

## REVIEW

# Donor leukocyte infusions for the treatment of relapsed acute leukemia after allogeneic stem cell transplantation

AW Loren and DL Porter

Bone Marrow and Stem Cell Transplant Program, University of Pennsylvania Abramson Cancer Center, Philadelphia, PA, USA

**Allogeneic stem cell transplantation (SCT) offers the only hope for cure for many adults with acute leukemia. Unfortunately, many patients relapse and die of their disease even after transplantation. Although in some cases, allogeneic SCT is effective because the intensive conditioning therapy eradicates all malignant cells, it has long been recognized that the adoptive transfer of donor immunity plays a critically important role in the induction and maintenance of remission. Recognition of the graft-versus-leukemia (GVL) effect of allogeneic SCT has prompted attempts at remission re-induction by adoptive immunotherapy with donor lymphocyte infusions (DLIs) in patients with relapsed disease after allogeneic SCT. In some cases, DLI-induced remissions are sustained and patients cured when no other treatment modality was effective. This review discusses the rationale, biology, complications and future applications of DLI in acute leukemia patients after allogeneic SCT.**

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The theoretical principle of treatment of acute leukaemia by adoptive immunotherapy is to permit allogeneic, immunologically competent cells to act against the host's leukemic cells... (Mathe *et al.*<sup>3</sup>)

### Introduction: the graft-versus-leukemia effect in allogeneic stem cell transplantation

Several classic experiments first identified allogeneic SCT as a successful method of adoptive immunotherapy.<sup>1,2</sup>

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Correspondence: Dr AW Loren, Bone Marrow and Stem Cell Transplant Program, University of Pennsylvania Abramson Cancer Center, 16 Penn Tower, 3400 Spruce Street, Philadelphia, PA 19104, USA.

E-mail: alison.loren@uphs.upenn.edu

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Leukemic mice transplanted with non-identical marrow grafts developed an 'immune reaction' with a 'secondary syndrome' (now recognized as graft-versus-host disease, GVHD) that was protective against relapse. This GVL effect was the rationale for some of the first human bone marrow transplants.<sup>3</sup> Several retrospective studies provided indirect evidence for GVL activity in humans (Table 1).

For instance, Weiden *et al.*<sup>4</sup> observed that patients who developed GVHD after allogeneic SCT were 2.5 times less likely to suffer leukemic relapse than those without GVHD. Subsequent registry analyses confirmed that GVHD is protective against relapse and also showed that syngeneic donors increased relapse risks.<sup>5,6</sup> Additionally, T cell depletion of the allograft increases the risk of relapse (most markedly in patients with CML), suggesting that donor T cells are important effectors of the GVL response.<sup>5</sup>

Given the importance of GVL activity, the effects of DLI were tested in patients with relapsed disease after SCT. One of the earliest reports in 1990 provided indisputable evidence for a direct and potent GVL effect associated with DLI in three patients with CML.<sup>7</sup> Subsequently, numerous studies have confirmed that DLI induces complete remissions in 70–80% of patients with relapsed CML in chronic phase,<sup>8–11</sup> the majority of which are durable molecular remissions.<sup>12,13</sup>

Unfortunately, DLI has been less effective in treating diseases other than CML. It is not known why DLI has disease specificity. Possible explanations for lack of response to DLI could include lack of expression of recognizable tumor-specific antigens, lack of co-stimulatory molecules on malignant cells, inhibition of T cell activation or function, weak tumor cell killing, or tumor burdens and/or growth patterns that out pace the GVL effect. In fact one of the major limitations of immunotherapy is its relatively slow onset of action. After donor cell infusion, the T cells must recognize antigen, become activated, proliferate to a critical number of effectors and target the malignant cells before cell killing can occur. In CML patients receiving DLI, an average of 40 days was required to see a response, and some responses occurred as late as 10–12 months post-DLI. Unfortunately, patients with relapsed acute leukemia may progress rapidly and die before a GVL effect is evident. Thus, rapidly growing diseases may appear to be less responsive to immunotherapy, although, in fact, it is possible that these

diseases simply outpace the ability of the T cells to control them.

### DLI for AML

Despite promising results with CML, the use of DLI for AML has been disappointing. GVL activity is weak after conventional SCT for patients with AML,<sup>5,6</sup> and thus it is not surprising that DLI for relapsed AML after transplant has not been efficacious. Several early case series do describe occasional remissions after DLI for relapsed AML.<sup>14–16</sup> In multicenter retrospective analyses, complete response rates for recipients of DLI alone for relapsed AML were 15–29% (Table 2).<sup>9,10</sup> Unfortunately, many of these remissions were not durable. In one study with long-term follow-up, 4 of the 15 patients who achieved remission relapsed shortly after therapy; event-free survival was only 31% at 3 years.<sup>12</sup> Interestingly, there are a surprisingly large number of patients whose post-DLI relapses are characterized by extramedullary disease in the absence of marrow involvement.<sup>21</sup> This observation suggests that AML can recur either in a sanctuary site or in a clone of

cells that possess a surface complement of proteins that escapes immunologic recognition or attack,<sup>18,22–24</sup> and also calls into question the activity of GVL in the central nervous system.

Because of the latency of the GVL effect, more recent trials studying patients with acute leukemias have administered DLI in association with chemotherapy, either as consolidation after achieving a remission or during a chemotherapy-induced nadir. Two illustrative prospective trials have been reported that include patients with relapsed advanced myeloid malignancies (AML, accelerated or blast phase CML, or advanced myelodysplastic syndrome (MDS))<sup>18,20</sup> who were treated with induction chemotherapy, followed 7–14 days later by a defined dose of G-CSF mobilized peripheral blood mononuclear cells as the source of DLI. Results in these two studies were similar: complete remissions were achieved in 47% (27/57) and 63% (10/16) patients respectively (Figure 1). Overall survival was 19 and 31% at 2 years. However, several important findings should be highlighted. For patients who achieved complete remission after chemotherapy and DLI, 1- and 2-year survival rates were approximately 50 and 40%, respectively, compared to a 1-year survival of 0–5% in non-responders. Therefore, remission induction is an important predictor of long-term outcomes. For patients whose disease can be controlled, there does appear to be a potent antileukemic effect of DLI. The most important predictor of survival was time from transplant to relapse; 1-year survival for patients relapsing more than 6 months after SCT was about 50% compared to 0–10% for patients relapsing less than 6 months from transplant (Figure 2).<sup>18,20</sup> Thus, for the group of patients who relapse within 6 months after SCT, novel manipulations of DLI or other therapy should be considered.

### DLI for ALL

The existence of a GVL effect in ALL is well established.<sup>5,6</sup> In fact, one of the first recipients of DLI was a child

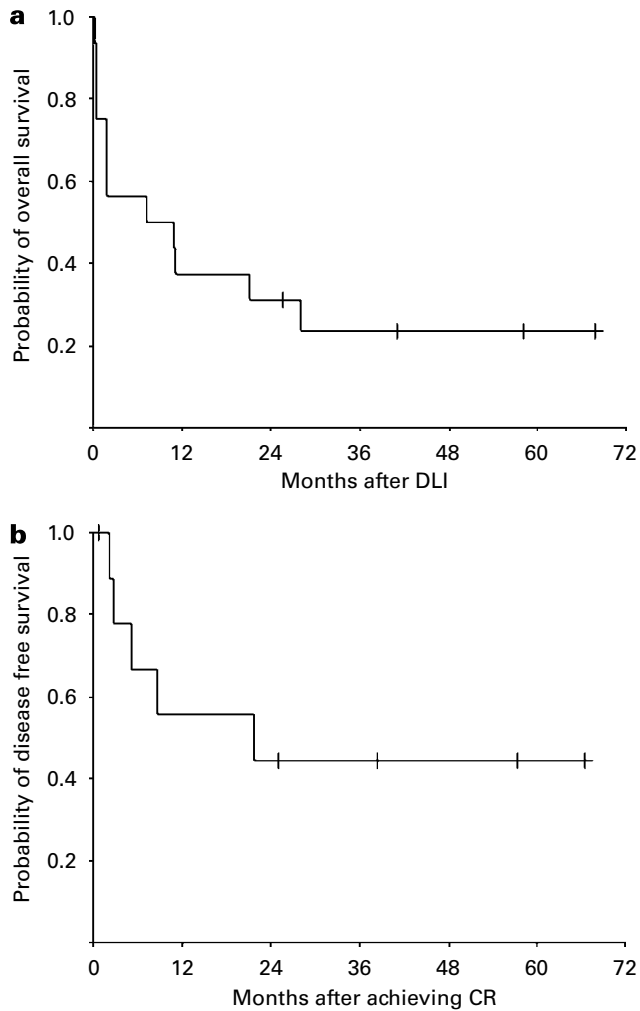
**Table 1** Evidence of a graft-versus-leukemia effect in clinical transplantation

- Anecdotal reports describe patients with relapsed leukemia after allogeneic stem cell transplantation who achieve complete remission with a flare of graft-versus-host disease or after withdrawal of immunosuppression
- Relapse rates are lowest in patients who develop graft-versus-host disease after allogeneic stem cell transplantation
- Relapse rates are higher after syngeneic stem cell transplantation when compared to matched sibling stem cell transplantation
- T-cell depletion of the donor graft as graft-versus-host disease prophylaxis results in increased relapse rates, especially for patients with CML
- Donor lymphocyte infusions can re-induce sustained complete remissions for patients with relapsed leukemia after allogeneic stem cell transplantation

**Table 2** Selected trials of DLI, with or without chemotherapy, for relapsed AML and ALL

Study	Treatment	AML (n)	AML outcomes	ALL (n)	ALL outcomes
Collins <i>et al.</i> <sup>9</sup>	DLI alone	46	6/39 (15%) CR	15	2/11 (18%) CR
Collins <i>et al.</i> <sup>9</sup>	Pre-DLI chemotherapy	7	4/7 (57%) DFS	4	1/4 (25%) DFS
Kolb <i>et al.</i> <sup>10</sup>	DLI alone	19	5/17 (29%) CR	22	0/12 (0%) CR
Kolb <i>et al.</i> <sup>10</sup>	Pre-DLI chemotherapy	8	4/8 (50%) CR	17	9/17 (53%) CR
Shiobara <i>et al.</i> <sup>17</sup>	DLI alone	21	2/4 (50%) DFS at 2 years 8/21 (38%) Resp 7% DFS at 2 years	23	3/9 (33%) DFS at 2 years 6/23 (25%) CR 5% DFS at 2 years
Choi <i>et al.</i> <sup>18</sup>	Pre-DLI chemotherapy	16	10/16 (63%) CR 31% DFS at 2 years		
Choi <i>et al.</i> <sup>18</sup>	Pre-DLI chemotherapy			10	7/10 (70%) CR 1/7 (14%) DFS
Collins <i>et al.</i> <sup>19</sup>	Pre-DLI chemotherapy			29	13/29 (45%) CR 1/13 (8%) DFS
Collins <i>et al.</i> <sup>19</sup>	DLI alone			15	2/15 (13%) CR
Levine <i>et al.</i> <sup>20</sup>	Pre-DLI chemotherapy	65	27/57 (47%) CR 19% DFS at 2 years		

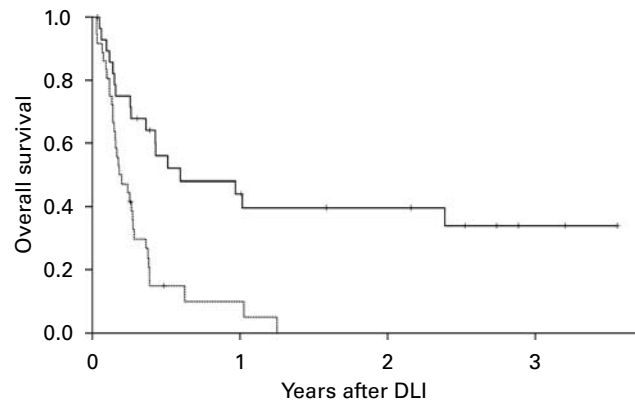
Abbreviations: DFS = disease-free survival; DLI = donor lymphocyte infusion; Resp = response.



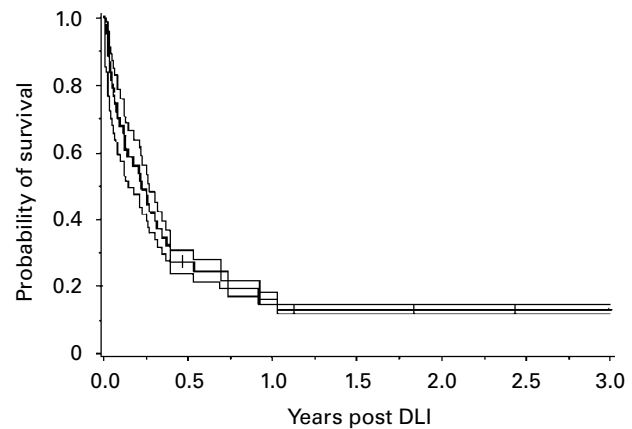
**Figure 1** (a) Probability of overall survival for 16 recipients of chemotherapy and DLI for relapsed AML. (b) Probability of disease-free survival for 10 of the 16 patients above who achieved complete remission after chemotherapy and DLI.<sup>18</sup> Reprinted from Choi *et al.*<sup>18</sup>

with relapsed ALL,<sup>15</sup> who remains in remission 15 years following the infusion.<sup>25</sup> However, in general, DLI for ALL has been disappointing. The European Group for Blood and Marrow Transplantation reported that no patient with relapsed ALL achieved remission from DLI alone and the median survival was less than 6 months after DLI; in addition, all patients who received chemotherapy or were in CR relapsed a median of 15 months after treatment.<sup>10</sup>

Similarly, outcomes in retrospective<sup>19</sup> and prospective<sup>18</sup> studies have been poor. Collins *et al.*<sup>19</sup> provided a combined analysis of retrospectively ( $n=27$ ) and prospectively treated ( $n=17$ ) patients culled from 27 centers. Fifteen patients received no pre-DLI chemotherapy, while the remainder were treated primarily with a vincristine/prednisone-based regimen and received DLI at their nadir. Sixteen patients received GCSF-mobilized donor products, 12 patients did not and this information was not available for the remainder. Patients had uniformly poor outcomes, and pre-DLI chemotherapy did not seem to impact on survival. Although GVHD was frequent, long-term remis-



**Figure 2** Overall survival after chemotherapy and DLI for relapsed advanced myeloid malignancy. Overall survival is dependent on time from transplant to relapse; the estimated 2-year survival for patients whose time to relapse was >6 months (solid line) is 44% at 1 year compared to 10% at 1 year for patients relapsing within 6 months of transplant.<sup>20</sup>



**Figure 3** Actuarial survival with 95% confidence intervals in 44 patients with relapsed ALL patients treated with DLI.<sup>19</sup> Reprinted from Collins *et al.*<sup>19</sup>

sion was not, and overall survival was 13% at 3 years (Figure 3). Only three patients were long-term disease-free survivors and 26/44 died of disease-related complications. More recently, a prospective trial using idarubicin-based chemotherapy followed by GCSF-stimulated DLI at nadir in 10 patients with relapsed ALL also resulted in the majority of patients developing GVHD.<sup>26</sup> Only one patient was a long-term leukemia-free survivor.

It has been speculated that failure of DLI for ALL may be due to induction of T-cell anergy by ALL cells<sup>27</sup> or their inadequate expression of important co-stimulatory molecules or adhesion molecules.<sup>28,29</sup> Likewise, ALL resistance to killing by natural killer cells has also been reported.<sup>30</sup> Ruggeri *et al.*<sup>30</sup> found a 0% probability of relapse by 5 years for 20 AML patients whose donors had antirecipient natural killer cell clones following haploidentical T cell depleted transplantation. By contrast, the probability of relapse at 5 years for 14 ALL patients whose donors had antirecipient natural killer cell clones was 85%. These

findings highlight the difficulty in generating effective strategies to employ immunotherapy against relapsed ALL.

### DLI after non-myeloablative SCT

Nonmyeloablative allogeneic SCT (NST) seeks to exploit the potent GVL effect after allogeneic SCT while minimizing conditioning regimen toxicity. However, relapse rates tend to be high due to patient selection and the reduced intensity conditioning therapy, or in some cases the use of serotherapy (for example, alemtuzumab or antithymocyte globulin) resulting in *in vivo* T-cell depletion.<sup>31,32</sup> In these attenuated conditioning regimens, DLI has become increasingly important to maximize the GVL effect.<sup>32–37</sup> In addition, DLI is typically employed after NST when complete lymphohematopoietic chimerism is not achieved.<sup>33,38,39</sup> Conversion to full chimerism with DLI has occurred in approximately 35% of cases.<sup>40,41</sup> Unfortunately, there are notably few reports of successful DLI for relapsed acute leukemia after NST, though in some cases complete remissions have been achieved.<sup>40,41</sup> Since DLI in acute leukemia may be most effective for patients with minimal disease burdens, the role of prophylactic DLI after NST is being studied.<sup>36,37,42</sup> At doses of approximately  $1–10 \times 10^6$  CD3<sup>+</sup> cells/kg, recipients of prophylactic DLI appear more likely to achieve full chimerism and high response rates, though GVHD was a common complication. In one report using  $1–5 \times 10^6$  CD3 cells/kg 120 days after NST, 2 year overall survival was estimated at 40% for 75 patients with high-risk AML and MDS.<sup>36</sup> Interestingly, there was no difference between patients with favorable and unfavorable disease characteristics.

### Unrelated donor lymphocyte infusions

DLIs are being used with increasing frequency after unrelated donor SCT, though there are less data regarding anticipated outcomes after unrelated DLI (UDLI) than there are after related donor DLI (reviewed in Loren and Porter<sup>43</sup>). There has been concern that UDLI would carry an increased risk of significant GVHD (due to the higher degree of minor histocompatibility antigen disparity between recipient and donor) and it is largely unknown if UDLI can provide similar, less or even enhanced GVL activity compared to matched related DLI.

A number of studies have included recipients of UDLI,<sup>9,10,13,19,44–46</sup> but in most cases specific outcomes after UDLI compared to sibling DLI have not been

provided. The two large registry studies suggested that UDLI and related DLI result in similar outcomes but included small number of patients,<sup>9,10</sup> and the other study using DLI for relapsed CML found no significant difference in outcomes using matched sibling or unrelated donor DLI.<sup>47</sup>

There has been a retrospective analysis of recipients of UDLI identified through the National Marrow Donor Program database that included 23 patients with relapsed AML and 7 patients with relapsed ALL.<sup>48</sup> The median UDLI cell dose administered was  $1.0 \times 10^8$  mononuclear cells (MNC)/kg and 21% of patients were HLA-mismatched. Outcomes are summarized in Table 3. Notably, complete remissions were achieved in 42% of patients with AML, and in 2 of 4 evaluable patients with ALL. The incidence of grade II–IV acute GVHD was 25% and chronic GVHD occurred in 41% of patients, both in keeping with data from large series of related DLI. The estimated probability of disease-free survival at 1 year after CR was 23% for AML and 30% for ALL. There was no association of mononuclear cell dose with GVHD, response, survival or disease-free survival. Only a longer time interval from transplant to relapse and transplant to UDLI was associated with improved survival and disease-free survival respectively. There was also no obvious association between acute and chronic GVHD and disease response, in contrast to several,<sup>9,10,49</sup> though not all,<sup>50</sup> related DLI studies.

It should be noted that comparisons of UDLI to related DLI for patients with acute leukemia are difficult, largely because of the small numbers of patients studied, the relatively short follow-up in most studies and the retrospective nature of the data. Nevertheless, the complete remission rates for recipients of UDLI for acute leukemia appeared at least similar if not higher than rates reported in related DLI studies.<sup>9,10</sup> Given these data, and the generally accepted poor outcome after relapse from unrelated donor SCT, UDLI appears to be an appropriate approach for patients who relapse with leukemia after unrelated donor marrow grafting.

### DLI: composition and dose

#### Cellular components

Recent evidence suggests that cells other than T cells—both donor and host derived—are important for both GVL and GVHD, and thus it is not surprising that the cellular composition of the product influences the activity of DLI. Most commonly, the DLI product is obtained by leuka-

**Table 3** Response and toxicity after unrelated donor leukocyte infusion for treatment of relapsed acute leukemia<sup>47</sup>

Indication	N	CR (%)	DFS (%)	aGVHD (%) grade II–IV	cGVHD (%) extensive	Aplasia (%)
AML	23	42	23	35	40	8
ALL	4	50	30	67	40	0

Abbreviations: aGVHD = acute graft-versus-host disease; ALL = acute lymphocytic leukemia; AML = acute myelogenous leukemia; cGVHD = chronic graft-versus-host disease; CR = complete response; DFS = disease-free survival; N = number of patients.

pheresis of unstimulated peripheral blood, containing approximately  $2 \times 10^8$  mononuclear cells, of which about 50% are CD3+ cells, but also includes B-cells, dendritic cells and others.<sup>51</sup> Since the precise effector of GVL is unknown, unfractionated blood mononuclear cell infusions have the benefit of providing a spectrum of alloreactive and other immune accessory cells. This may increase the likelihood of a response although it is reproducibly associated with significant toxicity from GVHD.

An ideal cellular product would maximize GVL and minimize GVHD. CD4+ cells may be most critical for GVL induction without GVHD, and CD4+ -enriched DLI has been effective in treating patients with relapsed CML and myeloma with minimal GVHD.<sup>52</sup> A subsequent small, randomized trial of prophylactic CD4+ -enriched versus unmanipulated DLI in patients with AML/MDS who had previously received T-cell depleted transplants showed improved leukemia-free survival and less GVHD in recipients of the CD4+ -enriched products compared to recipients of unmanipulated DLI.<sup>53</sup>

Another controversial aspect of DLI composition is the effect of G-CSF priming of the donor prior to apheresis. Because marrow aplasia is a rare complication of DLI, it was logical to assume that increasing the stem cell component of the product reduces this risk. However, a small comparative study from the Seattle group failed to demonstrate a difference in incidence of aplasia using G-CSF-mobilized DLI.<sup>44</sup> More recently, there has been growing interest in the effects of G-CSF on T cell subsets and dendritic cells, spurred in part by retrospective studies and a meta-analysis,<sup>54–56</sup> demonstrating an increased risk of chronic GVHD and perhaps an improved survival rate in recipients of G-CSF-mobilized peripheral blood stem cells compared to bone marrow grafts. In addition to a marked increase in CD34+ stem cells, G-CSF priming in normal donors also increases the yield of CD3+ cells by almost threefold,<sup>51</sup> and has important effects on cytokine expression including increased levels of IL-10, transforming growth factor- $\beta$  and IFN- $\alpha$ .<sup>57</sup> This change in cytokine profile after G-CSF promotes Th2-type differentiation, increases T<sub>regs</sub> and expands immature APCs, all of which may reduce the incidence of acute GVHD. Hypotheses to explain the observation that peripheral blood stem cell grafts may result in increased chronic GVHD include the thought that the same cytokines that favor Th2 differentiation may also increase the fibrosis frequently seen as a component of chronic GVHD<sup>57</sup> and that there is a threshold effect such that higher number of T cells in the G-CSF-stimulated graft may increase the risk of chronic GVHD.<sup>58</sup> Whether G-CSF-mobilized DLI will affect GVL induction remains to be determined.

#### Cell dose

It is difficult to interpret data regarding dosing of DLI. Studies may report the dose as total mononuclear cells, total nucleated cells or total CD3+ cells. Even comparing similar cellular products is difficult because the range of DLI dose varies greatly even within individual reports. Most trials use  $1–3 \times 10^8$  MNC/kg though the range may vary between 0.1 and  $15 \times 10^8$  MNC/kg. Most of these

trials include too few patients to study appropriately the relationship between dose and outcome for acute leukemia.

However the relationship between cell dose and GVHD has been best studied in patients with relapsed CML. When DLI was given at a low cell dose ( $1–10 \times 10^6$  CD3 cells/kg), followed by dose escalation if necessary, complete remissions were ultimately achieved in 86% of patients and only one patient developed acute GVHD.<sup>50</sup> Thus, although T cell doses of less than  $1 \times 10^7$  per kg of recipient body weight are unlikely to induce GVL, a dose escalation strategy clearly limits GVHD. As might be anticipated, patients with molecular and cytogenetic relapses of CML were more likely to respond to lower T cell doses, while patients with frank hematologic relapse required sequentially higher cell doses. It should be emphasized, however, that this strategy may take 6–12 months, and thus is only practical for patients with indolent diseases. Patients with acute leukemias experience rapid disease progression before an effective GVL response can be generated. A dose-escalation approach for acute leukemia would seem feasible only for patients with minimal disease burdens (such as high-risk patients achieving remission after SCT).

From available data, no obvious dose–response relationship has been identified for diseases other than CML.<sup>9,10,14,20,48</sup> Similarly, there appears to be no relationship between DLI dose and GVHD.<sup>9,48</sup> Most studies include patients who received a relatively high dose of donor cells, and it is possible that in these cases the T cell dose was already above a threshold for either GVHD or GVL. In one Japanese study,<sup>59</sup> GVHD was more frequent with nucleated cells doses  $> 5 \times 10^7$ /kg (and no recipient of  $< 7 \times 10^7$  cells/kg developed lethal GVHD), though there was no such relationship identified between cell dose and GVL. These authors suggest a starting dose of  $1 \times 10^7$  T cells/kg. However, the incidence of GVHD in this population may not be generalizable. As noted in Figure 3, separating GVHD and GVL continues to be a challenge in the majority of cases. Although many investigators use a lower dose for UDLI than for matched sibling DLI, limited retrospective data have identified neither a higher risk of GVHD for UDLI nor a relationship between UDLI dose and either response or toxicity.<sup>48</sup>

## Complications of DLI

### *Graft-versus-host disease*

The most significant and common complication after DLI is acute and chronic GVHD, developing in 40–60% of evaluable patients. It is important to note that in most studies of DLI, GVHD correlates with GVL response. For instance, in a North American analysis, over 90% of complete responders developed acute GVHD and 88% of responders developed chronic GVHD. Of 23 patients who did not experience GVHD, only three achieved a complete remission.<sup>9</sup> In some cases, DLI-induced GVHD may be quite severe: 20–35% of DLI recipients can be anticipated to develop grade III–IV acute GVHD. Furthermore, acute GVHD contributed to death in almost 10% of patients.<sup>60</sup> Interestingly, DLI from an unrelated donor carries the same risk of GVHD as related donors.<sup>48</sup>

Although the target organs of acute GVHD after DLI are the same as those after SCT, the clinical manifestations may differ. A hepatic variant of liver GVHD characterized by marked elevations of serum aminotransferase levels was observed in 15% patients who received DLI at Johns Hopkins University.<sup>61</sup> Typical skin, liver and intestinal acute GVHD manifestations also occur after DLI; in one report of 81 DLI recipients following reduced intensity allogeneic SCT, skin, liver and intestinal GVHD developed in 26, 8 and 14%, respectively.<sup>62</sup> Likewise, typical findings of chronic GVHD can be seen following DLI<sup>62</sup> and on occasion both acute and chronic GVHD can develop simultaneously following DLI.<sup>52</sup>

### Marrow aplasia

Pancytopenia has been reported in 18–50% of recipients of DLI,<sup>9,10</sup> but sustained marrow aplasia occurs in only 2–5% of patients. In the treatment of relapsed acute leukemias, chemotherapy is frequently used prior to DLI, making it difficult to attribute the pancytopenia to chemotherapy, disease or the DLI itself.<sup>63,64</sup> The mechanism of pancytopenia after DLI is poorly understood. One hypothesis is that DLI destroys residual host hematopoiesis prior to recovery of donor hematopoiesis. Indeed, lack of residual donor chimerism in the CD34+ cell pool is predictive of marrow aplasia after DLI.<sup>65</sup> However, mechanisms other than destruction of host hematopoiesis may be responsible for pancytopenia, because the use of CD34+ cell-enriched donor cells as the source of DLI does not seem to prevent pancytopenia.<sup>44</sup> Marrow aplasia may also be a manifestation of GVHD, analogous to transfusion-associated GVHD. Sometimes engraftment studies can differentiate marrow GVHD from hematopoietic failure, but it can be very challenging to separate these two etiologies. Marrow aplasia may be reversed successfully with infusion of additional donor stem cells,<sup>8,11</sup> particularly if due to marrow failure. However, in most cases, pancytopenia after DLI resolves without therapy.

### Future directions

Although DLI is a highly effective salvage strategy for relapsed CML after allogeneic SCT, there is a clear need for improvement in the acute leukemias. Many strategies are under active investigation to try to augment the efficacy of DLI for these and other diseases (Table 4).

One approach taken at the University of Pennsylvania involves the *ex vivo* expansion and activation through co-stimulation of donor T cells for GVL induction. The hypothesis that drives these studies is that donor T cells may not be appropriately activated *in vivo* to induce an antitumor response—perhaps due to  $T_{regs}$  or suppressive cytokines in the product, lack of host APCs to present antigen, lack of co-stimulatory ligands on tumor cells or insufficient numbers of cytotoxic effector cells. To overcome these obstacles, T cells are expanded *ex vivo* and co-stimulated via exposure to magnetic beads coated with anti-CD3 and anti-CD28. This is expected to enhance the antitumor potential of donor T cells.<sup>66</sup> In the setting of

**Table 4** Newer approaches to donor leukocyte infusions

- *Ex vivo* activation and expansion of donor T cells through co-stimulation
- Generation and infusion of tumor-specific T cells
- Generation and infusion of minor histocompatibility antigen-specific T cells
- Low-dose DLI followed by dose escalation
- Infusion of selected T cell subsets (that is, after CD8+ cell depletion or CD4+ cell selection)
- Inactivate alloreactive T cells (that is, through transduction of suicide genes into donor T cells, photochemical inactivation, chemotherapy inactivation, irradiation)
- Infusion of T regulatory cells
- Generation and infusion of Th2-type T cells
- Manipulation of antigen presenting cells to maximize GVL or minimize GVHD

Abbreviations: DLI = donor lymphocyte infusion; GVL = graft-versus-leukemia; GVHD = graft-versus-host disease.

autologous SCT, administration of *ex vivo* co-stimulated T cells can reverse both *in vivo* and *in vitro* functional T cell defects in patients with non-Hodgkin's lymphoma,<sup>67</sup> and can enhance immune reconstitution and response to vaccinations in patients with myeloma.<sup>68</sup> In a phase I trial, conventional DLI was followed by escalating doses of *ex vivo* co-stimulated donor T cells (known as activated DLI or aDLI) for relapsed acute leukemias (ALL,  $n=7$ ; AML,  $n=4$ ), CML in blast phase ( $n=1$ ), CLL ( $n=1$ ), non-Hodgkin's lymphoma ( $n=2$ ), Hodgkin's disease ( $n=1$ ), myeloma ( $n=1$ ) and lymphoblastic lymphoma ( $n=1$ ).<sup>69</sup> Eight of 17 evaluable patients achieved a complete remission including 2 patients with AML, 4 patients with ALL, 1 patient with CLL and 1 patient with NHL (mantle cell lymphoma). Although four complete responders subsequently relapsed, four were alive in remission a median of 23 months after aDLI including two with acute leukemia. Activated DLI did not result in excessive toxicity: five patients developed grade I–II acute GVHD, two patients developed grade III acute GVHD and four patients developed chronic GVHD. No patients died of complications related to GVHD. Overall, the response rates were impressive in diseases that historically have not responded well to unmanipulated DLI, suggesting that aDLI may offer an advantage for GVL induction. Future studies with aDLI will include further dose escalation, repetitive dosing of aDLI to minimize late recurrences, and attempts at activation and expansion of tumor-specific T cells.

Ultimately, the use of tumor-specific DLI is likely to be the most effective method of adoptive immunotherapy to maximize GVL activity with limited toxicity from GVHD. Unfortunately, in the majority of circumstances, the target antigens for GVL induction are not known. Furthermore, malignant stem cells appear to differ in their susceptibility to immunologic control. Reasons for this relative resistance to immunotherapy may include inadequate cell surface presentation of molecules that can be recognized by the donor T cells, failure to express co-stimulatory molecules,<sup>70</sup> sanctuary sites, a rapid proliferative rate of the malignancy or other mechanisms.<sup>71</sup> Furthermore, there may also be an

important component of humoral immunity that must develop.<sup>72,73</sup>

The actual target for a GVL reaction may be leukemia-specific<sup>74,75</sup> or possibly directed against minor histocompatibility antigens differentially expressed on hematopoietic cells.<sup>76</sup> The strongest support of the former is that GVL may exist in the absence of clinical GVHD.

It is attractive to hypothesize that hematopoietic-specific minor histocompatibility antigens (mHags) are targets for tumor-specific DLI. CTLs have been generated against mismatched mHags that demonstrate leukemia-specific cell lysis *in vitro*.<sup>77,78</sup> In three recipients of DLI for relapsed CML, an increase in mHag HA-1 and HA-2-specific CD8+ T cells was noted.<sup>78</sup> Unfortunately, only a small minority of patients will have polymorphic differences with their HLA-matched donor for HA-1 and HA-2 mHags, restricting the potential use of these targets to limited number of patients.

Other minor histocompatibility antigens may be critical and may be the same antigens that are the targets of GVHD. This might explain why in most (though not all) studies, GVHD is strongly associated with GVL. An excellent example of this effect is observed when female donors are used for male recipients. Y-chromosome encoded proteins are some of the best-studied mHags. Since they are unique to the recipient, they can function as mediators of both GVL and GVHD which can be quite potent.<sup>72,73,79</sup> It is likely that in cases with GVL but without demonstrable GVHD, the effect is still mediated by the same antigens but in a subclinical fashion.

Another potential target antigen for patients with myeloid malignancies is proteinase 3, which is the target of autoimmunity in Wegener's granulomatosis and is overexpressed in myeloid leukemias. Cytotoxic T cells specific for proteinase 3 and the HLA-A2 restricted proteinase 3 peptide PR1 have been found to lyse selectively CML cells *in vitro*.<sup>80</sup> A strong correlation has been identified between PR1-specific CTLs and clinical response after interferon treatment and allogeneic BMT.<sup>81</sup> Therefore, one can envision unique vaccination strategies using proteinase 3,<sup>82</sup> but these findings also raise the possibility of using PR1-specific T cells for selective adoptive immunotherapy.

A similar role has been hypothesized for the Wilm's tumor protein, WT1. WT1 is an endogenous host protein that is overexpressed in blast phase CML as well as in a majority of patients with AML and MDS.<sup>83,84</sup> WT1 could serve as a potential myeloid leukemia tumor antigen. Vaccine strategies using WT1 have been explored in animal models (reviewed in Rosenfeld *et al.*<sup>83</sup>), and it is logical to begin testing WT1-specific T cells generated and expanded *in vitro* as tumor-specific DLI. Other potential tumor associate antigens that may serve as targets for immunotherapy include NY-ESO-1, a cancer-testis antigen found also preferentially on myeloma cells,<sup>85</sup> and the melanoma-associate antigen PRAME, an HLA-24 restricted antigen on AML cells.<sup>86</sup>

Generation of tumor-specific DLI can be quite laborious, and at least presently, this technology is confined to a limited number of research laboratories. Nevertheless, donor T cells with tumor specificity have been identified,

and expanded in some cases. Exciting preliminary data suggest that they may be useful as adoptive immunotherapy. The feasibility of this approach was first demonstrated by Falkenburg *et al.*<sup>87</sup> Donor T cells reactive against recipient CML mononuclear cells were isolated and expanded in culture and used successfully to induce remission in a patient with relapsed accelerated phase CML; notably this patient had not responded to conventional DLI. Although not yet used clinically, Montagna *et al.*<sup>88</sup> have shown that it is possible to generate leukemia-specific cytotoxic T cells from 10/11 patient/donor pairs using donor-derived dendritic cells pulsed with blasts from patients with leukemia and MDS. These principles are also being applied to solid tumors with tumor-infiltrating lymphocytes such as melanoma<sup>89</sup> and renal cell carcinoma.<sup>90</sup> As the target antigens and effector cells for GVL induction become better characterized, new techniques for cell selection and expansion will allow tumor-specific adoptive immunotherapy to become reality.

Alternative strategies to minimize GVHD after DLI include methods to inactivate alloreactive T cells, such as genetically engineering them to contain a suicide gene. Donor lymphocytes transduced with the herpes simplex thymidine kinase (*HSV-TK*) gene are sensitive to treatment with ganciclovir.<sup>91,92</sup> *HSV-TK*-modified T cells have successfully induced complete remissions in patients with both relapsed leukemia and EBV-associated post-transplant lymphoproliferative disorder (PTLD). Acute GVHD after DLI was treated with ganciclovir, which resulted in a decrease in the number and activity of alloreactive cells as well as a decrease in the number of cells containing the *HSV-TK* gene. This strategy is now being tested in larger clinical trials using DLI to treat relapsed disease after allogeneic BMT.

Recent investigations have focused on the regulatory subset of CD4+ T cells, hypothesizing that these cells are important in suppressing anti-host immune responses.<sup>93</sup> These findings have been confirmed in animal models, which have shown that donor-derived CD4+ CD25+ immunoregulatory T cells are educated in the host thymus and induce tolerance with a concomitant reduction in GVHD.<sup>94,95</sup> The role of T<sub>reg</sub> infusions as DLI remains to be determined.

To modulate GVHD after NST, Fowler *et al.*<sup>96</sup> infused *ex vivo*-expanded Th2 cells. They found that Th2 cell infusions resulted in enhanced lymphocyte recovery without an apparent increase in GVHD. It is not known if these cells possess GVT activity or might function only to limit GVHD without inhibiting GVT effects of other effector cells.

Investigations into the complementary roles of both host and donor dendritic cells are ongoing. It appears that host APCs are critical in initiating alloimmunity (both GVHD and GVL effects) and that there is a dynamic relationship between host APCs and donor T<sub>regs</sub>, which mediates both phenomena.<sup>97</sup> In animal models with complete donor chimerism, there was no GVL effect observed, whereas mixed chimeras with detectable host APCs did experience GVL.<sup>98</sup> Donor APCs also appear to play an important role in GVHD.<sup>99,100</sup> Thus, manipulations of dendritic cells both in the graft and in the recipient may be important as well.

## Summary

The fact that donor leukocyte infusions can cure leukemia is a powerful and direct demonstration that GVL effects exist, and that adoptive immunotherapy is a potent treatment for patients with leukemia. Although highly active in patients with CML who relapse after allogeneic SCT with molecular, cytogenetic or chronic phase disease, the effectiveness of DLI in patients with more aggressive diseases such as blast phase CML or acute leukemia remains disappointing. However, great progress has been made in understanding the cellular and cytokine effector mechanisms of DLI and in exploring ways to manipulate the graft itself via both nonspecific *ex vivo* activation and by tumor-specific culture. Creative alteration of the components of the graft and/or of the host cells offers opportunities to achieve the ultimate goal of improving GVL and minimizing GVHD. The population of patients eligible for allogeneic SCT is continually growing, particularly because of the increasing number of patients who are candidates for non-myeloablative transplants. These transplants are associated with an even higher risk of relapse and graft failure. Thus, there is an ever-increasing need to study and improve on DLI for acute leukemia. Future work should focus on unifying the definition of the components and cell doses of DLI, defining a dose/efficacy and a dose/toxicity relationship, and developing ways to improve the efficacy of DLI either via graft manipulation or host conditioning. Most importantly, all patients in whom DLI is planned for leukemic relapse after allogeneic SCT should be enrolled in a clinical trial to maximize our understanding of this powerful immunotherapy.

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## Conflict of interest

The authors have no conflicts of interest to declare.

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