)

versus 22% in older patients, and this was confirmed in multivariate analysis.

As to the second point, we are reporting an actuarial survival rate of 78% at 7 years for AML in CR1, using a relatively mild conditioning regimen. Our observation that IdCSA can strongly influence leukemia relapse⁹ suggests that the GvHD prophylaxis has a significant impact on control of leukemia in the context of this conditioning regimen. It could possibly be more difficult to detect the impact of GvHD prophylaxis in the setting of high-dose TBI. Our results seem superior to those reported from international registries¹⁷ or recently from the MRC-AML 10 study (56% survival, 34% relapse).²⁰ Our unit is primarily a referral center, and we tend to see more and more patients with high-risk factors such as delayed remissions or chromosomal abnormalities. Despite this trend in the past 3 years, after this randomized trial was closed, we have treated 22 consecutive AML patients in CR1 with CSA + MTX and have confirmed a very encouraging actuarial survival rate of 94% (unpublished data). Among the possible reasons for the extremely low TRM in our center we would like to point out a high number of nucleated bone marrow cells—in this trial, $4.5 \times 10^8/\text{kg}$ —which is achieved by obtaining 20 to 25 mL/kg of donor body weight in small aliquots,²¹ and a high number of nucleated cells has been recently reported to be a strong predictor of favorable outcome also in the unrelated setting.²² In addition we have significantly reduced mortality caused by CMV by treating patients with a high number of CMV-antigen positive cells with combined ganciclovir plus foscarnet therapy.¹² Therefore we believe that factors such as a total fTBI of less than 10 Gy, low-dose immunosuppression, high marrow cell dose, and aggressive therapy of CMV have all contributed to determine the very low mortality.

Regarding patients with disease beyond CR1, the major problem remains leukemia relapse. Relapse can be reduced by increasing the conditioning regimen, but this is associated with greater TMR, as shown in one of the few randomized trials comparing two different TBI regimens: patients receiving 12 or 15.75 Gy had a relapse rate of 35% versus 12% ($P = .06$), and a TMR of 12% versus 32% ($P = .04$), resulting in equal overall survival rates.⁵ In the same study the incidence of moderate-severe aGvHD was 21% versus 48% ($P = .02$). The alternative option is to reduce GVHD prophylaxis, which in our hands was effective in CR1, and has also been reported to be successful for patients in first relapse.²³ Alternative strategies such as the use of radiolabelled monoclonal antibodies are being investigated.²⁴

There is increasing interest in tailoring the therapy on the major prognostic factors for AML patients. Several reports have suggested that some FAB subtypes may be cured with high-dose cytosine arabinoside in the intensification [M2 with t(8;21) and inv 16]²⁵ or with new chemotherapeutic approaches [M3 with t(15;17)].²⁶ In part it is possible that the brilliant results obtained by the GIMEMA group²⁶ may completely change the approach to the latter AML subtype. However, the recent EBMT analysis has shown that allogeneic BMT is superior in controlling the disease also in t(8;21) and inv 16 in which chemotherapy alone has a favorable effect.^{25,27} Therefore, if the TRM could be kept at 20% for patients above 30 years of age and consistently below 10% for younger individuals, then allogeneic BMT in CR1 will be a therapeutic option for more patients with an HLA

identical sibling. For patients over the age of 45, relatively mild conditioning regimens are being tested with encouraging results.²⁸ However, the problem of advanced disease remains and requires alternative strategies to control leukemia relapse.

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