Long-term survival after unrelated donor marrow transplantation for aplastic anaemia after optimized conditioning regimen: a retrospective multicentre cohort study

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Summary

Background Almost all acquired severe aplastic anaemia is immune mediated and characterised by hypocellular bone marrow and ≥2 affected haematopoietic lineages. The optimal preparartive regimen for unrelated donor transplantation remains to be established. We aimed to study long-term outcomes after unrelated donor transplantation for severe aplastic anaemia with de-escalation of cyclophosphamide (Cy) dose in steps of 50 mg/kg (150, 100, 50, 0 mg/kg) in combination with total body irradiation (TBI) 2 Gy, anti-thymocyte globulin (ATG) and fludarabine.

Methods Ninety-six patients with severe aplastic anaemia aged ≤65 years with adequate organ function enrolled on a trial of human leukocyte antigen (HLA)-matched or 1 HLA-locus mismatched unrelated donor marrow transplantation conducted between 02/2006 and 12/2013 in the United States (NCT00326417). Exclusion criteria were Karnofsky performance status of less than 60, clonal cytogenetic abnormalities and inherited marrow failure syndormes. The primary outcome was day-100 engraftment (achievement of absolute neutrophil recovery to at least 0.5×10^9/L without subsequent decline) and day-100 survival. The trial determined the lowest effective Cy dose as 50 mg/kg (n = 38) for day-100 engraftment and survival. Cy dose 100 mg/kg (n = 41) was also acceptable. Accrual to Cy doses 150 mg/kg (n = 15) and 0 mg/kg (n = 3) was terminated early for toxicities. The current study is an extended follow up of patients enrolled on the trial (NCT00326477) and includes 76 of 96 patients alive ≥1 year after transplantation. There were 20 deaths in the first year after transplantation (Cy 0 mg/kg [n = 2], Cy 50 mg/kg [n = 1], Cy 100 mg/kg [n = 10], Cy 150 mg/kg [n = 7]). Patients were followed prospectively from transplantation and data reported using standardized data collection forms until death, loss to follow up or last contact through November 2023. The incidence of graft failure was calculated using the cumulative incidence estimator and the probability of survival using the Kaplan–Meier estimator.

Findings The median follow up of the cohort is 8.02 (IQR) 5.16–10.12) years. With Cy 50 mg/kg, there was one graft failure and five deaths ≥1 year after transplantation. With Cy 100 mg/kg there was only one late death and no graft failure. The 8-year probabilities of survival were 85.0% (95% CI 67.3–93.5) and 75.6% (95% CI 59.4–86.1) after Cy 50 mg/kg and 100 mg/kg, respectively, P = 0.31. With Cy 0 mg/kg and 150 mg/kg, there were no graft failures or death ≥1 year after transplantation. Regardless of Cy dose 12 of 15 patients aged ≥50 years died.

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Interpretation Cy 50 mg/kg or 100 mg/kg with TBI 2 Gy, ATG and fludarabine are effective conditioning regimens for unrelated donor marrow transplants for aplastic anaemia. Identification of an optimized transplantation approach for patients aged ≥50 years is needed.

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Keywords: Severe aplastic anaemia; Haematopoietic cell transplant; Survival; Graft failure

Research in context

Evidence before this study

There is agreement the regimen for unrelated donor marrow transplantation should be based on a combination of fludarabine and cyclophosphamide (Cy) probably with low dose total body irradiation (TBI) and anti-thymocyte globulin (ATG) to reduce the risk for graft failure, chronic graft-versus-host disease and death. We searched PubMed for articles on clinical trials for unrelated donor marrow transplantation for severe aplastic anaemia containing long-term clinical efficacy and safety data between January 2016 and December 2023 and found none. A trial of unrelated donor marrow transplantation for severe aplastic anaemia in the United States was conducted between 2006 and 2013 (NCT00326417). This trial concluded Cy dose 50 mg/kg was the most desirable day-100 engraftment and survival although Cy 100 mg/kg was also effective.

Added value of this study

Follow up of patients enrolled on a trial (NCT00326417) beyond the frist year after transplantation allowed us to examine whether engraftment could be sustained beyond the first year post-transplant period, and report on late organ toxicities and survival after lowering the Cy dose to 50 and 100 mg/kg. Knowledge of long-term outcomes are relevant for counseling patients and for decision-making.

Implications of all the available evidence

With a median follow up of 8 years, we confirmed sustained engraftment and excellent survival ≥75% after unrelated donor marrow transplantation with Cy doses 50 mg/kg and 100 mg/kg with TBI (2 Gy), ATG and fludarabine. This supports our hypothesis that the *in vitro* synergism between Cy and fludarabine allowed for reductions in Cy dose.

Introduction

The conventional treatment algorithm for acquired severe aplastic anaemia prioritizes transplantation of marrow from an human leukocyte antigen (HLA)-matched sibling for patients aged ≤40 years.[1] Those without HLA-matched siblings are treated with immunosuppressive therapy (IST), a combination of equine-derived anti-thymocyte globulin (ATG), cyclosporine and eltrombopag. The 4-year disease recurrence rate after IST is in the range of 40%.[2,3] Recurrence typically occurs within six months of starting IST when eltrombopag is discontinued and when the dose of cyclosporine is reduced in responders.[3] For patients who fail IST and do not have an HLA-matched sibling, unrelated donor marrow transplantation is an option.[4–6] In a trial of unrelated donor marrow transplants in the United States between 1994 and 2004 that de-escalated the dose of total body irradiation (TBI) in combination with cyclophosphamide (Cy 200 mg/kg) and equine-ATG (90 mg/kg), the optimal minimum TBI dose was 2 Gy.[4] With a median follow up of seven years, 61% of patients who received HLA-matched and 40% of patients who received HLA-mismatched unrelated marrow transplants were alive.[4] Graft failure occurred in 5% of patients. The most common toxicity (grades 3 or higher) affected the lungs (diffuse alveolar injury) and generally occurred about three weeks after transplantation.[4] In an effort to optimize engraftment and survival and minimize toxicity, a follow-up trial of unrelated donor marrow transplantation was carried out between 2006 and 2013 in the United States (NCT00326417). This trial sought to de-escalate the Cy dose in steps of 50 mg/kg (150, 100, 50, 0 mg/kg), given in combination with TBI 2 Gy, ATG (9 mg/kg [rabbit-derived] or 90 mg/kg [equine-derived]) and fludarabine (120 mg/kg).[7,8] Fludarabine having been added as a less toxic approach to preserve immunosuppression in the setting of Cy dose de-escalation. The trial (NCT00326417) identified a Cy dose of 50 mg/kg as the most desirable dose for day 100 engraftment and survival, although a Cy dose of 100 mg/kg also resulted in excellent short-term survival.[7] In the Cy 50 mg/kg and 100 mg/kg cohorts the likelihood of being engrafted and alive at day 100 was 92% (35 of 38) and 85% (35 of 41), respectively.[7] At the time of our initlal report,[7] the median follow-up of the Cy 50 mg/kg cohort was 17 months (IQR 12–24) and that of the Cy 100 mg/kg cohort, 24 months (IQR 24–25). The corresponding 1-year probabilities of survival were 97.4% (95% CI 83.8–99.6) and 80.5% (95% CI 64.8–89.7), respectively.[7] The most common

early toxicity was pulmonary (grade 3 or 4 dyspnea or hypoxia requiring mechanical ventilation).[7] A Cy dose of 150 mg/kg resulted in excess toxicities with seven deaths in fourteen patients, and Cy 0 mg/kg resulted in graft failure in all three patients enrolled at this dose level.[8] The aim of the current study was to describe long-term outcomes of patients in the trial of unrelated donor transplantation with de-escalation of Cy dose (NCT00326417).[7,8] Pateints were followed for a median of 8.02 (interquartile range [IQR] 5.16–10.12) years from transplantation, using data reported to an observational registry, the Centre for International Blood and Marrow Transplant Research (CIBMTR).

Methods

Study design and participants

Between 02/2006 and 12/2013 the primary trial (NCT00326417) enrolled 97 patients with severe aplastic anaemia. Patients were eligible for the primary trial if they were aged ≤65 years with severe aplastic anaemia (bone marrow cellularity less than 25% and ≥2 haematopoietic lineages are affected [absolute reticulocyte count <60 × 10^9/L, absolute neutrophil count (ANC) < 0.5 × 10^9/L, platelets <20 × 10^9/L]), had adequate organ function and an unrelated donor HLA matched at the allele level for HLA-A, B, C and DRB1 or mismatched at 1 HLA-locus.[7,8] Exclusion criteria were Karnofsky performance status of less than 60, clonal cytogenetic abnormalities, Fanconi anaemia or other inherited marrow failure syndromes. One patient withdrew consent and was excluded. All remaining patients (n = 96) received TBI (2 Gy, single fraction), ATG (rabbit-derived 9 mg/kg or equine-derived 90 mg/kg), fludarabine (120 mg/m^2) and Cy. The Cy dose started at 150 mg/kg and was de-escalated in steps of 50 mg/kg (to 100 mg/kg, 50 mg/kg and 0 mg/kg). All patients received bone marrow as a source of stem cells. Graft versus host disease (GVHD) prophylaxis consisted of a calcineurin inhibitor (cyclosporine or tacrolimus) and methotrexate. Seventy-six of 96 patients were alive ≥1 year after transplantation, representing the population of interest in the current analysis. There were 20 deaths in the first year after transplantation (Cy 0 mg/kg [n = 2], Cy 50 mg/kg [n = 1], Cy 100 mg/kg [n = 10], Cy 150 mg/kg [n = 7]).

Proceedures

Patients were followed prospectively from transplantation and data reported using standardized data collection forms until death, loss to follow up or last contact through November 2023. Data were abstracted from standardized data collection forms available at www.cibmtr.org. Follow-up information beyond one year was available through the CIBMTR. The CIBMTR collects patient-level data from U.S. transplant centres annually for five years and then biannually for as long as the centre maintains contact with the patient.

Ethics

Written informed consent for the trial and reporting to the CIBMTR was obtained by respective transplant centres. The Institutional Review Board of the National Marrow Donor Program approved this study.

Statistics

The primary outcomes of interest were graft failure and survival. Secondary outcomes of interest were chronic GVHD and organ function. Graft failure was defined as failure to achieve an ANC ≥0.5 × 10^9/L, or a sustained decrease below that level after having achieved ANC ≥0.5 × 10^9/L, or a myeloid donor chimerism ≤5% or poor graft function leading to a second transplant.[9] Chronic GVHD was diagnosed according to previously described criteria.[10] Late organ (cardiac, pulmonary, hepatic and renal) complications were abstracted from standardized data collection forms.

The incidences of graft failure and chronic GVHD were calculated using the cumulative incidence estimator to accommodate competing risk of death.[11] Probability of overall survival was calculated using the Kaplan–Meier estimator.[12] 95% confidence intervals (CI) were calculated using the Greenwood formula. Comparison of probability estimates was performed using Gray's test for cumulative incidence of graft failure and the log-rank test for overall survival. P-values are two-sided. Continuous variables are shown as median with interquartile range (IQR). Recipients of second transplants were censored at the date of second transplantation for all endpoints except survival. Surviving patients were censored at last follow-up. Late organ complications are described. Analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC).

Role of the funding source

The funder of the study had no role in study design, in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the manuscript for publication.

Results

Patient and transplant characteristics

Between 02/2006 and 12/2013 the primary trial (NCT00326417) enrolled 97 patients with severe aplastic anaemia. One patient withdrew consent and was excluded. Table 1 shows the characteristics of 96 patients enrolled on the primary trial by Cy dose received. Cy dosing started at 150 mg/kg (n = 14) and was de-escalated in steps of 50 mg/kg to 100 mg/kg (n = 41), 50 mg/kg (n = 38) and 0 mg/kg (n = 3). The median age at transplantation of patients who received Cy doses of 0 mg/kg, 50 mg/kg, 100 mg/kg or 150 mg/kg were 22 years, 25 years, 18 years and 18 years, respectively. One of three patients who received a Cy dose of 0 mg/kg and three of thirteen patients who received Cy at 150 mg/kg

Characteristics	Cy 0 mg/kg	Cy 50 mg/kg	Cy 100 mg/kg	Cy 150 mg/kg
Number	3	38	41	14
Age, years				
Median (IQR)	22 (17–61)	25 (17–39)	18 (11–25)	18 (15–23)
<18 years	1	11	21	7
18–40 years	1	18	14	4
>40 years	1	9	6	3
Sex				
Male	1	19	21	5
Female	2	19	20	9
Race				
Asian	2	1	2	1
African American	_	3	2	2
Caucasian	1	30	34	11
Other	__	4	3	__
IST before transplant				
Yes	3	35	41	13
No	0	3	0	1
Donor-recipient HLA-match				
Matched A, B, C, DRB1	2	31	27	10
Single locus mismatch	1	7	14	4
Time, diagnosis to BMT, median, (IQR) years	1.42 (0.58–5.17)	0.68 (0.40–1.24)	0.87 (0.72–1.38)	1.50 (0.58–1.67)
Total infused nucleated cell dose, median (IQR) x 10^8/kg	1.08 (0.03–2.35)	2.89 (1.63–4.15)	3.08 (1.84–4.79)	2.31 (1.98–3.60)
Acute grade II-IV GVHD				
Yes	__	13	12	3
No	3	25	29	10
Not evaluable	__	__	__	1
Chronic GVHD				
Yes	1	17	17	4
No	2	21	24	9
Not evaluable	__	__	__	1
Second transplant				
Yes	3	2	4	1
No	__	36	37	12
Not evaluable	__	__	__	1
Follow up, surviving patients, median, (IQR) years	8.67	7.27 (4.58–8.12)	9.11 (5.05–11.03)	10.41 (5.16–14.09)

IST, immunosuppressive therapy; GVHD, graft versus host disease; IQR, Interquartile range; Cy dose 0 mg/kg—values shown are median (range); Not evaluable, subject died the day before infusion of marrow graft.

Table 1: **Characteristics of patients by Cy dose.**

received marrow grafts from unrelated donors mismatched at 1 HLA-locus. One patient who received Cy 150 mg/kg died one day before marrow infusion. Seven of 38 patients who received a Cy dose of 50 mg/kg, and 14 of 41 patients who received a Cy dose of 100 mg/kg received marrow grafts from donors mismatched at 1 HLA-locus. Pre-transplant transfusion data were incomplete. The median follow-up times for patients who received Cy doses 50 mg/kg, 100 mg/kg or 150 mg/kg were 7.27 (IQR 4.58–8.12), 9.11 (IQR 5.05–11.03) and 10.41 (IQR 5.16–14.09) years, respectively (Table 1). Only one patient who received Cy dose 0 mg/kg is alive, follow up, 8.67 years.

Outcomes

At the time of our initial report,[7] 37 of 38 patients who received Cy dose of 50 mg/kg were alive. On extended follow-up in the 50 mg/kg Cy dose cohort, there was one graft failure four years after transplantation, and five deaths that occurred at 1.5, 1.8, 4.2, 5.2 and 12.2 years after transplantation, respectively. The 8-year incidence of graft failure was 13.9% (95% CI 4.9–27.4) and overall survival was 85.0% (95% CI 67.3–93.5), Figs. 1 and 2. All early and late graft failures (n = 4) occurred among 31 of 38 HLA-matched transplants. The median infused total nucleated cells (TNC) was 3.10 (IQR 2.99–5.79) x

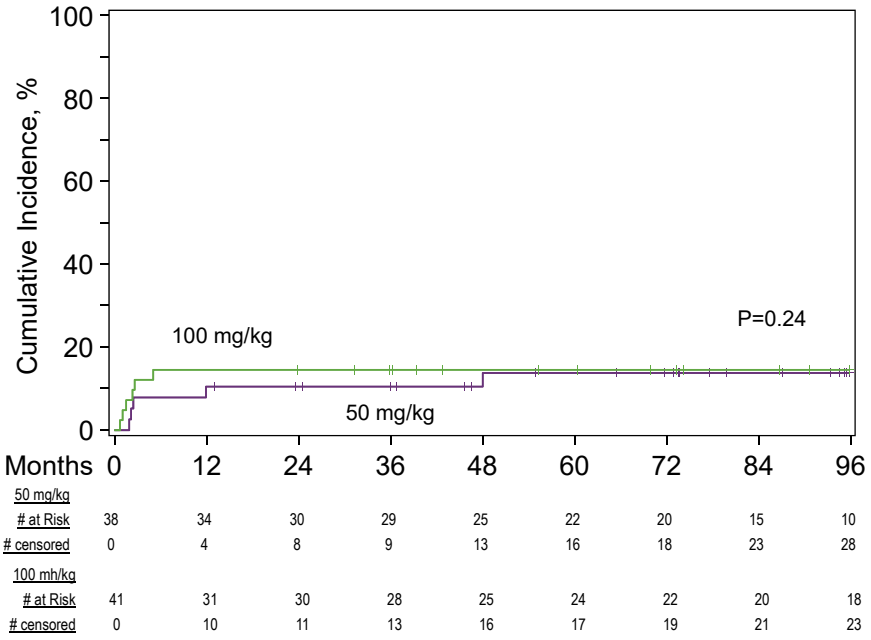

Fig. 1: Graft failure. The 8-year cumulative incidence of graft failure in the Cy 50 mg/kg dose cohort was 13.9% (95% CI 4.9–27.4). The 8-year cumulative incidence of graft failure in the Cy 100 mg/kg dose cohort was 14.6% (95% CI 5.9–27.3).

10^8/kg in the four patients with graft failure compared to 2.69 (IQR 1.43–4.12) x 10^8/kg in the remaining 34 patients without graft failure in this dose cohort. There were a total of six deaths. Three deaths occurred among 7 of 38 HLA-mismatched transplants and the remaining three deaths occurred among 31 of 38 HLA-mached transplants. In the six patients that died, the median infused TNC was 3.25 (IQR 2.11–5.38) x 10^8/kg

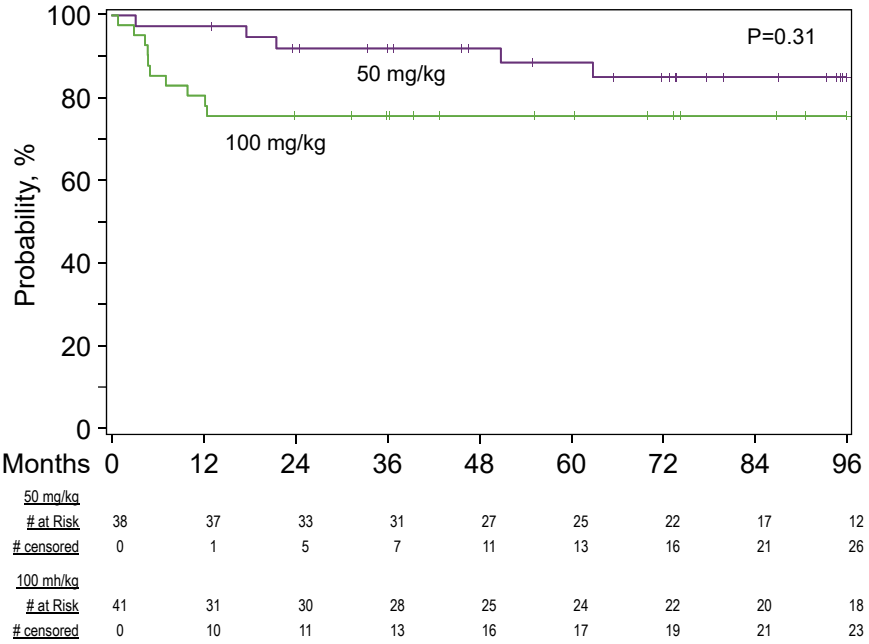

Fig. 2: Overall survival. The 8-year probability of overall survival in the Cy 50 mg/kg dose cohort was 85.0% (95% CI 67.3–93.5). The 8-year probability of overall survival in the Cy 100 mg/kg dose cohort was 75.6% (95% CI 59.4–86.1).

compared to 2.88 (IQR 1.39–4.14) x 10^8/kg in the remaining 32 patients who are alive in this dose cohort. Three of five late deaths (beyond the first year after transplantation) were after 1 HLA-locus mismatched transplants (1.5 years, 1.8 years and 5.2 years from chronic GVHD [n = 2] and sepsis [n = 1]). Two patients developed chronic GVHD of moderate severity 1.4 and 1.5 years after transplantation. The 8-year incidence of chronic GVHD was 42.1% (95% CI 26.1–57.3%). There were eight organ-related complications beyond the first year after transplantation, including liver cirrhosis (n = 1, 1.25 years), myocardial infarction (n = 1, 3.8 years), congestive heart failure (n = 1, 8.7 years), acute renal failure requiring dialysis (n = 1, 3.8 years), avascular necrosis (n = 1, 9 years), gonadal dysfunction in children pre-pubertal at the time of transplant (n = 2, 1.4 and 3.9 years), and cancer (n = 1, pancreatic adenocarcinoma 3.1 years). The causes of death are presented in Table 2. Of the three deaths attributed to chronic GVHD, two were in patients aged 54 and 58 years and one in a patient aged 9 years at transplantation. Two deaths that were not GVHD-related also occurred in older patients, aged 59 and 66 years at transplantation. Eleven of 38 patients had EBV reactivation by PCR assay (any degree) and 4 reported EBV-posttransplant lymphoproliferative disease. There were no EBV-associated deaths.

Cy dose	Age, years	Survival time	Cause of death
0 mg/kg	22	0.31 years	Adult respiratory distress syndrome
0 mg/kg	61	0.55 years	Cytomegalovirus pneumonia
50 mg/kg	9	1.80 years	Chronic graft versus host disease
50 mg/kg	19	0.27 years	Secondary graft failure
50 mg/kg	54	5.24 years	Chronic graft versus host disease
50 mg/kg	58	1.47 years	Chronic graft versus host disease
50 mg/kg	59	12.20 years	Multiorgan failure Chronic renal insufficiency and congestive heart failure
50 mg/kg	66	4.24 years	Pancreatic adenocarcinoma
100 mg/kg	2	0.83 years	Secondary graft failure
100 mg/kg	5	8.57 years	Adult respiratory distress syndrome (Dyskeratosis Congenita)
100 mg/kg	15	1.04 years	Secondary graft failure
100 mg/kg	18	0.40 years	Chronic graft versus host disease
100 mg/kg	26	0.39 years	Secondary graft failure
100 mg/kg	38	1.02 years	Chronic graft versus host disease
100 mg/kg	54	0.59 years	Secondary graft failure
100 mg/kg	55	0.25 years	Secondary graft failure
100 mg/kg	55	0.08 years	Primary graft failure
100 mg/kg	56	0.37 years	Acute graft versus host disease
100 mg/kg	63	0.42 years	Sepsis (bacterial)
150 mg/kg	9	Died 1 day before marrow infusion	Pulmonary failure (after cyclophosphamide)
150 mg/kg	15	0.37 years	Pulmonary failure
150 mg/kg	17	0.25 years	Adult respiratory distress syndrome
150 mg/kg	20	0.22 years	Multiorgan failure
150 mg/kg	44	0.41 years	Pneumonia (parainfluenza type 3)
150 mg/kg	52	Died 1 day after marrow infusion	Adult respiratory distress syndrome (diffuse alveolar injury on autopsy)
150 mg/kg	61	0.17 years	Primary graft failure Cardiac failure

Cy, cyclophosphamide; Survival time, interval between transplantation and death.

Table 2: **Causes of death.**

At the time of our initial report,[7] 31 of 41 patients who received Cy dose of 100 mg/kg were alive. On extended follow up of Cy dose of 100 mg/kg cohort there was no graft failure beyond the first year after transplantation and one death at 8.6 years after HLA-matched transplantation in a child with underlying dyskeratosis congenita that was unrecognized pre-transplant (aged 5 years at transplantation). The 8-year incidence of graft failure was 14.6% (95% CI 5.9–27.3) and overall survival was 75.6% (95% CI 59.4–86.1), Figs. 1 and 2. Of the six early graft failures, five graft failures occurred among 27 of 41 HLA-matched transplants and one graft failure among 14 of 41 HLA-mismatched transplants. The median infused TNC was low in the six patients with early graft failure, 1.65 (IQR 1.42–2.44) x 10^8/kg compared to 3.65 (IQR 2.43–5.01) x 10^8/kg in the 35 patients without graft failure. There were a total of 11 deaths. Nine deaths occurred among 27 of 41 HLA-matched transplants and the remaining two deaths among 14 of 41 HLA-mismatched transplants. The median infused TNC was low, 1.80 (IQR 1.36–7.04) x 10^8/kg in the 11 patients who died compared to 3.61 (IQR 2.03–5.01) x 10^8/kg in the 30 patients who are alive in this dose cohort. One patient was diagnosed with severe chronic GVHD of the lungs 7 years after transplantation. There were 6 organ-related complications beyond the first year after transplantation including pulmonary (n = 2, adult respiratory distress syndrome, 8.0 years and restrictive airway disease, 1.9 years), chronic renal failure requiring dialysis (n = 1, 15.0 years), avascular necrosis (n = 1, 1.5 years), gonadal dysfunction (n = 1, aged 9 years at transplantation, 2.6 years), and malignancy (n = 1, myelodysplastic syndrome 8.9 years). The 8-year survival among the 41 patients who received Cy 100 mg/kg was 75.6% (95% CI 59.4–86.1%), Fig. 2. The causes of death are presented in Table 2. For the 11 deaths in this dose cohort, the median infused TNC dose was 1.80 (IQR 1.36–7.04) and two transplants were mismatched at 1 HLA-locus. Seventeen of 41 patients had EBV reactivation by PCR assay and 3 reported EBV-posttransplant lymphoproliferative disease. There were no EBV-associated deaths.

Accrual at Cy doses 0 mg/kg and 150 mg/kg was stopped early after meeting prespecified day 42 or day 100 stopping rules, and few patients were evaluable for late complications.[8] With extended follow up, only one of three patients who received no Cy (0 mg/kg) is alive 8.7 years after the first transplant after a successful second transplant for graft failure, and no late organ

complications have been reported. Two patients in this dose cohort received low dose marrow grafts (TNC $\leq 1.0 \times 10^8$/kg) but were HLA-matched to their donors. Seven of fourteen patients who received Cy at a dose of 150 mg/kg are alive with sustained engraftment at a median follow up of 10.4 (IQR 5.2–14.1) years. There were seven deaths at this Cy dose level, five within 3 months of transplantation, from pulmonary toxicity (n = 5), cardiac and multiorgan failure (n = 2). Infused TNC was 2.00×10^8/kg for the patient who died from cardiac failure. One patient aged 18 years at transplantation who received a Cy dose 150 mg/kg developed gonadal dysfunction 1.2 years later. No other late organ complications were reported. EBV viremia was not detected after Cy doses of 0 mg/kg or 150 mg/kg.

Nineteen of 96 patients (20%) in our trial were aged ≥40 years and 15 of these (79%) were aged ≥50–65 years at transplantation. The 8-year probability of survival in patients aged ≥50 years was 26.7% (95% CI 8.2–51.0) compared to 85.0% (95% CI 76.3–91.9) in patients younger than 50 years ($P < 0.0001$). There were four patients aged 40–49 years and one death occurred in a 44-year-old who received Cy 150 mg/kg. Accrual to this dose was terminated for early mortality.

Discussion

An earlier multicentre trial of unrelated donor marrow transplantation for severe aplastic anaemia established a minimum TBI dose of 2 Gy, when combined with Cy dose of 200 mg/kg and ATG to achieve sustained engraftment, and resulted in 62% survival at 5 years.[4] Using that platform, our trial (NCT00326417) deescalated the dose of Cy while adding fludarabine, based on the hypothesis that reducing the dose of Cy would decrease non-haematologic toxicity while fludarabine would provide the immunosuppression necessary to achieve sustained engraftment.[7,8] The results showed that a Cy dose of 50 mg/kg was the most desirable for day 100 engraftment and survival based on our original statistical design which used a Bayesian dose-finding algorithm based on the approach introduced by Thall and Cook. The desirability of a dose was defined geometrically as the Euclidean distance between the dose's posterior means of engraftment and fatality without graft failure and the ideal point (1.0) on the trade-off counter corresponindg to 100% engraftment and 0% death without graft failure.[7,13] A higher Cy dose of 100 mg/kg was also associated with excellent day 100 engraftment and survival.[7] Extended follow up of patients beyond the trial period, the focus of the current analysis showed sustained engraftment and survival ≥75% at a median follow-up of 8 years among patients given Cy doses of 50 mg/kg or 100 mg/kg. These observations confirm our hypothesis that the addition of fludarabine (to Cy doses of 50 or 100 mg/kg) allows for sufficient immunosuppression to achieve sustained engraftment and survival for several years after transplantation.

Although the Bayesian method picked the Cy dose 50 mg/kg as the most desirable for short-term (day 100) outcomes, there was one graft failure and five deaths that occurred beyond the first year after transplantation compared to one death in the Cy dose 100 mg/kg cohort. Three of five deaths in the Cy dose 50 mg/kg cohort occurred ≥4 years after transplantation underscoring the need for continued surveillance beyond the immediate post-transplantation period. Chronic GVHD was the predominant cause of death in this cohort, both early[7] (within the first year after transplantation) and in the later period. In contrast, in the Cy 100 mg/kg dose cohort, there were no graft failures beyond the first year after transplantation and one late death from pulmonary complications in a patient with dyskeratosis congenita. While inherited bone marrow failure syndromes (IBMFS) were excluded from the trial, the diagnosis of dyskeratosis congenita was apparently made only after transplantation. Late deaths secondary to pulmonary complications in dyskeratosis congenita are likely part of the disease pathophysiology.[14] Germline mutations in *TERT* or *TREC* as seen in dyskeratosis congenita can cause both isolated aplastic anaemia and pulmonary fibrosis.[15]

A natural history study and reports on aplastic anaemia transplants have shown higher mortality for adults compared to children.[16–18] The adverse effect of age on survival is particularly strong in patients older than 50 years at transplantation.[17,18] We also observed an adverse effect of age (≥50 years) on survival and cannot recommend unrelated donor marrow transplantation using the current approach for these patients. In both the Cy 50 mg/kg and 100 mg/kg cohorts deaths were common in patients aged ≥50 years at transplantation, although the timing of death varied by Cy dose. After a Cy dose of 50 mg/kg, deaths were more frequent in a later period (≥1 year after transplantation), and all deaths except one occurred in patients aged older than 50 years at transplantation. Four of seven patients aged ≥50 years who received Cy dose 50 mg/kg died. After Cy dose 100 mg/kg, all except one death occurred early (i.e., within 6 months after transplantation). Five patients aged ≥50 years received Cy 100 mg/kg, and all have died; graft failure was the predominant cause of death. There were two patients aged ≥50 years at transplantation who received Cy 150 mg/kg, and both died early, one from cardiac failure after primary graft failure and one from diffuse acute lung injury associated with transplant conditioning, even before graft infusion. One patient who did not receive Cy (0 mg/kg) who was aged ≥50 years, had secondary graft failure and died from cytomegalovirus pneumonia. The 15 patients aged ≥50 years in the current report may have had co-existing co-morbidities that would have added to their mortality risk. The haematopoietic cell transplant comorbidity index that is routinely assessed now was not the norm

when this trial was designed.[19] Others have identified Karnofsky performance scores less than 90 and transplantation of grafts from unrelated donors as risks for mortality.[18] We were unable to examine for an effect of performance score. Approximately half of the patients aged ≥50 years in our study had Karnofsky performance scores of 70 or 80 and the remainder, scores of 90 or 100. With 12 deaths in 15 patients (80%) aged ≥50 years at transplantation our approach to cure severe aplastic anaemia for this subset of patients failed.

Another report on late complications and long-term outcome after transplantation for severe aplastic anaemia carried out between 1995 and 2006 included 542 1-year survivors of unrelated donor transplantation.[20] Common late effects in that report included gonadal dysfunction, growth disturbance, avascular necrosis, hypothyroidism and cataracts, most consistent with the frequent use of higher dose TBI containing regimens.[20] That report also showed that the incidence of late effects increased with time after transplantation.[20] In the current analysis with a median follow up of 8 years, approximately 20% of patients, regardless of Cy dose, reported late effects, in liver, lungs, heart and kidneys. The fact that we observed fewer endocrinopathies is likely related to the low doses of TBI employed in our trial. However, late effects were not completely prevented, their development presumably related to interactions between the various components of the conditioning regimen. In our cohort there were no pregnancies reported (pregnant females or males having fathered offspring) in those whose current age is ≥18 years.

The incidence of chronic GVHD was similar after Cy doses of 50 mg/kg and 100 mg/kg and comparable to that reported after Cy doses of 200 mg/kg.[4] Consistent with other reports on late outcomes after transplantation for severe aplastic anaemia, GVHD-associated deaths were common in the current analysis.[7,20–22] The absence of late deaths from chronic GVHD in the Cy 100 mg/kg cohort is difficult to explain other than from the early loss of patients from other complications.[7] The incorporation of novel agents to prevent GVHD may mitigate GVHD risks in patients with diseases that do not benefit from graft-versus-tumor effect.

Our study has several limitations. First, we used data collected through an observational transplant registry, the CIBMTR, albeit with standardized data collection forms. These forms collect data generated in the course of standard clinical care, which differs from clinical trials where outcomes of interest and timing of assessments are set prior to the initiation of the trial. Sustaining a rigorous clinical trial type infrastructure for long-term follow up of trial participants is prohibitively expensive, forcing us to study late effects retrospectively, using registry data. Second, the number of patients enrolled in our trial was modest, thus limiting our ability to detect significant differences if such differences existed. While the primary trial was powered to identify an optimal dose of Cy based on day 100 outcomes, it was not powered for later outcomes, including survival at later timepoints. Consequently this analysis was exploratory. Third, the trial did not collect marrow characteristics other than the infused TNC dose nor did the trial specify minimum required TNC dose for marrow harvest. Fourth, the trial did not collect data on the number of pre-transplant immunosuppression cycles or the response rate or duration of remission. Nevertheless, follow-up beyond the pre-determined trial period has allowed us to collect informative data. Using Cy doses of 50 mg/kg or 100 mg/kg (instead of the conventional Cy 200 mg/kg dose) combined with TBI 2 Gy, fludarabine 120 mg/m^2 and ATG (rabbit derived 9 mg/kg or equine derived 90 mg/kg) allows for sustained engraftment and long-term survival in patients with aplastic anaemia transplanted from HLA-matched or 1 locus HLA-mismatched unrelated bone marrow donors. The lowest Cy dose for early engraftment and survival was 50 mg/kg but close long-term surveillance is required as most deaths occurred beyond the first year after transplantation. The 8-year survival of ≥75% of our cohort is comparable to the reported 1-year survival of 81% after HLA haploidentical related donor transplantation, for which long-term survival remains to be determined.[23] Early referral for transplantation is encouraged, with reports of comparable long-term survival after HLA-matched sibling, unrelated donor marrow and HLA haploidentical related donor transplantation, although most patients have been aged <50 years at transplantation.[23–25] Donor selection for unrelated donor transplantation should adhere to guidelines with a preference for donors aged 18–29 years who are HLA-matched to the recipient at a minimum at HLA-A, -B, -C and -DRB1.[26,27]

In conclusion, de-escaltion of Cy dosing (Cy 50 mg/kg and 100 mg/kg) with TBI 2 Gy, fludarabine and ATG showed sustained engraftment and survival ≥75% at a median follow up of 8 years. While early mortality was higher with Cy 100 mg/kg than with Cy 50 mg/kg, with longer follow up Cy 100 mg/kg proved to be an acceptable dose, particularly when considering long-term engraftment and survival for patients younger than 50 years at transplantation. The high mortality in patients older than 50 years support an unmet need to identify an optimal approach for unrelated donor transplantation for this subset of patients. There is also a need for further improvement in GVHD prevention as GVHD-associated deaths were common. Cy doses of 0 mg/kg or 150 mg/kg cannot be recommended. We recommend continued patient surveillance beyond the early post-transplant period as graft failure and deaths can occur several years after transplantation.

Contributors

ME and HJD designed the study that was approved by JHA, JT, SA, MEH, JK, EL, JMM, RN, MAP, SDR and MMH. ME and JK prepared

and analysed the data. The underlying data have been verified by ME, JK and HJD. ME drafted the manuscript. JHA, JT, SA, MEH, JK, EL, JMM, RN, MAP, SDR, MMH and HJD critically reviewed the manuscript. All authors have seen and approved the final manuscript.

Data sharing statement

The study data set is available upon request at https://www.cibmtr.org/refernce center/publist/pubdsdownload/pages/default.aspx.

Declaration of interests

MAP: Consulting fees from Pfizer, Cargo, Noavartis, Bluebird Bio, Vertex, Medexus, Equillium. Honoraria serving as DSMB member (Autlolos).

SDR: Serves on the Community Advisory Board (SIRPant Immunotherapeutics) and stock options (SIRPant Immunotherapeutics).

Other authors declare no competing interest.

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