Published in final edited form as:

Lancet Haematol. 2015 September ; 2(9): e367–e375. doi:10.1016/S2352-3026(15)00147-7.

Cyclophosphamide conditioning in patients with severe aplastic anaemia given unrelated marrow transplantation: a phase 1–2 dose de-escalation study

Prof. Paolo Anderlini, MD, **Juan Wu, MS**, **Iris Gersten, MS**, **Marian Ewell, ScD**, **Prof. Jakob Tolar, MD**, **Prof. Joseph H Antin, MD**, **Roberta Adams, MD**, **Sally Arai, MD**, **Gretchen Eames, MD**, **Mitchell E Horwitz, MD**, **Prof. John McCarty, MD**, **Ryotaro Nakamura, MD**, **Prof. Michael A Pulsipher, MD**, **Prof. Scott Rowley, MD**, **Eric Leifer, PhD**, **Shelly L Carter, ScD**, **Nancy L DiFronzo, PhD**, **Prof. Mary M Horowitz, MD**, **Prof. Dennis Confer, MD**, **Prof. H Joachim Deeg, MD**[*], and **Prof. Mary Eapen, MD**[*]

University of Texas, MD Anderson Cancer Center, Houston, TX, USA (Prof P Anderlini MD); Emmes Corporation, Rockville, MD, USA (J Wu MS, I Gersten MS, M Ewell ScD, S L Carter ScD); University of Minnesota, Minneapolis, MN, USA (Prof J Tolar MD); Dana Farber Cancer Institute, Boston, MA, USA (Prof J H Antin MD); Phoenix Children's Hospital, Phoenix, AZ, USA (R Adams MD); Stanford Hospital and Clinics, Stanford, CA, USA (S Arai MD); Cooks Children's Hospital, Fort Worth, TX, USA (G Eames MD); Duke University, Durham, NC, USA (M E Horwitz MD); Virginia Commonwealth University, Richmond, VA, USA (Prof J McCarty MD); City of Hope National Medical Center, Duarte, CA, USA (R Nakamura MD); University of Utah, Salt Lake City, UT, USA (Prof M A Pulsipher MD); Hackensack University Medical Center, Hackensack, NJ, USA (Prof S Rowley MD); National Heart, Lung, and Blood Institute, Bethesda, MD, USA (E Leifer PhD, N L DiFronzo PhD); Medical College of Wisconsin, Milwaukee, WI, USA (Prof M M Horowitz MD, Prof M Eapen MD); National Marrow Donor Program, Minneapolis, MN, USA (Prof D Confer MD); and Fred Hutchinson Cancer Research Center, Seattle, WA, USA (Prof H J Deeg MD)

Summary

Background—The optimum preparative regimen for unrelated donor marrow transplantation in patients with severe aplastic anaemia remains to be established. We investigated whether the combination of fludarabine, anti-thymocyte globulin, and total body irradiation (TBI) would enable reduction of the cyclophosphamide dose to less than 200 mg/kg while maintaining engraftment and having a survival similar to or better than that with standard regimens using a cyclophosphamide dose of 200 mg/kg (known to be associated with significant organ toxicity) for

Correspondence to: Prof Paolo Anderlini, Department of Stem Cell Transplantation and Cellular Therapy, Room FC5.3062, University of Texas, MD Anderson Cancer Center, 1515 Holcombe Blvd, Unit 0423, Houston, TX 77030-4095, USA, panderli@mdanderson.org.

[*]Contributed equally

See Online for appendix

Contributors

PA, MEw, SLC, EL, HJD, MMH, and MEa designed the study. PA, HJD, and ME drafted the manuscript. JW prepared and analysed the data. IG, MEw, JT, JHA, RA, SA, GE, MEH, JM, RN, MAP, SR, EL, SLC, NLD, DC, and MMH critically reviewed the manuscript. All authors have seen and approved the final manuscript.

Declaration of interests

We declare no competing interests.

unrelated donor transplantation for severe aplastic anaemia. We have previously shown that cyclophosphamide at 150 mg/kg resulted in excess toxicity and its omission (0 mg/kg) resulted in unacceptable graft failure (three of three patients had secondary graft failure). Here we report results for the 50 mg/kg and 100 mg/kg cohorts.

Methods—In a multicentre phase 1–2 study, patients (aged ≤65 years) with severe aplastic anaemia, adequate organ function, and an unrelated adult marrow donor HLA matched at the allele level for HLA A, B, C, and DRB1 or mismatched at a single HLA locus received bone marrow grafts from unrelated donors. All patients received antithymocyte globulin (rabbit derived 3 mg/kg per day, intravenously, on days −4 to −2, or equine derived 30 mg/kg per day, intravenously, on days −4 to −2), fludarabine (30 mg/m^2 per day, intravenously, on days −5 to −2), and TBI (2 Gy). Cyclophosphamide dosing 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). The primary endpoint was the selection of the optimum cyclophosphamide dose based on assessments of graft failure (primary or secondary), toxicity, and early death during 100 days of follow-up after the transplant; this is the planned final analysis for the primary endpoint. This trial is registered with ClinicalTrials.gov, number NCT00326417.

Findings—96 patients had bone marrow transplant. At day 100, 35 (92%) of 38 patients were engrafted and alive in the cyclophosphamide 50 mg/kg cohort and 35 (85%) of 41 in the 100 mg/kg cohort. Cyclophosphamide 50 mg/kg and 100 mg/kg resulted in posterior means for fatality without graft failure of 0·7% (credible interval 0–3·3) and 1·4% (0–4·9), respectively. Three patients (8%) had graft failure with cyclophosphamide 50 mg/kg and six (15%) with cyclophosphamide 100 mg/kg. Four (11%) patients had major regimen-related toxicity with cyclophosphamide 50 mg/kg and nine (22%) with cyclophosphamide 100 mg/kg. The most common organ toxicity was pulmonary (grade 3 or 4 dyspnoea or hypoxia including mechanical ventilation), and occurred in three (8%) and four (10%) patients given cyclophosphamide 50 mg/kg and 100 mg/kg, respectively.

Interpretation—Cyclophosphamide at 50 mg/kg and 100 mg/kg with TBI 2 Gy, fludarabine, and anti-thymocyte globulin results in effective conditioning and few early deaths after unrelated donor transplantation for severe aplastic anaemia. These doses of cyclophosphamide provide a framework for further regimen optimisation strategies.

Funding—US National Heart, Lung, and Blood Institute and National Cancer Institute.

Introduction

Unrelated donor bone marrow transplantation (BMT) is an option for the treatment of patients with severe acquired aplastic anaemia who do not have HLA-matched siblings and sustained responses to immunosuppressive therapy.[1,2] Outcome after unrelated donor BMT has improved over the past decades,[3,4] presumably because of closer donor–recipient HLA matching and improved supportive care. The optimum preparative regimen remains to be established. Ideally, the conditioning regimen should maximise engraftment while minimising organ toxicity. However, with current transplant conditioning, graft rejection and regimen-related toxicity continue to adversely affect patient outcome.

Conditioning regimens for unrelated donor BMT in patients with aplastic anaemia are typically combinations of cyclophosphamide, anti-thymocyte globulin, and total body

irradiation (TBI).[1,2,5] Several of these combinations were assessed in a large retrospective review of 154 patients from the Japan Marrow Donor Program.[5] 56% of patients were alive at 5 years, with 11% of patients rejecting the graft overall. Because of the retrospective, non-randomised nature of the study, no combination could be clearly identified as the preferred one. In a landmark prospective multicentre study, Deeg and colleagues[6] assessed TBI dose de-escalation in 87 patients. The optimum TBI dose was 2 Gy when given with cyclophosphamide (200 mg/kg) and horse anti-thymocyte globulin (90 mg/kg). Graft failure occurred in four (5%) of 81 assessable patients. Overall, 48 (55%) of 87 patients were alive at the last follow-up; the dose-limiting toxicity was diffuse pulmonary injury.[6] 1 year survival in patients who received fully HLA-matched grafts was 75% for those younger than 20 years of age, and 50% for older patients.[6] More recently, fludarabine has been incorporated into conditioning regimens as an alternative to low-dose TBI and as a strategy to reduce the dose of cyclophosphamide.[7–12] The European Blood and Marrow Transplant Group tested a fludarabine-based, low-dose cyclophosphamide regimen (1200 mg/m^2, equivalent to about 30 mg/kg in an adult of average size) in 38 patients with severe acquired aplastic anaemia.[7] They reported an actuarial 2 year survival of 73%, with younger patients (up to 14 years) showing a lower risk of rejection (5% *vs* 32%; p=0·03) and improved survival (84% *vs* 61%; p=0·2) compared with older patients. The high proportion of rejections (particularly in older patients) prompted the European Blood and Marrow Transplant Group to suggest an increase in the cyclophosphamide dose to 120 mg/kg.[8] A similar fludarabine-based, low-dose cyclophosphamide (1200 mg/m^2) approach was adopted in a multicentre study from the UK, albeit with incorporation of alemtuzumab in lieu of anti-thymocyte globulin.[12] The investigators reported an overall survival at 2 years of 95%, although graft rejection after unrelated donor transplants was 15%.

Research in context

Evidence before the study

Patients with severe aplastic anaemia who do not respond to immunosuppressive therapy and do not have suitably HLA-matched related donors can be treated successfully with unrelated donor marrow transplantation. However, there is no consensus for the optimum preparative regimen for transplantation in these patients. A conditioning regimen of low-dose total body irradiation (TBI) in combination with cyclophosphamide and anti-thymocyte globulin is widely regarded as the standard for patients with severe acquired aplastic anaemia undergoing unrelated donor marrow transplantation in the USA. More recently, the European Group for Blood and Marrow Transplantation (EBMT) proposed a different approach that used fludarabine, cyclophosphamide, and anti-thymocyte globulin. Another approach developed in the UK included fludarabine, cyclophosphamide, and alemtuzumab as the preparative regimen. These data suggested that the addition of fludarabine to the preparative regimen could be beneficial to facilitate engraftment.

Added value of this study

Although cyclophosphamide is a key part of the preparative regimen for patients with severe aplastic anaemia, the optimum dose remains undefined. In view of the known

toxicities (eg, cardiac, hepatic, and haemorrhagic cystitis) linked to high-dose (ie, 200 mg/kg) cyclophosphamide, a reduction of the cyclophosphamide dose would be desirable. Based on the in-vitro synergism between fludarabine and cyclophosphamide, we hypothesised that there would be a cyclophosphamide-sparing effect of fludarabine with lower regimen-related toxicity and engraftment and survival would be similar to or better than those reported with cyclophosphamide (200 mg/kg), antithymocyte globulin, and low-dose TBI. In our phase 1–2 trial, cyclophosphamide dosing was de-escalated in combination with fludarabine, anti-thymocyte globulin, and low-dose TBI (2 Gy). The trial design maximised the clinical value of each patient (a desirable feature in rare diseases such as severe acquired aplastic anaemia) with a Bayesian approach.

Implication of all the available evidence

This study confirms the value of fludarabine as part of the conditioning regimen for patients with severe acquired aplastic anaemia undergoing unrelated marrow transplant. It also supports the cyclophosphamide-sparing effect originally hypothesised for fludarabine. With regard to cyclophosphamide dosing, we noted that complete omission of cyclophosphamide resulted in unacceptable graft failure, and cyclophosphamide 150 mg/kg resulted in excess toxicity. Although regimen-related toxicity was similar to and graft failure somewhat higher than that reported with cyclophosphamide, antithymocyte globulin, and low-dose TBI, 1-year survival with cyclophosphamide 50 mg/kg and 100 mg/kg was 97·4% and 80·5%, respectively. Outcomes with cyclophosphamide at 50 mg/kg seem noteworthy, although the two groups differed for median age of patients and HLA matching of donor and recipient, which might have affected the results. These data identify desirable cyclophosphamide doses in this setting and provide a framework for further regimen optimisation strategies, ideally through randomised studies.

In the current phase 1–2 study, the Blood and Marrow Transplant Clinical Trials Network (BMT CTN protocol 0301) investigated whether the addition of fludarabine to a 2 Gy TBI, anti-thymocyte globulin, and cyclophosphamide regimen would enable the reduction of the cyclophosphamide dose to less than 200 mg/kg while maintaining engraftment and survival rates similar to or superior to those reported by Deeg and colleagues.[6] We present here a follow-up to our initial report of this study.[19]

Most current regimens include cyclophosphamide, but its role and optimum dose in fludarabine-containing combinations is not established. In view of the known in-vitro synergism between fludarabine and cyclophosphamide,[13,14] we hypothesised that there would be a cyclophosphamide-sparing effect when the two drugs are combined. Srinivasan and colleagues[15] reported results in 26 patients with transfusion-dependent nonmalignant haematological disorders who were transplanted from HLA-matched relatives after conditioning with fludarabine (125 mg/m^2) plus cyclophosphamide (120 mg/kg) with or without inclusion of anti-thymocyte globulin. All patients were engrafted and two died, and overall survival was 77% at a median of 21 months after transplantation. Preliminary data suggest that survival might be improved by reducing the cyclophosphamide dose, a desirable outcome in view of the known toxicities linked to high-dose cyclophosphamide.[16–18]

Methods

Study design and participants

This trial was a phase 1–2 single-arm, dose-optimisation study of cyclophosphamide in patients with severe acquired aplastic anaemia, as defined by Peinemann and colleagues,[1] and was done in bone marrow transplant centres in the USA. Patients were eligible for inclusion in the study if they were 65 years old or younger, had adequate organ function, and had an unrelated adult donor HLA matched at the allele-level for HLA A, B, C, and DRB1 or mismatched at a single HLA locus. Exclusion criteria were Karnofsky performance status of less than 60, clonal cytogenetic abnormalities, Fanconi's anaemia, or other marrow failure syndromes. Bone marrow was the only stem cell source allowed because of the poor outcome reported in patients with severe acquired aplastic anaemia transplanted with peripheral blood cells.[20,21]

Institutional review boards at all participating centres provided ethics approval. All patients provided written informed consent in accordance with the Declaration of Helsinki.

Procedures

Between Feb 22, 2006, and Aug 1, 2007, 21 patients were accrued to phase 1 of the study. All patients were given a fixed dose of anti-thymocyte globulin (rabbit-derived thymoglobulin 3 mg/kg per day, intravenously, on days −4 to −2 or horse-derived ATGAM [Pharmacia & Upjohn, New York, NY, USA] 30 mg/kg per day, intravenously, on days −4 to −2), fludarabine (30 mg/m^2 per day, intravenously, on days −5 to −2), and TBI (2 Gy from a linear accelerator at ≤0·2 Gy/min on day −1). In phase 1, each of the four doses of cyclophosphamide was tested for adequate safety and graft retention. The doses were tested with six patients at each dose except at the 0 mg/kg dose, which was closed after accrual of three patients (planned accrual was six) because of secondary graft failure (table 1).[19]

The Bayesian approach assigned cyclophosphamide 150 mg/kg as the most desirable dose for phase 2 of the study. Phase 2 opened in Nov 5, 2007, and closed to accrual on Dec 2, 2013, with a final enrolment of 96 patients (table 1). The starting cyclophosphamide dose was 150 mg/kg (50 mg/kg per day, intravenously, on days −4 to −2), and was to be de-escalated depending on engraftment and toxicity. Eight patients were accrued to the cyclophosphamide dose 150 mg/kg; thereafter, this dose was closed because of excess toxicity (seven of 14 patients died: four from organ failure, two from adult respiratory distress syndrome, and one from infection).[19] The Bayesian approach next assigned cyclophosphamide 100 mg/kg as the most desirable dose compared with 50 mg/kg. Therefore, patients were enrolled to the 100 mg/kg dose in cohorts of six, with enrolment paused until all patients in a cohort had their day 100 assessments. Although this approach ensured that safety was maximum, it slowed accrual because of the long pauses. Therefore, 2 years after the start of phase 2 (Jan 14, 2010), the data safety monitoring board allowed enrolment of patients to cyclophosphamide 50 mg/kg and 100 mg/kg. Patients were preferentially assigned to cyclophosphamide 100 mg/kg, but when accrual to this dose was paused, patients were assigned to cyclophosphamide 50 mg/kg. The last 20 patients were preferentially assigned to cyclophosphamide 50 mg/kg to balance accrual to the two doses.

One patient withdrew consent before transplant and could not be analysed (table 1). After refinement of the cyclophosphamide dose, a total of 68 patients were allocated to the two remaining cohorts.

Outcomes

The primary endpoint was the selection of the optimum cyclophosphamide dose based on assessments of graft failure (primary or secondary), regimen-related toxicity, and early death during 100 days of follow-up after the transplant. Neutrophil engraftment was defined as the achievement of an absolute neutrophil count of at least $0{\cdot}5 \times 10^9$ cells per L for three consecutive measurements. Primary graft failure was defined by the absence of neutrophil recovery to at least $0{\cdot}5 \times 10^9$ cells per L by 100 days after transplant. Secondary graft failure was defined as initial neutrophil engraftment followed by subsequent decline in the absolute neutrophil count to less than $0{\cdot}5 \times 10^9$ cells per L for three consecutive measurements that was unresponsive to growth factor therapy. Regimen-related toxicity was assessed weekly until day 100 and scored according to the Bearman grading system[22] and supplemented with the US National Cancer Institute's Common Terminology Criteria for Adverse Events (version 3.0).[23] Using either scale, for some organs and adverse events (ie, bladder, CNS, gastrointestinal organs, and thrombotic microangiopathy), only grades 4 and 5 toxicities seemed clinically meaningful so grades 0–3 were bundled. For all other organs, grades 3, 4, and 5 were judged to be clinically relevant so grades 0–2 were bundled. Major regimen-related toxicity was defined as grade 3 pulmonary, cardiac, renal, oral mucosal, or hepatic events and grade 4 events for other organ systems. Early death was defined as death before day 100 after transplant. Causes of death were attributed by use of the scheme developed by Copelan and colleagues.[24]

The secondary endpoints were survival after transplant, secondary graft failure, and acute graft-versus-host disease of grades 2–4, and chronic graft-versus-host disease. Survival was defined as the time from transplant to death from any cause. Patients who were alive were censored at the last follow-up. Acute and chronic graft-versus- host disease was graded according to the BMT CTN manual of procedures.[25] Graft failure was not regarded as a competing risk for graft-versus-host disease unless it resulted in death.

Epstein-Barr virus (EBV) DNA quantitative PCR was done on peripheral blood samples at least every 2–4 weeks beginning at engraftment until days 90–100. Rituximab (375 mg/m^2) was administered for EBV DNA of 1000 copies per mL or higher detected on two consecutive occasions. This cutoff was endorsed by the trial investigators and consistent with reported data.[26] Treatment of persistent viraemia or EBV-post-transplant lymphoproliferative disorder was done in accordance with local institutional protocols.

Statistical analysis

The primary hypothesis was that, with high probability, the optimum cyclophosphamide dose could be selected with a Bayesian dose-finding algorithm based on the approach used by Thall and Cook.[27] The dose-finding algorithm was started by specifying β prior distributions for the respective engraftment and death without graft failure rates at each cyclophosphamide dose. The trade-off contours for desirability shown in the appendix used

the L^p norm method.[28] For the four cyclophosphamide doses (0 mg/kg, 50 mg/kg, 100 mg/kg, 150 mg/kg) tested, the previous distributions corresponded to prior mean probabilities for engraftment of 0·70, 0·80, 0·90, and 0·95, and for fatality without graft failure of 0·05, 0·10, 0·20, and 0·30. After a cohort of six patients had been enrolled and followed up, the endpoint data from the cohort were used to update the β-binomial posterior distributions for the engraftment and fatality without graft failure rates for the dose that the cohort received. 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 contour, corresponding to 100% engraftment and 0% death without graft failure.

All the secondary analyses were exploratory, and no formal statistical comparisons were done. Kaplan-Meier survival curves were constructed for each cyclophosphamide dose. Cumulative incidences for acute graft-versus-host disease, chronic graft-versus-host disease, and graft failure were constructed for each cyclophosphamide dose with death as a competing risk. Recipients of second transplants were censored at the date of second transplant for all endpoints except survival. Statistical analyses were done with SAS (version 9.3). The desirability computation and cumulative incidence analyses were done with R software (version 2.15.1).

This trial is registered with ClinicalTrials.gov, number NCT00326417.

Role of the funding source

The funder did not participate in the study design, or data gathering, analyses, or interpretation. The US National Institutes of Health appointed a committee to review and approve the study protocol, and appointed a data safety monitoring board. The corresponding and senior authors had full access to all the data and the final responsibility to submit for publication.

Results

We previously published the results of patients treated with cyclophosphamide 0 mg/kg and 150 mg/kg.[19] Table 2 shows the characteristics of patients who received cyclophosphamide 50 mg/kg or 100 mg/kg. In the cyclophosphamide 50 mg/kg cohort, six patients were enrolled during phase 1 and 32 during phase 2; and in the cyclophosphamide 100 mg/kg cohort, six patients were enrolled during phase 1 and 35 during phase 2. The median age at transplantation of patients who received cyclophosphamide 50 mg/kg or 100 mg/kg was 20·6 years (range 0·5–65·9). The median age of patients in the cyclophosphamide 50 mg/kg and 100 mg/kg groups were 24·5 years and 17·6 years, respectively (table 2; p=0·10). Seven (18%) patients given cyclophosphamide 50 mg/kg and 14 (34%) given cyclophosphamide 100 mg/kg had HLA mismatches (ie, 7/8 HLA matched; table 2; p=0·10). We did not obtain data about pretransplant transfusion history. Several of the patients in our study were treated at institutions other than the institution where the transplant was done and transfusion data were not readily available or thought to be accurate. The median follow-up of the surviving patients given cyclophosphamide 50 mg/kg and 100 mg/kg was 17 months (IQR 12–24, range 4–26) and 24 months (24–25; range 12–51), respectively.

Day 100 outcomes (graft failure, major regimen-related toxicity, and early death) for patients who received cyclophosphamide 50 mg/kg or 100 mg/kg are summarised in table 3. The computed desirability with the Bayesian method was 0·524 and 0·216 for cyclophosphamide doses 50 mg/kg and 100 mg/kg, respectively (appendix). A higher value indicates higher desirability. Cyclophosphamide 50 mg/kg had a posterior mean for engraftment of 91·2% (95% credible interval [CrI] 82·6–98·5) and a posterior mean for fatality without graft failure of 0·7% (0–3·3). Cyclophosphamide 100 mg/kg had a posterior mean for engraftment of 85·7% (95% CrI 75·3–95·1) and a posterior mean for fatality without graft failure of 1·4% (0–4·9).

The cumulative incidence of graft failure at 12 months was 11·7% (95% CI 3·5–25·4) for cyclophosphamide 50 mg/kg and 14·6% (5·9–27·2) for cyclophosphamide 100 mg/kg (figure 1). Eight of nine patients had secondary graft failure; one patient had primary graft failure with cyclophosphamide 50 mg/kg. The median time to secondary graft failure was 2·4 months (range 1·9–11·9) with cyclophosphamide 50 mg/kg, and 2·0 months (0·7–5·0) with cyclophosphamide 100 mg/kg. In the cyclophosphamide 50 mg/kg cohort, three patients had graft failure before day 100 and one patient had graft failure after day 100. Of the four patients who had graft failure at the 50 mg/kg dose, two received a second transplant and both are alive. Two patients did not receive a second transplant, and one is alive. Six patients had graft failure at the 100 mg/kg dose, and all six died, three after a second transplant. The remaining patients in the cyclophosphamide 50 mg/kg and 100 mg/kg groups had donor chimerism of more than 95% in peripheral blood or bone marrow at day 100 and day 365. Long-term (ie, >1 year) chimerism was not a prespecified outcome and, therefore, was not a required test for patients enrolled on the trial. All three patients treated at the 0 mg/kg dose received second transplants, and one is alive. No graft failure was reported in patients given cyclophosphamide 150 mg/kg.

Regimen-related toxicity according to organ system is shown in table 4. As in Deeg and colleagues,[6] the most common organ toxicity was pulmonary (grade 3 or 4 dyspnoea or hypoxia including mechanical ventilation); three (8%) patients had regimen-related toxicity with cyclophosphamide 50 mg/kg and four (10%) with cyclophosphamide 100 mg/kg. Grade 3 renal toxicity (requiring dialysis) and mucositis occurred with cyclophosphamide 100 mg/kg but not with 50 mg/kg. Thrombotic microangiopathy was noted at both doses. None of the patients needed dose reductions of their conditioning regimens and none discontinued the conditioning regimen because of drug-related toxicities.

Nine patients who received cyclophosphamide 50 mg/kg had grade 2 (n=6) or 3 acute graft-versus-host disease (n=3) and 11 patients who received cyclophosphamide 100 mg/kg had grade 2 (n=7) or 3 (n=4) acute graft-versus-host disease; no grade 4 acute graft-versus-host disease was reported in either group; incidence of grade 2–4 acute graft-versus-host disease at day 100 was 23·7% (95% CI 10·0–37·4) and 26·8% (13·0–40·6), respectively. Eight patients who received cyclophosphamide 50 mg/kg developed chronic graft-versus- host disease (five mild, two moderate, and one severe) and 13 patients who received cyclophosphamide 100 mg/kg developed chronic graft-versus-host disease (ten mild, two moderate, and one severe); 1 year incidence of chronic graft-versus-host disease was 22·5% (95% CI 10·3–37·5) at 50 mg/kg and 31·7% (18·1–46·2) at 100 mg/kg.

Median follow-up after transplantation was 17 months (IQR range 12–24; 4–26) and 24 months (24–25, 12–51) for patients receiving cyclophosphamide 50 mg/kg and 100 mg/kg, respectively. Overall survival at 1 year for patients receiving 50 mg/kg and 100 mg/kg was 97·4% (95% CI 82·8–99·6) and 80·5% (64·8–89·7), respectively (figure 2).

Three (8%) of 38 patients died in the cyclophosphamide 50 mg/kg cohort from graft failure (n=1) and chronic graft-versus-host disease (n=2); and ten (24%) of 41 patients died in the 100 mg/kg cohort from graft failure (n=6), acute or chronic graft-versus-host disease (n=3), and bacterial sepsis (n=1). All deaths before day 100 were due to primary or secondary graft failure (cyclophosphamide 50 mg/kg, n=1; cyclophosphamide 100 mg/kg, n=2).

EBV reactivation by PCR assay (any degree) was detected in 11 (29%) patients receiving 50 mg/kg of cyclophosphamide and in 17 (41%) patients receiving 100 mg/kg. 24 (86%) of 28 patients had EBV viraemia of 1000 copies per mL or higher, and 20 patients received rituximab (at least 375 mg/m^2, intravenously). EBV-post-transplant lymphoproliferative disorder was reported with cyclophosphamide 50 mg/kg in four (11%) patients and with cyclophosphamide 100 mg/kg in three (7%) patients. No deaths were attributable to EBV viraemia or EBV-post-transplant lymphoproliferative disorder. Because only a few patients received equine-derived antithymocyte globulin (table 2), no subgroup analysis was possible for EBV-post-transplant lymphoproliferative disorder (or other endpoints).

Discussion

We noted that cyclophosphamide at 50 mg/kg and 100 mg/kg with TBI 2 Gy, fludarabine, and antithymocyte globulin allowed for effective conditioning and excellent short-term survival after unrelated donor BMT in patients with severe aplastic anaemia.

Unrelated donor transplantation for severe acquired aplastic anaemia has developed greatly over the past few decades. Data from the Center for International Blood and Marrow Transplant Research (Milwaukee, WI, USA) suggest that outcomes after unrelated donor BMT for severe acquired aplastic anaemia have improved progressively, showing a 3 year survival probability of 76% during 2000–08 compared with 61% before 2005, and 32% before 1998.[21] The previous multicentre trial, done from 1994 to 2004, established a minimum TBI dose of 2 Gy, when used in combination with cyclophosphamide 200 mg/kg and anti-thymocyte globulin, to achieve sustained engraftment of marrow from unrelated donors.[6] On that backbone regimen, we attempted to de-escalate the dose of cyclophosphamide by adding fludarabine to the regimen in the current trial. The cyclophosphamide dose de-escalation approach was based on the hypothesis that the inclusion of fludarabine would provide a cyclophosphamide-sparing effect and that reducing the cyclophosphamide dose would decrease non-haematological toxicity while maintaining sustained engraftment.

Results of the current trial provide important, clinically relevant information. We confirmed our hypothesis that dose de-escalation of cyclophosphamide to as low as 50 mg/kg or 100 mg/kg, in combination with fludarabine, low-dose TBI, and anti-thymocyte globulin allowed engraftment and early survival that compared favourably with data reported with alternative

regimens.[6,12,21] The approach we adopted also assigned cyclophosphamide 50 mg/kg as the most desirable dose. However, complete omission of cyclophosphamide resulted in graft failure in all patients tested, and cyclophosphamide 150 mg/kg was associated with fatal toxicities.[19] We hypothesise that the addition of fludarabine might have led to the excess deaths with 150 mg/kg mainly because of pulmonary and renal toxicity on the basis of the reported synergism between fludarabine and cyclophosphamide.[13,14]

Long-term survival was a secondary endpoint. Although 1 year survival was 97% and 80% with cyclophosphamide 50 mg/kg and 100 mg/kg, respectively, the mean follow-up of the 38 patients taking cyclophosphamide 50 mg/kg was 17 months, with only 16 patients followed up for 2 years or longer, and 15 patients followed up for between 1 year and 2 years after transplantation; seven patients were followed up for 4–11 months. 39 of 41 patients taking cyclophosphamide 100 mg/kg were followed up for 2 years or longer; the remaining two patients were followed up for longer than 1 year. Because all cases of secondary graft failure in the current trial occurred within the first year after transplantation we do not anticipate many more patients developing secondary graft failure. However, deaths might continue to occur and will be monitored and reported as a planned secondary outcome. Although almost all patients had received immunosuppressive treatment before transplantation, and the median interval between diagnosis and transplantation was longer than 6 months, identified as a key prognostic factor,[30] day 100 and 1 year survival were excellent for young adults after unrelated donor transplantation.

Graft failure rates were somewhat higher than those reported with cyclophosphamide 200 mg/kg, TBI 2 Gy, and anti-thymocyte globulin,[6] but not the other regimens for unrelated donor transplantation.[7,21,31] Salvage with second transplants was disappointing, emphasising the need for optimised first transplant regimens. Other investigators have suggested that a longer interval between transplantations and good performance scores predict better survival after second transplantation in patients who sustained graft rejection as well but had a shorter interval between transplant and worse performance scores.[32]

Although a third of patients had EBV reactivation or EBV-post-transplant lymphoproliferative disorder, presumably related to the high dose of anti-thymocyte globulin, no deaths were attributed to EBV-post-transplant lymphoproliferative disorder, presumably because of the close monitoring and prompt intervention, unlike in previous trials, which showed mortality directly attributed to viral (ie, EBV) reactivation ranging from 2·5% to 4%.[6,8] This trial was powered only for a Bayesian desirability comparison between the different doses of cyclophosphamide for the primary endpoint (graft failure and survival at day 100 after transplantation).

No significant differences were noted in performance score, age, or HLA match between the treatment groups, but there might have been differences in unmeasured or unknown factors. The small sample size prevents us from doing exploratory subset analyses. Although the protocol allowed the enrolment of patients up to 65 years of age, the median age was much lower at 21 years. This is reflective of clinical practice—most unrelated donor transplants are offered to children and young adults. The median age of patients in the Japanese and European reports was 17 years and 14 years, respectively.[5,7] Therefore, the generalisability

to older patients of outcomes after unrelated donor transplantation for severe acquired aplastic anaemia cannot be ascertained from this study or the above studies.[5,7]

Transplantation from HLA-mismatched adult unrelated donors for non-malignant haematological diseases has been associated with a doubling in graft failure rates and an 8% absolute difference in overall survival.[31] Because most transplants were HLA matched in the cyclophosphamide 50 mg/kg cohort, the desirability of this dose might at least partly be attributable to HLA matching rather than the dose itself.

In conclusion, in this phase 1–2 trial, we identified cyclophosphamide 50 mg/kg as the most desirable dose in combination with TBI 2 Gy, fludarabine 120 mg/m^2, and anti-thymocyte globulin for engraftment and early survival for unrelated donor transplantation in patients with severe acquired aplastic anaemia. A dose of 100 mg/kg, although slightly less desirable, also provided promising early results. These cyclophosphamide doses should be assessed further, ideally in the context of a randomised trial.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This trial was fully supported by grant number U10HL069294 from the US National, Heart, Lung and Blood Institute and the National Cancer Institute. EL and NLD are US Government employees (National Heart, Lung, and Blood Institute). We thank the clinical investigators who entered this study and managed patients; and Ellen Parker (Emmes Corporation, Rockville, MD, USA) for her assistance in the preparation of the manuscript. The findings reported in this paper are those of the authors.

References

1. Peinemann F, Grouven U, Kröger N, Pittler M, Zschorlich B, Landge S. Unrelated donor stem cell transplantation in acquired severe aplastic anemia: a systematic review. Haematologica. 2009; 94:1732–42. [PubMed: 19648165]
2. Bacigalupo A, Marsh JCW. Unrelated donor search and unrelated donor transplantation in the adult aplastic anaemia patient aged 18–40 years without an HLA-identical sibling and failing immunosuppression. Bone Marrow Transplant. 2013; 48:198–200. [PubMed: 23178545]
3. Maury S, Balère-Appert ML, Chir Z, et al. 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; 92:589–96. [PubMed: 17488681]
4. Viollier R, Socié G, Tichelli A, et al. Recent Improvement in outcome of unrelated donor transplantation for aplastic anemia. Bone Marrow Transplant. 2008; 41:45–50. [PubMed: 17982502]
5. 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. [PubMed: 12130489]
6. Deeg HJ, 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; 108:1485–91. [PubMed: 16684959]
7. Bacigalupo A, Locatelli F, Lanino E, et al. Fludarabine, cyclophosphamide and anti-thymocyte globulin for alternative donor transplants in acquired severe aplastic anemia: a report from the EBMT-SAA Working Party. Bone Marrow Transplant. 2005; 36:947–50. [PubMed: 16205733]

8. Bacigalupo A, Locatelli F, Lanino E, Marsh J, Socié G, Passweg J. Fludarabine, cyclophosphamide, antithymocyte globulin, with or without low dose total body irradiation, for alternative donor transplants, in acquired severe aplastic anemia: a retrospective study from the EBMT-SAA working party. Haematologica. 2010; 95:976–982. [PubMed: 20494932]
9. Lee JH, Choi SJ, Lee JH, et al. Non-total body irradiation containing preparative regimen in alternative donor bone marrow transplantation for severe aplastic anemia. Bone Marrow Transplant. 2005; 35:755–61. [PubMed: 15735661]
10. Kang HJ, Shin HY, Park JE, et al. Successful engraftment with fludarabine, cyclophosphamide, and thymoglobulin conditioning regimen in unrelated transplantation for severe aplastic anemia: a Phase II prospective multicenter study. Biol Blood Marrow Transplant. 2010; 16:1582–88. [PubMed: 20685256]
11. Anderlini P, Acholonu SA, Okoroji GJ, et al. Fludarabine, cyclophosphamide, and antithymocyte globulin for matched related and unrelated allogeneic stem cell transplant in severe aplastic anemia. Leuk Lymphoma. 2011; 52:137–41. [PubMed: 20939697]
12. Marsh JC, Gupta V, Lim Z, et al. Alemtuzumab with fludarabine and cyclophosphamide reduces chronic graft-versus-host disease after allogeneic stem cell transplantation. Blood. 2011; 118:2351–57. [PubMed: 21518925]
13. Plunkett W, Gandhi V, Huang P, et al. Fludarabine: pharmacokinetics, mechanism of action, and rationales for combination therapies. Semin Oncol. 1993; 20(suppl 7):2–12. [PubMed: 8235690]
14. Yamauchi T, Nowak BJ, Keating MJ, et al. DNA repair initiated in chronic lymphocytic leukemia lymphocytes by 4-hydroperoxycyclophosphamide is inhibited by fludarabine and clofarabine. Clin Cancer Res. 2001; 7:3580–89. [PubMed: 11705880]
15. Srinivasan R, Takahashi Y, McCoy JP, et al. Overcoming graft rejection in heavily transfuses and allo-immunised patients with bone marrow failure syndromes using fludarabine-based haematopoietic cell transplantation. Br J Haematol. 2006; 133:305–14. [PubMed: 16643433]
16. Goldberg MA, Antin JH, Guinan EC, Rappeport JM. Cyclophosphamide cardiotoxicity: an analysis of dosing as a risk factor. Blood. 1986; 68:1114–18. [PubMed: 3533179]
17. McDonald GB, Slattery JT, Bouvier ME, et al. Cyclophosphamide metabolism, liver toxicity, and mortality following hematopoietic stem cell transplantation. Blood. 2003; 101:2043–48. [PubMed: 12406916]
18. Payne H, Adamson A, Bahl A, et al. Chemical- and radiation-induced haemorrhagic cystitis: current treatments and challenges. Br J Urol. 2013; 112:885–97.
19. Tolar J, Deeg HJ, Arai S, et al. Fludarabine-based conditioning for marrow transplantation from unrelated donors in severe aplastic anemia: early results of a cyclophosphamide dose de-escalation study show life-threatening adverse events at predefined cyclophosphamide dose levels. Biol Blood Marrow Transplant. 2012; 18:1007–11. [PubMed: 22546497]
20. Schrezenmeier H, Passweg JR, Marsh JCW, et al. Worse outcome and more chronic GHVD with peripheral blood progenitor cells than bone marrow in HLA-matched sibling donor transplants for young patients with severe acquired aplastic anemia. Blood. 2007; 110:1397–400. [PubMed: 17475907]
21. Eapen M, Le Rademacher J, Antin JH, et al. Effect of stem cell source on outcomes after adult unrelated donor transplantation in severe aplastic anemia. Blood. 2011; 118:2618–21. [PubMed: 21677312]
22. Bearman SI, Appelbaum FR, Buckner CD, et al. Regimen-related toxicity in patients undergoing bone marrow transplantation. J Clin Oncol. 1988; 6:1562–68. [PubMed: 3049951]
23. [accessed July 16, 2015] Common Terminology Criteria for Adverse Events v3.0 (CTCAE). http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/ctcaev3.pdf
24. Copelan E, Casper JT, Carter SL, et al. A scheme for defining cause of death and its application in the T cell depletion trial. Biol Blood Marrow Transplant. 2007; 13:1469–76. [PubMed: 18022577]
25. Blood and Marrow Transplant Clinical Trials Network. [accessed Aug 17, 2015] Technical Manual of Procedures, version 3.0. Mar 19. 2013 https://web.emmes.com/study/bmt2/public/MOP/BMT%20CTN%20Technical%20MOP%20v3.pdf

26. Coppoletta S, Tedone E, Galano B, et al. Rituximab treatment for Epstein-Barr Virus DNAemia after alternative-donor hematopoietic stem cell transplantation. Biol Blood Marrow Transplant. 2011; 17:901–07. [PubMed: 20950702]
27. Thall PF, Cook JD. Dose-finding based on efficacy-toxicity tradeoffs. Biometrics. 2004; 60:684–93. [PubMed: 15339291]
28. Cook, JD. Efficacy-toxicity trade-offs based on L^p norms. University of Texas Department of Biostatistics Working Paper Series. Vol. 29. Berkeley Electronic Press; 2006.
29. Coscarelli Schag C, Heinrich RL, Ganz PA. Karnofsky Performance Status revisited: reliability, validity and guidelines. J Clin Oncol. 1984; 2:187–93. [PubMed: 6699671]
30. Bacigalupo A, Socié G, Hamladji RM, et al. Current outcome of HLA identical sibling vs. unrelated donor transplants in severe aplastic anemia: an EBMT analysis. Haematologica. 2015; 100:696–702. [PubMed: 25616576]
31. Horan J, Wang T, Haagenson M, et al. Evaluation of HLA matching in unrelated hematopoietic stem cell transplantation for nonmalignant disorders. Blood. 2012; 120:2918–24. [PubMed: 22829628]
32. Horan JT, Carreras J, Tarima S, et al. Risk factors affecting second HLA-matched sibling donor transplantation for graft failure in severe acquired aplastic anemia. Biol Blood Marrow Transplant. 2009; 15:626–31. [PubMed: 19361755]

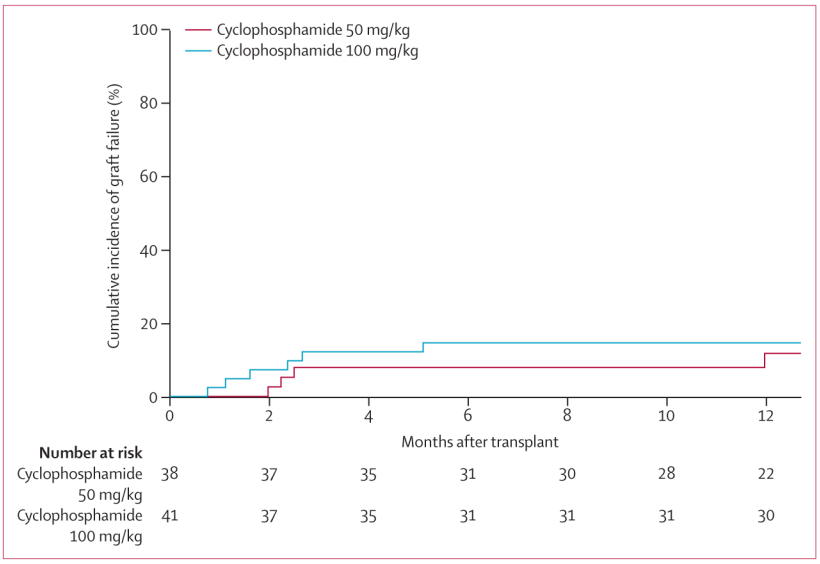

Figure 1.

Cumulative incidence of graft failure by cyclophosphamide dose Number at risk

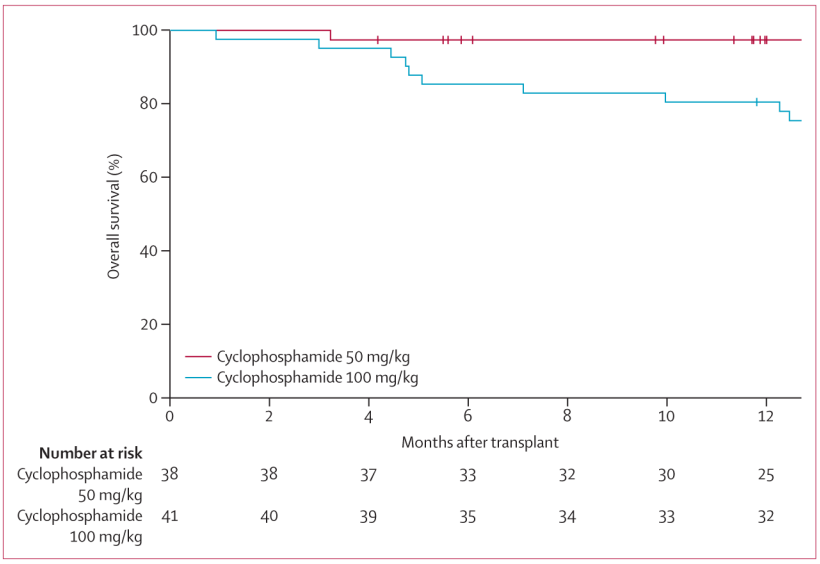

Figure 2.
Overall survival by cyclophosphamide dose Number at risk

Table 1

Accrual of patients during phases 1 and 2

	Number of patients
Phase 1	
Cyclophosphamide 150 mg/kg	6
Cyclophosphamide 100 mg/kg	6
Cyclophosphamide 50 mg/kg	6
Cyclophosphamide 0 mg/kg	3
Phase 2	
Cyclophosphamide 150 mg/kg	8
Cyclophosphamide 100 mg/kg	35
Cyclophosphamide 50 mg/kg*	33
Total	96

*One patient withdrew consent before transplantation.

Table 2

Characteristics of patients in the cyclophosphamide 50 mg/kg and 100 mg/kg cohorts

	Cyclophosphamide 50 mg/kg (n=38)	Cyclophosphamide 100 mg/kg (n=41)
Age (years)		
Median (range)	24·5 (0·5–65·9)	17·6 (1·9–63·3)
<18	11 (29%)	21 (51%)
18–40	18 (47%)	14 (34%)
>40	9 (24%)	6 (15%)
Sex		
Male	19 (50%)	21 (51%)
Female	19 (50%)	20 (49%)
Recipient's cytomegalovirus status		
Positive	17 (45%)	26 (63%)
Negative	21 (55%)	15 (37%)
Race		
White	30 (79%)	34 (83%)
African-American	3 (8%)	2 (5%)
Asian	1 (3%)	2 (5%)
Other or unknown	4 (11%)	3 (7%)
Ethnic origin		
Hispanic	8 (21%)	6 (15%)
Non-Hispanic	29 (76%)	32 (78%)
Unknown or not answered	1 (3%)	3 (7%)
Karnofsky performance status[29]	38 (100%)	41 (100%)
100	12 (32%)	18 (44%)
90	17 (45%)	15 (37%)
80	7 (18%)	3 (7%)
70	2 (5%)	4 (10%)
60	0 (0%)	1 (2%)
Immunosuppressive therapy before transplant		
Yes	35 (92%)	41 (100%)
No	3 (8%)	0 (0%)
Time between diagnosis to transplant (months; median, range)	8·2 (1·2–298·4)	10·4 (1·6–132·6)
Donor–recipient HLA match		
Matched for HLA A, B, C, DRB1	31 (82%)	27 (66%)
Single HLA-locus mismatch	7 (18%)	14 (34%)
Type of anti-thymocyte globulin administered		

	Cyclophosphamide 50 mg/kg (n=38)	Cyclophosphamide 100 mg/kg (n=41)
Rabbit-derived	29 (76%)	33 (80%)
Horse-derived	9 (24%)	7 (17%)
None	0 (0%)	1 (2%)
Total nucleated cell dose (infused) × 10^8/kg * (median, IQR)	2·89 (1·63–4·15)	3·08 (1·84–4·79)
Follow-up of surviving patients (months; median, IQR)	17 (12–24)	24 (24–25)

Data are number (%), unless otherwise indicated.

* No significant difference between the two groups (p=0·17).

Table 3

Outcomes at day 100 in the cyclophosphamide 50 mg/kg and 100 mg/kg cohorts

	Cyclophosphamide 50 mg/kg (n=38)	Cyclophosphamide 100 mg/kg (n=41)
Graft failure, primary and secondary	3 (8%)	6 (15%)
Survival	37 (97%)	39 (95%)
Major regimen-related toxicity * (grade 3 or higher)	4 (11%)	9 (22%)
Alive and engrafted	35 (92%)	35 (85%)

Data are number (%).

* Defined as severity of grade 4 in any organ system or grade 3 for pulmonary, cardiac, renal, oral, mucosal, or hepatic toxicity, in keeping with the approach adopted in Deeg and colleagues.[6]

Table 4

Regimen-related toxicities in the cyclophosphamide cohorts according to organ system until day 100 after transplant

	Cyclophosphamide 0 mg/kg (n=3)	Cyclophosphamide 50 mg/kg (n=38)	Cyclophosphamide 100 mg/kg (n=41)	Cyclophosphamide 150 mg/kg (n=14)	Total (n=96)
Cardiac toxicities *					
Grade 0–2	3 (100%)	38 (100%)	41 (100%)	13 (93%)	95 (99%)
Grade 3	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Grade 4	0 (0%)	0 (0%)	0 (0%)	1 (7%)	1 (1%)
Bladder toxicities †					
Grade 0–3	3 (100%)	38 (100%)	41 (100%)	14 (100%)	96 (100%)
Grade 4	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Renal toxicities *					
Grade 0–2	3 (100%)	38 (100%)	38 (93%)	11 (79%)	90 (94%)
Grade 3	0 (0%)	0 (0%)	3 (7%)	2 (14%)	5 (5%)
Grade 4	0 (0%)	0 (0%)	0 (0%)	1 (7%)	1 (1%)
Pulmonary toxicities *					
Grade 0–2	3 (100%)	35 (92%)	37 (90%)	11 (79%)	86 (90%)
Grade 3	0 (0%)	3 (8%)	3 (7%)	1 (7%)	7 (7%)
Grade 4	0 (0%)	0 (0%)	1 (2%)	2 (14%)	3 (3%)
Hepatic toxicities *					
Grade 0–2	3 (100%)	38 (100%)	40 (98%)	12 (86%)	93 (97%)
Grade 3	0 (0%)	0 (0%)	1 (2%)	1 (7%)	2 (2%)
Grade 4	0 (0%)	0 (0%)	0 (0%)	1 (7%)	1 (1%)
CNS toxicities †					
Grade 0–3	3 (100%)	38 (100%)	41 (100%)	14 (100%)	96 (100%)
Grade 4	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Stomatitis or mucositis *					
Grade 0–2	3 (100%)	38 (100%)	38 (93%)	14 (100%)	93 (97%)

	Cyclophosphamide 0 mg/kg (n=3)	Cyclophosphamide 50 mg/kg (n=38)	Cyclophosphamide 100 mg/kg (n=41)	Cyclophosphamide 150 mg/kg (n=14)	Total (n=96)
Grade 3	0 (0%)	0 (0%)	3 (7%)	0 (0%)	3 (3%)
Grade 4	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Gastrointestinal toxicity[†]					
Grade 0–3	3 (100%)	38 (100%)	41 (100%)	14 (100%)	96 (100%)
Grade 4	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Thrombotic microangiopathy[†]					
Grade 0–3	3 (100%)	37 (97%)	40 (98%)	14 (100%)	94 (98%)
Grade 4	0 (0%)	1 (3%)	1 (2%)	0 (0%)	2 (2%)
Overall					
Grade ≥4	0 (0%)	4 (11%)	9 (22%)	6 (43%)	19 (20%)

Data are number (%). The frequency of toxicities in each column does not add up to the total number of patients (overall) because individual patients might have had more than one organ toxicity. Percentages do not add up to 100% because of rounding.

[*] Grades 0–2 captured in one category.

[†] Grades 0–3 captured in one category.

Lancet Haematol. Author manuscript; available in PMC 2016 September 02.