Biol Blood Marrow Transplant xxx (2015) 1–6

Biology of Blood and Marrow Transplantation

journal homepage: www.bbmt.org

ASBMT American Society for Blood and Marrow Transplantation

Pretransplantation Minimal Residual Disease Predicts Survival in Patients with Mantle Cell Lymphoma Undergoing Autologous Stem Cell Transplantation in Complete Remission

Q6 Andrew J. Cowan [1], Philip A. Stevenson [1], Ryan D. Cassaday [1], Solomon A. Graf [1], Jonathan R. Fromm [2], David Wu [2], Leona A. Holmberg [1], Brian G. Till [1], Thomas R. Chauncey [1,3], Stephen D. Smith [1], Mary Philip [1], Johnnie J. Orozco [1], Andrei R. Shustov [1], Damian Green [1], Edward N. Libby III [4], William I. Bensinger [1], Mazyar Shadman [1], David G. Maloney [5], Oliver W. Press [1], Ajay K. Gopal [1,*]

Q1 [1] *Fred Hutchinson Cancer Research Center, University of Washington, Seattle, Washington*
[2] *Department of Laboratory Medicine, University of Washington, Seattle, Washington*
[3] *VA Puget Sound Health Care System, Seattle, Washington*
[4] *Division of Medical Oncology, Department of Medicine, University of Washington, Seattle, Washington*
[5] *Transplantation Biology Department, Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, Washington*

Article history:
Received 25 June 2015
Accepted 31 August 2015

Key Words:
Mantle cell lymphoma
Transplantation
Minimal residual disease
Predictors
Survival

A B S T R A C T

Autologous stem cell transplantation (ASCT) is standard therapy for mantle cell lymphoma (MCL) in remission after induction chemotherapy, with the best results for patients in complete remission (CR). We hypothesized that evaluation of minimal residual disease (MRD) before ASCT could further stratify outcomes for these patients. Patients with MCL who underwent ASCT in clinical CR between 1996 and 2011 with pretransplantation MRD testing were eligible. Presence of a clonal IgH rearrangement, t(11; 14) by PCR or positive flow cytometry from blood or bone marrow, was considered positive. An adjusted proportional hazards model for associations with progression-free (PFS) and overall survival (OS) was performed. Of 75 MCL patients in CR, 8 (11%) were MRD positive. MRD positivity was associated with shorter OS and PFS. The median OS for MRD-negative patients was not reached, with 82% survival at 5 years, whereas for the MRD-positive patients, median OS was 3.01 years (hazard ratio [HR], 4.04; $P = .009$), with a median follow-up of 5.1 years. The median PFS for MRD-negative patients was not reached with 75% PFS at 5 years, whereas for MRD-positive patients, it was 2.38 years (HR, 3.69; $P = .002$). MRD positivity is independently associated with poor outcomes after ASCT for MCL patients in CR.

INTRODUCTION

Mantle cell lymphoma (MCL) is an aggressive lymphoma, representing approximately 6% of all lymphomas in the United States, with a median survival of greater than 5 years [1,2]. The majority of patients with MCL are male and present at a median age of 68 [2,3]. The t(11; 14) (q13; q32) chromosomal translocation is characteristic of MCL and juxtaposes the *CCND1* gene in close proximity to the immunoglobulin heavy chain (*IGH*) gene locus, resulting in overexpression of cyclin D1 [4]. Although some patients may present with indolent disease, in general, MCL has an aggressive course, and most patients are found to have stage III to IV disease [2]. Historically, MCL has been associated with a poorer prognosis than many other lymphomas [2]. Over the last decade, there has been dramatic improvement in the management of patients with MCL with the advent of advances in transplantation, targeted novel therapies, and improved understanding of the molecular biology of MCL.

Typically, front-line management of MCL takes a risk-adapted approach, reserving the most intensive approach with high-dose therapy followed by autologous stem cell transplantation (ASCT) for younger, fitter patients, and treating elderly patients with less toxic regimens [2,5]. Several important clinical trials have established ASCT as a standard of care for MCL. The first randomized study by

Financial disclosure: See Acknowledgments on page 5.

* Correspondence and reprint requests: Ajay K. Gopal, MD, 825 Eastlake Ave E, MS: G3-200, Seattle, WA 98109-1023.
E-mail address: agopal@u.washington.edu (A.K. Gopal).

http://dx.doi.org/10.1016/j.bbmt.2015.08.035
1083-8791/

Dreyling et al. in 2005 compared induction chemotherapy with CHOP followed by autologous transplantation or interferon alpha (IFN) maintenance, and described a progression-free survival (PFS) of 54% for ASCT versus 25% for IFN at 3 years, a statistically significant difference [6]. Subsequent studies performed by the Nordic Lymphoma Group, the Cancer and Leukemia Group B, and the European MCL Network have confirmed prolonged disease control with intensive induction and ASCT, although not in the context of a randomized trial [7-9]. Accordingly, major societies, such as the European Society for Blood and Marrow Transplantation and European MCL Network have concluded that upfront ASCT for patients in remission after induction immunochemotherapy should be the standard of care for younger patients with MCL [10].

Although intensive induction followed by ASCT results in durable remissions for many, a significant proportion have disappointing outcomes. Investigations into predictors for patients with MCL undergoing ASCT have been studied; among those studied have been the Mantle Cell Lymphoma International Prognostic Index (MIPI), positron emission tomography–computed tomography (PET-CT) imaging, and measurement of minimal residual disease (MRD). The MIPI was created using a regression analysis of a clinical trial database and was shown to be a useful prognostic tool [11,12]. More recent studies have confirmed that the MIPI is also predictive of outcomes after transplantation, and when applied to the results of the Nordic MCL2 trial, both the MIPI and the simplified MIPI were demonstrated to be superior to the International Prognostic Index in predicting survival after ASCT [13-15]. In several series, PET-CT assessment before transplantation has been shown to be a good predictor of outcomes after transplantation [16-19]. Taken together with other data in non-Hodgkin lymphoma, the recent update of the Lugano Classification included PET-CT with standard staging, emphasizing the importance of this new modality in staging and assessment of disease response [20].

Limited data exist regarding the use of polymerase chain reaction (PCR) amplification of *IGH* rearrangements or flow cytometry–based MRD measures and outcome in MCL [21-23]. These studies only included patients in prospective clinical trials using patient-specific PCR assays, and few data exist regarding the application of this strategy to an unselected population of MCL patients undergoing ASCT when routine clinical testing is employed. We sought to address the hypothesis that clinical MRD testing would be predictive of outcome by analyzing the association of standard MRD assay results with outcomes in an unselected cohort of MCL patients undergoing ASCT in CR at our institution.

METHODS

Patients

Sequential patients older than 18 years of age with confirmed diagnosis of MCL who underwent ASCT between 1996 and 2011 at the Fred Hutchinson Cancer Research Center, University of Washington Medical Center, and Veterans Affairs Puget Sound Healthcare System (Seattle, WA) were eligible. Patients treated on an investigational study had signed a consent form authorized by the human subjects committee of the University of Washington and/or the institutional review board of the Fred Hutchinson Cancer Research Center in accordance with the Declaration of Helsinki. Separate institutional approval was also obtained to gather retrospective data from patient records and databases.

Study Variables

We collected baseline demographic data and patient characteristics at the time of diagnosis and at the time of ASCT, including age, presence of B symptoms (fever, night sweats, or >10% unintentional weight loss), number and type of treatments received before ASCT; and after transplantation, whether or not patients received maintenance rituximab. Simplified MIPI scores were calculated using data available at time of diagnosis [11]. Data on type of MRD analysis specimen and type of analysis were collected; presence of a clonal *IGH* rearrangement or t(11; 14) *IGH/CCND1* fusion by PCR or positive flow cytometry from blood, bone marrow, or apheresis products before transplantation was scored as MRD positive. Complete remission (CR), overall survival (OS), and PFS were defined by standard criteria [24].

Treatment

For this retrospective study, patients who received either conventional conditioning regimens or radioimmunotherapy-based regimens were included. Conventional regimens comprised either carmustine, etoposide, cytarabine, and melphalan; busulfan, melphalan, and thiotepa; or cyclophosphamide with or without etoposide in combination with 12 Gray of total body irradiation, as previously documented [25,26]. Radioimmunotherapy was given either alone or with increasing doses of fludarabine or with cyclophosphamide and etoposide [27-29].

Flow Cytometry

Multiparameter flow cytometry was used to assess MRD and is described in detail elsewhere [30-32]. Specimens were prepared by prelysis (with ammonium chloride) or simultaneously lysed and fixed (with ammonium chloride and formaldehyde), stained with antibodies, and run on the flow cytometer as described in prior publications from our group [33]. Data were analyzed using custom, in-house software as described elsewhere [33]. The sensitivity to detect minimal residual disease is ~ 1 in 10,000 cells and dependent on the number of analyzed events by flow cytometry, which typically was greater than 200,000 viable events per sample.

PCR-based MRD Assessment

Purification of DNA for PCR used either EZ1 Advanced XL solution (Qiagen, Venlo, Netherlands) or Puregene DNA Isolation kit (Gentra Systems, Minneapolis, MN). Samples were analyzed for B cell clonality by PCR using testing for *IGH* and, in some cases, kappa light chain gene rearrangement employing a method developed by the European BIOMED-2 Concerted Action study group [34]. PCR products are subsequently evaluated by capillary electrophoresis. The sensitivity of detecting a clonal *IGH* or kappa light chain gene is approximately 2% to 5% and is dependent on the background number of B or plasma cells in the sample, in addition to the amount and quality of input DNA.

The assay used for detection of t(11; 14), (*IGH-CCND1*), uses a nested PCR with primers directed against the major translocation cluster of breakpoints in the *CCND1* locus on chromosome 11, coupled with consensus primers directed against the J regions of the *IGH* gene on chromosome 14. PCR products are evaluated by gel electrophoresis and visualized with ethidium bromide staining. The sensitivity for detection of the t(11; 14) fusion by nested-PCR ranges in this clinical assay ranges from 1 in 10,000 to 1:30,000, dependent on the amount and quality of the input DNA.

Statistical Considerations

Survival analysis using Kaplan-Meier curves were generated to estimate the OS and PFS from the time of ASCT. MRD, along with clinical factors was evaluated in an adjusted Cox proportional hazards regression model for associations with PFS and OS.

RESULTS

Patient Characteristics

We identified 75 patients with MCL who were in a CR at the time of ASCT and who subsequently underwent ASCT between the years 1996 and 2011. The baseline demographics and patient characteristics of all patients, as well as those with MRD-positive and -negative statuses, are depicted in Table 1. The median age at the time of transplantation was 58 years (range, 38 to 71) for all patients and the majority of the patients were male. The median time from diagnosis to initial induction treatment was 1 month (range, 0 to 12 months), and a minority of patients (n = 17) had B symptoms at diagnosis (23%). Of evaluable patients, 26 (41%) had a simplified MIPI between 0 and 2, and 37 (59%) had a simplified MIPI greater than 2 (59%). The majority of patients received rituximab within 3 months of ASCT. Initial induction chemotherapy regimens included HyperCVAD in 37 patients (49%), CHOP in 35 (47%), CVP in 1 (1%),