

Non-radiotherapy conditioning with stem cell transplantation from alternative donors in children with refractory severe aplastic anemia

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Summary:

Conditioning including total body/lymphoid irradiation is widely used to prevent graft rejection in patients with refractory severe aplastic anemia (SAA) undergoing hemopoietic cell transplantation (HCT) from alternative donors and or after graft manipulation. To reduce regimen-related toxicity we transplanted three children with refractory SAA after conditioning with radiotherapy-free regimens. Conditioning included fludarabine 175–180 mg/m² in all patients. In addition, patient 1 (failing two previous grafts) received thiotepa 10 mg/kg and Campath-1H 60 mg/m²; patient 2 cyclophosphamide 120 mg/kg, thiotepa 15 mg/kg and OKT-3 0.1 mg/kg/day for 4 weeks; and patient 3 cyclophosphamide 120 and ATG 90 mg/kg. Stem cell source was unmanipulated marrow from the same unrelated donor as for the two previous transplantations in patient 1 and CD34⁺-purified peripheral blood stem cells from an HLA-matched unrelated donor and from the haploidentical mother in patients 2 and 3. Only patient 1 received graft-versus-host disease (GVHD) prophylaxis with cyclosporine A and mycophenolate mofetil. Follow-up is now 30, 51, and 15 months. None of the patients developed GVHD. All patients have normal counts with complete donor chimerism. Fludarabine-based conditioning is powerfully immunosuppressive and may be used for children with refractory SAA undergoing HCT from alternative donors even after rejection following previous HCT.

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severe aplastic anemia (SAA) not responding to immunosuppressive therapy (IST).^{1–3} As most of the patients with SAA lack an HLA-compatible family donor HCT using mismatched related or matched unrelated donors might be an appropriate alternative treatment. However, graft failure and graft-versus-host disease (GVHD) are two of the greatest barriers to potentially curative HCT for SAA. With conventional transplants from HLA-identical siblings or unrelated donors, engraftment failures occur in 5–25% of patients with SAA.^{2–6} In addition, 20–52% of these patients experience severe acute GVHD after unrelated-donor transplantation.^{4,6} In a retrospective data analysis of the European Group for Blood and Marrow Transplantation graft rejection and GVHD were reported in 26% and 28% of 269 patients with SAA allografted from alternative donors.⁷ In pediatric studies the incidence of graft failure and grade II–IV acute GVHD was 2.4–3% and 20–28%, respectively, after unrelated-donor transplantation for malignant and nonmalignant diseases.^{8,9} Others have shown that more than 80% of children may have grade II–IV acute GVHD after matched unrelated-donor transplantation.¹⁰ The risk of graft failure and GVHD increases with the number of HLA class I/II mismatches.^{6,9,11} The risk of graft failure may also increase with the number of pretransplant transfusions due to allosensitization.^{5,12} We have previously shown that stable engraftment without increasing the risk of GVHD can be achieved by transplantation of large numbers of highly purified CD34⁺ cells in children with refractory SAA.¹³ Limited experience, however, exists with nonradiotherapy based conditioning in children with SAA receiving allografts from unrelated or haploidentical donors or after previous HCT. We here present three children with refractory SAA who were allografted from alternative donors (two after allograft manipulation) after conditioning with fludarabine-based regimens.

Hemopoietic cell transplantation (HCT) from an HLA-identical sibling is the treatment of choice for children with

Patients and methods

As all three patients lacked an HLA-identical family donor and did not respond to one or more courses of standard IST, they were selected for alternative donor transplantation. In order to avoid TLI/TBI we used a nonradiotherapy based ‘chemotherapy only’ conditioning. All transplants were performed after informed consent was obtained. In

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patients 1 and 2 serological typing was carried out for class I and molecular typing for class II antigens. In patient 3 class I/II typing was performed by high-resolution molecular typing. In all three patients chimerism studies were repeatedly carried out at particular time points after transplantation.

Case reports

Patient 1

An 11-year-old boy with SAA received an allograft consisting of highly purified CD34⁺ peripheral stem cells from a matched unrelated donor after conditioning with antilymphocyte globulin (0.75 ml/kg/day on days -6 through -3), cyclophosphamide (CY) (60 mg/kg on days -3 and -2), thiotepa (TT) (250 mg/m² on days -5 and -4) and TLI (2 × 2.5 Gy on day -1) as previously described,^{13,14} but developed *de novo* SAA after a common viral infection. At the age of 13 years he was successfully retransplanted with highly purified CD34⁺ cells from the same donor after conditioning with antithymocyte globulin (ATG) (5 mg/kg/day on days -5 through -2) and CY (60 mg/kg on days -3 and -2). Two and a half years later his counts again decreased (white blood count (WBC) 3.3 × 10⁹/l; Hb 12.6 g/dl; platelets 28 × 10⁹/l). Despite IST with cyclosporine A (CyA) his counts rapidly deteriorated within the next 3 months (WBC 2.4 × 10⁹/l, Hb 6.5 g/dl; platelets 3 × 10⁹/l). Marrow aspiration disclosed severe hypocellularity. Variable number of tandem repeat (VNTR) analysis carried out on different marrow subpopulations (CD19⁺, CD3⁺, CD14⁺ cells, granulocytes) showed that DNA isolated from granulocytes and monocytes was of donor origin. In contrast, DNA isolated from CD19⁺ and CD3⁺ cells was of recipient origin at 3–9%. Since this patient failed two previous allografts containing highly purified CD34⁺ stem cells, we used unmanipulated marrow for the third transplantation. Conditioning consisted of fludarabine (35 mg/m²/day) on days -7 through -3, Campath-1H (10 mg/m²/day) on days -8 through -4 and -1, and TT (10 mg/kg) on day -2. The patient received 5 × 10⁸/kg nucleated cells. The graft contained 3.18 × 10⁶ CD34⁺ cells/kg and 0.85 × 10⁸ CD3⁺ cells/kg. GVHD prophylaxis with CyA and mycophenolate mofetil was initiated on days -1 and 0, respectively. Prompt engraftment with absolute neutrophil count (ANC) >0.5 × 10⁹/l and platelet count >20 × 10⁹/l was observed occurring on days +10 and +13. GVHD prophylaxis was gradually tapered and stopped 1 year after transplantation. Chimerism analysis showed full hemopoiesis of donor origin during the entire post transplant follow-up. The patient is alive and well with normal counts after now 2 1/2 years. GVHD was not observed.

Patient 2

A 4-year-old girl presented with thrombocytopenia, which progressed to SAA. Her family history was positive with regard to medullary thyroid carcinoma. The patient as well as other family members (mother, brother) were found to have a RET protooncogene mutation known to cause

medullary thyroid carcinoma and multiple endocrine neoplasia (MEN) type IIa. At the age of 11 years she became transfusion-dependent. One year later (May 1995), she showed a short lasting partial remission after a first IST course according to the SAA 94 protocol,¹⁵ but became again transfusion-dependent. Since she did not respond to a second IST course given in October 1998, CyA administration was stopped by April 1999. The patient developed hemosiderosis of the liver with highly elevated serum ferritin levels necessitating a continuous desferrioxamin therapy. As the patient did not have an HLA-identical sibling donor an international search for a potential unrelated donor was initiated by the 'Deutsche Knochenmarkspendeteile' (DKMS; Tübingen, Germany) and an HLA-matched unrelated male donor was found. Complete blood count (CBC) before HCT showed a WBC count of 1.5 × 10⁹/l (ANC 0.7 × 10⁹/l), Hb of 8.5 g/l and a platelet count of 12 × 10⁹/l. Conditioning consisted of fludarabine (30 mg/m²/day) on days -7 through -2, TT 15 mg/kg on day -6, CY (60 mg/kg/day) on days -3 and -2, and anti-CD3 antibody OKT-3 (0.1 mg/kg/day) for 23 days. Donor peripheral CD34⁺ cells were purified using the CliniMACS device as described previously.¹⁴ In April 2000 the patient received a total of 9.6 × 10⁶/kg CD34⁺ peripheral stem cells and 21.5 × 10³/kg CD3⁺ cells. Stable sustained hemopoietic engraftment with ANC >0.5 × 10⁹/l and platelet count >20 × 10⁹/l occurred on days 9 and 17, respectively. GVHD prophylaxis was not given. Chimerism studies disclosed 100% male donor cells. More than 4 years after HCT the patient is in excellent clinical condition with normal counts.

Patient 3

An 11-year-old girl presented with mucocutaneous bleeding. CBC showed a WBC count of 3.0 × 10⁹/l (ANC: 0.9 × 10⁹/l), Hb of 11.1 g/l and a platelet count of 5 × 10⁹/l. The marrow aspirate was almost completely acellular with only lymphocytes, plasma cells and stroma cells detectable. EBV-PCR was repeatedly found positive in peripheral blood and marrow, whereas EBV serology was negative. The girl developed intracranial hemorrhage with transient monoparesis of the left upper extremity. IST according to the SAA 94 protocol¹⁵ with antilymphocyte globulin, methylprednisolone and CyA was commenced, but marrow aspiration 2 months after initiation of IST still revealed severe hypocellularity. The girl had to receive platelet as well as packed red blood cell transfusions every 3–4 days (21 packed red blood cell and 29 platelet transfusions were given within a 3-month period). As the patient lacked an HLA-identical family donor an unrelated donor search was started, but was unsuccessful. The patient's mother, however, was found to be identical with regard to HLA class I and haploidentical with regard to HLA class II antigens by high-resolution typing (patient: A 03,25; B 07,35; Cw 04,07; DRB1 0901,1501; DQB1 0303,0602; mother: A 03,25; B 07,35; Cw 04,07; DRB1 01,1501; DQB1 05,06). Peripheral CD34⁺ cells from the patient's mother were mobilized, collected, and isolated as previously described.¹⁴ Conditioning consisted of fludarabine (35 mg/m²/day) on days -7 through -3, ATG (30 mg/kg/

day) on days -7 through -5, and CY (60 mg/kg/day) on days -4 and -2. A total of 8.59×10^6 CD34⁺ cells/kg were infused with 8.4×10^3 CD3⁺ cells/kg. No pharmacologic GVHD prophylaxis was given. Engraftment was prompt and chimerism analysis showed permanent 100% donor chimerism. The patient is alive without GVHD and normal CBC 15 months after HCT.

Discussion

For more than 30 years TBI has been included into conditioning regimens because of its excellent immunosuppressive and antitumor activity.¹⁶ Particularly in patients with refractory SAA who need alternative donor HCT with or without graft manipulation, TBI/TLI is considered an essential immunosuppressive tool for prevention of graft rejection.^{5,12,17,18} However, TBI/TLI carries the risk of significant late regimen-related toxicity. Secondary malignant neoplasms (SMN) are considered the most serious transplant-related complication. The estimated cumulative incidence to develop a solid SMN following allogeneic HCT ranges from 3.8 to 6.7% at 10–20 years post-transplant.¹⁹ Of note risk rates continue to rise with elapsed time since transplantation and no plateau has been reached so far. This is of particular interest when children have to undergo HCT for a nonmalignant disease such as SAA. In a large series by the Fred Hutchinson Cancer Research Center patients receiving TBI for conditioning had a 25-year incidence of SMN of 21% compared to 10% for patients without TBI.¹⁹ For patients younger than 10 years at HCT this difference was even more prominent. Patients in this age group who received TBI had an incidence of SMN of 19% compared with 5% for patients who did not receive TBI. The high risk to develop an SMN together with other TBI-related late effects^{19–22} prompted us to consider an alternative, radiotherapy-free conditioning regimen for children with refractory SAA. Deeg *et al*,²³ however, have shown that conditioning with CY/ATG alone is insufficient for a stable sustained engraftment in patients with SAA transplanted from alternative donors. As all our three patients were at high risk to develop GVHD or graft failure (the first patient had undergone two previous HCT with highly purified CD34⁺ peripheral blood stem cells, the second had received a large number of transfusions over a period of 9 years and the third received an allograft from her haploidentical mother) an intensive immunosuppressive conditioning regimen was necessary.

Fludarabine, a purine nucleoside analogue, that requires intracellular phosphorylation to its biologically active form has broad antitumor activity against a variety of hematologic malignancies.^{24,25} Due to its immunosuppressive potency fludarabine has been included into reduced-intensity nonmyeloablative conditioning regimens for malignant as well as nonmalignant diseases.^{26–30} However, experiences with fludarabine-based conditioning in children with refractory SAA undergoing alternative donor transplantation is limited.³¹ Chan *et al*³¹ reported on five children with refractory SAA who received fludarabine, CY and ATG as conditioning before alternative donor HCT. GVHD prophylaxis consisted of methylprednisolone

(for approximately 2 months) and CyA or tacrolimus for 6 months. All patients engrafted. Four of them developed acute GVHD and two chronic extensive GVHD. Follow-up was less than 1 year in all patients at the time the paper was published.

Other nonmyeloablative conditioning regimens have used melphalan, thiotepea, low-dose busulfan, or monoclonal antibodies such as Campath-1H or OKT3 in addition to fludarabine^{29,30,32–34} to further increase the immunosuppressive activity of the conditioning regimen. However, no clear superiority of one of these regimens has been shown and large multicenter trials comparing activity and toxicity of these different conditioning regimens have not been performed so far. Although toxicity of nonmyeloablative conditioning regimens is generally low, GVHD remains a significant cause of morbidity and mortality following reduced-intensity HCT. Incidence of grade II–IV acute GVHD is reported to be as high as 40%.^{26,32} We, in contrast, did not observe acute or chronic GVHD in one of our patients; in two of them due to the fact that they received CD34⁺ highly purified stem cells.

Our preliminary observations in three children with refractory SAA show that fludarabine-based conditioning is powerfully immunosuppressive and ensures stable engraftment in patients who undergo HCT from alternative donors even after graft manipulation or graft failure from previous HCT with purified CD34⁺ stem cells. By using fludarabine-based conditioning we could avoid administration of TBI/TLI in children. This might have a positive impact on preservation of fertility and preventing development of SMN in patients who are possibly cured from their underlying nonmalignant hematologic disease.

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