CLINICAL RESEARCH

Partial T Cell-Depleted Allogeneic Stem Cell Transplantation following Reduced-Intensity Conditioning Creates a Platform for Immunotherapy with Donor Lymphocyte Infusion and Recipient Dendritic Cell Vaccination in Multiple Myeloma

Henriëtte Levenga,[1] Nicolaas Schaap,[1] Frans Maas,[2] Bennie Esendam,[2] Hanny Fredrix,[2] Annelies Greupink-Draaisma,[2] Theo de Witte,[1] Harry Dolstra,[2] Reinier Raymakers[1]

Allogeneic stem cell transplantation (SCT) in multiple myeloma (MM) may induce a curative graft-versus-myeloma (GVM) effect. Major drawback in unmanipulated reduced-intensity conditioning (RIC) SCT is the risk of severe and longstanding graft-versus-host-disease (GVHD). This study demonstrates that transplantation with a partial T cell-depleted graft creates a platform for boosting GVM immunity by preemptive donor lymphocyte infusion (DLI) and recipient dendritic cell (DC) vaccination, with limited GVHD. All 20 MM patients engrafted successfully. Chimerism analysis in 19 patients evaluable at 3 months revealed that 7 patients were complete donor, whereas 12 patients were mixed chimeric. Grade II acute GVHD (aGVHD) occurred in 7 patients (35%) and only 4 patients (21%) developed chronic GVHD (cGVHD). Fourteen patients received posttransplantation immunotherapy, 8 preemptive DLI, 5 patients both DLI and DC vaccination, and 1 patient DC vaccination only. DC vaccination was associated with limited toxicity, and none of these patients developed GVHD. Importantly, overall treatment-related mortality (TRM) at 1 year was low (10%). Moreover, the overall survival (OS) is 84% with median follow-up of 27 months, and none of the patients died from progressive disease. These findings illustrate that this novel approach is associated with limited GVHD and mortality, thus creating an ideal platform for adjuvant immunotherapy.

Biol Blood Marrow Transplant 16: 320-332 (2010)

KEY WORDS: Multiple myeloma, Reduced-intensity conditioning, Donor lymphocyte infusion, Dendritic cell vaccination, Graft-versus-myeloma

INTRODUCTION

Allogeneic stem cell transplantation (SCT) may cure patients with multiple myeloma (MM) because of a graft-versus-myeloma (GVM) effect. Myeloablative (MA) conditioning has been limited by a high treatment-related mortality (TRM), and at present, reduced-intensity conditioning SCT (RIC-SCT) following autologous SCT seems a promising approach. Importantly, TRM following RIC-SCT is reduced from 30% to 40% to 10% to 20% [1]. However, 3 prospective trials comparing autologous transplantation followed by RIC-SCT versus double autologous SCT showed contradictory results in clinical outcome [2-4]. The study by Bruno et al. [2] showed a superior overall survival (OS) for autologous SCT followed by allogeneic RIC-SCT. In line with this study, Rosiñol et al. [4] observed a trend toward a longer progression-free survival (PFS) for patients treated with auto/RIC-SCT, but no significant differences in event-free survival (EFS) and OS. In contrast, the Intergroupe Francophone du Myelome (IFM) observed no differences in EFS and OS comparing double autologous SCT versus auto/RIC-SCT in high-risk patients. Although these differences in outcome may be explained by different inclusion criteria and treatment schedules, they illustrate that improvement of the

From the [1]Department of Hematology; and [2]Central Hematology Laboratory, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands.

Financial disclosure: See Acknowledgments on page 332.

Correspondence and reprint requests: Henriëtte Levenga, MD, Department of Hematology, Radboud University Nijmegen Medical Centre, Geert Grooteplein 8, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands (e-mail: t.levenga@hemat.umcn.nl).

Received June 4, 2009; accepted October 7, 2009

1083-8791/10/163-0004$36.00/0
doi:10.1016/j.bbmt.2009.10.006

GVM effect, without the toxicity and morbidity of graft-versus-host disease (GVHD) after allogeneic RIC-SCT, is a prerequisite to further establish this therapeutic approach.

Previously, we showed that partial T cell-depleted allogeneic SCT followed by preemptive donor lymphocyte infusion (DLI) resulted in long-term complete remission (CR) in about one-third of MM patients [5]. In this cohort of 24 patients, 1-year TRM after MA conditioning was 29%. But a continuous CR in 7 MM patients after preemptive DLI with a median follow-up of 8.6 years encouraged us to investigate partial T cell-depleted allogeneic SCT in the RIC setting, combined with preemptive immunotherapy with DLI. The major advantage of T cell-depleted grafts is reduction of severe and prolonged GVHD, but effective posttransplantation immunotherapy is essential to overcome the higher rate of relapse. As a novel approach we incorporated recipient-derived dendritic cell (DC) vaccination in the posttransplantation strategy for patients with residual disease after two preemptive DLI dosages.

DC are the professional antigen-presenting cells (APCs) of the immune system, and are essential for the induction of antigen-specific T cell immunity. In the setting of allogeneic SCT and DLI, alloreactive T cell responses targeting minor histocompatibility antigens (MiHA) on malignant cells of the recipient can be induced directly by recipient-derived DC and indirectly by donor-derived DC because of crosspresentation [6]. Boosting GVM immunity by vaccination with donor-derived DC loaded with hematopoiesis-restricted MiHA seems most ideal, but this approach is hampered by the limited number of known MM-expressed MiHA. Studies in mouse models demonstrated that recipient DC play a pivotal role in the initiation of alloreactive $CD8^+$ T cell-mediated immunity against leukemia [7,8]. Moreover, the presence of recipient DC in the setting of mixed chimerism has a positive impact on the effectiveness of DLI [9]. Because recipient DC and myeloma tumor cells are both derived from the hematopoietic system, immune responses induced by recipient-derived DC may enhance GVM with limited GVHD in other tissues, like mucosa, liver, and skin.

Here, we show the results of partial T cell-depleted RIC-SCT after autologous transplant for MM, with limited GVHD and a low 1-year TRM of 10%. Furthermore, we investigated the feasibility of generating mature recipient-derived DC from cryopreserved apheresis products, the immunogenicity of the vaccine, and the toxicity of recipient-derived DC vaccination. Our study indicates that partial T cell-depleted RIC-SCT is feasible, results in excellent engraftment, and offers opportunities for posttransplantation cellular immunotherapy with DLI in some patients combined with DC vaccination. Importantly, our approach keeps open the treatment with novel agents (bortezomib and lenalidomide) in case of progressive or relapsed disease even in combination with DLI.

MATERIALS AND METHODS

Transplantation Procedure

From January 2006 to May 2008, 20 patients have been included in a pilot study of partial T cell-depleted, allogeneic RIC-SCT for MM. All patients were pretreated for symptomatic MM with induction chemotherapy and high-dose melphalan (HDM), followed by autologous SCT (conform HOVON-50 or HOVON-65 studies or standard induction scheme at that time) [10]. Patients <65 years with an HLA-identical sibling donor were offered upfront allogeneic RIC-SCT within 6 months after autologous transplant, regardless of risk factors or disease status. Before RIC-SCT, autologous PBMC were collected by apheresis, washed to deplete platelets, and cryopreserved for posttransplant DC vaccination (Figure 1). The conditioning regimen consisted of cyclophosphamide (Cy) 1200 mg/m^2 .v. in combination with fludarabine (Flu) 30 mg/m^2 on each of 4 consecutive days (days −5, −4, −3, and −2 before SCT). Donor stem cell grafts were depleted from T and B cells by anti-CD3 and anti-CD19 immunomagnetic beads (Miltenyi Biotec, Bergisch Gladbach, Germany). Following depletion, $CD3^+$ T cells were added back to generate a stem cell graft containing a fixed number of 0.5×10^6 T cells/kg body weight of recipient. GVHD prophylaxis consisted of Cyclosporine A (CsA) 3 mg/kg/day .v. starting on day −1 until CsA could be taken orally. CsA was administered orally at a dose of 6 mg/kg/day until 8-10 weeks after SCT followed by a gradually tapering off in 4 weeks. Acute and chronic graft-versus-host disease (aGVHD, cGVHD) were classified grade I-IV and limited or extensive, respectively, according to the criteria described by Glucksberg [11] and Shulman [12].

Evaluation of Response and chimerism Analysis

Responses were evaluated according to the response criteria for MM described by Durie et al. in 2006 [13]. Bone marrow (BM) aspirates during posttransplantation immunotherapy were performed in patients receiving DC vaccination. Lambda free light chains were measured using the serum free light chain (FLC) assay (Freelite, Birmingham, UK). For measuring kappa free light chains, we used the ELISA assay, as described by Lamers et al. [14]. This ELISA was shown to parallel FLC kappa assay, with lower absolute values. To define CR, the FLC ratio was measured with the Freelite assay for both lambda and kappa free light chains. The data were analyzed in December 2008.