T-Cell–Depleted Reduced-Intensity Transplantation Followed by Donor Leukocyte Infusions to Promote Graft-Versus-Lymphoma Activity Results in Excellent Long-Term Survival in Patients With Multiply Relapsed Follicular Lymphoma


ABSTRACT

Purpose
Follicular lymphoma (FL) is an indolent disorder that is treatable but considered incurable with chemotherapy alone. The curative potential of allogeneic transplantation using conventional myeloablative conditioning has been demonstrated, but this approach is precluded in the majority of patients with FL because of excessive toxicity. Thus, reduced-intensity conditioning regimens are being explored.

Patients and Methods
This study reports the outcome of 82 consecutive patients with FL who underwent transplantation using fludarabine, melphalan, and alemtuzumab for in vivo T-cell depletion. Patients were heavily pretreated, having received a median of four lines of prior therapy, and 26% had experienced treatment failure with previous autologous transplantation. Median patient age was 45 years, and 52% of patients received stem cells from unrelated donors.

Results
With a median follow-up time of 43 months, the nonrelapse mortality was 15% at 4 years (8% for sibling and 22% for unrelated donor transplantations), acute grade 2 or 3 graft-versus-host disease (GVHD) occurred in 13%, and the incidence of extensive chronic GVHD was only 18%. Although relapse risk was 26%, this was significantly reduced where mixed chimerism had been converted to full donor chimerism by the use of donor lymphocyte infusion (DLI; \( P = .03 \)). In addition, 10 (77%) of 13 patients given DLI for relapse after transplantation experienced remission, with nine of these responses being sustained. Current progression-free survival at 4 years was 76% for the whole cohort (90% for those with sibling donors and 64% for those with unrelated donors).

Conclusion
The excellent long-term survival with associated low rates of GVHD and the frequency and durability of DLI responses make this an extremely encouraging strategy for the treatment and potential cure of FL.


INTRODUCTION

Although most patients with follicular lymphoma (FL) readily respond to chemoradiotherapy and some experience a long remission after high-dose treatment with autologous stem cell support, few remain disease free long term. Allogeneic transplantation with myeloablative conditioning has been successful in some, particularly younger patients and patients with HLA-matched related donors, and a graft-versus-lymphoma effect has been demonstrated.1-3 However, the prevalence of this disease in older age groups and the disparity between the treatment-related mortality of transplantation and that of chemotherapy alone, makes the use of such techniques in an indolent malignancy unappealing for most.

More recently, allogeneic transplantation using reduced-intensity conditioning has been developed and applied to patients previously considered ineligible because of age or comorbidity for myeloablation. Early data on reduced-intensity transplantation (RIT) in lymphoma suggested efficacy but with heterogeneity of histology and short...
follow-up.\textsuperscript{1-9} More recently, data on FL with lengthier follow-up has been reported. These series are largely based on a strategy of stem-cell return without T-cell depletion. While maximizing an early graft-versus-host disease (GVHD) risk, with most studies reporting chronic extensive GVHD in more than 40% of patients, with its associated morbidity/mortality.\textsuperscript{10-12} An alternate approach is partial T-cell depletion at transplantation, with a program of adoptive immunotherapy with donor lymphocyte infusions (DLIs) targeted at those whose disease recurs. Apart from reducing GVHD, particularly because HLA-matched or -mismatched unrelated donors are frequently required, this approach may be well-suited to the treatment of an indolent malignancy, where rapid establishment of an allogeneic effect might be less important and where responses to DLI are commonly seen. We report a series of 82 consecutive patients with FL who received transplantation with a T-cell–depleted reduced-intensity regimen at nine centers.

### Patients and Methods

**Patients**

Patients were treated initially on a prospective multicenter study using the regimen described here in hematologic malignancy from 1998. The protocol was ethically approved, and all patients gave written consent. On completion of the prospective study, clinicians elected to continue using this protocol, approved by their local institutions. Patients gave signed consent to data collection for research. Exclusion criteria for transplantation were creatinine clearance less than 40 mL/min, left ventricular ejection fraction less than 40%, and ALT more than 3 times the upper limit of normal. Patients had Eastern Cooperative Oncology Group performance status ≤ 2 and Karnofsky performance status ≥ 80%. All patients who received transplantation for biopsy-proven FL, with no evidence of histologic transformation, were eligible for this analysis, and 82 consecutive patients were included, who received transplantation from 1998 to 2009 at nine centers. Nineteen of these patients have been previously reported and are included with more than 5 years of additional follow-up.\textsuperscript{3} All diagnostic biopsies were assessed by specialist hematopathologists. Patient characteristics are listed in Table 1.

### Conditioning Regimen

Conditioning was fludarabine (30 mg/m²/d) on days −7 to −3, melphalan (140 mg/m²) on day −2, and alemtuzumab to provide in vivo T-cell depletion. The standard alemtuzumab dose was 100 mg (20 mg/d on days −8 to −4), given to 60 patients (73%), with 22 patients receiving less than 100 mg (60 mg, n = 8; 40 mg, n = 6; 30 mg, n = 5; 20 mg, n = 3). Cyclosporine (3 mg/kg/d intravenous) was given from day −1 and tapered from 3 months. Stem-cell source was bone marrow (n = 11; 13%) or peripheral blood after granulocyte colony-stimulating factor mobilization, and stem cells were infused unmanipulated.

### Supportive Care/Monitoring

Patients received antimicrobial prophylaxis and cytomegalovirus (CMV) surveillance as previously described.\textsuperscript{13} Antifungal prophylaxis and granulocyte colony-stimulating factor were given to all but 12 patients. Blood was assayed for chimerism by polymerase chain reaction analysis of minisatellite regions (either on unseparated mononuclear cells or on T-cell, B-cell, and myeloid lineages). Disease status was monitored from 3 months, and patients were eligible for treatment with DLI in the presence of mixed chimerism (MC) or residual disease from 6 months. DLIs were concomitantly in the presence of greater than grade 1 GVHD. The standard starting dose was 1 × 10⁹/kg, with further DLIs given every 3 months at three-fold dose escalation. DLIs could be administered at any time for progressive disease, with the dose varying according to donor type and time from transplantation.

### Study End Points

End points were engraftment, CMV reactivation, occurrence of GVHD, incidence of relapse and response to therapy, and incidence and cause of mortality. Neutrophil engraftment was defined as a count of ≥ 0.5 × 10⁹/L, and platelet engraftment was defined as an unsupported count of ≥ 20 × 10⁹/L, on the first of 2 consecutive days.

GVHD was assessed according to consensus guidelines.\textsuperscript{14,15} Nonrelapse mortality (NRM) was defined as death without relapse. Chemotherapy sensitivity before transplantation required at least partial remission by standard computed tomography criteria.\textsuperscript{16} Positron emission tomography scans were performed in two centers, with complete remission (CR) requiring physiologic fluorodeoxyglucose uptake in addition to CR by computed tomography. Relapse was defined as the recurrence of morphologically or radiologically detectable disease, and death after relapse was ascribed to disease.

Progression-free survival (PFS) was measured from transplantation until relapse or death. Patients in ongoing remission after DLI given for progression or relapse were censored at last follow-up for current PFS (cPFS).

Actuarial curves were estimated according to the Kaplan-Meier method for overall survival (OS) and cPFS, and the cumulative incidence (CI) method was used for NRM and relapse risk (RR), with relapse as a competing risk for the former and death in remission as a competing risk for the latter. The log-rank test was used to compare subgroups for OS and cPFS, and the method
of Gray was used for NRM and RR. \( P \leq .05 \) was considered significant. Data were analyzed in May 2009.

### RESULTS

**Engraftment and GVHD**

Eighty (98%) of 82 patients engrafted, with a median time to neutrophil engraftment of 12 days (range, 10 to 23 days). Two patients underwent autologous reconstitution (one sibling donor and one unrelated donor). Median time to platelet engraftment was 12 days (range, 7 to 40 days), with three patients dying before platelet regeneration.

Without DLI, grade 2 or 3 acute GVHD occurred in 11 (13%) of 82 patients, with no occurrence of grade 4 GVHD (Table 2). Chronic GVHD occurred in 23 of 76 assessable patients, with limited GVHD in eight patients (all cutaneous) and extensive GVHD in 15 patients (cutaneous, \( n = 8 \); cutaneous and GI, \( n = 4 \); oral, \( n = 1 \); pulmonary, \( n = 1 \); neurologic, \( n = 1 \)). The 4-year CI of extensive chronic GVHD was 18%. After DLI, nine patients developed GVHD requiring systemic immunsuppression (cutaneous, \( n = 5 \); GI, \( n = 6 \); pulmonary, \( n = 2 \)). The CI of extensive chronic GVHD occurring before DLI and/or any post-DLI GVHD was 32% at 4 years. At the time of reporting, only seven of these patients remained on immunsuppression.

### NRM and Infection

Nonrelapse death occurred in 12 patients at a median of 8 months (range, 1 to 13 months), with varying causes (infection related, \( n = 6 \) [Gram-negative sepsis, \( n = 3 \]; fungal infection, \( n = 2 \); adenoviral pneumonia, \( n = 1 \); COPD, \( n = 1 \); encephalopathy, \( n = 1 \); idiopathic pneumonitis, \( n = 2 \)). Estimated NRM was 12% at 1 year and 15% at 4 years. Three patients had siblings donors and nine patients had unrelated donors, with 4-year NRM rates of 8% and 22%, respectively (\( P = .08 \); Fig 1).

There was a trend to increased NRM in patients with significant GVHD (acute GVHD \( \geq \) grade 2 or any chronic GVHD), with 4-year NRM of 27% in patients with GVHD and 9% in patients without GVHD (\( P = .06 \)). NRM at 4 years was 19% in the presence of a failed prior autograft and 14% without a failed prior autograft (\( P = .55 \)). Patients with chemotherapy-sensitive disease before RIT had a 4-year NRM of 14%, and patients with chemotherapy-resistant disease had a 4-year NRM of 29% (\( P = .26 \)).

Other nonfatal opportunistic infection occurred as follows: GI tract (norovirus, \( n = 7 \); adenovirus, \( n = 1 \); rotavirus, \( n = 2 \); microsporidium, \( n = 1 \)); respiratory tract (respiratory syncytial virus, \( n = 7 \); influenza A, \( n = 3 \); parainfluenza III, \( n = 6 \); metapneumovirus, \( n = 1 \)); fungal, \( n = 6 \); cutaneous (herpes simplex virus 1, \( n = 6 \); herpes simplex virus 2, \( n = 2 \); dermatomal varicella zoster virus, \( n = 5 \); molluscum contagiosum, \( n = 1 \)); other (Pneumocystis carinii pneumonia, \( n = 1 \); Mycobacterium tuberculosis, \( n = 1 \)). One patient had Epstein-Barr virus post-transplantation lymphoproliferative disorder. CMV reactivation occurred in 33 (70%) of 47 at-risk patients, with neither end-organ disease nor deaths attributable to CMV.

### Relapse

Relapse or progression occurred in 19 patients at a median of 8 months (range, 1 to 43 months) after transplantation. Estimated RR was 16% at 1 year and 26% at 4 years. There was no significant association with chemotherapy sensitivity before transplantation, with 4-year RRs of 24% in chemotherapy-sensitive patients (\( n = 74 \)) and 43% in chemotherapy-resistant patients (\( n = 7 \); \( P = .17 \)). There was no significant association with prior autologous transplantation (RR: 37% with failed autograft \( v \) 21% without; \( P = .18 \)), donor type (RR: 25% sibling \( v \) 28% unrelated; \( P = .75 \)), or the presence of significant pre-DLI GVHD (RR: 15% with acute GVHD \( \geq \) grade 2/any chronic GVHD \( v \) 32% without; \( P = .15 \)).

### DLI for Relapse

Twenty-five DLIs were given to 13 patients for recurrence in escalating doses from 1 to \( 10 \times 10^6 \)/kg. Ten patients (77%) experienced remission, and three patients had no response; these three patients died at 27, 36, and 105 days from DLI. Of the 10 patients who achieved CR, four patients had GVHD after DLI, rituximab therapy.
was given before DLI to six patients, and one patient received radiotherapy. Five of the six responders given rituximab had already received this treatment before transplantation. Responses were durable in nine of 10 patients, with one patient experiencing relapse before responding to further DLI. In these 10 patients, CR is currently ongoing at a median of 44 months (range, 12 to 74 months) after last DLI.

**DLI for MC**

DLIs were given from 6 months for MC alone. Chimerism data were available in 64 of 82 patients for this decision point, and 33 patients had MC without progression or relapse. Of these patients, 28 received one to four DLIs. The starting dose was $1 \times 10^6$/kg in 19 (68%) of 28 patients, with the remainder starting at $5 \times 10^5$ to $1 \times 10^6$/kg, depending on donor type/HLA matching. Seventeen patients had full donor chimerism (four with GVHD after DLI), one patient died, and 10 patients had ongoing MC. There was a significantly increased RR in patients with persistent MC (after DLIs or where DLIs were not given) compared with patients with full donor chimerism (after DLIs or where DLIs were not required). Four-year RR was 14% in patients with full donor chimerism ($n = 48$) and 40% in patients with persistently MC ($n = 16; P = .012$; Fig 2). In the 28 patients given DLIs for MC, relapse occurred in only one of 17 patients who converted to full donor chimerism versus four of 11 patients who had persistent MC (4-year RR: 10% v 36%, respectively; $P = .03$).

**Survival**

At 1 and 4 years, OS rates were 80% and 76%, respectively, and cPFS rates were 78% and 76%, respectively. The presence of a sibling donor and the absence of a prior autograft had a favorable effect on both OS and cPFS, whereas presence of persistent MC, prior exposure to rituximab, age more than 45 years at transplantation, chemotherapy sensitivity before transplantation, and significant GVHD (grade 2 to 4 acute/chronic GVHD) were not significant.

Four-year cPFS rates were 90% with sibling donors versus 64% with unrelated donors ($P = .012$; Fig 3A); 57% with prior autograft versus 83% without ($P = .016$); 78% with chemotherapy-sensitive disease versus 57% with chemotherapy-resistant disease ($P = .133$); 76% with prior rituximab versus 70% without ($P = .638$); 69% if transplantation was received at age greater than 45 years versus 83% in younger patients ($P = .258$); and 74% with GVHD versus 78% without-out ($P = .89$). Only donor type remained significant in multivariate analysis ($P = .035$).

Four-year OS with sibling donors was 90% compared with 63% with unrelated donors ($P = .012$; Fig 3B) and 57% in the presence of a prior autograft compared with 83% without prior autograft ($P = .017$). Only donor type remained significant in multivariate analysis ($P = .037$).

**DISCUSSION**

Allogeneic transplantation in FL offers the potential for cure, but minimal morbidity/mortality is required if it is to be considered as anything other than salvage treatment once less aggressive treatments have been exhausted. The use of myeloablative conditioning has historically been associated with substantial NRM (30% to 40%) and morbidity, although almost all patients have had HLA-matched sibling donors. Subsequent reports have suggested improvement in outcome in patients receiving transplantation more recently, but younger patients with matched sibling donors again predominate.
FL occurs primarily in older patients, and there has been interest in the use of reduced-intensity regimens, with Khouri et al reporting an OS rate of 85% in a group of 47 chemotherapy-sensitive patients, all but two of whom had sibling donors. Outcomes have been less favorable in patients with chemotherapy-resistant/transformed FL, and the use of unrelated donors has been associated with significant GVHD and substantial NRM.10

The relative merits of myeloablative and reduced-intensity conditioning are difficult to analyze in retrospective comparisons,12,20 and the issue remains unresolved. The rationale for myeloablative conditioning, however, becomes less compelling in the presence of potent graft-versus-malignancy, and in this setting, myeloablation would have to be demonstrably superior to RIT to make the enhanced toxicity worthwhile.

This report addresses the issue of T-cell depletion in reduced-intensity conditioning. The published series that have reported extended follow-up with RIT in this disease have been primarily with T-cell–replete regimens. Although impressive disease control can be achieved, particularly in patients with chemotherapy-sensitive disease, the rates of acute and chronic GVHD are substantial, especially in older patients who receive transplantations from unrelated donors. This translates into significant NRM, with Rezvani et al10 reporting death from nonrelapse causes in the majority of patients with unrelated donors. In this report, we describe the long-term outcomes using a strategy of initial T-cell depletion followed by DLI to promote full donor chimerism and a graft-versus-lymphoma effect.

The patients had undergone extensive pretreatment, having had a median of four lines of therapy before RIT, with failed autologous transplantation in 26%, and proceeded to RIT at a median of 4 years from diagnosis. The outcome in the 39 patients (46%) with a related donor is excellent, with a 4-year cPFS rate of 90%, only 8% NRM at 4 years, and low rates of GVHD. For patients with unrelated donors, despite the presence of HLA mismatch in 23% and prior autograft failure in 42%, there was a modest NRM of 22%, and cPFS at 4 years was 64%. This represents the best outcomes reported to date.

It is well recognized that GVHD contributes significantly to both procedure-related death and poor quality of life in survivors.21 The conditioning regimen in this report, which uses the monoclonal antibody alemtuzumab to facilitate in vivo T-cell depletion, was associated with only 13% grade 2 or 3 and no grade 4 acute GVHD, despite the majority of patients having HLA-matched or -mismatched unrelated donors. Extensive chronic GVHD in the absence of DLI was 18% at 4 years and, including any GVHD requiring systemic therapy after DLI, was 32% at 4 years, with only seven patients remaining on immunosuppression at the time of reporting. This compares favorably to data from series of transplantation in FL without T-cell depletion.

For such a strategy to improve outcome, however, gains from reduction in GVHD must be assessed against loss of disease control. It is postulated that prompt use of DLI in early relapse in diseases such as this, where DLI responses have been demonstrated in addition to the use of DLI in the presence of MC to minimize risk of relapse, may overcome any adverse effect of delaying immune reconstitution. In this way, augmentation of alloreactivity and increased exposure to GVHD is targeted only at a subset of patients. Of note, in the current series, a substantial proportion of patients (30 of 82 patients; 37%) remained alive and in remission having received no DLI, and 21 of these patients had experienced no significant GVHD.

For disease recurrence, DLI responses were encouraging, both in rate of response and in durability. CR occurred in 10 (77%) of 13 patients, and only one patient required further DLI for disease progression; responses occurred in 40% of patients without the development of GVHD. These responses seem durable, with ongoing CR at a median of 44 months from last DLI, suggesting that effective salvage can be achieved in many patients whose disease recurs after transplantation.

The issue of conversion of MC is controversial, with the theoretical reduction in RR that may follow overcoming tolerance to host hemopoiesis yet to be definitively proven. This study demonstrates a significant reduction in RR in the presence of full donor status versus MC (RR at 4 years: 13% vs 40%, respectively), where the patients had full donor chimerism either with or without requiring posttransplantation donor lymphocytes. In the group given DLIs for MC alone at 6 months, this effect was maintained, with patients converting to full donor status having a significant reduction in RR compared with patients who exhibited persistent MC. Furthermore, 13 (76%) of 17 patients who converted to full donor status with DLI did so without developing clinical GVHD. This demonstrates that the use of DLI after RIT to convert MC can result in significant reduction in relapse and that this protection is conferred without clinical GVHD in the majority of patients, as expected from published data demonstrating reduction in GVHD risk with DLI given subsequent to the transplantation episode.28,29 Furthermore, in the minority of patients in whom GVHD developed, GVHD was usually readily controlled and of limited duration. The reduction in RR is not converted into a survival advantage because patients who experience relapse have excellent response to DLI. There is a suggestion that GVHD rates are higher in the relapse setting relative to that of converting MC, as would be expected from the higher doses of T cells used in the context of disease recurrence, although the numbers are too small for meaningful analysis.

This relation of chimerism to relapse has not been the experience of others. Khouri et al found no association between MC and relapse, although no chimerism data are reported beyond 90 days, and the median T-cell chimerism seemed to be spontaneously improving between 30 and 90 days, reaching a median of 99% by this time point. It may be that a proportion of these patients would have continued to a full conversion, as has been our experience in some patients, particularly those with GVHD, a scenario more prevalent in a T-cell–replete setting.

Finally, the issue of increasing recipient age and transplantation outcome is an important one. Although no significant difference was seen in this cohort when analyzed by age greater than 45 years (median age), the numbers and heterogeneity involved preclude more detailed analysis, and further studies are required.

Therefore, we propose a strategy of partial T-cell depletion at the time of transplantation using a reduced-intensity conditioning regimen, with prompt DLI to convert MC or to treat recurrence should this occur before MC has been assessed/treated or in the event of failure of low doses of DLI to convert. In this way, transplantations using HLA-matched or -mismatched related or unrelated donors can be effectively delivered to older patients with FL, with modest GVHD rates and NRM and with the use of DLI delayed to attenuate its GVHD risks and targeted only to those who require such treatment. Long-term survival is impressive, despite advanced disease status, and as a result of the excellent outcome data in sibling transplantations and the modest morbidity and mortality from such conditioning, consideration should be given to introducing transplantation earlier in the
current management algorithms, ideally in the context of a random-
ized prospective study.

**AUTHORS’ DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST**

The author(s) indicated no potential conflicts of interest.

**AUTHOR CONTRIBUTIONS**

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**REFERENCES**

2. van Besien K, Sobocinski KA, Rowlings PA, et al: Allogeneic bone marrow transplantation for low-
tation for refractory and recurrent low-grade non-
intensity allogeneic transplantation regimen for re-
lative allogeneic hematopoietic transplantation as 
8. Robinson SP, Goldstone AH, Mackinnon S, et al: Chemoresistant or aggressive lymphoma predicts for a poor outcome following reduced-intensity allogene-
eloablative allogeneic hematopoietic cell transplan-
ter nonmyeloablative conditioning with fludarabine, cyclophosphamide, and rituximab. Blood 111:5530-
5536, 2008
pared to myeloablative conditioning. Biol Blood Mar-
row Transplant 14:238-245, 2008
able long-term survival after reduced-intensity allogene-
17. Toze CL, Shepherd JD, Connors JM, et al: Allogeneic bone marrow transplantation for low-
18. van Besien K, Loberiza FR Jr, Bajurinaite R, et al: Comparison of autologous and allogeneic hema-
plantation with nonmyeloablative or myeloablative conditioning regimens for treatment of lymphoma and chronic lymphocytic leukemia. Blood 111:446-
452, 2008
plantation and functional status of long-term survi-
cyte infusion in indolent lymphomas relapsed after allogeneic stem cell transplantation. Bone Marrow Transplant 32:1159-1163, 2003
23. Russell NH, Byrne JL, Faulkner RD, et al: Donor lymphocyte infusions can result in sustained remissions in patients with residual or relapsed lymphoid malignancy following allogeneic haemo-
poietic stem cell transplantation. Bone Marrow Transplant 36:437-441, 2005
26. Ingram W, Devereux S, Das-Gupta EP, et al: Outcome of BEAM-autologous and BEAM-
escalated donor lymphocyte infusions following re-
calating doses of donor leukocytes for relapse of chronic myeloid leukemia after bone marrow trans-
plantation: Separation of graft-versus-leukemia re-

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**JOURNAL OF CLINICAL ONCOLOGY**

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