# Allogeneic bone marrow transplantation for children with myelodysplastic syndrome

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Six children with myelodysplastic syndrome underwent allogeneic bone marrow transplantation (BMT) from their HLA-identical siblings. Ages ranged from six to 16 years. French-American British (FAB) diagnosis was refractory anemia with excess blasts (RAEB) in three, RAEB in transformation (RAEB-t) in one and chronic myelomonocytic leukemia (CMML) in two cases. Two patients had progressed to leukemia before BMT. All patients received busulfan and cyclophosphamide as a conditioning regimen. Antithymocyte globulin (ATG) was administered to two of them due to the multiple transfusion history. Graft versus host disease (GvHD) prophylaxis consisted of cyclosporine-methotrexate. Engraftment was documented in all patients except one who underwent a second infusion of bone marrow cells. She died in the early post-transplant period with pancytopenia and veno-occlusive disease of the liver. Two patients died from disease recurrence. Three patients are alive >12 months post-transplant, two are in remission and one just relapsed at +16 months and is now being prepared for a second bone marrow transplant. The only significant factor for favorable outcome was short duration between diagnosis to transplant in the two patients in remission.

*Key words: myelodysplastic syndrome, children, bone marrow transplantation.*

Myelodysplastic syndrome (MDS) comprises a heterogeneous group of clonal stem cell disorders characterized by ineffective hematopoiesis and morphologic abnormalities usually involving all three blood lineages. Its occurrence is relatively unusual in childhood[1,2]. The reported incidence of MDS among all leukemias varies from one to 16 percent. In pediatrics, only three percent or less of hematological malignancies have been diagnosed as MDS; however, this figure is accepted to be an underestimated representation of its real occurrence[3,4].

The syndrome is highly aggressive in children and may progress to acute myelocytic leukemia (AML) in a short time period. The response to chemotherapy is limited, usually complicated by prolonged periods of aplasia, and most remissions are of short duration[1,2]. The goal of treatment is eradication of the preleukemic clone and reconstitution of normal hematopoiesis. Allogeneic bone marrow transplantation (alloBMT) has proved effective in this regard, representing the only curative treatment for MDS in young patients at present, with reported disease-free survival rates of 50-65 percent for patients <20 years of age[5-12]. However, a suitable bone marrow family donor is not available for the majority of patients. In such cases intensive chemotherapy followed by autologous stem cell rescue may also be considered[13]. Recent studies suggest that ineffective hematopoiesis, excessive bone marrow apoptosis, and an aberrant immune response play a role in the pathogenesis of MDS[14]. Therefore, in selected patients the use of trophic agents (amifostine); recombinant growth-factors; anti-apoptotic treatment; differentiation inducers such as hexamethylene bisacetamide, 5-azacytidine[14], and high-dose methyl prednisolone[15]; or immunosuppressive treatment (cyclosporine; antithymocyte globulin has been of benefit. Meanwhile, alloBMT from

a HLA-matched sibling remains the best treatment modality for children with MDS[7,8,10].

Here we describe six children with MDS who underwent alloBMT from tehir HLA-identical sibling donors at our institution, using a busulfan-based conditioning regimen.

## Material and Methods

### *Patients' Characteristics*

Between March 1995 and January 1999, six children with MDS underwent alloBMT from their HLA-identical, mixed lymphocyte culture (MLC) non-reactive siblings at our institution. Table I shows the patient characteristics including age, sex, clinical findings, peripheral blood and bone marrow blasts, karyotype analysis, and French American British (FAB) classification. Transplant characteristics are listed in Table II. The ages at time of transplantation ranged fram six to 16 years. Four patients were female and two males. None was diagnosed as sceondary MDS. Patients were classified according to the FAB cooperative group criteria. Diagnosis at the time of presentation was refractory anemia with excess blasts (RAEB) in three patients, RAEB in transformation (RAEB-t) in one and chronic myelomonocytic leukemia (CMML) in the remaining two children.

Pre-BMT cytogenetic studies were obtained from marrow aspirates in all patients. Trisomy 8 was detected in one patient with CMML, and 45,XX,-15,11(q21) in one.

Two out of six patients were in remission at the time of transplant. Four patients were treated with conventional chemotherapy before BMT. The remaining two patients (Case 3, Case 5) were not treated with chemotherapy before BMT due to the low number of BM blasts (<10%).

Table I. Patients' Characteristics at Diagnosis

| Case | Age at diagnosis years/sex | FAB | Hepatomegaly/ Splenomegaly | EMD | Hb (g/dl) | WBC ($x10^9$/L) | Platelet ($x10^9$/L) | PB blast | BM blast | MCV (fl) | Cytogenetics |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 12/F | RAEB-t | 13/7 | Gingival | 5.3 | 80 | 68 | 12 | 28 | 90 | 45,XX,-15,11 (q21) |
| 2 | 9/M | RAEB | 0/0 | – | 6.4 | 2.3 | 220 | 0 | 9 | 80 | NA |
| 3 | 7/F | RAEB | 7/0 | – | 5.6 | 0.8 | 19 | 0 | 10 | 82 | NA |
| 4* | 4/F | RAEB | 2/0 | – | 5 | 2.3 | 180 | 2 | 4 | 90 | 46,XX |
| 5 | 8/M | CMML | 0/0 | – | 11 | 6.7 | 36 | 24 mono | 4 | 112 | 47,XY,+8 |
| 6 | 14/F | CMML | 0/splen.** | – | 9 | 18.7 | 180 | 30 mono | 7 | – | NA |

* syngeneic.

** splen: splenectomy, six months before the diagnosis of myelodysplastic syndrome.

FAB: French American British classification. EMD: extramedullary disease. RAEB: refractory anemia with excess blasts. RAEB-t: RAEB in transformation. CMML: myelomonocytic leukemia.

Table II. Patients' Characteristics at the Time of Transplantion

| Case | Age at BMT (yrs) | Interval Dx-BMT (mo) | Disease status at BMT | Sex (donor/ recipient) | ABO (donor/ recipient) | Nucleated cell ($x10^8$/kg) | Conditioning regimen | GVHD prophylaxis | Engraft (+day) PNL/plt | Outcome (mo) |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 16 | 50 | Disease present | M/F | A/A | 4 | BU/CY | CsA+Mtx | 16/23 | AML (+4) exitus |
| 2 | 11 | 22 | CR, | F/M | O/O | 3.7 | BU/CY | CsA+Mtx | 14/17 | AML (+16) alive |
| 3 | 7 | 4 | Disease present | F/F | AB/AB | 4 | BU/CY/ATG | CsA+Mtx | 14/19 | CR (+16) |
| 4* | 6 | 24 | CR, | F/F | O/O | 4 | BU/CY | CsA | 12/14 | ALL (+12) exitus |
| 5 | 8 | 3 | Disease present | F/M | O/O | 5 | BU/CY | CsA+Mtx | 16/18 | Cr (+13) |
| 6 | 16 | 17 | Disease present | F/F | A/A | 3 | BU/CY/ATG | CsA+pred | – | exitus |

* syngeneic.

BMT: bone marrow transplantation. Dx: diagnosis. GVHD: graft versus host disease. PNL/plt: polymorphonuclear leukocytes/ plateles. BU/CY: busulfan/cyclophosphamide. CsA+mtx: cyclosporin A+methotrexate. AML: acute mvelocytic leukemia. CR: complete remission. ATG: antithymocyte globulin. ALL: acute lymphocytic leukemia. pred: prednisolone.

Case 4 progressed to acute lymphocytic leukemia (ALL) 16 months after initiation of chemotherapy for MDS and underwent BMT from her twin sister following eight months of ALL treatment. Another child (Case 2) transformed to AML four months after diagnosis of MDS AlloBMT was performed from his HLA-identical sibling following 22 months of chemotherapy on AML protocol. One splenectomized patient with CMML (Case 6) was transplanted for chemoresistant disease; at the time of transplantation, she had been suffering from persistent fevers, pancytopenia and hemolytic anemia.

*Conditioning Regimen*

Conditioning regimen consisted of: busulfan (Bu) 16 mg/kg of total dose given orally in 16 divided doses every six hours from day -9 to day -6 and cyclophosphamide (Cy) 50 mg/kg/day given intravenously (i.v.) on days -5 to day -2. Antithymocyte globulin (ATG) was added to the conditioning regimen in two patients due to multiple transfusion history, at a dose of 30 mg/kg/day on days -5 to -3. Anticonvulsive therapy as seizure prophylaxis during Bu administration was employed in all patients. In addition, hyperhydration and administration of mesna were done according to the standard schedule to reduce the risk of hemorrhagic cystitis during Cy administration.

*Marrow Transplantation*

Donor marrow was infused on day 0, and median nucleated marrow cell dose was $4x10^8$/kg (range $3\text{-}5x10^8$/kg). Written consent was obtained from parents before transplant.

All donors were HLA-identical, MLC non-reactive siblings (one identical twin). There were two sex mismatched; none of the recipient-donor pairs showed ABO incompatibility.

*Graft-Versus Host Disease (GvHD) Prophylaxis*

Graft versus host disease (GvHD) prophylaxis consisted of cyclosporin A (CsA) administered i.v. starting on day -2, at a dose of 3 mg/kg/day for the first two to three weeks and subsequently perorally (p.o.) at a dose of 6 mg/kg/day for six months after transplant, and short-term methotrexate (MTX) at a dose of 10 mg/m$^2$ on days 1, 3 and 6. MTX was omitted from the protocol in a patient at high risk of developing hepatic venoocclusive disease (VOD). One patient who received syngeneic BM cells was not given GvHD prophylaxis.

*Supportive Care*

Patients were isolated in hepa-filtered rooms at day -10 and received oral antibiotics for enteric decontamination. For Pneumocytis carinii prophylaxis, all patients received TMX 3 days a week, starting a week after engraftment. Irradiated and filtered blood products were used in all. Other supportive care measures included weekly infusions of intravenous immunoglobulins (200 mg/kg), acyclovir for herpes simplex virus (HSV) and cytomegalovirus (CMV) prophylaxis, and fluconazole. In addition, low molecular weight heparin, vitamin E and ursodeoxycolic acid were used for VOD prophylaxis in one splenectomized chemoresistant patient.

*Engraftment*

Hematopoietic engraftment was documented by either karyotype analysis on peripheral blood (PB) or BM cells, or DNA analysis by polymerase chain reaction (PCR) amplification of STR regions. White blood cell enfragtment was defined as the first of three consecutive days when neutrophils reached $>0.5x10^9$/L and platelet engraftment as the first of three consecutive days when the unsupported platelet count was $>50x10^9$/L.

## Results

Transplant characteristics and the outcome of six patients are reported in Table II.

*Engraftment*

Duration of cytopenia and blood requirements were unremarkable except in one case with graft failure. Five patients achieved prompt and complete engraftment as evidenced by rising blood counts (neutrophil engraftment at 12-16 days with a median of 14 days). Platelet engraftment took place at 14-23 days (median 18). Donor engraftment was documented in four patients by karyotype or DNA analysis.

*GvHD*

None of the patients developed >grade II acute or chronic GvHD.

*Regimen Related Toxicity*

Grade I-II mucositis was documented in five patients, and Case 1 developed necrotizing and hemorrhagic (grade IV) mucositis. Nausea and

vomiting were infrequent. No significant central nervous system or renal toxicity was observed. Moderate VOD occurred in one patient.

*Patient Outcome*

Of six patients, three are alive, two in complete remission with a follow-up period of 12 and 16 months and a Karnofsky score of 90-100 percent. One patient just relapsed at +16 months during the preparation of this manuscript. The patient has received remission-induction treatment and will be prepared for a second BMT. Serial karyotype and molecular analysis demonstrated a full chimerism of donor cells in two surviving patients; complete disappearance of the characteristic chromosome abnormality (Trisomy 8) was demonstrated in Case 5, following BMT.

Two of six patients died with recurrent disease at four and 12 months after BMT. Another case (Case 6) with CMML, who had resistant disease, pancytopenia, persistant high grade fevers and hemolytic anemia at transplant did not engraft. Not surprisingly, she had a very complicated post-transplant course with continuous fevers, worsening hemolytic anemia, CNS hemorrhage, VOD, platelet refractoriness, and graft failure necessitating a second bone marrow boost from the same donor at day +50. No conditioning regimen was used. She died of pulmonary compromise, infection and hemorrhage 32 days after her second transplant. BM aspiration at the time of death showed recurrent disease with infiltration of the marrow with >30% percent blasts. Postmortem examination of liver and lung tissues was consistent with VOD and hemorrhage.

## Discussion

Myelodysplastic syndromes (MDS) run an aggressive course in children. Most pediatric cases show features associated with poor prognosis, and AML develops within a few months[16]. The syndrome comprises a heterogeneous group of clinical disorders such as monosomy 7 and juvenile chronic myelogeneous leukemia (JCML), in which clinical behavior can vary substantially with overlapping features and similarities to adult MDS[1,16]. BMT offers a good chance of cure for such patients if a suitable donor is present[7,8].

The effect of age on the outcome of transplantation in patients with MDS is well documented. In most disease categories, the survival after alloBMT is better in younger patients due to less GvHD and better ability to tolerate the toxicity of chemotherapy. Similarly, patients with MDS less than 20 years of age are reported to achieve a disease-free survival (DFS) of 50 to 65 percent[5-12].

The cohice of the conditioning regimen also has an impact on transplantation success. Conditioning regimens with busulfan have been used successfully for eradication of the malignant clone in MDS[7,8,17], and are generally preferred in children over total body irradiation to avoid late sequelae of irradiation. In the present study, the conditioning regimen was busulfan-based due to its strong efficacy and acceptable toxicity. All patients received busulfan and cyclophosphamide (BuCy); in addition, ATG was added to the regimen in two hypertransfused patients to prevent graft rejection. The regimen was generally well tolerated in all children. In three patients, the myeloablative regimen was not sufficient to eradicate the clone since two died of early recurrence, and one with resistant disease.

Several investigators have suggested the use of intensified conditioning regimens to potentially eradicate stem cells and the abnormal clone. The addition of melphalan to the classical BuCy regimen has resulted in >70 percent survival rates with acceptable toxicity[7]. Likewise, an Ara-C, busulfan and melphalan combination has also led to a favorable outcome after BMT in children[17]. However, large-scale randomized pediatric series are missing since the syndrome is rarely diagnosed in children.

Disease duration has also been suggested as a significant factor for transplant success although not as important as the impact of age[7,12]. It has been shown that patients transplanted after five years of diagnosis had a less favorable outcome than the patients transplanted at earlier phases of disease[12]. In the present study, in the patients with relapse and resistant disease, the interval from diagnosis to transplant was long (17-50 months), as opposed to that of relapse-free patients (3-4 months). Longer disease duration, and therefore, more advanced stages of the disease, may have contributed to the higher incidence of treatment-related mortality and the higher relapse risk. Two of our surviving patients were in the early stage of their disease suggesting the important role of transplant timing in patients with MDS as in other hematological malignancies.

Other factors that may contribute to the adverse effect on BMT outcome have been reported as the presence of BM fibrosis, an increased number of BM blasts and secondary MDS[7,8]. One of our patients with a fatal outcome had an increased number of blasts (Case 1) in the bone marrow; none had BM fibrosis. All of our patients were de novo cases with no secondary MDS.

Extramedullary involvement has also been suggested as a poor prognostic factor in MDS; however, its effect on BMT outcome is not well known. In a recent study, pretransplant diagnosis of extramedullary disease was associated with poor outcome in a group of patients less than two years old with AML (n=34) and MDS (n=6)[10]. One of our patients who presented with gingival involvement and massive organomegaly at initial diagnosis had a poor outcome with early BM relapse at +4 months. At the time of BMT and at relapse she was free of extramedullary disease. In another study, granulocytic sarcoma after BMT was demonstrated as a poor risk factor in a group of patients with AML, MDS and chronic myelocytic leukemia (CML)[18]. Thus, the role of extramedullary involvement on BMT outcome in patients with MDS has yet to be defined.

The role of cytogenetic analysis in prognosis determination in patients with MDS has been clearly demonstrated by the international prognostic scoring system[19]. Certain cytogenetic abnormalities such as del5(q), del(20q), -Y (as sole abnormalities), or normal cytogenetics are considered as good factors, whereas the presence of 3 karyotype abnormalities or monosomy 7 or del (7q) suggest a poor prognosis[19]. The application of this scoring system to pediatric cases is not defined. Numerical abnormalities, in which a whole chromosome is either lost or gained (monosomy 7, trisomy 8) are typically seen in pediatric ases[1]. In the present report, one of our patients carried trisomy 8 abnormality in his hematopoietic cells; he is currently in remission >12 months post-transplant. However, due to the small number of cases in pediatrics, the effect of cytogenetic abnormalities on BMT outcome is not clearly defined.

In the present report we have described the outcome of six children with MDS treated with alloBMT. The only significant factor was the disease duration between relapsed and relapse-free patients. Two patients alive in compelete remission were transplanted very early after diagnosis without previous chemotherapy. In spite of the small number of patients, the present study suggests early transplantation as a favorable prognostic factor.

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