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REVIEW

T cell therapies following hematopoietic stem cell transplantation: surely there must be a better way than DLI?

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Advances in the past few years have significantly improved adoptive immunotherapy strategies available following autologous and allogeneic hematopoietic stem cell transplantation (HSCT). Minimal residual disease, relapsed disease and viral infections remain a significant cause of mortality in patients undergoing HSCT. Novel therapies are critically needed to overcome these management dilemmas, while sparing the graft-versus-tumor effect and avoiding graft-versus-host disease. This review focuses on the T-cell strategies currently available to allay disease while minimizing toxicities in patients who have undergone HSCT.
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Introduction

Hematopoietic stem cell transplantation (HSCT) remains the treatment of choice for many malignant and non-malignant diseases that affect the hematopoietic and immune systems. Adoptive T-cell immunotherapies offer ways to treat residual disease, viral infections and new malignancies after HSCT. However, the ability to balance the graft-versus-leukemia (GVL) effect with the detriments of graft-versus-host disease (GVHD) is at the forefront of concern when using these therapies. This review outlines these numerous T-cell immunotherapy strategies and their role in manipulating disease after HSCT.

Targets for T cell therapy

Target tumor antigens
Significant improvements have been made in T-cell immunotherapies, thanks to the identification of novel immunogenic tumor proteins by screening tumor-derived expression libraries or tumor cells using autologous sera.[1] The identification of these antigens and subsequent mapping of specific epitopes recognized by $CD4^+$ and $CD8^+$ T cells has allowed the development of strategies to augment tumor antigen-specific T-cell responses.[2]

Potential target antigens of hematologic malignancies fall into three distinct molecular categories. First, the vast majority of targets arise from major histocompatibility (MHC) class I- or II-associated peptides, including viral antigens, translocation peptides, mutated proto-oncogene peptides, cancer-testis antigens and minor histocompatibility antigens. Second, glycoproteins at the cell surface of clonotypic T-cell receptor of T-cell tumors lead them vulnerable to antibody targets.[3] Last, secreted proteins, such as the clonal Ig of multiple myeloma, offer another potential tumor-antigen target.

Alloantigens
Alloantigens that differ between donor and recipient are targets for T-cell recognition. MHC molecules arise in the setting of a mismatched donor and recipient; whereas, minor histocompatibility antigens (mHA) are peptides derived from normal cellular proteins that arise when different polymorphisms are present in donor and recipient. Thus far, data from donor lymphocyte infusions (DLIs) illustrate that responses to DLIs coincide with the increase in immune responses directed against these mHA.[4,5] Differences in mHA have been shown to be involved in both GVL and GVHD responses.[6] Moreover, the pattern of tissue expression of mHA varies, and those selectively expressed on hemopoietic cells or on particular lineages would provide specific targets for recognition.

Minor histocompatibility antigens, HA-1 and HA-2, targeted by donor cytotoxic T lymphocyte (CTL) clones have shown restricted specificity to recipient hematopoietic tissue, suggesting their use as recipient-restricted leukemia antigens.[7] HA-1 and HA-2 frequencies following DLIs have been linked to the occurrence of GVHD and disease remission in patients with chronic myelogenous leukemia (CML) and multiple myeloma, implicating a role for them in both GVHD and the GVL effect.[4] An early-phase clinical vaccine trial with HA-1 or HA-2 peptides after HSCT is currently in progress and should help shed light on any association between GVL and GVHD in relation to these peptides.[8]

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Tumor-associated antigens

Tumor-associated antigens (TAA) are those antigens expressed in cancer cells, but at low levels, or not at all, in normal tissues. Cancer–testis antigens (CTAs) are proteins with restricted expression among tumor cells and germinal tissues that are overexpressed in some cases of lymphoma, thereby making them potential cancer immunotherapeutic targets.[9–12] One example is NY-ESO-1, which elicits both specific $CD4^+$ and $CD8^+$ T-cell-mediated immune responses in patients with multiple myeloma.[13] NY-ESO-1 has been shown to be expressed in more than 60% of patients with poor-prognosis multiple myeloma at diagnosis and immunotherapeutic trials to treat residual disease after HSCT are now underway.[14,15]

To date, very few leukemia-associated antigens have been identified. PR1 is a CTL epitope peptide that has been shown to induce immune responses mediated by high-affinity $CD8^+$ T lymphocytes in human leucocyte antigen (HLA)-A2-positive patients with CML or acute myelogenic leukemia (AML).[16] A phase I/II clinical trial using peptide vaccination with PR1 is currently accruing HLA-A2-positive patients with AML, CML or myelodysplastic syndrome, in which immunological and clinical responses will be evaluated.[17]

A melanoma-associated antigen, PRAME (preferentially expressed antigen of melanomas), is recognized by CTL in an HLA-A24-restricted manner. High levels of this TAA in AML patients have been found to be associated with favorable clinical outcome and induced strong T-cell responses, thereby making it a potential target for immunotherapy.[18] This finding suggests that the expression of distinct tumor-specific TAAs on AML blasts might allow the immune system improved eradication of minimal residual disease following intensive chemotherapy.[18]

One of the most widely studied leukemia-associated proteins, as a potential target for immunotherapy, is the BCR–ABL fusion protein associated with CML. This fusion protein results from the reciprocal translocation t(9;22)(q34;q11) and the most common chimeric BCR–ABL mRNA (b3a2). Peptides derived from the fusion region have been found to bind to several HLA alleles and have been shown to elicit T cells that recognize peptide-pulsed target cells *in vitro*.[19–21] Recently, the modification of an amino acid at the HLA-binding anchor residues in BCR–ABL peptides showed that CTLs could be elicited against these altered peptide ligands while producing greater cytotoxicity without loss of the original sequence specificity.[21] Unfortunately, these CTLs failed to lyse specifically fresh CML cells, thereby limiting their clinical utility.

Viral antigens

Many viral-associated cancers possess highly immunogenic, unique epitopes that are available targets for a T-cell response. Virus-transformed cells may express a range of viral antigens that can be presented for CTL recognition, unlike most malignant cells that probably express small quantities of a single modified peptide as a CTL target. These virus-transformed cells are potentially much more susceptible to T-cell immunotherapy than tumor cells transformed by other mechanisms. For example, latent Epstein–Barr virus (EBV) infection is associated with many malignancies, including some cases of non-Hodgkin's lymphoma (NHL), Burkitt's lymphoma, NK cell lymphoma, lymphoproliferative disease (LPD), Hodgkin's lymphoma and nasopharyngeal carcinoma,[22–24] thereby making adoptive T-cell strategies targeting EBV an attractive option for EBV-related malignancies.

Clinical applications using cytotoxic T cells after HSCT

Ex vivo expanded CTLs have been used in clinical trials in the treatment of relapsed/refractory disease or viral infections after HSCT (Tables 1–3).

Table 1 Nonspecific T-cell immunotherapies

Type of therapy	*Mechanism*	*Clinical applications*	*Advantages*	*Disadvantages*	*Clinical trials*
Donor lymphocyte infusions	Unmanipulated donor T cells	Treat residual or recurrent disease post HSCT	Simple and cheap	Major risk for GVHD	
Allodepleted T cells	CD25 immunotoxin to selectively deplete alloreactive donor T cells	Improve T-cell recovery post HSCT	Spares virus and leukemia-specific T cells	Depletes regulatory T cells as well	Amrolia *et al.*;[102,103] Andre-Schmutz *et al.*[104]
Ex vivo expanded T cells	CD3/CD28 activated T cells	Improve lymphocyte recovery after autologous HSCT for NHL	Faster absolute lymphocyte recovery than controls	Incomplete restoration of T-cell number and function	Laport *et al.*[72]
Suicide gene transfection	HSV–TK-gene	Treatment of relapsed disease post HSCT	Ability to destroy cells if GVHD occurs	Immunogenic; impairs cell function, can lead to EBV infection; cannot use ganciclovir prophylaxis	Sauce *et al.*[65]
	Caspase9	Treatment of relapsed disease post HSCT	Ability to destroy cells if GVHD occurs	Unknown – clinical trial development underway	Straathof *et al.*[68]

Abbreviations: GVHD = graft-versus-host disease; HSCT = hematopoietic stem cell transplantation; NHL = non-Hodgkin lymphoma; HSV–TK = herpes simplex virus-thymidine kinase; EBV = Epstein Barr virus.

Table 2 Specific T-cell immunotherapies

Type of therapy	*Mechanism*	*Clinical applications*	*Advantages*	*Disadvantages*	*References*
Antigen-specific CTLs					
Anti-tumor	EBV-specific CTLs	Treatment of relapsed or refractory EBV+ lymphomas or EBV-LPD post HSCT	Specific to EBV	Prolonged culture periods *in vitro* and may lack tumor specific CTL	Rooney *et al.*;[86] Gottschalk *et al.*;[87] Gustafsson *et al.*;[105] Papadopoulos *et al.*;[80] Heslop *et al.*[106]
	LMP2-specific CTLs	As above	Improved tumor specificity	Prolonged culture periods *in vitro*	Bollard *et al.*[107]
Antiviral	CMV-CTLs	Treatment or prophylaxis of CMV infections post HSCT	Clear active CMV and immune reconstitution to CMV	May have prolonged culture periods *in vitro*	Walter *et al.*;[81] Einsele *et al.*;[108] Riddell *et al.*;[90] Cobbold *et al.*;[109] Peggs *et al.*[42]
	ADV-CTLs	Treatment of adenovirus infections post HSCT	Clearance of Adv	Efficacy for treating ADV disease not yet proven	Feuchtinger *et al.*[94]
	Trivirus-CTLs	Treatment of EBV, CMV or adenovirus infections post HSCT	Single culture and clearance of multi viruses *in vivo*	Prolonged culture periods *in vitro*	Leen *et al.*[96]
CIK cells	CTLs with CD3+ CD56+ phenotype	Treatment of lymphomas in autologous HSCT setting	Easy to generate	May be more useful in treating MRD than active disease	Leemhuis *et al.*[77]
Tumor-associated antigen therapy	PR1 vaccination	Treatment for HLA-A2+ myeloid leukemias and MDS	Specific to certain leukemias	Clinical trial underway; limited to HLA-A2 patients	Qazilbash *et al.*[110]
	NY-ESO-1	Treatment of multiple myeloma	Present in 60% with poor prognosis	Clinical trial underway	Szmania *et al.*[15]
Infusion of functional subsets	CD4+ Th2 cells generated via CD3/CD28 costimulation with IL-4	Treatment of B and T lymphomas and myeloid leukemias in attempt to separate GVL effect from GVHD after HSCT	Could offer therapy for relapsed diseases not usually cured by conventional DLI	Does not change risk of GVHD	Fowler *et al.*;[60,111] Introna *et al.*[78]

Abbreviations: ADV = adenovirus; CIK = cytokine-induced killer; CMV = cytomegalovirus; CTL = cytotoxic T lymphocyte; EBV = Epstein–Barr virus; HSCT = hematopoietic stem cell transplantation; LMP2 = latent membrane protein 2; MDS = myelodysplastic syndrome.

Table 3 Donor lymphocyte infusions in treatment of multiple myeloma after allogeneic HSCT

Therapy	*Patients*	*Results*	*Toxicities*	*Reference*
DLI after non-myeloablative allogeneic HSCT	48 relapsed MM; 15 persistent MM	24/63 (38.1%) responded 12 (19%) CR 12 (19%) PR Median DFS of CR patients 27.8 months	Acute GVHD 38.1% Chronic GVHD 42.9% 7 (11.1%) died from TRM	Van de Donk *et al.*[46]
DLI ± chemotherapy after allogeneic HSCT	25 relapsed or persistent MM	2/22 responded to DLI alone 3 responded to DLI + chemotherapy 9 received additional DLI: 2 CR 3 PR	13/25 acute GVHD 11/21 chronic GVHD All responders developed GVHD	Salama *et al.*[48]
DLI after reduced intensity conditioning allogeneic HSCT	11 residual MM; 8 relapsed/ progressed MM	1 CR; 8 PR Only 2 still in PR to date	7/8 responders developed GVHD 4 deaths attributed to GVHD	Peggs *et al.*[50]

Abbreviations: CR = complete response; DLI = donor lymphocyte infusion; GVHD = graft-versus-host disease; MM = multiple myeloma; PR = partial response; TRM = treatment-related mortality.

Unmanipulated donor T cells

Perhaps the most successful T-cell immunotherapy to date has been the use of unmanipulated allogeneic donor T cells, following allogeneic HSCT. DLI has been used for many years to treat relapses of myeloid leukemias following allogeneic HSCT by providing further GVL effect to

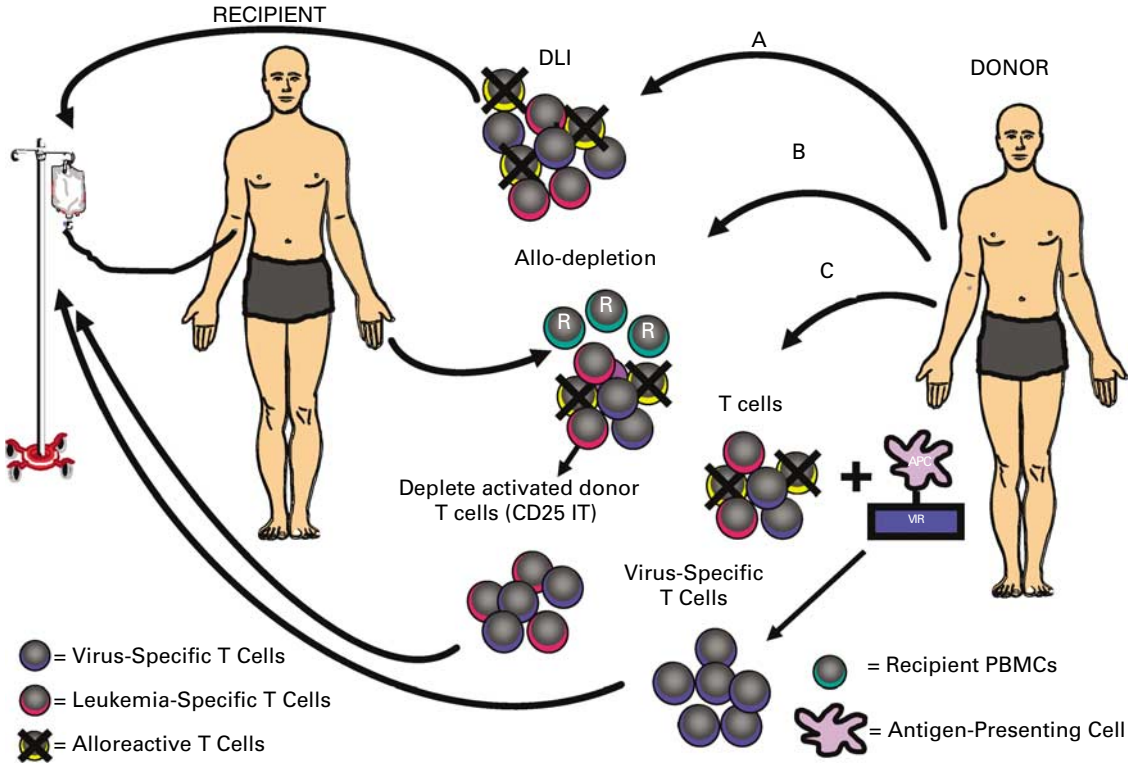

Figure 1 Three major forms of adoptive T-cell strategies following HSCT are shown. (A) Unmanipulated DLIs directly from donor to recipient involves the adoptive transfer of all T cells, including viral- and leukemia-specific T cells along with alloreactive T cells. (B) Allodepletion allows the selective depletion of donor-derived alloreactive T cells that are activated after coculture with irradiated recipient-derived mononuclear cells or B cells. This strategy utilizes CD25 immunotoxin to bind to the activated $CD25^+$ donor T cells and selectively remove them from the final 'allodepleted' product. The aim is that the remaining T cells in the allodepleted product infused to the patients provide viral- and leukemia-specific activity without inducing alloreactivity. (C) Antigen-specific T cells (e.g. targeting viruses) are expanded after exposure to APCs expressing the viral antigens.

eradicate residual disease (Figure 1). DLI is most effective in patients with relapsed CML resulting in hematologic and cytogenetic responses of approximately 70–80% for patients in chronic phase.[25] DLI is intermediately effective for relapse of AML with between 15 and 40% of patients achieving complete remission following DLI alone,[26] but is rarely successful for patients with relapsed acute lymphoblastic leukemia (ALL). The comparative lack of efficacy of DLI in the acute leukemia patients as opposed to CML patients is unclear and could be related to a lack of antigen expression on tumor cells, alterations of other molecules that contribute to T-cell recognition or the difficulty overcoming a growing tumor burden with immunotherapy alone. This modality of therapy is limited by potentially fatal complications that arise from alloreactive T cells also present in the lymphocyte infusion.[27] In other words, with the benefits of the GVL effect comes a high risk of GVHD, with up to 50% patients experiencing significant GVHD after treatment with DLI.[28]

Nevertheless, studies evaluating CML responses to DLI have found that up to 55% of patients who do not experience GVHD may have a disease response,[29,30] demonstrating that GVL can be separable from GVHD. However, now that we are in the era of imatinib, it has been questioned whether this tyrosine kinase inhibitor alone can produce durable molecular remissions in CML patients following HSCT. Weisser *et al.*[31] reported that imatinib therapy alone resulted in a higher incidence of relapse and inferior leukemia-free survival ($P = 0.006$ and 0.016, respectively), following HSCT compared to patients who received DLI.

In an attempt to improve upon the dismal results that relapsed ALL patients achieve following DLIs, Choi *et al.*[32] recently conducted a prospective study to determine whether G-CSF-stimulated DLI could induce higher remission rates. Of ten patients, seven achieved a complete remission at a median of 25 days; however, only one remains alive in complete response (CR) 907 days following DLI. Two patients died in CR of GVHD, and the remaining four relapsed at a median of 153 days. Thus, the use of DLI alone, or primed with G-CSF, does not seem to change the lack of a significant GVL effect in ALL patients.

The first reports of successful DLI in the treatment of lymphoma were in patients who developed EBV-associated LPD (EBV–LPD). Investigators administered unmanipulated DLI to HSCT recipients with established EBV–LPD following allogeneic HSCT.[33] The rationale for this strategy was that this malignancy only occurs in immunosuppressed patients and is normally controlled by an EBV-specific T-cell response. Most EBV-seropositive individuals have a high frequency of EBV-specific precursors, so that transfer of unmanipulated DLI should be able to restore the immune response to EBV. In this setting, the overall

response rate was high, with 20 of 22 patients attaining complete remissions.[34] Other centers have seen lower response rates to DLI, possibly reflecting differing patient populations or suboptimal EBV-specific immune responses in donors.[35]

There have also been several groups who have used DLI for the treatment of residual Hodgkin's and non-Hodgkin's lymphoma following allogeneic HSCT.[36–39] While the response rates in low-grade lymphomas have been very promising (over 60%), the response for high-grade lymphomas has been low.[36–39] The existence of significant graft-versus-Hodgkin's lymphoma activity following allogeneic HSCT has proven difficult to establish and may be hindered by the particularly high treatment-related mortality rates in this heavily pretreated patient population. However, the use of submyeloablative regimens could offer promise for decreasing the toxicity-related deaths for these patients. Furthermore, transplant registry-based studies report that relapse rates are lower in HL patients who develop GVHD after allogeneic HSCT versus those who do not.[40,41] Based on this information, DLI was administered to 16 patients with residual disease or disease progression of HL following a reduced-intensity conditioning regimen resulting in nine disease responses, including eight CRs. However, severe, acute GVHD developed in six and chronic GVHD in five.[42] More recently, Anderlini *et al.*[43] reported on nine patients with advanced HL who received DLI for disease persistence or progressive disease, following allogeneic HSCT and observed a response rate of 44%. All but one patient developed GVHD following DLI. The median $CD3^+$ cell dose was 77.5×10^6 cells/kg (range, 5–285) and no correlation was observed between $CD3^+$ cell dose and disease response.

DLI has also been evaluated in patients with relapsed or persistent metastatic myeloma following allogeneic HSCT. DLI alone has been shown to induce response rates in 40–67% of patients with metastatic myeloma.[44–49] In a recent study, prolonged survival was seen in patients who responded to DLI; however, the incidences of significant acute and chronic GVHD were high (38 and 43%, respectively), and the occurrence of GVHD was the only significant prognostic factor for response to DLI.[46] It is also important to note that while a graft-versus-myeloma effect has been shown in several case reports and small patient populations, they must be interpreted carefully as many patients were receiving either interferon for immunological modulation[47,48] or corticosteroids for GVHD prophylaxis, which has anti-myeloma properties, at the time of DLI. Further investigation of allogeneic approaches for lymphomas and myeloma are clearly warranted; however, the development of strategies to maximize efficacy and minimize the toxicity of these T-cell therapies is crucial.

One frequently raised question is the association between T-cell dose infused and the development of GVHD. To attempt to answer this question, Peggs *et al.*[50] conducted a dose-escalation study using DLI after HSCT for patients with hematologic malignancies. They showed that DLI doses in excess of 1×10^7 cells/kg from sibling donors were associated with a significantly increased risk of GVHD ($P < 0.0001$). The risk of GVHD was even more common and occurred at lower T-cell doses in patients with unrelated donors.[50]

Another approach to minimize GVHD while maximizing the GVL effect is the selective depletion of $CD8^+$ T cells from either the allograft or from donor lymphocytes, which has been shown to efficiently reduce the incidence and severity of GVHD.[51,52] Recently, a new method of $CD8^+$ T-cell depletion for this purpose was described using a $CD8^+$ murine monoclonal antibody-coated high-density microparticle, which allows for the rapid depletion of $CD8^+$ T-cells from apheresis products using gravity sedimentation.[53] Alyea *et al.*[53] conducted a pilot study using this $CD8^+$ T-cell depletion technique in nine patients (three multiple myeloma, two chronic lymphocytic leukemia and one NHL) and demonstrated their ability to deplete the product of 99.3% $CD8^+$ T cells with a median recovery of 75% $CD4^+$ cells. All patients achieved a complete molecular remission without any infusional toxicities and only one patient developed GVHD.

Allodepleted T cells

Selective depletion of alloreactive T cells (cells that express activation markers in response to alloantigen) from the donor product provides another approach to dealing with the issue of alloreactivity (Figure 1). Our group and others have used an anti-CD25 immunotoxin (RFT5-SMPT-dgA immunotoxin generated by linking a murine anti-CD25 monoclonal antibody to a deglycosylated ricin α chain) to deplete alloreactive lymphocytes at the time of HSCT.[48,54,55] Preclinical studies have shown that this method can deplete alloreactive cells whilst preserving T cells reactive with cytomegalovirus (CMV), EBV and tumor antigens such as PR1 and HA-1.[56] Early-phase clinical trials have shown that this approach can be safely used to improve T-cell recovery after haploidentical SCT with a low incidence of significant GVHD.[54] Specifically, Amrolia *et al.*[54] reported the results from a dose-escalation study using allodepleted T cells following haploidentical transplantation in 16 patients with a median age of nine years (range, 2–58), treated mostly for high-risk hematologic malignancies.[56] Each patient was scheduled to receive three infusions of allodepleted donor T cells on days 30, 60 and 90 after HSCT. Eight patients received dose level 1 (10^4 cells/kg/dose) and eight patients received dose levels 10^5 cells/kg/dose. Only two patients developed significant acute GVHD, followed by extensive, chronic GVHD, with death in one of those patients from liver failure associated with GVHD and adenovirus. Patients at dose level 1 had T-cell reconstitution consistent with other patients undergoing haploidentical HSCT without allodepleted T-cell add back. However, patients at dose level 2 showed significantly improved T-cell recovery time, particularly at 3–5 months after HSCT, which is most often the time period in which patients die of infection following haploidentical HSCT.[56]

Infusion of functional subsets

As another approach to overcome the risk of GVHD with DLI, investigators have evaluated specific subsets of lymphocytes, such as CD4-selected cells,[57] CD8-depleted

cells,[58] or functionally defined subsets such as Th2 cells,[59] in the hope that GVL and GVHD may be separated. In animal models, the culture of functionally defined T-cell subsets, such as Tc2 or Th2 cells, has allowed antitumor effects to be produced in the absence of alloreactivity. A recent phase I clinical trial at the NIH evaluated the feasibility of infusing donor CD4 Th2 cells generated *ex vivo* using CD3/CD28 costimulation in the presence of IL-4 and IL-2 in patients with hematologic malignancies, who received G-CSF-mobilized matched sibling donor HSCT following reduced intensity conditioning regimens.[60] Nineteen patients received no additional cells, and the remaining 28 received these Th2-like cells in a dose-escalation fashion (5, 25 or 125×10^6 cells/kg). The Th2 cohort had accelerated lymphocyte reconstitution with acute GVHD and overall survival similar in both the Th2 and non-Th2 cohorts.[60]

Suicide genes

Another potential solution to alloreactivity from unmanipulated T cells is to transduce the donor T cells with a suicide gene, so that they can be ablated if GVHD occurs. The herpes simplex virus 1–thymidine kinase (HSV–TK) gene has been used in several clinical trials without significant acute toxicity. Bonini *et al*.[61] used an HSV–TK-modified DLI to treat EBV–LPD or relapsed leukemia after allogeneic T-cell-depleted HSCT in a small population of patients and showed the modified cells had antitumor efficacy, persisted in the circulation and could be eliminated by the administration of ganciclovir. Tiberghien *et al*.[62] administered HSV–TK-positive T cells in conjunction with a T-cell-depleted graft to 12 HSCT recipients and also confirmed the persistence of HSV–TK cells; however, results in subsequent studies in which HSV–TK donor T cells administered following non-T-cell-depleted grafts have not shown the persistence of the gene-modified cells in most patients.[63] Limitations have arisen, such as a reduction in the immune function of the gene-modified T cells and immunogenicity of these cells, which can lead to the destruction of TK-expressing lymphocytes.[64] A phase I study of 12 patients who received donor T cells transduced with the HSV–TK gene soon after transplant resulted in three patients with EBV–LPD, likely due to impaired EBV reactivity in the product.[65] The use of CD3 and CD28 for T-cell activation can potentially overcome this problem of activation-induced cell death because CD28 costimulation provides T cells with signals to prevent the induction of anergy and promotes IL-2 production and clonal expansion.[66] New non-immunogenic suicide genes, based on inducible Fas and Caspase9, also have been developed.[66–68] These suicide genes are involved in T-cell apoptosis, and their activation is dependent on a dimerization process that can be activated by a nontoxic chemical inducer of dimerization (CID). These molecules have been fused to a human FK506-binding protein to allow conditional dimerization, and *in vitro* and *in vivo* studies demonstrated that T cells transduced with a retroviral vector coding for the dimerizable gene can be selectively eliminated after the exposure to the CID.[68]

Ex vivo *expanded T cells*

Over the past few years several studies have correlated faster recovery of lymphocyte counts with improved outcome after autologous transplant for NHL, HL, AML and multiple myeloma.[69–71] CD3/CD28 costimulated T cells have also been administered safely to patients with relapsed or refractory non-Hodgkin's lymphoma patients following high-dose chemotherapy with $CD34^+$-selected autologous HSCT.[72] Thus far, this approach has been shown to be feasible and associated with a rapid recovery of lymphocytes, but without clear data showing antitumor activity.[72] Another group recently reported a phase I study using *ex vivo* activated DLI for 18 patients with relapse of aggressive malignancies after HSCT.[73] All patients received induction chemotherapy and conventional DLI, followed 12 days later by activated DLI (dose range, 1×10^6–1×10^8 $CD3^+$ cells/kg). Eight patients achieved complete remission, including four of seven with ALL, two of four with AML, one of two with NHL and one with CLL. Seven patients developed acute GVHD and four chronic GVHD.[73] Thus, while these numbers are small, the adoptive transfer of costimulated activated T cells may allow durable remissions in diseases that usually are not cured by conventional DLI alone without significant increases in GVHD.

Cytokine-induced killer cells

Expanded cells also have been used in the autologous setting to treat relapse or to augment T-cell function against minimal residual disease. Cytokine-induced killer (CIK) cells are a unique population of $CD3^+$ $CD56^+$ CTLs generated from peripheral blood mononuclear cells (PBMC), stimulated with appropriately timed anti-CD3, interferon-γ and interleukin-2.[74–76] A clinical trial included nine heavily pretreated patients with advanced Hodgkin's and non-Hodgkin's lymphoma treated with escalating doses of CIK cells without significant toxicity. Two patients achieved partial responses and two had stabilization of disease.[77] Given these results, this approach may have greater activity in the setting of minimal residual disease. Another group has also examined the role of CIK cells in cord blood. *In vitro* studies showed that CIK cells with strong cytotoxic activity against a variety of tumor target cell lines including B and T lymphomas and myeloid leukemias can be expanded from mononuclear cells derived from cord blood using anti-CD3, interferon-γ and interleukin-2.[78] However, further studies are warranted to demonstrate the feasibility and reproducibility of *ex vivo*-expanding cord-blood-derived cytotoxic T cells.

Antigen-specific CTLs

Adoptive immunotherapeutic approaches using CTLs have been found to be beneficial in treating relapse of some malignancies and viral infections after HSCT.[33,79,80] In order to develop immunotherapies using CTLs, potential target antigens on tumor cells must first be identified to minimize the risk of GVHD. To target a tumor, it must not only contain unique proteins capable of providing epitopes for specific immune responses, but also present candidate peptides frequently enough and for sufficient duration to

engage responder T cells. Either the tumor cell or a specialized antigen-presenting cell (APC) must express MHC antigens and costimulatory molecules, such as CD28, to induce T-cell activation. The T-cell response is then influenced by the specific type of APC that determines whether there is effector and memory T-cell generation or development of T-cell tolerance.[81]

EBV-specific CTLs

An ongoing balance exists in normal seropositive individuals between the EBV viral load and the immune defense mechanisms. However, following transplantation, when cytotoxic T-cell numbers and/or activity are suppressed, the EBV-infected B cells expressing a type 3 latency are able to proliferate unchecked, which results in accumulation of EBV-infected B cells in the body and enhanced viral replication as demonstrated by elevated levels of EBV DNA detected in the peripheral blood by polymerase chain reaction.[82] These changes reflect the loss of CTL activity and in some patients, uncontrolled EBV driven B-cell lymphoproliferation occurs leading to development of EBV–LPD.

The humanized murine CD20 antibody rituximab (Rituxan; IDEC Pharmaceuticals, San Diego, CA, USA and Genentech Inc., San Francisco, CA, USA) has offered a promising strategy for the treatment of EBV–LPD after HSCT.[83] However, there are potential hazards of anti-CD20 therapy. First, the efficacy of anti-CD20 is due to the successful depletion of the EBV-infected B cells. However, the underlying EBV-specific cytotoxic T-cell deficiency is not restored with this therapy. In addition, the profound B cell depletion may further exacerbate immunodeficiency in transplant recipients although eventual recovery should occur as CD20 is not expressed on B-cell precursors. Finally, anti-CD20 therapy may result in selection of a CD20-negative population of proliferating B cells.[84]

Because the tumor cells in EBV–LPD are highly immunogenic expressing all nine latent cycle EBV antigens, including the immunodominant EBNA3 antigen, EBV–LPD is an excellent model to evaluate EBV-specific CTLs and restore the underlying T-cell deficiency in this disease. Moreover, lymphoblastoid cell lines generated by infecting donor lymphocytes with a laboratory strain of EBV serve as excellent APCs for the expansion of EBV-specific CTL *in vitro*.

Our group has used donor-derived EBV-specific CTLs as prophylaxis for EBV–LPD in over 60 patients who received a T-cell-depleted HSCT or were transplanted for an EBV-associated malignancy.[85–87] Gene marking of donor CTLs showed persistence of infused CTLs for as long as seven years.[88] None of these patients developed LPD, compared to 11.5% of a historical, non-treated control group.[86] However, one of the limitations of EBV-specific CTL therapy includes the development of escape mutants.[89] An additional limitation is that since post transplant lymphoproliferative disease requires immediate treatment, CTLs must be available at diagnosis. The generation of EBV-specific CTLs requires two to three months, although this time can be reduced by using EBV antigen-loaded dendritic cells as APCs. However, with the advent of anti-CD20 therapy, the use of EBV-specific CTL after HSCT are probably better utililized for high-risk patients (e.g. patients with Wiscott–Aldrich syndrome or patients with pre-exisiting EBV-positive lymphomas) or for patients with EBV–LPD who are refractory to CD20 antibody therapy.

Another approach against EBV-related malignancies is the use of CTLs targeting the immunosubdominant latent membrane protein (LMP) 2. We have generated LMP2-specific CTL lines in 19 patients with EBV-positive HL or EBV-positive B-cell or T/NK-cell NHL.[90,91] Using LMP2-specific tetramers, a significantly increased number of LMP2-specific CTLs were detected in the LMP2-CTL lines compared to EBV-CTL lines generated with genetically unmodified LCL from the same patients. Patients received doses ranging from 4×10^7 to 1.2×10^8 CTLs/m^2, and no immediate toxicity was observed. Seven of eight patients without radiological evidence of disease who received CTLs as adjuvant therapy after HSCT or chemotherapy remain well up to 36 months following CTL infusion.[91] We have also seen complete clinical responses in five of six patients treated with active disease. Although this study is ongoing, immunotherapy with autologous LMP2-CTLs appears well tolerated in patients with relapsed EBV-positive HL/NHL and infused LMP2-CTL cells can accumulate to tumor sites and induce clinical responses.

Multivirus-specific CTLs

The use of donor-derived, virus-specific CTLs offers an alternative or adjuvant means of treating serious, life-threatening viral infections that often occur in HSCT recipients. Three of the most commonly seen viral infections after HSCT includes EBV and CMV, which usually indicate reactivations of latent infections, and adenovirus; however, antiviral therapies have limited efficacies and are associated with significant toxicities, making adoptive immunotherapy strategies an attractive option. CTLs targeting EBV, CMV or adenovirus have been generated in our and others' laboratories and used with success for the treatment and prevention of these viral infections in HSCT recipients.[79,85,92–95] Because it is not feasible to expand individual CTL lines against all three viruses on every patient, we have now focused on the generation of trivirus-specific (EBV, CMV and adenovirus) CTLs for use in HSCT recipients.[96] Using a chimeric adenovirus-CMVpp65 vector to modify EBV-expressing B-cell lines, a single culture of donor PBMCs was able to generate CTLs specific for EBV, CMV and adenovirus (Figure 1). Eleven HSCT recipients received trivirus-specific CTLs (median, 5×10^7 cells/m^2) without any significant toxicities or GVHD. All individuals with active EBV, CMV or adenovirus infection had rapid reduction in viral titer with resolution of symptoms and corresponding increase in virus-specific CTL expansion *in vivo*.[96] Therefore, this study demonstrated that it is possible for APCs in a single culture consistently to generate effector T cells directed to at least three viruses, and that *in vivo* the trivirus-specific cells expand and are active against all three viruses. Furthermore, restoring the immunity to three viruses simultaneously obviates the need for continued prophylaxis using pharmacotherapy, thereby offering a

potentially cost effective approach to protect HSCT recipients from these potentially lethal viruses. Although the current methodology to generate multi-virus specific CTL is over 6 weeks, having validated the approach, there is no doubt that – as for any technology – methodological improvements will further simplify the exact process used.

Tumor-infiltrating lymphocytes

Cellular-based antitumor immunity is initiated by $CD8^+$ CTLs containing T-cell receptor (TCR) that recognize specific tumor-associated peptides bound to class I MHC molecules for which the TCR determines the specificity of this binding. Recently, Rosenberg's group derived MART-1 (melanoma antigen-reactive) tumor-infiltrating lymphocyte (TIL) clones from the tumors of five patients and TCR genes were isolated from these clones.[97] They proposed that the highest-avidity TCR-identified, CD8-independent DMF5 clone, generated from a TIL infusion that mediated tumor regression clinically, has potential for allogeneic TCR gene therapy in metastatic melanoma patients. This TCR was able to transform non-reactive donor $CD8^+$ and $CD4^+$ PBMCs and TILs to recognized MART-1-expressing tumors, as well as produce high levels of multiple immunologically relevant cytokines and lyse tumor cells *in vitro*.[97]

Adoptive transfer of autologous TILs has also been shown to mediate tumor regression in 50% of lymphodepleted patients with metastatic melanoma; however, they are difficult to generate.[98,99] A cancer vaccination trial used autologous PBMC from nine gp100-vaccinated patients with metastatic melanoma were stimulated *ex vivo* with gp100:209–217(210 M) peptide and adoptively transferred back to the patient along with high-dose IL-2 and cancer vaccination; however, no clinical response occurred.[100]

A major limitation of this approach is that patients must have pre-existing tumor-reactive cells that can be expanded *ex vivo*. In many patients, especially those with non-melanoma malignancies, it is difficult to identify these tumor-reactive lymphocytes. As a way around this limitation, one group showed that they were able to confer tumor recognition by autologous lymphocytes from peripheral blood by using a retrovirus that encodes a TCR.[101] The adoptive transfer of these cells in 15 patients with metastatic melanoma resulted in durable engraftment at levels exceeding 10% of peripheral blood lymphocytes for at least two months after infusion, and they observed high levels of circulating, engineered cells one year after infusion in two patients that demonstrated objective regression of tumor lesions.[97]

Future considerations

Adoptive T-cell immunotherapies offer a multitude of strategies to influence the immune system in an attempt to treat relapsed or persistent malignant disease after HSCT. While DLI remains the mainstay of adoptive immunotherapy following HSCT, many new approaches have been developed in the past 10 years, allowing for more specific responses to relapsed or persistent leukemias and tumors, or viruses that affect patients following HSCT. However, many clinical studies have also identified limitations of such strategies, including inadequate persistence or expansion *in vivo*. With better understanding of the optimum methodology for T-cell product generation, identification of further antigen targets and enhancement of gene therapy approaches to augment the function of adoptively transferred T cells, these immunotherapy strategies may find a more defined role in the therapy of malignant disease and infection following HSCT. The generation of viral-specific CTLs, methods to augment the DLI approach and the use of peptide vaccination are just a few of the newer methods being employed to assist the never-ending battle against malignant disease and viral infections, while preventing the devastating effects of GVHD in these patients.

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