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Fludarabine-Based Conditioning for Marrow Transplantation from Unrelated Donors in Severe Aplastic Anemia: Early Results of a Cyclophosphamide Dose Deescalation Study Show Life-Threatening Adverse Events at Predefined Cyclophosphamide Dose Levels

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Abstract

Excessive adverse events were encountered in a Phase I/II study of cyclophosphamide (CY) dose deescalation in a fludarabine-based conditioning regimen for bone marrow transplantation from unrelated donors in patients with severe aplastic anemia. All patients received fixed doses of antithymocyte globulin, fludarabine, and low-dose total body irradiation. The starting CY dose was 150 mg/kg, with deescalation to 100 mg/ kg, 50 mg/kg, or 0 mg/kg. CY dose level 0 mg/kg was closed due to graft failure in 3 of 3 patients. CY dose level 150 mg/kg was closed due to excessive organ toxicity (n = 6) or viral pneumonia (n = 1), resulting in the death of 7 of 14 patients. CY dose levels 50 and 100 mg/kg remain open. Thus, CYat doses of 150 mg/kg in combination with total body irradiation (2 Gy), fludarabine (120 mg/m^2), and antithymocyte globulin was associated with excessive organ toxicity.

Keywords

Bone marrow transplantation; Stem cell transplantation; Matched unrelated donor; Antithymocyte globulin

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INTRODUCTION

Unrelated donor bone marrow transplantation (BMT) is an important therapy for patients with severe acquired aplastic anemia (SAA) who lack suitable related donors and who fail to respond or who experience recurrence after immunosuppressive therapy [1]. Outcomes of unrelated donor BMT have improved over the last few decades [2,3], presumably due to closer donor–recipient HLA matching and improved supportive care. However, the optimal preparative regimen remains unknown.

Kojima et al. [4] reported on 154 patients who underwent BMT after conditioning with various regimens. Five-year overall survival was 56%, and 11% of the patients experienced graft failure. The conditioning regimens included total body irradiation (TBI; 200–1000 Gy or limited field irradiation), cyclophosphamide (CY; 120–200 mg/kg), and antithymocyte globulin (ATG). Deeg and coworkers [5,6] evaluated a TBI dose deescalation regimen in 87 patients and found that the optimum TBI dose was 200 cGy given along with CY (200 mg/kg) and horse ATG (ATGAM 90 mg/kg).[5,6] Graft failure occurred in 5% of patients. Overall, 55% of patients were alive; the dose-limiting toxicity was diffuse pulmonary injury. Recently, the need for TBI has been called into question, and fludarabine (FLU) has been incorporated in some conditioning regimens as a possible alternative to low-dose TBI [7–11]. Although most regimens still use CY, the drug's role and dose in combination regimens have not been well established [1].

The Blood and Marrow Transplant Clinical Trials Network (BMT CTN) designed a multicenter Phase I/ II study to identify the optimal dose of CY in a BMT preparative regimen that also incorporates FLU, ATG, and low-dose TBI [12]. To notify the transplantation community of these critical interim results, we now report serious adverse events in the 2 of 4 study cohorts with the highest (150 mg/kg) and the lowest (0 mg/kg) CY dose levels. The trial continues to accrue patients to the 50 mg/kg and 100 mg/kg dose levels.

PATIENTS AND METHODS

BMT CTN 0301 is a Phase I/II study evaluating the optimal dose of CY within a preparative regimen of FLU, ATG, and TBI before unrelated BMT for SAA. The trial is registered at www.clinicaltrials.gov (NCT00326417). The study protocol has been approved by the Institutional Review Board at each participating center. Written informed consent is obtained in accordance with the Declaration of Helsinki before the initiation of conditioning therapy. Eligibility criteria are SAA, age up to 65 years, adequate organ function, and an available unrelated adult marrow donor matched for HLA-A, -B, -C, and -DRB1 or mismatched at a single locus. Transplantation of peripheral blood progenitor cells is prohibited, in view of the inferior patient outcome with this stem cell product in related donor transplantation [13], as was recently confirmed in a study of unrelated donor transplantation [14]. Patients with Fanconi anemia or other marrow failure syndromes are excluded.

All patients receive a fixed dose of ATG (either Thymoglobulin 3 mg/kg i.v. or ATGAM 30 mg/kg i.v. daily on days −4 to −2), FLU (30 mg/m^2 i.v. daily on days −5 to −2), and low-dose TBI (200 cGy on day −1) (Figure 1). Graft-versus-host disease prophylaxis is provided by a calcineurin inhibitor (cyclosporine or tacrolimus) and methotrexate. Day 0 is the day of marrow infusion.

The Phase I component of the trial tested 4 CY dose levels—150 mg/kg (50 mg/kg/day on days −4 to −2), 100 mg/kg (on days −3 to −2), 50 mg/kg (on day −2), and 0 mg/kg—to establish the optimal CY dose. The intention was to test each dose level in 6 patients, unless

graft failure or toxicity boundaries were crossed, and then enroll additional patients to the optimal dose level in the Phase II portion of the trial, using adaptive Bayesian criteria to rank the desirability of the CY doses (Figure 1). Patients were evaluated for graft failure and early mortality at days 42 and 100. The aim of the safety monitoring was to minimize the number of patients treated at a dose level at which the probability of engraftment is too low or the probability of severe toxicity (including death) is too high, and to maximize the number of patients treated at the "optimal" dose. Stopping guidelines were devised incorporating both endpoints. The Phase II portion of the trial was designed to refine the dose selection and to provide a more precise estimate of efficacy at the optimal dose.

RESULTS AND DISCUSSION

The study opened for accrual in January 2006. As of June 26, 2011, a total of 61 patients had been enrolled. Characteristics of patients enrolled at the 0 mg/kg and 150 mg/kg CY dose levels are summarized in Table 1. The Phase I portion was completed (21 patients treated) in August 2007. CY dose level 0 mg/kg was closed in August 2007 during Phase I, because of an excessive number of graft failures. All 3 enrolled patients (all treated with Thymoglobulin) developed secondary graft failure (at days +29, +52, and +84), defined as initial neutrophil engraftment followed by a decline in the neutrophil count to $< 0.5 \times 10^9$/L for 3 consecutive measurements on different days, unresponsive to growth factor therapy. All 3 patients received a second allograft. One patient is alive at 23 months after BMT, and 2 patients died, one due to interstitial pneumonia on day +114 and the other from adult respiratory distress syndrome on day +200 (Table 1).

At the completion of Phase I, CY dose level 150 mg/ kg was chosen as the likely optimal dose, and 8 additional patients were accrued at this level (total n = 14). This dose level was closed in March 2008 because of excessive organ toxicity. Seven of the 14 patients died (including 7 of the last 8 enrolled). Four died from organ failure, 2 from acute respiratory distress syndrome, and 1 from pneumonia due to parainfluenza virus type 3 infection (Table 1). Accrual continues at CY 100 mg/kg (currently 35 patients). Enrollment is also allowed at the CY 50 mg/kg dose level (currently 13 patients), whereas CY 100 mg/kg has been paused to allow follow-up of the enrolled patients through the predefined 42- and 100-day monitoring points. To ensure that the CY 50 mg/kg dose level undergoes adequate testing and to achieve a more balanced accrual in the 2 remaining groups, we have proposed a revised patient allocation plan that favors the latter dose level to the study Data Safety Monitoring Board.

The excessive graft failures occurred when CY was omitted from the regimen. One of the 3 transplants was mismatched at the HLA-DRB1 locus, and the other 2 patients received relatively low graft cell doses. Graft rejection is a recognized complication in these settings [1]. The lethal organ toxicities (primarily pulmonary) seen in the CY 150 mg/kg cohort were unexpected, given that the safe use of similar (and even higher) doses of CY has been reported in other transplantation regimens and settings [1,4]. Thus, this toxicity might have been related to the other agents in the conditioning regimen. Of note, pulmonary toxicity is the main adverse effect of even low-dose TBI [5,6]. Furthermore, the administration of FLU with high-dose CY may lead to interactions that magnify the known effects of low-dose TBI.

In conclusion, our early analysis of this trial has revealed 2 important findings regarding the regimen tested in this study. First, the omission of CY was associated with a higher-than-expected secondary graft failure rate, although 2 of 3 patients in this group received lower-than-recommended cell doses. Second, a CY dose of 150 mg/kg was associated with higher-than-expected transplantation-related mortality. The current data suggest caution in

combining 150 mg/ kg CY, ATG, FLU, and TBI (or in omitting the CY entirely) in transplantation regimens targeting SAA, and highlight the need for well-controlled and carefully monitored multicenter studies of new dosing regimens before widespread implementation.

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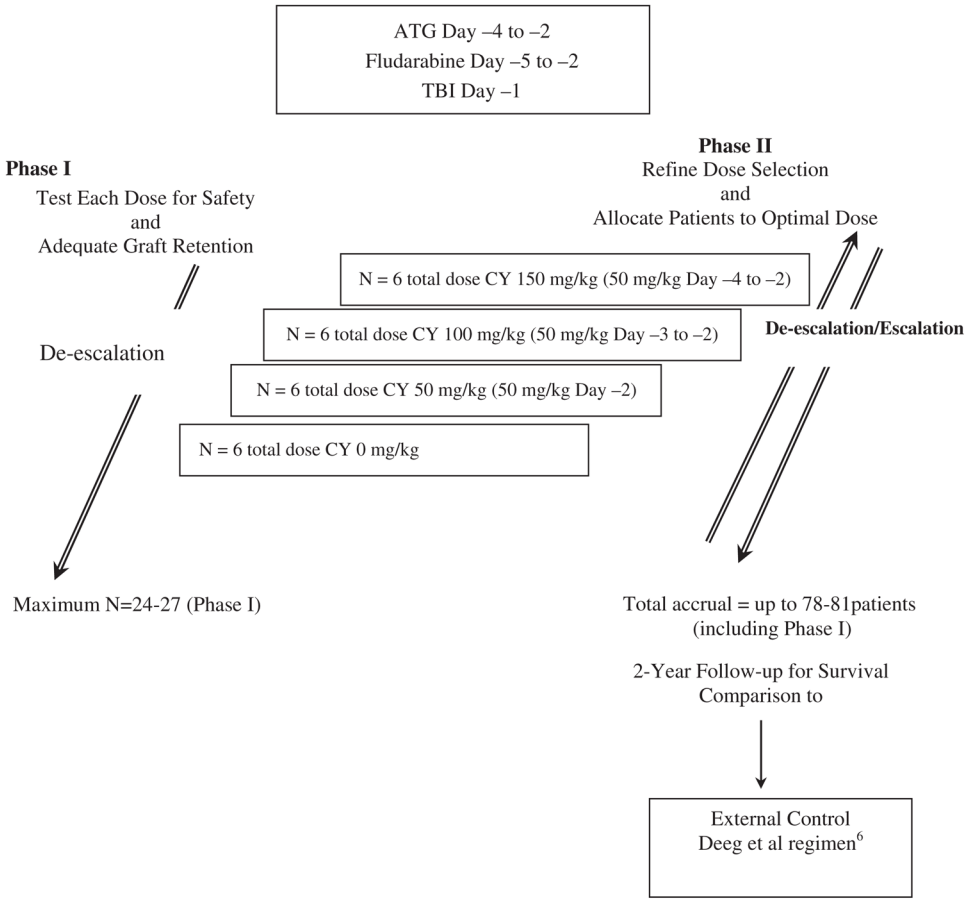

Figure 1. Study design

BMT CTN: Blood and Marrow Transplant Clinical Trials Network; CY: Cyclophosphamide; ATG: antithymocyte globulin; TBI: Total Body Irradiation.

Table 1

Patient Characteristics and Outcomes

Patient	CY Dose, mg/kg	Time from Diagnosis to BMT, Months	Age, Years	HLA Match	Previous Immunosuppressive Treatment	TNC Dose Infused, $\times 10^7$/kg	Recipient–Donor Sex Match	Recipient–Donor CMV Match	Engraftment/ Graft Failure	Survival Status	Cause of Death
1	0	17	22	7/8	ATG, S	23.5	M/M	P/N	Engrafted at day 22; secondary graft failure at day 29	Died, day 114	ARDS (diffuse alveolar damage at postmortem)
2	0	62	17	8/8	ATG, S, CSA	10.8	F/M	P/P	Engrafted at day 23; secondary graft failure at day 84	Alive, day 732	—
3	0	7	61	8/8	ATG, CSA	0.29	F/F	P/P	Engrafted at day 24; secondary graft failure at day 52	Died, day 200	CMV pneumonia
4	150	5	16	8/8	ATG, S, CSA	36.0	M/M	P/P	Engrafted, day 21	Alive, day 838	—
5	150	7	5	8/8	No treatment	30.0	F/M	P/N	Engrafted, day 20	Alive, day 742	—
6	150	7	8	8/8	ATG, S, CSA	55.4	F/F	N/N	Engrafted, day 26	Alive, day 704	—
7	150	19	18	8/8	ATG, S, CSA	44.5	F/F	P/N	Engrafted, day 22	Alive, day 763	—
8	150	21	18	8/8	ATG, CSA, MMF	19.8	F/F	P/N	Engrafted, day 26	Alive, day 703	—
9	150	12	15	8/8	ATG, S, CSA	38.8	F/F	N/P	Engrafted, day 31	Died, day 135	Pulmonary failure
10	150	44	17	7/8	Androgens, ATG, S, CSA, sirolimus	32.1	F/F	P/U	Engrafted, day 22	Died, day 92	ARDS
11	150	77	20	7/8	Unknown	12.1	M/F	N/N	Engrafted, day 20	Died, day 82	Multiorgan failure
12	150	15	61	8/8	ATG, CSA,	20.0	M/M	P/N	Primary graft failure by day 42	Died, day 62	Cardiac failure
13	150	15	23	8/8	ATG, CSA, alemtuzumab	19.7	M/F	N/N	Engrafted, day 23	Alive, day 817	—
14	150	7	44	8/8	ATG, S, CSA	21.9	F/F	P/P	Engrafted, day 26	Died, day 148	Parainfluenza virus type 3 pneumonia
15	150	12	52	8/8	ATG, CSA	23.1	F/M	P/N	NA	Died, day 1	ARDS (diffuse acute lung injury postmortem)
16	150	20	9	7/8	ATG, S, CSA	NA	M/F	P/P	NA	Died 1 day before planned BMT	Pulmonary failure
17	150	8	15	7/8	ATG, S, CSA	0.07	F/M	N/N	Engrafted, day 22	Alive, day 897	—

CSA indicates cyclosporine; S, corticosteroids; ATG, antithymocyte globulin; MMF, mycophenolate mofetil; SAA, severe aplastic anemia; CMV, cytomegalovirus; P, positive; N, negative; U, unknown; ARDS, adult respiratory distress syndrome; IP, interstitial pneumonia; BMT, bone marrow transplantation; CY, cyclophosphamide; TNC, total nucleated cells; NA, not applicable.

The transplantation in patient 1 was mismatched at the HLA-DRB1 locus. Engraftment was defined as the achievement of an absolute neutrophil count (ANC) $\geq 0.5 \times 10^9/L$ for 3 consecutive measurements on different days. Primary graft failure was defined by a lack of neutrophil engraftment, that is, ANC $< 0.5 \times 10^9/L$ on 3 consecutive measurements on different days by 100 days after transplantation. See the text for the definition of secondary graft failure.