



William Wolk. *Edge of the Pond*. Oil on canvas, 24" × 36".

Donor lymphocyte infusions show promise as a treatment approach for patients with relapsed hematologic malignancies following allogeneic transplantation.

Donor Lymphocyte Infusions to Treat Hematologic Malignancies in Relapse After Allogeneic Blood or Marrow Transplantation

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Background: *Patients with hematologic malignancies in relapse after allogeneic bone marrow transplantation can be treated by infusing leukocytes from the original stem cell donor.*

Methods: *The published literature on donor lymphocyte infusion (DLI) was reviewed.*

Results: *DLI induces complete remissions in the majority of patients with chronic myeloid leukemia (CML) in early-stage relapse and in less than 30% of patients with relapsed acute leukemia, myelodysplasia, and multiple myeloma. DLI-induced remissions of chronic phase CML are durable, but as many as half of patients with other diseases ultimately relapse. Complications of DLI include acute and chronic graft-vs-host disease (GVHD) and aplasia, which induce profound immunosuppression and susceptibility to opportunistic infections. There is a strong correlation of GVHD and disease response.*

Conclusions: *Novel methods of augmenting the antitumor efficacy of DLI and of dissociating the graft-vs-leukemia effect from GVHD are needed. These studies will require an improved understanding of the cellular and molecular mechanisms of alloreactivity and the development of novel agents to control the nature and intensity of the alloimmune response.*

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Submitted October 15, 2001; accepted January 3, 2002.

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This research is supported by PHS grant P01-CA15396A1 from the National Cancer Institute, a Translational Research Award from the Leukemia and Lymphoma Society of America, and the Amy Strelzer Manasevitz Award from the National Marrow Donor Program. Dr Fuchs is a fellow of the Cancer Research Institute, and Dr Luznik is a fellow of the Cure for Lymphoma Foundation.

Introduction

Over the past 35 years, allogeneic blood or marrow transplantation (alloBMT) has emerged as an effective and potentially curative therapy of a variety of drug-resistant hematologic malignancies. Marrow transplantation was initially developed as a procedure to rescue atomic bomb survivors from marrow aplasia induced by high-dose total body irradiation (TBI). Naturally, the anti-leukemic effect of alloBMT was initially ascribed to the myeloablative conditioning regimen, and allogeneic

ic marrow was used because it is a tumor-free source of stem cells for rescue. Graft-vs-host disease (GVHD) was a major cause of transplant-related mortality, but circumstantial evidence suggested that donor T cells, the mediators of GVHD, could also induce a therapeutic “graft-vs-leukemia” (GVL) effect. Definitive evidence for the GVL effect was finally provided when donor lymphocyte infusions (DLIs) alone were shown to be able to induce complete remissions of chronic myeloid leukemia (CML) in relapse after alloBMT.

The advent of DLI has brought about a paradigm shift in the field of BMT. The potency of the GVL effect, especially against CML, is now well recognized. Myeloablative conditioning is no longer deemed necessary for the eradication of malignancy. In nonmyeloablative allogeneic stem cell transplantation (SCT), immunosuppressive conditioning is administered to allow donor cell engraftment, and the primary therapeutic modality is donor T cells. In light of the increasing role of adoptive immunotherapy in the treatment of hematologic malignancies, we review the history of DLI, current results and complications of treatment, and future directions to enhance the efficacy and reduce the toxicity of adoptive immunotherapy with allogeneic T cells.

History of Adoptive Immunotherapy of Malignancy

The notion that immunocompetent cells, contained within adult bone marrow or peripheral blood, are capable of mediating an antitumor effect was first validated experimentally in 1957 by Barnes and Loutit.¹ In these experiments, leukemic animals that were lethally irradiated and reconstituted with allogeneic marrow had a lower tumor burden following transplantation than similarly treated animals that were reconstituted instead with syngeneic marrow. This observation led Mathé and colleagues² to speculate that leukocyte transfusions could mediate antitumor effects in cancer-bearing recipients. To test this hypothesis, pooled white cell products from patients with CML were transfused, in doses ranging from 6.3 to 120×10^{10} cells, into 21 nontransplanted patients with end-stage acute leukemia.³ Complete and partial disease responses occurred in 6 and 3 patients, respectively, lasting from 7 days to as long as 4 months. There was a strong temporal and statistical correlation between disease response and acute GVHD; that is, the reduction of blast cells from the blood coincided with the onset of GVHD, and 6 of the 7 patients who developed GVHD responded. Finally, disease response was also correlated with cell dose, with 8 of 9 responses, and all 6 complete responses occurring among the 13 patients who received $\geq 10^{11}$ leukocytes. Since the

patients had not previously received a bone marrow transplant and were infused with cells from multiple donors, it is possible that immunologic rejection of the transfused leukocytes by the patients' immune systems accounted for the transient nature of the antitumor response. Nevertheless, the results illustrate two of the important characteristics of DLI that remain true today. First, there is a relationship between the infused cell dose and the likelihood of a clinically significant antitumor response. Second, the antitumor response mediated by donor immunocompetent cells goes hand in hand with GVHD.

Nearly 25 years elapsed before peripheral blood leukocytes were again transfused into patients in a deliberate attempt to treat hematologic malignancies. In the interim, however, progress was being made in the laboratory. In 1973, Bortin and colleagues⁴ attempted to quantify the immunologic antitumor effect, which they called “GVL effect,” of donor lymphocytes. In their model, significant GVL effects were induced in lethally irradiated leukemic mice by transfusing them with marrow plus spleen cells from unprimed, major histocompatibility complex (MHC)-mismatched, but not from MHC-identical, allogeneic donors. In a later study, these investigators found that GVL effects could be obtained by priming MHC-identical donors with irradiated leukemia cells of the recipient prior to adoptive transfer, but only at the cost of increased GVHD.⁵ Likewise, Weiden and colleagues⁶ reported that long-term, stable radiation chimeras were relatively resistant to the induction of GVHD by a transfusion of unsensitized lymphocytes from the original marrow donor. However, if the donors were subjected to repeated skin grafts from the chimeras to sensitize lymphocytes against host histocompatibility antigens, significant and lethal GVHD followed lymphocyte transfusion. The authors speculated that the resistance of stable chimeras to the induction of GVHD by unprimed DLI could be accounted for by a “cellular resistance” mechanism, perhaps the existence of regulatory T cells. Taken together, the studies demonstrate that an observable GVH response is not the inevitable outcome of transfusing lymphocytes into immunologically tolerant recipients, and that attempts to augment the GVL effect by priming the donor against host tissues usually result in increased GVHD.

Meanwhile, in the clinic, evidence for a clinically significant GVL effect of alloBMT was emerging. In a retrospective review of transplants for acute leukemia, Weiden and colleagues^{7,8} found a statistically significant inverse correlation between the risk of relapse and the incidence of either acute or chronic GVHD. These findings led to an attempt to use GVHD as adoptive immunotherapy, ie, to decrease the risk of leukemic

Table 1. — Response of Chronic Myeloid Leukemia to DLI

Disease Stage	Europe ²⁸	North America ¹⁴	Total
Cytogenetic relapse	40/50 (80%)*	3/3 (100%)	43/53 (81%)
Hematologic relapse	88/114 (77%)	25/34 (74%)	113/148 (76%)
Transformed phase	13/36 (36%)	5/18 (28%)**	18/54 (33%)
All	141/200 (71%)	33/55 (60%)	174/255 (68%)

* Complete responses/total evaluable (%).
 ** Includes 4 of 12 complete remissions for patients in accelerated phase and 1 of 6 complete remissions for patients in blast crisis.

relapse after alloBMT by adding peripheral blood lymphocytes to the marrow inoculum or to shorten the duration of posttransplant immunosuppression. While both approaches increased the incidence and severity of GVHD, there was no corresponding decrease in the risk of leukemia relapse.⁹ These disappointing results were balanced by encouraging reports of donor leukocyte transfusions for the treatment of relapsed hematologic malignancies after alloBMT. The first patient to receive DLI for a hematologic malignancy in relapse after BMT was a boy with acute lymphocytic leukemia (ALL) that was resistant to chemotherapy and cytokines. He ultimately obtained a sustained complete remission of his disease by receiving multiple transfusions of lymphocytes from his sister, the original bone marrow donor.¹⁰ Then, Kolb and colleagues¹¹ reported on 3 patients with relapsed CML who failed to respond to treatment with interferon alfa (IFN- α) but who obtained complete remissions with the combination of IFN- α plus DLI. Thus, the era of adoptive immunotherapy to treat posttransplant relapse of hematologic malignancies was born.

Clinical Results of Donor Lymphocyte Infusions

Chronic Myeloid Leukemia

CML in chronic phase is highly susceptible to GVL effects mediated by allogeneic T cells, as evidenced by a 5.14-fold increased risk of relapse associated with T-cell depletion of donor marrow prior to HLA-identical alloBMT.¹² It is therefore not surprising that donor T-cell infusions induce remissions in the majority of patients with chronic phase CML in relapse after alloBMT. Table 1 presents the results obtained from databases in Europe and North America on the treatment of relapsed CML with DLI from related donors.^{13,14} Perhaps the most striking feature of Table 1 is that the likelihood of obtaining a remission declines with advancing disease stage. Thus, DLI induced cytogenetic complete remissions in 81% of patients whose

relapse was detectable only by cytogenetic analysis, but in only 33% of patients whose disease had transformed to either accelerated phase or blast crisis. For patients who relapse after BMT into chronic phase CML, responses to DLI are also durable (Fig 1). In one study, 44 of 57 patients receiving DLI for relapsed chronic phase CML achieved a “molecular remission,” as defined by the failure to detect *Bcr-Abl* gene transcripts by polymerase chain reaction (PCR).¹⁵ Only 4 of the 44 patients converted to a PCR-positive state, and 3-year survival of this group was 95%. Therefore, over 70% of patients who are treated with DLI for relapsed, chronic phase CML may be expected to obtain a durable, molecular remission.

DLI has also been used to treat patients with CML who relapse after unrelated donor BMT.¹⁶ In these instances, T cells were either freshly obtained from the original stem cell donor or were taken from specimens that were cryopreserved after T-cell depletion of the donor marrow at the time of transplantation. Among 25 patients with relapsed CML identified from the National Marrow Donor Program database, 12 (48%) obtained remission, including 7 of 12 patients in chronic phase, 4 of 12 patients in advanced phase CML, and 1 patient with advanced phase CML who received chemotherapy prior to DLI. Eight of the 12 patients who achieved cytogenetic complete remissions were also tested by PCR, and all 8 were found to be in a molecular remission.

The next most powerful predictor of the response of CML to DLI, following disease stage, is T-cell dose, expressed as the number of CD3+ cells per kilogram of recipient body weight. In an attempt to induce remissions of CML without simultaneously inducing GVHD,

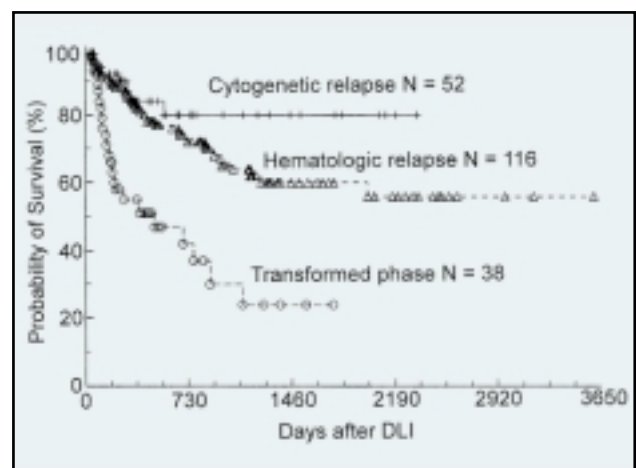


Fig 1. — Survival after donor lymphocyte infusions (DLI) for chronic myeloid leukemia, by phase of disease. European Group for Blood and Marrow Transplantation (EBMT) study follow-up, August 1998. Copyright 1999 by the American Society of Clinical Oncology. Reprinted with permission.

Mackinnon and colleagues¹⁷ developed a strategy of treating relapsed CML after HLA-identical sibling BMT by infusing a low dose of donor T cells and subsequently escalating the dose if a remission was not achieved. In this study of 22 patients, an initial T-cell dose below 10^7 cells/kg was given to 10 patients, and none achieved a complete remission. Twelve patients were treated with a starting dose of 10^7 CD3+ cells/kg. Eight of these patients achieved a remission with this dose only, and 3 additional patients achieved a remission following subsequent infusions of 5×10^7 or 10^8 T cells/kg. In all, 18 of 22 patients achieved a molecular remission, and only 8 patients developed GVHD. Of note, GVHD developed in only 1 of the 8 patients who achieved a remission with a single dose of 10^7 T cells/kg. A subsequent study compared a single bulk dose regimen (BDR; median dose 1.5×10^8 T cells/kg) with escalating doses as required (EDR; $10^7 \rightarrow 5 \times 10^7 \rightarrow 10^8$ T cells/kg).¹⁸ EDR was found to have a non-significantly higher overall remission rate (91% vs 67%, $P=.70$) and a lower rate of GVHD (10% vs 44%, $P=.011$). Interestingly, the incidence of acute and chronic GVHD was lower using the EDR strategy, even when comparable total lymphocyte doses were compared. Based on these studies, a reasonable strategy for the treatment of early-stage CML in relapse after alloBMT is to administer 10^7 donor T cells/kg and escalate only if a remission is not achieved. For unrelated DLI, patients who received fewer than 10^7 T cells/kg did not achieve remission, and there was no correlation between cell dose (above 10^7 cells/kg) and achieving a complete remission or acute or chronic GVHD.¹⁶

An understanding of the kinetics of GVL and GVH responses is critical in deciding if a dose of DLI has failed to induce a remission and when to administer the next dose of DLI. In a survey of North American BMT programs,¹⁴ the median time from DLI to achievement of remission was 85 days, although the times for assessing disease status after DLI were not standardized. However, a kinetic analysis of 3 CML patients treated with DLI showed a lag phase of 5 to 13 weeks before any increase in donor chimerism or decrease in *Bcr-Abl* transcripts occurred.¹⁹ After this lag phase, there was a critical switch period of 4 to 5 weeks to achieve full donor chimerism and eradication of *Bcr-Abl*+ cells. Thus, responses to DLI in early-stage CML may not be observed for as long as 3 months, and complete responses may take over 4 months to occur.

A variety of strategies may be employed for early-stage CML patients who do not respond to DLI alone or for patients with advanced-stage CML who are unlikely to respond. IFN- α alone induces responses in approximately one third of patients with relapsed chronic phase CML,²⁰ and IFN- α has been given together with

DLI as initial therapy of relapsed CML after alloBMT. However, this combination was found to be less effective than DLI alone as initial therapy. One patient with chronic phase CML failed to respond to DLI with or without in vivo treatment with interleukin-2 (IL-2), but the patient did respond to lymphocytes that were preactivated in vitro with IL-2 and treated in vivo with subcutaneous IL-2.²¹ Finally, leukemia-reactive cytotoxic T-cell lines, which lyse leukemia cells but not normal patient or donor lymphocytes, have been generated and used to achieve remission in a patient with accelerated phase CML.²² This observation supports the hypothesis that leukemia cells express antigens, not shared by normal blood cells, that can be targeted to produce effective GVL responses without GVHD.

In light of ability of DLI to induce remissions in the majority of patients with relapsed, early-stage CML, a strategy of depleting T cells from the donor stem cell graft to reduce the rate of complications arising from alloBMT and using DLI to prevent or treat relapse was tested.²³ As expected, T-cell depletion of donor stem cells reduced GVHD and transplant-related mortality after BMT for CML but at the cost of an increased risk of relapse. However, DLI was able to salvage the vast majority of relapses, and overall survival ranged from 72% to 89%.^{24,26}

Diseases Other Than CML

These diseases do not respond to DLI as well as early-stage CML responds. In general, less than 30% of patients with relapsed acute leukemia, myelodysplasia, and multiple myeloma achieve complete responses to DLI.^{13,14,27} Worse yet, as many close to half or more of patients who do achieve a complete response may be expected to relapse after DLI.^{28,29} The primary goal of future research, as discussed below, should be directed to augmenting the efficacy of adoptive immunotherapy against these diseases.

Acute Leukemia and Myelodysplasia

Because of the large number of acute leukemia patients who relapse after alloBMT, there has been a substantial experience in the treatment of these patients with DLI (Tables 2 and 3).^{14,30,31} Patients with relapsed acute leukemia do approximately as well with DLI as patients with advanced-stage CML but consistently worse than patients with early-stage CML. Using median doses of $\geq 10^8$ T cells/kg, DLI alone induces complete remissions in 8% of patients with ALL and 22% of patients with acute myeloid leukemia (AML). When patients who receive chemotherapy prior to DLI are included, complete response rates are signifi-

Table 2. — Response of Acute Myeloid Leukemia, Acute Lymphocytic Leukemia, and Myelodysplasia to DLI Alone

Disease	Europe ³⁰	North America	Total
Acute myeloid leukemia	12/42 (29%)	6/39 (15%) ¹⁴	18/81 (22%)
Acute lymphocytic leukemia	1/22 (5%)	2/15 (13.3%) ³¹	3/37 (8%)
Myelodysplasia	3/9 (33%)	2/5 (40%) ¹⁴	5/14 (36%)

Table 3. — Complete Response Rates of Acute Myeloid Leukemia, Acute Lymphocytic Leukemia, and Myelodysplasia After DLI, With or Without Prior Chemotherapy

Disease	Europe ³⁰	North America	Total
Acute myeloid leukemia	28/66 (42%)	13/46 (28%) ¹⁴	41/112 (37%)
Acute lymphocytic leukemia	16/41 (39%)	11/44 (25%) ³¹	27/85 (33%)
Myelodysplasia	4/9 (44%)	2/6 (33%) ¹⁴	6/15 (40%)

cantly higher, ranging from 33% to 37%. These response rates include patients who received DLI in the nadir of blood counts after chemotherapy as well as patients who received DLI as consolidation of a chemotherapy-induced remission, and the response rates are similar to the complete response rates obtained by chemotherapy alone in the treatment of relapsed acute leukemia after alloBMT.³² However, follow-up of ALL patients reveals few, if any, long-term survivors (Fig 2), although 1 has been reported.¹⁰ Relapse occurs in approximately one quarter to one half of patients with AML in remission after DLI, leaving a long-term survival rate of approximately 10% to 15% (Fig 2). It is not clear whether administration of induction chemotherapy at the time of relapse improves long-term survival following DLI. A randomized trial with analysis performed on an intent-to-treat basis is needed to answer this question.

In the report of Collins et al,¹⁴ the median time to remission of acute leukemia following DLI was 34 days, substantially shorter than the 12 to 16 weeks to induction of remission in patients with relapsed chronic phase CML. It is possible that the kinetics of donor T-cell activation is faster in patients with relapsed AML than in CML patients. However, it is also possible that delayed responses to DLI are not observed in acute leukemia patients because patients with delayed responses develop disease complications and die before a remission can be achieved.

The risk of relapse after a DLI-induced remission is higher for patients with acute leukemia than for patients with CML. In a follow-up study of patients who entered a DLI-induced remission,²⁹ relapse occurred in 4 of 15 patients with AML, 3 of 4 patients with ALL, and 1 of 3 patients with myelodysplasia. The median time from remission to relapse was 10 months, and the prob-

ability of event-free survival of patients with a DLI-induced remission was 52% at 1 year, 38% at 2 years, and 31% at 3 years.

Few treatment options are available for acute leukemia patients who either fail to respond to DLI or relapse from a DLI-induced remission. Some patients who failed to respond to DLI alone were induced into remission by donor lymphocytes that have been activated in vitro and/or in vivo (in the patient) with recombinant IL-2.³³ Alternatively, patients may undergo a second bone marrow transplant procedure using the same donor, but second BMT is toxic, especially if performed within 6 months of the first transplant. Among 170 patients in Europe receiving second bone marrow transplants for acute leukemia, the 5-year probability for transplant-related mortality, leukemia-free survival, and relapse were 46%, 25%, and 59%, respectively. Grade II or higher acute GVHD occurred in 59% of patients, and chronic GVHD occurred in 32% of patients. Factors associated with an improved outcome included diagnosis, interval to relapse after first BMT >292 days, acute GVHD after first SCT, use of TBI with second SCT, acute GVHD after second SCT, and use of bone marrow as the stem cell source for the second transplant. Three-year leukemia-free survival was 52% for patients whose relapse occurred >292 days after the first SCT and who were in remission prior to the second transplant procedure. Thus, patients with these characteristics should be considered for a second BMT as the procedure of choice instead of DLI.

Fewer patients with relapsed myelodysplasia have been treated with DLI. However, because myelodyspla-

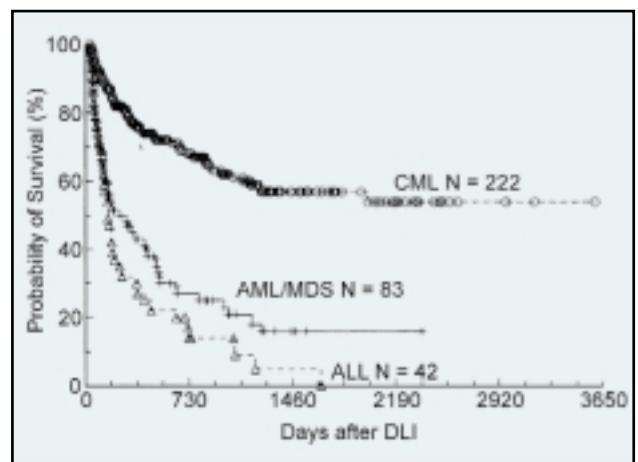


Fig 2. — Survival after donor lymphocyte infusions (DLI) for recurrent leukemia. EBMT study follow-up, August 1998. MDS, myelodysplastic syndrome. Copyright 1999 by the American Society of Clinical Oncology. Reprinted with permission.

Table 4. — Response of Multiple Myeloma to DLI

Reference	No. of Patients	Prior Chemotherapy	Complete Responses	Partial Responses	Continuous Complete Remission	Acute GVHD	Chronic GVHD
Lokhorst et al ³⁶	27	13	6	8	5	15	7
Salama et al ²⁷	25	3	7	2	2	13	11
Total	52	16	13 (25%)	10 (19%)	7 (13%)	28 (54%)	18 (35%)

GVHD = graft-vs-host disease

sia progresses more slowly than AML, the rate of complete remissions may be higher. The natural history of myelodysplasia patients who enter into a remission following DLI has not been described.

Multiple Myeloma

Several case reports and case series have established that DLI alone can induce complete remissions in patients with multiple myeloma.³⁴⁻⁴² Reports containing larger numbers of patients (Table 4) illustrate that DLI has modest effects against this disease. Complete responses to DLI, with or without preceding chemotherapy, are achieved in 25% of patients, with partial responses occurring in an additional 19% of patients. Only 13% of patients, or slightly greater than 1 in 8, were alive and free of disease more than 1 year after DLI. In both studies, response to chemotherapy prior to DLI and a dose of $\geq 10^8$ T cells/kg correlated with the development of a complete response after DLI. Complete remissions came at a substantial cost. In the study of Salama et al,²⁷ 6 of 7 responding patients developed acute GVHD, and 3 of 4 evaluable responders developed chronic GVHD. Moreover, 3 of 4 patients with remissions longer than 1 year had either grade III-IV acute GVHD or extensive chronic GVHD, and 2 of these 4 patients relapsed anyway. For reasons that are unclear, it is not uncommon for relapses to occur in extramedullary sites.^{41,43-46}

Lymphoma and Chronic Lymphocytic Leukemia

Clinical data suggest the existence of a graft-vs-lymphoma effect of alloBMT,^{47,48} yet there is a paucity of experience in the use of DLI to treat lymphoma in relapse after alloBMT. In the North American registry,¹⁴ DLI failed to induce responses in any of 6 patients with non-Hodgkin's lymphoma or in 2 patients with Hodgkin's disease. In isolated case reports, at least 3 patients with low-grade, follicular non-Hodgkin's lymphoma in relapse after alloBMT have obtained complete remissions after receiving DLI. In these cases, respons-

es were slow and evolved over several months.^{49,50} Chronic lymphocytic leukemia (CLL) also appears to be responsive to allogeneic donor T cells.⁵¹ At least 1 patient has obtained a remission following DLI as treatment of persistent disease following alloBMT.⁵² Other CLL patients have obtained complete remissions, including molecular complete remissions, following discontinuation of posttransplant immunosuppression.^{53,54} Experience with adoptive immunotherapy for patients with lymphoma or CLL will undoubtedly increase as more of these patients receive allogeneic SCT following non-meloablative conditioning.

Viral Diseases and Virus-Induced Malignancy

Patients with progressive disease from adenovirus or respiratory syncytial virus after allogeneic SCT have been treated with DLI.⁵⁵ One patient who received DLI for plasma cell leukemia and concomitant respiratory syncytial virus pneumonia experienced improvement in respiratory symptoms concomitant with the disappearance of respiratory syncytial virus antigen in bronchoalveolar lavage fluid and nasal swabs.⁵⁶ T-cell clones specific for cytomegalovirus (CMV) have been generated from donor cells *ex vivo* and infused into patients to reconstitute anti-CMV immunity after alloBMT.^{57,58} Persistence of T-cell clones in the circulation for up to 12 weeks was documented, and no patient receiving CMV-specific T cells later developed CMV disease.

Epstein-Barr virus-induced lymphoproliferative disease (EBV-LPD) is a polyclonal proliferation of B cells that occurs after solid organ or SCT. The proliferating B cells are usually, but not always, of donor origin. DLI is highly active in the treatment of EBV-LPD, and complete eradication of disease often follows the infusion of as few as 10^6 donor T cells/kg.⁵⁹ The high frequency of EBV-specific memory T cells, as much as 1 in 1,000, in the circulation of EBV-immune individuals may account for the rapid (within 14 to 30 days) and complete disappearance of bulky disease with such small doses. More recently, EBV-specific T-cell lines have been generated *ex vivo* from donor blood and infused to prevent or

treat EBV-induced lymphoma in the recipients of alloBMT.^{60,61} In principle, banks of virus-specific T-cell lines or clones could be established from normal donors and taken “off the shelf” to treat patients with viral infections after BMT, as long as the T cells recognize viral antigens in the context of HLA molecules that are shared by both the T-cell donor and the recipient.⁶² It is unknown whether adoptively transferred, haploidentical T cells could escape rejection by the host immune system for a sufficient period of time to eradicate virally infected cells, or whether GVHD-associated immunodeficiency⁶³ would suppress antiviral immunity.

Complications of Donor Lymphocyte Infusions

Graft-vs-Host Disease

In the North American database,¹⁴ acute or chronic GVHD develops in approximately 60% and 61%, respectively, of patients receiving DLI for relapsed hematologic malignancies. The risk of developing acute GVHD correlates with the donor T-cell dose among recipients of related,¹⁷ but not unrelated,¹⁶ DLI. Doses below 10^7 T cells/kg generally do not induce GVHD but, with the exception of DLI for EBV-LPD (vide supra), also do not have significant antitumor activity. Acute GVHD occurs in approximately 10% of patients receiving 10^7 T cells/kg, in 20% to 30% of patients receiving $3\text{--}5 \times 10^7$ T cells/kg, and in $\geq 50\%$ of patients receiving $\geq 10^8$ T cells/kg. There is a strong temporal association and statistical correlation between the development of GVHD and the induction of a disease response. For instance, in the study of Collins et al,¹⁴ 42 (93%) of 45 complete responders developed acute GVHD, and 36 (88%) of 41 assessable complete responders developed chronic GVHD. Chronic phase CML is the one exception to the gener-

al rule that remission goes hand in hand with GVHD since, as noted above, most patients who receive a dose of 10^7 T cells/kg achieved a remission without GVHD. Also, remissions were achieved in approximately half of European patients with chronic phase CML who did not develop GVHD after DLI (Table 5). Among the total population of patients receiving DLI, 3-year leukemia-free survival is greater for patients who develop chronic GVHD than for those who do not.⁶⁴

Analysis of the European database shows that approximately 55% of chronic phase CML patients developed acute GVHD in response to DLI. This GVHD was mild (grade I) in 18%, moderate (grade II) in 24%, and severe (grades III and IV) in 13%. Nonetheless, GVHD was the primary cause of death in only 4 of 135 European patients and 8 of 140 North American patients treated with DLI, or less than 10% of patients who develop the syndrome. It is therefore possible that GVHD after DLI is easier to control than GVHD after BMT. In animal models, the incidence and severity of DLI-induced GVHD are inversely proportional to the time interval between BMT and DLI, and delayed infusions of donor lymphocytes are capable of inducing potent antileukemia effects and conversion from mixed to full donor hematopoietic chimerism without significant GVHD.^{65,67} The increased toxicity of DLI in the peritransplant period probably results from the synergistic toxicities of BMT conditioning and allogeneic T cells.⁶⁸ Gut damage from BMT conditioning allows translocation of microbial products such as bacterial lipopolysaccharide, from the gut lumen into the systemic circulation.^{69,70} Lipopolysaccharide acts in concert with T-cell-derived interferon gamma (IFN- γ) to induce macrophage production of tumor necrosis factor alpha,⁷¹ a cytokine that has been implicated in GVHD-associated mortality. With delayed DLI, donor T cells may be activated by histocompatibility differences between donor and host, but in the absence of conditioning associated tissue damage, the anti-host reaction may be more confined to the hemolymphoid compartment,^{65,66} and GVHD-associated mortality is thereby reduced.

In light of the potential for dissociating GVL effects from GVHD by delaying T-cell administration, an overall strategy of performing a T-cell-depleted BMT followed by delayed, prophylactic DLI (as opposed to salvage DLI, as discussed above for CML) has been tested.^{72,73} However, caution should be taken against using this strategy indiscriminately since T-cell depletion increases the risk of fatal graft rejection. Even if recovery of autologous hematopoiesis ensues, the patient has been sensitized to all tissues of the same donor and will reject the DLI, rendering it therapeutically useless. Patients who may be at increased risk for graft rejection

Table 5. — GVHD and Response of Chronic Phase Chronic Myeloid Leukemia to DLI

Grade of GVHD	No. of Patients		
	Studied	Responding	%
0	93	47	51
I	38	29	76
II	51	46	90
III	19	16	84
IV	8	6	75

$P \leq .0001$.

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tion, including those who have received extensive prior transfusions while immunocompetent, or recipients of HLA-mismatched stem cells, should receive non-T-cell-depleted BMT, with DLI reserved for treatment of relapse.

Aplasia

DLI-induced pancytopenia is somewhat less common than GVHD after DLI, occurring in approximately 18% to 50% of patients. In general, the aplasia resulting from DLI is mild and transient, and blood counts recover without specific treatment. In 2% to 5% of patients, aplasia is more prolonged. The extent of residual host hematopoiesis predicts the occurrence of DLI-induced aplasia,⁷⁴ which results from the destruction of normal host hematopoietic elements by allogeneic donor lymphocytes. The risk of DLI-induced aplasia is highest among patients with hematologic relapse of chronic phase CML, lower among patients with relapsed multiple myeloma or acute leukemia, and lowest among patients with CML in cytogenetic or molecular relapse only. Approximately one third of patients with hematologic relapse of chronic phase CML develop aplasia after DLI. Complications of aplasia include infection, bleeding, and anemia with increased blood transfusion requirements.

In light of the high likelihood of spontaneous blood count recovery, supportive care is usually adequate treatment of DLI-induced aplasia. Patients who fail to recover blood counts after 1 or 2 weeks may be treated with recombinant human growth factors, such as granulocyte colony-stimulating factor (G-CSF). Patients who fail to respond to G-CSF may be considered candidates for donor stem cell infusion. To avoid the risk of inducing GVHD, a T-cell-depleted bone marrow product may be the preferred stem cell source. Some patients do not respond to stem cell infusion, and mortality from aplasia is estimated to be 4% of all patients who develop the syndrome. An immunologic “graft-vs-stroma” has been described in animal models,⁷⁵ and this may in part account for the failure of patients with DLI-induced aplasia to respond to stem cell infusions. Treatment of prospective DLI donors with G-CSF to increase the stem cell content of the DLI has not been shown to decrease the risk of DLI-induced aplasia.⁷⁶

Infection

Patients who receive DLI for relapsed hematologic malignancy are at high risk of infection. Contributing causes to infection risk include the underlying malignancy with myelophthisis, immunocompromise from prior treatment, DLI-induced aplasia, and GVHD and its treatment. Patients who enter DLI-induced aplasia or

who develop GVHD should be considered as candidates for appropriate prophylactic measures against *Pneumocystis carinii* pneumonia as well as viral and other fungal diseases.

Alternatives to DLI in the Treatment of Hematologic Malignancies in Relapse After AlloBMT

Patients with post-BMT relapse of acute leukemia may be treated with salvage chemotherapy or with intensive conditioning followed by a second SCT. Aggressive chemotherapy for patients who relapse within 100 days after BMT is associated with a high mortality and a low rate of complete remissions.⁷⁷ Complete remissions can be induced in as many as half or more of acute leukemia patients who relapse more than 1 year after BMT, but median survival of treated patients is still less than 1 year, and there are few, if any, long-term survivors.^{32,77} Second BMT is quite toxic and should not be considered for patients whose relapse occurs within 6 months of the first BMT. Radich et al⁷⁸ have reported that second BMT is associated with a 36% risk of death within 100 days of transplant, a 70% risk of relapse in the remainder of patients, and a long-term disease-free survival rate of 14%. Among 18 patients with chronic phase CML in relapse after alloBMT, IFN- α induced cytogenetic complete responses in 6 patients,²⁰ but long-term remissions are rare. These results are inferior to DLI alone, which should be considered the standard of treatment for CML in early phase relapse after alloBMT. Filgrastim has also induced remissions in patients who relapse after alloBMT.⁷⁹⁻⁸¹ In many but not all of the patients, remission was associated with the discontinuation of immunosuppression and the development of GVHD.

Efforts to Minimize GVHD and Enhance Antitumor Efficacy

A number of approaches have been taken to reduce the incidence and/or severity of GVHD after DLI. As previously mentioned, one method of reducing the risk of GVHD among patients with early-stage CML is to administer an initial dose of 10^7 T cells/kg and escalate the dose only as required to obtain a remission.¹⁷ This dose escalation approach has not been rigorously evaluated in patients with other hematologic malignancies in relapse after alloBMT. Another approach is to insert a “suicide gene” into donor T cells ex vivo prior to DLI and pharmacologically induce the death of the transduced T cells after the desired therapeutic effect has been achieved. In an initial clinical trial, the herpes simplex virus thymidine kinase gene

(HSV-TK) was inserted into donor lymphocytes using a retroviral vector. HSV-TK+ cells were transfused into 8 patients with relapsed hematologic malignancies or EBV-LPD after alloBMT.⁸² GVHD, which occurred in 3 patients, was readily controlled by ganciclovir-induced elimination of the transduced cells. Despite the initial positive results, 8 of 24 patients subsequently treated with this approach developed an immune response against HSV-TK, leading to elimination of the transduced cells. One patient who developed chronic GVHD became partially resistant to ganciclovir treatment, possibly due to cell cycle dependence of HSV-TK-mediated killing.⁸³ To some extent, these problems may be solved through the development of alternate suicide gene systems.^{83,84} An alternative to suicide gene insertion is to infuse irradiated donor T cells, which retain short-term cytotoxic potential but are unable to clonally expand in the recipient.⁸⁵

Another approach to the prevention of GVHD after DLI relies on selective depletion of lymphocyte subpopulations. CD4+ and CD8+ T cells have been postulated to play different roles in the induction of GVL vs GVHD, and a randomized trial has shown a reduced risk of GVHD following CD8+ T-cell-depleted alloBMT without a significant impairment in leukemia-free survival.⁸⁶ Following an initial encouraging report of CD8+ T-cell-depleted DLI for patients with relapsed CML,⁸⁷ a clinical trial of CD8+ T-cell-depleted DLI containing defined doses of CD4+ T cells was undertaken in 40 patients with various relapsed hematologic malignancies.⁸⁸ Acute or chronic GVHD occurred in 6 (22%) of 27 evaluable patients receiving 0.3×10^8 CD4+ cells/kg but in 6 (55%) of 11 patients receiving $\geq 10^8$ CD4+ cells/kg, for an overall GVHD incidence of 32%. Disease responses occurred in 15 of 19 patients with early-stage CML, in none of 5 patients with advanced-stage CML, in 5 of 6 patients with multiple myeloma, and in 1 of 7 patients with other diseases. These results suggest that CD8+ T cells can be depleted from the DLI product to reduce GVHD without significantly compromising antitumor efficacy, but a trial that randomizes patients to no depletion vs CD8+ T-cell depletion would be required to definitely resolve this issue.

Cytokines and growth factors play an important role in the development and manifestations of GVH reactions. It has been postulated that prototypic type I helper cytokines, including IL-2 and IFN- γ , promote acute GVHD,⁸⁹ whereas prototypic type II cytokines, including IL-4 and IL-10, suppress GVHD,^{90,94} but conflicting data exist.⁹⁵ For example, the effect of IL-10 on GVHD in a mouse model depended on the dose. A high dose of exogenous IL-10 accelerated GVHD mortality, whereas a low dose of IL-10 was protective.⁹⁶ In vitro stimulation of CD8+ T cells in the presence of IL-12 or

IL-4 causes them to differentiate into cells that secrete type I or type II cytokines, respectively, and either of these populations can induce GVL effects with reduced GVHD.⁹⁷ It should be noted, however, that cytokine secretion profiles of CD4+ and CD8+ T cells in vivo are not as distinct or as stable as was initially proposed based on the early in vitro studies.⁹⁸ For example, the majority of IL-4-producing T cells in mice also produce IFN- γ ,⁹⁹ IL-10 production can be found in either IL-4+ or IFN- γ + T cells,¹⁰⁰ and individual CD8+ T cells retain the ability to proliferate and effectively switch cytokine profiles in vivo in response to local stimuli.¹⁰¹ These studies reveal that T-cell cytokine production is heterogeneous and flexible and that T cells that have been polarized in vitro may not behave as predicted when administered as DLI.

Other cytokines may influence GVHD susceptibility through effects on nonlymphoid tissues. Peritransplant administration of IL-11 or keratinocyte growth factor prevents GVHD after alloBMT by protecting the gastrointestinal mucosa from cytotoxic damage,¹⁰²⁻¹⁰⁴ and keratinocyte growth factor has been shown to downregulate GVHD by mechanisms that are independent of gastrointestinal cytoprotection.¹⁰⁵ Collectively, these studies demonstrate that cytokines and growth factors profoundly influence GVH reactions and may have a role in the prevention of DLI-induced GVHD.

Another approach to the prevention of GVHD after BMT or DLI relies on differential tissue expression of histocompatibility antigens. The targets of GVHD in HLA-identical alloBMT are minor histocompatibility antigens (miHAs), polymorphic antigens that are inherited independently of the MHC and that demonstrate a broad or restricted tissue distribution. GVHD is considered to be the consequence of an immune response against immunodominant miHAs with a wide tissue distribution, including expression on skin, liver, and the gastrointestinal tract. In contrast, antigens that are expressed only on blood cells could, in theory, induce an exclusive lymphohematopoietic GVH reaction, including GVL, without the induction of GVHD. Several groups have endeavored to identify hematopoietic lineage-restricted miHAs with a goal of generating cytotoxic T-lymphocyte (CTL) lines or clones for infusion into patients with hematologic malignancies expressing these antigens. Cytotoxic T cells specific for miHAs have been demonstrated in the blood of HLA-identical BMT recipients,¹⁰⁶⁻¹¹⁰ and Goulmy and colleagues¹¹¹⁻¹¹³ were able to generate miHA-specific CTL lines and clones from patients with severe GVHD. CTL clones were used to identify five miHAs, dubbed HA-1, -2, -3, -4, and -5, that are recognized by T cells in association with HLA-A1 and HLA-A2. At least two of these antigens, HA-1 and HA-2, are expressed only on cells of the hematopoietic lineage.

Paradoxically, a mismatch between donors and recipients for one of these antigens, HA-1, was found to be associated with a higher risk of GVHD following HLA-identical BMT,¹¹⁴ an observation that may not have been expected if the immune response against HA-1 was directed solely against blood cells. A potential explanation for this apparent paradox is that donor T cells, specific for a hematopoietic lineage-restricted miHA, can induce the phenomenon of “epitope spreading,” in which destruction of host blood cells leads to the release of ubiquitous miHAs and activation of T cells specific for these antigens.¹¹⁵ Thus, a strategy of immunizing DLI donors against hematopoietic lineage-restricted antigens may fail to separate GVHD from GVL unless the antigen-specific T cells are purified to near homogeneity before they are transfused.

Enhancing GVL

The GVL response is a complex, multistep process that involves repeated cycles of activation of donor T cells by antigen on competent antigen-presenting cells (APCs), clonal expansion of the activated T cells, and differentiation of these cells into helper and/or cytotoxic effectors. Major obstacles to a curative GVL response may include the size of the leukemic burden, the inability of some leukemia cells to serve as effective APCs for T-cell activation,¹¹⁶ a low frequency of T cells reactive to host miHAs (as low as 1 in 10⁵ T cells) or leukemia-specific antigens, and an even lower frequency of leukemia-reactive memory T cells, which may be easier to activate than naive T cells. These obstacles suggest the development of the following strategies to enhance the GVL effect of DLI: (1) Reduce the leukemic burden prior to DLI, (2) augment the immunogenicity of leukemia cells, and (3) increase the precursor frequency of leukemia-reactive naïve and/or memory T cells in the DLI, and (4) promote the in vivo clonal expansion of leukemia-reactive T cells.

Reduce the Leukemic Burden Prior to DLI

The use of chemotherapy to induce remissions of acute leukemia prior to DLI has been discussed above. As yet, there is no conclusive evidence that the combination of chemotherapy plus DLI is superior to DLI alone for the induction of long-term remissions of hematologic malignancies in relapse after alloBMT. To the extent that the chemotherapy induces mucositis, it might be expected to exacerbate GVHD, unless the DLI is administered after healing of the gastrointestinal tract. Minimally toxic biologic agents with significant antiactivity are playing an increasing role in the treatment of hematologic malignancies in relapse after alloBMT. These include STI-571 (Gleevec), a *Bcr-Abl*

tyrosine kinase inhibitor for CML, rituximab (Rituxan), a chimeric monoclonal antibody against CD20 for follicular lymphoma, and gemtuzumab ozogamicin (Mylotarg), a toxin-conjugated monoclonal antibody against CD33 for AML. It will be interesting to see whether these compounds enhance disease-free survival when given in conjunction with DLI.

Augment the Immunogenicity of Leukemia Cells

T cells require at least two signals for efficient activation to effector function. Signal 1 is generated by ligation of the T cell's antigen receptor by a complex of the antigenic peptide within the groove of an MHC molecule, and signal 2 is a co-stimulatory signal delivered by the APC. According to the two signal models of T-cell activation,¹¹⁷ T-cell recognition of antigen in the absence of co-stimulation leads to antigen-specific T-cell tolerance. Leukemia cells may lack immunogenicity for donor T cells because of inadequate expression of MHC (signal 1) and/or co-stimulatory molecules (signal 2). Expression of MHC antigens can be upregulated by in vivo administration of pro-inflammatory cytokines such as IFN- γ , but this might also be expected to augment MHC antigen expression on the inducing cells and target tissues of GVHD. In mouse models, T cells may be rendered tolerant of antigens that are generated uniquely by tumor cells,^{118,119} suggesting that defective T-cell co-stimulation rather than insufficient antigen is the culprit behind the failure of an effective antitumor immune response. Leukemia cells have been engineered to express higher levels of co-stimulatory molecules by transfection with the genes for B7-1 and/or B7-2, the ligands for the T-cell co-stimulatory receptor CD28.^{120,121} However, it is worth noting that in at least one model, tolerance of tumor-specific T cells was induced not by the tumor cells themselves, but by host APCs.¹²² Thus, engineering tumor cells to express co-stimulatory molecules may not have the desired effects.¹²³ T-cell co-stimulation is augmented by ligation of CD40 on the surface of an APC, and treatment of follicular lymphoma cells¹²⁴ or B-lineage CLL cells¹²⁵ with agonistic anti-CD40 antibodies enhances their stimulatory capacity in allogeneic mixed lymphocyte reactions. Since in vivo blockade of CD40-CD40L (CD154) interactions prevents the induction of GVHD,¹²⁶ one might expect that in vivo administration of agonistic CD40 antibodies might also exacerbate GVHD after DLI.

Increase the Frequency of Leukemia-Reactive Naive and/or Memory T Cells in the DLI

Even when the recipient's tumor cells are capable of serving as effective APCs, as in chronic phase CML, several cycles of T-cell activation and clonal expansion

may be required before any GVL effects can be manifested. When donor T cells contain a high frequency of memory T cells against tumor-specific antigens, as is the case for EBV-LPD,¹²⁷ the anamnestic response can rapidly eradicate a substantial tumor burden, often within 21 days, and with as few as 10^6 donor T cells/kg.⁵⁹ If CTL lines or clones could be generated against leukemia-specific or hematopoietic lineage-restricted antigens, they could induce rapid antitumor responses against aggressive, rapidly proliferating hematologic malignancies. Warren and colleagues¹²⁸ have utilized T-cell clones obtained from bone marrow transplant recipients to identify 17 distinct human minor H antigens, 12 of which are expressed on hematopoietic lineage cells only. HLA-A*0201-restricted CD8+ T cells specific for the hematopoietic lineage-restricted miHAs, HA-1 and HA-2, have been generated by stimulating donor T cells with autologous dendritic cells pulsed with HA-1 or HA-2 peptides.¹²⁹ Moreover, an miHA-specific CD8+ CTL clone was found to inhibit the engraftment of human myeloid leukemia cells in severe combined immunodeficient (SCID) mice,¹³⁰ raising the possibility that such clones could mediate antitumor effects in humans. Leukemia-specific T-cell lines and clones can be generated by culturing donor T cells with irradiated leukemia cells taken from the recipient prior to transplant, and leukemia-specific CTL lines were used to induce remission in a patient with accelerated phase CML after alloBMT. It is worth noting that, depending on the affinity of the CD8+ T cell for its antigen, CD4+ T-cell helper activity may be required to maintain the persistence of CD8+ T-cell clones in vivo.^{58,131,132} Thus, polyclonal lines containing both CD4+ and CD8+ leukemia-specific T cells may be preferred over T-cell clones for administration to patients with aggressive leukemias in relapse after alloBMT.

The most compelling rationale for the adoptive immunotherapy of cancer is that the responding T cells are taken from healthy donors who have not been exposed to, and should not be immunologically tolerant of, tumor-specific antigens. This raises the possibility of augmenting the GVL effect by vaccinating the BMT or DLI donor with tumor-specific antigens from the patient.¹³³ Unfortunately, with the exception of the immunoglobulin idiotype of multiple myeloma^{134,135} or non-Hodgkin's lymphoma,¹³⁶ tumor-specific antigens are exceedingly difficult to identify. Moreover, cancer cells are capable of evading immune surveillance against a single or small number of tumor-specific epitopes by losing epitope expression through mutation.¹³⁷ These problems are reduced to a minimum by using vaccines that contain the patient's tumor cells, but these cells also express the patient's miHAs, and pretransplant immunization of the marrow donor with recipient cells exacerbates GVHD after alloBMT.^{5,138} Moreover, the

effect of autologous tumor cell vaccines on GVL and GVHD after DLI has not been rigorously examined. Thus, while tumor-specific vaccines represent an alluring strategy for enhancing GVL without exacerbating GVHD, substantial progress in antigen identification and vaccine formulation must occur before the strategy can be applied to the setting of alloBMT.

Promote the In Vivo Clonal Expansion of Leukemia-Reactive T Cells

The factors that control the burst size of T lymphocytes in responding to an antigenic stimulus are, at best, incompletely understood. IL-2 stimulates the proliferation of antigen-activated T cells, and administration of exogenous IL-2 together with DLI has induced remissions in patients who fail to respond to DLI alone.³³ T-cell expansion is also profoundly influenced by a variety of homeostatic mechanisms, some of which evolved to regulate the size of the total T-cell pool. T cells transferred into T-cell-deficient nude mice proliferate substantially more than the same cells transferred into a T-cell-replete host, implying that host T cells actively regulate the expansion of adoptively transferred T lymphocytes independent of their specificity for antigen.¹³⁹ This observation suggests that depletion of host T cells, eg, with cytotoxic chemotherapy or irradiation, may facilitate a GVL effect of DLI by effectively creating "space" for donor T-cell expansion. In apparent confirmation of this hypothesis, tumor-specific transgenic T cells, which could be tracked in vivo, expand significantly more on transfer into tumor-bearing recipients of lethal conditioning and syngeneic BMT than on transfer into tumor-bearing but nontransplanted controls. However, nonspecific depletion of host T cells would also be expected to facilitate GVH reactions, and cyclophosphamide pretreatment of recipients does exacerbate DLI-induced GVHD.¹⁴⁰

A potentially more promising approach to enhancing the expansion of leukemia-reactive T cells comes from the finding that progressively growing tumors induce a population of CD4+/CD25+ T cells that suppress antitumor immunity¹⁴¹ and that depletion of these cells facilitates immune-mediated eradication of tumors.¹⁴² These intriguing results raise the possibility that tumor-induced suppressor T cells can be selectively targeted for elimination, but the effect of this manipulation on GVHD remains to be determined.

Conclusions

DLI is an established therapy of hematologic malignancies in relapse after allogeneic SCT. DLI induces sustained complete remissions in more than 60% of

patients with CML in early-stage relapse but in fewer than 20% of patients with acute leukemia, multiple myeloma, and lymphoma. A reasonable strategy to obtain a molecular remission of chronic phase CML without GVHD is to administer escalating doses of T cells at intervals as required until a remission is achieved. Augmentation of the efficacy of DLI against other diseases should be a main focus of future research.

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