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EXPERT REVIEWS

Clinical options after failure of allogeneic hematopoietic stem cell transplantation in patients with hematologic malignancies

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Disease recurrence is the single most common cause of death after allogeneic or autologous hematopoietic stem cell transplantation (HSCT). Disease status and chemosensitivity at the time of transplantation, as well as the development of graft-versus-host disease (GVHD), are factors known to influence the risk of relapse post-HSCT. Both acute and chronic GVHD have been associated with decreased relapse rates; however, owing to toxicity, overall survival is not consistently improved in these patients. Furthermore, there is a transient period of immunodeficiency after HSCT, which may permit residual malignant cells to proliferate early in the post-transplant course, before the donor immune system can establish a graft-versus-tumor response. Patients who fail an initial HSCT have an extremely poor outcome; therefore, maneuvers to prevent, identify and treat recurrent disease as early as possible in these situations are necessary. Strategies to distinguish graft-versus-tumor from GVHD, to enhance both general and disease-specific immune reconstitution after transplantation, and to increase donor-mediated anti-host immune reactions are being investigated in clinical trials. Single agent nontoxic post-HSCT chemotherapy, cellular therapies and second allogeneic HSCT using reduced intensity regimens are among the modalities under investigation.

Keywords: allogeneic HSCT • dendritic cells • donor leukocyte infusion • graft-versus-leukemia effect • killer immunoglobulin-like receptor • leukemia • lymphoma • natural killer cells • relapse

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Learning objectives

Upon completion of this activity, participants should be able to:

- Analyze the risk for disease recurrence following HSCT
- Evaluate donor leukocyte infusions in the treatment of patients with relapse after HSCT
- Discuss second allogeneic HSCT in the treatment of patients with relapse after HSCT
- Describe other novel therapies for patients with disease recurrence after HSCT

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Identification of patients at high risk of relapse

The underlying disease, disease status at transplantation and cytogenetic alterations in malignant cells are predictive of disease recurrence after transplantation. More recent studies have demonstrated that identification of minimal residual disease during leukemia therapy and at the time of transplantation identifies patients with greatest risk of relapse. Relapse rates after allogeneic hematopoietic stem cell transplantation (HSCT) vary by disease type, disease status at transplantation, and on the presence of graft-versus-host disease (GVHD) after HSCT. For patients with acute leukemia, relapse rates range from 25 to 90%; for myelodysplastic syndromes the rates are between 20 and 90%; and for juvenile myelomonocytic leukemia the rates approximate 60% [1–4]. Many trials have established the utility of minimal residual disease (MRD) measurements at various time points throughout leukemia therapy in predicting which patients will need HSCT as consolidation therapy [5–7]. Similarly, MRD at the time of transplant is also predictive of outcome after HSCT [8]. These studies suggest that monitoring of MRD post-transplantation will allow physicians to identify patients who have persistent disease who may benefit from early maneuvers to enhance graft-versus-leukemia effect to abort progression to hematologic relapse. MRD has been shown to be predictive of outcome in acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML) and chronic myeloid leukemia (CML) [9]. Other investigators have demonstrated that declines in post-transplant donor chimerism studies identify patients at high risk of disease recurrence, suggesting that chimerism studies may be a useful surrogate marker for centers without access to MRD technology or for patients for whom MRD markers cannot be established [10–12]. Improved methods of identification of patients at high risk for disease recurrence after HSCT will allow transplant physicians to employ adjuvant post-transplant antitumor therapies (**Box 1**).

Minimal disease determination

The role, methodologies and optimal tissue source for MRD determination before and after allogeneic HSCT are yet to be fully determined [13]. Peripheral blood and bone marrow have been used in patients to identify persistently low levels of disease, with approximately 75% of samples yielding concordant results. Peripheral blood in some cases may be less sensitive than marrow samples in detecting low levels of disease; however, the easy access of peripheral blood makes this tissue more readily available for ongoing monitoring [14]. Detection of elevated levels of MRD below the detection of microscopy predicts patients with a high risk of relapse. Most studies demonstrate that ALL patients with marrow MRD levels above 1% have an increased risk of relapse rate. MRD assays using markers such as WT1 in AML have also been successful in identifying relapse after allogeneic HSCT. MRD allows early detection of disease recurrence, which may allow early interventions such as rapid withdrawal of immunosuppression or the administration of cellular therapies to treat disease recurrence before it is overt. Unfortunately, not all children are able to have effective MRD markers determined [15,16].

Withdrawal of immunosuppression

Reduction of immunosuppression is typically the initial intervention performed once disease recurrence is identified after transplantation. Remission rates approaching 84% were observed in patients with chronic phase CML, but only 10% for AML, and 0% for ALL and advanced phase CML [17]. This intervention can be performed in all patients with nonlymphoid malignancies, irrespective of hematopoietic stem cell source. Alternatively, development of less intense immunosuppressive agents and other agents that cause less tissue damage and inflammation may reduce the risks of GVHD post-transplantation, making reduction in immunosuppression less dangerous.

Donor leukocyte infusions

Building on observations that patients who developed acute or chronic GVHD had lower relapse rates, donor leukocyte infusions (DLIs) have been proven to induce remissions post-transplantation in patients with relapsed hematologic malignancies. These observations have been most consistently observed in patients with CML, and to a lesser extent in AML, multiple myeloma and myelodysplasia [18–20]. Responses have been observed anecdotally in patients with lymphoid malignancies such as ALL, chronic lymphocytic leukemia, non-Hodgkin lymphoma and Hodgkin lymphoma. However, some patients experience disease progression while others develop pancytopenia and/or GVHD. DLIs are not currently obtainable from cord blood grafts and are therefore limited to recipients of peripheral blood or bone marrow grafts.

Typically obtained by leukapheresis of unstimulated peripheral blood or by simple phlebotomy, unmanipulated DLI

product will contain other cell types in addition to $CD3^+$ cells, such as dendritic cells, B cells, monocytic cells and natural killer (NK) cells, providing a spectrum of alloreactive and other accessory cells that might play a role in graft-versus-tumor (GVT) effect [21]. By contrast, granulocyte colony-stimulating factor (G-CSF)-stimulated peripheral blood cells for DLI have also been used and the efficacy and toxicity of unstimulated versus stimulated DLI still remains to be determined [22]. An optimal CD3 cell dose for use in DLI is not established and DLI cell doses are reported either in mononuclear cell dose/kg or in $CD3^+$ dose/kg. However, some studies have demonstrated less risk of development of GVHD if a dose-escalating strategy is used for DLI [23]. By contrast, Fozza *et al.* demonstrated that in patients with CML, the incidence of GVHD was not significantly different in patients receiving less then 1×10^7 $CD3^+$/kg compared with the patients who received doses greater then 1×10^7 $CD3^+$/kg [23]. DLI dose can also be influenced by disease burden at the time of administration, such that patients with molecular relapse of CML might require a lower dose than patients with hematologic relapse.

Timing of DLI and the time it takes to observe the effects from the DLI are important factors influencing the effectiveness of this strategy. Patients with relapses occurring more than 6 months post-transplantation have higher chances of responding to DLI [24]. Disease response following DLI can be seen between 40 days and up to 1 year following DLI. In more indolent diseases, sequential DLIs given over a longer period of time may lead to remission. However, in more aggressive diseases, debulking chemotherapy might be necessary to reduce the initial large disease burden as well as allowing time for DLI to exert its effects. Choi *et al.* demonstrated 31% survival at 2 years in patients with relapsed AML post-HSCT when treated with cytarabine, idarubicin and etoposide followed by G-CSF-primed DLI. In further support of the aforementioned data, Choi *et al.* found 55% overall survival at 1 year in patients who were treated for relapse, which occurred greater than 6 months post-transplantation, as opposed to 0% survival at 1 year in patients treated for relapse that occurred within 6 months following HSCT [25]. Another predictor of DLI success is the tumor burden at the time DLI is administered. Patients with evidence of molecular relapse at the time of DLI have better responses even in malignancies not typically viewed as responsive to DLI such as ALL, which might support careful screening of patients for detection of molecular relapse [24]. There are few studies comparing survival rates in patients treated with or without DLI for relapses following HSCT. In a large retrospective study, Schmid *et al.* demonstrated improved survival rates in patients with AML who received DLI post-HSCT relapse compared with patients who did not receive DLI (21 vs 9%; $p < 0.001$) [26]. In this study, lower tumor burden, remission with favorable karyotype and relapse occurring more then 5 months following HSCT were identified as favorable predictors for long-term survival. Choi *et al.* studied ten ALL patients with relapse following HSCT who were treated with debulking chemotherapy followed by G-CSF-primed DLI, showing high rates of complete remission (seven patients), which were not durable however [27]. In a combination retrospective and prospective analysis of ALL-relapsed post-HSCT patients, Collins *et al.* reported no difference in survival whether patients received pre-DLI chemotherapy or not [28].

Box 1. Post-transplant interventions for recurrent disease.

Non-alloreactive
- Salvage chemotherapy
- Second autologous HSCT

Alloreactive
- Cytotoxic T lymphocytes
- Natural killer cells
- Dendritic cells

Allogeneic HSCT
- Withdrawal of immunosuppression
- Donor leukocyte infusions
- Reduced intensity regimen

HSCT: Hematopoietic stem cell transplantation.

The major complications with DLI are the development of GVHD and cytopenias; marrow aplasia is quite rare. GVHD develops in up to 40–60% of patients who receive DLI. The development of GVHD does not always correlate with GVT activity [29], leading some investigators to use immunosuppression around DLI administration [30,31]. Pancytopenia following DLI occurs in up to 50% of cases. A total of 62% of patients demonstrated measurable clinical response.

There is emerging evidence of DLI product manipulation with regard to $CD4^+CD25^+$ regulatory T cells and that the administration of low-dose IL-2 to patients with DLIs may offer some protective effects [32,33]. In order to make DLI more effective by changing the host environment, Miller *et al.* created a lymphopenic environment prior to DLI with fludarabine and cyclophosphamide to allow for *in vivo* lymphocyte expansion. A total of 15 patients with acute leukemia relapses post-HSCT received intervention and were compared with the historic controls, however, they developed significantly more GVHD [34].

Donor leukocyte infusions may convert mixed donor–host chimerism to full donor chimerism as a surrogate measure to prevent relapse. Orti *et al.* reported a study of 28 patients who received CD8-depleted DLI with a CD4 dose of 4×10^6 at 6 months or greater if they had mixed peripheral chimerism or persistent hematologic malignancy [35]. In eight out of 16 patients treated for mixed chimerism, stable full chimerism was established. Meyer *et al.* reported 20 patients who received DLI following a reduced intensity conditioning regimen with *in vivo* T-cell depletion with alemtuzumab, followed by infusion of dose escalated $CD8^+$ depleted DLI post-HSCT [36]. A total of 13 patients received DLI and 12 of them converted to full donor chimerism, whereas only one out of seven patients who did not receive DLI converted to full donor chimera. Overall, DLI is an effective form of immunotherapy in patients with CML who relapse following HSCT, with remission rates of approximately 80%. The results in patients

with acute leukemias and myelodysplasia are disappointing with remission rates in 15–25% of patients and often the responses are not durable.

Second allogeneic transplantation

Patients who have failed an initial HSCT have chemorefractory disease, making additional chemoradiotherapy unlikely to be curative. Allogeneic HSCT offers a potentially therapeutic GVT effect; the GVT may vary according to donor type, graft source and post-HSCT immunosuppression. Second allogeneic HSCT is typically performed after withdrawal of immunosuppression and administration of DLI; chemotherapy is typically administered to patients with rapidly progressive leukemias such as ALL. The efficacy of second allogeneic HSCT depends on several factors, such as underlying malignancy, patient age and performance status, type of conditioning regimen employed, and time interval between first and second HSCT. Historically, second transplantation has had poor outcomes, complicated by high relapse rates due to chemorefractory malignancies and excessive rates of regimen-related toxicity. In a large Center for International Blood and Marrow Transplant Research (CIBMTR) retrospective study of patients with hematologic malignancies undergoing second allogeneic HSCT, transplant-related mortality was 30% and the relapse rate was 42%, yielding an overall survival rate of 28% at 5 years post-HSCT [37]. Smaller series have confirmed these results, with the degree of tumor burden at HSCT being identified as prognostic. Similar outcomes have been reported for patients undergoing allo-HSCT after an initial autologous HSCT [38–42].

With the advent of reduced intensity and non-myeloablative conditioning regimens, second transplantation has been increasingly offered to older patients and those with comorbid conditions, including those who failed an initial allogeneic or autologous HSCT [43,44]. Fludarabine, a potent immunosuppressive chemotherapeutic agent, has become incorporated into many reduced intensity regimens because it has a favorable organ toxicity profile. These regimens typically rely more heavily on a GVT effect than the upfront heavy cytoreductive chemoradiotherapy characteristic of ablative regimens. Recent smaller studies have suggested that treosulfan may be a feasible alternative to busulfan in conditioning regimens, suggesting that treosulfan-containing regimens have lower pulmonary and liver toxicity than busulfan-containing regimens in patients with hematologic malignancies such as ALL, AML or MDS [45–46]. Further studies with treosulfan are needed to define its role; treosulfan is still under investigation in the USA.

In order to maximize the GVT effect, graft manipulation to alter the cellular content of the hematopoietic grafts has been studied in an effort to increase the content of immunologically active cells, which may increase GVT effect, or to remove other populations such as $CD3^+$ cells, which are associated with GVHD [47–49]. Investigators have used immunomagnetic columns with magnetic beads attached to antibodies in an attempt to separate cell populations in the hematopoietic graft by enriching the graft for certain cell populations such as NK cells or DCs. In clinical trials, $CD3^-$-depleted grafts allow haploidentical HSCT to be performed with acceptable GVHD risk [50–53]. This strategy allows T cells to be partially depleted from the donor graft, while allowing the graft to contain immunologically active cells such as NK cells, monocytes and DCs. Combined $CD3^+$–$CD19^+$ depletion allows the hematopoietic graft to have a balanced reduction in both T and B lymphocyte content, partly in an attempt to reduce the incidence of post-transplant lymphoproliferative disorder. Recent studies in adults and children suggest that this latter graft manipulation strategy results in excellent outcomes after haploidentical HSCT, with overall survival rates approaching 50%. While relapse rates are lower than with traditional regimens and unmanipulated grafts, disease recurrence remains the most common reason for transplant failure.

Newer methodologies of graft manipulation have focused on enriching grafts for γδ T lymphocytes, a lymphocyte population thought to eradicate malignant cells with a lower risk of GVHD [54–56]. Other investigators have employed a strategy of depleting hematopoietic grafts of $CD4^+$ and $CD8^+$ cells as a surrogate to enrich grafts for γδ T lymphocytes; however, little clinical data is available on this strategy. Currently in Europe, an antibody against αβ T cells has been developed so that haploidentical grafts can be enriched for γδ T lymphocytes. No such antibody is yet available for clinical use in the USA.

Second transplants using unrelated donor grafts and cord blood units have been successful. However, coordinating donor and recipient schedules is more difficult with an unrelated donor than for related donors or cord blood units. Cord blood units have limited volumes and cell content, and the majority of centers do not have the ability to manipulate the graft content of these units.

Novel strategies

Cytotoxic T lymphocytes

In transplants where recipient and donor are matched at MHC, minor histocompatibility antigens (mHA) can be recognized by donor T cells. By analyzing immunologic reactions occurring with DLI administration facilitating GVT effect in patients who responded to DLI, it was determined that both autosomal and H-Y mHA play an important role. mHA are generally widely expressed in all tissue types but some are limited to cells of hematopoietic origin, including leukemic stem cells [57]. By studying patients with responses following DLI, it has been demonstrated that the T-cell response associated with GVT is polyclonal and is directed against several antigens. Studies looking at donor host autosomal mHA disparities that demonstrated GVT activity have yielded inconsistent results. By contrast, retrospective analyses examining male recipient female donor pairs showed lower relapse rates suggesting that T-cell responses to H-Y antigens are potent in generating GVT activity [58,59]. Warren *et al.* reported data in seven patients with advanced MDS or acute leukemia beyond first remission who were treated with $CD8^+$ mHA CTL clones upon post-transplant relapse (median time 7 months post-HSCT) [60]. Patients underwent immunosuppression withdrawal followed by administration of cytoreductive chemotherapy followed by three cytotoxic T lymphocyte infusions on day 0, 4 and 11 administered at escalating doses (3.3×10^7, 3.3×10^8 and 3.3×10^9). If patients did not develop GVHD they were eligible for an additional three infusions performed at weekly intervals followed by a 2-week course of recombinant IL-2. Three patients

developed grade 3 or 4 pulmonary toxicity, which was CTL dose dependent. Three patients developed GVHD but the causality role of transferred CTL could not be established. Five out of seven patients developed complete morphologic remission and three of the five patients had persistent disease following chemotherapy administration. However, all five patients subsequently relapsed and of note is that transferred CTLs could not be detected following 21 days postadministration. Norde *et al.* examined seven patients with myeloid malignancies who received DLI pre-emptively or therapeutically, with respect to development of LRH-1-specific $CD8^+$ T cells [61]. Some authors have previously demonstrated the presence of LRH-1 on the myeloid leukemic progenitor cells. Functional analysis of LRH-1 CTLs from two patients demonstrated effective targeting of LRH-1-positive leukemic $CD34^+$ stem cells from both CML and AML patients [61].

Another strategy of generating CTLs against antigens presented on leukemic cells has been attempted by genetically modifying T cells to introduce antigen receptors capable of recognizing leukemic cells. Cooper *et al.* generated T lymphocytes engineered to express chimeric antigen receptors (CARs) specific for the CD19 molecule that may be able to prevent or treat leukemia relapse in B-ALL patients as these cells almost invariably express CD19 [62]. Furthermore, it was recently reported by Micklethwaite *et al.* that it is feasible to generate CTL lines from peripheral blood or cord blood units that recognize Epstein–Barr virus (EBV), cytomegalovirus and adenovirus and also provide antileukemic activity by transgenic expression of a CAR targeting CD19 expressed on B-ALL [63]. This combination CTL can provide antiviral and antileukemic activity in patients transplanted for high risk B-ALL. Stimulation of CTL-native T-cell receptor by viral antigen would increase their antileukemic activity.

B cells and antibodies have also been shown to play a role in tumor immunity after HSCT. Wu *et al.* identified antibodies reactive with CML cells that were identified from patients who responded to DLI treatment of relapse following HSCT [64]. Target proteins in this cohort of patients were also identified and generation of antibodies to these targets coincided with patients attaining remission. Antibody responses have also been demonstrated in patients with myeloma. As cord blood grafts are being increasingly used in HSCT, methodology to consistently generate antiviral and antitumor CTLs or other immunologically active cell lines will be important.

In an attempt to overcome anergy and to enhance antitumor activity of infused T cells, investigators have infused *ex vivo* activated $CD3^+$ cells for patients with recurrent disease after HSCT [65]. Donor cells, activated through costimulation with anti-CD3 and anti-CD28 coated beads, were administered to 18 patients in a Phase I trial, with dosing ranging from 1×10^6 to 1×10^8 $CD3^+$/kg. Eight patients achieved remission, and developed acute GVHD and four developed chronic GVHD. Four survived at a median follow-up of 23 months, demonstrating that infusion of *ex vivo* activated T lymphocytes can be performed with long-term antitumor effects and without excessive GVHD.

Epstein–Barr virus-specific cytotoxic T lymphocytes have long-term use in treating and preventing EBV-mediated post-transplant lymphoproliferative disorder in patients undergoing hematopoietic and solid organ transplantation. Studies have demonstrated rapid efficacy in treating detectable and radiographically measurable disease, by effective prevention of EBV reactivation, and long-term persistence of donor-derived EBV-specific T cells in recipients of allogeneic HSCT [66–68]. This experience serves as a model for generation of T cells generated to recognize antigens expressed on a malignancy for post-transplant prevention and treatment of disease recurrence.

NK cells

During the first month after allogeneic HSCT, the predominant lymphocyte population detected in the peripheral blood is NK cells, comprising approximately 20% of the lymphocytes in the peripheral blood. NK cells express the immunophenotype $CD3^+CD56^+$ and kill target cells in an MHC-unrestricted fashion, without prior stimulation or antigen recognition.

In one large retrospective study of 1770 patients undergoing T-cell-repleted HSCT from HLA-matched and mismatched unrelated donors for hematologic malignancies, investigators found that recipient homozygosity for HLA class B or class C killer immunoglobulin-like receptor (KIR) epitopes identified a group who were at lower risk of disease recurrence when receiving grafts from mismatched donors only. This finding was not observed in recipients of matched unrelated donor grafts. These findings suggest that NK cell alloreactivity, mediated via HLA class I expression, may play an antitumor role after allogeneic HSCT [69].

Interactions occur between KIRs expressed on the NK cell and the HLA class I molecule on the surface of cells. KIRs can be inhibitory or activating, however, the majority are inhibitory and prohibit self recognition [70]. Since the 1990s, several studies of haploidentical HSCTs have found that NK cells could enhance hematopoietic engraftment and decrease disease recurrence for patients with hematologic malignancies receiving T-cell-depleted grafts from mismatched family member donors through a graft-versus-leukemia effect. Early studies of haploidentical transplantation have demonstrated antileukemia NK cell activity [71–74]. In addition, NK cell infusion after allogeneic and autologous HSCT demonstrated that these infusions could be safely performed [72,75–77].

The effect of KIR-ligand mismatching on transplant outcomes in patients receiving unrelated donor or matched sibling donor transplantation is less clear. Some authors have reported an improvement in disease-free survival attributable to lower relapse rates, while others have demonstrated no difference between recipients of KIR-mismatched grafts and those with matched grafts [78–81]. In these latter studies, the grafts were T-cell replete, which may have overshadowed any NK cell effect [82]. Other authors have demonstrated that the NK cell effect is greater in T-cell-depleted transplantation. In the unrelated donor setting, it is important to point out that a KIR-ligand mismatch requires a mismatch in class I HLA antigens, which is known to increase GVHD and decrease overall survival. It is unclear whether a KIR-ligand mismatch can overcome this adverse effect.

Recent studies in adults with refractory malignancies have shown that *in vivo*-expanded mismatched-related donor NK cells demonstrated NK cell expansion only in those patients who

received an immunoablative lymphocyte-depleting regimen. More recent studies of haploidentical NK cell infusions in pediatric patients with AML demonstrate that with postinfusion IL-2 administration, NK cells can persist for a median of 10 days; none of the ten patients developed disease recurrence at a median time of 964 days [83].

B-cell lymphoblasts appear to be resistant to the lytic effects of NK cells. In order to overcome this resistance, investigators are generating human NK cells with enhanced cytotoxicity by coculturing NK cells *ex vivo* with irradiated leukemia cell line K562 modified to express a membrane-bound form of IL-15. Clinical trials are underway to investigate the safety and efficacy of this approach [84]. Other investigators have studied ways to use gene therapy to enhance NK cell activity against targets resistant to them, such as B lymphoblasts.

Genes encoding KIR and their HLA class I ligands are inherited independently; therefore, individuals may express an inhibitory KIR gene but not its corresponding ligand. In one study in children undergoing autologous transplantation, the authors found that disease recurrence rates were lowest for those with two mismatched pairs (0%) followed by one mismatched pair (50%), with the highest rates (100%) observed for those with no KIR–HLA ligand mismatch [85]. This report suggests that KIR-ligand mismatch in autograft recipients may be used as a prognostic factor and that NK cells may be effective in post-transplant immunomodulation to decrease relapse rates. *In vitro* studies suggest that NK cell cytotoxicity is observed in cell lines representative of Ewing sarcoma, rhabdomyosarcoma, neuroblastoma and osteosarcoma [86].

Dendritic cells

Dendritic cells are antigen-presenting cells originating from hematopoietic cells, differentiating along two different pathways into immunologically active cells: plasmacytoid DC or myeloid DC. Plasmacytoid DCs secrete type 1 interferon, to activate NK and NKT cells, thus initiating immune responses to viruses. Myeloid DCs comprise Langerhans cells, which are located in skin and mucosal surfaces and interface with the external environment. There are currently no standard clinical applications of DCs although clinical trials are underway. DCs may be limited in their effectiveness in T-cell lymphopenic environments since they exert their influence by activating T-cell populations [87–92].

Donor vaccination

Because of the risk of GVHD associated with DLI, investigators have considered vaccination of donors prior to stem cell collection in an effort to enhance specific antitumor immunity in the donor immune system. In order to successfully vaccinate the donor, the tumor antigen must be known, a current limitation for many malignancies. Alternatively, the patient-specific tumor could be used to develop a lysate to be used in the vaccine, which could be a labor intensive, expensive process. In either event, following vaccination, the graft would be harvested and presumably the immunity transferred to the recipient following transplantation. In one study of five donor-recipient sibling pairs, in which the recipients were undergoing HSCT for myeloma, donors were immunized with a recipient-derived myeloma idiotype protein conjugated to keyhole limpet hemocyanin, an immunogenic carrier. Following transplantation, recipients continued to receive booster vaccines with the idiotype protein. Of the three pairs evaluable, all three converted to complete remission post-HSCT and no adverse events associated with the immunizations were observed. Interestingly, in all three recipients, the T-cell responses to the idiotype protein were not detected pre-HSCT, but were detected for up to 18 months after HSCT. This study suggests that vaccination of donors may be potentially useful in solid tumors and hematologic malignancies when the tumor antigen is well defined [93]. However, potential risks to the donor need to be carefully considered.

Novel pharmacotherapeutic options (tyrosine kinase inhibitor, demethylating agents, rituximab)

Following allogeneic HSCT, specific chemotherapeutic agents may be employed to slow disease progression once disease recurrence is confirmed, to allow time for donor identification for second transplant procedure, pre-transplant evaluation, and for recovery from the first transplant procedure. Patients whose malignancies contain the Philadelphia chromosome may receive tyrosine kinase inhibitors [94,95]. Imatinib and dasatinib have been well tolerated prophylactically during leukemia induction and post-transplant, with cytopenias being the most commonly observed toxicity. Longer follow-up is necessary to ascertain the effects of post-transplant tyrosine kinase inhibitor administration on overall survival. In adults with MDS/AML, post-transplant use of demethylating agents have been used successfully [96]. In 45 patients treated, the most common toxicities were hematologic, particularly thrombocytopenia. 1-year event-free survival was 58%. In addition, the anti-CD20 antibody rituximab has been well-tolerated in patients with non-Hodgkin's lymphoma who tumors express CD20 antigen [97]. However, single-agent administration is unlikely to reinduce remission in patients with aggressive malignancies.

Antibody therapy

Recently, a novel group of agents have been studied in an effort to enhance immunologic activity against the tumor, by developing bispecific T-cell engaging antibodies, termed BiTE [98]. These T cells are bispecific for a target antigen on the surface of malignant cells and for CD3, expressed on the surface of T cells. This bispecificity enables them to connect a T cell to a cancer cell independent of any T-cell receptor specificity. Two novel agents are currently being studied – one in non-Hodgkin's lymphoma and B-cell-precursor ALL (targeting CD19) and another in carcinomas (targeting epithelial cell adhesion molecule). Initial results of a Phase II trial of blinatumomab demonstrate that T cells engaged by blinatumomab are able to locate and eradicate tumor cells in the bone marrow. Three pediatric patients with B-cell-precursor ALL, recurrent after allogeneic transplantation, received blinatumomab and attained complete remission, all achieving MRD levels below the detection limit in the bone marrow [99]. Adverse events are generally mild and consist of leukopenia, lymphopenia, chills, pyrexia and elevated C-reactive protein, most of which resolve with ongoing treatment. All are considered investigational in the USA.

Expert commentary & five-year view

Over the last 40 years, transplant physicians have made significant strides in improving patient outcomes, largely through better supportive care to prevent and to treat infections, to reduce regimen-related toxicity, and to lower the risk of GVHD though improved HLA typing and donor availability. Disease recurrence remains a significant cause of treatment failure for transplant recipients. Immunotherapy for relapse following HSCT has evolved from quite nonspecific therapy utilizing multiple cell types such as what is administered with unmanipulated DLI, to highly specific genetically modified CTLs that are capable of treating both infections as well as providing antileukemic activity and without significant risk of developing GVHD, to the identification of novel cell immunologically active cell populations and new graft processing technologies, leading us closer to the ultimate goal of transplantation separation of GVHD from GVT.

In the future, HSCT will be increasingly utilized as advances in HLA typing and supportive care make transplantation safer and as the number of potential donors increase. Increasingly, HSCT will become incorporated into the treatment regimen of patients as MRD and other markers are used to identify patients likely to fail standard treatments earlier in their treatment course. As transplantation becomes safer, disease recurrence will increasingly become the primary cause of death after HSCT, making it the primary factor to overcome to improve overall transplant success.

In order to reduce the rates of relapse, transplant physicians will be increasingly working to identify the best time to perform transplantation in the course of a patient's treatment. Developing means to measure MRD for each patient will become standard and physicians will use these results to optimize the timing of transplantation and to counsel patients on transplant outcomes.

Second allogeneic HSCT will continue to be an integral part of the salvage therapy for patients with recurrent disease after transplantation. Reduced intensity regimens will be selected to contain agents that are effective for the individual patient by screening the patient's malignant cells for resistance mechanisms. Prior to transplantation, patients at high risk of disease recurrence will have CTLs manufactured and cryopreserved for administration post-transplant either prophylactically or in response to MRD measurements. Post-transplant immunomodulatory manipulation through graft composition, cytokines and other cellular therapies will be increasingly explored as the roles of various cell populations in preventing or treating disease recurrence are studied. NK cells will be increasingly utilized post-transplantation and may regularly be administered to high-risk patients at specified time points after HSCT. As cancer-specific antigens are identified, cytotoxic T cells specific for a patient's malignancy may be generated prior to HSCT for administration post-transplantation with a low risk of GVHD. This intervention will expand the field of personalized medicine.

As cord blood grafts are likely to increase quantitatively as a common stem cell source, cord blood banks will begin keeping aliquots to generate CTLs against the patients' malignancy at the time the unit is dispensed to the transplant center.

In some settings, transplant physicians may purposefully select a donor, mismatched with the recipient so that there is a KIR-ligand mismatch, allowing NK cell cytotoxic effects to eradicate residual leukemia cells, particularly in patients with myeloid malignancies and those receiving a T-cell-depleted graft from a mismatched family member. Transplantation using donors from mismatched family members will increase since nearly all patients have such a donor, these donors are readily available and highly motivated, and scheduling of stem cell collection and transplantation is easier for patients with refractory disease or unpredictable disease treatment courses. The role of NK cell alloreactivity will be explored in studies of T-cell-replete grafts from matched-related or volunteer-related donors.

As regimen-related toxicity rates decline, more patients will undergo second allogeneic HSCT for disease recurrence. Second transplantation will likely incorporate novel graft manipulation strategies and donor stem cell sources. Haploidentical grafts are readily available and easy to schedule in HSCT in the course of the treatment of patients with refractory malignancies. Donor-recipient KIR incompatibility will be increasingly utilized, making mismatched donors the preferred donors for second HSCT, particularly if KIR incompatibility exists. Multidimensional treatment modalities, incorporating novel chemotherapeutic agents, identification of early disease recurrence, novel graft manipulation strategies and identifying measures to optimize the post-transplant antitumor response, will most likely be effective in reducing relapse rates after transplantation.

Key issues

- Disease recurrence is the single most common cause of death after allogeneic and autologous hematopoietic stem cell transplantation (HSCT).
- Reduced intensity conditioning regimens are most commonly used in patients with comorbid conditions or requiring second allogeneic HSCT in an effort to reduce treatment-related mortality.
- Mechanisms to enhance donor T-cell alloreactivity, such as withdrawal of immunosuppression or donor leukocyte infusions, induce antileukemia effects in some patients, more commonly those with myeloid malignancies, but at the risk of graft-versus-host disease.
- Specific alloreactive cellular therapies such as cytotoxic T lymphocytes are investigational and hampered by the clear identification of antigens specific to the malignancy.
- Natural killer adoptive cellular therapy may represent a new treatment option to be studied in inducing remission in patients post-HSCT, most commonly in those with myeloid malignancies.
- Dendritic cells exhibit potential antitumor effects but at the risk of causing GVHD, but are dependent on the presence of T cells for their influence to be exerted.
- Post-transplant immunostimulatory options are the most likely adjuvant treatments to study to decrease relapse after HSCT.

References

Papers of special note have been highlighted as:
• of interest
•• of considerable interest

1 Doney K, Gooley TA, Deeg J, Flowers ME, Storb R, Appelbaum FR. Allogeneic hematopoietic cell transplantation with full-intensity conditioning for adult acute lymphoblastic leukemia: results from a single center, 1998–2006. *Biol. Blood Marrow Transplant.* DOI:10.1016/j.bbmt.2010.12.699 (2010) (Epub ahead of print).

2 Kroger N, Zabelina T, van Biezen A *et al.* Allogeneic stem cell transplantation for myelodysplastic syndrome with bone marrow fibrosis. *Haematologica* 90(2), 291–297 (2011).

3 Hubel K, Weingart O, Naumann F *et al.* Allogeneic stem cell transplantation in adult patients with acute myelogenous leukemia: a systematic analysis of international guidelines and recommendations. *Leuk. Lymphoma* 52(3), 444–457 (2011).

4 Locatelli F, Nollke P, Zecca M *et al.* Hematopoietic stem cell transplantation (HSCT) in children with juvenile myelomonocytic leukemia (JMML): results of the EWOG-MDS/EBMT trial. *Blood* 105(1), 410–419 (2005).

5 Stow P, Key L, Chen X *et al.* Clinical significance of low levels of minimal residual disease at the end of remission induction therapy in childhood acute lymphoblastic leukemia. *Blood* 115(23), 4657–4663 (2010).

6 Shook D, Coustan-Smith E, Ribeiro RC *et al.* Minimal residual disease quantitation in acute myeloid leukemia. *Clin. Lymphoma Myeloma* 9(Suppl. 3), S281–S285 (2009).

7 Campana D. Role of minimal residual disease monitoring in adult and pediatric acute lymphoblastic leukemia. *Hematol. Oncol. Clin. North Am.* 23(5), 1083–1098 (2009).

8 Lankester AC, Bierings MB, van Wering ER *et al.* Preemptive alloimmune intervention in high-risk pediatric acute lymphoblastic leukemia patients guided by minimal residual disease level before stem cell transplantation. *Leukemia* 24(8), 1462–1469 (2010).

9 Lee S, Kim YJ, Chung NG *et al.* The extent of minimal residual disease reduction after the first 4-week imatinib therapy determines outcome of allogeneic stem cell transplantation in adults with Philadelphia chromosome-positive acute lymphoblastic leukemia. *Cancer* 115(3), 561–570 (2009).

10 Holle W, Beck JF, Dueckers G *et al.* Clinical relevance of serial quantitative analysis of hematopoietic chimerism after allogeneic stem cell transplantation in children for severe aplastic anemia. *Bone Marrow Transplant.* 33(2), 219–223 (2004).

11 Bader P, Kreyenberg H, Hoelle W *et al.* Increasing mixed chimerism defines a high-risk group of childhood acute myelogenous leukemia patients after allogeneic stem cell transplantation where pre-emptive immunotherapy may be effective. *Bone Marrow Transplant.* 33(8), 815–821 (2004).

12 Bader P, Kreyenberg H, Hoelle W *et al.* Increasing mixed chimerism is an important prognostic factor for unfavorable outcome in children with acute lymphoblastic leukemia after allogeneic stem-cell transplantation: possible role for pre-emptive immunotherapy? *J. Clin. Oncol.* 22(9), 1696–1705 (2004).

13 Bacher U, Haferlach T, Fehse B, Schnittger S, Kroger N. Minimal residual disease diagnostics and chimerism in the post-transplant period in acute myeloid leukemia. *Scientific World Journal* 11, 310–319 (2011).

14 Stahl T, Badbaran A, Kroger N *et al.* Minimal residual disease diagnostics in patients with acute myeloid leukemia in the post-transplant period: comparison of peripheral blood and bone marrow analysis. *Leuk. Lymphoma*, 51(10), 1837–1843 (2010).

15 Diez-Campelo M, Perez-Simon JA, Perez J *et al.* Minimal residual disease monitoring after allogeneic transplantation may help to individualize post-transplant therapeutic strategies in acute myeloid malignancies. *Am. J. Hematol.* 84(3), 149–152 (2009).

16 Bader P, Willasch A, Klingebiel T. Monitoring of post-transplant remission of childhood malignancies: is there a standard. *Bone Marrow Transplant.* 42(Suppl. 2), S31–S34 (2008).

17 Elmaagacli AH, Beelen DW, Trenn G *et al.* Induction of a graft-versus-leukemia reaction by cyclosporine A withdrawal as immunotherapy for leukemia relapsing after allogeneic bone marrow transplant. *Bone Marrow Transplant.* 23, 771–777 (1999).

18 Deol A, Lum LG. Role of donor lymphocyte infusion in relapsed hematological malignancies after stem cell transplantation revisited. *Cancer Treat. Rev.* 36(7), 528–538 (2010).

19 Leis J, Porter DL. Unrelated donor leukocytes infusions to treat relapse after unrelated donor bone marrow transplantatian. *Leuk. Lymphoma* 43 (1), 9–17 (2002).

20 Takami A, Okumura H, Yamazaki H. Prospective trial of high-dose chemotherapy followed by infusions of peripheral blood stem cells and dose-escalated donor lymphocytes for relapse leukemia after allogeneic stem cell transplantation. *Int. J. Hematol.* 82(5), 449–455 (2005).

21 Loren AW, Porter DL. Donor Leukocyte infusions for the treatment of relapsed acuteleukemia after allogeneic stem cell transplantation. *Bone Marrow Transplant.* 41, 483–493 (2008).

22 Ringden O, Karlsson H, Olsson R *et al.* The allogeneic graft-versus-cancer effect. *Br. J. Haematol.* 147, 614–633 (2009).

23 Fozza C, Szydlo RM, Abdel-Rehim MM *et al.* Factors for graft-versus-host disease after donor lymphocyte infusions with an escalating dose regimen: lack of association with cell dose. *Br. J. Haematol.* 136, 833–836 (2006).

24 Raiola AM, Van Lint MT, Valbonesi M *et al.* Factors predicting response and graft-versus-host disease after donor lymphocyte infusion: a study on 593 infusions. *Bone Marrow Transplant*. 31, 687–693 (2003).

25 Choi S-J, Lee J-H, Lee J-H *et al.* Treatment of relapsed acute myeloid leukemia after allogeneic bone marrow transplantation with chemotherapy followed by G-CSF primed donor leukocyte infusion: high incidence of isolated extramedullary relapse. *Leukemia* 18, 1789–1797 (2004).

26 Schmid, C, Labopin M, Nagler A *et al.* Donor lymphocyte infusion in the treatment of first hematological relapse after allogeneic stem-cell transplantation in adults with acute myeloid leukemia: a retrospective risk factors analysis and comparison with other strategies by the EBMT Acute Leukemia Working Party. *J. Clin. Oncol.* 25, 4938–4945 (2007).

• **Reports the European Group for Blood & Marrow Transplantation's clinical experience with donor lymphocyte infusion (DLI) for treatment of relapsed acute myeloid leukemia (AML) after hematopoietic stem cell transplantation (HSCT) in 399 patients, comparing those who received DLI with those who did not receive DLI. Factors that predicted a response to DLI included a lower tumor**

burden at relapse, female gender, favorable cytogenetics and remission at the time of DLI administration.

27 Choi SJ, Lee JH, Lee JH *et al.* Treatment of relapsed acute lymphoblastic leukemia after allogeneic bone marrow transplantation with chemotherapy followed by G-CSF-primed donor leukocyte infusion: a prospective study. *Bone Marrow Transplant.* 36, 163–169 (2005).

28 Collins RH Jr, Goldstein S, Giralt S *et al.* Donor leukocyte infusions in acute lymphocytic leukemia. *Bone Marrow Transplant.* 26, 511–516 (2000).

29 Tomblyn M, Lazarus HM. Donor lymphocyte infusions: the long and winding road: how should it be traveled? *Bone Marrow Transplant.* 42, 569–579 (2008).

30 Huang X, Wang Y, Liu D. Modified donor lymphocyte infusion (DLI) for the prophylaxis of leukemia relapse after hematopoietic stem cell; transplantation in patients with advanced leukaemia – feasibility and safety study. *J. Clin. Immunol.* 28, 390–397 (2008).

31 Loren AW, Porter DL. Donor leukocyte infusions after unrelated donor hematopoietic stem cell transplantation. *Curr. Opin. Oncol.* 18, 107–114 (2006).

32 Zorn E, Mohseni M, Kim H *et al.* Combined $CD4^+$ donor lymphocyte infusion and low-dose recombinant IL-2 expand FOXP3 regulatory T cells following allogeneic hematopoietic stem cell transplantation. *Biol. Blood Marrow Transplant.* 15(3), 382–388 (2009).

33 Hicheri Y, Bouchekioua A, Hamel Y *et al.* Donor regulatory T Cells identified by FOXP3 expression but also by the membranous $CD4^+CD127^{low/neg}$ phenotype influence graft-versus-tumor effet after donor lymphocyte infusion. *J. Immunother.* 31, 806–811 (2008).

34 Miller JS, Weisdorf D, Burns L *et al.* Lymphodepletion followed by donor lymphocyte infusion (DLI) causes significantly more acute graft-versus-host disease than DLI alone. *Blood* 110, 2761–2763 (2007).

35 Orti G, Lowdell M, Fielding A *et al.* Phase I study of high-stringency CD8 depletion of donor leukocyte infusions after allogeneic hematopoietic stem cell transplantation. *Transplantation* 88, 1312–1318 (2009).

36 Meyer RG, Wagner EM, Konur A *et al.* Donor CD4 T cells convert mixed to full donor T-cell chimerism and replenish the CD52-positive T-cell pool after alemtuzumab-based T-cell-depleted allo-transplantation. *Bone Marrow Transplant.* 45, 668–674 (2009).

37 Ringden O, Labopin M, Gorin NC *et al.* The dismal outcome in patients with acute leukaemia who relapse after an autograft is improved if a second autograft or a matched allograft is performed. Acute Leukaemia Working Party of the European Group fro Blood and Marrow Transplantation (EBMT). *Bone Marrow Transplant.* 25 (10), 1053–1058 (2000).

38 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 Transplant.* 36(2), 157–162 (2005).

39 Ringden O, Laabopin M, Frassoni F *et al.* Allogeneic bone marrow transplant or second autograft in patients with acute leukemia who relapse after an autograft. Acute Leukaemia Working Party of the European Group for Blood and Marrow Transplantation (EBMT). *Bone Marrow Transplant.* 24(4), 389–396 (1999).

40 Eapen M, Giralt SA, Horowitz MM *et al.* Second transplant for acute and chronic leukemia relapsing after first HLA-identical sibling transplant. *Bone Marrow Transplant.* 34, 721–777 (2004).

41 Freytes CO, Lazarus HM. Second hematopoietic SCT for lymphoma patients who relapse after autotransplantation: another autograft or switch to allograft? *Bone Marrow Transplant.* 44(9), 559–569 (2009).

42 Martino R, Caballero MD, de la Serna J *et al.* Low transplant-related mortality after second allogeneic peripheral blood stem cell transplant with reduced-intensity conditioning in adult patients who have failed a prior autologous transplant. *Bone Marrow Transplant.* 30, 638 (2002).

43 Bashey A, Owzar K, Johnson JL *et al.* Reduced-intensity conditioning allogeneic hematopoietic cell transplantation for patients with hematologic malignancies who relapse following autologous transplantation: a multi-institutional prospective study from the Cancer and Leukemia Group B (CALGB trial 100002). *Biol. Blood Marrow Transplant.* 17(4), 558–565 (2010).

44 Nemecek ER, Guthrie KA, Sorror ML *et al.* Conditioning with treosulfan and fludarabine followed by allogeneic hematopoietic cell transplantation for high-risk hematologic malignancies. *Biol. Blood Marrow Transplant.* 17(3), 341–350 (2010).

45 Casper J, Wolff D, Knauf W *et al.* Allogeneic hematopoietic stem-cell transplantation in patients with hematologic malignancies after dose-escalated treosulfan/fludarabine conditioning. *J. Clin. Oncol.* 28(20), 3344–3351 (2010).

46 Guinan E, Luzmik L, Handgretinger R *et al.* Preservation of immune repertoire by selective depletion of haploidentical grafts. *Biol. Blood Marrow Transplant.* 16(Suppl. 1), S68–S74 (2010).

47 Waller EK, Giver CR, Rosenthal H *et al.* Facilitating T-cell immune reconstitution after haploidentical transplantation in adults. *Blood Cells Mol. Dis.* 33(3), 233–237 (2004).

48 Bryant GL, Gillespie GY, Lopez RD *et al.* Preclinical evaluation of ex vivo expanded/activated γδ T cells for immunotherapy for glioblastoma multiforme. *J. Neurooncol.* 101(2), 179–188 (2010).

49 Bethge WA, Haegele M, Faul C *et al.* Haploidentical allogeneic hematopoietic cell transplantation in adults with reduced-intensity conditioning and CD3/CD19 depletion: fast engraftment and low toxicity. *Exp. Hematol.* 34(12), 1746–1752 (2006).

50 Bethge WA, Faul C, Bornhäuser M *et al.* Haploidentical allogeneic hematopoietic cell transplantation in adults using CD3/CD19 depletion and reduced intensity conditioning: an update. *Blood Cells Mol. Dis.* 40(1), 13–19, (2008).

•• **This clinical trial demonstrates the safety and feasibility of performing haploidentical HSCT in adult recipients of peripheral blood grafts using CD3/CD19 depletion and a reduced intensity conditioning regimen. Neutrophil engraftment occurred at a median of 12 days, and the incidence of grade 2–4 acute graft-versus-host disease (GVHD) was 48%.**

51 Handgretinger R, Chen X, Pfeiffer M *et al.* Cellular immune reconstitution after haploidentical transplantation in children. *Biol. Blood Marrow Transplant.* 14(Suppl. 1), 59–65 (2008).

52 Chen X, Hale GA, Barfield R *et al.* Rapid immune reconstitution after a reduced-intensity conditioning regimen and a CD3-depleted haploidentical stem cell graft for paediatric refractory hematological malignancies. *Br. J. Haematol.* 135(4), 524–532 (2006).

53 Chaleff S, Otto M, Barfield RC *et al.* A large-scale method for the selective depletion of αβ T lymphocytes from PBSC for allogeneic transplantation. *Cytotherapy* 9(8), 746–754 (2007).

•• **This preclinical study demonstrates that peripheral blood stem cell grafts for allogeneic transplantation can be enriched for γδ cells by secretively depleting the graft of αβ T lymphocytes. These authors highlight the importance of developing novel graft manipulation strategies to improve the allo-immune effect after transplantation to reduce disease recurrence rates.**

54 Otto M, Barfield RC, Martin WJ *et al.* Combination immunotherapy with clinical-scale enriched human γδ T-cells, hu14.18 antibody, and the immunocytokine Fc-IL7 in disseminated neuroblastoma. *Clin. Cancer Res.* 11(23), 8486–8491 (2005).

55 Otto M, Barfield RC, Iynegar R *et al.* Human γδ T cells from G-CSF-mobilized donors retain strong tumoricidal activity and produce immunomodulatory cytokines after clinical-scale isolation. *J. Immunother.* 28(1), 73–78 (2005).

56 Godder KT, Henslee-Downey PJ, Mehta J *et al.* Long term survival in acute leukemia patients recovering wit increased γδ T cells after partially mismatched related donor bone marrow transplantation. *Bone Marrow Transplant.* 39(12), 751–757 (2007).

57 Miller JS, Warren EH, van den Brink MRM. NCI First international workshop on the biology, prevention and treatement of relapse after allogeneic hematopoietic stem cell transplantation: report from the committee on the biology underlyinh recurrence of malignant disease following allogeneic HSCT: graft-versus-tumor/leukemia reaction. *Biol. Blood Marrow Transplantation* 16(5), 565–586 (2010).

58 Randolph SS, Gooley TA, Warren EH. Female donors contribute to a selective graft-versus leukemia effect in male recipients of HLA-matched, related hematopoietic stem cell transplant. *Blood* 103, 347–352 (2004).

59 Stern M, Brand R, de Witte T *et al.* Female-versus-male alloreactivity as a model for minor histocompatibility antigens in hematopoietic stem cell transplants. *Am. J. Transplant.* 8, 2149–2157 (2008).

60 Warren EH, Fujii N, Akatsuka Y *et al.* Therapy of relapsed leukemia after allogeneic hematopoietic cell transplantation with T cells specific for minor histocompatibility antigens. *Blood* 115(19), 3869–3878 (2009).

61 Norde WJ, Overes IM, Maas F *et al.* Myeloid leukemic progenitor cells can be specifically targeted by minor histocompatibility antigen LRH-1-react cytotoxic T cells. *Blood* 113(10), 2312–2323 (2008).

62 Cooper LJN, Topp MS, Serrano LM *et al.* T-cell clones can be rendered specific for CD19: toward the selective augmentation of the graft-versus-B-lineage leukemia effect. *Blood* 101(4), 1637–1644 (2002).

63 Micklethwaite KP, Savoldo B, Hanley PJ *et al.* Derivation of human T lymphocytes from cord blood and peripheral blood with antiviral and antileukemic specificity from a single culture as protection against infection and relapse after stem cell transplantation. *Blood* 115(12), 2695–2703 (2010).

64 Wu CJ, Yang XF, McLaughlin S *et al.* Detection of a potent humoral response associated with immune-induced remission of chronic myelogenous leukemia. *J. Clin. Invest.* 106, 705–714 (2000).

65 Porter DL, Levine BL, Bunin N *et al.* A Phase 1 trial of donor lymphocyte infusions expanded and activated *ex vivo* via CD3/CD28 costimulation. *Blood* 107(4), 1325–1331 (2006).

66 De Angelis B, Dotti G, Quintarelli C *et al.* Generation of Epstein–Barr virus-specific cytotoxic T lymphocytes resistant to the immunosuppressive drug tacrolimus (FK506). *Blood* 114(23), 4784–4791 (2009).

67 Helsop HE, Savoldo B, Rooney CM. Cellular therapy of Epstein–Barr-virus-associated post-transplant lymphoproliferative disease. *Best Pract. Res. Clin. Haematol.* 17(3), 401–413 (2004).

68 Bollard CM, Savoldo B, Rooney CM, Helsop HE. Adoptive T-cell therapy for EBV-associated post-transplant lymphoproliferative disease. *Acta Haematol.* 110(2–3), 139–148 (2003).

69 Hsu KC, Gooley T, Malkki M *et al.* KIR ligands and prediction of relapse after unrelated donor hematopoietic cell transplantation for hematologic malignancy. *Biol. Blood Marrow Transplant.* 12(8), 828–836 (2006).

70 Leung W, Iyengar R, Triplett B *et al.* Comparison of killer Ig-like receptor genotyping and phenotyping for selection of allogeneic blood stem cell donors. *J. Immunol.* 174(10), 6540–6545 (2005).

71 Bachanova V, Burns LJ, McKenna DH *et al.* Allogeneic natural killer cells for refractory lymphoma. *Cancer Immunol. Immunother.* 59(11), 1739–1744 (2010).

72 Triplett B, Handgretinger R, Pui CH *et al.* KIR-incompatible hematopoietic-cell transplantation for poor prognosis infant acute lymphoblastic leukemia. *Blood* 107(3), 1238–1239 (2006).

73 Symons HJ, Leffell MS, Rossiter ND *et al.* Improved survival with inhibitory killer immunoglobulin receptor (KIR) gene mismatches and KIR haplotype B donors after nonmyeloablative, HLA-haploidentical bone marrow transplantation. *Biol. Blood Marrow Transplant.* 16, 533–542 (2010).

74 Pende D, Marcenaro S, Falco M *et al.* Anti-leukemia activity of alloreactive NK cells in KIR ligand-mismatched haploidentical HSCT for pediatric patients: evaluation of the functional role of activating KIR and redefinition of inhibitory KIR specificity. *Blood* 113, 3119–3129 (2009).

75 Miller JS, Soignier Y, Panoskaltsis-Mortari A *et al.* Successful adoptive transfer and *in vivo* expansion of human haploidentical NK cells in patients with cancer. *Blood* 105(8), 3051–3057 (2005).

76 Rizzieri DA, Storms R, Chen DF *et al.* Natural killer cell enriched donor lymphocyte infusions from a 3–6/6 HLA matched family member following non-myeloablative allogeneic stem cell transplantation. *Biol. Blood Marrow Transplant.* 16(8), 1107–1114 (2010).

77 Sanchez CJ, Le Treut T, Boehrer A *et al.* Natural killer cells and malignant haemopathies: a model for the interaction of cancer with innate immunity. *Cancer Immunol. Immunother.* 60(1), 1–13 (2010).

78 Locatelli F, Pende D, Maccario R *et al.* Haploidentical hematopoietic stem cell transplantation for the treatment of high-risk leukemias: how NK cells make the difference. *Clin. Immunol.* 133, 171–178 (2009).

79 Clausen J, Wolf D, Petzer AL *et al.* Impact of natural killer cell dose and donor killer-cell immunoglobulin-like receptor (KIR) genotype on outcome following human leucocyte antigen-identical hematopoietic stem cell transplantation. *Clin. Exp. Immunol.* 148(3), 520–528 (2007).

80 Geibel S, Locatelli F, Lamparelli T *et al.* Survival advantage in KIR ligand incompatibility in hematopoietic stem cell transplantation from unrelated donors. *Blood* 102(3), 814–819 (2003).

81 Lowe EJ, Turner V, Handgretinger R *et al.* T-cell alloreactivity dominates natural killer cell alloreactivity in minimally T-cell-depleted HLA-non-identical paediatric bone marrow transplantation. *Br. J. Haematol.* 123(2), 323–326 (2003).

82 Rubnitz J, Inaba H, Ribeiro RC *et al.* NKAML: as pilot study to determine the safety and feasibility of haploidentical natural killer cell transplantation in childhood acute myeloid leukemia. *J. Clin. Oncol.* 28(6), 955–959 (2010).

•• **This novel pilot study demonstrated the safety, feasibility and transient engraftment of haploidentical NK cells in children with AML after treatment with an immunosuppressive regimen of cyclophosphamide and fludarabine. All patients were in first remission at the time of NK cell infusion, which was administered as consolidation therapy. The 2-year survival rate was 100%.**

83 Rubnitz JE, Inaba H, Dahl G *et al.* Minimal residual disease-directed therapy for childhood acute myeloid leukemia: results of the multicentre AML02 trial. *Lancet Oncol.* 11(6), 543–552 (2010).

•• **This nontransplant multicenter trial in pediatric patients with AML highlights the importance of a multifaceted approach to therapy to improve overall survival. The authors demonstrate that prophylactic antibiotics, a thorough identification of leukemia cell characteristics at diagnosis, and the routine monitoring of minimal residual disease to guide therapy led to a 3-year event-free survival rate of 63%.**

84 Fujisaki H, Kakuda H, Shimasaki N *et al.* Expansion of highly cytotoxic human natural killer cells for cancer cell therapy. *Cancer Res.* 69(9), 4010–4017 (2009).

85 Leung W, Handgretinger R, Iyengar R *et al.* Inhibitory KIR-HLA receptor-ligand mismatch in autologous haematopoietic stem cell transplantation for solid tumor and lymphoma. *Br. J. Cancer* 97(4), 539–542 (2007).

86 Cho D, Shook DR, Shimasaki N *et al.* Cytotoxicity of activated natural killer cells against pediatric solid tumors. *Clin. Cancer Res.* 16(15), 3901–3909 (2010).

87 Moyer JS, Maine G, Mulé JJ. Early vaccination with tumor-lysate-pulsed dendritic cells after allogeneic bone marrow transplantation has antitumor effects. *Biol. Blood Marrow Transplant* 12(10), 1010–1019 (2006).

• **This preclinical study in mice demonstrated that dendritic cell vaccines could be safely administered in the study animals, resulting in tumor-specific disease responses while not exacerbating GVHD.**

88 Markey KA, Banovic T, Kuns RD *et al.* Conventional dendritic cells are the critical donor APC presenting alloantigen after experimental bone marrow transplantation. *Blood* 113(22), 5644–5649 (2009).

89 Mukaide H, Adachi Y, Koike-Kiriyama N *et al.* Immunotherapy for malignant tumors using combination of allogeneic intra-bone marrow-bone marrow transplantation, donor lymphocyte infusion and dendritic cells. *Int. J. Oncol.* 30(6), 1309–1315 (2007).

90 Van Tendeloo VF, Van de Velde A, Van Driessche A *et al.* Induction of complete and molecular remissions in acute myeloid leukemia by Wilms' tumor 1 antigen-targeted dendritic cell vaccination. *Proc. Natl Acad. Sci. USA* 107(31), 13824–13829 (2010).

91 Feuchtinger T, Opherk K, Bicanic O *et al.* Dendritic cell vaccination in an allogeneic stem cell recipient receiving a transplant from a human cytomegalovirus (HCMV)-seronegative donor: induction of a HCMV-specific T(helper) cell response. *Cytotherapy* 12(7), 945–950 (2010).

92 Neelapu SS, Munshi NC, Jagannath S *et al.* Tumor antigen immunization of sibling stem cell transplant donors in multiple myeloma. *Bone Marrow Transplant.* 36(4), 315–323 (2005).

93 Wright MP, Shepherd JD, Barnett MJ *et al.* Response to tyrosine kinase inhibitor therapy in patients with chronic myelogenous leukemia relapsing in chronic and advanced phase following allogeneic hematopoietic stem cell transplantation. *Biol. Blood Marrow Transplant.* 16, 639–646 (2010).

94 Ribera JM, Oriol A, González M *et al.* Concurrent intensive chemotherapy and imatinib before and after stem cell transplantation in newly diagnosed Philadelphia chromosome-positive acute lymphoblastic leukemia. Final results of the CSTIBES02 trial. *Haematologica* 95(1), 87–95 (2010).

95 Kang BW, Moon JW, Chae YS, Kim JC, Kim SN, Sohn SK. Pre-emptive therapy with nilotinib after second allogeneic transplantation in a Philadelphia chromosome-positive acute lymphoblastic leukemia patient with high risk of relapse. *Acta Haematol.* 123(4), 242–247 (2010).

96 de Lima M, Giralt S, Thall PF *et al.* Maintenance therapy with low-dose azacitidine after allogeneic hematopoietic stem cell transplantation for recurrent acute myelogenous leukemia or myelodysplastic syndrome: a dose and schedule finding study. *Cancer* 116(23), 5420–5431 (2010).

97 Pfeiffer M, Stanojevic S, Feuchtinger T *et al.* Rituximab mediates *in vitro* antileukemic activity in pediatric patients after allogeneic transplantation. *Bone Marrow Transplant.* 36(2), 91–97 (2005).

98 Baeuerle PA, Reinhardt C. Bispecific T-cell engaging antibodies for cancer therapy. *Cancer Res.* 69(12), 4941–4933 (2009).

99 Handgreting R, Zugmaier G, Henze G, Kreyenberg H, Lang P, von Stackelberg. Complete remission after blinatumomab-induce donor T-cell activation in three pediatric patients with post-transplant relapsed acute lymphoblastic leukemia. *Leukemia* 25, 181–184 (2011).

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Clinical options after failure of allogeneic hematopoietic stem cell transplantation in patients with hematologic malignancies

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Activity Evaluation
Where 1 is strongly disagree and 5 is strongly agree

	1	2	3	4	5
1. The activity supported the learning objectives.					
2. The material was organized clearly for learning to occur.					
3. The content learned from this activity will impact my practice.					
4. The activity was presented objectively and free of commercial bias.					

1. A 65-year-old woman who has been treated with chemotherapy for acute myeloid leukemia (AML) and is now being considered for hematopoietic stem cell transplantation (HSCT) presents. The patient and her husband are concerned regarding the possibility of relapse in this patient.
Which of the following statements regarding the risk for relapse after allogeneic HSCT is most accurate?

- ☐ **A** The relapse rate for acute leukemia after HSCT is less than 25%
- ☐ **B** Minimal residual disease (MRD) is predictive of outcome in chronic myeloid leukemia (CML) but not AML
- ☐ **C** Peripheral blood may be less sensitive than marrow samples in detecting MRD
- ☐ **D** MRD levels less than 1% predict a higher risk for relapse among patients with AML

2. The patient from question #1 undergoes HSCT but then develops evidence of relapse. What should you consider regarding the use of donor leukocyte infusions (DLIs)?

- ☐ **A** DLI is most effective for patients with AML
- ☐ **B** Relapses more than 6 months post-transplantation have higher chances of responding to DLI
- ☐ **C** The most significant complication of DLI is marrow aplasia
- ☐ **D** Remission rates of acute leukemias approach 80% after DLI

3. You also consider a second allogeneic HSCT for this patient. Which of the following statements regarding this therapy is most accurate?

- ☐ **A** It should be performed prior to DLI
- ☐ **B** It should be avoided among older patients with comorbid conditions
- ☐ **C** Fludarabine should be avoided because of its negative organ toxicity profile
- ☐ **D** Removing $CD3^+$ cells in the graft reduces the risk for GVHD

4. What else should you consider regarding novel strategies to treat this patient?

- ☐ **A** Cytotoxic T-lymphoctes (CTLs) are the primary lymphocyte population detected in the peripheral blood in the month following HSCT
- ☐ **B** Killer immunoglobulin-like receptors (KIRs) on NK cells function purely as activating receptors
- ☐ **C** KIR-ligand donor–recipient mismatch may increase the risk for GVHD
- ☐ **D** Infusions of dendritic cells improve outcomes among patients with relapse after HSCT