Biol Blood Marrow Transplant xxx (2016) 1–7

Biology of Blood and Marrow Transplantation

journal homepage: www.bbmt.org

ASBMT American Society for Blood and Marrow Transplantation

Immunologic Autograft Engineering and Survival in Non-Hodgkin Lymphoma

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Article history:
Received 23 September 2015
Accepted 19 January 2016

Key Words:
Autograft absolute lymphocyte count
Apheresis machine
Non-Hodgkin lymphoma
Autologous peripheral hematopoietic blood stem cell transplantation

A B S T R A C T

Retrospective studies have reported that the collected and infused autograft absolute lymphocyte count (A-ALC) affects clinical outcomes after autologous peripheral hematopoietic stem cell transplantation (APHSCT). We hypothesized that manipulation of the apheresis machine to target a higher A-ALC dose would translate into prolonged progression-free survival (PFS) in patients with non-Hodgkin lymphoma (NHL) undergoing APHSCT. Between December 2007 and October 2010, we performed a double-blind, phase III, randomized study randomly assigning 122 patients with NHL to undergo collection with the Fenwal Amicus Apheresis system with our standard settings (mononuclear cells offset of 1.5 and RBC offset of 5.0) or at modified settings (mononuclear cells offset of 1.5 and RBC of 6.0). The primary endpoint was PFS. Neither PFS (hazard ratio [HR] of modified to standard, 1.13; 95% confidence interval [CI], .62 to 2.08; $P = .70$) nor overall survival (OS) (HR modified to standard, .85; 95% CI, .39 to 1.86; $P = .68$) were found to differ by collection method. Collection of A-ALC between both methods was similar. Both PFS ($P = .0025$; HR, 2.77; 95% CI, 1.39 to 5.52) and OS ($P = .004$; HR, 3.38; 95% CI, 1.27 to 9.01) were inferior in patients infused with an A-ALC $< .5 \times 10^9$ lymphocytes/kg compared with patients infused with an A-ALC $\geq .5 \times 10^9$ lymphocytes/kg, regardless of the method of collection. We did not detect significant differences in clinical outcomes or in the A-ALC collection between the modified and the standard Fenwal Amicus settings; however, despite physician discretion on primary number of collections and range of cells infused, higher A-ALC infused dose were associated with better survival after APHSCT.

INTRODUCTION

Poor clinical outcomes observed after high-dose chemotherapy with autologous peripheral hematopoietic blood stem cell transplantation (APHSCT) have been attributed to the inability of the high-dose chemotherapy to eradicate residual tumor [1]. Our group has found that failure to recover absolute lymphocyte count by day 15 (ALC-15) after APHSCT is associated with poorer clinical outcomes not only in hematologic malignancies, but also in solid tumors [2-14]. We also have found that the amount of infused autograft absolute lymphocyte count (A-ALC) not only affects ALC-15 recovery, but also that an infused A-ALC $\geq .5 \times 10^9$ cells/kg is associated with better clinical outcomes after APHSCT [15-19]. The discovery of the impact of infused A-ALC on clinical outcomes provided the impetus to develop new methods to increase the number of infused A-ALCs.

Our institution has used 3 different apheresis machines for the collection of stem cells: the COBE Spectra (Terumo BCT, Lakewood, CO), the Fenwal Amicus (Fenwal Inc, Lake Zurich, IL), and the Fenwal CS3000 Plus (Baxter Healthcare, Deerfield, IL). In patients with non-Hodgkin lymphoma (NHL), we found superior clinical outcomes after APHSCT when stem cells were collected with the COBE Spectra than with either the Fenwal Amicus or the Fenwal CS3000 Plus [20]. Collections were done using the apheresis machines' standard settings. This benefit was attributed to the COBE Spectra being able to collect more A-ALCs than the other 2 apheresis machines [20]. As our institution moved to the

Financial disclosure: See Acknowledgments on page 6.

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http://dx.doi.org/10.1016/j.bbmt.2016.01.024
1083-8791/

Fenwal Amicus for the use of stem cell collection, we performed a study to evaluate the impact of modifying the Fenwal Amicus settings to enhance collection of A-ALC [21]. Thus, we conducted a double-blind, phase III, randomized study to assess progression-free survival (PFS) among NHL patients who underwent collection by modified Fenwal Amicus parameters compared with those of NHL patients who underwent collection with our standard Fenwal Amicus parameters.

METHODS

Study Design and Objectives

This double-blind, randomized, phase III clinical trial in patients with NHL who were to undergo APHSCT was performed to assess whether the PFS was significantly increased among those whose apheresis was done using a modification to a standard Fenwal Amicus settings relative to those whose apheresis was done using standard Fenwal Amicus settings by increasing the collection of A-ALC. Our standard Fenwal Amicus apheresis settings (StdC) were mononuclear cells (MNC) offset = 1.5 and red blood cells (RBC) offset = 5.0 and the modified Fenwal Amicus apheresis settings (ModC) were MNC offset = 1.5 and RBC = 6.0. We used 5-hour procedure time as the endpoint for hematopoietic progenitor cell collections. We have previously found that high inlet flow rates with high ($>35 \times 10^9$/L) per procedure WBC results in poor yields. So, if WBC counts are $>35 \times 10^9$/L, we use 65 mL/minute as the inlet rate. If the WBC $< 35 \times 10^9$/L, we use 90 mL/minute inlet rates. For this study, there were 447 collections. The median volume of whole blood processed was 15,842 mL (range, 10,291 mL to 22,865 mL), the mean was 16,584 mL, and the standard deviation was 2136 mL. The secondary aims included assessing whether PFS differed with respect to the content of the apheresis product (namely, A-ALC, autograft nature killer cell count [A-NKC], autograft CD3 count [A-CD3C], autograft CD4 count [A-CD4C], and autograft CD8 count [A-CD8C]). Approval for the study was obtained from the Mayo Clinic institutional review board and was in accordance with federal regulations and the Declaration of Helsinki. Written informed consent was obtained from all patients. Progress was reviewed by the Mayo Clinic Data and Safety Monitoring Board every 6 months. This study was registered with ClinicalTrials.gov, number NCT00566228.

Inclusion and Exclusion Criteria

This trial enrolled individuals ≥ 18 years of age with NHL who were candidates for APHSCT and had Eastern Cooperative Oncology Group performance status of 0 or 1. Individuals were excluded from participation if they had medical comorbidities contradictory for undergoing apheresis or APHSCT, required bone marrow harvesting to collect stem cells, had an active uncontrolled infection requiring antibiotic treatment, did not show chemosensitivity by either partial response or complete response, or were participating in any APHSCT study not using the standard BEAM conditioning regimen for NHL. Pregnant women were not eligible for participation. Women of child-bearing potential also underwent a serum pregnancy test within 7 days of registration and agreed to use effective contraception during treatment.

Randomization and Masking

A dynamic allocation procedure [22] was used to randomly assign an equal number of patients to the 2 collection approaches so that the marginal distribution of stratification factors, namely, International Prognostic Index score (<2 versus ≥2) and positron emission tomography (PET) scan results (positive versus negative) before APHSCT were balanced between the collection approaches. The treating physicians and patients were blinded to the collection approach the patients were randomly assigned.

Protocol Treatment and Clinical Protocol Assessments

Within 4 weeks of study entry, patients underwent the institutional standard pre-APHSCT testing including a complete medical examination with complete blood cell counts (CBC) and blood chemistries; blood typing; infection work-up; bilateral bone marrow biopsy; cerebrospinal fluid analysis; urinalysis; PET scan; computer tomography (CT) scans of the chest, abdomen, and pelvis; chest x-ray, echocardiogram, and electrocardiogram.

Patients received 10 μg/kg of granulocyte-colony stimulating-factor (G-CSF) daily for 5 to 7 consecutive days by subcutaneous injection alone or in conjunction with .24 mg/kg of plerixafor for up to 4 consecutive days by subcutaneous injection. On day 4 after the start of G-CSF, if the peripheral blood $CD34^+$ count were less than 10 cells/μL, plerixafor was added that evening and collections were initiated the after day. If plerixafor was not used, when the patient reached a peripheral CD34 count of 10 cells/μL or greater, stem cell collection began. Apheresis collections were to be performed daily. At least 2×10^6 CD34 cells/kg were to be collected. Additional collections were at the discretion of the transplantation team. Patients for whom mobilization of peripheral stem cells failed or stem cell collections failed to gather at least 2×10^6 CD34 cells/kg were allowed to choose to either undergo a second mobilization/apheresis or discontinue study participation. Hematopoietic progenitor cells products were cryopreserved and infused using our institution standard operation procedures [23].

In the week before transplantation, all patients received the BEAM conditioning regimen (300 mg/m^2 carmustine intravenously (i.v.) once on day −6 followed by 100 mg/m^2 etoposide i.v., and 100 cytarabine mg/m^2 i.v. twice daily on days −5 to −2; and then 140 mg/m^2 melphalan i.v. once on day −1).

After stem cell infusion, hematopoietic engraftment was monitored daily using CBC until both hematologic (neutrophil $\geq .5 \times 10^9$ cells/L for 3 consecutive days and platelets $\geq 50 \times 10^9$ cells/L for the first 3 consecutive days and no platelets transfusions for 7 days) and immunologic engraftment (ALC $\geq .5 \times 10^9$ cells/L) were documented or for a maximum of 30 days. Hereafter, disease status was assessed every 3 months by complete medical examination, CBC, blood chemistry, and PET or CT scans following the guidelines from the International Harmonization Project on Lymphoma [24].

A-ALC and Autograft Lymphocyte Subsets Assessment

The infused A-ALC for each apheresis unit collection was calculated as follows: A-ALC = % collection lymphocytes × (absolute WBC/kg) [15,16].

For the analysis of the autograft lymphocyte subset analysis, patients' autograft samples for each apheresis collection were collected and studied by flow cytometric analysis (Supplemental Appendix I).

Statistical Analysis

PFS was defined as the time from the date of infusion to disease progression, relapse, or death from any cause. Patients alive without disease progression or relapse were censored at their last disease evaluation or at their secondary primary cancer diagnosis, whichever occurred first, when estimating PFS. *Overall survival (OS)* was defined at the time from infusion to death from any cause. Survival curves for OS and PFS were analyzed using the approach of Kaplan-Meier [25].

The sample size was chosen under the assumption that the 1-year PFS rate when using the standard Amicus setting is 50%, 5 to 6 eligible patients could be enrolled per month, and the follow-up period after the close of enrollment would be at least 2 years. With a sample size of 63 eligible patients per arm, a 2-sided alpha = .05 log-rank test will have a 90% chance of detecting a 50% decrease in the hazard of disease progression when apheresis is done with modified Amicus setting relative to apheresis with standard Amicus setting. This is equivalent to an increase in the 1-year PFS rate from 50% to 70%.

Log-rank test and Cox models [26] were used to assess whether PFS or OS differed with respect to apheresis collection method. The Wilcoxon rank test was used to assess associations between categorical and continuous variables. Spearman rank correlation coefficients were used to examine the association between continuous variables.

For the autograft lymphocyte subset and clinical outcome analysis, the number of A-NKC, A-CD3C, A-CD4C, and A-CD8C cells/kg within each apheresis product were determined. For each of these parameters, a patient was classified as being above or below the parameter's median across the entire infusion cohort. Patients were also classified as being above or below the prespecified A-ALC value of $.5 \times 10^9$ cells/kg [15,16]. Stratified Cox models with treatment arm as the stratification factor were then used to assess whether PFS or OS differed with respect to A-ALC, A-NKC, A-CD3C, A-CD4C, and A-CD8C.

Role of Funding Source

The study was funded by the Goldman Philanthropic Partnership/Partnership for Cures and by the Predolin Foundation; neither the Goldman Philanthropic Partnership/Partnership for Cures or the Predolin Foundation played any additional role in the study or had access to the data presented here.

RESULTS

Study Cohort

Enrollment to this trial began on December 10, 2007 and was terminated on October 12, 2010 because of slow accrual and lack of funds. There were 122 individuals (ModC, 62; StdC, 60) enrolled onto this trial, 4 short of the accrual goal. One patient (ModC) cancelled participation before the start of peripheral stem cell mobilization because of relapse in the