Survival Advantage of Treosulfan Plus Fludarabine Before Allogeneic Hematopoietic Cell Transplantation for Older or Comorbid Patients With Myeloid Malignancies

Ioanna Sakellari¹, Eleni Gavriliaki¹, Despina Mallouri¹, Ioannis Batsis¹, Christos Varelas¹, Sofia Tagara¹, Zoi Bousiou¹, Maria Papathanasiou¹, Anna Vardi¹, Apostolia Papalexandri¹, Chrysanthi Vadikoliou¹, Anastasia Athanasiadou¹, Chrysavgí Lalyanní¹, Asimina Fylaktou², Konstantinos Antoniadis³, Achilles Anagnostopoulos¹

¹Hematology Department – BMT Unit, G Papanicolaou Hospital, Thessaloniki, Greece
²National Peripheral Histocompatibility Center, Department of Immunology, Hippokration General Hospital, Thessaloniki, Greece
³Aristotle University of Thessaloniki, School of Health Sciences, School of Dentistry, Thessaloniki, Greece

ABSTRACT

We have previously shown an advantage of a myeloablative conditioning regimen with reduced toxicity (Fludarabine 150 mg/m², Treosulfan 42 g/m², FluTreo) compared to a reduced-intensity regimen. We aimed to determine long-term safety and efficacy of FluTreo. We prospectively studied consecutive patients who received FluTreo in our center (2014-2019) on the basis of age (≥50 years), hematopoietic cell transplantation comorbidity index (HCT-CI) ≥2, or both. FluTreo recipients were then compared to a historical control group. We studied 68 FluTreo recipients, with a median age of 58.5 years and HCT-CI of 3. We calculated cumulative incidence (CI) of acute (grade 2-4) and moderate/severe chronic graft-versus-host disease (GVHD) (29.9% and 25%, respectively). The 3-year CI of treatment-related mortality was 19.1%, associated only with acute GVHD (P < .001). With a median follow-up of 27.3 (range 5.7-84.5) months in surviving patients, the 3-year overall survival (OS) was 56.6%, and disease-free survival (DFS) was 54.9%. Median survival has not yet been reached. Among pretransplantation and transplantation factors, only HCT-CI was associated with DFS and OS (P = .022 and P = .043, respectively). FluTreo recipients aged ≥50 with HCT-CI ≥2 had favorable DFS and OS compared with patients aged ≥50 with HCT-CI <2 after myeloablative conditioning. Our real-world study confirms that HCT with FluTreo expands the transplant population with favorable outcomes compared to previously used conditions. The choice of HCT in patients of a rather older age and comorbidity index needs to be revisited.

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Treosulfan is a structural analog of busulfan incorporated as part of a conditioning regimen before allogeneic hematopoietic cell transplantation (HCT) over the last 2 decades [1–4]. Although it was initially used in pediatric protocols, its use in adult patients is continuously expanding given its strong myeloablative action and lower organ toxicity, when compared with busulfan [5,6]. These properties have rendered treosulfan an ideal candidate for older or comorbid patients in whom HCT remains the only curative option.

Indeed, myeloid malignancies, primarily involving acute myeloid leukemia (AML) and high-risk myelodysplastic syndrome (MDS) are diseases of the elderly, with poor survival rates without an HCT, especially in AML patients [7]. More importantly, patients with therapy-related AML and patients who develop AML as a progression of MDS or myeloproliferative neoplasm (sAML) are considered a high-risk group of AML. This group is particularly characterized by poor prognosis because of adverse cytogenetics or molecular characteristics, older age with comorbidities, and frailty because of prior malignancy and chemotherapy [8,9]. Given these unique characteristics, most patients who were given the opportunity of HCT have undergone transplantation with reduced-intensity regimens (RIC). In addition to its utility in patients considered unsuitable for standard myeloablative HCT because of comorbidities, RIC has also been used as an alternative approach in older patients who could otherwise be eligible for transplantation [10]. However, available RIC regimens suffer from...
significant limitations with increased relapse and treatment-related mortality (TRM) rates [11–15].

The choice of the proper regimen plays a very important role in the success of HCT because it considers precisely both patient and disease characteristics. To overcome limitations of RIC regimens, treosulfan has been increasingly studied, showing a particularly favorable acute organ toxicity profile in patients with AML or MDS [16-20]. More than that, our group has shown a survival advantage in older AML and MDS patients, with a reduced toxicity combination of treosulfan plus fludarabine (Fludarabine 150 mg/m² and treosulfan 14 g/m² for 3 days [FluTreo or FT14]) compared to a busulfan-based RIC regimen [21]. These results have been confirmed by a recent meta-analysis of 6 comparative studies showing favorable survival along with reduced acute graft-versus-host disease (GVHD) [22]. Compelling evidence of noninferiority have been also provided by a recent phase 3 randomized clinical trial using treosulfan 10 g/m² for 3 days or FT10 [23].

It should be noted, however, that the follow-up of existing studies for treosulfan-based regimens is rather short, with a maximum follow-up of 2 years. It should be noted that doses of treosulfan vary among studies, with the majority using 10, 12, or 14 g/m² for 3 days. In addition, the patient population that would benefit from such a regimen has not yet been clearly defined. Hematopoietic cell transplantation comorbidity index (HCT-CI) [24] and age are currently suggested for selection of reduced toxicity regimen [23]. Importantly, the favorable profile of treosulfan-based myeloablative regimens raises the question of their utility in older patients who would otherwise be candidates for standard myeloablative regimens. Up to now, only 1 recent study of MDS patients has compared treosulfan-based regimens with standard myeloablative regimens showing favorable results [25].

Taking into account the open questions in this field, we hypothesized that the reduced toxicity regimen (Fludarabine 150 mg/m², Treosulfan 42 g/m² [FluTreo]) would lead over time to a stable survival advantage over previously used treatment alternatives in older patients or patients with comorbid myeloid malignancies.

METHODS

Study population

We performed a real-world study of consecutive adult patients who underwent HCT from January 2014 to January 2020 in our Joint Accreditation Committee-BMT & EBMT–accredited unit with the FluTreo conditioning regimen. According to our unit’s standard operating procedures, the regimen was used in patients who met the following criteria:

1. Age at HCT ≥50 years, HCT-CI ≥2, or both
2. Diagnosis of a myeloid malignancy
3. Available sibling or matched or mismatched unrelated donor (molecular typing of HLA-A, -B, -C, -DR, and -DQ)

To further understand the long-term safety and efficacy of FluTreo, we used a historical control group. The characteristics of this group were selected to represent a comparable patient population that would have been transplanted with a standard myeloablative before the introduction of FluTreo in our practice. Therefore FluTreo recipients aged ≥50 with HCT-CI ≥2 were compared to patients aged ≥50 with HCT-CI ≤2 who were transplanted with a standard myeloablative regimen between 1990-2013 (busulfan plus cyclophosphamide [BuCy]).

To assess comorbidity risk, we used the HCT-CI published by Sorror et al. [24] in 2005. HCT-CI was assessed retrospectively for patients who underwent transplantation until 2005, and prospectively for patients who underwent transplantation since 2006. Disease phase was defined as early as defined in first complete remission, late in other complete remission, and advanced in relapsed refractory patients.

Our institutional review board approved this study, and all patients gave a written informed consent in accordance with the Declaration of Helsinki.

Conditioning regimens

FluTreo regimen consisted of fludarabine 30mg/m² (intravenously for 5 consecutive days) and treosulfan 14 g/m² (intravenously infused over 1 hour for 3 consecutive days). Before the introduction of FluTreo, standard myeloablative conditioning was BuCy consisting of intravenous busulfan 0.8 mg/kg 4 times per day during 2-hour infusions for 4 consecutive days, and cyclophosphamide at 60 mg/kg/d for 2 consecutive days. Patients with unrelated donors received 5 mg/kg of rabbit antithymocyte globulin (ATG) [26]. To avoid reactions to ATG, methylprednisolone 80 mg every 8 hours was used on the ATG infusion days and was quickly tapered.

Similar standard operating procedures were implemented in patients after FluTreo and after the standard BuCy. Prophylactic granulocyte colony-stimulating factor after transplantation was routinely used in all regimens. Supportive care also was composed of prophylactic platelet transfusion if platelet counts decreased to <20 x 10⁹/L or prophylactic red blood cell transfusion if hemoglobin levels decreased to <8 g/dL. All patients received supportive treatment against bacterial, fungal, and viral infections. Trimethoprim-sulfamethoxazole was used as prophylaxis for Pneumocystis jiroveci infection. Patients also received preemptive treatment for cytomegalovirus and Epstein-Barr virus reactivation according to close molecular monitoring.

GVHD prophylaxis

BuCy recipients received a combination of a calcineurin inhibitor (cyclosporine [CSA] or tacrolimus), along with short-term 4 post-transplantation methotrexate doses of either 5 mg/m² (on days 1, 3, 6, and 11) after unrelated transplants, or 15 mg/m² (on days 1; 10 mg/m², 3, 6, 11) after sibling transplants. FluTreo recipients received CSA and mycophenolate mofetil until day 45 post-transplant (sibling and unrelated donor) for GVHD prophylaxis. Plasma CSA concentration was maintained at between 100 and 200 ng/ml, until day 90 in sibling transplants and 150 in unrelated donor transplants. CSA dose was tapered off by 5% every week if there were no signs of chronic GVHD. Assessment and grading of acute GVHD was performed according to the classic Glucksberg criteria, whereas chronic GVHD was assessed and graded according to the 2014 National Health Institute criteria [27,28].

Chimerism

Chimerism evaluation in unfractionated bone marrow with short tandem repeat fragment analysis was performed regularly (on days 14, 30, 60, and 90) in both groups. Donor chimerism >95% was defined as complete donor chimerism.

Statistical analysis

Data were analyzed using the statistical program SPSS 22.0 (IBM SPSS Statistics for Windows, Version 22.0; IBM Corp, Armonk, NY). Descriptive statistics were performed using median and range for continuous variables and frequency for categorical variables. Continuous variables were assessed for normality and compared using a t-test or Mann-Whitney test. The following factors were studied: age, type of disease/donor/graft, phase at transplant, HCT-CI score, CD34 cells infused, infections, cumulative incidence (CI) of GVHD, and TRM, overall (OS), and disease-free survival (DFS). Categorical variables were compared using chi-square test. For survival analysis the Kaplan-Meier method was used and survival curves were compared using a log-rank test. Cox regression analysis was performed for univariate and multivariate predictors of survival, with time-dependent covariates computed through SPSS analysis. Cumulative incidence (CI) of competing events analysis was performed using the EZR software (http://www.jichi.ac.jp/satana/statmed.html) [29]. Statistical significance was assessed by the Gray test and Fine and Gray regression modeling. The level of statistical significance was defined at 0.05.

RESULTS

Study population

We studied 68 FluTreo recipients, with a median age of 58.5 (25-70) years, transplanted for de novo AML (31 patients, 46%), secondary AML (either therapy-related AML or sAML) (20, 30%), MDS (14, 21%), myeloproliferative neoplasms (1 with chronic myeloid leukemia/CML and 1 with chronic myelomonocytic leukemia/CMMML, 3%). The majority was transplanted in early (45/68, 66%), while 18 (26%) in late, and 5 (8%) in advanced phase. Grafts were peripheral blood stem cells from unrelated (33 matched, 49%, and 10 mis-matched, 15%) and sibling (25, 36%) donors. Median HCT-CI was 3 (0-7), and previous lines of treatment 3 (1-6).

Engraftment and Chimerism

Donor engraftment occurred in all patients, with full donor chimerism achieved in all patients at median 30 (12-90) post-
transplant days. In the FluTreo group, neutrophil engraftment was achieved at day +10 (8-12) and platelet +11 (9-20), with 7 median days of neutropenia.

Toxicity

Mucositis grade 1-2 according to World Health Organization (WHO) grading was observed in 49/68 (72%) patients, grade 1-2 hyperbilirubinemia in 11 (16%) and grade 1-2 renal toxicity in 6 patients (9%). No grade 3-4 organ associated toxicities were noted.

Treatment for bacterial infection received 19 patients (28%) and post-discharge 21 patients (32%) were treated for infections. Twenty-one patients (32%) experienced cytomegalovirus reactivation (7 infections), 27 (40%) Epstein-Barr virus (1 post-transplant lymphoproliferative disease/PTLD), 1 (1%) herpes zoster infection, 1 (1%) probable lung aspergillosis, and 1 (1%) lung tuberculosis.

Relapse

Relapse was observed in 15 (22%) patients at median 5 (range 2-22) months post-transplant. Among them, 8 patients (12%) were transplanted for secondary AML, 4 (6%) for de novo AML, 2 (3%) for MDS, and 1 (1%) for CMML. MDS and secondary AML received azacytidine. Nine AML patients (13%) received chemotherapy and subsequently, 4 (6%) of them who responded to chemotherapy received donor lymphocyte infusions (DLIs). Three relapsed patients (4%) remain alive at 11 (range 7-37) months post-transplant.

GVHD and TRM

We calculated acceptable rates of CI for acute (gr 2-4) GVHD (29.9%). Similarly, 3-year CI of moderate/severe chronic GVHD was 25% (Figure 1A). CI of chronic GHVD was associated with age (p<0.001), and mis-matched donors (p=0.042). Interestingly, 3-year CI of TRM was only 19.1% (Figure 1B). Among pre-transplant and transplant factors, TRM was associated with HCT-CI >3 (p=0.029).

Survival

With a median follow-up of 27.3 (range 5.7-84.5) months in surviving patients, 3-year OS was 56.6%, and DFS was 54.9%. Median survival has not been reached yet. Among pretransplantation and transplantation factors, HCT-CI was associated with DFS and OS (p= .022 and p= .043, respectively). Patients with HCT-CI ≤2 had significantly higher OS (71.2% versus 45.1%, p= .019), and DFS (70.3% versus 40.8%, p= .006) at 3 years (Figure 2). It should be also noted that diagnosis of secondary AML did not impact transplantation outcomes. Among post-transplantation factors, acute GVHD (p= .045) and relapse (p< .001) were associated with OS. In the multivariate model, relapse (p= .002) was an independent predictive factor of OS.

Comparison of FluTreo with historical control group

To further understand the long-term safety and efficacy of FluTreo, we utilized our historical control group. As expected, the percentage of patients with age 50 years or older that were able to undergo HCT with a standard myeloablative conditioning was significantly lower compared to FluTreo conditioning (17% versus 81%, p< .001). Interestingly, the same was true for patients with secondary AML (6% versus 21%, p= .034).

To achieve a balanced comparison, we compared FluTreo recipients aged ≥50 with HCT-CI ≤2 to a historical control group of patients aged ≥50 with HCT-CI ≤2 that were transplanted with a standard BuCy myeloablative regimen. As
showed in Table 1, patients in the FluTreo group (n = 25) had significantly increased age compared to patients in the BuCy group (n = 45), although both groups had an age ≥50. No other significant differences were found in transplant characteristics. Despite the increased age in FluTreo, patients with FluTreo had significantly higher OS (Figure 3A, 70.2% versus 40.3%, P = .024), and DFS (Figure 3B, 69.0% versus 29.3%, P = .005) at 3 years. Differences in survival measures may be attributed to significantly increased CI of TRM in FluTreo recipients compared to BuCy (16.2% versus 27.9%, P = .014).

**DISCUSSION**

Our real-world study confirms that HCT with FluTreo expands the transplant population in unfit comorbid patients with outcomes comparable to standard myeloablative conditioning, even in secondary AML. HCT-CI remains a major determinant of morbidity and mortality in this patient population. Therefore the choice of HCT in patients of a rather older age and comorbidity index needs to be refined in the manner of an individualized risk adapted strategy.

Indeed, the number of HCT performed for AML continues to rise over the last decade [30], potentially reflecting advances in donor availability and current conditioning regimens of reduced toxicity. The more sophisticated ambition toward more personalized conditioning that achieves longer DFS without the burden of TRM has largely been hindered by challenging recruitment in prospective randomized trials [31]. The comparison between different intensity regimens is also puzzling due to variabilities in relapse and toxicity between different regimens [32,33]. Categorization of intensity in the current era may be improved with implementation of the transplant conditioning intensity (TCI) score [34]. Taken together, the limitations in the available literature highlight the importance of robust real-world data with comparisons of the standard myeloablative conditioning of a high TCI score to a widely used reduced toxicity regimen like FluTreo or FT14, of an intermediate TCI score.

Trehosulfan, initially used in the treatment of solid tumors, is a bifunctional alkylating prodrug with strong activity against hematopoietic stem cells [35]. Its use over the last 2 decades as a conditioning regimen was expanded even to the nonmalignant setting because of its reduced toxicity. It combines anti-leukemic and myelosuppressive characteristics, and its immunosuppressive properties make it ideal for enabling stem cell engraftment. Moreover, it provokes limited adverse events accompanied with lower TRM [1]. A dose escalation study in nonpediatric patients concluded that a treosulfan dose of 42 g/m² in combination with fludarabine 150 mg/m² is effective conditioning with regard to safety, toxicity, and myeloablation [36]. Therefore we used this regimen in the present study.

This real-world study highlights long-term safety and efficacy of FluTreo or FT14, aiming to further emphasize the benefits offered by this regimen in everyday clinical practice. These include not only the expansion of patients offered the opportunity toward an HCT in terms of age, HCT-CI, and high-risk AML, but also the potential benefits of a reduced toxicity regimen over the standard myeloablative regimen in older adults. These benefits have so far been highlighted only in the MDS population by a recent study [25]. Our study further suggests a benefit for de novo and, most importantly, secondary AML that is traditionally associated with poor prognosis [37]. Because patients with secondary AML represent 10% to 30% of the AML group [38], it becomes necessary to optimize outcomes. In a large systemic analysis performed by the EBMT–Acute Leukemia Working Group, OS and DFS were 45% and 39%, respectively, in sAML patients undergoing HCT [39]. Although RIC regimens have offered better toxicity profiles, this advantage

<table>
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<th>Patients characteristics</th>
<th>FluTreo (n = 25)</th>
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<td>Previous lines of treatment</td>
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Continuous variables are expressed as median (range).

HCT-CI indicates hematopoietic cell transplantation–comorbidity index; AML, acute myeloid leukemia; MDS, myelodysplastic syndrome; CML, chronic myeloid leukemia.

**Figure 3.** Survival measures for patient receiving treosulfan plus fludarabine compared to patients receiving standard BuCy myeloablative conditioning. All patients had HCT-CI ≥2 and age ≥50 (n = 25 in FluTreo versus 45 in BuCy). Patients with FluTreo had significantly higher overall survival (A, 70.2% versus 40.3%, P = .024), and disease-free survival (B, 69.0% versus 29.3%, P = .005) at 3 years. HCT-CI, hematopoietic cell transplantation–comorbidity index; BuCy, busulfan plus cyclophosphamide.
has been outweighed by the increased relapse rate [33]. Reduced toxicity regimens like FluTreo seem to offer a balance between toxicity and relapse irrespective of age, especially in patients with HCT-CI ≥ 2.

Comorbidity assessment remains an understudied but important issue in this field. The standard assessment tools of the EBMT score and the HCT-CI have been questioned in these subgroups of patients, because a number of comorbidities is less strongly associated with mortality after RIC than after MAC [40]. In our study of the reduced toxicity regimen, HCT-CI remained a strong predictor of TRM, DFS, and OS, highlighting a patient population with poor outcomes. These outcomes were similar in the recent randomized trial where patients with HCT-CI > 3 did not show a survival benefit from treosulfan compared to busulfan [23]. This patient population may benefit from a more personalized approach integrating next-generation sequencing and novel biologic agents into the complex treatment algorithm [31].

Our study has some limitations. First, the comparative analysis was performed retrospectively. Additionally, although it included a long treatment period, the number of patients is limited by its single-center nature. Despite these limitations, the robust standard operating procedures of a single center over the years validate the quality of presented data.

In conclusion, treosulfan plus fludarabine offers favorable outcomes in older and comorbid patients, with myeloid malignancies minimizing TRM and maximizing the survival benefit. It is important that well-designed studies with proper patient stratification are conducted with a larger patient population and a longer follow-up period to ascertain the ideal conditioning regimen in a personalized approach.

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