Induction of Autologous Graft-versus-Host Disease: Results of a Randomized Prospective Clinical Trial in Patients with Poor Risk Lymphoma


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ABSTRACT
The results of blood or marrow transplantation in patients with chemorefractory aggressive lymphoma, that is, those not responding to conventional-dose chemotherapy at the time of transplant, have been poor. The relapse rate has been high after autologous bone marrow transplant, whereas allogeneic transplantation has been associated with excessive transplant-related toxicity. Administration of cyclosporine after autologous transplantation can induce an autoreactive syndrome that resembles graft-versus-host disease (GVHD). This syndrome, named autologous graft-versus-host disease, has clear antitumor activity in animal models that can be enhanced by the addition of cytokines such as interferon and interleukin-2. A randomized, prospective study was conducted to evaluate the antitumor effect of autologous graft-versus-host disease induced with cyclosporine, and augmented by the administration of interferon and interleukin-2 in patients with chemorefractory Hodgkin and aggressive non-Hodgkin lymphomas. Fifty-one patients were randomized, 24 to the autologous GVHD induction arm, and 27 to the noninduction arm after autologous transplant using mobilized peripheral blood stem cell (PBSC) grafts. There were no differences in treatment-related mortality, overall and event-free survival (OS, EFS) between both groups; however, in the induction arm, GVHD developed only in 4 patients. The administration of oral cyclosporine followed by interleukin-2 and interferon is generally not well tolerated, and does not appear to be an effective method to induce autologous GVHD in patients receiving autologous PBSC grafts.

KEY WORDS
Autologous graft-versus-host disease ● Graft-versus-host disease ● Lymphoma ● Hodgkin lymphoma ● Autologous stem cell transplant

INTRODUCTION
The use of high-dose chemotherapy or chemoradiotherapy with autologous stem cell rescue is a common strategy to treat patients with relapsed or refractory lymphoma. However, it is clear that this approach does not cure all patients, especially those transplanted with chemorefractory disease, that is, not responding to conventional-dose chemotherapy at the time of transplant [1-4]. This has triggered an interest in evaluating the graft-versus-tumor (GVT) effect of allogeneic bone marrow transplantation (BMT) in these diseases in an attempt to exploit the immune response against the neoplastic cells [1,3,5-7]. However, many patients will lack an HLA matched donor, and transplant-related toxicity has been high in chemoresistant lymphoma patients. Therefore, immunotherapy utilizing autologous graft-versus-host disease...
(GVHD) induction is an attractive approach for these high-risk patients.

The administration of cyclosporine after an autologous peripheral blood stem cell (PBSC) or BMCT can induce an autoimmune clinical syndrome that closely resembles acute GVHD (aGVHD) in up to 80% of treated patients [8-11]. This syndrome, autologous GVHD, is a mild, self-limited disease that generally involves only the skin. Histologic changes in the skin during autologous GVHD are identical to those of allogeneic GVHD. In rodent models, autologous GVHD induced by treatment with cyclosporine is mediated by autoreactive lymphocytes directed against class II histocompatibility (HLA-DR or Ia) antigens [12]. These autoreactive lymphocytes also lyse MHC class II positive tumor cells in vivo. Tumor cell lysis was increased with gamma interferon (γ-IFN) and interleukin-2 (IL-2) [13,14]. The mechanism of this effect appears to be different for the 2 cytokines: γ-IFN increases class II expression on the tumor, thereby enhancing tumor cell recognition [13], whereas IL-2 augments the effector mechanisms [14-16]. Because most hematopoietic malignancies express MHC class II antigens, autologous GVHD could potentially produce a clinical immunologic antitumor effect without significantly increasing posttransplant toxicity. Preliminary clinical studies also suggested that autologous GVHD might improve disease-free survival (DFS) [17,18]. To determine if this approach provides an antitumor benefit, a randomized prospective clinical trial was conducted comparing induction of autologous GVHD to standard therapy in patients with chemotherapy resistant aggressive lymphomas.

**PATIENTS, MATERIALS, AND METHODS**

**Study Group**

All patients receiving autologous transplants for chemoresistant Hodgkin lymphoma (HL) or aggressive non-Hodgkin lymphoma (NHL) were eligible for inclusion in this study. Low-grade lymphoma, such as follicular grade 1 or 2 or monocytoid B cell lymphomas were excluded. Chemoresistant disease was defined as: (1) progressive disease developing during or within 6 weeks of completing initial induction therapy, or (2) failure to achieve at least an overall partial response (greater than at least a 50% reduction in tumor size assessing the products of the perpendicular diameters of all measurable lesions) to conventional salvage therapy following relapse. Patients required an adequate yield from mobilized peripheral blood harvest. To ensure patient safety, patients had to have adequate organ function (renal, cardiac, and pulmonary, and absence of fever) to participate in the trial posttransplant.

**Treatment**

Peripheral blood stem cells were collected after mobilization with cyclophosphamide 2.5 g/m² and granulocyte-colony stimulating factor (G-CSF) (10 μg/kg/day). Target yield of apheresis was >5 × 10⁶ CD34 cells/kg with a minimum of 2 × 10⁶ CD34 cells/kg. After collection, patients were randomized to the treatment arm or to observation. All patients received busulfan and cyclophosphamide or cyclophosphamide and total body irradiation (TBI) preparative regimen (if there had been prior radiotherapy total body irradiation was not used). Patients assigned to the GVHD induction arm started cyclosporine (Neoral) at 2 mg/kg twice a day (i.v. formulation was given to those unable to take pills) starting on the day of the BMT and continued until γ-IFN and IL-2 were completed. γ-IFN started when the total white count was >200 cells/mL for 2 consecutive days posttransplant and it was given at a dose of 0.025 mg/m² subcutaneous every other day for 10 doses. The dose of IL-2 started 2 days later and the dose was 1 × 10⁶ units/m² subcutaneous for 18 days. G-CSF was given after the transplant until WBC was 1000 for 3 days, 10,000/mm³ on 1 occasion, or 5000/mm³ on 2 occasions. Should the WBC drop to <1000 on γ-IFN, G-CSF was restarted until the white count was consistently over 1000.

If clinical Stage I GVHD [19] was diagnosed, cyclosporine, γ-IFN, and IL-2 were discontinued. Clinical GVHD was confirmed by biopsy. GVHD was treated according to the standard practice at the Johns Hopkins Hospital at that time. Cyclosporine levels were not followed because previous studies revealed that a fixed dose of the drug was capable to induce autologous GVHD [8-12]. Cyclosporine was adjusted only for renal failure according to the following schedule: creatinine >2.2 mg/dL, decrease dose by 25%; creatinine >3.0, decrease dose by 75%, and creatinine >4.0; hold drug. The only dose modification of γ-IFN or IL-2 anticipated in this study was discontinuation of therapy. This was done if cyclosporine was discontinued because of GVHD or unexpected toxicity of γ-IFN and/or IL-2. IL-2 and γ-IFN could be held for 48 hours beyond their anticipated administration time point to assess whether a particular toxicity was related to these drugs.

**Evaluation**

Prior to returning home after the transplant, patients were assessed for response with computed tomography scans of chest, abdomen, and pelvis, plus other sites of disease. Bone marrow biopsy in patients with previous bone marrow involvement and bone marrow aspirate for tumor marker studies in patients who had a known tumor marker pre-BMT were also preformed.
Statistical Design

Based on preliminary data, the study was designed to detect a 40% improvement in 1-year DFS from 10% to 50% in patients randomized to receive autologous GVHD induction. Twenty-five patients per arm were required to detect this difference with 80% power using a 2-sided 0.05 alpha-level test. Even if the improvement were modestly less (ie, 35%), this sample size would detect the difference with relatively high power. A 20% improvement in DFS was considered to be clinically important. However, to detect a 20% improvement with the same power would require 75 patients in each arm. Such a study would require a cooperative group study rather than a single-institution trial.

Two analyses of the randomized trial were performed. The first was based on the intention-to-treat principle. It compared the outcome in all patients randomized to the intervention arm versus all patients randomized to the other arm. A second analysis of results was also performed. The “per-protocol” analysis compared patients randomized to the treatment arm who actually received the treatment. They were compared to those in the control group who met eligibility criteria to receive IL-2 and γ-IFN. These consisted of screening for renal function, liver function, pulmonary function, and absence of fever.

The design of the study allowed for early termination (based on 1 interim analysis halfway through enrollment) because of evidence of engraftment failure, unexpectedly high transplant-related mortality (TRM), or poor relapse-free survival. To terminate early for efficacy (as measured by disease-free survival), the interim $P$-value would have had to be $\leq 0.005$.

Event-free survival (EFS) was compared between the 2 arms of the study using a competing risks analysis, where TRM was considered a competing risk. Cumulative incidence of relapse at 12 months and their 95% confidence intervals were calculated in addition to a $P$-value testing the difference in relapse between the 2 groups [20]. The “per-protocol” analysis was performed in the same manner. Kaplan-Meier methods were used for evaluating time to event (defined as relapse or death) and overall survival (OS). Incidence of early mortality (<60 days from randomization) was compared in the 2 arms using a Fisher’s exact test to compare proportions, and 95% confidence intervals were calculated using an exact binomial procedure. OS was defined as the time from study entry until death by any cause. EFS was defined as the time from study entry until relapse or death. OS was defined as the time from study entry until death by any cause.

Ethical Principles

The study was approved by the Johns Hopkins Institutional Review Board, and all patients signed informed consent. A data safety monitoring committee supervised the study.

RESULTS

Patients

Between 10/14/1997 and 4/16/2002, 54 patients were screened and 51 patients were enrolled. Twenty-four patients were randomized to the treatment arm and 27 to the control arm. Table 1 shows the characteristics of the groups.

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<th>Table 1. Demographics</th>
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<tr>
<td>17 Bu-Cy/10 Cy-TBI</td>
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<td>Bone marrow involvement</td>
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<td>Failed to achieve a PR to salvage therapy following relapse</td>
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<td>Progressive disease less than 6 weeks after completing induction</td>
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W indicates White; AA, African-American; DLBC, diffuse large cell; NHL, non-Hodgkin lymphoma; HL, Hodgkin lymphoma; XRT, radiation therapy; PR, partial response; Bu-Cy, busulfan and cyclophosphamide; Cy-TBI, cyclophosphamide and total body irradiation; GVHD, graft-versus-host disease.
Autologous GVHD

Four patients (16%) in the treatment arm developed a clinically significant rash with biopsy-proven GVHD. The first patient developed skin GVHD (stage 2, grade I) while on cyclosporine so did not receive γ-IFN and IL-2. The patient is in a complete remission 6 years after transplant. The second patient received 5 doses of γ-IFN and 6 doses of IL-2 and then developed autologous skin GVHD (stage 2, grade I), but succumbed to VOD. The third patient developed flu-like symptoms and skin GVHD (stage 2, grade I) while on γ-IFN and IL-2 so the drugs were discontinued. This patient died of relapsed lymphoma 6 years after transplant. The fourth patient developed skin GVHD (stage 2, grade I) while on cyclosporine so did not receive γ-IFN and IL-2. The patient died of acute respiratory distress syndrome. No GVHD was observed in the control arm.

Survival and Relapse Analysis

As of April 11, 2006, 34 patients have died, 18 of 27 (66%) in the control arm and 16 of 24 (66%) in the GVHD induction arm. Based on a competing risks intent-to-treat analysis, the 12-month incidence of relapse in the control and treated groups are 63% and 50%, respectively ($P = .35$) (Table 2). The cumulative incidence of relapse is shown in Figure 1. TRM was 21% in the treatment group and 7% in the control group ($P = .23$).

In the “per-protocol” analysis, there are 18 patients in the treated group who completed treatment and they were compared to 25 patients in the control group who would have been eligible to begin the experimental treatment. The per-protocol analysis showed no difference in the incidence of relapse as shown in Figure 2, with GVHD induction and control groups having 12-month incidence of relapse of 61% and 60%, respectively ($P = .84$) as described in Table 2. There were no differences in EFS (death or relapse) or OS between both groups by either analysis (“per protocol” or intent to treat) (Figure 3).

Adverse Events

Of the 24 patients who started cyclosporine, 6 did not begin IL-2 or γ-IFN: 1 because of renal failure, 1 because of renal failure and skin GVHD, 1 because of capillary leak syndrome, 1 because of skin GVHD, 1 because of VOD of the liver, and 1 because of VOD renal failure. Eight patients stopped IL-2 and/or γ-IFN early: because of eosinophilia (15,390/mm$^3$, $n = 1$), flu-like symptoms ($n = 2$), fever ($n = 1$), capillary leak syndrome and renal failure ($n = 1$), VOD and skin GVHD ($n = 1$), mental status changes ($n = 1$), and flu-like symptoms and skin GVHD ($n = 1$). Ten patients tolerated the entire induction therapy (ie, cyclosporine, γ-IFN, and IL-2). Seven deaths occurred prior to day 60: 5 among patients randomized to autologous GVHD and 2 in the control group ($P = \ldots$)}
23); however, only 1 of these patients received γ-IFN and IL-2. Four of the 7 deaths were related to known preparative-regimen related toxicities (adult respiratory distress syndrome, interstitial pneumonitis, VOD/liver failure), whereas 3 patients died of progressive lymphoma.

**DISCUSSION**

The group of patients with refractory disease included in the present trial historically has a very poor outcome. Philip et al. [21] studied 100 such patients with intermediate-grade or high-grade NHL. Thirty-four percent had disease that had been refractory to primary chemotherapy, and 66% had had a complete remission with primary chemotherapy but later relapsed. After high-dose therapy and bone marrow transplantation, the actuarial 3-year DFS was zero in the refractory group, 14% in the resistant-relapse group, and 36% in the sensitive-relapse group. Even when posttransplant therapy may improve the outcome of these patients [4], the results are far from satisfactory. Our experience is similar with poor results in this group of patients. Aksentijevich et al. [3] reported that patients with resistant diffuse large cell NHL at the time of BMT, only 12.5% and 19.1% of patients survived 3 years following allo- or auto-stem cell transplantation (SCT), respectively (P = .08). Aksentijevich et al. [3] reported that patients with resistant diffuse large cell NHL at the time of BMT, only 12.5% and 19.1% of patients survived 3 years following allo- or auto-stem cell transplantation (SCT), respectively (P = .08). Akpek et al. [1] analyzed the outcome of 157 consecutive patients with relapsed or refractory HL, who underwent SCT between March 1985 and April 1998. Disease status before SCT (sensitive relapse if responding to conventional-dose therapy or resistant disease if not) was an independent predictor of EFS and relapse (P < .0001).

GVHD is associated with a GVT as evidenced by a decreased relapse rate after allogeneic SCT [22]. In animal models and exploratory clinical trials, autologous GVHD also appears to induce a GVT effect [10,11]. Autologous GVHD has been observed using many “induction” regimens. Ratanatharathorn et al. [23] reported on the use of cyclosporine and α-IFN in a small clinical trial [23]. The study showed that this approach was feasible, with a majority of patients developing autologous GVHD after BMT. Cyclosporine-induced autologous GVHD has been studied intensively by our group [8,10-12,14,24-27]. From the early clinical studies, it was clear that the administration of cyclosporine could predictably induce skin GVHD after BMT [8,10,11]. Jones et al. reported that 5 of 5 patients with lymphoma developed autologous GVHD after exposure to cyclosporine. Later on, patients with leukemia that received a nonpurged [11] or purged [10] SCT exposed to cyclosporine also developed autologous GVHD in high proportions (close to 80%). Vogelsang et al. [18] reported a clinical trial on patients with hematologic malignancies receiving cyclosporine and IFN to induce autologous GVHD in patients receiving 4HC-purged marrow grafts. Treatment with cyclosporine and γ-IFN after BMT was well tolerated and did not impair engraftment. EFS with a median of 964 days of follow-up was 44%. Clinically significant GVHD was seen in 20% of cases.

In the current study, only 4 patients developed clinically apparent GVHD. This is in marked contrast to our previous autologous GVHD trials where the majority of patients developed clinical evidence of GVHD [5,8,18]. In animal studies of autologous GVHD, immunologic effector cells and antitumor
activity was seen only in those animals actually developing the syndrome [12,14]. Thus, the lack of clinically significant antitumor effect in the current trial may have resulted from the inability to induce the syndrome in most patients. The relatively low rate of observed autologous GVHD in the present study may result from the use of mobilized peripheral blood instead of BM. Previous studies have also found a much lower incidence of autologous GVHD utilizing mobilized peripheral blood grafts rather than BM [17,26]; this primarily appears to be the result of the infusion of a large number of T cells and monocytes that may downregulate the development of autologous GVHD [17,26,28,29]. Mobilized peripheral blood contains approximately 10 times more T cells than do bone marrow grafts [30]. Interestingly, animal studies suggest that the transfer of mature T cells along with the graft can modify the ability to induce autologous GVHD [31-33]. Indeed, it has been reported that autologous GVHD will not occur unless T cells are removed from the peripheral blood graft [17,34]. The use of oral cyclosporine (instead of parenteral) may also play an important role, as changes in bioavailability of the drug in the thymus may prevent the induction of autoreactive T cells [35]. Other factors such as the criteria for diagnosis of GVHD and the use of biopsies in patients without symptoms can also affect the expected frequency of GVHD [11,18,36,36-39]. In the current study, patients had biopsies only when they developed clinical evidence of GVHD and strict criteria (standard at our institution) for the diagnosis of skin GVHD were followed (lymphocytic infiltrate with dyskeratosis). Certainly, it is possible that a GVT was present but not detected because of the sample size (see the Statistical Design section) or because of a “better than expected” outcome in the control arm [21]. This study was designed to detect a 40% improvement in 1-year DFS from 10% to 50% in patients randomized to receive autologous GVHD induction. The idea behind this ambitious goal was that if a large difference was obtained, we would be confident that the effect found was substantial, which would justify expanding rapidly into other patient groups. Conversely, if the effect was less clear (as it happened), a much larger patient population would be needed to detect any benefit. Further studies would require cooperative group trials as the 1 currently conducted by the Children’s Oncology Group that hopefully will help to clarify this issue.

REFERENCES


