Busulfan, Cyclophosphamide and Melphalan as Conditioning Regimen for Bone Marrow Transplantation in Children with Myelodysplastic Syndromes

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As typical disorders of the elderly, myelodysplastic syndromes (MDSs) are relatively unusual in childhood. Nevertheless, up to 17% of cases of pediatric acute myeloid leukemia may have a preleukemic phase. In young patients, the goal of treatment is eradication of the preleukemic malignant clone and reconstitution of normal hematopoiesis. Allogeneic bone marrow transplantation (BMT) has proved to be capable of this, but the optimal conditioning treatment to achieve eradication remains to be defined. Between May 1989 and June 1993, eight consecutive pediatric patients with MDS received a marrow transplant from an HLA-identical, mixed lymphocyte culture (MLC) nonreactive sibling. Diagnosis at time of presentation was refractory anemia with excess of blasts (RAEB) in two patients, RAEB in transformation (RAEB-t) in three, and juvenile chronic myelogenous leukemia (JCML, the pediatric counterpart of adult chronic myelomonocytic leukemia) in the remaining three children. Conditioning regimen consisted of busulfan, cyclophosphamide and melphalan, three alkylating agents potentially capable of killing also dormant preleukemic stem cells. The preparative regimen was very well tolerated, and all patients engrafted promptly. Six out of eight patients (75%) are alive and well with a median observation time of 20 months (range 8–34 months). Serial karyotype monitoring and molecular analyses have demonstrated a full chimerism of donor cells and the complete disappearance of trisomy 8 detected before transplant in three cases. All surviving patients have a Karnofsky score of 100%. One boy, affected by RAEB-t with monosomy 7 resistant to treatment with low-dose ara-C, relapsed 11 months after BMT, evolved in AML and died from progressive leukemia. Another patient with RAEB died on day +95 after BMT due to interstitial pneumonia of unclear etiology. This study confirms that allogeneic BMT is the treatment of choice in pediatric patients with MDS, and suggests that the employed conditioning regimen is a safe and effective means for eradicating the preleukemic malignant clone. Particularly noteworthy is that the three children with JCML obtained a complete remission and one of them is now a long-term survivor.

INTRODUCTION

A large body of evidence indicates that myelodysplastic syndromes (MDSs) represent steps in the multiphasic evolution of acute myeloid leukemia (AML) (1,2). Studies of clonality have shown a variable pattern of clonal proliferation in MDSs and this heterogeneity has also been observed in AML, thus confirming the preleukemic nature of MDS (1). In addition, patients with MDS have been found to carry the same molecular lesions observed in those with AML, in particular point mutations in cellular proto-oncogenes or genes relevant for cell proliferation and cell death (1). Different combinations of these molecular lesions likely explain the variable clinical course of these patients (3,4).

MDSs are typical disorders of the elderly, relatively unusual in childhood, the reported median age at presentation being 60–65 years. However, Blank and Lange (5) suggested that up to 17% of cases of pediatric AML have a preleukemic phase and MDSs seem to account for about 3% of childhood hematologic malignancies.

Myelodysplasia in children is often characterized by an aggressive clinical course and response to chemotherapeutic agents is limited and complicated by prolonged periods of aplasia (5–8). Moreover, most remissions are of short duration (9). Therefore, children affected by MDS and with an HLA-histocompatible sibling are to be considered elective candidates to allogeneic bone marrow transplantation (BMT). Published reports in the overall population of MDS patients receiving BMT evidence a probability of disease-free survival at 5 years approaching 40% (10–13). In one study on pediatric patients treated with allogeneic BMT, four out of eight children survived in complete remission (11).

We report on eight children with MDS treated with BMT, using an HLA-identical sibling as donor, after an original conditioning regimen consisting of busulfan (BU), cyclophosphamide (CY), and melphalan (L-PAM). The good outcome of these children suggests that the employed conditioning regimen is particularly effective in suppressing the preleukemic clone.

PATIENTS AND METHODS

Patients' Characteristics

Between May 1989 and June 1993, eight consecutive pediatric patients affected by MDS received a marrow transplant from an HLA-identical, mixed lymphocyte culture (MLC) nonreactive sibling at the Departments of Pediatrics of the Universities of Pavia and Bologna, Italy.

Patients were classified as having *de novo* MDS according to the French–American–British (FAB) Cooperative Group criteria (14), and previously reported criteria for the diagnosis of juvenile chronic myelomonocytic leukemia (JCML) (15). This disorder is considered the pediatric equivalent of adult chronic myelomonocytic leukemia (CMML), sharing similar clinical/biological characteristics and karyotype abnormalities (6,15–17). Diagnosis at time of presentation was refractory anemia with excess of blasts (RAEB) in two patients, RAEB in transformation (RAEB-t) in three and JCML in the remaining three children.

Details of patients' age, sex, peripheral blood findings, marrow fibrosis and blasts, karyotype analysis, FAB classification, treatment prior to transplant, interval between diagnosis and transplant and disease status at BMT are reported in Table 1.

Pre-BMT cytogenetic studies were obtained from marrow

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LEUKEMIA

Table 1 Patients' Characteristics

UPN	Age (yrs)	Sex	FAB	Peripheral Blood Counts at Diagnosis				Bone Marrow at Diagnosis		Karyotype	Previous Treatment	Interval Dx–BMT (months)	Disease Status at BMT
				Hb (g/dl)	WBC ($\times 10^9$/l)	PLT ($\times 10^9$/l)	% Blasts	% Blasts	Fibrosis				
054	11	M	RAEB-t	6	3.1	38	5	28	No	45,XY,–7	Low-dose Ara-C	2	Disease present
083	10	M	RAEB-t	6.2	13.2	5	3	25	No	47,XY,+8	Ara-C + DNM	4	CR
087	1	M	JCML	8	11.2	32	0	6	No	46,XY	Ara-C + DNM	4	Disease present
091	18	F	RAEB	10	0.7	113	0	10	Yes	47,XX,+8	None	24	Disease present
116	10	M	RAEB-t	7.8	8.4	57	6	26	Yes	46,XY	Ara-C + DNM	5	Disease present
122	8	M	RAEB	8.5	4.6	104	0	18	No	46,XY	Low-dose Ara-C	5	CR
140	3	M	JCML	9	36.9	32	4	20	No	47,XY,+8	None	3	Disease present
144	5	M	JCML	11.5	11.1	45	5	4	No	46,XY	Low dose Ara-C	3	Disease present

UPN, unique patient number; Ara-C, Cytosine arabinoside; DNM, Daunomycin; RAEB, refractory anemia with excess blasts; RAEB-t, refractory anemia with excess blasts in transformation; JCML, juvenile chronic myelomonocytic leukemia; Dx, diagnosis, CR, complete remission.

aspirates in all patients. Non-random simple chromosomal abnormalities (one translocation or loss/gain of one chromosome) were detected in three out of the five cases of RAEB and RAEB-t (two trisomy 8 and one monosomy 7) and in one out of the three children with JCML (trisomy 8). At the time of transplant, two patients (one with RAEB and the other with RAEB-t) had attained a complete hematological remission after low-dose infusional cytosine arabinoside (ara-C) therapy and intensive chemotherapy (ara-C + daunomycin) respectively, while four patients were transplanted for chemoresistant disease (two with JCML and two with RAEB-t). The remaining two patients affected by RAEB and JCML had received only supportive therapy.

Conditioning Regimen

Conditioning regimen consisted of: BU 16 mg/kg given orally in 16 divided doses every 6 h from day −7 to day −4; CY 60 mg/kg/day i.v. on days −3 and −2, and L-PAM 140 mg/m^2 i.v. single dose on day −1. Anticonvulsant therapy for seizure prophylaxis during BU administration was employed in three patients. To reduce the risk of hemorrhagic cystitis during Cy administration, patients received hyperhydration and Mesna according to the standard schedule.

Donor marrow was infused on day 0 and median nucleated marrow cell dose was 3.5×10^8/kg (range 2.1–7.8). Patients' parents gave written informed consent before transplant.

Graft-Versus-Host Disease Prophylaxis

Graft-versus-host disease (GVHD) prophylaxis consisted of Cyclosporine-A (Cs-A) administered intravenously, starting on day −1, at a dose of 1–3 mg/kg/day for the first 21–28 days and subsequently p.o. at a dose of 6 mg/kg/day for 6 months after transplant. Acute and chronic GVHD were classified according to previously described criteria (18,19). A-GVHD of grades II–IV was treated with steroids, anti-lymphocyte globulin, Cs-A or a combination of these drugs.

Supportive Therapy

Patients were nursed in laminar air-flow isolation and received oral antibiotics for enteric decontamination. As a *Pneumocystis carinii* pneumonia prophylaxis, all patients received oral cotrimoxazole at standard dosage, starting from the day of engraftment. To reduce risk of infections, the children received a commercial immunoglobulin preparation intravenously at a dosage of 400 mg/kg every week starting 2 days prior to transplant and ending at day 120 after BMT. To prevent endogenous reactivation of herpes simplex virus (HSV), and human cytomegalovirus (HCMV), patients received prophylactic intravenous Acyclovir at a dose of 750 mg/m^2 from day −2 to day +30. The expression of pp65 HCMV matrix protein was investigated twice weekly and cultures of throat swabs, blood and urine were routinely performed to detect HCMV reactivation.

Definitions

Haematopoietic and lymphoid engraftment was documented by karyotype analysis on marrow cells and peripheral blood lymphocytes, by red blood cell antigens phenotyping and using the amplification of variable numbers of tandem repeats (VNTR) by polymerase chain reaction (PCR) (20). White blood cell (WBC) engraftment was defined as the first of 3 consecutive days when neutrophils (PMN) were $>0.5 \times 10^9$/l and platelet (PLT) engraftment as the first of 3 consecutive days when the unsupported PLT count was $>50 \times 10^9$/l.

Regimen-Related Toxicity

Regimen-related toxicity (RRT) was graded according to the criteria previously reported by Bearman *et al.* (21). Hepatic veno-occlusive disease (VOD) was diagnosed using previously described parameters (22); interstitial pneumonia (IP) was diagnosed when bilateral interstitial/alveolar infiltrates on chest X-rays were associated with hypoxemia (pO_2 on room air $\leq$65 mm Hg).

RESULTS

The outcome of the eight patients is reported in Table 2.

Engraftment

All patients engrafted promptly and the median time needed to achieve more than 0.5×10^9/l neutrophils was between 10 and 21 days (median, 16 days). Return of platelet count to $>50 \times 10^9$/l occurred in 22 to 49 days (median, 31 days). Donor engraftment was documented in all patients using one of the methods reported above.

GVHD

Four out of the eight patients developed grade II–III A-GVHD, with skin and gastrointestinal involvement in three children and skin rash associated with moderate liver function abnormalities in the fourth. Three of the remaining four patients presented only grade I skin A-GVHD, while the last never showed signs of the disease. In all patients with A-GVHD, signs and symptoms of the disease disappeared after appropriate immunosuppressive therapy.

Two of the three patients with previous mild to moderate A-GVHD developed limited C-GVHD at day +125 and day +80, respectively. Both children were treated with steroids and Cs-A, that resolved the disease in one case and controlled the symptoms in the other child.

Regimen-Related Toxicity

The preparative regimen was generally very well tolerated. All patients had mild to moderate (grade I–II) mucositis; nausea and vomiting were only seen occasionally. Reversible hemorrhagic cystitis was observed in two patients, while hepatic VOD developed in one patient but completely resolved with conventional treatment. No significant central nervous system or renal toxicities were noted. One patient, with C-GVHD requiring immunosuppressive treatment, developed lethal idiopathic IP with dyspnea and hypoxemia 3 months after transplant. Investigations to detect HCMV reactivation did not permit us to identify this virus as the cause of clinical lung disease. Notwithstanding intensive supportive treatment, the patient presented progressive respiratory failure and died on day +95 after BMT. At autopsy, his pathology did not show evidence of lung involvement caused by C-GVHD and findings were consistent with severe pulmonary interstitial inflammatory infiltrate and moderate fibrosis of unclear etiology.

Table 2 Patients' Outcome

UPN	Donor sex	Sex Mismatch between Donor and Recipient	A-GVHD	C-GVHD	Outcome
054	F	Yes	III, SG	Limited	Relapse 334 days, death 352 days
083	M	No	0	No	Alive and well +34 months
087	M	No	I, S	No	Alive and well +30 months
091	M	Yes	II, SG	No	Alive and well +27 months
116	M	Yes	II, SG	No	Alive and well +15 months
122	F	Yes	II, SL	Limited	IP, death 95 days
140	F	Yes	I, S	No	Alive and well +9 months
144	M	No	I, S	No	Alive and well + 8 months

UPN, unique patient number; A-GVHD, acute GVHD; C-GVHD, chronic GVHD; S, skin; G, gut; L, liver; IP, interstitial pneumonitis.

Patients' Outcome

Six out of the eight patients (75%) are alive and well with a median observation time of 20.5 months (range 8–34 months). In all patients peripheral blood cell count, marrow cellularity and morphological features are normal. Serial karyotype monitoring and molecular analysis have demonstrated a full chimerism of donor cells and the complete disappearance of the characteristic chromosomal abnormality (trisomy 8) detected before transplant in three cases. All surviving patients have a Karnofsky score of 100%.

One boy, affected by RAEB-t with monosomy 7 resistant to treatment with low-dose ara-C, relapsed 11 months after marrow transplant, notwithstanding development of grade III A-GVHD and limited C-GVHD. He subsequently developed AML and died from progressive leukemia. As reported above, another patient with RAEB died on day +95 after BMT due to IP of unclear etiology. At time of death, the child was in complete hematological remission and he was receiving immunosuppressive therapy (i.e. Cs-A and steroids) owing to limited C-GVHD.

DISCUSSION

Clinical and biological heterogeneity is an intrinsic characteristic of myelodysplasia, some subtypes being associated with short survival and others with a more indolent prolonged course (3,4). Due to the wide variation in survival between patients belonging to the same FAB subgroup of MDSs, alternative attempts to assess prognosis and to refine the prognostic value of FAB classification have been elaborated (23–25). Prognostic factors reported to be associated with short survival include pancytopenia, high blast counts in peripheral blood and bone marrow, and karyotype abnormalities (23–25).

Most reported pediatric cases of MDS show features associated with poor prognosis and can be classified in those MDS subsets that are characterized by a more aggressive clinical course (5–7). This justifies intensive treatments aimed at eradicating the malignant clone and reconstituting normal hematopoiesis (26). Chemotherapy has been found to induce hematological remission in young patients (27–30). However, duration of remission has generally been short and results in terms of overall survival in children are poor and comparable to those observed in adults (5–7).

Allogeneic marrow transplant represents at present the only curative treatment for myelodysplasia in young patients and previous reports have emphasized that a significant number of patients with MDS can be successfully treated by allogeneic BMT (10–13,26,30). In the group of patients published by DeWitte *et al.* (10), the reported disease-free survival was about 50%, the better results being observed in patients affected by MDS with low proliferative capacity, i.e. refractory anemia (RA) and some cases of RAEB, and in patients at a more advanced stage of disease, but in complete hematological remission at time of transplant. In the largest and most recent update of the Seattle data (13) on patients with MDS given allogeneic BMT, the probabilities of disease-free survival, relapse, and non-relapse mortality at 4 years were 41, 28 and 43%, respectively. Young age (under 40), absence of blast excess, and short disease duration were found to be good prognostic factors.

Even though previous studies included few pediatric cases, the only report specifically addressing the question of BMT in children with MDS is that published by Guinan *et al.* (11). In this study eight children with MDS were transplanted with an HLA-identical sibling after a conditioning regimen consisting of fractionated total body irradiation (TBI) and chemotherapy. Both the drugs employed and the doses of radiotherapy delivered differed among the patients, and GVHD prophylaxis was heterogeneous. There were no relapses, while four patients died of rejection and acute or chronic GVHD.

In the present study, eight children with MDS received allogeneic BMT after homogeneous myeloablative therapy and GVHD prophylaxis treatment. We chose a busulfan-based

regimen due to its strong efficacy and acceptable toxicity in treating AML (31–33). In addition, as the use of L-PAM appears to substantially reduce the relapse rate after BMT (34), we speculated that by adding this drug to the classical 'little BU-Cy' regimen proposed by Tutschka *et al.* (32), the killing of malignant cells could be further increased. Moreover, a conditioning regimen consisting of three alkylating drugs, that have a non-cell-cycle active action, appears potentially capable of eradicating stem cell disorders, in which at least a portion of clonogenic cells are dormant out of cycle. Furthermore, this conditioning regimen has the advantage of reducing the risk of radiation-induced severe late sequelae.

The employed conditioning regimen was well-tolerated, with only one patient dying of transplant-related IP of unclear etiology. Relapse was observed in only one child with RAEB-t and monosomy 7, both factors considered to be adverse prognostic features for patients with MDS. Although the small number of patients does not allow us to draw any firm conclusion, the reported disease-free survival of six out of eight children after this original regimen is encouraging and confirms that allogeneic BMT may represent the elective treatment for young patients with myelodysplasia. Recently, two reports on the outcome of groups of patients (mainly adults) with MDS transplanted after a busulfan-based myeloablative therapy (35,36) proved that this preparative regimen has an acceptable RRT. Nonetheless, disease-free survival was excellent only in the study of Ratanatharathorn *et al.* (36) (78 ± 10%, after BMT with HLA-identical sibling), whereas the high incidence and severity of acute or chronic GVHD and five relapsing patients determined a disease-free survival of only 35% in the study reported by Nevill *et al.* (35).

Finally, it is particularly worth noting that all children with JCML obtained a complete remission, and one of them is now a long-term survivor. This MDS that represents, albeit with some distinctive features, the pediatric counterpart of adult CMML, was found to be curable with allogeneic BMT after a TBI-containing regimen (37), while the combination of BU and CY did not eradicate the malignant clone in a recent case report (38). Our data suggest that a conditioning regimen consisting of BU, Cy and L-PAM is enough to secure achievement of remission in JCML and probably to eradicate the single genetically altered clone that dominates blood cell production.

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