Statistical analysis

Statistical analyses were performed using SPSS statistical software (SPSS Co., Tokyo, Japan). OS was calculated by the Kaplan–Meier method.

This retrospective analysis was performed in accordance with the Helsinki Declaration of 1975.

Results

Engraftment

All 18 patients showed engraftment. The median number of days until the neutrophil count exceeded 500/μL was 16 (range 11–21). The median number of days until the reticulocyte count exceeded 10% was 20 (range 9–89), and the median number of days until the platelet count exceeded $>50\,000$ was 28 (range 15–170). Among the 18 who achieved neutrophil recovery, one subsequently experienced a sustained decline in the neutrophil count. He received a second transplant from the same donor (HLA-matched sibling) after conditioning including ATG + -CY + Flu. Tacrolimus and MTX were given as GVHD prophylaxis.

Complications

GVHD

Grade 2–4 acute GVHD occurred in five patients (grade 3 in 4, grade 4 in 1). The day-100 probability of grade 2–4 acute GVHD was 27.8%. Limited and extensive cGVHD occurred in three and three patients, respectively, at a median of 10 months (4–25). The five-yr probability of cGVHD was 16.7%. One died from interstitial pneumonia complicated with extensive cGVHD. One developed extensive cGVHD as scleroderma followed by mild arthrogryposis in his extremities. The remaining one patient with extensive cGVHD and three patients with limited cGVHD recovered completely. Other complications were neutropenic fever in 15, hemorrhagic cystitis in four, and thrombotic microangiopathy, posterior reversible encephalopathy syndrome, and nephrotic syndrome in one each.

Infectious complications

Infectious complications included viral cystitis in two, central venous catheter-related MRS infection in one, fungal pneumonia in one, and zoster in one patient. All recovered without sequelae. In a deceased patient with cGVHD, the cause of interstitial pneumonia was not determined.

Survival

The surviving patients were followed-up for a median of 46 months (range 10–153). None died within 100 days after BMT. One patient died because of interstitial pneumonia complicated with cGVHD at five months post-BMT. The five-yr probability of OS with a median follow-up of 42 months was 94% (95% CI: 83–105) (Fig. 1). KPS in most of the surviving patients was 100. One patient had a score of 50, and two had a score of 90. The patient who had a KPS of 50 had congenital AA complicated with cerebral palsy and mental retardation prior to BMT. Her KPS did not change with BMT.

Discussion

In general, it would be preferable to enroll patients with AA in clinical trials to improve the outcome of the disease. However, clinical trials take time and the advances in transplant procedures can be quite rapid. Where clinical trials are not feasible, data from observational studies may be the best-available evidence to guide practice (1). Therefore, we treated our patients with a best-available evidence strategy, i.e., treatment was based on the best donor source, conditioning regimen, GVHD prophylaxis, and supportive care at the time of transplant. Because almost all Japanese are covered by public medical insurance, it was possible to choose a best-available evidence strategy.

We report here the clinical outcome for allogeneic BMT in 18 consecutive children with AA. Because OS at five yr was good, this best-available evidence strategy was acceptable. The probability of FFS at 10 yr in children with AA who received an allogeneic BMT from an HLA-matched family donor between 1984 and 1998 was reported to be 97% (9). Kojima et al. also reported an excellent result for BMT from an

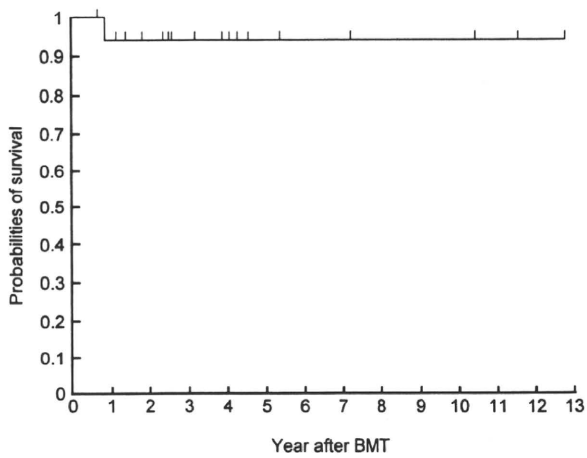

Fig. 1. OS in children undergoing BMT for AA.

unrelated donor in the Nagoya area (7), which is compatible with our findings. However, in a multi-center trial conducted between 1994 and 2004, FFS in 33 children who received BMT from an HLA-matched sibling was reported to be 69.7% when these patients received IST with ATG and CsA prior to BMT (10). In another study conducted between 1997 and 2004, FFS in 31 children who received BMT from an alternative donor was reported to be 83.9% (11), which is also compatible with our results. We obtained a good outcome based on various advances regarding BMT for AA, as described later.

High-resolution HLA typing has improved the outcome for matched unrelated donor BMT in patients with AA, and long-term survival has reached 61% (12). Maury et al. reported that survival after unrelated SCT for severe AA has improved significantly over the past 15 yr, mainly because of better HLA matching (13). The results for young patients who are fully HLA-matched with their donor at the allelic level are comparable to those observed after SCT from a related donor (13). One of our patients who was transplanted in 1997 died because of interstitial pneumonia complicated with chronic extensive GVHD. This patient received serologically matched unrelated BM. Because we did not have a technique for matching HLA based on DNA typing at that time, this patient might have received mismatched marrow by DNA typing. Since the end of 1997, we have been able to type HLA based on a DNA technique. This could support our good outcome for BMT thereafter.

As shown in Fig. 2, we started BMT with low-dose irradiation regimens in 1992. We shifted to non-irradiation-based conditioning regimens in 2003 based on reports by Chan (14) and others (6, 15). Later, this was also supported by a report by Kahl (16). In 2004, we used Flu as a conditioning regimen for the first time. This particular patient developed severe AA after acute hepatitis, and she did not have an HLA-matched sibling or unrelated donor. Therefore, she received BMT from her HLA-mismatched mother. At that time, a Flu-based conditioning regimen was adopted based on the report by Chan. Meanwhile, we started BMT with an unrelated donor using a Flu-based conditioning regimen in 2006. There have been several reports regarding Flu-based conditioning regimens that showed promising results, with good engraftment, tolerable toxicity, and minimal GVHD (17–21). This seems to be a consistent finding in BMT from alternative donors based on our experience and these other reports (12, 22). Although no report has shown that Flu-based conditioning is definitely superior to others, it would be reasonable to choose this approach to avoid possible toxicities by irradiation. Currently, we choose an HLA-matched sibling as a donor with CY + ATG conditioning. If an HLA-matched sibling is not available, an HLA-mismatched family donor or HLA-matched unrelated donor with a Flu-based conditioning regimen is chosen.

A previous report demonstrated that survival after SCT from alternative donors is significantly

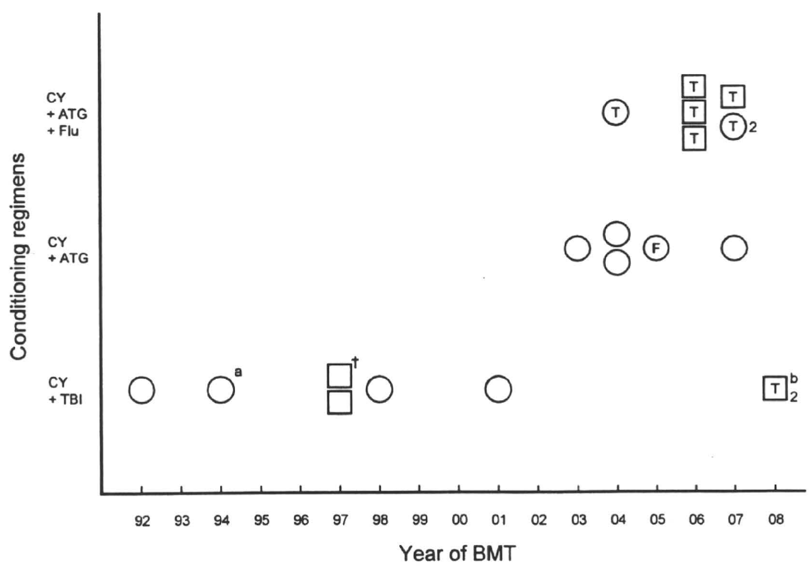

Fig. 2. Changes in donor sources, conditioning regimens, and GVHD prophylaxis. Open circle, related donor; open square, unrelated donor; F, graft failure; T, tacrolimus; 2, second transplant; †, dead patient; a, received additional ATG; b, received additional Flu + ATG.

lower, with five-yr survival rates of 56% in the largest study from Japan with 158 patients including children, in contrast to family donors, (23) and survival rates of 39% and 36% were reported for matched unrelated and mismatched unrelated donors in the Center for International Blood and Marrow Transplant Research, respectively (24). However, several recent reports have shown an improved outcome for alternative donor transplantation. In 2006, Kennedy-Nasser et al. reported that alternative donor transplantation was as effective as matched sibling donor transplantation (25). Perez-Albuerne et al. also reported that unrelated donor transplantation is an acceptable alternative for children; early referral for transplantation and identification of an HLA-matched (allele-level) donor offer the best outcome (26). Kosaka et al. reported excellent results in BMT from alternative donors as a second-line therapy after initial IST failed (11). Since 2006, we have transplanted five patients from unrelated donors (Fig. 2). Four of these five were successfully transplanted even from mismatched unrelated donors. This success is likely because of the use of Flu-based conditioning, as mentionedearlier.

The present best-available evidence strategy was acceptable, and most patients benefited from this strategy. A better understanding of the HLA combinations for GVHD or the type of amino acid substitution in HLA molecules (27) or antibody screening for HLA (28) should lead to further success in BMT from alternative mismatched donors.

Conflict of interest

The authors have no conflicts of interest to declare.

References

1. Lee SJ, Joffe S, Artz AS, et al. Individual physician practice variation in hematopoietic cell transplantation. J Clin Oncol 2008: 26: 2162–2170.
2. Kurre P, Johnson FL, Deeg HJ. Diagnosis and treatment of children with aplastic anemia. Pediatr Blood Cancer 2005: 45: 770–780.
3. Davies JK, Guinan EC. An update on the management of severe idiopathic aplastic anemia in children. Br J Haematol 2007: 136: 549–564.
4. Karnofsky DA, Burchenal JH. The clinical evaluation of chemotherapeutic agents in cancer. Evaluation of Chemotherapeutic Agents. New York: Columbia University Press, 1949.
5. Locatelli F, Bruno B, Zecca M, et al. Cyclosporin A and short-term methotrexate versus cyclosporine A as graft versus host disease prophylaxis in patients with severe aplastic anemia given allogeneic bone marrow transplantation from an HLA-identical sibling: Results of a GITMO/EBMT randomized trial. Blood 2000: 96: 1690–1697.
6. Storb R, Etzioni R, Anasetti C, et al. Cyclophosphamide combined with antithymocyte globulin in preparation for allogeneic marrow transplants in patients with aplastic anemia. Blood 1994: 84: 941–949.
7. Kojima S, Inaba J, Yoshimi A, et al. Unrelated donor marrow transplantation in children with severe aplastic anaemia using cyclophosphamide, anti-thymocyte globulin and total body irradiation. Br J Haematol 2001: 114: 706–711.
8. Barnard DR, Rogers ZR. Blood component therapy. In: Altman AJ, ed. Supportive Care of Children with Cancer, 3rd edn. Baltimore, MA: The Johns Hopkins University Press, 2004: pp. 39–57.
9. Kojima S, Horibe K, Inaba J, et al. Long-term outcome of acquired aplastic anaemia in children: Comparison between immunosuppressive therapy and bone marrow transplantation. Br J Haematol 2000: 111: 321–328.
10. Kobayashi R, Yabe H, Hara J, et al. Preceding immunosuppressive therapy with antithymocyte globulin and cyclosporin increases the incidence of graft rejection in children with aplastic anaemia who underwent allogeneic bone marrow transplantation from HLA-identical siblings. Br J Haematol 2006: 135: 693–696.
11. Kosaka Y, Yagasaki H, Sano K, et al., on behalf of the Japan Childhood Aplastic Anemia Study Group. Prospective multicenter trial comparing repeated immunosuppressive therapy with stem-cell transplantation from an alternative donor as second-line treatment for children with severe and very severe aplastic anemia. Blood 2008: 111: 1054–1059.
12. Deeg H, O'donnell M, Tolar J, et al. Optimization of conditioning for marrow transplantation from unrelated donors for patients with aplastic anemia after failure of immunosuppressive therapy. Blood 2006: 108: 1485–1491.
13. Maury S, Balère-Appert ML, Chir Z, et al., on behalf of the French Society of Bone Marrow Transplantation and Cellular Therapy (SFGM-TC). Unrelated stem cell transplantation for severe acquired aplastic anemia: Improved outcome in the era of high-resolution HLA matching between donor and recipient. Haematologica 2007: 92: 589–596.
14. Chan KW, Li CK, Worth LL, et al. A fludarabine-based conditioning regimen for severe aplastic anemia. Bone Marrow Transplant 2001: 27: 125–128.
15. Kröger N, Zabelina T, Renges H, et al. Long-term follow-up of allogeneic stem cell transplantation in patients with severe aplastic anemia after conditioning with cyclophosphamide plus antithymocyte globulin. Ann Hematol 2002: 81: 627–631.
16. Kahl C, Leisenring W, Deeg H, et al. Cyclophosphamide and antithymocyte globulin as a conditioning regimen for allogeneic marrow transplantation in patients with aplastic anemia: A long-term follow-up. Br J Haematol 2005: 130: 747–751.
17. Kang HJ, Shin HY, Choi HS, Ahn HS. Fludarabine, cyclophosphamide plus thymoglobulin conditioning regimen for unrelated bone marrow transplantation in severe aplastic anemia. Bone Marrow Transplant 2004: 34: 939–943.
18. Bacigalupo A, Locatelli F, Lanino E, et al., for the Severe Aplastic Anemia Working Party of the European Group for Blood. Fludarabine, cyclophosphamide and anti-thymocyte globulin for alternative donor transplants in acquired severe aplastic anemia: A report from the EBMT-SAA Working Party and Marrow Transplantation (SAA WP-EBMT). Bone Marrow Transplant 2005: 36: 947–950.
19. Kumar R, Prem S, Mahapatra M, et al. Fludarabine, cyclophosphamide and horse antithymocyte globulin conditioning regimen for allogeneic peripheral blood stem cell transplantation performed in non-HEPA filter rooms for multiply transfused patients with severe aplastic anemia. Bone Marrow Transplant 2006: 37: 745–749.
20. George B, Mathews V, Viswabandya A, Kavitha ML, Srivastava A, Chandy M. Fludarabine and cyclophosphamide

based reduced intensity conditioning (RIC) regimens reduce rejection and improve outcome in Indian patients undergoing allogeneic stem cell transplantation for severe aplastic anemia. Bone Marrow Transplant 2007: 40: 13–18.

21. George B, Mathews V, Viswabandya A, Kavitha ML, Srivastava A, Chandy M. Fludarabine-based reduced intensity conditioning regimens in children undergoing allogeneic stem cell transplantation for severe aplastic anemia. Pediatr Transplant 2008: 12: 14–19.
22. Urban C, Benesch M, Sykora KW, Schwinger W, Lackner H. Non-radiotherapy conditioning with stem cell transplantation from alternative donors in children with refractory severe aplastic anemia. Bone Marrow Transplant 2005: 35: 591–594.
23. Kojima S, Matsuyama T, Kato S, et al. Outcome of 154 patients with severe aplastic anemia who received transplants from unrelated donors: The Japan Marrow Donor Program. Blood 2002: 100: 799–803.
24. Passweg J, Pérez W, Eapen M, et al. Bone marrow transplants from mismatched related and unrelated donors for severe aplastic anemia. Bone Marrow Transplant 2006: 37: 641–649.
25. Kennedy-Nasser AA, Leung KS, Mahajan A, et al. Comparable outcomes of matched-related and alternative donor stem cell transplantation for pediatric severe aplastic anemia. Biol Blood Marrow Transplant 2006: 12: 1277–1284.
26. Pérez-Albuerne ED, Eapen M, Klein J, et al. Outcome of unrelated donor stem cell transplantation for children with severe aplastic anemia. Br J Haematol 2008: 141: 216–223.
27. Kawase T, Matsuo K, Kashiwase K, et al., for The Japan Marrow Donor Program. HLA mismatch combinations associated with decreased risk of relapse: Implications for the molecular mechanism. Blood 2009: 113: 2851–2858.
28. Gutman JA, Mckinney SK, Pereira S, et al. Prospective monitoring for alloimmunization in cord blood transplantation: "virtual crossmatch" can be used to demonstrate donor-directed antibodies. Transplantation 2009: 87: 415–418.