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Success of allogeneic marrow transplantation for children with severe aplastic anaemia

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SUMMARY

Allogeneic marrow transplantation offers curative therapy for children with severe aplastic anaemia (SAA). We report the outcomes of 148 children with SAA who received human leucocyte antigen (HLA)-matched related marrow grafts between 1971 and 2010. Patients were divided into 3 groups, reflecting changes in conditioning and graft-versus-host disease (GVHD) prophylaxis regimens that occurred over time. Patients in Group 1 were conditioned with cyclophosphamide (CY; 200 mg/kg) followed by "long" (102 days) methotrexate (MTX). Patients in Groups 2 and 3 received CY alone (Group 2) or combined with anti-thymocyte globulin (Group 3) followed by "short" (days 1, 3, 6, and 11) MTX and ciclosporin (until day 180). With a median follow-up of 25 years, the 5-year survivals were 66%, 95%, and 100% for Groups 1, 2, and 3, respectively (overall $p<0.0001$). The 3-year estimates of graft rejection were 22%, 32%, and 7%, respectively. The probabilities of grades III-IV acute and 2-year chronic GVHD were 15%, 0%, and 3%, and 21%, 21%, and 10%, respectively. Advances in preparative and GVHD prophylaxis regimens, and supportive care during the past 40 years have led to improved outcomes for children with SAA. These results confirm the use of allogeneic marrow transplantation for children with SAA who have HLA-matched related donors.

Keywords

Bone marrow transplantation; Paediatric aplastic anaemia; Haematopoietic cell transplantation

INTRODUCTION

Acquired severe aplastic anaemia (SAA) is a rare, potentially fatal, haematological disorder characterized by pancytopenia and bone marrow aplasia or hypoplasia (Young, 1995). Allogeneic marrow transplantation offers curative therapy for SAA patients and is the treatment of choice for younger patients with human leucocyte antigen (HLA)-matched related donors (Doney *et al*, 1997; Fouladi *et al*, 2000; Pulsipher *et al*, 2011). Over the past 4

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AUTHORSHIP AND DISCLOSURES

L.M.B designed the study, researched and analysed data, and wrote the manuscript. A.E.W, H.J.D., M.E.D.F., P.J.M., P.A.C., K.D., F.R.A. and R.S. provided interpretation and analysis of data, edited the manuscript. B.E.S. performed statistical analyses and edited the manuscript. R.S. was the principal investigator, providing support, and takes primary responsibility for the paper.

decades, progress has been made in the prevention and treatment of graft rejection and graft-versus-host disease (GVHD), based on preclinical animal studies (Santos & Owens, Jr., 1969; Storb *et al*, 1970a; Storb *et al*, 1969; Deeg *et al*, 1982; Storb *et al*, 1989; Stucki *et al*, 1998). This report describes how sequential improvements in marrow transplantation for SAA have led to significantly improved outcomes, particularly for children who have received HLA-matched related marrow grafts at our centre since 1971.

DESIGN AND METHODS

Patients

We retrospectively reviewed the clinical records of 148 paediatric patients with SAA who received HLA-matched related marrow grafts at the Fred Hutchinson Cancer Research Center (FHCRC) between May 1971 and September, 2010 (Table 1). Two patients who received marrow grafts from their identical twins were excluded. Results were analysed as of January, 2011. The median age of patients was 12.8 (range, 1.8–19) years and the median time from diagnosis was 1.2 (range, 0.0–108) months. All patients underwent chromosome fragility testing to exclude underlying inherited marrow failure disorders. Diagnoses established at referring institutions were confirmed at the FHCRC by review of outside marrow specimens and repeat marrow aspirates and biopsies. Patients or their parents signed forms approved by the FHCRC Institutional Review Board documenting informed consent to participate in the clinical trials.

Preparative regimen and postgrafting immunosuppression

For purposes of the analysis, patients were divided into 3 groups, reflecting changes in conditioning and GVHD prophylaxis regimens that occurred over time. All patients were conditioned with cyclophosphamide (CY; 50 mg/kg intravenously (IV) × 4 days). Group 1 included 98 patients (1971–1984) given CY followed by “long” methotrexate (MTX; n=95; 15 mg/m^2 day 1, 10 mg/m^2 days 3, 6, and 11, then weekly until 102 days following marrow infusion) or ciclosporin (CSP; n=3) (Storb *et al*, 1977). Group 2 included 19 patients (1981–1988) who received CY followed by “short” (15 mg/m^2 day 1, 10 mg/m^2 days 3, 6, and 11) MTX and CSP (until day +180) (Storb *et al*, 1986a). There was one additional patient included in Group 2 who received a transplant in 1995 with CY alone due to a positive skin test to antithymocyte globulin (ATG). Group 3 included 31 patients (1989–2010) who received CY and horse ATG (30 mg/kg/day × 3 days) followed by “short” MTX and CSP (Storb *et al*, 1994). From 1981–1984 patients were randomized between MTX (Group 1) and MTX/CSP (Group 2) (Storb *et al*, 1986a). MTX/CSP was shown to be superior to MTX alone, and therefore all patients received MTX/CSP from that point on. Thirty multiply-transfused patients were given donor buffy coat (non-mobilized peripheral blood leucocytes) infusions in addition to marrow as part of a prospective study aimed at reducing the incidence of graft rejection (Storb *et al*, 1982).

GVHD grading and treatment

Diagnosis and clinical grading of acute and chronic GVHD were performed according to established criteria and both complications were treated as previously described (Przepiorka *et al*, 1995; Sullivan *et al*, 1991; Koc *et al*, 2002). Patients were not evaluated for acute GVHD if they died before engraftment or for chronic GVHD if they died before day 80 after marrow transplantation.

Analyses of donor engraftment

Graft rejection was defined as either failure to reach a granulocyte count > 1 × 10^9/l for at least 3 consecutive days or by a progressive decrease in peripheral blood counts after initial

engraftment, together with recurrent marrow aplasia. In addition, the disappearance of donor haematopoietic cells and reappearance of T lymphocytes of host origin were interpreted to represent graft rejection. In patients with sex-mismatched grafts, donor chimerism was evaluated by marrow cytogenetics and, more recently, using fluorescence *in situ* hybridization (FISH). In all other patients, informative erythrocyte antigens, erythrocyte enzyme polymorphisms, and leucocyte enzyme polymorphisms served as donor and recipient markers through the 1980s. Beginning in the 1990s, variable number tandem repeat polymorphisms were used to monitor donor chimerism (Martin, 2009).

Statistical methods

Median, range, and proportions were used to summarize descriptive data, as appropriate. Overall survival was estimated by the Kaplan-Meier method with time to death as the primary outcome and censoring at last follow-up. Cumulative incidence estimates were calculated for the probabilities of acute and chronic GVHD, and rejection. Prevalence of chronic GVHD was estimated according to methods previously described (Pepe *et al*, 1991). Death was treated as a competing risk event for all outcomes (Kalbfleisch & Prentice, 1980). Comparisons among groups for time-to-event endpoints were based on likelihood ratio tests from Cox regression models. Comparisons of time to rejection among patients who rejected, and time between first and second marrow transplantation among patients receiving a second transplant for rejection were by Wilcoxon two-sample test. All reported P-values are 2-sided and were considered statistically significant when ≤ 0.05.

RESULTS

Engraftment

Overall, 30 patients (20%) rejected their first marrow graft. The 3-year estimates of graft rejection were 22% for Group 1, 32% for Group 2, and 7% for Group 3 (overall p = 0.04; Fig 1A). Of the 30 patients who rejected their first grafts, 24 received second marrow grafts (Group 1, n=17; Group 2, n=5; Group 3, n=2) and 3 received third marrow grafts (Group 1, n=1; Group 2, n=1; Group 3; n=1) due to rejections of first and then second grafts, respectively. No graft rejections have occurred in patients who had marrow transplantation after 1993 and no deaths have occurred due to graft rejection since 1987.

The median number of days to first graft rejection was 35 (range, 18–588) for Group 1 and 210 (range, 51–414) days for Groups 2 and 3 (P = 0.001). As a result, the time between first and second marrow transplantation was longer for patients in Groups 2 and 3 (median days to second marrow transplantation = 237) compared to Group 1 (median days to second marrow transplantation = 39 days; P = 0.004). Of note, three patients experienced pancytopenia at 25, 11, and 14 years following marrow transplantation. All had donor engraftment. The aetiology of the pancytopenia was unknown in two patients. One patient died of hepatitis C-related hepatocellular carcinoma with continued pancytopenia and the other patient was given a course of immunosuppression with resolution of the cytopenia. The third patient had recurrence of paroxysmal nocturnal haemoglobinuria (PNH) and received a second transplant following nonmyeloablative conditioning with resolution of the PNH.

GVHD

The estimated probabilities of Grade II acute GVHD were 6%, 11%, and 36% for Groups 1, 2, and 3, respectively (overall P = 0.002) and the estimated probabilities of Grades III-IV acute GVHD were 15%, 0%, and 3 % for Groups 1, 2, and 3, respectively (overall P = 0.01; Fig 2A–B). The median onset of grades II–IV acute GVHD was day 24 (range, 8–64) in Group 1 and day 29 (range, 24–56) in Group 3. Two patients in Group 2 developed grade II–

IV acute GVHD at day 10 and 18 following marrow transplantation. The 2-year estimates of extensive chronic GVHD were 21%, 21%, and 10% for Groups 1, 2, and 3, respectively (overall P = 0.14; Fig 2C). The median onset of extensive chronic GVHD was day 146 (range, 84–421) in Group 1 and day 245 (range, 88–335) in Group 2. Three patients in Group 3 were diagnosed with extensive chronic GVHD at days 83, 89, and 91, respectively, following marrow transplantation. The prevalence curves (Fig 3A–C) describe extensive chronic GVHD onset, its resolution, and eventual discontinuation of all immunosuppressive therapy, using previously described methods (Pepe *et al*, 1991). Of the 30 patients with donor buffy coat infusions following bone marrow transplantation, 27 were in Group 1. Within Group 1, the cumulative incidence of chronic GVHD was 14% without donor buffy coat and 41% with donor buffy coat (p=0.01).

Malignancy after transplantation

Twelve of the 148 patients (8%) developed cancer after marrow transplantation and all were in Group 1 (Fig 4). Specifically, 5 patients developed skin cancer (squamous cell (n=4), squamous cell and basal cell (n=1) carcinoma) at 8, 15, 20, 26, and 31 years after marrow transplantation. All 5 of the patients who developed skin cancer had a history of chronic skin GVHD. In addition, 2 patients developed leukaemia of host origin [acute lymphoblastic leukaemia (ALL; n=1), acute myeloid leukaemia (AML; n=1)] 6 months after marrow transplantation, and one patient developed myelodysplastic syndrome (MDS) of donor origin 27 years after marrow transplantation. The patient who developed MDS had remission induced after second haematopoietic cell transplantation. Four patients developed solid tumours [cervical (n=1), breast (n=2), hepatitis C-associated hepatocellular carcinoma (n=1)] 17, 27, 30, and 32 years after marrow transplantation.

Overall survival

With a median follow up of 25.3 (range, 0.3–37.2) years for living patients, the 5-year survival estimates were 66% for Group 1, 95% for Group 2, and 100% for Group 3 (overall P < 0.0001; Fig 5). The primary causes of death were graft rejection (n=18; 12%) and infections with and without GVHD (n=17; 11%). Importantly, the 5-year survival after graft rejection has improved from 24% to 100% following second marrow transplantation (overall P = 0.0001; Fig 1B). In addition, death due to infections with or without GVHD has decreased over the past 40 years with all of the deaths due to infections with or without GVHD occurring in Group 1. Five patients (3%) died of cancer [(ALL (n=1), metastatic squamous cell carcinoma (n=1), metastatic cervical cancer (n=1), metastatic breast cancer (n=1), and hepatitis C associated hepatocellular carcinoma (n=1)]. Four other patients died of human immunodeficiency virus (HIV)/hepatic failure (n=1), idiopathic interstitial pneumonitis (n=1), suicide (n=1), and an unknown cause (n=1). One patient rejected the graft and developed acute myeloid leukaemia 6 months after graft rejection. This patient was counted as a death due to graft rejection.

Twenty-three patients acquired hepatitis C, presumably related to blood product transfusions before or after marrow transplantation [Group 1 (n=15), Group 2 (n=7), and Group 3 (n=1)] (Strasser *et al*, 1999). In addition, 1 patient had hepatitis B (Group 1) and one patient had HIV (Group 1). Two of the 25 patients with viral infections died as a result [Hepatitis C (n=1), HIV n=1)]. All patients with hepatitis B, C, or HIV were transplanted before 1990.

DISCUSSION

SAA is a life-threatening bone marrow failure disorder characterized by a hypocellular marrow and pancytopenia. Allogeneic marrow transplantation using HLA-matched related donors provides curative therapy for patients with SAA and is the preferred therapy for

paediatric patients (Kobayashi *et al*, 2006; Locasciulli *et al*, 2007). Long-term survivals of approximately 80–90% following marrow transplantation have been reported in several studies that included children (Schrezenmeier *et al*, 2007; Locasciulli *et al*, 2007; Kennedy-Nasser *et al*, 2006; Pulsipher *et al*, 2011). Here we report a single centre series of 148 children with SAA who received HLA-matched related marrow grafts over the past 4 decades.

In the current paediatric cohort, 5-year survival has significantly improved, from 66% to 100%, during the past 4 decades. One important factor contributing to the improvement in survival was the understanding and abrogation of marrow graft rejection. Patients in Group 1 had the lowest survival rate due to a high incidence of graft rejection and an inability to deal with rejections effectively. Preclinical canine studies and clinical observations suggested that rejections were largely due to transfusion-induced sensitization to minor histocompatibility antigens (Storb *et al*, 1970b; Storb *et al*, 1977). Rejection rates were lower in patients who had not received transfusions before marrow transplantation (Storb *et al*, 1980). Subsequent experimental studies showed that the risk of sensitization to minor antigens was reduced by leucocyte depletion and by *in vitro* irradiation (2000 cGy) of the transfusion products (Bean *et al*, 1991). These methods have now become standard clinical practice in the treatment of newly diagnosed SAA and probably contributed to the lower rejection risk in more recently transplanted patients. At the same time, preclinical studies led to the inclusion of ATG in the conditioning regimen, as a means to eliminate T cells in the recipient responsible for graft rejection. ATG plus CY was first shown to facilitate second marrow grafts after initial graft rejection (Storb *et al*, 1987). As a consequence, the overall mortality associated with graft rejection has been eliminated over the past 40 years due to the success of second marrow transplantation. This is highlighted by the fact that there have been no patient deaths due to graft rejection since 1987.

The encouraging results with second marrow transplantation prompted the introduction of CY/ATG as a conditioning regimen for initial marrow transplantation beginning in 1988 (Group 3) (Storb *et al*, 1994; Storb *et al*, 2001). With this change, rejection declined from 32% to 7% (p=0.05). It is likely; however, that increasing use of leuco-depleted irradiated blood products before marrow transplantation contributed to the decreased rejection rate. A randomized study comparing CY to CY/ATG conditioning in 134 paediatric and adult patients, conducted at 29 centres during a 7-year period did not show outcome differences between the two study arms and was prematurely closed because of slow accrual. Rejection rates in both arms were 18% and 16%, and 5-year survivals were 74% and 80%, respectively (Champlin *et al*, 2007).

Prevention of acute GVHD is a second area of progress. In the early 1970s, “long” MTX (102 days) was the only regimen used for GHVD prevention based on preclinical canine studies. CSP monotherapy was then introduced in the late 1970s but was shown convincingly in prospective randomized trials to be no better than MTX (Storb *et al*, 1988). However, canine studies showed that a short course of MTX combined with CSP was superior to either drug alone (Deeg *et al*, 1982), and these preclinical results were confirmed in subsequent clinical trials (Storb *et al*, 1986a; Storb *et al*, 1986b). Accordingly, the rate of grades III–IV acute GVHD declined from 15% with MTX to 3% grade III (no grade IV) with MTX/CSP. These observations have been confirmed by others (Locatelli *et al*, 2000). The increase in grade II acute GVHD among patients in Group 3 was primarily due to an increased diagnostic sensitivity for GVHD of the upper intestinal tract, reflecting the aggressive use of endoscopy for evaluation of gut symptoms at our centre, as previously reported (Martin *et al*, 2004). Our study supports this finding. Of the 31 patients in Group 3, 11 were diagnosed with grade II acute GVHD. Of these 11 patients, 7 had grade II GVHD that was endoscopy-proven stage 1 gut GVHD. In the remaining 4 patients, the incidence of

grade II acute GVHD was 13% compared to 6% in Group 1 and 11% in Group 2, which was not significantly different. Therefore, it is possible that the incidence of grade II acute GVHD was underestimated in patients transplanted in Groups 1 and 2.

The incidence rates of chronic GVHD have remained low throughout the three time periods except for a period during the late 1970s and early 1980s when transfused patients received combined marrow and buffy coat grafts (Storb *et al*, 1982). Combined grafts were administered to overcome the problem of graft rejection. While the manoeuvre was effective in decreasing graft rejection, it was associated with higher incidences of chronic GVHD (Storb *et al*, 1983). Introduction of the CY/ATG regimen permitted the return to marrow as the sole source of stem cells, and the cumulative incidence of chronic GVHD since 1988 has decreased to 10%. A recent combined analysis of adult and paediatric patients with SAA at our centre suggested that limiting the graft to 2.0 to 2.5 × 10^8 total nucleated cells (corrected for donor's peripheral blood leucocyte count)/kg of recipient's actual weight could reduce the incidence of chronic GVHD further without increasing the rejection risk (Kahl *et al*, 2005). A recent retrospective analysis of combined European Group for Blood and Marrow Transplantation (EBMT)/ Center for International Blood & Marrow Transplant Research (CIBMTR) data emphasized that outcomes were worse and the risk of chronic GVHD was higher with peripheral blood progenitor grafts compared to marrow, especially in patients who were younger than 20 years (Schrezenmeier *et al*, 2007).

A third area of major progress has been advances in the management of infectious diseases after transplantation (Goodman *et al*, 1992; Boeckh *et al*, 1996; Limaye *et al*, 2001). In particular, measures to prevent infection included the use of trimethoprim-sulfamethoxazole or other prophylaxis regimens for prevention of *Pneumocystis jiroveci* pneumonia, prophylactic fluconazole for prevention of yeast infections, and acyclovir for prevention of herpes simplex virus and varicella reactivation. In addition, preemptive treatment with ganciclovir or foscarnet was used if cytomegalovirus was detected.

Several studies have evaluated the effectiveness of non-transplant alternative therapies for patients with SAA with immune suppression alone and reported overall survivals of 60–80% (Scheinberg *et al*, 2008; Bacigalupo *et al*, 2000; Rosenfeld *et al*, 1995; Pulsipher *et al*, 2011). CSP and ATG are most commonly used and are currently the treatment of choice for patients who lack HLA-matched related donors. Initial response rates to immunosuppressive therapy range from 60 to 75%, but 10–35% will fail to respond or relapse (Scheinberg *et al*, 2008; Pulsipher *et al*, 2011). Attempts to improve initial response rates by increasing immune suppression with the addition of mycophenolate mofetil or sirolimus to ATG/CSP have not had a major impact (Scheinberg *et al*, 2006). A recent EBMT study compared the outcomes of 2479 patients with SAA who received first-line treatment with marrow transplantation (n=1567) or immunosuppressive therapy (n=912) (Locasciulli *et al*, 2007). Survival was significantly better in patients who received marrow transplantation and has improved over time, similar to findings at our centre. Survival for patients receiving immunosuppressive therapy has also improved over the past 2 decades, albeit not to the same extent. Of note, however, recent studies indicate that determination of pre-treatment telomere length and reticulocyte counts identifies patients who are less likely to respond to immunosuppressive therapy and, therefore, should be considered for transplantation early in the disease course (Scheinberg *et al*, 2010; Scheinberg *et al*, 2009a). Immunosuppressive therapy also carries the risk of clonal evolution to PNH, MDS, or AML (Scheinberg *et al*, 2008; Scheinberg *et al*, 2009b). In our cohort of 148 children, median follow-up 25 years, 12 patients (8%) developed malignancies following transplantation. Four of the 12 patients had solid tumours (breast cancer (n=2), cervical cancer, or hepatitis C-associated hepatocellular carcinoma), which may or may not have been late effects from the transplantation procedure. In addition, in the patient who developed AML, retrospective analysis revealed

abnormal cytogenetics on the patient's pre-transplant marrow identical to those identified at the time of AML diagnosis, suggesting that this patient presumably had MDS rather than acquired SAA at the time of transplantation (Appelbaum *et al*, 1984). A detailed discussion of late effects and quality of life in patients with SAA was reviewed by Sanders *et al*, (2011). In general, patients with SAA have normal growth, development, and quality of life.

In summary, this study demonstrated significant improvement in survival over the past 40 years among children with SAA who received HLA-matched related marrow grafts. The reasons for this improvement include decreased incidences of graft rejection and improved survivals after second marrow transplantation for graft rejection, decreased rates of grades III–IV acute GVHD, a low incidence of chronic GVHD with marrow grafts, and improved supportive care. This study confirms the efficacy of allogeneic marrow transplantation as primary therapy for children with SAA who have HLA-matched related donors.

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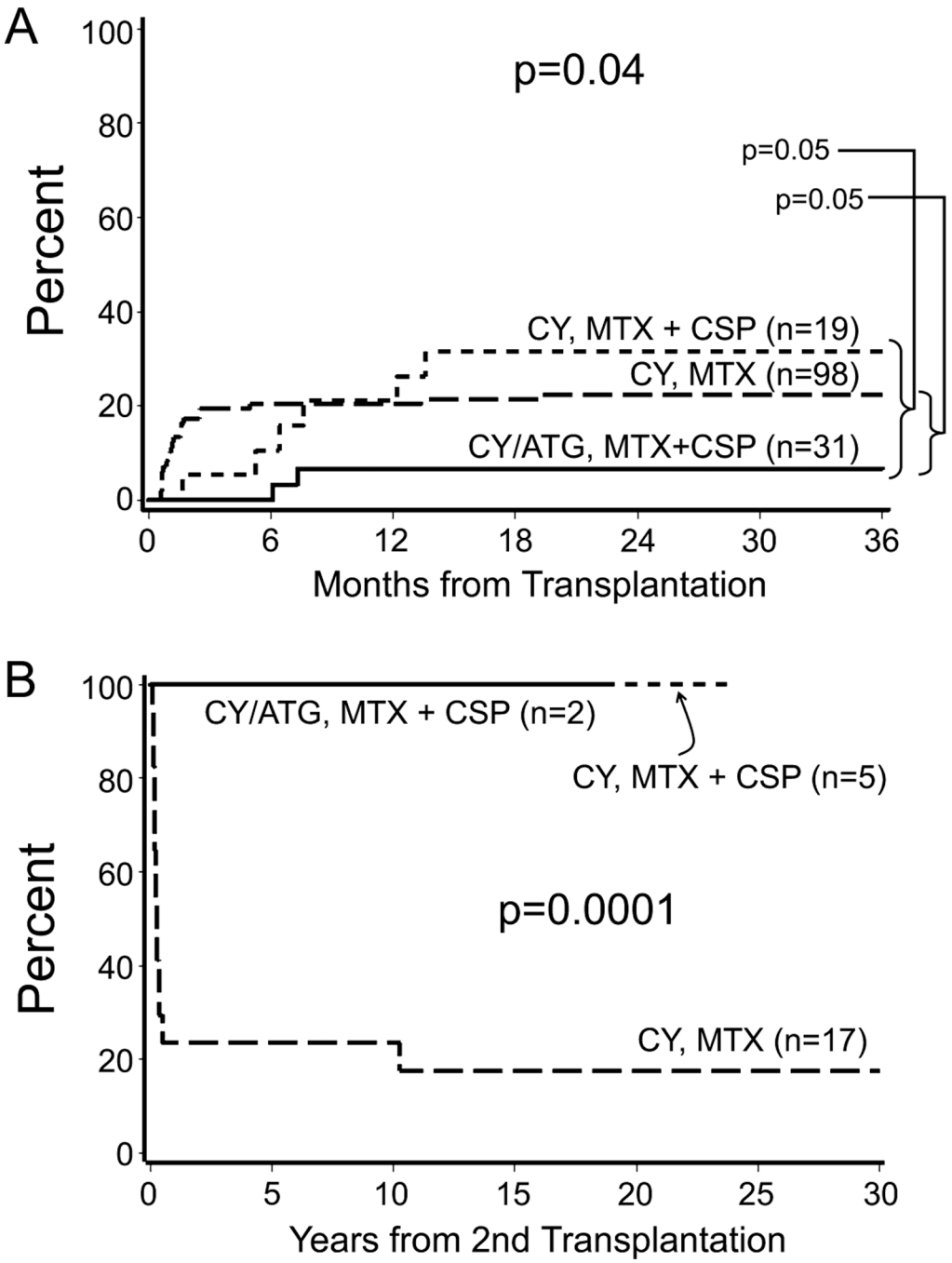

Fig 1.

Incidence of (**A**) graft rejection according to conditioning regimen and graft-versus-host disease (GVHD) prophylaxis over time in 148 children with severe aplastic anaemia (SAA) and (**B**) survival following second marrow transplantation for graft rejection according to the conditioning regimen and GVHD prophylaxis over time in 24 patients with SAA. ATG=anti-thymocyte globulin; CSP=ciclosporin; CY = cyclophosphamide; MTX=methotrexate.

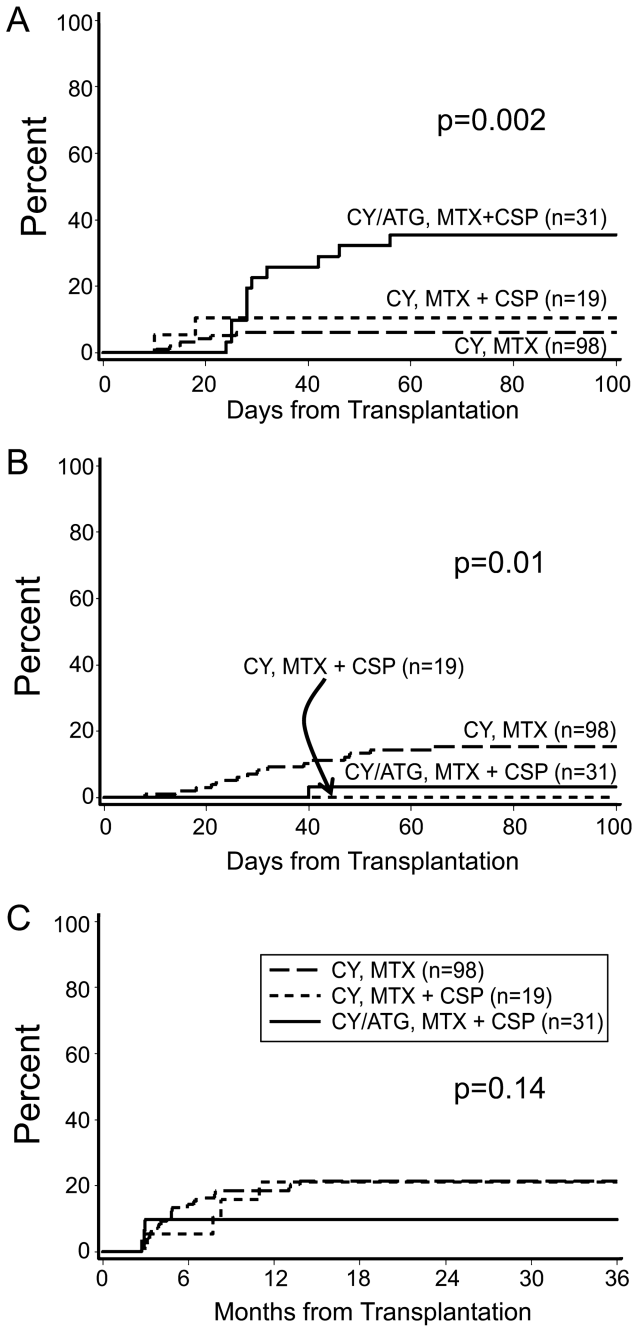

Fig 2.

Cumulative incidence of (**A**) Grade II acute graft-versus-host disease (GVHD) (**B**) Grade III–IV acute GVHD (**C**) and chronic GVHD in 148 children with severe aplastic anaemia. ATG=anti-thymocyte globulin; CSP=ciclosporin; CY = cyclophosphamide; MTX=methotrexate.

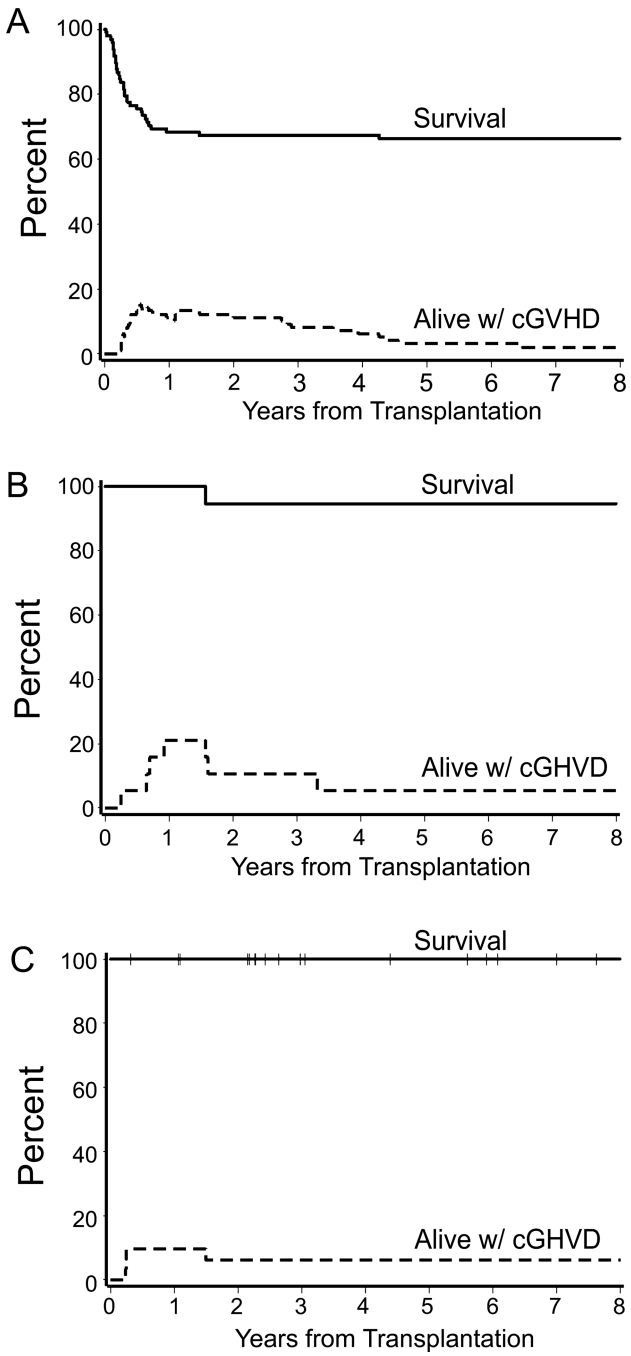

Fig 3.

Survival (upper curves) and prevalence of chronic graft-versus-host disease (lower curves) among patients in (**A**) Group 1 (CY, MTX n=98) (**B**) Group 2 (CY, MTX + CSP n= 19) and (**C**) Group 3 (CY/ATG, MTX + CSP n=31) in 148 children with severe aplastic anaemia. ATG=anti-thymocyte globulin; CSP=ciclosporin; CY = cyclophosphamide; MTX=methotrexate.

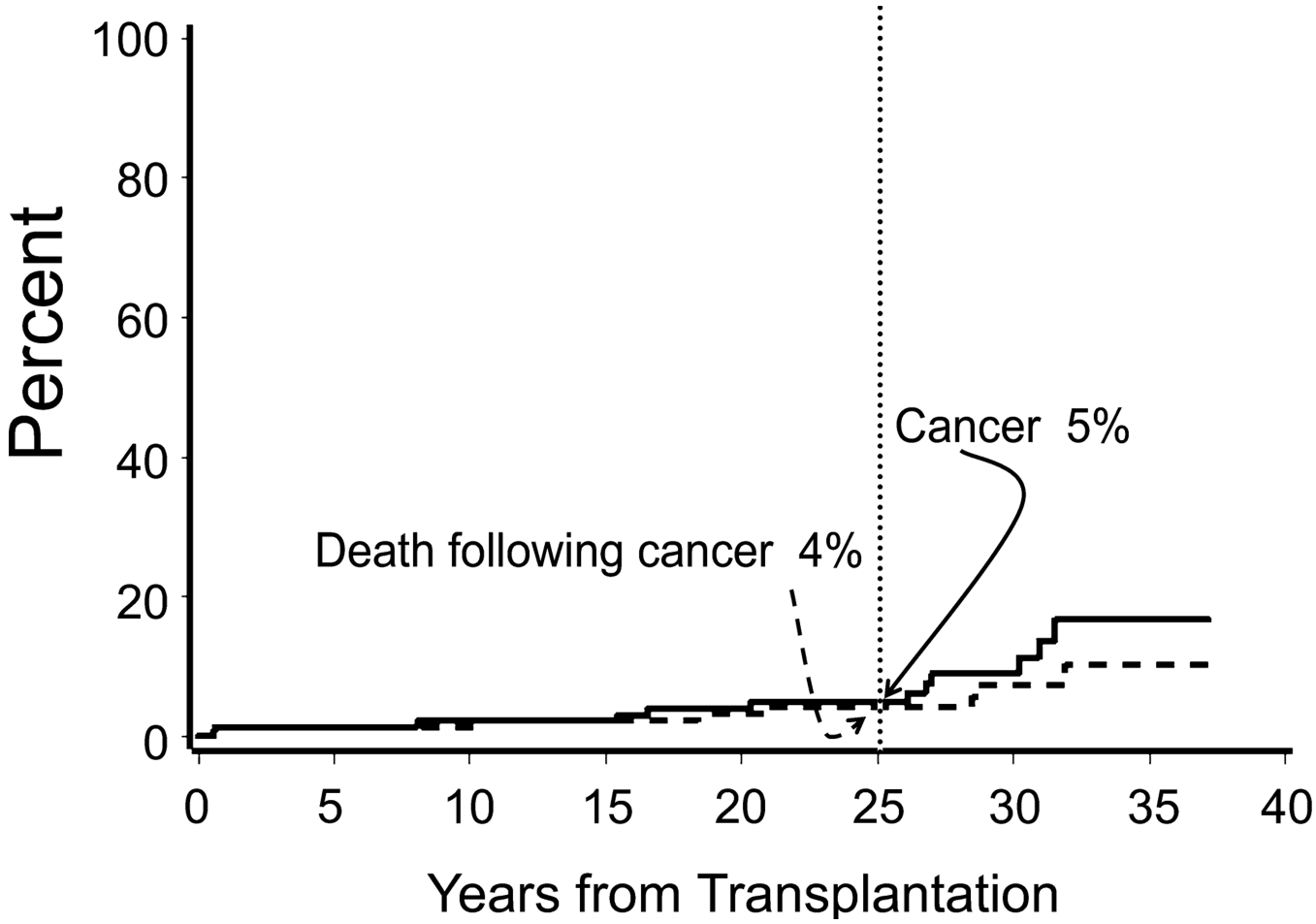

Fig 4.
Cumulative incidence of post-transplant cancer and cancer-related mortality in 148 children with severe aplastic anaemia.

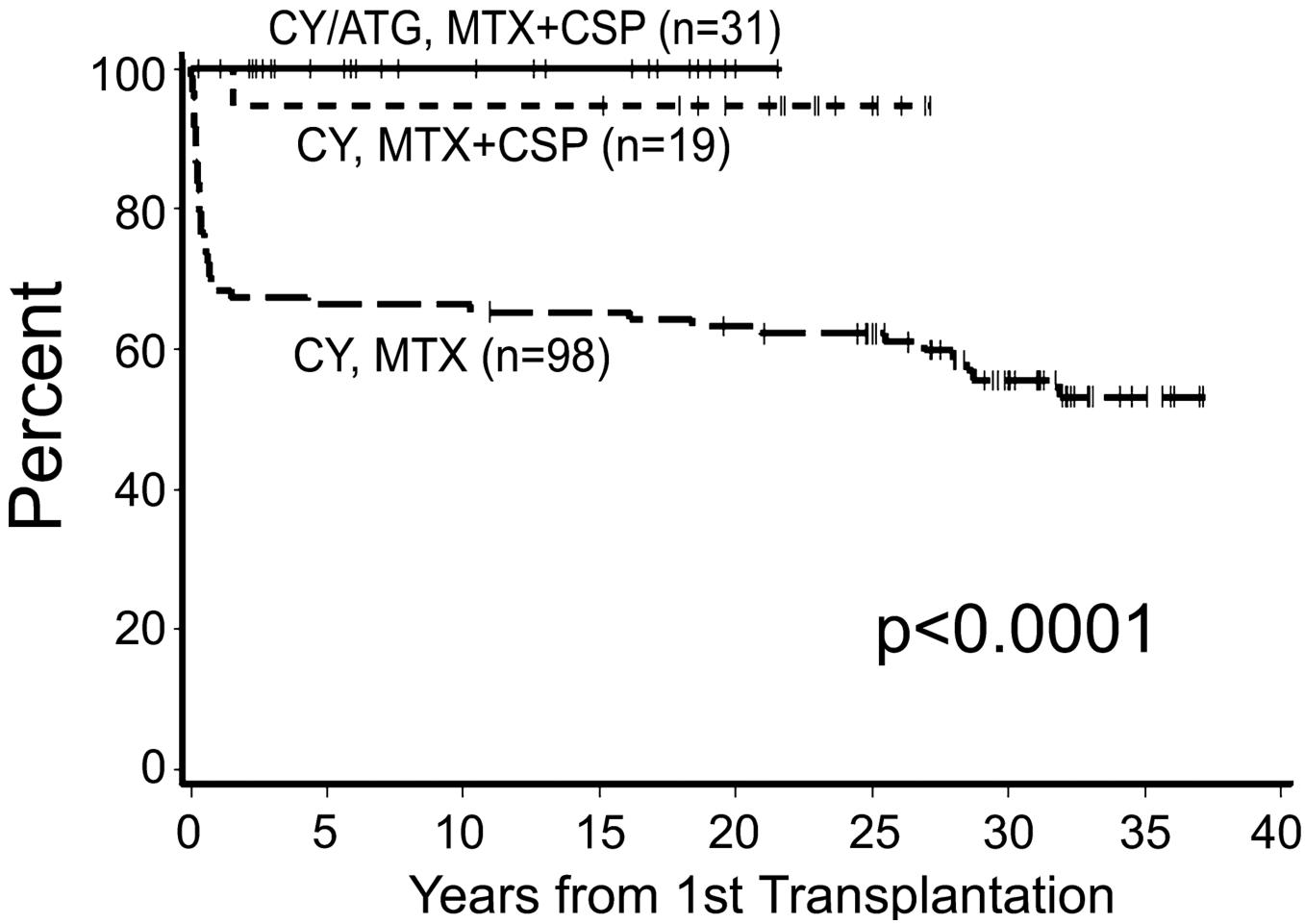

Fig 5.
Overall survival according to the conditioning regimen and graft-versus-host disease prophylaxis used in 148 children with severe aplastic anaemia.
ATG=anti-thymocyte globulin; CSP=ciclosporin; CY = cyclophosphamide; MTX=methotrexate.

Table 1

Characteristics (n=148)

Group denomination, number of patients	Group 1 (n=98)	Group 2 (n=19)	Group 3 (n=31)
Years of transplantation	1971–1984	1981–1988 *	1989–2010
Age, years			
Median (range)	13.1 (1.9–19)	12.5 (1.8–18.8)	10.9 (2.0–18.3)
Gender, n (%)			
Male	56 (57%)	8 (42%)	20 (65%)
Female	42 (43%)	11 (58%)	11 (35%)
Aetiology of aplastic anaemia, (n)			
Unknown	81	19	26
Hepatitis	7	0	5
Drugs/chemicals	8	0	0
Paroxysmal nocturnal haemoglobinuria	2	0	0
Number of patients who received transfusions before marrow transplantation, n (%)			
Red blood cell	75 (77%)	15 (79%)	28 (90%)
Platelet	71 (72%)	14 (74%)	28 (90%)
Number of patients who received treatment before marrow transplantation, n (%)			
Androgens	35 (36%)	3 (16%)	0 (0%)
Steroids	55 (56%)	7 (37%)	6 (19%)
ATG or other agent	0 (0%)	1 (5%)	3 (10%)
Months from diagnosis to marrow transplantation			
Median, (range)	1 (0–108)	1.4 (0.3–5.8)	1.2 (0.4–24.0)
Preparative regimen	CY	CY	CY/ATG
GVHD prophylaxis	MTX (n=95) CSP (n=3)	MTX/CSP (n=19)	MTX/CSP (n=31)
Nucleated marrow cell dose ($\times 10^8$/kg)			
Median, (range)	3.7 (0.6–15.5)	2.7 (0.7–9.6)	2.8 (0.9–6.2)
Follow-up living patients, years			
Median, (range)	31.1 (11–37.2)	23 (15.2–27.1)	6.1 (0.3–21.5)

ATG=anti-thymocyte globulin; CSP=ciclosporin; CY = cyclophosphamide, GVHD=graft versus host disease; kg=kilogram; MTX=methotrexate; n=number

* There was one patient included in group 2 who received a transplant in 1995 following conditioning with CY and CSP/MTX for GVHD prevention. This patient had a positive skin test to ATG and was therefore unable to receive ATG.