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# Reduced-intensity conditioning regimen with thiotepa and fludarabine followed by allogeneic blood stem cell transplantation in haematological malignancies

EP Alessandrino, P Bernasconi, AA Colombo, D Caldera, L Malcovati, D Troletti, L Vanelli, M Varettoni, F Montanari and M Lazzarino

*Bone Marrow Transplantation Unit, Division of Haematology, IRCCS Policlinico San Matteo, Pavia, Italy*

**Summary:**

**The aim of this study was to investigate thiotepa (TT) and fludarabine (Fluda) as a preparative regimen for allogeneic peripheral stem cell transplant in patients not eligible for a standard myeloablative regimen due to comorbidities and/or poor performance status. TT was given at a dose of 10 mg/kg over 2 days and Fluda at 125 mg/m$^2$ over 5 days. In all, 21 patients (14 male, seven female; 10 acute leukaemia, eight myelodysplastic syndrome, two non-Hodgkin's lymphoma, one Hodgkin's disease) were treated. The median age was 51 years (range 30–55 years). All patients achieved full donor-type chimaerism. Adverse events included mild nausea and vomiting in two patients and a slight increase of serum amylase in three. A total of 13 patients received RBC transfusions (median 6 U, range 1–23), and all received platelets (median 4 U, range 1–27). Four patients died of nonrelapse causes and five of relapse. The 1-year probabilities of transplant-related mortality and relapse were 19 and 29%, respectively. In total, 12 patients remain in complete remission (median follow-up: 786 days). The 3-year overall survival probability was 58%. We conclude that this regimen is feasible and well tolerated.**
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Transplant-related toxicity and mortality (TRM) are high with conventional allografts, particularly in patients with advanced age, comorbidities, previous heavy treatment and/or poor performance status.[1–3] Nonmyeloablative regimens are used for the treatment of patients who are considered ineligible for myeloablative conditioning.[4–7]

Correspondence: Dr EP Alessandrino, Bone Marrow Transplantation Unit, Division of Haematology, IRCCS Policlinico San Matteo, Viale Golgi N. 19, Pavia 27100, Italy; E-mail: alessandrino@smatteo.pv.it
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There are at least two types of nonmyeloablative regimens:[8] one type is a true nonablative conditioning, which does not eradicate recipient haematopoiesis, such as regimens including standard chemotherapy doses or low doses of total body irradiation, which can be given without stem cell support and a mixed chimaerism is generally detected early after transplant;[9–11] the other type of conditioning, for which the term reduced-intensity conditioning seems to be more appropriate, is generally based on the use of an alkylating agent and a purine analogue, and requires stem cell support, but has a lower extra-haematological toxicity than conventional myeloablative regimens.[12–14]

Based on the observation that patients with aggressive disease may relapse rapidly after a truly nonablative conditioning, reduced-intensity conditioning regimens have been utilized for allogeneic transplantation in a variety of patients not qualifying for a standard myeloablative preparative regimen but considered to be at risk of relapse. Following a preliminary study including six patients,[15] we started a phase two study on patients affected with haematological malignancies aimed at evaluating the combination thiotepa and fludarabine (TT–Fluda) given as a preparative regimen for allogeneic peripheral haematopoietic stem cell transplantation. TT is an alkylating agent with a prevalent haematopoietic stem cell toxicity and an antitumoral activity. When TT is given at the dose of 180 mg/m$^2$, it requires haematopoietic stem cell support, while extrahaematological toxicities involving skin, liver and central nervous system (CNS) may be seen only at dosages above 500 mg/m$^2$.[16,17] TT was supplemented with fludarabine at the dose of 125 mg/m$^2$ over 5 days. Fluda, which is extensively used in several reduced-intensity or nonmyeloablative schedules,[4–6,13] was given to induce immunosuppression and to enhance the antileukaemic effect of TT. We have hypothesized that the TT–Fluda combination could induce adequate immunosuppression to achieve a stable donor-haematopoietic chimaeric status with acceptable toxicity.

This report focuses on the feasibility and toxicity of TT–Fluda combination in 21 patients affected with various haematological malignancies who were poor candidates for conventional myeloablative conditioning regimen due to advanced age or comorbidities.

## Patients, materials and methods

Between February 2000 and December 2003, 21 patients underwent reduced-intensity allogeneic haematopoietic stem cell transplantation with TT–Fluda. The protocol was approved by the institutional ethical committee. Inclusion criteria were diagnosis of haematological malignancy with indication for allogeneic transplant, available HLA-identical related donor providing peripheral blood progenitor cells (PBPC), age between 50 and 60 years or younger than 50 but with contraindications for standard conditioning regimen due to comorbidities including cardiac ejection fraction less than 50%, carbon monoxide diffusion capacity less than 50%, abnormal hepatic function tests or extensive prior chemotherapy. After giving informed signed consent, patients received as a conditioning regimen TT–Fluda, rescued by allogeneic peripheral stem cell infusion from an HLA-identical sibling donor. TT was given at the dose of 5 mg/kg intravenously over 3 h on days −8 and −7, Fluda at the dose of 25 mg/m$^2$ over 1 h on days −6, −5, −4, −3 and −2 before stem cell infusion. Allogeneic peripheral blood stem cells were infused at day 0 or +1 through a central venous catheter.

GvHD prophylaxis consisted of cyclosporin A (Cs-A) at the dosage of 1.5 mg/kg/day continuous infusion from day −5 and methotrexate (MTX) 10 mg/m$^2$ on day +1 i.v. and 8 mg/m$^2$ on days +3, +6 and +11 after transplant. After the engraftment, Cs-A was administered orally at the dose of 3 mg/kg twice a day. Doses were adjusted to maintain plasma trough level concentrations between 150–350 mg/dl. From day +60 in the absence of acute (aGvHD) or chronic GvHD (cGvHD), the Cs-A dose was tapered by 20% every 2 weeks until withdrawal.

### *Donor characteristics*

The median donor age was 48 years (range 32–57 years), 10 male, 11 female. All donors except one were HLA identical siblings; one patient received the transplant from a class I antigen-mismatched related donor. Stem cells were mobilized by G-CSF 5 $\mu$g/kg twice a day; cells were collected by leukaphaereses after or 5 days G-CSF therapy in order to harvest a minimum dose of $4 \times 10^6$ CD34+ cells/kg of recipient body weight.

### *Supportive care*

All patients were treated in a single room with HEPA filtration until engraftment; they received irradiated blood products. Patients received colistin and neomycin as gut prophylaxis. Oral amphotericine B was given as a fungal prophylaxis; in patients treated with high doses of steroids for aGvHD, fluconazole 400 mg/day was added. Trimethoprim-sulphametoxazole was given for *Pneumocystis carinii* prophylaxis two times a week for 1 year after transplant from the time of engraftment. Patients were monitored until day +100 after transplant for CMV antigenaemia; positive antigenaemia was defined as two or more positive cells out of 200 000 peripheral blood cells. Patients with CMV reactivation were treated with ganciclovir 5 mg/kg twice daily; the therapy was stopped after two consecutive negative tests.

### *Engraftment and chimaerism analysis*

Granulocytic engraftment was defined as the first of 3 consecutive days with a neutrophil count $\geqslant 0.5 \times 10^9$ and platelet engraftment as the first of 6 days with an unsupported platelet count $\geqslant 20 \times 10^9$/l. Peripheral blood and marrow were analysed for haematopoietic donor–recipient chimaerism at the time of engraftment and at days +30, +60, +90, +180 and +360 by minisatellite PCR amplification or XY-FISH. The PCR analysis was performed by Genescan Analysis, using the 377 PE applied Biosystem. The following minisatellite regions were evaluated as follows: D3S 1349, FGA, D12S676, D19S246, D19S253, D20S85, D2S165, D2S160, D2S367, D2S125, D2S206 and D2S117. XY-FISH was carried out in sex-mismatched donor recipient pairs as reported previously.[18]

### *Cytometric analysis of lymphocyte subsets*

Immunophenotypic analysis of lymphocyte subsets was performed on peripheral blood samples collected at the time of engraftment, at days +30, +60, +90, +180 and +360 post transplant. Peripheral blood lymphocyte subsets were studied by direct immunofluorescence and flow cytometry using a lyse-no-wash sample processing of total peripheral blood. The following monoclonal antibodies were utilized: anti-CD45, anti-CD3, anti-CD4, anti-CD8, anti-CD45RA, anti-CD45RO, anti-CD16, anti CD56 and anti-CD19.

### *Toxicity*

Regimen-related toxicity was graded according to the Seattle criteria.[19] All nonhaematological organ dysfunctions were evaluated from day −8 until day +30. aGvHD and cGvHD were classified according to published guidelines.[20,21]

### *Statistical analysis*

Overall survival, transplant-related mortality and probability of relapse were plotted using the method of Kaplan and Meier. Overall survival was calculated from transplantation until death from any cause. TRM was measured from the date of transplant until death occurring without evidence of relapse. The actuarial probability of relapse was calculated from transplantation until relapse. Patients who died in complete remission (CR) were censored at the time of death.

## Results

A total of 21 patients (14 male and seven female) received stem cell transplants from a related donor. The median age was 51 years ranging from 30 to 55 years. All patients were affected with haematological malignancies: eight with myelodysplastic syndrome (MDS), 10 with acute leukaemia

(AL), two with non-Hodgkin's lymphoma (NHL), one with Hodgkin's disease (HD).

Among MDS patients, three were affected with refractory anaemia (RA), one with RA with ringed sideroblasts (RARS), three with RA with excess of blasts (RAEB) and one with chronic myelomonocytic leukaemia in advanced phase. All MDS patients had active disease and received transplants as a front-line therapy. AL patients were classified as acute lymphoblastic leukaemia one case, acute myeloid leukaemia (AML) nine cases, five of whom had a previous history of MDS (AML-MDS). Six AL patients were in their first CR, two were in partial remission (PR) and two had refractory disease. Among patients with lymphoma, one patient had a refractory mantel cell lymphoma with bulky splenomegaly and the other had a follicular lymphoma in CR with a previous history of autologous transplant. One patient with HD was in relapse after autologous transplantation. The main characteristics of patients and risk factors leading to their inclusion in the study are shown in Table 1. The median number of total nucleated infused cells was $14.2 \times 10^8$/kg (range 3.54–37) with a median number of CD34+ cells equal to $6.8 \times 10^6$/kg (range 4–32.47) and median number of CD3+ cells equal to $3.41 \times 10^8$/kg (range 1.2–4.7).

### *Engraftment and chimaerism analysis*

Neutrophil engraftment was successful in all patients. The time to an absolute neutrophil count (ANC) $\geqslant 0.5 \times 10^9$/l ranged from 11 to 19 days (median value 13 days). In patients with normal peripheral blood counts before transplant (eight cases), duration of severe neutropenia (ANC $< 0.5 \times 10^9$/l) was 12 days (range 9–14 days) starting from day −1 to +3. Five patients had severe thrombocytopenia before transplant. In cases with normal platelet counts at the beginning of conditioning regimen, platelet recovery occurred at day +9 (range 5–12 days). The duration of platelet transfusion dependency was 4 days (range 2–13 days). One patient with a major ABO incompatibility developed red cell aplasia associated with thrombocytopenia. She died at day +60 of aGvHD grade IV (Table 2).

Out of 21 patients, 18 achieved 100% donor chimaerism at the engraftment, while three patients displayed a mixed chimaerism (1, 2.7, 5% of recipient cells) on peripheral blood. All patients reached full donor engraftment at day +30 in both peripheral blood and marrow.

### *Cytometric analysis of lymphocyte subset*

Peripheral blood samples were evaluated at the time of engraftment, on days +30, +60, +90, +180 and +360 post transplant. The analysis of CD3+CD4+ lymphocytes showed values exceeding the threshold of $0.4 \times 10^9$/l from day +180 post transplant. From this time, a progressive increase in CD4+CD45RA+ cells was observed. CD8+ lymphocytes showed a progressive expansion from day +30 post transplant, associated with an

**Table 1** Clinical characteristics of patients

| *UPN* | *Age/sex* | *Diagnosis* | *Comorbidities* | *Interval diagnosis – HSCT (months)* | *Disease status at transplant* | *Blood cell count at HSCT* | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | *ANC ($\times 10^9$/l)* | *Plt ($\times 10^9$/l)* | *Hb (g/l)* |
| 417 | 48/M | Mantle cell lymphoma | Hepatitis B | 29 | Refractory disease | 1.86 | 38 | 13 |
| 476 | 54/M | B-ALL | — | 8 | I CR | 2 | 549 | 11.4 |
| 486 | 50/M | AML | — | 13 | I CR | 5.5 | 168 | 14.6 |
| 490 | 54/M | AML-MDS | — | 12 | I CR | 2.9 | 144 | 15 |
| 498 | 44/M | AML-MDS | Previous sepsis | 11 | Refractory disease | 0.25 | 12 | 7.5 |
| 512 | 52/M | RA | — | 2 | Untreated disease | 0.4 | 26 | 8.7 |
| 519 | 48/F | AML | Previous invasive fungal infection | 4 | I CR | 4.3 | 322 | 15.3 |
| 527 | 53/F | AML-MDS | Diabetes mellitus | 5 | I CR | 3.34 | 181 | 11.6 |
| 539 | 44/M | AML-MDS | Perianal fistula and apical granuloma | 12 | I CR | 5.8 | 102 | 15.3 |
| 541 | 50/F | RAEB | Previous CNS bleeding | 5 | Untreated disease | 1.55 | 61 | 8.2 |
| 555 | 55/F | RAEB-2 | Mitral stenosis | 3 | Untreated disease | 0.4 | 7 | 8.6 |
| 558 | 54/M | CMML | Liver function abnormalities | 5 | Untreated disease | 1.9 | 38 | 11.6 |
| 453 | 35/M | HD | Previous autograft | 41 | Refractory disease | 7.4 | 215 | 10.6 |
| 578 | 51/M | RA | — | 45 | Untreated disease | 1.81 | 13 | 8.2 |
| 591 | 54/F | AML-MDS | — | 6 | PR | 2.62 | 61 | 10 |
| 461 | 30/F | Follicular NHL | Previous autograft | 44 | III CR | 4.48 | 173 | 11.5 |
| 610 | 48/M | AML-MDS | Previous history of toxic hepatitis | 6 | PR | 5.33 | 310 | 13 |
| 614 | 51/F | RAEB | — | 13 | Untreated disease | 0.35 | 109 | 8.1 |
| 618 | 52/M | AML-MDS | — | 11 | Untreated disease | 8.4 | 140 | 7.9 |
| 646 | 44/M | RARS | Haemochromatosis-associated liver function abnormalities | 38 | A | 0.65 | 137 | 7.9 |
| 659 | 54/M | RA | Cardiac arrhythmia | 6 | Untreated disease | 2.4 | 60 | 7.7 |

**Table 2** Haematological recovery and post-transplant outcome

| *UPN* | *Characteristics of cell infused* | | *Engraftment* | | *Transfusion requirement* | | *Severe neutropenia (days)* | *Days of fever* | *aGvHD* | *cGvHD* | *Outcome* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | *TNC* ($\times 10^8$/kg) | *CD34+* ($\times 10^6$/kg) | *ANC* $>0.5 \times 10^9$/l | *PLT* $>20 \times 10^9$/l | *RBC* (*Units*) | *PLT* (*Units*) | | | | | |
| 417 | 4.1 | 6.75 | 13 | 17 | 0 | 7 | 13 | 0 | I (skin) | Extensive | Splenic relapse at day +266 |
| 476 | 10.87 | 7.37 | 14 | 9 | 1 | 2 | 14 | 2 | I (skin) | Limited | Alive in CR at day +1121 |
| 486 | 19.50 | 4.4 | 11 | 11 | 0 | 1 | 12 | 23 | IV (gut) | NE | Dead due to aGvHD at day +41 |
| 490 | 15.70 | 6.14 | 13 | 15 | 0 | 1 | 13 | 1 | No | No | Alive in CR at day +1066 |
| 498 | 18 | 9 | 13 | NR | 13 | 27 | 17 | 5 | I (skin) | NE | Medullary relapse at day +31 |
| 512 | 14.58 | 4.59 | 11 | 9 | 5 | 5 | 12 | 1 | No | Limited | Alive in CR at day +954 |
| 519 | 10.90 | 14.1 | 11 | 3 | 0 | 1 | 9 | 7 | No | No | Alive in CR at day +925 |
| 527 | 12.70 | 8.94 | 12 | 3 | 0 | 1 | 12 | 0 | No | Extensive | Sudden death syndrome at day +440 |
| 539 | 20.00 | 4 | 13 | 10 | 0 | 2 | 10 | 2 | No | No | Alive in CR at day +806 |
| 541 | 21.48 | 10.44 | 13 | 9 | 6 | 4 | 10 | 0 | No | Limited | Alive in CR at day +801 |
| 555 | 21.50 | 4.73 | 15 | 15 | 6 | 15 | 15 | 0 | No | NE | Dead due to heart failure at day +47 |
| 558 | 18.70 | 7.52 | 15 | 15 | 4 | 4 | 15 | 0 | No | Limited | Alive in CR at day +690 |
| 453 | 6 | 7.2 | 11 | 7 | 0 | 1 | 9 | 0 | No | NE | Nodal relapse at day +76 |
| 578 | 12.60 | 6.8 | 16 | NR | 13 | 11 | 16 | 2 | No | Limited | Alive in CR at day +575 |
| 591 | 3.54 | 32.47 | 17 | 13 | 2 | 8 | 21 | 1 | No | No | Medullary relapse at day +34 |
| 461 | 12.6 | 5.79 | 12 | 5 | 1 | 1 | 13 | 1 | No | Limited | Alive in CR at day +431 |
| 610 | 10.80 | 9.97 | 12 | 8 | 0 | 1 | 10 | 2 | I (skin) | Limited | Alive in CR at day +407 |
| 614 | 18.60 | 4.8 | 14 | NR | 23 | 52 | 11 | 5 | IV (gut) | NE | Dead of acute GvHD at day +77 |
| 618 | 6.7 | 5.8 | 19 | 12 | 20 | 4 | 19 | 5 | No | No | Medullary relapse at day +139 |
| 646 | 9.80 | 7.3 | 13 | 12 | 19 | 10 | 15 | 5 | No | NE | Alive in CR at day +150 |
| 659 | 37.00 | 4.33 | 14 | 12 | 9 | 6 | 9 | 1 | No | NE | Alive in CR at day +89 |

inversion of CD4+ to CD8+ cell ratio in the first year after transplant. CD16+CD56+ NK cells showed rapid recovery, with a median value of $0.18 \times 10^9$/l at day +90 post transplant. Analysis of CD19+ B cells showed a persistent low value up to day +180 post transplant; then, a progressive increase was documented that was, however, inadequate to reach a normal value one year after transplant (Figure 1).

### *Transplant-related toxicity*

The conditioning regimen was well tolerated. Mild nausea and vomiting (grade I) occurred in two patients. In three cases, we noticed a slight increase of amylases (grade I). No evidence of renal or liver toxicity was found. No evidence of mild or severe mucositis was noted.

Of 21 patients, 15 (71%) experienced CMV infection. Three of them showed only a transient positivity for CMV antigenaemia, which did not require specific treatment. In the remaining 12 cases, specific therapy with ganciclovir was given until two negative antigenaemia assays were obtained. Six of these patients had more than one episode of CMV infection (range 2–4) (in all cases within day +100).

A total of 15 patients had febrile episodes during the neutropenic phase, with a median duration of 2 days (range 1–23 days). In total, 13 patients received a red blood cell transfusion, with a median requirement of six packed red cell units, ranging from 1 to 23. All patients received a platelet transfusion, with a median requirement of four units (range 1–27).

Six out of 21 patients developed aGvHD; in four cases, a mild GvHD skin target was noted, whereas in two cases a grade IV gut involvement occurred. cGvHD was observed in nine out of 14 evaluable patients. Seven patients developed limited cutaneous involvement, while in two cases extensive disease was noted (Table 2).

Four patients died without signs of relapse: two of aGvHD grade IV, one with mitral stenosis died of heart failure at day +47 and one with extensive cGvHD plus diabetes died of sudden death syndrome 1 year after transplant.

### *Disease progression and relapse*

The median follow-up was 431 days (range: 41–1121 days). Relapse occurred in five of 21 patients. Two patients had refractory lymphoma, one with a mantel cell lymphoma and one with HD had a splenic and nodal relapse, respectively, at days +76 and +266. Three patients had AML-MDS (two of whom with refractory disease and one with a PR) experienced early disease progression after a transient phase of complete donor chimaerism. The probability of relapse was 29% at 1 year after transplant.

### *Survival analysis*

At present, 12 patients (six MDS with untreated disease, five with AL in complete or PR, one with NHL in CR) are alive in CR after a median follow-up of 786 days (range 89–1121 days). TRM was 19% at 1 year after transplant

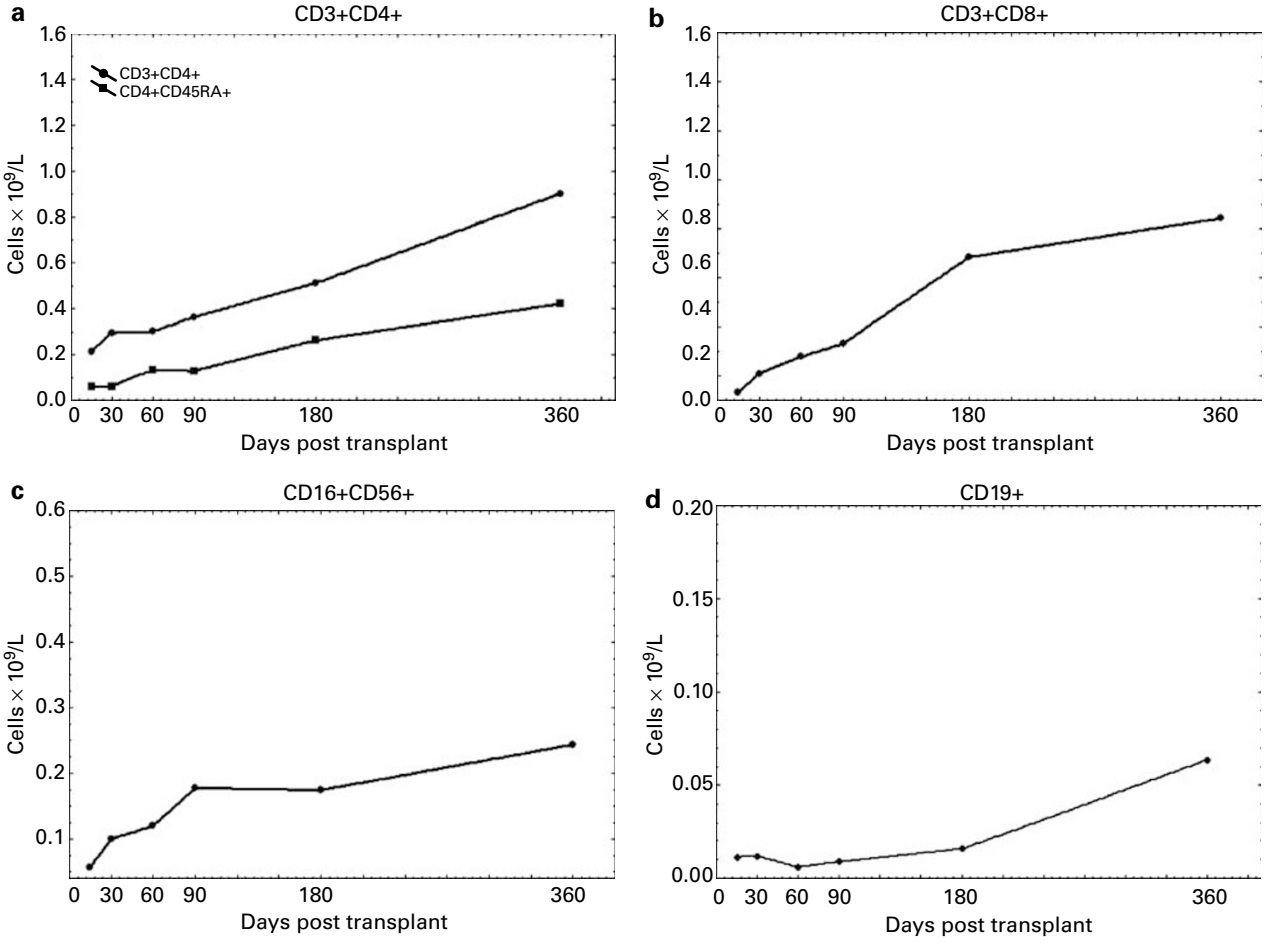


**Figure 1** Median lymphocyte-subset counts in PBPC recipients during the first year after transplantation. Naïve CD4 T cells were defined as CD4 + CD45RA + T cells; natural killer (NK) cells were defined as CD3−CD16 + CD56 +; B-cell lymphocytes were identified with the CD19 + monoclonal antibody marker. The absolute lymphocyte-subset count represents the median values of 21 patients' analyses performed at engraftment on days 30, 60, 90, 180 and 360 post transplant. (**a**) Median values of mature T cells, CD3 + CD4 + (black circle), and naïve T cells, CD4 + CD45RA + (black square). (**b**) Median CD8 T-cell values. (**c**) Median NK cell values. (**d**) Median B-cell values.

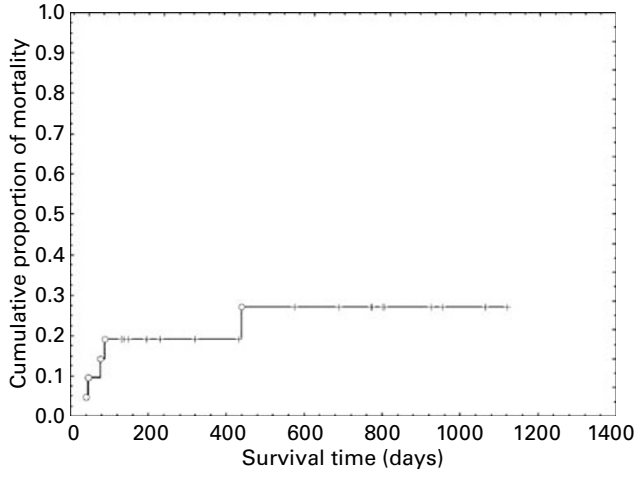


**Figure 2** Transplant-related mortality.

(Figure 2). Event-free survival was 51% 3 years after transplant. The actuarial probability of survival at 3 years after transplant was 58% (Figure 3).

## Discussion

The age limit in our study was lower than that reported in other studies, where reduced-intensity procedures were performed in subjects up to 65–70 years old.[13,14,22] Although our inclusion criteria could have favourably biased the evaluation of the conditioning regimen toxicity, 13 patients were over 50 years old or had severe comorbidities contraindicating standard conditioning regimens. The TRM at 1 year was 19%, which compares favourably with that of myeloablative approaches.[3,23] The TRM is not different from that observed in other studies on patients treated with reduced-intensity conditioning regimens such as busulphan–Fluda, melphalan–Fluda or treosulphan–Fluda, where TRM rates were 15–30%.[6,13,24,25] In this study no cases of major extrahaematological toxicity were observed. The conditioning was well tolerated without any relevant sign of liver or pulmonary dysfunction as reported in other studies.[6,13]

TT is an alkylating agent used for patients with breast cancer at the dose of 500–800 mg/m$^2$.[26] The dose given in our protocol is 10 mg/kg ideal body weight, which is six-

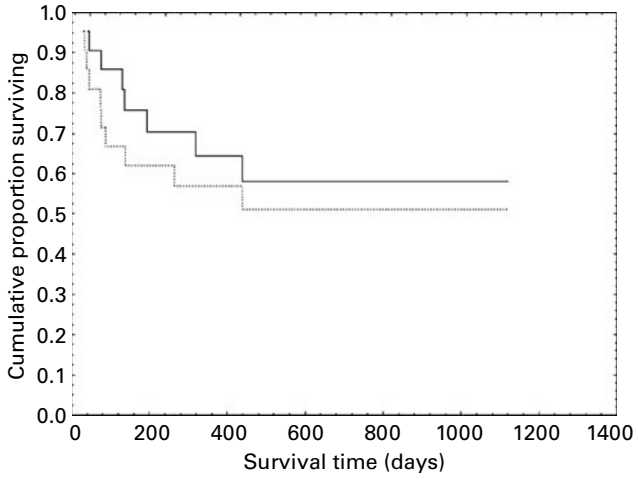


**Figure 3** Survival rates. Overall survival (continuous line) and event-free survival (broken line).

fold greater than standard dose levels and corresponds to 400 mg/m$^2$. The extrahaematological toxicity of TT, involving skin, CNS, liver, gut and occasionally heart, is usually seen only at dosages above 500 mg/m$^2$.[16,17] This may explain the lack of extrahaematological toxicity observed in our series. Fluda, extensively used in several reduced-intensity or nonmyeloablative schedules,[4–6,13] was given at the dose 125 mg/m$^2$ to induce immunosuppression and to enhance the antileukaemic effect of TT. Despite the low nonhaematological toxicity observed in this study, the median duration of neutropenia was 11 days, which is similar to that observed after myeloablative transplants. This strong myelosuppressive activity, associated with immunosuppression provided by Fluda, may explain the high incidence of early stable full donor chimaerism observed in this study.

Rates of aGvHD and cGvHD were comparable to those reported in other studies.[6,13,24] However, two patients died of severe aGvHD and one patient died of sudden death syndrome, with signs of extensive cGvHD 1 year after transplant. Severe aGvHD and cGvHD still remains a problem and novel preventive regimens deserve to be tested.

We observed CD4+ T-cell values permanently above $0.4 \times 10^9$/l from day +180 post transplant. An early increase of CD4+CD45RA+ naïve T cells from day +90 post transplant was noted, which is higher than that observed after standard allogeneic peripheral blood stem cell transplant.[27] The early appearance of naïve T cells may be an expression of limited thymic damage, and may confirm the low extrahaematological toxicity of our combination. In addition, a rapid recovery of NK cells, as observed after standard allogeneic peripheral blood stem cell transplantation,[27] was noticed, which may have helped to sustain a graft-versus-leukaemia (GvL) effect.[28]

It is difficult to evaluate the antineoplastic activity of TT–Fluda, because of patient population heterogeneity, diagnosis and disease status at transplant. In patients with refractory disease (four cases), who experienced an early relapse after transplant, TT–Fluda did not provide adequate antitumour activity. In this subset of patients, it seems advisable to give additional chemotherapy to reduce tumour burden and or post-transplant donor lymphocyte infusion in an attempt to enhance a GvL effect.

From the data reported here, the TT–Fluda conditioning regimen is feasible and well tolerated. Although the present study did not address the efficacy of such an approach, the low relapse rate observed in specific subsets of patients such as MDS or AL in CR or PR, encourages further prospective evaluation.

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