Clofarabine and Treosulfan as Conditioning for Matched Related and Unrelated Hematopoietic Stem Cell Transplantation: Results from the Clo3o Phase II Trial

Clofarabine and Treosulfan as conditioning for allogeneic hematopoietic stem cell transplantation from matched related and unrelated donors: results from the phase II trial "*Clo3o*"

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ABSTRACT

We conducted a phase II study to evaluate tolerability and efficacy of Clofarabine and Treosulfan as conditioning regimen prior to allogeneic hematopoietic stem cell transplantation (allo-HSCT). Primary objective was evaluation of the cumulative incidence of non-relapse mortality (NRM) on day +100. Forty-four patients (37 acute myeloid leukemias, 5 acute lymphoblastic leukemias, 3 myelodysplastic syndromes) were enrolled. Conditioning regimen was with Clofarabine 40 mg/m^2 (day -6 to -2) and Treosulfan 14 g/m^2 (day -6 to -4). Allogeneic haematopoietic stem cells were derived from a sibling (n=23) or a well-matched unrelated donor (n=21). Graft versus host disease (GvHD) prophylaxis consisted of Thymoglobuline, Rituximab, Cyclosporine and short course Methotrexate. The regimen allowed rapid engraftment and was well tolerated with a 2-year transplant related mortality of 18%. Cumulative incidences of grade 2-4 acute and chronic GvHD were 16% and 19% respectively. 2-year overall survival (OS), progression free survival and relapse incidence were 51%, 31% and 50% respectively. A significant difference emerged between patients with "low/intermediate" vs "high/very high" disease risk index (1-year OS 78% and 24%). Treosulfan and Clofarabine combination is feasible, safe and allows prompt engraftment. The considerable relapse incidence in patients with poor prognostic risk factors is still a major issue and could be addressed through the modulation of *in vivo* T-cell depletion.

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) can be curative for patients with hematological malignancies. The ideal conditioning regimen before allogeneic HSCT has not yet been established. Reduced-intensity conditioning (RIC) regimens emerged more than 15 years ago with the aim of decreasing the toxicity and morbidity related to HSCT. Unfortunately, the trade-off for RIC has been an increase in disease recurrence and a high incidence of chronic graft-versus-host disease (cGvHD) with its considerable impact on late transplantation-related mortality (TRM) and quality of life. Progressively, the concept of RIC switched to the concept of reduced-toxicity conditioning (RTC), based on the combination of Fludarabine and an alkylating agent, which currently represent the back-bone in conditioning regimens for HSCT performed world-wide.

Treosulfan is a water-soluble bi-functional alkylating cytotoxic agent often considered as an alternative agent to Busulfan in conditioning regimens because of its well-known advantages, such as low non-hematologic toxicity profile, broad stem cell toxicity, and immunosuppressive as well as anti-leukemic activity. In the last decade, the combination of Treosulfan and Fludarabine proved to be feasible and efficient in several types of malignancies, including acute myeloid leukemia (AML) and myelodysplastic syndromes*(1-3)*.

Clofarabine is a second-generation purine nucleoside analogue; it requires intracellular phosphorylation to be activated and is resistant to deamination. In addition to inhibiting DNA polymerase, Clofarabine also acts as an inhibitor of cellular ribonucleotide reductase. It has been documented that Clofarabine has significant anti-leukemic activity, particularly in relapsed acute lymphoblastic leukemia (ALL), and the drug is approved for the treatment of pediatric ALL patients after at least two prior regimens. Clofarabine was also studied in relapsed AML. Direct induction of apoptosis by activation of caspase 9 and direct interaction with the mitochondrial membrane may also play a role in this superior anti-leukemic effect. Hand-foot syndrome and reversible liver function abnormalities are the two main adverse events of the drug described in the literature *(4)*.

With the aim of identifying a better RTC regimen, both in terms of efficacy and tolerability, we investigated a novel combination of drugs and here we present the results of a prospective, multicenter phase II trial ("Clotreo", EudraCT 2008-006972-

31), testing the use of a conditioning regimen with Clofarabine replacing Fludarabine, in combination with Treosulfan and antithymocyte globulin (Thymoglobuline), in 44 patients affected by acute leukemia or high-risk myelodysplastic syndrome.

MATERIALS AND METHODS

Patients and Donors

This prospective phase II study was conducted at five bone marrow transplant centers in Italy (Milan, Udine, Torino, Bolzano and Pavia). The study was approved by the institutional review board of each participating center. Patients had to be between 1 and 70 years of age, with an available human leukocyte antigen (HLA) matched related or unrelated donor. HLA-compatibility among donor-recipient pairs was assessed by ten loci molecular typing (HLA-A,-B,-C,-DRB1,-DQB1), with no more than a two allele disparity allowed.

Additional eligibility criteria included creatinine clearance above 50 ml/min, alanine aminotransferase $\leq$ 2.5 times the upper normal limit, a Karnofsky performance status $\geq$ 80%, an hematopoietic cell transplantation-comorbidity index (HCT-CI) according to Sorror of less than 4*(5)*. Patients with a previous HSCT were excluded. From November 2009 to November 2013, we enrolled 44 patients (median age 47 years), 36 affected by acute myeloid leukemia, 5 by acute lymphoblastic leukemia and 3 by myelodisplastic syndrome. Comorbidities at time of transplantation were evaluated according to HCT-CI. Patients were stratified by disease type and status at the time of transplantation, according to the disease risk index (DRI) validated by Armand and collaborators *(6)*. 27 patients belonged to the "low-intermediate" group, while 17 were at "high-very high" risk. Patient, disease and transplantation characteristics are summarized in **Table 1**.

Conditioning Regimen and GvHD Prophylaxis

All patients received Treosulfan 14 g/m^2 for 3 days (day -6 through -4) and Clofarabine 40 mg/m^2 for 5 days (day -6 through -2).

GvHD prophylaxis consisted of *in vivo* T cell-depletion by Thymoglobuline for 3 days (day -4 through -2) at two different dosages according to HLA matching: 1.5 mg/kg/day for patients with a 10/10 donor, 2.5 mg/kg/day for patient-donor pairs with any

mismatch. All patients received a single dose of Rituximab at 200 mg/m^2 on day -1 for *in vivo* B cell-depletion; Cyclosporine from day -1 (target plasmatic levels between 150 and 250 ng/ml) and short-course Methotrexate (15 mg/m^2 on day +1, 10 mg/m^2 on day +3 and +6) with folinic rescue were additionally used for GvHD prophylaxis.

In the absence of GvHD or disease relapse Cyclosporine was tapered to discontinuation, starting at month +3 after HSCT in patients with a "high-very high" DRI, and at month +6 in patients with "low-intermediate" DRI, with the aim of maximally exploiting the graft versus leukemia effect exerted by the donor's immune system, selectively in those patients with the highest probability of disease recurrence.

Donor Graft

Peripheral blood stem cells were obtained from donors using standard mobilization protocols and apheresis techniques. A median of 6.0x10^6 CD34^{pos}/kg (range 1.3-14.4) were infused. If peripheral blood mobilization was not possible and in all pediatric patients, bone marrow (BM) was the stem cell source instead.

Supportive Care

Microbial, fungal and viral prevention, together with the treatment of infectious complications were performed according to institutional transplant guidelines, following international recommendations*(7) (8) (9)*. Allogeneic recipients have been screened for the presence of CMV in peripheral blood samples 1 time/week from HSCT to at least 100 days after HSCT. Diagnostic tests to determine the need for preemptive treatment included the detection of CMV DNA by quantitative polymerase chain reaction (PCR). Ganciclovir was used first-line for preemptive treatment of CMV. For prevention of EBV-related post transplant lymphoproliferative disease, patients were monitored every two weeks for EBV DNA load using a blood EBV PCR assay. Testing for galactomannan in serum was performed every week, by adopting PCR-based diagnostics.

Acute GvHD was graded according to the consensus criteria*(10)* and cGvHD was classified according to the NIH criteria *(11)*. GvHD was treated as per institutional protocols, considering the EBMT-ELN recommendations*(12)*.

Evaluation of response

Neutrophil engraftment was defined as neutrophil counts $\geq 0.5x10^9$/L for more than three consecutive days; platelet engraftment was defined as platelet counts $\geq 20x10^9$/L for more than three consecutive days in the absence of transfusions. Toxicity after HSCT was graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE v.3). Post-transplantation disease follow-up comprised monthly marrow evaluations for the first three months after HSCT, and thereafter every three months for the first year. Response and relapse were determined by standard hematological criteria. Hematopoietic chimerism was assessed on BM aspirate samples by performing in parallel short-tandem repeats.

Statistical analysis

Categorical variables are expressed as proportions, and continuous variables are expressed as the median with the respective range. Comparisons between groups were performed with the chi-square and Mann-Whitney tests for categorical and continuous variables, respectively. Outcomes were calculated from the date of transplantation. Progression-free survival (PFS) was defined as the probability of being alive and progression-free at any time; non-relapse mortality (NRM) was defined as death without evidence of disease progression or relapse. Disease progression or relapse was treated as a competing event in the NRM analyses. The incidence of acute (aGvHD) and chronic cGvHD (cGvHD) was estimated considering disease progression or relapse as competing events. Only patients alive at day +100 after transplant were evaluated for cGvHD. Cumulative incidence of engraftment was calculated using death before engraftment as a competing event. The Kaplan-Meier method was used for survival analyses, hazard ratios were estimated with their respective 95% confidence intervals (CIs). The cumulative incidence method with competing risk was used for the NRM, relapse/progression, GvHD and engraftment analyses. *P* values $\leq .05$ were considered significant. The statistical software packages SPSS version 16.0 (SPSS Inc., Chicago, Ill) and R version 2.12.0 (R Foundation for Statistical Computing, Vienna, Austria) were used.

RESULTS

Engraftment and chimerism

93% of the patients achieved primary engraftment, with a median time for neutrophil recovery of 14 (range, 10-27) days, while that for platelet recovery was 12 (range, 7-117) days. The median time for the platelet count to reach $50x10^9$/L was 16 (range, 11-156) days. A total of 3 patients died before neutrophil recovery. One patient experienced secondary graft failure two months after HSCT, concomitant to a severe invasive fungal infection: he was rescued by a BM-derived stem cells boost from the original donor. The patient is currently alive, in good clinical condition, in complete remission, and with normal peripheral blood counts.

Full donor chimerism (> 95% donor chimerism) was documented in 100% of assessed patients at day +30 and confirmed at the following evaluations in patients maintaining a complete disease remission.

Toxicity

Table 2 summarizes all adverse events observed during conditioning and after HSCT graded > 2. Overall, reversible hepatic damage and body weight gain were the most frequent side effects, the latter occurring in 14/44 subjects (32%), mainly due to liquid retention and thus usually managed with maximal diuretic stimulation. Skin rash after Clofarabine administration was frequently observed, but was reversible and of low severity in the vast majority of cases (only 3 patients presented severe cutaneous lesions). Five patients experienced an increase in creatinine levels, with a maximal severity grade of 2.

With regard to infectious events, febrile neutropenia occurred in > 70% of patients, 8 subjects suffered from septic shock, which was ultimately fatal in 7 patients. Pneumonitis was diagnosed in five patients, being the cause of death in one case only. One patient was diagnosed with a proven fungal pneumonia that was successfully treated with a surgical lobectomy. Seven patients experienced EBV reactivation, but only two of them required treatment and no EBV related lymphoma occurred. Reactivation of cytomegalovirus occurred in 23 patients (52%), with a median time of onset of 28 days (range 6-52) from transplantation.

Finally, one patient died during the conditioning regimen because of massive cerebral hemorrhage.

Other adverse events were infrequent and of lower severity (see Table 2).

Overall, the 1-year NRM incidence was 18 (95% CI: 7-30) (**Figure 1**).

Acute and chronic graft-versus-host disease

Grades 2, 3 and 4 acute GvHD occurred in one, four and one patient respectively. The cumulative incidence of grades 2–4 acute GvHD was 16% (95% CI: 4-28), while the cumulative incidence of grades 3–4 acute GvHD was 13% (95% CI: 2-24). The median time of diagnosis was at 54 days post transplant (range 11-111). The cumulative incidence of chronic GvHD (all grades) was 19% (95% CI: 5-33). Overall, 7 patients presented with chronic GvHD, 5 after a family donor transplant and 2 after an unrelated donor transplant.

Outcome

With a median follow-up of 27 (range, 0-61) months after allogeneic HSCT, the 1-year overall survival (OS), progression-free survival (PFS) and relapse incidence were 60% (95% CI: 52-68), 41% (95% CI: 34-48) and 41% (95% CI: 26-56), respectively. The corresponding figures at 2 years were 51% (95% CI: 43-59), 31% (95% CI: 24-39) and 50% (95% CI: 34-67) respectively (**Figure 2A**). At 1 year, 18 patients had relapsed (41%) within a median time of 3.4 (range, 1–9.5) months after transplantation. Three additional relapses occurred at 12.5, 20.6 and 20.9 months.

As expected, patients belonging to the 'high-very high' DRI group displayed significantly lower OS and PFS rates, as compared with those with a 'low-intermediate' DRI: 24% (95% CI: 11-37) *versus* 78% (95% CI: 70-86) and 9% (95% CI: 1-17) *versus* 59% (95% CI: 50-69), respectively at 1 year ($p<0.0001$; **Figure 2B**), and this was consistently related to a higher relapse rate in patients affected by advanced diseases: 68% (95% CI: 41-95) versus 26% (95% CI: 9-43); $p=0.002$; **Figure 3C**.

Five patients with overt disease relapse received either a single (n=2) or two (n=3) donor lymphocyte infusions, with a documented response in only one of them. Five additional patients who relapsed underwent a second allogeneic HSCT from a different donor.

DISCUSSION

In an era in which allogeneic HSCT is considered the potential curative treatment not only for the majority of hematological malignancies, but also for other non-malignant diseases, such as genetic or autoimmune disorders, there is a continuous search for

novel conditioning regimens that will reduce HSCT-related toxicity while retaining maximal anti-malignancy effect. Although advances in transplantation approaches over the past few decades have led to markedly improved outcomes after allogeneic HSCT, the mortality due to disease recurrence remained largely unchanged*(13)*. However, approaches for reducing relapse, such as more intensive conditioning regimens, could also increase toxicities without improving overall outcomes*(14)*.

The combination of Fludarabine with an alkylating agent has become more and more popular in the last decade; the available safety and efficacy data favor such a combination over that of double alkylating agent regimens such as Busulfan-Cyclophosphamide*(15)*.

Treosulfan is a new generation alkylating agent with a myeloablative effect on committed and non-committed stem cells, as extensively investigated in preclinical studies. Moreover, it has potent immunosuppressive activity, which makes it an attractive candidate for its use in conditioning regimens before allo-HSCT, as compared to Busulfan. Of note, Treosulfan toxicity profile is favorable thanks to its limited extramedullary toxicity.

The combination of Fludarabine and Treosulfan was explored in patients not eligible for standard myeloablative conditioning, and data are rapidly emerging. This regimen is associated with consistent engraftment and favorable survival in the range of 40-80%. Promising results were seen in myelodysplastic syndrome and leukemia in remission.

While Fludarabine primarily acts as an immunosuppressant, the more recently synthetized purine nucleoside analog Clofarabine has demonstrated higher anti-leukemic activity. Clinical trials using Clofarabine within the conditioning regimen prior to allo-HSCT allowed elucidation of its role in terms of anti-leukemic potential, immunosuppressive and engrafting-promoting activity. The first published trial using Clofarabine (in association with Cytarabine) in transplant conditioning reported an unacceptably high rate of poor donor chimerism*(16)*.

Here, we report results of a multicenter trial (‘Clo3o’, EudraCT 2008-006972-31) investigating a new conditioning regimen based on Clofarabine substituting Fludarabine. In our study Clofarabine was combined with Treosulfan and both engraftment and chimerism data were encouraging. Recently, other authors reported good engraftment even with Clofarabine and Busulfan based conditioning, inferring that the immunosuppressant potential of Clofarabine is enough to guarantee

engraftment and full donor chimerism*(17) (18-20)*. From literature data, Clofarabine-based conditioning regimens display a NRM ranging from 26% to 32% at 1 year. Of note, all studies included either Busulfan or Melphalan in the conditioning schedule*(21) (22) (23)*. In our study, Clofarabine and Treosulfan allowed a favorable toxicity profile, with a 1-year NRM of 18%.

By incorporating Clofarabine in the conditioning regimen our main aim was to improve disease control and reduce relapse incidence but we observed opposing results. While patients belonging to the 'low-intermediate' DRI group experienced an encouraging 59% of PFS at 1 year, with a 79% OS, subjects with a 'high-very high' DRI displayed 9% of PFS with 24% OS at 1 year. All patients treated in our trial, regardless of HLA matching, received *in vivo* T and B-cell depletion, by Thymoglobulin and Rituximab respectively, besides Cyclosporine and short course Methotrexate. The dose of Thymoglobulin was adjusted according to HLA disparity. This robust immunosuppressive regimen resulted in promising (even lower than expected) acute and chronic GvHD incidence. Indeed, *in vivo* T-cell depletion has been recently shown to lower the incidence of chronic GvHD among patients in complete remission from acute leukemia who received peripheral blood stem cells from an HLA-identical sibling*(24)*. However, we speculate that the disappointing high relapse rate observed in patients with advanced disease in our experience could be at least partially explained by our GvHD prophylaxis policy. Accordingly, to reduce the relapse incidence, high-risk patients may benefit from a less aggressive GvHD prophylaxis, namely a modulation of *in vivo* T-cell depletion, especially when receiving a graft from an HLA-fully matched sibling donor.

To our knowledge, this is the first study combining Clofarabine and Treosulfan as conditioning prior to allogeneic HSCT and has demonstrated a favorable toxicity profile. The considerable relapse incidence in patients with poor prognostic risk factors is still a major issue.

Still, options for decreasing the risk of disease relapse are limited thus far, with little chance of further implementing the efficacy and safety profiles of current conditioning regimens. Given these limitations, prophylactic or pre-emptive post HSCT strategies targeting minimal residual disease will play a dominant role in future clinical trials.

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Figures and tables

Table 1. Patients, disease and transplant characteristics

	All transplants	MRD transplants	MUD transplants
Total number	**44**	**22**	**22**
Patient characteristics			
Median age at HSCT, years (range)	47 (13-69)	43 (13-61)	50 (16-69)
Male sex, n (%)	22 (50%)	9 (41%)	13 (59%)
Disease diagnosis, n (%)			
Acute myeloid leukaemia	36 (82%)	17 (77%)	19 (86%)
Myelodisplastic syndrome	3 (7%)	3 (14%)	0
Acute lymphoblastic leukaemia	5 (11%)	2 (9%)	3 (14%)
Status at transplant, n (%)			
First complete remission	16 (36%)	8 (36%)	8 (36%)
Other complete remission	9 (20%)	4 (18%)	5 (23%)
Active disease	16 (36%)	7 (32%)	9 (41%)
Upfront	3 (8%)	3 (14%)	0
Comorbidities (HCT-CI) § , n (%)			
0	15 (34%)	10 (45%)	5 (23%)
1-2	12 (27%)	5 (23%)	7 (32%)
3-4	17 (39%)	7 (32%)	10 (45%)
Disease Risk Index (DRI) ¶, n (%)			
Low	1 (2%)	1 (4%)	0
Intermediate	26 (59%)	14 (64%)	12 (55%)
High	13 (30%)	5 (23%)	8 (36%)
Very High	4 (9%)	2 (9%)	2 (9%)
CMV serostatus (host/donor) , n			
negative/negative	2	0	2
negative/positive	2	1	1
positive/negative	11	3	8
positive/positive	29	18	11
Donor-recipient HLA matching, n (%)‡			
MRD			
(10/10)	--	22 (100%)	--
MUD			
(8/10)	--	--	1 (4%)
(9/10)	--	--	7 (32%)
(10/10)	--	--	14 (64%)

MRD: matched related donor; MUD: matched unrelated donor; HSCT: haemopoietic stem cell transplantation; GvHD: graft-versus-host disease; HLA: human leukocyte antigen; DRI: Disease Risk Index; HTC-CI: Comorbidity Index; CMV: cytomegalovirus. ‡Donor-recipient HLA matching: at 4-digit for 5 HLA loci (HLA-A, -B, -C, -DRB1, -DQB1). ¶ Disease Risk Index (DRI) according to Armand and collaborators. **§** Comorbidities at time of transplantation were evaluated according to the Comorbidity-Index by Sorror et al.

Table 2. Toxicities

Adverse event	n (%)	Max CTCAE grade
Febrile neutropenia	32 (73)	3
Liver enzymes	12 (27)	4
Septic shock	8 (18)	5
Mucositis	6 (14)	4
Pneumonia	5 (11)	5
Skin lesions[§]	3 (7)	4
CNS infection	3 (7)	4
Hematuria/cystitis	2 (5)	3
Nausea	1 (2)	3
Pleural effusion	1 (2)	3
VOD	1 (2)	3
DVT	1 (2)	3
Arrhythmia	1 (2)	3
CNS bleeding	1 (2)	5
Microangiopathy	1 (2)	3
Hypocalcemia	1 (2)	3

[§] Rash, erytrodermia, ulcerations; CNS: central nervous system; VOD: veno occlusive disease; DVT: deep vein thrombosis.

Figure 1

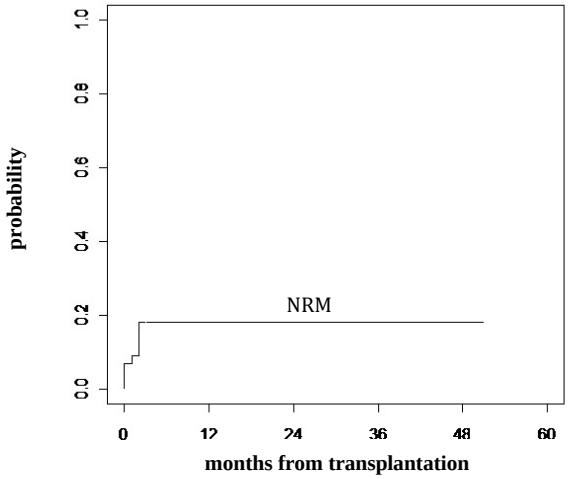
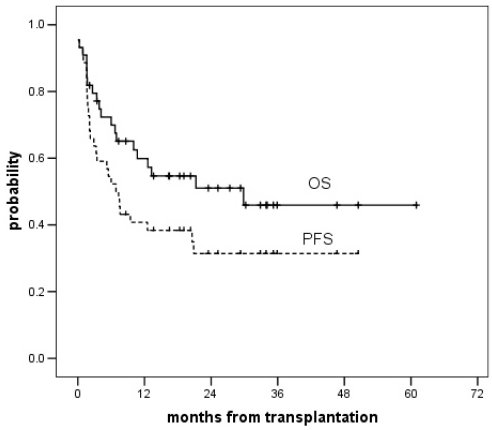
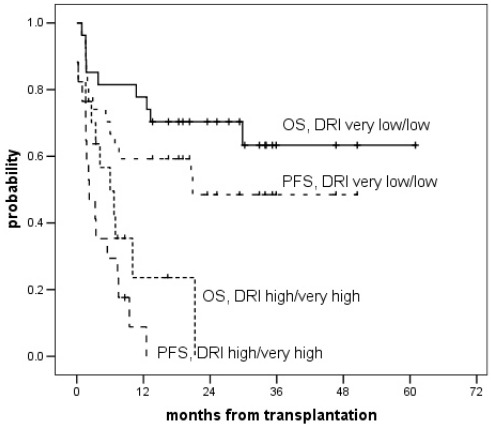


Figure 2

A



B



C