

Review

The graft-versus-lymphoma effect: clinical review and future opportunities

BW Butcher and RH Collins Jr

Department of Internal Medicine, Hematopoietic Cell Transplantation Program, University of Texas Southwestern Medical Center, Dallas, TX, USA

Summary:

Numerous lines of preclinical and clinical evidence support the existence of a graft-versus-leukemia effect, but less evidence supporting a comparable graft-versus-lymphoma effect exists. We review here current clinical data addressing the graft-versus-lymphoma effect, including comparisons of autologous, syngeneic, and allogeneic transplantation; responses to immunomodulation; and responses to nonmyeloablative stem cell transplantation. Despite several limitations of the data, we believe that there is sufficient evidence suggesting a significant graft-versus-lymphoma effect. In addition, we discuss approaches for clinical management of lymphoma patients, opportunities for mechanistic studies afforded by donor leukocyte infusions and nonmyeloablative transplantation, and suggestions for clinical studies to further define the magnitude and applicability of the graft-versus-lymphoma effect.

Bone Marrow Transplantation (2005) 36, 1–17.

doi:10.1038/sj.bmt.1705008

Published online 16 May 2005

Keywords: graft-versus-lymphoma effect (GVL); non-myeloablative stem cell transplantation (NST); donor leukocyte infusion (DLI); non-Hodgkin's lymphoma (NHL); Hodgkin's disease (HD)

For over two decades, scientists and clinicians have recognized the existence of a graft-versus-tumor effect, whose role has become increasingly important in the treatment of hematologic malignancies. Initially suggested by numerous animal studies and corroborated by considerable retrospective analysis of clinical data, this effect is most directly supported by durable responses to withdrawal of immunosuppression, administration of donor leukocytes, and more recently, nonmyeloablative stem cell transplantation. Most studies to date have investigated the graft-versus-leukemia effect, while comparable studies of a graft-versus-lymphoma effect have been limited. Current data regarding

graft-versus-lymphoma activity, however, have increasingly supported the likelihood of its existence. We review below the most recent evidence, composed of case reports and small series, larger retrospective analyses, and few prospective studies, suggesting a graft-versus-lymphoma effect.

Background

The antitumor capacity of allogeneic immune cells was first demonstrated by the experiments of Barnes *et al.*,¹ in which leukemic mice were irradiated and rescued by allogeneic or syngeneic bone marrow. In this classic study, allogeneic transplantation conferred a lower relapse risk than syngeneic transplantation, a result recapitulated in a number of murine and canine models.^{2–4} In the 1970s and 1980s, growing laboratory data were complemented by clinical observations suggesting graft-versus-tumor activity following human allogeneic transplantation in patients with leukemia.⁵ Specifically, lower relapse rates were observed in recipients of allogeneic rather than syngeneic transplantation,⁶ lower relapse rates were observed in allograft recipients who developed graft-versus-host disease (GVHD) than in those who did not,^{7,8} and increased relapse rates were observed following T-cell depletion of the donor graft.⁹ Horowitz *et al.*¹⁰ confirmed these findings in a large analysis of International Bone Marrow Transplant Registry (IBMTR) data.

Clinicians appreciative of this graft-versus-tumor activity attempted to harness it by withdrawing immunosuppression and administering donor leukocyte infusions (DLIs) to patients with recurrent or persistent disease following transplantation.^{11,12} Complete responses in a significant percentage of patients led to broad application of this approach,^{13–16} which has enjoyed its greatest success in the treatment of chronic myelogenous leukemia (CML), where durable responses in chronic phase relapse are approximately 75%.^{17–22} Donor leukocyte infusions have also been utilized, with varying degrees of success, in the treatment of acute lymphocytic leukemia,²³ acute myelogenous leukemia and myelodysplasia,²⁴ myelofibrosis,^{25,26} and multiple myeloma.^{27–29}

The success of DLI in the treatment of leukemia demonstrates the therapeutic benefit of interactions between donor-derived leukocytes and host tumor cells. Indeed, the elimination of tumor cells by adoptive therapy has resulted in a strategy that both exploits the antitumor capacity of allogeneic cells and reduces the toxicities of

Correspondence: Dr RH Collins Jr, Department of Internal Medicine, Hematopoietic Cell Transplantation Program, University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, TX 75390, USA; E-mail: robert.collins@utsouthwestern.edu
Received 24 November 2004; accepted 12 January 2005
Published online 16 May 2005

conventional transplantation.^{30–32} In such nonmyeloablative transplants, patients receive relatively nontoxic preparative regimens that are immunosuppressive enough to allow engraftment of the donor cells that ultimately effect antitumor activity.^{33,34} Long-lasting remissions have been observed in a variety of hematologic malignancies treated by this approach.^{35,36} Thus, clinical data supporting a graft-versus-leukemia effect have included reduced relapse rates following allogeneic *vs* autologous transplantation and responses to immunomodulation and nonmyeloablative transplantation. We examined the literature for similar lines of evidence supporting a comparable graft-versus-lymphoma effect.

Comparison of autologous, syngeneic, and allogeneic transplantation

Retrospective analyses consistently demonstrate significantly lower relapse rates for lymphoma patients receiving allogeneic rather than autologous transplantation; however, the higher treatment-related mortality characteristic of the former tempers this advantage, yielding similar overall survivals for the two strategies (see Table 1). The existence of a graft-versus-lymphoma effect was first suggested by Jones *et al*³⁷ in a series of 118 consecutive patients with non-Hodgkin's lymphoma and Hodgkin's disease (HD). An outcomes assessment of 38 allograft recipients and 80 autograft recipients showed a significantly lower relapse rate among allograft recipients, although a

statistically significant difference in event-free survival between the two groups was not appreciated given the higher treatment-related mortality associated with allogeneic transplantation. A prospective study published 3 years later analyzed the outcomes of 66 consecutive patients with poor prognosis lymphoma, with priority for allogeneic transplantation given to patients younger than 55 years with a major histocompatibility complex-matched or one-antigen-disparate sibling donor.³⁸ Univariate analysis of the data suggested that the probability of disease progression remained significantly lower among allograft recipients, although, as in the Jones study, a statistically significant difference in progression-free survival was not apparent.

An analysis of 429 bone marrow transplants for non-Hodgkin's lymphoma between 1986 and 1997 in Ontario yielded similar results.³⁹ In this retrospective study, 385 patients received autologous transplants, while 44 patients received allogeneic transplants for persistent marrow involvement or inadequate stem cell harvest. Allogeneic transplantation was associated with a significantly lower risk of relapse at 3 years (6 *vs* 41%, $P=0.0006$). The disparate group sizes and heterogeneity of the cohort were addressed by randomly matching two autologous transplant recipients to one allogeneic transplant recipient in terms of age, disease status at transplant, disease histology, and year of transplant. In this matched comparison, survival was equivalent between the two groups, but the relative risk for relapse after allogeneic transplantation was 0.190, corroborating the results of preceding studies.^{40,41}

Table 1 Comparison of allogeneic and autologous bone marrow transplantation, selected series

Reference	Histology	Type of transplant	Number of patients	OS	DFS/PFS	Relapse rate	TRM
43	Follicular lymphoma	Purged autologous	131	62% at 5 years	39% at 5 years	43% at 5 years	14% at 5 years
		Unpurged autologous	597	55% at 5 years	31% at 5 years	58% at 5 years	8% at 5 years
		Allogeneic	176	51% at 5 years	45% at 5 years	21% at 5 years	30% at 5 years
42	Lymphoblastic lymphoma	Autologous	128	44% at 5 years	39% at 5 years	56% at 5 years	5% at 5 years
		Allogeneic	76	39% at 5 years	36% at 5 years	34% at 5 years	25% at 5 years
118	NHL and HD	Autologous	24	57% at 3 years	51% at 3 years	30% at 3 years	29%
		Allogeneic	14	43% at 3 years	43% at 3 years	38% at 3 years	29%
119	HD	Autologous	104	37% at 10 years overall; 44% in pts with sensitive disease	26% EFS at 10 years overall; 33% in pts with sensitive disease	60% at 10 years overall; 51% in pts with sensitive disease	NS
		Allogeneic	53	30% at 10 years overall; 63% in pts with sensitive disease	27% EFS at 10 years overall; 44% in pts with sensitive disease	53% at 10 years overall; 34% in pts with sensitive disease	NS
39	NHL	Autologous	385	62% at 3 years	52% at 3 years	41% at 3 years	6%
		Allogeneic	44	71% at 3 years	71% at 3 years	6% at 3 years	23%
38	NHL	Autologous	35	NS	24% at 2 years	69%	NS
		Allogeneic	31	NS	47% at 2 years	20%	NS
41	NHL	Autologous	101	NS	46%	35%	14% NRM
		Allogeneic	101	NS	49%	29%	28% NRM
37	NHL and HD pts in sensitive relapse	Autologous	61	NS	41% EFS	46%	13% NRM
		Allogeneic	19	NS	47% EFS	18%	42% NRM

OS = overall survival; DFS = disease-free survival; EFS = event-free survival; PFS = progression-free survival; TRM = treatment-related mortality; NRM = non-relapse mortality; NHL = non-Hodgkin's lymphoma; HD = Hodgkin's disease; NS = not stated.

Note: The Bierman *et al*⁴⁴ paper is referred to extensively in the text but is not included in this table given that its data are primarily expressed in terms of relative risk rather than actuarial statistics.

Studies focusing on single lymphoma histologies, such as lymphoblastic lymphoma, produce similar findings. Although recipients of allogeneic transplantation had a significantly lower 5-year relapse rate than recipients of autologous transplantation (34 vs 56%, $P = 0.004$), higher treatment-related mortality offset any potential survival benefit.⁴² The lower relapse rate for allogeneic transplant recipients held true after adjusting for the possible confounding effects of age, sex, Karnofsky score at the time of transplantation, disease stage at transplantation, bone marrow involvement at the time of transplantation, and several other factors.

Principle drawbacks to this group of studies include lack of randomization and the potential for selection bias (see Table 2). It has also been suggested that the higher relapse rates associated with autologous transplantation result from failure to purge the graft of tumor cells prior to infusion. van Besien *et al*⁴³ addressed this hypothesis by analyzing data collected from 904 patients with follicular lymphoma undergoing allogeneic ($n = 176$), purged autologous ($n = 131$), and unpurged autologous ($n = 597$) transplantation between 1990 and 1999. In all, 5-year recurrence rates were 21, 43, and 58% after allotransplantation, purged autotransplantation, and unpurged autotransplantation, respectively, indicating a benefit to stem cell purging. As demonstrated previously, allogeneic transplantation conferred the lowest risk of relapse and the highest treatment-related mortality, resulting in a similar 5-year overall survival rate to autologous transplantation.

Perhaps the most provocative study to date comparing transplantation outcomes in patients with non-Hodgkin's lymphoma was published by the Lymphoma Working Committee of the IBMTR and the European Group for Blood and Bone Marrow Transplantation.⁴⁴ In this retrospective analysis of 3376 patients receiving syngeneic, autologous (with or without purging), or allogeneic (with or without T-cell depletion) transplantation, no significant difference in relapse rate was observed between syngeneic and allogeneic transplantation for any disease histology. Patients receiving unpurged autografts for low-grade

disease had a five-fold greater risk of relapse than recipients of syngeneic transplants and a two-fold greater risk than recipients of purged autografts. Among patients with intermediate- or high-grade disease, however, there was no significant difference in relapse risk between purged and unpurged autologous transplants. According to the authors, these results failed to demonstrate a graft-versus-lymphoma effect but suggested reinfusion of tumor cells as the cause of increased relapse rates in recipients of autologous transplants with low-grade disease.

These conclusions must be interpreted cautiously, however, given several drawbacks to the study design and data analysis. Importantly, the marked heterogeneity of the patient groups relative to histology and specific disease characteristics suggests that the difference in treatment outcome more likely resulted from differences in patient characteristics rather than the source of stem cells. For example, greater than 70% of patients undergoing autologous transplantation had chemosensitive disease, while this was true for fewer than 40% of allogeneic transplant recipients.⁴⁵ Of the allogeneic transplant recipients, 50% had high-grade histology compared with only 30% of syngeneic and 10% of autologous transplant recipients, and bone marrow involvement was present in 70% of allogeneic transplant recipients, but only 36% of syngeneic and 10% of autologous transplant recipients. These differences are consistent with general clinical experience: allografts are typically held in reserve until other treatments have failed, whereas syngeneic and autologous transplants, given their comparatively lower toxicity, are more likely to be used earlier in the disease course. This stands in marked contrast to the classic graft-versus-leukemia analysis on which the design of this study is partially based, where patient populations were relatively homogeneous, with all acute leukemia patients in first remission and all CML patients in chronic phase.¹⁰ Of course, the difference in lymphoma relapse rates with varying types of transplantation can only be answered by a properly designed prospective controlled trial, and such a trial is not likely to be carried out.

Table 2 Drawbacks of previous studies

Comparison of autologous, syngeneic, and allogeneic transplantation

- Lack of randomization
- Significant potential for selection bias
- Marked heterogeneity of the patient cohort
- Failure to purge autologous grafts of tumor cells prior to infusion (in many studies)

Response to immunomodulation

- Evidence limited to case reports and small series
- Confounding explanations for disease response include prior or concomitant administration of chemotherapy, radiation, rituximab, and/or corticosteroids

Response to nonmyeloablative transplantation

- Wide variation in regimen components and intensity, GVHD prophylaxis, and application of DLI
- Marked heterogeneity of patient groups relative to histology and specific disease characteristics
- Limited data reporting (eg response rates, actuarial statistics, treatment-related toxicities) by histologic subtypes
- Largest series to date remain in abstract form with limited data and short follow-up
- Registry analyses and retrospective single institution studies subject to referral bias, physician preference, and variability in patient selection and supportive care
- Lack of randomized, prospective trials

Response to immunomodulation

A graft-versus-lymphoma effect is most convincingly demonstrated by the durable resolution of residual or progressive disease after allografting in response to withdrawal of immunosuppression or DLI administration.⁴⁶ Such evidence has been limited largely to brief, yet compelling, case reports and small series. Aoyama *et al*⁴⁷, for example, present the case of a 33-year-old Japanese man diagnosed with diffuse large B-cell lymphoma, stage IV, with invasion of the central nervous system. The patient was initially treated with aggressive chemotherapy, but nevertheless failed two autologous transplants and relapsed only 16 days following a nonmyeloablative allogeneic transplant. Cyclosporine A was discontinued on day +32, and a DLI was given on day +40. At 8 days after DLI, the patient developed grade III acute GVHD involving the skin and liver, and on day +220, the patient developed biopsy-proven chronic GVHD of the liver and lung. At 4 months after DLI, there was neither symptomatic nor radiological evidence of tumor, and at the time of publication, 15 months post transplant, the patient remained free of lymphoma and in good clinical condition.

Similarly, Mandigers *et al*⁴⁸ used real-time quantitative PCR with primers flanking the characteristic chromosomal translocation of follicular lymphoma to quantify objectively the graft-versus-lymphoma effect. The patient studied was a 48-year-old female with a low-grade follicular lymphoma who, after failing two previous treatment regimens, was given a high-dose preparative regimen and a T-cell-depleted allogeneic transplant. At 22 months following transplant, a relapse involving the lymph nodes and bone marrow was diagnosed. A single infusion of lymphocytes from the original donor resulted in durable complete remission, including a lymphoma-negative bone marrow, as measured by PCR.

Table 3 lists several other examples of a graft-versus-lymphoma effect mediated by the withdrawal of immunosuppression or the administration of DLI. These techniques have been effective against numerous lymphoma histologies, and in some cases, have resulted in durable remissions lasting over 7 years. The table also suggests the frequency with which acute and chronic GVHD occur in the setting of responders, although the correlation between these two variables is often not explicitly presented in the literature. Nevertheless, as in a previous analysis,²⁰ a strong temporal association between the development of GVHD and the induction of disease response appears to exist.

Despite the compelling nature of these reports, there are often confounding explanations for disease response (see Table 2). First, the administration of DLI is frequently preceded or accompanied by other agents with activity against lymphoma, including chemotherapy, radiation, and rituximab.⁴⁹ Secondly, corticosteroids used to treat GVHD accompanying DLI administration may also possess antilymphoma activity. Nevertheless, durable responses in often heavily pretreated and refractory patients are unlikely to be explained by these agents.

Response to nonmyeloablative stem cell transplantation

Limitations of the current literature

Numerous series have reported promising response rates to nonmyeloablative transplantation in patients with lymphoma. The current literature is limited, however, by the heterogeneity of nonmyeloablative transplantation regimens and the patients who receive them, the relatively limited reporting and short follow-up characteristic of most studies, and the current lack of randomized trials (see Table 2).

Approaches to nonmyeloablative transplantation vary significantly from study to study. Fludarabine-based regimens, often in combination with other chemotherapies and post transplantation immunosuppression, may differ considerably in intensity, but are generally well-tolerated and consistently result in engraftment.^{50–52} Conditioning with fludarabine and melphalan, for example, frequently results in full donor chimerism and a risk of GVHD comparable to traditional myeloablative regimens, while conditioning with fludarabine and low-dose cyclophosphamide is more often associated with mixed chimerism and a comparatively low incidence of GVHD. Low-dose total body irradiation in various doses, either alone or in combination with immunosuppressive chemotherapy, has also emerged as an effective strategy for nonmyeloablative transplantation.⁵³ Selected patients receive DLI for persistent disease, mixed chimerism, or relapse after transplantation, but the precise criteria for DLI administration are not always clear, and if criteria are present, the degree of compliance is not always certain.

Cohorts in these studies are typically composed of patients with significant differences in baseline pretransplantation characteristics, including age, previous chemotherapies, chemosensitivity of disease, status at the time of transplantation, and donor type/degree of compatibility. Many registry analyses and retrospective single-institution studies also include a number of different lymphoma subtypes, making conclusions regarding specific entities difficult to draw. Frequently, many patients have undergone radiotherapy, prior autologous transplantation, or even prior allogeneic transplantation.

Small series have served as the foundation for more detailed and systematic studies of nonmyeloablative transplantation, yet the largest series to date remain in abstract form with limited data and short follow-up. Registry analyses and retrospective single-center experiences are subject to referral bias, physician preference, and variability in patient selection and standards of supportive care, reinforcing the need for randomized, prospective trials. Despite these limitations, results from studies examining the role of nonmyeloablative transplantation in the treatment of several lymphoma histologies are encouraging and are summarized below and in Table 4.

Chronic lymphocytic leukemia

An increasing amount of data has indicated that allogeneic transplantation is a potentially curative approach

Table 3 Response to withdrawal of immunosuppression and donor leukocyte infusions, selected series

<i>Histology</i>	<i>Reference</i>	<i>Number of patients^a</i>	<i>Withdrawal of IS?</i>	<i>DLI given?</i>	<i>GVHD present?</i>	<i>Result</i>	<i>Duration/median follow-up^b</i>
CLL	120	10	Yes (10 pts)	Yes (7 pts)	70% of pts, unspecified	7 CRs, 2PRs (90%)	4–84 months (median 21 months)
	121	2	Yes (1 pt)	Yes (1 pt)	NS	2 CRs (100%)	12–18 months (median 15 months)
	122	12	No	Yes	NS	3 CRs, 1 PR (33%)	2–19 months (median 6 months)
	49	1	Yes (with no effect)	Yes	100% chronic GVHD	CR (100%)	20 months
	123	1	Yes	Yes	100% chronic GVHD	CR (100%)	18 months
Follicular/Low grade	124	7 (5 with follicular, 2 SLL)	No	Yes (1pt received a second DLI)	43% acute GVHD 83% chronic GVHD	5 CR, 2 PR (100%)	43–89+ months (median 65+ months)
	125	2	Yes (1 pt)	Yes (1 pts)	50% acute GVHD 50% chronic GVHD	2 CRs (100%)	12–17 months
	126	13	No	Yes	50% acute GVHD 38% chronic GVHD	8 CRs (62%)	15–39 months (median 31 months)
	59	2	Yes	No	100% chronic GVHD	2 CR (100%)	NS
	50	1	No	Yes	100% chronic GVHD	CR (100%)	37 months
	48	1	No	Yes	100% chronic GVHD	CR (100%)	> 12 months
	127	2	No	Yes	100% chronic GVHD	2 CRs (100%)	6–23+ months (median 14.5+ months)
	128	2	Yes (2 pts)	Yes (1 pt)	50% acute GVHD	1 PR (50%)	6 months
Aggressive lymphoma (including diffuse large B cell and transformed low-grade lymphoma)	47	1	Yes (with no effect)	Yes	100% acute GVHD 100% chronic GVHD	CR (100%)	15 months
	50	1	Yes	Yes	100% cGVHD	CR (100%)	25 months
	128	5	Yes (5 pts)	Yes (2 pts)	aGVHD	1 CR, 1 PR (40%)	20+ months
	82	1	No	Yes	None	1 CR (100%)	NS
	126	6	No	Yes	100% acute GVHD 100% chronic GVHD	CR (100%)	27 months
Lymphoblastic	128	2	Yes	No	50% acute GVHD	1 CR (50%)	24+ months
Mantle cell	65	1	No	Yes	NS	1 CR (100%)	45+ months
	63	2	No	Yes	NS	1 CR (50%)	45+ months
	126	4	No	Yes	100% acute GVHD, 100% chronic GVHD	1 CR (25%)	28+ months
	90	1	No	Yes	None	1 CR (100%)	12+ months
	80	1	No	Yes (pt received more than 2 DLIs)	100% acute GVHD 100% chronic GVHD	CR (100%)	20 months
	129	1	Yes	No	100% acute GVHD	CR (100%)	4 months (died of aspergillus infection)
	60	1	Yes	No	100% chronic GVHD	CR (100%)	35+ months
Hodgkin's disease	130	9	No	Yes (4 pts received more than 1 DLI)	88% acute GVHD	4 CR (44%)	7 months (4–9 months)
	126	4	No	Yes	100% acute GVHD, 100% chronic GVHD	1 CR (25%)	16 months
	90	5	No	Yes	40% acute GVHD	2 CR (40%)	33.5+ months (33–34+ months)
	131	3	No	Yes	100% acute GVHD	1 CR (33%)	43 months

Table 3 Continued

<i>Histology</i>	<i>Reference</i>	<i>Number of patients^a</i>	<i>Withdrawal of IS?</i>	<i>DLI given?</i>	<i>GVHD present?</i>	<i>Result</i>	<i>Duration/median follow-up^b</i>
	52	4	No	Yes	75% acute GVHD 25% chronic GVHD	3 CRs, 1 PR (100%)	11–20+ months (median 13 months)
Burkitt's lymphoma	132	1	Yes	No	100% chronic GVHD	1 CR (100%)	5 months

^aThe number of patients refers to only those patients in the study who underwent withdrawal of immunosuppression or administration of donor leukocyte infusion, although, in some instances, a larger number of patients was included in the report. Accompanying GVHD, clinical response, and median follow-up statistics also refer only to those patients in the reports who received withdrawal of immunosuppression or donor leukocyte infusion.

^bAll follow-up data presented in days, weeks, or years were converted and rounded to the nearest month for consistency in the table.

IS = immunosuppression; DLI = donor leukocyte infusion; GVHD = graft-versus-host disease; CR = complete response; PR = partial response; CLL = chronic lymphocytic leukemia.

for chronic lymphocytic leukemia (CLL),⁵⁴ although the substantial treatment-related mortality associated with myeloablative therapy has restricted its broad application. Several recent studies of nonmyeloablative transplantation for CLL have demonstrated significantly reduced morbidity and mortality with preservation of durable clinical and molecular remissions.⁵⁵ In four of the largest series to date, 2-year overall survival for recipients of nonmyeloablative allografts ranges from 68 to 80%, with 40–71% of patients achieving complete remissions. These data are comparable to those reported in a number of studies investigating myeloablative allogeneic transplantation.^{56–58} Since CLL is primarily a disease of older patients, many of whom cannot tolerate myeloablative preparative regimens, nonmyeloablative transplantation has considerable promise given its reduced toxicity, apparent efficacy, and potential for cure.

Follicular and low-grade lymphoma

Conventional allogeneic transplantation for low-grade lymphoma is associated with a 40% chance of long-term disease-free survival but has a high rate of treatment-related mortality. In a study of 20 patients with follicular or small-cell lymphocytic lymphoma undergoing nonmyeloablative allogeneic transplantation with or without rituximab, all patients achieved complete remission, and none had relapsed after a median follow-up of 21 months.⁵⁹ Overall and progression-free survival at 2 years were both 84%, but most patients had nonbulky chemosensitive disease, and follow-up was relatively short for a disease with a propensity for late relapse. In the context of larger studies of nonmyeloablative transplantation, where data for individual subjects are often difficult to obtain, follicular lymphomas have generally high complete response (CR) rates, often approaching 100%. Although the number of patients with indolent lymphoma in these studies is small and follow-up is often comparatively short, these data are promising. Prospective trials with long-term follow-up are necessary to define the role of nonmyeloablative allogeneic transplantation in the treatment of low-grade disease.

Mantle cell lymphoma

Evidence of a graft-versus-mantle cell lymphoma (MCL) effect has been reported in several patients following myeloablative allografting.^{60–62} Khouri *et al*⁶³ studied the efficacy of nonmyeloablative transplantation in 18 MCL patients with progressive or recurrent disease after conventional chemotherapy or autologous transplantation. Following conditioning with fludarabine-containing regimens, donor cell engraftment occurred in all patients, mortality at 100 days was 0, and 94% of patients achieved CR. At a median follow-up period of 26 months, three patients had relapsed, although one was reintended into CR with DLI. A contemporaneous study by Dasgupta *et al*⁶⁴ had less promising results, with only 48% of patients alive and disease-free at a median of 21.5 months post transplant. Given that MCL is incurable with conventional chemotherapy and that no plateau exists in the survival curve following autologous transplantation, these preliminary results are encouraging.

Aggressive lymphoma (including diffuse large B-cell lymphoma and transformed low-grade lymphoma)

To date, no large series investigating the efficacy of nonmyeloablative transplantation specifically in diffuse large cell or transformed low-grade lymphoma exist, and individual cases must be drawn from larger reports that include various histologies. Complete remission rates in these reports range from 50 to 100%, although the majority has fewer than five patients, many of whom received conditioning regimens of differing intensity. Escalón *et al*⁶⁵ described a cohort of 10 patients with diffuse large-cell lymphoma, who had failed prior autologous transplantation despite having chemosensitive or stable disease. Following nonmyeloablative allografting, all patients achieved durable complete remissions, with only one death, which was secondary to pulmonary *Aspergillus* infection at 10.5 months post transplantation.

In another study, 18 patients with aggressive lymphoma were treated with a debulking autograft followed by a nonmyeloablative allograft. At a median follow-up of 22 months, eight patients were alive in complete remission,

Table 4 Response to nonmyeloablative stem cell transplantation, selected series

Histology	Reference	Number of Patients ^a	CRs/PRs (best response)	Median follow-up (Range)	PFS or EFS	OS	GVHD (of those assessable)	TRM	Notes		
CLL	133	222	NS	NS	NS	68% at 2 years	NS	22% at 2 years	10 pts received rituximab with chemotherapy		
	120	17	71% CR 23% PR	21 months (11–84 months)	60% PFS at 2 years	80% at 2 years	Acute – 41% Chronic – 60%	22% at 2 years			
	121	4	100% CR	14 months (10–22 months)	49% EFS at 22 months ^b	73% at 22 months ^b	Acute – 58% Chronic – 39% ^b	NS			
	122	77	69% CR 22% PR	18 months (1–44 months)	56% EFS at 2 years	72% at 2 years	Acute (grades II-IV) – 34% Chronic – 58% (risk estimate at 1 yr)	18% at 1 year		No relapses after onset of cGVHD; all but one of the pts who developed cGVHD reached CR	
	134	30	40% CR 53% PR	24 months (7–43 months)	67% PFS at 2 years	72% at 2 years	Acute (grade II-IV) – 56% Chronic – 75%	15% at 2 years		Late CR occurred up to 2 years post-tx; 10 of 12 CRs developed GVHD; 15 related and 15 unrelated donors	
	135	15	80% CR, 7% PR	5.3 months	77% EFS	84%	Acute – 52% Chronic – 71%	NS			
	136	14	58.3% CR, 17% PR	19 months (5–39 months)	62% PFS at 2 years	70% at 2 years	Acute – 57% Chronic – 50%	22% at 2 years			
	Follicular/low grade	137	20	NS	19 months (5–52 months) ^b	85% current PFS	85%	Acute (grade II-IV) – 20% Chronic – 36% ^b		NS	11 pts had early withdrawal of IS, 5 received DLIs
		65	5	100% CR	24+ months (19–35+ months)	95% PFS at 3 years ^b	NS	Chronic – 50% ^b		NS	
		73	3	100% CR	10 months (2–32+ months)	NS	NS	Acute – 100% Chronic – 66%		NS	Patients received high-dose BEAM with no stem cell support for debulking, then NST with allograft
121		3	100% CR	14 months (10–22 months)	49% EFS at 22 months ^b	73% at 22 months ^b	Acute – 58% Chronic – 39% ^b	NS			
74		8	100% CR	15+ months (6–22+ months)	70% at 1 year ^b	70% at 1 year ^b	Acute – 50% Chronic – 75%	NS			
67		52	NS	9 months ^b	54% PFS at 2 yr	65% at 2 yr	48% (not categorized)	31% at 2 yr			
126		13	62% CR	23 months	NS	55% at 2 years, inclusive of all pts	Acute (grade II-IV) – 33% Chronic – 33% ^b	NS	Planned DLIs were part of the NST regimen		
59		20	100%	21 months (5–46 months)	84% at 2 years	84% at 2 years	Acute (grade II-IV) – 20% Chronic – 64%	NS	Nine pts received rituximab in addition to the chemotherapy		
Mantle cell		137	14	NS	19 months (5–52 months) ^b	68% current PFS	67%	Acute (grade II-IV) – 20% Chronic – 36% ^b	NS	11 pts had early withdrawal of IS, 5 received DLIs	
		65	5	100% CR	29+ months (12–45+ months)	95% at 3 years ^b	NS	Chronic – 50% ^b	NS		
	138	22	48% CR	16 months (1–54 months)	NS	NS	Acute (grade II-IV) – 23% Chronic – 36%	28% at 1 year	15 pts received campath as in vivo T cell depletion		
	63	18	94% CR	26 months (11–47 months)	82% EFS at 3 years	85.5% at 3 years	Acute – 17% Chronic – 36%	NS			

Table 4 Continued

Histology	Reference	Number of Patients ^a	CRs/PRs (best response)	Median follow-up (Range)	PFS or EFS	OS	GVHD (of those assessable)	TRM	Notes
Hodgkin's	82	9	56% CR 22% PR	NS	NS	NS	44.4% (not categorized)	NS	Two pts received chemotherapy prior to planned DLI Relapse rate approximately 50% at 4 years OS and PFS higher in pts without a previous ASCT. Nineteen pts received DLIs Early withdrawal of immunosuppression as part of regimen
	139	99	42% CR	NS	NS	NS	Acute – 44%	NS	
	140	41	NS	37.7 months (2.0–70.4 months)	34% PFS at 4 years	63% at 4 years	Acute (grade II-IV) – 12% Chronic – 10%	9% from transplant, additional 11% from DLI	
	141	10	80% CR 20% PR	12 months (1–21 months)	NS	NS	Acute – 20% Chronic – 56%	NS	
	142	5	40% CR	13 months (3–19 months)	61.5% PFS at 1 yr ^b	75.5% at 1 yr ^b	Acute – 40% Chronic – 20%	NS	
	67	52	NS	9 months ^b	42% PFS at 2 yr	56.3% at 2 yr	27% (not categorized)	17.3% at 2 yr	
	131	8	37.5% CR 37.5% PR	7 months (1–18 months)	NS	NS	Acute – 62.5% Chronic – NS	27%	
Aggressive lymphoma (including diffuse large B cell and transformed low-grade lymphoma)	137	15	NS	19 months (5–52 months) ^b	60% current PFS	69%	Acute (grade II-IV) – 20% Chronic – 36% ^b	NS	11 pts had early withdrawal of IS, 5 received DLIs
	65	10	100% CR	24.5+ months (18–52+ months)	95% at 3 years ^b	NS	Chronic – 50% ^b	NS	
	74	5	60% CR	5 months (5–13+ months)	70% at 1 year ^b	70% at 1 year ^b	Acute – 60% Chronic – 60%	NS	
	78	9	78% CR 11% PR	15 months (2–28 months)	57% PFS at 20 months ^b	53% at 24 months ^b	Acute – 77.8% Chronic – 33.3%	13% NRM at 10 months	
	77	5	20% CR	1 month (1–43+ months)	NS	NS	NS	NS	Four pts died early with progressive disease.
	67	62	NS	9 months ^b	12.9% PFS at 2 yr	46.7% at 2 yr	52% (not categorized)	36.7% at 2 yr	
	142	8	37.5% CR	5 months (1–28+ months)	61.5% PFS at 1 yr ^b	75.5% at 1 yr ^b	Acute- 0% Chronic – 0%	NS	
T cell	71	17	71% CR 6% PR	28 months (4–57.5 months)	64% PFS at 3 years	81% at 3 years	Acute – 35% Chronic – 50%	6% at 2 years	
	72	4	100% CR	36 months (8–84 months) ^b	NS	NS	Chronic – 100%	NS	
	74	2	100% CR	10 months (9–11+ months)	70% at 1 year ^b	70% at 1 year ^b	Acute – 100% Chronic – 100%	NS	

^aThe number of patients refers to only those patients in the study who underwent nonmyeloablative stem cell transplantation, although, in some instances, a larger number of patients was included in the report (including recipients of other therapies). Accompanying actuarial and transplant-related morbidity and mortality statistics also refer only to those patients in the reports who received nonmyeloablative stem cell transplantation.

^bStudies including several lymphoma histologies often present follow-up data and actuarial statistics based on the total study population rather than by unique histologies. Accordingly, data marked^b is inclusive of all patients in the study and not just those with the specific histology listed in the table.

CR = complete response; PR = partial response; PFS = progression-free survival; EFS = event-free survival; OS = overall survival; GVHD = graft-versus-host disease; TRM = treatment-related mortality; NRM = non-relapse mortality; CLL = chronic lymphocytic leukemia; DLI = donor leukocyte infusion; NST = nonmyeloablative stem cell transplantation; ASCT = autologous stem cell transplantation; IS = immunosuppression; NS, not stated.

with the best results occurring in patients with full donor chimerism and acute or chronic GVHD.⁶⁶ In a similar study of 62 aggressive histology lymphoma patients treated with a nonmyeloablative allograft, 1-year overall and progression-free survival rates were 52 and 32%, respectively.⁶⁷ In all, 47% of patients progressed at 1 year, and in light of this marked disease progression, the authors suggest that additional strategies may be required to control aggressive histology disease. Despite this, the subsequent response of some patients to DLI and the absence of relapse in patients receiving matched unrelated donor allografts lend credence to the existence of a graft-versus-lymphoma effect for aggressive histology disease.

Hodgkin's disease

Myeloablative allogeneic transplantation results in significantly lower relapse rates than autologous transplantation in the treatment of HD, but treatment-related mortality approaches 50% in some series. Although retrospective studies of nonmyeloablative transplantation for HD often include few patients and have short follow-up, they suggest that even in heavily pretreated patients with refractory disease, sustained complete remissions are achievable with low treatment mortality rates. In these studies, complete response rates have ranged from 25 to 100%, and responses are highly correlated with the development of GVHD. A retrospective analysis of 99 patients receiving nonmyeloablative allografts for relapsed or refractory HD showed a complete remission rate of 42%, comparable to the control group of 154 patients receiving myeloablative allografts.⁶⁸ In a similar study of 41 multiply relapsed and refractory Hodgkin's lymphoma patients conditioned with fludarabine, alemtuzumab (Campath-1H), and melphalan, projected 4-year overall and progression-free survival were 63 and 34%, respectively, with a treatment-related mortality of 20%.⁶⁹ Survival statistics were even more encouraging in the small subset of patients who had not undergone prior autologous transplantation. Thus, nonmyeloablative allogeneic transplantation remains an attractive strategy for the treatment of HD, particularly in high-risk patients who have already failed autologous transplantation.⁷⁰

Other histologies

Nonmyeloablative stem cell transplantation has also been studied in less common lymphoma histologies. Corradini and colleagues treated 17 patients with resistant or relapsed peripheral T-cell lymphomas with a conditioning regimen of thiotepa, fludarabine, and cyclophosphamide followed by allogeneic transplantation. After a median follow-up of 28 months, 71% of patients had achieved and maintained CR, with estimated 3-year overall and progression-free survival rates of 81 and 64%, respectively.⁷¹ Durable complete remissions have also been observed in small series of patients with Sezary syndrome and mycosis fungoides,⁷² marginal zone lymphoma,^{73–75} immunocytoma,⁷⁶ lymphoplasmacytic lymphoma,⁷⁴ anaplastic large-cell lymphoma, lymphoblastic lymphoma,⁷⁷ Burkitt's lymphoma,⁷⁸ and Waldenström's macroglobulinemia.⁷⁹

Role of prophylactic DLI in nonmyeloablative transplantation

In many studies of nonmyeloablative transplantation, DLIs are selectively given to patients for mixed chimerism, disease progression, and/or relapse following transplantation. Their role in augmenting the putative graft-versus-lymphoma effect remains elusive,⁸⁰ however, given the often incomplete nature of data reporting in the literature. A retrospective analysis by Bethge *et al*⁸¹ examined the responses of 53 patients with various hematologic malignancies to DLI following nonmyeloablative allogeneic transplantation. Of the 48 patients receiving DLI for residual or progressive disease, seven achieved CR and five achieved partial response (PR), yielding an overall response rate of 25%. Both the degree of chimerism and the development of GVHD were positively correlated with disease response. Although the responses of lymphoma patients cannot be distinguished from those with other malignancies, the authors conclude that, in general, DLI administration is a minimally toxic and efficacious treatment for some patients with persistent or progressive disease following unsuccessful nonmyeloablative transplantation.

Peggs *et al*⁸² similarly addressed the role of dose-escalated DLIs following nonmyeloablative transplantation in 46 lymphoma and myeloma patients with mixed chimerism ($n=14$), residual disease ($n=13$), or disease progression/relapse ($n=19$). In all, 70% of patients with HD achieved CR or PR, and response was significantly correlated with the development of GVHD. Of the five evaluable patients with non-Hodgkin's lymphoma ($n=4$) or CLL ($n=1$), four achieved CR or PR, with some responses occurring in the absence of GVHD. A third study examining the efficacy of prophylactic DLIs in 42 patients with advanced hematologic malignancies similarly concluded that they could convert patients to full donor chimerism and effect sustained remissions in some patients with chemoresistant disease.⁸³

Failure of prior autografts

Despite improved outcomes in lymphoma patients treated with myeloablative therapy and autologous transplantation, disease progression remains the principle cause of treatment failure.⁸⁴ Indeed, relapse after autografting confers a median survival of 10.5 and 3 months for patients with HD and non-Hodgkin's lymphoma, respectively.⁸⁵ Accordingly, one of the most compelling lines of evidence for the existence of a graft-versus-lymphoma effect is the success of allogeneic transplantation following failed autologous transplantation in patients with refractory disease. Myeloablative conditioning with allogeneic transplantation is generally precluded by high treatment-related mortality, and accordingly, several studies have investigated the efficacy of nonmyeloablative transplantation in this setting.^{86–89}

Escalón and colleagues studied 20 patients with recurrent non-Hodgkin's lymphoma after autologous transplantation, who underwent nonmyeloablative transplantation. Following fludarabine-based conditioning, all patients

engrafted, and 19 (95%) achieved complete remission. The remaining patient had progressive disease at day +115, was treated with DLI, developed GVHD, and achieved a complete remission at day +220. The estimated 3-year progression-free survival was 95%, although all patients included in the study had either chemosensitive or low-bulk stable disease at the time of relapse.⁶⁵

Branson *et al*⁶⁰ similarly studied the efficacy of nonmyeloablative transplantation in 38 high-risk patients with relapsed or refractory lymphoid malignancies following autologous transplantation. Following conditioning with alemtuzumab, fludarabine, and melphalan, 97% of patients engrafted, and no grade III/IV GVHD was observed. Of the 35 assessable patients, 14 received DLI for progressive or relapsed disease after transplantation; two patients with HD also received additional chemotherapy because of high tumor burden. Given the collectively poor prognosis of the patient cohort, the response rates were encouraging, and overall and progression-free survival at 14 months were 53 and 50%, respectively. Nevertheless, a significant number of postprocedure relapses and a progression-free survival curve without a plateau necessitate longer follow-up of this patient cohort. Thus, conflicting data exist regarding the efficacy of nonmyeloablative transplantation following failed autologous transplantation, with more encouraging results seen in patients with chemosensitive disease and minimal tumor burden.

The role of GVHD

Approximately two-thirds of patients receiving DLI for hematologic malignancies will develop acute and chronic GVHD. Reviews of North American and European databases have suggested a strong temporal association and statistical correlation between the development of GVHD and the induction of an antileukemic response. Addressing the association of GVHD and a graft-versus-lymphoma response requires the answers to two fundamental questions: of complete responders, how many developed acute GVHD and chronic GVHD, and of patients who did not develop GVHD, how many were complete responders. Unfortunately, the incomplete nature of data reporting in the literature is often not amenable to such an analysis.

Mohty *et al*⁶¹ investigated the correlation between the development of GVHD and disease relapse in 101 high-risk patients with a variety of diagnoses following nonmyeloablative transplantation with fludarabine, busulfan, and antithymocyte globulin. The cumulative incidence of disease progression or relapse at 1 year was 30% in patients who developed GVHD ($n=69$) and 55% in those who did not ($n=31$; $P=0.02$), suggesting a durable graft-versus-tumor effect closely associated with the development of GVHD. Similarly, a retrospective analysis of 124 transplant recipients for non-Hodgkin's lymphoma in Japan showed that the development of grade II-IV GVHD was strongly associated with a lower incidence of disease progression after transplantation (5.8 vs 29.7%, $P=0.0054$).⁹² Despite this association, the possibility of disease response in the absence of clinical GVHD and the

significant morbidity and mortality of GVHD⁹³ have resulted in a number of approaches to minimize its incidence or severity following DLI. These include delayed administration of DLI,⁹⁴ *ex vivo* insertion of suicide genes into donor T cells prior to DLI,⁹⁵ and selective depletion of specific lymphocyte subpopulations prior to DLI.⁹⁶ Thus, despite the frequent correlation between the incidence of GVHD and antitumor activity, the development of GVHD is neither necessary nor sufficient to evoke a graft-versus-lymphoma response. More complete data reporting is essential to address this question statistically.

Current clinical management

The current data collectively suggest a significant graft-versus-lymphoma effect that can be harnessed clinically, yet its applicability appears dependent on disease histology and individual patient and disease characteristics. Moreover, the role of clinical approaches that attempt to harness the graft-versus-lymphoma effect, such as nonmyeloablative transplantation, have yet to be compared with other clinical approaches in optimal fashion. The field offers significant opportunity for clinical research (discussed below), and we strongly encourage participation in clinical trials when possible. Nevertheless, the physician must make clinical decisions regarding management of lymphoma patients in the present tempered with the understanding that the existing data are far from perfect. We offer here suggestions about the current use of DLI and allogeneic transplantation for the treatment of lymphoma patients.

Donor leukocyte infusions

Patients with persistent or relapsed disease following allogeneic stem cell transplantation may be suitable candidates for DLI. Diseases that seem most responsive to DLI include CLL, follicular lymphoma, and MCL. Patients with slowly growing, less bulky disease may be treated with DLI alone without the need for pre-DLI treatment. Lower T-cell doses, such as 10^7 CD3⁺ cells/kg, might be used first, with higher doses, such as 10^8 CD3⁺ cells/kg, reserved for patients who have failed to respond after several weeks of observation. Some studies suggest lower T-cell doses might be more likely to mediate disease response (at least in CML) without causing GVHD.⁹⁷ Patients with more aggressive disease should likely be treated with debulking therapy prior to DLI administration and higher T-cell doses initially. Debulking therapy might include standard antilymphoma chemotherapeutic agents, rituximab, or radio-labeled monoclonal antibodies. Alemtuzumab should be avoided in this circumstance given its anti-T-cell activity.

In the setting of nonmyeloablative transplantation, DLI administration depends largely on the GVHD prophylaxis used following transplantation. In approaches using alemtuzumab, DLI, given several weeks after transplantation to ensure clearance of Campath, is much more likely to be required to achieve optimal disease control. Approaches using tacrolimus/methotrexate or cyclosporin/mycophenolate, in contrast, are less likely to

require subsequent DLI, as delayed disease responses often occur after discontinuation of these agents, often in association with development of chronic GVHD.³⁵

Allografting

Chronic lymphocytic leukemia. Allografting should be considered in CLL patients with a poor prognosis as defined by clinical characteristics and cytogenetic abnormalities. We generally favor myeloablative regimens in younger patients at this point because we believe that efficacy data for nonmyeloablative transplants are too preliminary to allow their general adoption; moreover, advances in supportive care seem to have lessened treatment-related mortality of myeloablative regimens for otherwise healthy younger patients. For older patients and those with significant comorbidities, one of several nonmyeloablative strategies might be employed with some expectation of success (see Table 4).

Follicular lymphoma. Allografting should be reserved for patients with poor prognoses and more advanced disease, such as first relapse and beyond. (In addition, one should consider autografting for patients in second remission given recent randomized data.⁹⁸) Again, we recommend myeloablative approaches for younger, healthier patients, and nonmyeloablative approaches, many of which appear promising, for older patients and those with significant comorbidities (see Table 4).

Mantle cell lymphoma. The role of autografting vs allografting in first remission disease is not clear based on current data, yet more advanced disease, such as disease in second remission, is very unlikely to be cured with autografting. We believe that the current data suggest utility in this setting for allografting and would recommend consideration of this approach, with myeloablative regimens in younger, healthier patients, and nonmyeloablative regimens in older patients and those with significant comorbidities.

Diffuse large B-cell lymphoma. Randomized data support the use of autografting in relapsed chemosensitive disease and first remission poor-risk disease.^{99,100} Allografting, in contrast, might be considered in the settings of primary refractory disease, relapse-refractory disease, or relapsed disease following autologous transplantation, although the data supporting a graft-versus-lymphoma effect are not as compelling with this histology. Regarding the use of nonmyeloablative transplantation in patients having relapsed after autografts, we emphasize that the group of patients supported by Escalón *et al*⁶⁵ was a highly selected group with fairly early relapses.

Hodgkin's Disease. Randomized data support the use of autografting in relapsed disease,¹⁰¹ although allografting may be considered in poor-risk patients, including those with chemotherapy refractory disease. The study by Peggs *et al* is encouraging in that it shows excellent disease-free survival at 4 years in patients treated with fludarabine,

melphalan, and alemtuzumab, yet this study currently remains in abstract form.

Other histologies. Data for other histologies are too limited to allow even tentative conclusions, although some data, such as those in peripheral T-cell lymphoma, are intriguing.⁷¹ The reader is referred to Table 4 for data that might be helpful in decision-making for individual patients.

Other approaches. As transplanters, we are sometimes guilty of thinking that a transplant, in one form or another, is the only suitable strategy for our patients, when other approaches, including palliative chemotherapy, are often more appropriate. Indeed, the authors have several patients who have been palliated for months to years with intermittent courses of well-tolerated oral chemotherapy. Other potential approaches include rituximab, radio-labeled antibodies, or experimental agents including bortezomib, which shows activity in MCL. Lastly, many patients are best served by a purely palliative approach that ensures that physical, emotional, and spiritual concerns are met.

Mechanism of the graft-versus-lymphoma effect

The mechanism of the graft-versus-lymphoma effect remains unknown but is thought to be principally mediated by T cells and possibly NK cells. The putative target antigens of T cells can be divided into three categories: alloantigens that are broadly expressed on both malignant cells and normal epithelial cells; alloantigens that are expressed on both malignant cells and normal hematopoietic cells; and antigens that are expressed solely by malignant cells, including peptides derived from tumor-specific proteins, viral proteins, or overexpressed differentiation antigens. Alloantigens expressed on both malignant and normal epithelial cells would be expected to elicit a graft-versus-lymphoma effect in close association with clinical GVHD, while alloantigens expressed on both malignant and normal hematopoietic cells, despite being alloreactive, might elicit a graft-versus-lymphoma reaction without causing clinical GVHD. The frequent association between lymphoma responses and GVHD raises the possibility that alloantigens are the target of the graft-versus-lymphoma effect, although this observation does not exclude the possibility of lymphoma-specific T-cell clones arising alongside alloreactive T-cell clones. Lymphoma responses observed in the absence of GVHD suggest the presence of either tumor-specific T-cell clones or alloreactive clones that are reactive only with hematopoietic tissue-restricted alloantigens. This cannot, however, rule out the presence of a broadly alloreactive T-cell clone that is not present in sufficient quantity to cause clinically apparent GVHD.

The definitive role of GVHD in the context of a human graft-versus-lymphoma effect is not yet certain, but in some murine models, the two phenomena can be distinctly separated.¹⁰² Pelot and colleagues showed that administration of nontolerant donor cells as early as 5 weeks post transplant leads to full or nearly full donor chimerism in 80% of recipients without concomitant

GVHD. This suggests that induction of mixed chimerism with nonmyeloablative conditioning effectively sets the stage for DLI to combat chronic hematologic malignancies without causing clinically significant GVHD. Preliminary data from the same group strongly suggest that establishment of mixed chimerism and sufficient time for host recovery after conditioning are associated with decreased incidence of GVHD in humans. A similar strategy has had modest success in canine models.^{103–105}

Although animal models are valuable, the use of DLI and nonmyeloablative transplantation in human subjects provides the unique opportunity to study the mechanism of the graft-versus-lymphoma effect *during* its evolution. For example, Marijt and colleagues have suggested that the hematopoiesis-restricted minor compatibility antigens (mHAgs) HA-1 and HA-2 expressed on the surface of malignant cells serve as target antigens for alloreactive donor T cells. Three mHAg HA-1- and/or HA-2-positive patients with relapsed chronic phase CML ($n=2$) or multiple myeloma ($n=1$) after allogeneic transplantation were treated with DLI from their respective mHAg HA-1- and/or HA-2-negative donors, and HLA-A2/HA-1 and HA-2 peptide tetrameric complexes were used to monitor anti-mHAg T cells.¹⁰⁶ The emergence of tetramer-positive CD8⁺ T cells 5–7 weeks after DLI was followed immediately by complete remission of disease and restoration of 100% donor chimerism in each patient. Moreover, tetramer-positive cytotoxic T cells arising during the clinical response specifically recognized HA-1- and HA-2-expressing malignant cells and inhibited the growth of leukemic precursor cells *in vitro*. The authors caution, however, that additional activated T-cell clones were present and may have contributed to the graft-versus-tumor effect. Takahashi *et al* have recently shown that this observation is not limited to hematologic malignancies. In some cases, minor histocompatibility antigen-specific T cells mediate pronounced graft-versus-tumor effects in patients with cytokine-refractory metastatic renal cell carcinoma following nonmyeloablative transplantation.¹⁰⁷

Bellucci *et al*¹⁰⁸ by screening a myeloma cDNA expression library with serum from four patients achieving complete response after DLI, identified antibodies to 13 target antigens of the graft-versus-myeloma response, five of which were present in more than one patient. These proteins were reactive with post-DLI serum but not with pre-DLI and pre-BMT serum and only minimally reactive with the sera of 20 healthy donors and 20 patients with chronic GVHD. The development of a sustained, high-titer antibody response to a number of myeloma-associated antigens, whose expression is significantly higher than in normal plasma cells, highlights their immunogenicity and identifies them as targets for further immunologic interventions. A similar strategy might be applied to lymphoma patients having undergone DLI to identify potential targets of a graft-versus-lymphoma reaction.

As highlighted above, most mechanistic studies of graft-versus-tumor responses focus on effector cells and the putative target antigens rather than characteristics of target tumor cells that determine susceptibility to immune effector mechanisms. To be an appropriate target of an immune response, a tumor cell must: process and present antigen on

the cellular surface in the context of an HLA molecule, express appropriate adhesion molecules to facilitate interaction with effector cells, and express functional apoptotic machinery. The tumor antigen also must be presented along with a costimulus, either by the tumor cell itself, or by host or donor antigen-presenting cells that have taken up tumor antigen. The tumor cell can escape death by subverting any of these key elements of the immune response.^{109,110}

In addition, recent studies have suggested that the relevant target cell population of a tumor may be the small subset of cells that serve as tumor stem cells.^{111,112} Studies of leukemia have suggested that tumor stem cells differ from other tumor cells and from their normal tissue stem cell counterparts; only the tumor stem cells are capable of transmitting cancer when transplanted into irradiated animals. Thus, one might envision future studies involving isolation of tumor stem cells and investigation of the characteristics that either promote or limit susceptibility to immune attack.

Conclusions and future considerations

Although it is important to appreciate the difficulties in interpreting the literature and to realize that current data must be considered preliminary, we believe that the literature strongly suggests the existence of a graft-versus-lymphoma effect. Additional research is essential to better define the effect, and this includes:

1. *Retrospective registry analyses.* Large-scale analyses of DLI and nonmyeloablative transplantation data will provide further insight into which diseases are vulnerable to the graft-versus-lymphoma effect. As much as possible, such studies should specifically address alternative explanations for disease response, including pre-DLI and post-DLI therapies, which may have intrinsic antitumor activity. The nonmyeloablative stem cell transplantation patients of greatest interest are those treated with less intensive preparative regimens, such as fludarabine and low-dose cyclophosphamide or fludarabine and total body irradiation, as prolonged responses are less likely to be explained by the chemotherapy than by graft-versus-lymphoma activity.
2. *Prospective phase II studies.* Longer follow-up of current studies and additional, larger scale multicenter trials will grant further insight into long-term disease activity and regimen toxicity of nonmyeloablative transplantation, laying the groundwork for prospective comparative trials. Smaller scale phase II studies may investigate the efficacy of variations to current nonmyeloablative regimens, such as the incorporation of monoclonal antibodies or radio-labeled antibodies.
3. *Prospective randomized studies.* Current data are sufficient to allow design of prospective, randomized trials comparing nonmyeloablative transplantation to autologous transplantation or conventional therapy in a variety of settings, including poor-prognosis CLL, follicular lymphoma in second remission, and MCL in first or subsequent remission. Randomized studies may also allow comparison of various nonmyeloablative

transplantation strategies, including less intensive vs more intensive nonmyeloablative regimens and nonmyeloablative transplantation with or without alemtuzumab.

4. *Studies of graft-versus-lymphoma mechanisms.* The above clinical studies will better define the role of nonmyeloablative transplantation in the management of various lymphoma histologies. Although a significant percentage of responders will certainly suffer from severe chronic GVHD, the purposeful induction of debilitating chronic GVHD has limited appeal as long-term therapy. Thus, it is critical that careful investigation of graft-versus-lymphoma mechanisms accompany clinical trials of DLI and nonmyeloablative transplantation. Again, we cannot overemphasize the unique opportunity that these therapies offer to study powerful antitumor mechanisms during their evolution. In addition, we stress the importance of intensively studying the characteristics of target tumor cells in an effort to elucidate factors that influence sensitivity to destruction by immune-mediated mechanisms.
5. *Potential clinical approaches based on improved pre-clinical understanding of graft-versus-lymphoma.* A variety of approaches are under investigation that seek to simultaneously enhance graft-versus-tumor activity and limit development of GVHD. These approaches include: delayed administration of DLI,⁹⁷ selective depletion of CD8⁺ T cells¹¹³ or alloreactive T cells from a donor T-cell inoculum,¹¹⁴ *ex vivo* insertion of suicide genes into donor T cells prior to DLI,¹¹⁵ use of antigen-specific T cells lines and clones,¹¹⁶ and immunization of the donor with tumor-specific protein before DLI.¹¹⁷ One can imagine that basic insights into graft-versus-lymphoma mechanisms might provide the rationale to emphasize one or another of these approaches in future clinical studies.

We are optimistic that such mechanistic and clinical studies will allow the graft-versus-lymphoma effect to realize its full potential, which at this point seems quite promising.

References

- 1 Barnes D, Loutit J, Neal F. Treatment of murine leukemia with X-rays and homologous bone marrow. *BMJ* 1956; **2**: 626–630.
- 2 Bortin MM, Rimm AA, Saltzsein EC. Graft-versus-leukemia: quantification of adoptive immunotherapy in murine leukemia. *Science* 1973; **79**: 811–813.
- 3 Bortin MM, Truitt RL, Rimm AA *et al.* Graft-versus-leukemia reactivity induced by alloimmunization without augmentation of graft-versus-host reactivity. *Nature* 1979; **281**: 490–491.
- 4 Weiden PL, Storb R, Tsoi MS *et al.* Infusion of donor lymphocytes into stable canine radiation chimeras: implications for mechanism of transplantation tolerance. *J Immunol* 1976; **1226**: 1212–1219.
- 5 Luznik L, Fuchs EJ. Donor lymphocyte infusions to treat hematologic malignancies in relapse after allogeneic blood or marrow transplantation. *Cancer Control* 2002; **9**: 123–137.

- 6 Gale RP, Champlin RE. How does bone-marrow transplantation cure leukaemia? *Lancet* 1984; **2**: 28–30.
- 7 Weiden PL, Flournoy N, Thomas ED *et al.* Antileukemic effect of graft-versus-host disease: contribution to improved survival after allogeneic marrow transplantation. *N Engl J Med* 1981; **304**: 1529–1533.
- 8 Weiden PL, Sullivan KM, Flournoy N *et al.* Antileukemic effect of chronic graft-versus-host disease in human recipients of allogeneic-marrow grafts. *N Engl J Med* 1979; **300**: 1068–1073.
- 9 Mitsuyasu RT, Champlin RE, Gale RP *et al.* Treatment of donor bone marrow with monoclonal anti T-cell antibody and complement for the prevention of graft-versus-host disease: a prospective, randomized, double-blind trial. *Ann Intern Med* 1986; **105**: 20–26.
- 10 Horowitz MM, Gale RP, Sonderl PM *et al.* Graft-versus-leukemia reactions after bone marrow transplantation. *Blood* 1990; **75**: 555–562.
- 11 Kolb HJ, Mittermuller J, Clemm C *et al.* Donor leukocyte infusions for treatment of recurrent chronic myelogenous leukemia in marrow transplant patients. *Blood* 1990; **76**: 2462–2465.
- 12 Slavin S, Naparstek E, Nagler A *et al.* Allogeneic cell therapy with donor peripheral blood cells and recombinant human interleukin-2 to treat leukemia relapse after allogeneic bone marrow transplantation. *Blood* 1996; **87**: 2195–2204.
- 13 Bar BM, Schattenburg A, Mensink EJ *et al.* Donor leukocyte infusions for chronic myeloid leukemia relapsed after allogeneic bone marrow transplantation. *J Clin Oncol* 1993; **11**: 513–519.
- 14 Drobyski WR, Keever CA, Roth MS *et al.* Salvage immunotherapy using donor leukocyte infusions as treatment for relapsed chronic myelogenous leukemia after allogeneic bone marrow transplantation: efficacy and toxicity of a defined T cell dose. *Blood* 1993; **82**: 2310–2318.
- 15 Collins Jr RH, Pinero LA, Nemunaitis JJ *et al.* Transfusion of donor buffy coat cells in the treatment of persistent or recurrent malignancy after allogeneic bone marrow transplantation. *Transfusion* 1995; **35**: 891–898.
- 16 Kolb HJ, Schattenberg A, Goldman JM *et al.* Graft-versus-leukemia effect of donor lymphocyte transfusions in marrow grafted patients. European Group for Blood and Marrow Transplantation Working Party Chronic Leukemia. *Blood* 1995; **86**: 2041–2050.
- 17 Hertenstein B, Wiesneth M, Novotny J *et al.* Interferon-alpha and donor buffy coat transfusions for the treatment of relapsed chronic myeloid leukemia after allogeneic bone marrow transplantation. *Transplantation* 1993; **56**: 1114–1118.
- 18 Porter DL, Roth MS, McGarigle C *et al.* Induction of graft-versus-host disease as immunotherapy for relapsed chronic myeloid leukemia. *N Engl J Med* 1994; **330**: 100–106.
- 19 van Rhee F, Lin F, Cullis JO *et al.* Relapse of chronic myeloid leukemia after allogeneic bone marrow transplant: the case for giving donor leukocyte transfusions before the onset of hematologic relapse. *Blood* 1994; **83**: 3377–3383.
- 20 Collins Jr RH, Shpilberg O, Drobyski WR *et al.* Donor leukocyte infusions in 140 patients with relapsed malignancy after allogeneic bone marrow transplantation. *J Clin Oncol* 1997; **15**: 433–444.
- 21 Porter DL, Collins Jr RH, Shpilberg O *et al.* Long-term follow-up of patients who achieved complete remission after donor leukocyte infusions. *Biol Blood Marrow Transplant* 1999; **5**: 253–261.
- 22 Dazzi F, Szydlo RM, Cross NC *et al.* Durability of responses following donor lymphocyte infusions for patients who

- relapse after allogeneic stem cell transplantation for chronic myeloid leukemia. *Blood* 2000; **96**: 2712–2716.
- 23 Atra A, Millar B, Shepherd V *et al*. Donor lymphocyte infusion for childhood acute lymphoblastic leukemia. *Bone Marrow Transplant* 2002; **29**: 63–66.
 - 24 Porter DL, Roth MS, Lee SJ *et al*. Adoptive immunotherapy with donor mononuclear cell infusions to treat relapse of acute leukemia or myelodysplasia after allogeneic bone marrow transplantation. *Bone Marrow Transplant* 1996; **18**: 975–980.
 - 25 Cervantes F, Rovira M, Urbano-Ispizua A *et al*. Complete remission of idiopathic myelofibrosis following donor lymphocyte infusion after failure of allogeneic transplantation: demonstration of a graft-versus-myelofibrosis effect. *Bone Marrow Transplant* 2000; **26**: 697–699.
 - 26 Devine SM, Hoffman R, Verma A *et al*. Allogeneic blood cell transplantation following reduced-intensity is effective therapy for older patients with myelofibrosis with myeloid metaplasia. *Blood* 2002; **99**: 2255–2258.
 - 27 Verdonck G, Vesole DH, Jagannath S *et al*. Graft-versus-myeloma effect: proof of principle. *Blood* 1996; **87**: 1196–1198.
 - 28 Lokhorst HM, Schattenberg A, Cornelissen JJ *et al*. Donor leukocyte infusions are effective in relapsed multiple myelomas after allogeneic bone marrow transplantation. *Blood* 1997; **90**: 4206–4211.
 - 29 Mehta J, Singhal S. Graft-versus-myeloma. *Bone Marrow Transplant* 1998; **22**: 835–843.
 - 30 Little MT, Storb R. The future of allogeneic hematopoietic stem cell transplantation: minimizing pain, maximizing gain. *J Clin Invest* 2000; **105**: 1679–1681.
 - 31 Carella AM, Champlin R, Slavin S *et al*. Mini-allografts: ongoing trials in humans. *Bone Marrow Transplant* 2000; **25**: 345–350.
 - 32 Slavin S, Nagler A, Naparstek E *et al*. Nonmyeloablative stem cell transplantation and cell therapy as an alternative to conventional bone marrow transplantation with lethal cytoreduction for the treatment of malignant and nonmalignant hematologic diseases. *Blood* 1998; **91**: 756–763.
 - 33 Slavin S, Morecki S, Weiss L *et al*. Nonmyeloablative stem cell transplantation: reduced-intensity conditioning for cancer immunotherapy – from bench to patient bedside. *Semin Oncol* 2004; **31**: 4–21.
 - 34 Mielcarek M, Storb R. Non-myeloablative hematopoietic cell transplantation as immunotherapy for hematologic malignancies. *Cancer Treat Rev* 2003; **29**: 283–290.
 - 35 Niederwieser D, Maris M, Shizuru JA *et al*. Low-dose total body irradiation (TBI) and fludarabine followed by hematopoietic cell transplantation (HCT) from HLA-matched or mismatched unrelated donors and postgrafting immunosuppression with cyclosporine and mycophenolate mofetil (MMF) can induce durable complete chimerism and sustained remission in patients with hematological disease. *Blood* 2003; **101**: 1620–1629.
 - 36 Slavin S, Nagler A, Shapira MY. Treatment of leukemia by alloreactive lymphocytes and nonmyeloablative stem cell transplantation. *J Clin Immunol* 2002; **22**: 64–69.
 - 37 Jones RJ, Ambinder RF, Piantadosi S, Santos G. Evidence of a graft-versus-lymphoma effect associated with allogeneic bone marrow transplantation. *Blood* 1991; **77**: 649–653.
 - 38 Ratanatharathorn V, Uberti J, Karanes C *et al*. Prospective comparative trial of autologous vs allogeneic bone marrow transplantation in patients with non-Hodgkin's lymphoma. *Blood* 1994; **84**: 1050–1055.
 - 39 Schimmer AD, Jamal S, Messner H, Keating A. Allogeneic or autologous bone marrow transplantation (BMT) for non-Hodgkin's lymphoma (NHL): results of a provincial strategy. *Bone Marrow Transplant* 2000; **26**: 859–864.
 - 40 Verdonck LF, Dekker AW, Lokhorst HM *et al*. Allogeneic vs autologous bone marrow transplantation for refractory and recurrent low-grade non-Hodgkin's lymphoma. *Blood* 1997; **90**: 4201–4205.
 - 41 Chopra R, Goldstone AH, Pearce R *et al*. Autologous vs allogeneic bone marrow transplantation for non-Hodgkin's lymphoma: a case-controlled analysis of the European Bone Marrow Transplant Group registry data. *J Clin Oncol* 1992; **10**: 1690–1695.
 - 42 Levine JE, Harris RE, Loberiza Jr FR *et al*. A comparison of allogeneic and autologous bone marrow transplantation for lymphoblastic lymphoma. *Blood* 2003; **101**: 2476–2482.
 - 43 van Besien K, Loberiza Jr FR, Bajorunaite R *et al*. Comparison of autologous and allogeneic hematopoietic stem cell transplantation for follicular lymphoma. *Blood* 2003; **102**: 3521–3529.
 - 44 Bierman PJ, Sweetenham JW, Loberiza Jr FR *et al*. Syngeneic hematopoietic stem-cell transplantation for non-Hodgkin's lymphoma: a comparison with allogeneic and autologous transplantation – the Lymphoma Working Committee of the International Bone Marrow Transplant Registry and the European Group for Blood and Marrow Transplantation. *J Clin Oncol* 2003; **21**: 3744–3753.
 - 45 Bishop MR. The graft-versus-lymphoma effect: fact, fiction, or opportunity? *J Clin Oncol* 2003; **21**: 3713–3715.
 - 46 Grigg A, Ritchie D. Graft-versus-lymphoma effects: clinical review, policy proposals, and immunobiology. *Biol Blood Marrow Transplant* 2004; **10**: 579–590.
 - 47 Aoyama Y, Yamamura R, Shima E *et al*. Successful treatment with reduced-intensity stem cell transplantation in a case of relapsed refractory central nervous system lymphoma. *Ann Hematol* 2003; **82**: 371–373.
 - 48 Mandigers CMPW, Meijerink JPP, Raemaekers JMM *et al*. Graft-versus-lymphoma effect of donor leucocyte infusion shown by real-time quantitative PCR analysis of t(14;18). *Lancet* 1998; **352**: 1522–1523.
 - 49 Nieto Y, Bearman SI, Shpall EJ *et al*. Intensive chemotherapy for progressive chronic lymphocytic leukemia administered early after a nonmyeloablative allograft. *Bone Marrow Transplant* 2001; **28**: 1083–1086.
 - 50 Nagler A, Slavin S, Varadi G *et al*. Allogeneic peripheral blood stem cell transplantation using a fludarabine-based low intensity conditioning regimen for malignant lymphoma. *Bone Marrow Transplant* 2000; **25**: 1021–1028.
 - 51 Rodriguez R, Parker P, Nademane A *et al*. Cyclosporine and mycophenolate mofetil prophylaxis with fludarabine and melphalan conditioning for unrelated donor transplantation: a prospective study of 22 patients with hematologic malignancies. *Bone Marrow Transplant* 2004; **33**: 1123–1129.
 - 52 Carella A, Cavaliere M, Lerma E *et al*. Autografting followed by nonmyeloablative immunosuppressive chemotherapy and allogeneic peripheral-blood hematopoietic stem-cell transplantation as treatment of resistant Hodgkin's disease and non-Hodgkin's lymphoma. *J Clin Oncol* 2000; **18**: 3918–3924.
 - 53 McSweeney PA, Niederwieser D, Shizuru JA *et al*. Hematopoietic cell transplantation in older patients with hematologic malignancies: replacing high-dose cytotoxic therapy with graft-versus-tumor effects. *Blood* 2001; **97**: 3390–3400.
 - 54 Dreger P, Montserrat E. Autologous and allogeneic stem cell transplantation for chronic lymphocytic leukemia. *Leukemia* 2002; **16**: 985–992.
 - 55 Jabbour E, Keating MJ, Champlin RE, Khouri IF. Stem cell transplantation for chronic lymphocytic leukemia: should not more patients get a transplant? *Bone Marrow Transplant* 2004; **34**: 289–297.

- 56 Khouri I, Keating MJ, Saliba R *et al.* Long-term follow-up of patients with chronic lymphocytic leukemia treated with allogeneic hematopoietic transplantation. *Cytotherapy* 2002; **4**: 217–221.
- 57 Doney KC, Chauncey T, Appelbaum FR *et al.* Allogeneic related donor hematopoietic stem cell transplantation for treatment of chronic lymphocytic leukemia. *Bone Marrow Transplant* 2002; **29**: 817–823.
- 58 Pavletic ZS, Arrowsmith ER, Bierman PJ *et al.* Outcome of allogeneic stem cell transplantation for B cell chronic lymphocytic leukemia. *Bone Marrow Transplant* 2000; **25**: 717–722.
- 59 Khouri IF, Saliba RM, Giralt SA *et al.* Nonablative allogeneic hematopoietic transplantation as adoptive immunotherapy for indolent lymphoma: low incidence of toxicity, acute graft-versus-host disease, and treatment-related mortality. *Blood* 2001; **98**: 3595–3599.
- 60 Sohn SK, Bensinger W, Holmberg L *et al.* High-dose chemotherapy with allogeneic or autologous stem-cell transplantation for relapsed mantle cell lymphoma: the Seattle experience. *Proc Am Soc Clin Oncol* 1998; **17**: 17a (abstract 64).
- 61 Corradini P, Ladetto M, Astolft M *et al.* Clinical and molecular remission after allogeneic blood cell transplantation in a patient with mantle cell lymphoma. *Br J Haematol* 1996; **94**: 376–378.
- 62 Adkins D, Brown R, Goodnough LT *et al.* Treatment of resistant mantle cell lymphoma with allogeneic bone marrow transplantation. *Bone Marrow Transplant* 1998; **21**: 97–99.
- 63 Khouri IF, Lee MS, Saliba RM *et al.* Nonablative allogeneic stem-cell transplantation for advanced/recurrent mantle cell lymphoma. *J Clin Oncol* 2003; **21**: 4407–4412.
- 64 Dasgupta RK, Morris E, Mackinnon S *et al.* Non-myeloablative stem cell transplantation (NST) for mantle cell lymphoma in the United Kingdom. A report of the clinical trials committee of the British Society for Blood and Bone Marrow Transplantation. *Blood* 2003; **102**: 79a (abstract 265).
- 65 Escalon MP, Champlin RE, Saliba RM *et al.* Nonmyeloablative allogeneic hematopoietic transplantation: a promising salvage therapy for patients with non-Hodgkin's lymphoma whose disease has failed a prior autologous transplantation. *J Clin Oncol* 2004; **22**: 2419–2423.
- 66 Scalzulli PR, Corsetti MT, Baltrami G *et al.* Nonmyeloablative allografting for advanced lymphoma: a higher than expected remission rate and rate disease control. *Blood* 2003; **102**: 467b (abstract 5596).
- 67 Robinson SP, Goldstone AH, Mackinnon S *et al.* Chemo-resistant or aggressive lymphoma predicts for a poor outcome following reduced-intensity allogeneic progenitor cell transplantation: an analysis from the Lymphoma Working Party of the European Group for Blood and Bone Marrow Transplantation. *Blood* 2002; **100**: 4310–4316.
- 68 Sureda A, Robinson S, Ruiz de Elvira C *et al.* Nonmyeloablative allogeneic stem cell transplantation significantly reduces transplant related mortality in comparison with conventional allogeneic transplantation in relapsed or refractory Hodgkin's disease: results of the European Group for Blood and Bone Marrow Transplantation. *Blood* 2003; **102**: 198a (abstr. 692).
- 69 Peggs KS, Thomson K, Chopra R *et al.* Long term results of reduced intensity transplantation in multiply relapsed and refractory Hodgkin's lymphoma: evidence of a therapeutically relevant graft-versus-lymphoma effect. *Blood* 2003; **102**: 198a (abstr. 694).
- 70 Porter DL, Stadtmauer EA, Lazarus HM. GVHD: graft-versus-host disease or graft-versus-Hodgkin's disease? An old acronym with new meaning. *Bone Marrow Transplant* 2003; **31**: 739–746.
- 71 Corradini P, Doderio A, Zallio F *et al.* Graft-versus-lymphoma effect is relapsed peripheral T-cell non-Hodgkin's lymphomas after reduced-intensity conditioning followed by allogeneic transplantation of hematopoietic cells. *J Clin Oncol* 2004; **22**: 2172–2176.
- 72 Molina A, Zain J, Arber DA *et al.* Is allogeneic hematopoietic stem cell transplantation (AHSCT) potentially curative in a group of refractory cutaneous T-cell lymphomas (CTCLs)? Durable clinical, cytogenetic, and molecular remissions after AHSCT for refractory Sezary syndrome and mycosis fungoides. *Blood* 2003; **102**: 476a (abstract 1737).
- 73 Buser AS, Heim D, Bucher C *et al.* High-dose chemotherapy using BEAM for tumor debulking without stem cell support followed by early allogeneic reduced intensity conditioning transplantation to induce a graft-versus-lymphoma effect in patients with high risk or refractory lymphoma. *Bone Marrow Transplant* 2004; **33**: 1011–1014.
- 74 Tanimoto TE, Kusumi E, Hamaki T *et al.* High complete response rate after allogeneic hematopoietic stem cell transplantation with reduced-intensity conditioning regimens in advanced malignant lymphoma. *Bone Marrow Transplant* 2003; **32**: 131–137.
- 75 Mitterbauer M, Kalhs P, Keil F *et al.* Continuous complete and molecular remission in two patients with refractory lymphoid malignancies after autografting followed by allogeneic stem cell transplantation with reduced intensity conditioning. *Br J Haematol* 2002; **118**: 132–135.
- 76 Bertz H, Illerhaus G, Veelken H, Finke J. Allogeneic hematopoietic stem cell transplantation for patients with relapsed or refractory lymphomas: comparison of high-dose conventional conditioning vs fludarabine-based reduced-intensity regimens. *Ann Oncol* 2002; **12**: 135–139.
- 77 Ballen KK, Becker PS, Emmons RVB *et al.* Low-dose total body irradiation followed by allogeneic lymphocyte infusion may induce remission in patients with refractory hematologic malignancies. *Blood* 2002; **100**: 442–450.
- 78 Corradini P, Teralla C, Olivieri A *et al.* Reduced-intensity conditioning followed by allografting of hematopoietic cells can produced clinical and molecular remissions in patients with poor-risk hematologic malignancies. *Blood* 2002; **99**: 75–82.
- 79 Maloney DG. Graft-vs-lymphoma effect in various histologies of non-Hodgkin's lymphoma. *Leuk Lymphoma* 2003; **44**: S99–S105.
- 80 Sohn SK, Baek JH, Kim DH *et al.* Successful allogeneic stem cell transplantation with prophylactic stepwise G-CSF primed-DLIs for relapse after autologous transplantation in mantle cell lymphoma: a case report and literature review on the evidence of GVL effects in MCL. *Am J Hematol* 2000; **65**: 75–80.
- 81 Bethge WA, Hegenbart U, Stuart M *et al.* Adoptive immunotherapy with donor lymphocyte infusions after allogeneic hematopoietic cell transplantation following nonmyeloablative conditioning. *Blood* 2004; **103**: 790–795.
- 82 Peggs KS, Thomson K, Hart DP *et al.* Dose-escalated donor lymphocyte infusions following reduced intensity transplantation: toxicity, chimerism, and disease responses. *Blood* 2004; **103**: 1548–1556.
- 83 Dey BR, McAfee S, Colby C *et al.* Impact of prophylactic donor leukocyte infusions on mixed chimerism, graft-versus-host disease, and antitumor response in patients with advanced hematologic malignancies treated with nonmyeloablative conditioning and allogeneic bone marrow

- transplantation. *Biol Blood Marrow Transplant* 2003; **9**: 320–329.
- 84 Fung HC, Cohen S, Rodriguez R *et al*. Reduced-intensity allogeneic stem cell transplantation for patients whose prior autologous stem cell transplantation for hematologic malignancy failed. *Biol Blood Marrow Transplant* 2003; **9**: 649–656.
- 85 Vose JM, Bierman PJ, Anderson JR *et al*. Progressive disease after high-dose therapy and autologous transplantation for lymphoid malignancy: clinical course and patient follow-up. *Blood* 1992; **80**: 2142–2148.
- 86 Tsai T, Goodman S, Saez R *et al*. Allogeneic bone marrow transplantation in patients who relapse after autologous transplantation. *Bone Marrow Transplant* 1997; **20**: 859–863.
- 87 Radich J, Gooley T, Sanders JE *et al*. Second allogeneic transplantation after failure of first autologous transplantation. *Biol Blood Marrow Transplant* 2000; **6**: 272–279.
- 88 Anderlini P, Giralt S, Andersson B *et al*. Allogeneic stem cell transplantation with fludarabine-based, less intensive conditioning regimens as adoptive immunotherapy in advanced Hodgkin's disease. *Bone Marrow Transplant* 2000; **26**: 615–620.
- 89 Nagler A, Or P, Naparstek E *et al*. Second allogeneic stem cell transplantation using nonmyeloablative conditioning for patients who relapsed or developed secondary malignancies following autologous transplantation. *Exp Hematol* 2000; **28**: 1096–1104.
- 90 Branson K, Chopra R, Kottaridis PD *et al*. Role of nonmyeloablative allogeneic stem cell transplantation after failure of autologous transplantation in patients with lymphoproliferative malignancies. *J Clin Oncol* 2002; **20**: 4022–4031.
- 91 Mohty M, Bay J-O, Faucher C *et al*. Graft-versus-host disease following allogeneic transplantation from HLA-identical sibling with antithymocyte globulin-based reduced-intensity preparative regimen. *Blood* 2003; **102**: 470–476.
- 92 Izutsu K, Kanda Y, Ohno H *et al*. Development of grade II to IV acute GVHD is associated with lower incidence of disease progression after unrelated bone marrow transplantation for non-Hodgkin's lymphoma. A study from the Japan Marrow Donor Program. *Blood* 2003; **102**: 490a (abstract 1783).
- 93 Mielcarek M, Martin PJ, Leisenring W *et al*. Graft-versus-host disease after nonmyeloablative vs conventional hematopoietic stem cell transplantation. *Blood* 2003; **102**: 756–762.
- 94 Johnson BD, Drobyski WR, Truitt RL. Delayed infusion of normal donor cells after MHC-matched bone marrow transplantation provides an antileukemic reaction without graft-versus-host disease. *Bone Marrow Transplant* 1993; **11**: 329–336.
- 95 Bonini C, Ferrari G, Verzeletti S *et al*. HSV-TK gene transfer into donor lymphocytes for control of allogeneic graft-versus-leukemia. *Science* 1997; **276**: 1719–1724.
- 96 Nimer SD, Giorgi J, Gajewski JL *et al*. Selective depletion of CD8+ cells for prevention of graft-versus-host disease after bone marrow transplantation. A randomized control trial. *Transplantation* 1994; **57**: 82–87.
- 97 Dazzi F, Szydlo RM, Craddock C *et al*. Comparison of single-dose and escalating-dose regimens of donor lymphocyte infusion for relapse after allografting for chronic myeloid leukemia. *Blood* 2000; **95**: 67–71.
- 98 Schouten HC, Qian W, Kvaloy S *et al*. High-dose therapy improves progression-free survival and survival in relapsed follicular non-Hodgkin's lymphoma: results from the randomized European CUP trial. *J Clin Oncol* 2003; **21**: 3918–3927.
- 99 Philip T, Guglielmi C, Hagenbeek A *et al*. Autologous bone marrow transplantation as compared with salvage chemotherapy in relapses of chemotherapy-sensitive non-Hodgkin's lymphoma. *N Engl J Med* 1995; **333**: 1540–1545.
- 100 Milpied N, Deconinck E, Gaillard F *et al*. Initial treatment of aggressive lymphoma with high-dose chemotherapy and autologous stem-cell support. *N Engl J Med* 2004; **350**: 1287–1295.
- 101 Schmitz N, Pfistner B, Sextro M *et al*. Aggressive conventional chemotherapy compared with high-dose chemotherapy with autologous haemopoietic stem-cell transplantation for relapsed chemosensitive Hodgkin's disease: a randomised trial. *Lancet* 2002; **359**: 2065–2071.
- 102 Pelot MR, Pearson DA, Swenson K *et al*. Lymphohematopoietic graft-vs-host reactions can be induced without graft-vs-host disease in murine mixed chimeras established with a cyclophosphamide-based nonmyeloablative conditioning regimen. *Biol Blood Marrow Transplant* 1999; **5**: 133–143.
- 103 Sandmaier BM, Fukuda T, Gooley T *et al*. Dog leukocyte antigen-haploidentical stem cell allografts after anti-CD44 therapy and reduced-intensity conditioning in a preclinical canine model. *Exp Hematol* 2003; **31**: 168–175.
- 104 Fukuda T, Storb R, Gooley T *et al*. DLA-haploidentical stem cell allografts after anti-CD44 therapy and nonmyeloablative conditioning: achievement of full donor chimerism by donor lymphocyte infusion. *Blood* 2003; **102**: 458a (abstract 1670).
- 105 Taranova AG, Georges GE, Yunusov M *et al*. Breaking tolerance in stable mixed chimeric dogs with low-dose TBI and donor or recipient lymphocyte infusions. *Blood* 2003; **102**: 76a (abstract 256).
- 106 Marijt WAE, Heemskerk MHM, Kloosterboer FM *et al*. Hematopoiesis-restricted minor histocompatibility antigens HA-1- or HA-2-specific T cells can induce complete remissions of relapsed leukemia. *Proc Natl Acad Sci* 2003; **100**: 2742–2747.
- 107 Takahashi Y, Mena O, Srinivasan R *et al*. Minor histocompatibility antigen (mHa) specific T-cells with cytotoxicity against autologous tumor cells can be isolated from patients with renal cell carcinoma having a GVT effect after nonmyeloablative hematopoietic cell transplantation (HCT). *Blood* 2003; **102**: 701a (abstract 2594).
- 108 Bellucci R, Wu CJ, Chiaretti S *et al*. Complete response to donor lymphocyte infusion in multiple myeloma is associated with antibody responses to highly expressed antigens. *Blood* 2004; **103**: 656–663.
- 109 Ganss R, Arnold B, Hammerling GJ. Mini review: overcoming tumor-intrinsic resistance to immune effector function. *Eur J Immunol* 2004; **34**: 22635–22641.
- 110 Mocellin S, Semenzato G, Mandruzzato S, Riccardo Rossi C. Part II: vaccines for haematological malignant disorders. *Lancet Oncol* 2004; **5**: 727–737.
- 111 Hope KJ, Jin L, Dick JE. Acute myeloid leukemia originates from a hierarchy of leukemic stem cell classes that differ in self-renewal capacity. *Nat Immunol* 2004; **5**: 738–743.
- 112 Jordan CT. Cancer stem cell biology: from leukemia to solid tumors. *Curr Opin Cell Biol* 2004; **16**: 708–712.
- 113 Giralt S, Hester J, Huh Y *et al*. CD8-depleted donor lymphocyte infusion as treatment for relapsed chronic myelogenous leukemia after allogeneic bone marrow transplantation. *Blood* 1995; **86**: 4337–4343.
- 114 Michalek J, Collins RH, Durrani HP *et al*. Definitive separation of graft-versus-leukemia- and graft-versus-host-specific CD4+ T cells by virtue of their receptor beta loci sequences. *Proc Natl Acad Sci* 2003; **100**: 1180–1184.
- 115 Bonini C, Ferrari G, Verzeletti S *et al*. HSV-TK gene transfer into donor lymphocytes for control of allogeneic graft-versus-leukemia. *Science* 1997; **276**: 1719–1724.
- 116 Falkenburg JH, Wafelman AR, Joosten P *et al*. Complete remission of accelerated phase chronic myeloid leukemia by

- treatment with leukemia-reactive cytotoxic T lymphocytes. *Blood* 1999; **94**: 1201–1208.
- 117 Kwak LW, Neelapu SS, Bishop MR. Adoptive immunotherapy with antigen-specific T cells in myeloma: a model of tumor-specific donor lymphocyte infusion. *Semin Oncol* 2004; **31**: 37–46.
- 118 Nachbaur D, Oberaigner W, Fritsch E *et al.* Allogeneic or autologous stem cell transplantation (SCT) for relapsed and refractory Hodgkin's disease and non-Hodgkin's lymphoma: a single-centre experience. *Eur J Haematol* 2001; **66**: 43–49.
- 119 Akpek G, Ambinder RF, Piantadosi S *et al.* Long-term results of blood and marrow transplantation for Hodgkin's lymphoma. *J Clin Oncol* 2001; **19**: 4314–4321.
- 120 Khouri IF, Lee M-S, Saliba RM *et al.* Nonablative allogeneic stem cell transplantation for chronic lymphocytic leukemia. *Exp Hematol* 2004; **32**: 28–35.
- 121 Casper J, Knauf W, Kiefer T *et al.* Treosulfan and fludarabine: a new toxicity-reduced conditioning regimen for allogeneic hematopoietic stem cell transplantation. *Blood* 2004; **103**: 725–731.
- 122 Dreger P, Brand R, Hansz J *et al.* Treatment-related mortality and graft-versus-leukemia activity after allogeneic stem cell transplantation for chronic lymphocytic leukemia using intensity-reduced conditioning. *Leukemia* 2003; **17**: 841–848.
- 123 Rondon G, Giralt S, Huh Y *et al.* Graft-versus-leukemia effect after allogeneic bone marrow transplantation for chronic lymphocytic leukemia. *Bone Marrow Transplant* 1996; **18**: 669–672.
- 124 Mandigers CMPW, Verdonck LF, Meijerink JPP *et al.* Graft-versus-lymphoma effect of donor lymphocyte infusion in indolent lymphomas relapsed after allogeneic stem cell transplantation. *Bone Marrow Transplant* 2003; **32**: 1159–1163.
- 125 Ho AYL, Devereux S, Mufti GJ, Pagliuca A. Reduced-intensity rituximab-BEAM-CAMPATH allogeneic haematopoietic stem cell transplantation for follicular lymphoma is feasible and induces durable molecular remissions. *Bone Marrow Transplant* 2003; **31**: 551–557.
- 126 Marks DI, Lush R, Cavenagh J *et al.* The toxicity and efficacy of donor lymphocyte infusions given after reduced-intensity conditioning allogeneic stem cell transplantation. *Blood* 2002; **100**: 3108–3114.
- 127 Mandigers CMPW, Raemaekers JMM, Schattenberg AVMB *et al.* Allogeneic bone marrow transplantation with T-cell-depleted marrow grafts for patients with poor risk relapsed low-grade non-Hodgkin's lymphoma. *Br J Haematol* 1998; **100**: 198–206.
- 128 van Besien KW, de Lima M, Giralt SA *et al.* Management of lymphoma recurrence after allogeneic transplantation: the relevance of the graft-versus-lymphoma effect. *Bone Marrow Transplant* 1997; **19**: 977–982.
- 129 Grigg A, Bardy P, Byron K *et al.* Fludarabine-based non-myeloablative chemotherapy followed by infusion of HLA-identical stem cells for relapsed leukaemia and lymphoma. *Bone Marrow Transplant* 1999; **23**: 107–110.
- 130 Anderlini P, Acholonu SA, Okoroji G-J *et al.* Donor leukocyte infusions in relapsed Hodgkin's lymphoma following allogeneic stem cell transplantation: CD3+ cell dose, GVHD, and disease response. *Bone Marrow Transplant* 2004; **34**: 511–514.
- 131 Porter DL, Luger SM, Duffy KM *et al.* Allogeneic cell therapy for patients who relapse after autologous stem cell transplantation. *Biol Blood Marrow Transplant* 2001; **7**: 230–238.
- 132 Grigg AP, Seymour JF. Graft versus Burkitt's lymphoma effect after allogeneic marrow transplantation. *Leuk Lymphoma* 2002; **43**: 889–892.
- 133 Dreger P, van Biezen A, Brand R *et al.* Reduced-intensity conditioning lowers treatment-related mortality (TRM) of allogeneic stem cell transplantation (SCT) for CLL: a retrospective study on 448 patients. *Blood* 2003; **102**: 197a (abstract 689).
- 134 Schetelig J, Thiede C, Bornhäuser M *et al.* Evidence of a graft-versus-leukemia effect in chronic lymphocytic leukemia after reduced-intensity conditioning and allogeneic stem-cell transplantation: the Cooperative German Transplant Study Group. *J Clin Oncol* 2003; **21**: 2747–2753.
- 135 Caballero MD, Martino R, León A *et al.* Nonmyeloablative transplant in patients with B-chronic lymphocytic leukemia (B-CLL): results of a prospective multicentre trial. *Blood* 2003; **102**: 433b (abstract 5462).
- 136 Sorror ML, Maris M, Storer B *et al.* Nonmyeloablative (NM) conditioning and allogeneic hematopoietic cell transplantation (HCT) with HLA-matched unrelated donor (URD) for patients (pts) with chemotherapy-refractory chronic lymphocytic leukemia (CLL). *Blood* 2003; **102**: 482a (abstr. 1757).
- 137 Khouri IF, Champlin RE. Nonmyeloablative stem cell transplantation for lymphoma. *Semin Oncol* 2004; **31**: 22–26.
- 138 Dasgupta RK, Morris E, Mackinnon S *et al.* Non-myeloablative stem cell transplantation (NST) for mantle cell lymphoma in the United Kingdom. A report of the clinical trials committee of the British Society for Blood and Marrow Transplantation. *Blood* 2003; **102**: 79a (abstract 265).
- 139 Sureda A, Robinson S, Ruiz de Elvira C *et al.* Nonmyeloablative allogeneic stem cell transplantation significantly reduces transplant related mortality in comparison with conventional allogeneic transplantation in relapsed or refractory Hodgkin's disease: results of the European Group for Blood and Marrow Transplantation. *Blood* 2003; **102**: 198a (abstract 692).
- 140 Peggs KS, Thomson K, Chopra R *et al.* Long term results of reduced intensity transplantation in multiply relapsed and refractory Hodgkin's lymphoma: evidence of a therapeutically relevant graft-versus-lymphoma effect. *Blood* 2003; **102**: 198a (abstract 694).
- 141 Cooney JP, Stiff PJ, Toor AA, Parthasarathy M. BEAM allogeneic transplantation for patients with Hodgkin's disease who relapse after autologous transplantation is safe and effective. *Biol Blood Marrow Transplant* 2003; **9**: 177–182.
- 142 Chakraverty R, Peggs K, Chopra R *et al.* Limiting transplantation-related mortality following unrelated donor stem cell transplantation by using a nonmyeloablative conditioning regimen. *Blood* 2002; **99**: 1071–1078.