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Allogeneic Hemopoietic Stem Cell Transplants in Patients with Acute Myeloid Leukemia (AML) Prepared with Busulfan and Fludarabine (BUFLU) or Thiotepa, Busulfan, and Fludarabine (TBF): A Retrospective Study

This is a pre print version of the following article:

Original Citation:

Availability:

This version is available <http://hdl.handle.net/2318/1732814> since 2020-03-05T22:32:05Z

Published version:

DOI:10.1016/j.bbmt.2019.12.725

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Allogeneic hemopoietic stem cell transplants in patients with acute myeloid leukemia (AML): conditioning with Busulfan Fludarabine (BU-FLU) compared to Thiotepa Busulfan Fludarabine (TBF) .

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Aknowledgements: This study was funded by AIRC, Milano Italy, FARITMO, Genova, Italy
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Running head: BU-FLU compared to TBF for allogeneic transplants in AML

ABSTRACT

Purpose: This is a multicenter comparison of two conditioning regimens in 454 patients with acute myeloid leukemia (AML) in remission: busulfan and fludarabine (BU-FLU) versus thiotepa, busulfan and fludarabine (TBF).

Patients and Methods: Eligible for this study were patients allografted between January 2008 and December 2018, with AML in first or second remission: 201 patients received a conditioning regimen based on busulfan and fludarabine (BU-FLU) whereas 253 received a combination of thiotepa, busulfan and fludarabine (TBF). The two groups (BU-FLU and TBF) were comparable for age ($p=0.13$), and adverse AML risk factors ($p=0.3$). The TBF group had more second remissions and more haploidentical grafts. The donor type included HLA identical siblings, unrelated donors and family haploidentical donors.

Results: The 5-year cumulative incidence of non-relapse mortality (NRM) was 19% for BUFLU and 22% for TBF and the 5-year cumulative incidence of relapse was respectively 30% and 16% respectively ($P=0.0004$). The 5-year actuarial survival was 51% for BU-FLU and 66% for TBF ($p=0.002$); it was 51% and 64% ($p=0.01$) when excluding patients receiving haploidentical grafts. In a multivariate Cox analysis, after correcting for patients age, disease phase, AML risk factors, year of transplant and donor type, the use of TBF reduced the risk of relapse compared to BU-FLU (HR 0.53, $p=0.03$) and the risk of death (HR 0.62, $p=0.03$). Patients' age over 50 years was the only variable predicting NRM (HR 1.50, $p=0.07$)

Conclusions: Superior survival of patients receiving TBF, as compared to BU-FLU is due to a reduced risk of relapse, with comparable NRM. The survival advantage is independent of donor type and AML risk factors.

INTRODUCTION

The combination of intravenous busulfan and fludarabine (BU-FLU), is considered a standard conditioning regimen for patients with acute myeloid leukemia (AML) undergoing an allogeneic transplant (HSCT) in first or second remission (1). Several retrospective studies have shown relatively low non relapse mortality (NRM) and encouraging leukemia control (2-6). Two of these studies (5,6) have compared retrospectively BUFLU with the

standard combination of busulfan and cyclophosphamide (BUCY): transplant mortality was reduced in patients receiving BUFLU, which translated in superior overall survival **(5,6)**.

A prospective multicenter randomized study, comparing BUFLU with BUCY in patients with AML, has also been conducted **(7)** : eligible patients were de novo AML, in complete remission, 40-65 years old, with and ECOG performance status of <3. The primary end point of the study was NRM at 1 year **(7)**. All patients received full dose busulfan (3.2 mg/kg/day x4), combined with fludarabine 160 mg/m², or cyclophosphamide (CY) 120 mg/kg. A total of 252 patients were assigned to receive BUFLU (n=127) or BUCY (n=125): the NRM at 1 year was 8% for BUFLU and 17% for BUCY (p=0.02); the cumulative incidence of relapse at 5 years was 38% in both groups (p=0.7), and the actuarial 5 year survival was 54% vs 55% (p=0.9) **(7)**. The conclusion of this study, was that BUFLU reduces NRM without compromising the antileukemic effect of the conditioning regimen, and should therefore be considered a standard of care **(7)**. The editorial which accompanied this publication, also that BUFLU should be considered the standard conditioning regimen for AML patients aged 40-65 years **(1)**.

The Spanish group headed by G Sanz, reported some years ago, the efficacy of a new conditioning regimen in patients grafted with cord blood units **(8)**: the combination they described was a modified BUFLU , with a reduction of the dose of busulfan (0.8 mg/kg/day x3 days), but with the addition of thiotepa (10 mg/kg)): they named this regimen TBF **(8)**. In a retrospective study Eurocord confirmed that TBF conferred a survival advantage for patients grafted with cord blood units **(9)**. The combination has been since then largely used in Europe as a preparative regimen for patients with hematologic malignancies **(10-12)**. A retrospective study by the European group for blood and marrow transplantation (EBMT) has compared patients with AML in first remission receiving TBF or BUFLU as a conditioning regimen **(13)**: the study included 25% of patients who received 4 doses of BU together with thiotepa and fludarabine (a variation compared to the original TBF). Relapse was significantly reduced in patients receiving TBF, but NRM was significantly increased, leading to comparable leukemia free survival **(13)**. However, when the authors excluded patients receiving TBF with 4 doses of busulfan, NRM was still greater for TBF patients, but not significantly, whereas relapse remained statistically inferior **(13)**.

Given this background we sought to test in a real life setting the role of TBF or BUFLU as a conditioning regimen for AML patients grafted in first or second remission. We are now reporting the results of this multicenter comparison in 454 AML patients.

METHODS

Eligibility. Eligible for this study were patients with acute myeloid leukaemia (AML), who received an allogeneic stem cell transplantation from January 2008 to December 2018 , aged 18 years or older, in first or second remission (CR1 or CR2) . Secondary AML, treatment related AML, and AML with trilineage dysplasia were also included. Excluded were patients with AML in CR1 or CR2 with European Leukemia Net (ELN) favourable genetic abnormalities **(14)**. Ten transplant centers in Italy and Israel contributed consecutive AML patients grafted in CR1 or CR2, and prepared with either TBF or BUFLU: 454 consecutive patients were enrolled, 201 for BUFLU and 253 TBF.

Patients. Characteristics of patients, donor, disease stage and transplant are summarized in **Table 1**. There were no significant differences in patients age and gender in the two groups, nor for AML adverse factors : the latter included complex karyotype, secondary AML, deletion of chromosome 7 , Flt3 ITD or failure to achieve remission after a first course of induction chemotherapy. The proportion of patients with AML adverse factors was 40% for BUFLU and 36% for TBF (p=0.3) **(Table 1)**

Donors. Donors included HLA-identical matched siblings (SIB), matched unrelated donors (MUD) and family HLA haploidentical members (HAPLO) **(Table 1)**. A haploidentical related donor (HAPLO) was chosen, when suitable HLA matched sibling or volunteer UD, were either temporarily or definitively unavailable. The proportion of HAPLO donors was higher in the TBF group.

Follow up. The median follow up was 467 days (5-3122) for BUFLU and 551 days (5-3073) for TBF **(Table 1)**.

Study. The study was approved by the Institutional Review Board of the Institute of Hematology, Gemelli Hospital (18 march 2019). All patients and donors provided written informed consent for registration and distribution of anonymous clinical data for research purposes at the National and International level.

Conditioning regimens. There were two conditioning regimens.

BUFLU: intravenous busulfan 3.2 mg/kg/ day (total dose 12.8, mg/kg), combined with fludarabine 40 mg/m²/day from day -6 through day -3, (total dose 160 mg/m²).

TBF: thiotepa 5 mg/kg days -6 and -5 (total 10 mg/kg), plus busulfan 3.2 mg/kg/day (total dose 9.6 mg/kg) combined with fludarabine 50 mg /m²/day on days -4,-3 and -2 (total dose 150 mg/m²)(TBF3) . For patients over 60 years of age, or for patients with

significant comorbidities, the dose of BU was reduced to 2 days instead of 3 days (TBF2): 186 patients received TBF3 and 67 patients received TBF2.

Stem cell source. On day 0, 232 patients received unmanipulated bone marrow cells, 219 received granulocyte-colony stimulating factor (G-CSF)-mobilized peripheral blood progenitor cells and 3 patients received cord blood cells

GvHD prophylaxis .

SIB transplants received a conventional cyclosporin (CsA) methotrexate (MTX) regimen. Patients grafted from UD, were given CSA, MTX with the addition of rabbit anti-thymocyte globulin (ATG) (Genzyme, Cambridge, MA, USA) 5-7.5 mg/kg. HAPLO grafts received post-transplant cyclophosphamide (PT-CY) 50 mg/kgx2, CSA and mycophenolate. Therefore patients grafted from UD received the same GvHD prophylaxis, whether prepared with BUFLU or TBF; the same was true for patients grafted from SIB or HAPLO donors.

Supportive care. Patients were given supportive care according to local standards of care, including monitoring, prophylaxis and treatment of bacterial, viral and fungal infections.

Statistical analysis. The NCCS11 package was used for Chi square tables, descriptive statistics, actuarial survival, cumulative incidence reports and multivariate Cox analysis. When calculating the cumulative incidence of NRM, relapse was the competing event, and viceversa. The log rank test was used for differences between survival curves. The Grays test was used for differences between cumulative incidence curves.

RESULTS

Engraftment and GvHD. The median time to a neutrophil count of $0.5 \times 10^9/l$ was 15 days (range 10-42) for BU-FLU, and 17 days (range 10-64) for TBF ($p=0.001$); graft failure was reported in one patient (TBF) as a cause of death.

The risk of acute GvHD grade II-IV was 22% (BUFLU) vs 19% (TBF) ($p=0.5$); the risk of moderate severe chronic GvHD was 22% vs 16% respectively ($p=0.2$).

Non relapse mortality (NRM). The 5-year cumulative incidence (CI) of NRM was 19% (95% confidence interval 14%-26%) for BUFLU and 22% (95% confidence interval 16%-30%) for TBF (Gray test = 0.8). In univariate analysis patients over the age of fifty years, had higher NRM (HR 1.53, $p=0.06$), and there was a borderline effect also in multivariate analysis (HR 1.50, $p=0.07$) (**Table 2**).

Relapse. The 5-year CI of relapse was 30% (95% confidence interval 23%-38%) for BUFLU and 15% (95% confidence interval 10%-21%) for TBF (Gray's test $p=0.0004$) (**Fig.1**). In univariate analysis factors predicting relapse were the conditioning regimen (HR 0.44, $p=0,0002$), and adverse AML risk factors (HR 1.64, $p=0.02$) (**Table 2**). In multivariate the use of TBF (HR 0.53, $p=0.03$) and AML risk factors (HR 1.51, $p=0.05$) remained independent predictive variables (**Table 2**), together with AML adverse factors (HR 1.1, $p=0.05$) . When excluding patients receiving HAPLO graft, the CI of relapse was 30% (95% confidence interval 24%-39%) for BUFLU and 17% (95% confidence interval 9%-29%) for TBF (Gray's test $p=0.008$).

When selecting only TBF, the CI of relapse at 5 years, was 14% for HAPLO and 16% for other donor types (sibling and unrelated) ($p=0.8$).

Survival. The 5-year actuarial survival of the entire group of 454 patients was 68% (95%CI 60-76%); it was 51% for BUFLU (95%CI 43-59%), and 68 % for TBF (95%CI 60-76%) $p=0,0007$ (**Fig.2**).

In univariate analysis factors predicting survival were the conditioning regimen, with a HR of 0.56 for TBF vs BUFLU ($p=0.0008$). In multivariate analysis, after correcting for AML risk factors, year of transplant, disease phase and donor type, TBF conditioning conferred a significant survival advantage with a HR of 0.62 ($p=0.03$), together with patients age over 50 (HR 1,39 $p=0.04$) (**Table 2**).

TBF proved superior to BUFLU also when excluding patients receiving HAPLO grafts (64% vs 51% $p= 0.01$) (**fig 3**). When selecting patients receiving only TBF, we found no differences in survival comparing HAPLO grafts versus transplant from others donors (67% vs 64% $p=0,2$) (**Fig 4**). We could not run the same analysis in patients receiving only BUFLU, due to the small number of HAPLO grafts prepared with BUFLU ($n=4$).

TBF3 compared with TBF2. We then compared patients receiving 3 days of busulfan (TBF3) ($n=186$) with patients receiving 2 days of busulfan (TBF2) ($n=67$) : the median age was 46 years (18-61) for TBF3 and 61 years (18-70) for TBF2 ($p<0.00001$) , the proportion of HAPLO grafts was respectively 76% vs 50% ($p=0.001$); the proportion of patients with AML CR2 (26% vs 28%, $p=0.8$) and with AML adverse factors (38% vs 27%, $p=0.08$). The median follow up was comparable (467 vs 547 days) ($p=0.8$). The cumulative incidence of relapse at 5 years was 15% (95%CI 10-22%) for TBF3 compared with 14% (95% CI 6%-29%) for the older TBF2 patients ($p=0.4$). The cumulative incidence of non relapse mortality at 5 years was respectively 21% (95%CI, 14-31%) versus 25% (95%CI 15-40% ($p=0.4$). The actuarial 5 year survival was 65% vs 68% ($p=0.8$).

Causes of death. We recorded 148 deaths, 82 in the BUFLU and 66 in the TBF group. Leukemia relapse was the most frequent cause of death in 70/454 patients (15%): it was recorded in 46 BUFLU (23%) and 24 TBF patients (9%) ($p=0.00005$). Transplant related causes of death were as follows in the BUFLU and TBF groups respectively: GvHD (16 and 11 patients), infections (5 and 18 patients), other transplant related (15 and 13 patients).

DISCUSSION

Relapse has remained a major problem in patients with acute myeloid leukemia undergoing an allogeneic HSCT **(15)** and has remained unchanged, unlike NRM which has been significantly reduced **(16)**. Intensification of the preparative regimen is one way to attempt a better control of leukemia: unfortunately reduction of leukemia relapse may come with increased NRM. In a prospective randomized study comparing total body irradiation (TBI) 12 Gy versus 15.75 Gy in AML patients **(17)**, relapse was reduced from 40% with 12Gy, to 15% with 15.75 Gy, but NRM was increased from 18% (12 Gy) to 38% (15.75 Gy). The net result was identical 10 year survival for both 12Gy and 15.75 Gy patients **(17)**. Thus, intensification of the conditioning regimen usually increases the risk of non relapse mortality.

In the present study we report a significant reduction of post-transplant leukemia relapse in AML patients in first or second remission, receiving TBF as compared to BUFLU, with no detrimental effect on non relapse mortality: this produces a 15% increase of 5-year survival. The TBF regimen is not really an intensification as compared to the conventional BUFLU, but rather a modification: instead of 4 doses of busulfan (in the BUFLU), the TBF regimen has 3 doses of intravenous BU, but combines BU with thiotepa, 2 very strong myeloablative agents, both capable of allowing engraftment in a mismatched animal model **(18,19)**. The combination of the two alkylating agents appears to be very effective: this is true also for the older patients receiving two days of busulfan. Indeed we could not find a significant difference in the risk of relapse or survival when comparing patients receiving 3 days or 2 days of busulfan, despite the fact that TBF2 patients were 15 years older, as compared to TBF3. This suggests that two days of busulfan combined with two days of thiotepa delivers enough myeloablation in patients with AML, and is

tolerated also in patients up to the age of 70. The TBF is currently used in Europe in a variety of transplant settings **(8-13)**, and we have reported encouraging results in HAPLO transplants **(20)**.

We are particularly impressed with the low incidence of leukemia relapse with TBF (15%) compared to BUFLU (30%), and this study, although retrospective, is a large multicenter, multinational real-life setting. The difference of relapse is not due to a poor performance of the BUFLU arm: indeed in the randomized trial BUFLU versus BUCY for patients with AML in first or second remission **(7)**, the cumulative incidence of relapse at 5 years in the BUFLU arm was 38%, whereas in this study it is 30%. We therefore confirm the relapse risk of BUFLU recorded in the prospective study, and we report a significantly inferior relapse risk with the TBF regimen, as also confirmed by the EBMT study **(13)**. The increased risk of NRM in the EBMT study for TBF patients, compared to BUFLU patients **(13)**, was possibly due to the inclusion of patients receiving 4 doses of BU in addition to thiotepa and fludarabine.

One important question concerns confounding factors: the proportion of patients with adverse AML risk factors, such as complex karyotype, secondary AML, deletion of chromosome 7, Flt3 ITD or failure to achieve remission after a first course of induction chemotherapy, were comparable in the BUFLU and TBF arm. There were more HAPLO transplants in the TBF arm, and this could have been a bias: however the difference in relapse was still there when excluding HAPLO grafts (30% for BUFLU vs 17% for TBF). Conversely, when selecting only patients receiving TBF there was no difference in relapse between HAPLO and other donor types. Another word of caution concerns pre-transplant minimal residual disease (MRD), which we did not have for this group of AML: in fact, despite controversies on the optimal method to determine MRD in AML, there is significant evidence that patients with a positive MRD have a higher risk of relapse as compared to patients with a negative MRD **(21)**. However, in this large number of patients, close to 500, collected from many centers, one would expect MRD positivity to be randomly distributed among the two groups, BUFLU and TBF.

Survival at 5 years was 66% for TBF, with 50% of patients over 50 years of age, compared to 51% for BUFLU: again the difference is not due to poor performance of the BUFLU arm, since in the randomized BUFLU versus BUCY study, the 5 year survival in the BUFLU arm was exactly the same: 51% **(7)**. The survival difference was still there when excluding HAPLO grafts (51% vs 64%). Finally, when selecting only TBF patients the 5-year survival was absolutely identical for patients receiving a graft from a HAPLO or

an other donor. This suggests that the difference in relapse and survival is not due to a difference in donor type or a difference in GvHD prophylaxis.

In conclusion, the conditioning regimen TBF appears to reduce the risk of relapse in AML patients in first or second remission undergoing an allogeneic HSCT, as compared to a conventional BUFLU. A prospective randomized trial comparing TBF and BUFLU in patients with AML, is currently being activated.

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Legend for Figures

Figure 1. Cumulative incidence of relapse for acute myeloid leukemia patients receiving thiotepa, fludarabine, busulfan (TBF) or busulfan fludarabine (BUFLU).

Figure 2. Actuarial 5 year survival for acute myeloid leukemia patients receiving thiotepa, fludarabine, busulfan (TBF) or busulfan fludarabine (BUFLU).

Figure 3. Actuarial 5 year survival for acute myeloid leukemia patients receiving the TBF regimen , stratified according to donor type : haploidentical family donors (HAPLO) or HLA matched related or unrelated donors (other donors).

Figure 4. Actuarial 5 year survival for acute myeloid leukemia patients receiving thiotepa, fludarabine, busulfan (TBF) or busulfan fludarabine (BUFLU), after exclusion of HAPLO grafts.

Suppl Figure 1