Disease relapse after haematopoietic stem cell transplantation: Risk factors and treatment

F. Dazzi* MD, PhD
Professor of Stem Cell Biology

C. Fozza MD
Clinical Research Fellow
Department of Haematology, Imperial College at Hammersmith Hospital, Du Cane Road, London W12 0NN, UK

Disease relapse is the commonest cause of treatment failure after allogeneic haematopoietic stem-cell transplantation. Adoptive immunotherapy based on donor lymphocyte infusions (DLI) has a prominent role in the management of disease recurrence. Although the highest remission rates are achieved in chronic myeloid leukaemia (CML), encouraging results have also been reported in chronic lymphoproliferative disorders. However, the experience of DLI in CML is not necessarily applicable to the management of lymphoproliferative diseases because of the heterogeneity of the conditioning regimens used in chronic lymphoid malignancies. We will review the role of DLI for different disease types in the context of conventional and reduced-intensity conditioning regimens. The factors influencing response and graft-versus-host disease as well as the optimal cell dose will be discussed. Finally, we will describe the main avenues currently being explored to improve the selectivity and efficacy of DLI.

Key words: allogeneic haematopoietic stem-cell transplantation; relapse; adoptive immunotherapy; donor lymphocyte infusions.

RELAPSE AFTER ALLOGENEIC HAEMATOPOIETIC STEM-CELL TRANSPLANTATION

Disease relapse is by far the commonest cause of treatment failure after allogeneic haematopoietic stem-cell transplantation (SCT) and, regardless of the type of transplant, it is a predictor for worse outcome.1,2 The relapse rate progressively decreases after transplant. Not considering the disease type, 82% of patients who are disease-free at 2 years after allografting are still in complete remission at 9 years.3 Even though

* Corresponding author. Tel.: +44 208 383 2134; Fax: +44 208 742 9335.
E-mail address: f.dazzi@imperial.ac.uk (F. Dazzi).
the probability of relapse is linked to several transplant-related variables, the most important risk factor is probably the disease type, with the lowest probability of relapse (10–30%) for patients with acute leukaemia in first remission or chronic myeloid leukaemia (CML) in first chronic phase. The incidence of disease recurrence becomes much higher in patients in second or later remission (40–50%) and in those with advanced disease (more than 70%). Regarding autologous SCT, the most consolidated experience has been achieved with patients affected by multiple myeloma (MM). In a retrospective analysis of 560 subjects with MM receiving autologous SCT, at a median follow-up of 23 months 52% of patients had relapsed or progressed. The median time for relapse was 30 months, with an actuarial risk of progression or relapse of 78% at 60 months after transplantation. After different lines of treatment, a disease response could be achieved in approximately one third of patients, with a median overall survival after relapse of 14 months.

The intensification of the conditioning regimen does not result in a substantial reduction of relapse rates and is accompanied by an increase in transplant-related mortality. On the other hand, a reduction of the intensity of the conditioning, although potentially associated with a lower transplant-related mortality, is invariably followed by an increase in relapse rates. The high mortality related to SCT procedures has been the major reason for exploring reduced-intensity conditioning (RIC) regimens. It is nowadays clear that in patients with MM, RIC transplant programmes are associated with lower transplant-related mortality but also with higher rates of disease progression and relapse when compared to myeloablative SCT.

Among transplant-related variables, T-cell depletion and incidence of acute graft-versus-host disease (GVHD) unequivocally influence the probability of post-transplant relapse. Patients who develop GVHD have lower probability of relapse and, conversely, those who receive a T-cell-depleted stem-cell preparation as a prophylaxis of GVHD exhibit an increased relapse rate.

Relapse should be distinguished from disease persistence because in the latter a period of complete remission cannot be documented. Disease persistence is usually associated with a worse outcome because it almost invariably signifies resistance to first-line therapy. Disease relapses after transplant vary in magnitude, and they can be detected at very low levels or at very advanced stages. Therefore, it is possible to classify relapses according to the disease burden, depending on the sensitivity of the method available for identification. This very much depends on the existence of chromosomal and molecular markers unique to the disease, whereby recurrence of the malignancy can be quantified at the molecular (molecular relapse), cytogenetic (cytogenetic relapse), or haematological (haematological relapse) level.

Where techniques are available, the detection of minimal residual disease (MRD) has an invaluable role in predicting the probability of full-blown relapse. From the methodological point of view, the most reliable tools for MRD analysis include flow-cytometric analysis of aberrant phenotypes and polymerase chain reaction (PCR) amplification of fusion transcripts and antigen-receptor genes. Moreover, the analysis of chimerism represents a surrogate disease marker in selected cases. From the operational point of view, the definition of disease relapse after allogeneic SCT is of fundamental importance because it does affect the outcome of the treatment. CML can be considered as a paradigm of the relevance of robust definitions of disease recurrence, as well as of the routine application of molecular monitoring in the post-transplant scenario. The availability of real-time PCR has facilitated the development of unanimous criteria to define molecular relapse whereby BCR-ABL/ABL ratio must exceed 0.02% in three samples, or 0.05%
in two samples, or show rising levels with the last two >0.02% over a minimum of 4 weeks.\textsuperscript{25,26}

A clear correlation between MRD positivity after allogeneic SCT and risk of disease recurrence has been demonstrated in patients with acute lymphoblastic leukaemia (ALL) of both T- and B-cell type\textsuperscript{27}, as well as in the Philadelphia chromosome-positive subtype.\textsuperscript{28} In addition, although less conclusive, data suggest that MRD monitoring in patients with acute myeloid leukaemia (AML) can be exploited not only as a risk stratification parameter to predict response to treatment\textsuperscript{29} but also for the early detection of post-transplant relapse.\textsuperscript{30–32}

The role of post-SCT MRD monitoring in chronic lymphoproliferative disorders is less defined, at least for their therapeutic management. Patients with chronic lymphocytic leukaemia (CLL) without PCR-detectable disease after treatment have a better outcome.\textsuperscript{33} The initial studies indicating the prognostic role of MRD after transplant in MM\textsuperscript{34} have recently been confirmed in the non-myeloablative setting.\textsuperscript{35} The limited reliability in quantifying MRD by PCR with consensus primers in this setting has led to the use of the kinetics of plasma-cell chimerism as a possible tool to quantify MRD after SCT.\textsuperscript{36}

The frequency for monitoring relapse is strictly dependent on the type of disease and, as a general rule, chronic leukemias require less frequent assessments than acute malignancies. However, the frequency may be limited by the type of biological sample required (i.e. bone-marrow aspirate) for the detection method. In CML the possibility of identifying the disease at the molecular level in the peripheral blood may allow a choice of the best timing for monitoring. However, even though quantitative PCR testing has become the standard strategy and clear correlations have been established between positive test results and probability of relapse, no absolute guidelines for monitoring exist.

**THERAPEUTIC OPTIONS FOR RELAPSE AFTER ALLOGENEIC HAEMATOPOIETIC STEM-CELL TRANSPLANTATION**

Several therapeutic options can be considered for patients relapsing after SCT. The most important factor determining the outcome of post-transplant relapse treatment is disease type, because the benefit of further treatment is questionable or at least extremely limited for some diseases.\textsuperscript{1,2,4} Another variable strongly influencing the response rate to any treatment is the latency between SCT and relapse, with higher response rates for patients in later relapse.\textsuperscript{4,37}

Since the first suggestion by Barnes et al\textsuperscript{38} more than 30 years ago that bone-marrow transplantation was associated with an anti-tumor effect not explained by pre-transplant chemotherapy or radiotherapy, it has been demonstrated that the therapeutic properties of allogeneic SCT are largely based on the adoptive immunotherapeutic activity of transplanted allogeneic T lymphocytes: the graft-versus-leukaemia (GVL) effect.\textsuperscript{39} Therefore a simple strategy is the discontinuation of the immunosuppressive prophylaxis for GVHD with a view to restoring GVL, but successes have been anecdotal.\textsuperscript{40}

A second SCT has been of benefit for some patients\textsuperscript{37,41}, but it involves a high transplant-related morbidity and mortality. The largest retrospective study reported the experience with 150 patients undergoing a second SCT for relapsed acute leukaemia or CML.\textsuperscript{37} The 5-year overall and disease-free survivals were 32% and 30%, respectively, with a risk of further relapse of 44% and a transplant-related mortality
of 45%. In a multivariate analysis, the best candidates for a second transplant appeared to be patients with acute leukaemia in remission before transplant, in whom the HLA-identical donor was female and who relapsed more than 1 year after the first transplant. The role of disease burden in identifying patients more likely to benefit from SCT, which is well known for first SCT, has been demonstrated also in patients undergoing second SCT for AML.

The experience acquired with RIC regimens has been exploited to reduce the toxicity in second transplants. Such an approach has been reported in 14 patients with acute leukaemia and myelodysplastic syndrome (MDS). The actuarial overall survival and leukaemia-free survival at 5 years were 60% and 26%, respectively. Transplants were well tolerated with no treatment-related deaths. The option of a re-induction regimen exclusively involving radiotherapy or chemotherapy may still be valid for patients with acute leukaemia. However, the outcome for such patients is still extremely poor because of the very low response rate and durability of remissions.

The advent of Imatinib (STI571) — an inhibitor of the Abl tyrosine kinase able to selectively block the proliferation of CML progenitor cells — has revolutionized the therapeutic approach to CML. Although more than 80% of newly diagnosed chronic-phase patients can achieve complete cytogenetic remissions, only a minority of them turn out to be disease-free when screened by quantitative PCR for BCR-ABL transcripts. However, the presence of a small disease burden at relapse is potentially the optimal situation for the use of Imatinib. The possible role of Imatinib in CML relapsing after allogeneic SCT has been studied in a series of 128 patients. The overall haematological response rate was 84% (98% for patients relapsing in chronic phase). The complete cytogenetic response rate was 58% for patients in chronic phase, 48% for accelerated phase and 22% for patients in blast crisis. Complete molecular responses were obtained in only 26% of patients, most of which were in chronic or accelerated phase. Interestingly, a recent retrospective study compared the clinical outcome in a small group of CML patients who had received donor lymphocyte infusions (DLI) or Imatinib for relapse after SCT and showed that, although the proportion of responders did not differ statistically, Imatinib was associated with a higher incidence of relapse and inferior leukaemia-free survival. Alpha-2a-interferon has been reported to induce durable cytogenetic remissions in a good proportion (57%) of a small cohort of patients (n = 14) with cytogenetic relapse following allogeneic SCT.

Adoptive immunotherapy based on DLI has been introduced in the treatment of post-SCT leukaemia recurrence in the early 1990s, and nowadays it represents one of the most consolidated therapeutic approaches to post-allografting relapse. The therapeutic principle relies on the evidence that donor T lymphocytes can exert a GVL effect which is potentially able to eradicate the residual host disease.

**DONOR LYMPHOCYTE INFUSIONS**

**Therapeutic efficacy**

**Disease type**

After several years of experience, it is well known that the best response to DLI can be achieved in patients affected by CML whereby response rates vary between 63 and 100% (Tables 1 and 2). It is also clear that the majority of molecular remissions after DLI are durable, and thus the majority of responding patients may prove to be cured (Figure 1). Factors predicting response after DLI have been recently analysed in
a total of 100 patients, who had received 593 infusions. Relapse type was the major predictive factor for response. Patients have been stratified in five groups (CML molecular relapse, CML cytogenetic relapse, CML chronic phase relapse plus complete remission of other disease after chemotherapy, CML in accelerated/blastic phase, resistant disease), and the proportion of responders was 100, 90, 75, 36 and 0%, respectively. Other factors associated with response were GVHD post-DLI, pancytopenia, and a diagnosis of CML. The actuarial probability of DLI-related mortality was 9% for fully matched siblings and 44% for alternative donor transplants. No data have been reported about a possible use of DLI in the context of syngeneic SCT. However, the high relapse rate even in the absence of T-cell depletion does not support its use.

Response rate have been shown to be lower in patients with AML and MDS (15–30%) and even worse in ALL patients. The estimated survival at 3 years after DLI in patients with AML/MDS and ALL has been reported to be less than 20%.

### Table 1. Response to donor lymphocyte infusions (DLI) for disease relapse after conventional stem-cell transplantation (SCT).a

<table>
<thead>
<tr>
<th>Author</th>
<th>Disease type</th>
<th>Number of patients</th>
<th>Complete response (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Huff et al56</td>
<td>EP-CML</td>
<td>17</td>
<td>77</td>
</tr>
<tr>
<td>Raiola et al58</td>
<td>EP-CML</td>
<td>42</td>
<td>86</td>
</tr>
<tr>
<td>Shiobara et al87</td>
<td>EP-CML</td>
<td>12</td>
<td>91</td>
</tr>
<tr>
<td>Porter et al73</td>
<td>EP-CML</td>
<td>12</td>
<td>58</td>
</tr>
<tr>
<td>Dazzi et al56</td>
<td>EP-CML</td>
<td>57</td>
<td>74</td>
</tr>
<tr>
<td>Alyea et al75</td>
<td>EP-CML</td>
<td>19</td>
<td>63</td>
</tr>
<tr>
<td>Collins et al55</td>
<td>EP-CML</td>
<td>37</td>
<td>76</td>
</tr>
<tr>
<td>Mackinnon et al77</td>
<td>EP-CML</td>
<td>17</td>
<td>82</td>
</tr>
<tr>
<td>Kolb et al72</td>
<td>EP-CML</td>
<td>67</td>
<td>79</td>
</tr>
<tr>
<td>Raiola et al58</td>
<td>AP-CML</td>
<td>14</td>
<td>36</td>
</tr>
<tr>
<td>Shiobara et al87</td>
<td>AP-CML</td>
<td>11</td>
<td>27</td>
</tr>
<tr>
<td>Porter et al73</td>
<td>AP-CML</td>
<td>12</td>
<td>33</td>
</tr>
<tr>
<td>Collins et al55</td>
<td>AP-CML</td>
<td>18</td>
<td>28</td>
</tr>
<tr>
<td>Huff et al56</td>
<td>AML</td>
<td>13</td>
<td>46</td>
</tr>
<tr>
<td>Shiobara et al87</td>
<td>AML</td>
<td>21</td>
<td>38</td>
</tr>
<tr>
<td>Porter et al73</td>
<td>AML</td>
<td>19</td>
<td>42</td>
</tr>
<tr>
<td>Collins et al55</td>
<td>AML</td>
<td>39</td>
<td>15</td>
</tr>
<tr>
<td>Kolb et al72</td>
<td>AML</td>
<td>17</td>
<td>29</td>
</tr>
<tr>
<td>Shiobara et al87</td>
<td>ALL</td>
<td>23</td>
<td>25</td>
</tr>
<tr>
<td>Collins et al59</td>
<td>ALL</td>
<td>40</td>
<td>25</td>
</tr>
<tr>
<td>Collins et al55</td>
<td>ALL</td>
<td>11</td>
<td>18</td>
</tr>
<tr>
<td>Kolb et al72</td>
<td>ALL</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>Huff et al56</td>
<td>MDS</td>
<td>12</td>
<td>15</td>
</tr>
<tr>
<td>Depil et al98</td>
<td>MDS</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>Shiobara et al87</td>
<td>MDS</td>
<td>11</td>
<td>45</td>
</tr>
<tr>
<td>Huff et al86</td>
<td>MM</td>
<td>20</td>
<td>30</td>
</tr>
<tr>
<td>Lokhorst et al61</td>
<td>MM</td>
<td>54</td>
<td>17</td>
</tr>
<tr>
<td>Salama et al60</td>
<td>MM</td>
<td>25</td>
<td>28</td>
</tr>
</tbody>
</table>

DLI, donor lymphocyte infusions; SCT, stem-cell transplantation; EP-CML, early-phase chronic myeloid leukaemia (CML in molecular, cytogenetic or haematological chronic phase relapse); AP-CML, advanced-phase chronic myeloid leukaemia (CML in accelerated or blastic phase relapse); AML, acute myeloid leukaemia; ALL, acute lymphoid leukaemia; MDS, myelodysplastic syndrome; MM, multiple myeloma.

a Only studies including at least ten patients with the same disease type have been considered.
Responses in patients affected by MM have been reported to be better than for patients with acute leukaemias but not as good as for CML patients. A recent analysis of 54 patients showed that in most cases responses are of short duration and associated with a high incidence of GVHD. The study reported an overall response rate of 52%, with 17% complete responses. Progression-free and overall survivals were 19 and 23 months, respectively. Acute and chronic GVHD occurred in 57% and 47% of patients, respectively, and turned out to be the best predictors for response.

Encouraging results have been shown for patients with relapsed chronic lymphoproliferative disorders or Hodgkin’s disease after conventional regimen allografting, but they should be confirmed in larger-scale clinical trials.

So far, there is no evidence for a role for DLI in the management of extramedullary relapses in any of the above-mentioned diseases. Supporting this notion is the fact that AML relapses after DLI-induced remissions often occur at these sites.

Table 2. Therapeutic efficacy of donor lymphocyte infusions (DLI) in different disease types.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Efficacy of DLI</th>
</tr>
</thead>
<tbody>
<tr>
<td>CML</td>
<td></td>
</tr>
<tr>
<td>• molecular/cytogenetic relapse</td>
<td>++ +</td>
</tr>
<tr>
<td>• chronic phase relapse</td>
<td>++ +</td>
</tr>
<tr>
<td>• accelerated phase relapse</td>
<td>+</td>
</tr>
<tr>
<td>• blastic phase relapse</td>
<td>–</td>
</tr>
<tr>
<td>AML</td>
<td>±</td>
</tr>
<tr>
<td>MDS</td>
<td>±</td>
</tr>
<tr>
<td>ALL</td>
<td>±</td>
</tr>
<tr>
<td>MM</td>
<td>+ ±</td>
</tr>
<tr>
<td>NHL</td>
<td>+</td>
</tr>
<tr>
<td>HD</td>
<td>+</td>
</tr>
</tbody>
</table>

CML, chronic myeloid leukaemia; AML, acute myeloid leukaemia; ALL, acute lymphoid leukaemia; MDS, myelodysplastic syndrome; MM, multiple myeloma; NHL, non-Hodgkin’s lymphoma; HD, Hodgkin’s disease.

Figure 1. The probability of survival is higher in patients who achieve molecular remission after donor lymphocyte infusions (DLI) (this research was originally published in Blood. Dazzi F, Szydlo R, Cross NCP et al. Durability of responses following donor lymphocyte infusions for patients who relapse after allogeneic stem cell transplantation for chronic myeloid leukemia. Blood 2000; 96: 2712–2716. © the American Society of Hematology).
DLI after reduced-intensity conditioning regimen allografting

DLI is a fundamental component in RIC transplant programmes\textsuperscript{65,66}, as this procedure relies almost exclusively on the graft-versus-host effects for the eradication of the underlying malignancy. In this specific context DLI is used not only to treat full-blown relapse but also, pre-emptively, at persistence of mixed chimerism, or prophylactically to eradicate undetectable MRD. Given the impact of disease stage on response to DLI, the latter approach may be more successful but still has to be tested in randomized clinical trials.

The data so far reported in the literature are from groups of patients who are heterogeneous both in terms of disease type and indication for DLI.\textsuperscript{67–70} Overall, the response rates vary between 25 and 62%. Interestingly, disease responses have been reported in 12 out of 19 patients with MM and 7 out of 10 of those with Hodgkin's lymphoma.\textsuperscript{69} Conversions from mixed to full donor chimerism have been described in 35–86% of evaluable patients. Acute grade II–IV and extensive chronic GVHD have been reported in 17–55% and 19–43% of evaluable patients\textsuperscript{67–70} and correlated with clinical responses.\textsuperscript{70} In summary, even though DLI seems to be a potential treatment strategy with acceptable toxicity for patients after RIC, data on its application in this specific clinical setting remain limited and should be tested in larger and more homogeneous cohorts of patient. However, the promising results observed with the combination of RIC regimen and DLI in diseases other than CML\textsuperscript{67–70} suggest broadening indications for DLI.

Treatment of relapses post-DLI

So far, the optimal management of relapses after a DLI-induced remission has not been fully identified. Recently a cohort of 13 CML patients (seven molecular, four cytogenetic and two haematological relapses) has been retrospectively analysed.\textsuperscript{71} Further DLI was used in the treatment of 11 patients, Imatinib in three and chemotherapy in two. The two patients with haematological relapse died of blastic disease. The cumulative response rate in the remaining patients was 100%, with two complete cytogenetic and nine molecular remissions. Nine patients were still in molecular remission at a median follow-up of 29 months; seven of them had received only DLI, one DLI and Imatinib, and one Imatinib monotherapy. The apparently promising role of DLI in the management of relapses after a DLI-induced remission should be confirmed in larger cohorts of patients and with a longer follow-up.\textsuperscript{71}

Administration regimens

Escalating dose regimen

The initial approach to administering DLI was to infuse random ‘bulk’ doses of lymphocytes collected on a single leukapheresis, containing variable numbers of CD3\textsuperscript{+} T cells (bulk dose regimen, BDR). This approach was however associated with a high incidence of acute and chronic GVHD.\textsuperscript{59,72–74} Two alternative strategies have been introduced to reduce the incidence of GVHD. The first is based on the selective depletion from the infusion of CD8\textsuperscript{+} lymphocytes, which are considered to be the most important effector cells of GVHD.\textsuperscript{75,76} The other strategy relies on the transfusion of donor lymphocytes in multiple aliquots, starting with low cell numbers and escalating the dosage until remission is achieved.\textsuperscript{77} The assumption underlying the use of an escalating dose regimen (EDR) is that the incidence of GVHD increases with the total cell dose administered. Therefore, the identification of the minimal cell dose necessary
to induce remission would minimize the GVHD risk. We adopted this approach at our institution and compared response rate and GVHD incidence associated with BDR or EDR in 48 consecutive CML patients who had post-SCT relapses at cytogenetic or haematological levels. Although the probability of achieving cytogenetic remission did not differ significantly between the two groups, the incidence of GVHD was much lower using EDR (Figure 2). When we considered subsets of patients who had received similar total cell doses, the incidence and severity of acute and chronic GVHD were significantly lower for patients treated by EDR. This suggests that the reduction in incidence of GVHD associated with EDR is dependent on the fact that lymphocytes are administered over a longer period rather than on the injection of a smaller cell dose.

Effective cell dose

A still-controversial point is represented by the identification of the optimal cell dose for DLI treatment. To address this question we investigated the factors affecting the

![Figure 2](image_url)

**Figure 2.** The probabilities of acute grade II–IV (A) and extensive chronic (B) graft-versus-host disease (GVHD) is reduced in patients receiving an escalating dose regimen (EDR) rather than bulk dose regimen (BDR) donor lymphocyte infusions (DLI) (this research was originally published in Blood. Dazzi F, Szydlo RM, Craddock C et al. Comparison of single-dose and escalating-dose regimens of donor lymphocyte infusion for relapse after allografting for chronic myeloid leukemia. Blood 2000; 95: 67–71. © the American Society of Hematology).
dose required to achieve remission (effective cell dose, ECD) in 68 patients treated with an escalating dose regimen. The cumulative proportion of remitters increased significantly at each dose level (Table 3). Whilst 73% of patients in molecular/cytogenetic relapse obtained molecular remission with a cell dose $\leq 10^7$ CD3$^+$ cells/kg, only 27% of recipients in haematological relapse did so. Also the donor type influenced the ECD, since 73% of patients transplanted from voluntary unrelated donors, as compared to 46% of sibling recipients, responded to the low cell dose ($\leq 10^7$ CD3$^+$ cells/kg). It appears therefore that DLI is subjected to a dose—response effect and that the ECD correlates with disease stage and donor type. This concept has important implications in the definition of refractoriness to DLI and in the use of an EDR. In fact, a premature discontinuation of dose escalations is likely to reduce the number of responders.

**Association regimens**

Patients with CML relapsing after SCT were recently studied for the therapeutic efficacy of the combination of DLI with Imatinib. Even though the data are obtained retrospectively and based on a small group of patients, the association regimen appeared to offer some advantage in terms of rapidity of remission as well as of actuarial overall and disease-free survival, especially in patients in accelerated phase. If confirmed, this result could be particularly relevant for patients relapsing in advanced-stage disease who traditionally have a poor response to either DLI or Imatinib.

Several other association regimens have been being tested over recent years. The efficacy of cytoreductive chemotherapy followed by DLI has been recently evaluated in patients with acute leukaemia relapsing after SCT, with quite encouraging results especially for those with ALL. In a recent phase I/II investigation low-dose thalidomide followed by DLI seemed to show a valid anti-myeloma effect associated with a low GVHD incidence. Also interleukin-2 has been evaluated as adjuvant therapy in conjunction with DLI in small groups of patients and has appeared to increase the response rate in a proportion of subjects who do not respond well to DLI alone. However, all these preliminary data regarding novel association regimens need to be tested in larger clinical studies.

**Side-effects**

GVHD constitutes the most threatening side-effect of DLI treatment. The occurrence of acute GVHD of grade II or higher and extensive chronic GVHD have been reported in 10—48% and 11—41% of treated patients, respectively (Table 4).

<table>
<thead>
<tr>
<th>Infused CD3$^+$ donor cells/kg</th>
<th>10$^6$</th>
<th>10$^7$</th>
<th>5 x 10$^7$</th>
<th>10$^8$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Responders (%)</td>
<td>41</td>
<td>44</td>
<td>41</td>
<td>72</td>
</tr>
<tr>
<td>Cumulative responders (%)</td>
<td>41</td>
<td>57</td>
<td>73</td>
<td>93</td>
</tr>
<tr>
<td>Total patients infused</td>
<td>41</td>
<td>50</td>
<td>27</td>
<td>18</td>
</tr>
</tbody>
</table>

Table 3. Proportion of responders to escalating cell doses in a series of 68 chronic myeloid leukaemia (CML) patients treated with donor lymphocyte infusions (DLI).
Recipient—donor sex mismatch, patient or donor cytomegalovirus (CMV) seropositivity, T-cell depletion at transplant, and advanced patient age have previously been reported as prognostic factors associated with acute GVHD in a group of CML patients, some of whom were treated with BDR and some with EDR. Moreover, we have recently investigated factors associated with the development of GVHD in 82 CML patients in relapse following conventional allografting exclusively treated with EDR DLI (manuscript in preparation). The development of both grade II—IV acute GVHD and extensive chronic GVHD was associated with two main factors: infusion of male recipients with female donor cells, and an interval between transplant and initiation of DLI <3 years. These factors are representative of the major mechanisms which contribute to GVHD after allogeneic SCT. In fact, a disparity at the level of minor histocompatibility antigens between donor and recipient has been identified as a major risk factor for GVHD.89 We have noticed that the total cell dose did not influence the development of acute and chronic GVHD, supporting the notion that, in order to maximize the number of responders, the donor lymphocyte dose could be needed to be increased indefinitely.

A further possible complication of DLI is pancytopenia, which in some cases may require a donor stem-cell top-up infusion. This complication, which has been reported in 15—20% of treated patients,55,78, is usually confined to patients in whom relapse of CML is detectable at the haematological level78 and in whom there is little evidence of donor haematopoiesis.90

**FUTURE DIRECTIONS**

Two main avenues are being explored in order to improve the outcome of post-transplant adoptive immunotherapy: the development of new systems aimed at the selective inhibition of GVHD, and the design of new strategies capable of improving the specificity of the DLI-mediated GVL effect. Donor lymphocytes have been transduced...
with the herpes simplex virus thymidine kinase (HSV-TK). When unnecessary or harmful, HSV-TK-transduced T cells can be selectively removed in vivo by ganciclovir. Such an approach has been successfully tested in the clinical setting, as demonstrated by the efficacy on GVHD and the marginal effect on the anti-tumour activity. This strategy is now seeking consolidation in clinical practice.

One of the most attractive attempts to design a leukaemia-specific adoptive immunotherapy is based on the crucial finding that complete remission can be induced in accelerated-phase CML by the administration of leukaemia-reactive cytotoxic T lymphocytes. The evidence that T cells specific for the haematopoiesis-restricted minor histocompatibility antigens HA-1- or HA-2 are associated with complete remissions of relapsed leukaemia has shed further light on the identification of the specific targets of the DLI-mediated GVL effect, thus suggesting a strategy to generate leukaemia-reactive cytotoxic T lymphocytes in vitro.

Apart from tumour-specific and minor histocompatibility antigens, some other molecules have been explored as possible targets of adoptive immunotherapy with promising results. T lymphocytes, retrovirally transduced with a Wilms-tumour-antigen-1-specific T-cell receptor, have been shown to kill leukaemia cells in vitro and in vivo. Similarly, cytotoxic T lymphocytes specific for PR1, an HLA-A2-restricted peptide derived from proteinase 3, have been demonstrated to kill leukaemia cells and to be associated with leukaemia remission.

### Practice points

- DLI can be considered the optimal therapeutic option for CML patients in early (e.g. molecular, cytogenetic or haematological chronic phase) relapse after allogeneic SCT
- The role of DLI for other diseases — compared to re-transplant, chemotherapy, cytokine therapy or a palliative approach — is uncertain
- The use of DLI within association protocols and RIC transplant programmes remains to be explored in the context of clinical trials
- To minimize the risk of GVHD, DLI should be given in escalating doses, starting from a maximum dose of $10^7$ CD3+ donor cells/kg
- DLI dose for CML patients should be increased until a response is achieved; premature interruption of dose escalation increases the proportion of non-responders
- DLI remains a successful option in cases of relapse after DLI-induced remissions

### Research agenda

- Genetic engineering of donor lymphocytes with suicide genes is being explored with the aim of selectively inhibiting the occurrence of post-DLI GVHD
- The generation of leukaemia-reactive cytotoxic T lymphocytes, directed towards minor histocompatibility antigens or WT1, is a potential strategy to increase the selectivity of GVL
SUMMARY

Disease relapse is the commonest cause of treatment failure following allogeneic SCT. Among the different therapeutic options DLI is probably the most consolidated strategy. High response rates can be produced in patients affected by CML, especially in molecular and cytogenetic relapse. The proportion of responders is much lower in patients with AML and MDS and, despite the evidence of a GVL effect, very few ALL patients benefit from the DLI treatment. The outcome has been reported to improve in patients affected by MM, although the responses appeared to be marred by their short duration and high incidence of GVHD. Encouraging results have been recently shown for patients with other chronic lymphoproliferative disorders or Hodgkin’s disease.

Among the different administration protocols, EDRs have been shown to greatly reduce the incidence of GVHD without impairing the probability of achieving remission. The EDR modality has also demonstrated that DLI is subject to a dose-response effect, thus suggesting the importance of escalating the dose to increase the number of responders. The best administration regimens in the context of association protocols and RIC transplant programmes still need to be identified.

REFERENCES


42. Hosing C, Saliba RM, Shahjahan M et al. Disease burden may identify patients more likely to benefit from second allogeneic hematopoietic stem cell transplantation to treat relapsed acute myelogenous leukemia. *Bone Marrow Transplantation* 2005; 36: 157–162.


51. Weisser M, Tischer J, Schnittger S et al. Comparison of donor lymphocyte infusions or imatinib mesylate for hematogenous chronic myelogenous leukemia who have relapsed after allogeneic stem cell transplantation. *Haematologica* 2006; 91: 663—666.


63. Russell NH, Byrne JL, Faulkner RD et al. Donor lymphocyte infusions can result in sustained remissions in patients with residual or relapsed lymphoid malignancy following allogeneic haemopoietic stem cell transplantation. *Bone Marrow Transplantation* 2005; 36: 437—441.


