

ORIGINAL ARTICLE

Substitution of cyclophosphamide and busulfan by fludarabine, treosulfan and melphalan in a preparative regimen for children and adolescents with Shwachman–Diamond syndrome

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Allogeneic hematopoietic stem cell transplantation (HSCT) is the only definitive treatment for severe bone marrow dysfunction and clonal disorders in patients diagnosed with Shwachman–Diamond syndrome (SDS). In an attempt to minimize regimen-related toxicity (RRT), we have initiated a fludarabine/treosulfan/melphalan-based pilot protocol avoiding the combination of busulfan and cyclophosphamide. Median age at transplantation was 9.6 years (range 1.5–17 years). All three patients received conditioning with fludarabine (30 mg/m²/day × 6), treosulfan (12 g/m²/day × 3) and melphalan (140 mg/m²/day × 1). CAMPATH-1H (0.1 mg/kg × 2) was added in two cases, while rabbit ATG (Genzyme; 3 × 2.5 mg/kg) was given to the cord blood recipient. One patient was transplanted with a non-manipulated marrow graft from an HLA-identical sibling, one with a marrow graft from a 10/10 matched unrelated donor, and one with a 9/10 matched unrelated umbilical cord blood (UCB) unit. Mean cell doses given were 3.6 × 10⁸ nucleated cells/kg BW for the bone marrow recipients and 4.2 × 10⁷ nucleated cells/kg BW for UCB recipient. Overall, two of three patients are alive and display 100% donor chimerism. Acute graft-versus-host disease grade II was seen in one patient, while no GVHD exceeding grade I occurred in the remaining two.

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Introduction

Shwachman–Diamond syndrome (SDS) is a rare autosomal recessive disease characterized by exocrine pancreatic dysfunction, metaphyseal dysostosis and varying degrees of marrow dysfunction with cytopenias.¹ Myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML) have been described as a major complication of this disorder.² Almost all SDS patients have a mutation in the SBDS gene located on chromosome 7.³ However, the natural course of the disease is variable.

Long-term survival of SDS patients correlates with the degree of bone marrow dysfunction. Survival is significantly shortened when patients develop aplastic anemia. In these cases, survival from diagnosis averages 14 years.⁴ A relatively small percentage of SDS patients progresses to MDS or acute leukemia. In the largest reported series to date, 20% developed pancytopenia and 6% progressed to MDS.⁵ Patients progressing to MDS or leukemic transformation have a poor treatment response to chemotherapy associated with a very unfavorable prognosis.⁶ The French Severe Chronic Neutropenia Registry lists 10 out of 71 patients who received HSCT for SDS between 1987 and 2004. The indications were bone marrow failure in five cases and MDS/AML in another five cases.⁷ Although HSCT is currently the only treatment providing cure for severe bone marrow dysfunction, reported outcomes after HSCT have been not very encouraging with less than 50% of patients noted to be alive.⁸ The rarity of the disease and the poor correlation between genotype and resulting phenotype have contributed to the controversy about the role and timing of HSCT in patients with SDS. An important reason for treatment failure is the high rate of regimen related toxicity (RRT) early after HSCT, especially affecting heart and lungs. Although the preparative regimens of these cases varied, most patients received a combination of cyclophosphamide and busulfan or total body irradiation (TBI). Cyclophosphamide has been added to preparative regimens predominantly for its potent immunosuppressive activities. However, specific metabolites of cyclophosphamide are associated with an increased toxicity and mortality after conditioning, especially in the combination with busulfan.⁹ Furthermore, SDS patients

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Table 1 Pretransplant characteristics

Patient no.	Sex	Age (years)	Allele 1	Allele 2	Qualification for TX	Pulmonary function
1	M	17	183–184TA → CT	258 + 2T → C	Pancytopenia	VC _{max} : 47%, FEV1: 20%
2	F	7	183–184TA → CT	258 + 2T → C	Pancytopenia/MDS	VC _{max} : 78%, FEV1: 80%
3	M	1	297–300delAAGA	258 + 2T → C	Pancytopenia	Not done

seem to be especially susceptible to cardiac toxicity, the dose-limiting effect of cyclophosphamide.¹⁰ These experiences provided the rationale to replace cyclophosphamide and busulfan by fludarabine, treosulfan and melphalan.

The goal of this pilot study was to determine, whether a combination of 180 mg/m² fludarabine, 36 g/m² treosulfan, and 140 mg/m² melphalan can provide donor-derived engraftment after allogeneic HSCT from either related or unrelated HLA-matched donors without an increased incidence and severity of transplant-related complications.

Patients, materials and methods

Patients represented three consecutive referrals of children with SDS to the BMT Unit of the Children's Hospital, Hannover Medical University. In all patients, disease-associated mutations in the SBDS gene were confirmed as described previously.³ Patients were transplanted with an HLA-identical sibling bone marrow graft, an unrelated 10/10 matched marrow, and 9/10 matched unrelated umbilical cord blood (UCB), respectively. After detailed explanation of the procedure and its risks, informed consent was obtained from the parents. Particular emphasis was placed on the option of continuing conventional disease management with transfusions and cytokine therapy. Pretransplant characteristics of the patients are summarized in Table 1.

HLA typing and donor/recipient matching

Molecular typing of patients and donors was performed by extracting DNA from whole blood samples. Alleles at the HLA-A, -B, -Cw, -DRB1, -DRB3, DRB4, -DRB5, -DQA1, -DQB1 and -DPB1 loci were identified by single strand conformational polymorphism (SSCP) analyses and sequence-based typing of polymerase chain reaction (PCR) products. Amplification and sequencing of HLA class I and class II were performed as described previously.^{11,12}

Transplantation regimen, graft-versus-host disease prophylaxis and supportive therapy

All three patients received conditioning with fludarabine (30 mg/m²/day on six consecutive days), intravenous treosulfan (12 g/m²/day on three consecutive days), and intravenous melphalan (140 mg/m²/day once). The preparative regimen included CAMPATH-1H (0.1 mg/kg/day on two consecutive days) for the bone marrow recipients and 2.5 mg/kg/day ATG (Genzyme) for the cord blood recipient. Two children received a non-manipulated bone marrow graft from either an HLA-identical sibling or a 10/10 matched unrelated donor. Mean cell doses transplanted were 3.6×10^8 nucleated cells/kg BW (range $2.9\text{--}4.2 \times 10^8$ /kg) for the marrow recipients. The UCB graft contained

4.2×10^7 /kg nucleated cells/kg BW. For graft-versus-host disease (GVHD) prophylaxis, all children received 3 mg/kg per day cyclosporine (CSA) intravenously from day -2. The two recipients of bone marrow received short-term methotrexate (MTX),¹³ while mycophenolate (MMF) was given to the cord blood recipient (15 mg/kg/dose bid). CSA was switched to the oral form as soon as possible and was intended to be tapered by 10% per week, starting at day +60 for the recipient of the related graft and on day +180 for the recipient of the unrelated graft. All patients were treated in positive-pressure isolation rooms and received non-absorbable oral antibiotics and a low-bacteria diet. Supportive therapy consisted of *Pneumocystis carinii* prophylaxis with oral cotrimoxazole starting after engraftment, a first generation cephalosporin as standard antibiotic prophylaxis, and defibrotide as a preventive agent against venous occlusive disease (VOD). CMV reactivation was monitored either by expression of the pp65 antigen or by quantitative PCR. Empiric broad-spectrum antibiotic therapy was started when patients became febrile, and antifungal therapy was added in the presence of either clinical evidence of fungal infection or fever persisting after 3 days of antibiotic therapy. All blood products administered after transplantation were irradiated with 30 Gy.

Neutrophil and platelet engraftment were defined as the first of three consecutive days with an absolute neutrophil count (ANC) of more than 0.5×10^9 /l and platelets of more than 50×10^9 /l, respectively. Red cell recovery was defined by reticulocyte counts of >20 /nl. Acute and chronic GVHD were graded according to the Seattle criteria.¹⁴

Monitoring of chimerism

Chimerism was documented by *in situ* Y chromosome hybridization of either bone marrow or blood samples in sex-mismatched donor/recipient pairs, and by analysis of a variable number of tandem repeat (VNTR) polymorphism as well as by microsatellite analysis of bone marrow and/or blood samples in the case of sex-matched pairs.

Results

Engraftment

The three patients were transplanted between 2004 and 2006. They received bone marrow from an HLA-identical sibling, a 10/10 matched unrelated donor, and a 9/10 matched UCB graft, respectively. All three had 100% donor-derived hematopoiesis. At the time this report is written, two of the three patients are alive. The third patient receiving UCB died from idiopathic pneumonitis syndrome (IPS) 98 days after transplantation. Neutrophil engraftment occurred in all patients after 24, 31, and 26 days,

Table 2 Post-transplant characteristics

Patient no.	Graft	Nucleated cells/kg	Neutrophil engraftment	RRT	Donor chimerism (%)	aGVHD	Follow-up
1	MRD BM	2.9×10^8	Day 24	Mucositis grade II	100	0	24 months
2	MUD BM	4.4×10^8	Day 31	Mucositis grade II	100	II	16 months
3	MUD UCB	3.4×10^7	Day 26	Lethal IPS	100	0	Died on day 98

respectively (Table 2). For the first two patients, platelet recovery was reached on day 23 and day 231. Red cell recovery was observed on day 16 and 21. The third patient remained transfusion-dependent for red cells and platelets until he died from IPS.

Graft-versus-host disease

The incidence of grade II–IV GVHD within this small cohort of children was low. The recipient of the unrelated marrow graft developed grade II acute GVHD. No patient received donor lymphocyte infusions.

Regimen-related toxicity

The first two children experienced no toxicities >grade I despite having SDS-associated decreased pulmonary function, when going into transplantation. The third patient was 18-months-old when he received his cord blood graft. In contrast to the first two patients, his pulmonary function was normal. However, respiratory distress was already observed during the preparative regimen, when ATG was given. X-ray exams of the chest revealed bilateral pleural effusions and diffuse interstitial densities of the lung parenchyma. Pleural effusions quickly resolved after diuretic treatment. Despite broad antiviral, antibacterial and antifungal coverage, the patient developed progressive respiratory insufficiency. Respiratory distress responded quickly to pulses of intravenous prednisone, but worsened again after discontinuation of the drug. Results from a bronchial lavage did not show any evidence for an infectious etiology. The patient soon required respiratory support. Treatment with etanercept did not result in improvement. The patient finally required high frequency oscillation and died soon after he developed diffuse pulmonary hemorrhage. A lung biopsy was taken *post mortem*. Histology showed profoundly altered lung tissue with multiple necrotic areas, diffuse pulmonary hemorrhage, and diffuse alveolar damage with hyaline membranes accompanied by bronchiolar inflammation and epithelia damage. Typical pulmonary changes observed as a side effect of alkylating agents such as fibrosis were not seen.

Discussion

We present first results of three children with SDS after HSCT who received a conditioning regimen based on fludarabine, treosulfan, and melphalan, thereby avoiding cyclophosphamide and busulfan or TBI. After a follow-up time of 9 and 20 months, two of the three children are alive, having experienced hardly any treatment-related toxicity and showing 100% donor-derived hematopoiesis. The third patient died 3 months after transplant from IPS. Having

lost one child from pulmonary toxicity, we are unable to conclude that this regimen has the potential to decrease the typical treatment-related toxicities seen in SDS patients undergoing HSCT such as cardiac and pulmonary toxicities.

Allogeneic hematopoietic stem cell transplantation (HSCT) is the only definitive treatment for severe bone marrow dysfunction and clonal disorders in patients diagnosed with SDS. To date, 34 HSCT procedures for SDS have been reported in 19 different publications.⁷ The majority of those patients received a preparative regimen consisting of cyclophosphamide, either in combination with busulfan or TBI. Most patients received unrelated bone marrow as a source of stem cells. More recently, a single report described three patients transplanted with UCB.¹⁵

Unfortunately, long-term outcome data are not available for most of the patients. At the time of reporting, <50% of cases were noted to be alive.⁸ Based upon the available literature, patients with SDS seem to experience more RRT with HSCT than other myelodysplastic disorders like Kostman syndrome or JMML.¹⁶ A considerable number of patients died early after transplantation. Causes of death included congestive heart failure and pulmonary hemorrhage. The special predisposition to this toxicity profile is poorly understood, but has been attributed to the cardiotoxicity mediated by cyclophosphamide.¹⁷ In some series, a risk of cardiomyopathy has been reported in 50% of SDS patients who had not undergone HSCT. This included cardiac fibrosis and necrotic areas found upon necroscopy.¹⁰ Concerning the optimal preparative regimen for SDS patients, consideration therefore should be given to new preparative regimens that have the potential to decrease cardiac and pulmonary toxicities.

On a molar basis and relative to other toxicities, cyclophosphamide was shown to be the most immunosuppressive anticancer drug of any type.¹⁸ Given at very high doses (200 mg/kg or greater) as a single agent as used in preparation regimens for bone marrow transplantation, cyclophosphamide rarely causes cumulative damage to the bone marrow, reflecting its stem cell-sparing properties.¹⁹ Therefore, this compound has been primarily used in bone marrow transplantation for its immunosuppressive and antileukemic properties. Dose-limiting, however, seems to be its cardiac toxicity.²⁰ Fludarabine, on the other hand, is a purine analog with known inhibitory effects on lymphocyte proliferation. This drug has shown very little extramedullary toxicity in dose ranges between 90 and 125 mg/m², although side effects involving the central nervous system are known to occur at higher doses.^{21,22} Vaughan and McDonald found a strong correlation between blood levels of various cyclophosphamide metabolites and VOD. It was postulated that the formation of these metabolites deplete the liver of glutathione (GSH), resulting in

significant toxicity.^{23,24} Fludarabine, however, is not known to deplete the GSH content. This might be of increasing importance in patients entering HSCT with pre-existing organ damage. The described observation formed a compelling argument for us to replace cyclophosphamide by fludarabine in a conditioning regimen for children and adolescents with SDS.

In contrast to cyclophosphamide, busulfan has a potent myeloablative effect. In combination, these two drugs are the basis of the most commonly used radiation-free preparative regimens in bone marrow transplantation.²⁵ Unfortunately, the combination of cyclophosphamide and busulfan is prone to mediate endothelial damage of the small liver venules, resulting in venous occlusive disease and pulmonary injury.²⁶ Treosulfan is the prodrug of an alkylating agent with hematotoxicity being dose-limiting. In phase I protocols using treosulfan in escalating dosages for autologous HSCT, observed side effects were mucositis, diarrhea and skin toxicity.²⁷ *In vitro* data have confirmed the pronounced effect of treosulfan against hematopoietic stem cells, and repeated dosing of treosulfan has been shown to be as effective as busulfan or TBI in inducing reliable donor-cell engraftment in preclinical models.²⁸ Additionally, antileukemic activity superior to equitoxic doses of cyclophosphamide or busulfan has been demonstrated in human acute lymphoblastic leukemia models.²⁹ As a result of its limited nonhematologic toxicity and robust myeloablative properties, we decided to substitute busulfan by treosulfan.

The choice of tailoring a preparative regimen for SDS patients by adding a second alkylating agent was based on the results of a preliminary study demonstrating the safety of combining melphalan with busulfan³⁰ and on the fact that the retrospective analysis of the EWOG-MDS group showed that a myeloablative preparative regimen including a second alkylator was associated with a better event-free survival (EFS) and a lower relapse rate in comparison with regimens including TBI.³¹ Similarly, poorer results of HSCT for SDS-associated myelodysplasia/acute leukemia have been reported. Indeed, the French experience with HSCT in SDS patients showed a significant difference in EFS between patients receiving HSCT for bone marrow aplasia and those undergoing the procedure for leukemic transformation. Although several risk factors are discussed to contribute to this observation, it becomes increasingly evident, that HSCT fails to correct the leukemia or to eliminate the myelodysplastic clones.⁷ As SDS is a rare disease and the subgroup of patients qualifying for HSCT is even smaller, we therefore decided to give melphalan as well to patients presenting with bone marrow failure only.

The first two patients enrolled on our pilot-protocol, one with bone marrow failure and one with MDS developed minimal toxicity. Unfortunately, the third patient developed severe IPS early in the post-transplant course. The first two patients were diagnosed with the 183–184TA → CT mutation on one allele. The third patient carried a c.297–300delAAGA deletion. The question of whether a certain genotype predisposes its carrier to a specific toxicity profile seems to be important, but cannot be answered at this point. However, this pilot study underscores the need to carefully collect data of these patients in order to optimize

current treatment protocols and to potentially adjust the therapy to the patient's genotype in the future.

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