Report

# Proceedings from the National Cancer Institute's Second International Workshop on the Biology, Prevention, and Treatment of Relapse after Hematopoietic Stem Cell Transplantation: Part III. Prevention and Treatment of Relapse after Allogeneic Transplantation

Marcos de Lima [1], David L. Porter [2], Minoo Battiwalla [3], Michael R. Bishop [4], Sergio A. Giralt [5], Nancy M. Hardy [6,*], Nicolaus Kröger [7], Alan S. Wayne [8,9], Christoph Schmid [10]

[1] Hematologic Malignancies and Stem Cell Transplant Program, University Hospitals Seidman Cancer Center, Case Western Reserve University, Cleveland, Ohio
[2] Blood and Marrow Transplantation, Hospital of the University of Pennsylvania, Philadelphia, Pennsylvania
[3] Hematology Branch, National Heart, Lung and Blood Institute, Bethesda, Maryland
[4] Section of Hematology/Oncology, University of Chicago Medicine, Chicago, Illinois
[5] Department of Medicine, Memorial Sloan-Kettering Cancer Center, New York, New York
[6] Experimental Transplantation Immunology Branch, National Cancer Institute, Center for Cancer Research, Bethesda, Maryland
[7] Department of Stem Cell Transplantation, University Medical Center Hamburg-Eppendorf, Hamburg, Germany
[8] Pediatric Oncology Branch, National Cancer Institute, Center for Cancer Research, Bethesda, Maryland
[9] Current: Children's Center for Cancer and Blood Diseases, Children's Hospital Los Angeles, Keck School of Medicine, University of Southern California, Los Angeles, California
[10] Klinikum Augsburg, University of Munich, Munich, Germany

A B S T R A C T

In the Second Annual National Cancer Institute's Workshop on the Biology, Prevention, and Treatment of Relapse after Hematopoietic Stem Cell Transplantation, the Scientific/Educational Session on the Prevention and Treatment of Relapse after Allogeneic Transplantation highlighted progress in developing new therapeutic approaches since the first relapse workshop. Recent insights that might provide a basis for the development of novel, practical clinical trials were emphasized, including utilization of newer agents, optimization of donor lymphocyte infusion (DLI), and investigation of novel cellular therapies. Dr. de Lima discussed pre-emptive and maintenance strategies to prevent relapse after transplantation, for example, recent promising results suggestive of enhanced graft-versus-tumor activity with hypomethylating agents. Dr. Schmid provided an overview of adjunctive strategies to improve cell therapy for relapse, including cytoreduction before DLI, combination of targeted agents with DLI, and considerations in use of second transplantations. Dr. Porter addressed strategies to enhance T cell function, including ex vivo activated T cells and T cell engineering, and immunomodulatory approaches to enhance T cell function in vivo, including exogenous cytokines and modulation of costimulatory pathways.

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## INTRODUCTION

Cancer relapse remains the major cause of treatment failure after allogeneic hematopoietic stem cell transplantation (AlloSCT). In 2009, the first National Cancer Institute—sponsored workshop on the biology, prevention, and treatment of relapse published extensive reviews of disease-specific prevention and treatment strategies [1,2]. Progress in prevention and treatment was emphasized in the second workshop as well and focused on ideas that might provide a basis for the development of novel, practical clinical trials. Use of new agents, optimal utilization of donor lymphocyte infusion (DLI) and immunomodulatory therapeutics, and investigation of targeted interventions (eg, genetically

modified donor cells) and of novel cellular therapies are areas of ongoing study in the field. Promising advances reported since the first workshop are discussed here.

## I. PREVENTION

Prevention is likely the most feasible and effective means of managing relapse after AlloSCT. In the case of acute leukemias, because even extraordinarily low-level minimal residual disease (MRD) is associated with a high risk of relapse, the goal of prevention should be to achieve an MRD-negative state [3]. While most clearly defined for leukemias, the goal of MRD-negative remission is also relevant to relapse prevention for indolent malignancies and after reduced-intensity AlloSCT, that is, in settings where remission is established some time after AlloSCT. Our ability to target prevention interventions at individuals whose cancers have the highest risk of relapse is improvingly rapidly, with emerging data from molecular, proteomic, and genomic tumor investigations leading to better-informed relapse risk

stratification [4] and increasingly sensitive means of detecting residual disease [5-7]. Precise application of pre-emptive strategies that permit intervention when the burden of disease is minimal could improve our ability to eradicate malignancy before overt relapse. Indeed, many investigational treatments—even with modest efficacy in established relapse—might significantly improve AlloSCT outcomes if applied in the preventive setting. Preventive therapy decisions pose a dilemma: Withholding potentially efficacious therapy until relapse is detected compromises the patient's chance of cure, yet administering potentially toxic therapy without evidence of relapse results in overtreatment for some. Toxicity is a major concern in preventive therapy, particularly in the early months after AlloSCT, when side effects (eg, myelosuppression, rash, diarrhea) and drug interactions present significant management challenges, yet also when relapse often occurs and intervention might be most effective [8].

Strategic aims of prevention include (1) improving disease control before AlloSCT, (2) increasing graft-versus-tumor (GVT) potency of the transplant, (3) maintaining disease control while the allograft matures, and (4) detecting and pre-empting an impending relapse (Table 1). Preventing relapse in individuals whose cancers are active or demonstrate high-risk biology may require the use of multiple strategies.

Pretransplantation approaches may permit use of agents with significant hematologic toxicity but require pharmacokinetic consideration of potential effects on donor stem cell and lymphocyte populations. Use of novel agents (targeting signaling pathways, growth factors, cell surface antigens, etc.) may deepen remissions through effects on cancer cells or the tumor microenvironment and thus improve outcomes. A role for novel agents in the pretransplantation setting is suggested by observations of improved AlloSCT outcomes after their use in "bridge" therapy, such as with tyrosine kinase inhibitors in Philadelphia chromosome–positive acute lymphoblastic leukemia (ALL) [9] and brentuximab vedotin in Hodgkin's lymphoma [10]; distinct toxicity profiles and unique mechanisms of action have led to investigation of incorporating monoclonal antibodies into reduced-intensity conditioning regimens, resulting in immunomodulatory as well as direct antitumor effects [11]. New cancer drugs with novel targets and innovative methods of drug delivery are entering the clinic at a phenomenal rate; their potential to permit or augment GVT is an important research opportunity.

Transplant modifications to potentiate GVT effects may incorporate donor selection tactics, immunotherapeutic maneuvers, and tumor-specific immunotherapies. Recent advances in our understanding of natural killer (NK) immunogenetic influences on transplantation outcome, including relapse risk (particularly in acute myelogenous leukemia [AML]) may yield opportunities to prevent relapse through donor selection based on KIR genotyping in the context of HLA mismatch [12]. Early withdrawal of immune suppression, with or without prophylactic DLI, is another consideration in patients at very high risk of relapse, but randomized trial data are lacking and there is significant risk of graft-versus-host disease (GVHD) [13]. Furthermore, when used to pre-empt impending or early leukemia relapse, these immunotherapeutic maneuvers appear to have limited activity outside of chronic myelogenous leukemia and result in considerable GVHD morbidity [14]. The morbidity of prophylactic DLI may be reduced in the setting of

**Table 1**
Strategies for Relapse Prevention

Improved preparative therapy
- Incorporating new drugs with stronger antileukemia activity and/or less toxicity without compromising dose intensity
- Examples under investigation: monoclonal antibodies (radiolabeled or not), clofarabine, and treosulfan

Graft engineering
- Allograft enrichment with leukemia- or lineage-specific cytotoxic T lymphocytes
- Graft depletion of alloreactive T cells
- NK cell enrichment or adoptive transfer

Pre-emptive treatment
- Monitoring for MRD (cytogenetics, PCR, flow cytometry, etc.)
- Intervention based on detection of MRD
- Therapeutic approaches: pharmacologic, immunologic, and cellular therapies

Early withdrawal of immunosuppression
- High risk of GVHD may offset reduced relapse risk

Maintenance
- Relapse risk defined by pretransplantation parameters (eg, advanced disease stage, presence of high-risk karyotype or genetic mutation, or detection of MRD before and/or after AlloSCT)
- Therapeutic approaches: pharmacologic, immunomodulatory, and cellular therapy
- Approaches under investigation (AML): azacitidine, FLT3 inhibitors

Ideal maintenance agent
- Documented activity against the disease
- Acceptable nonhematologic toxicity (will be tolerated early after transplant)
- Acceptable myelotoxicity (will not interfere with engraftment)
- Minimal drug interactions
- Will not inhibit GVT
- Will not worsen GVHD

Caveats to maintenance strategies
- Dose is likely to be lower than in other scenarios
- Dose escalation trials are essential and randomized trials ultimately necessary given multiples confounding variables

T cell–depleted allografts or mixed chimerism [15,16]. Interestingly, preliminary results of administering ex vivo activated DLI prophylaxis suggest fairly modest GVHD toxicity after reduced-intensity conditioning AlloSCT with alemtuzumab [17]. Efforts to optimize selective subset depletion of DLI (or allograft) continue, attempting to reduce risk of GVHD while maintaining protection from relapse [18].

There has been significant progress in developing tumor-targeted immunotherapies, including tumor vaccines, genetically modified T cells (discussed below in III. Strategies to Enhance T Cell Function), and selectively expanded antigen-specific T cells [19]. The early post-transplantation period may be an ideal time for their administration, when minimal tumor burden coincides with lymphopenia-induced homeostatic cytokine abundance and increased efficiency of antigen-specific lymphocyte proliferation [20]. The use of novel (eg, targeted) agents in maintenance therapy requires phase I evaluation of cumulative and overlapping toxicities (eg, with conditioning and immunoprophylaxis agents), with particular attention to effects on rapidly expanding progenitor and lymphocyte populations.

Maintenance therapeutics may be effective in relapse prevention, providing early tumor control and, potentially, immunomodulatory support for the development of an allogeneic immune response. Acute leukemia relapse poses a particularly great management challenge after AlloSCT due to rapid cell growth and disease progression once recurrence is detected; as such, maintenance approaches for acute leukemia may be informative in indolent malignancies as well. A phase I trial at M.D. Anderson defined a safe, low-dose azacitidine maintenance regimen (32 mg/m$^2$/day, days 1-5 of

30, beginning day +40 after AlloSCT), with preliminary results suggesting improved event-free and overall survival (OS) and less chronic GVHD [21]; an ongoing trial is examining 1 year of maintenance with this regimen (NCT00887068). Others have confirmed the favorable toxicity profile of low-dose azacitidine maintenance, with indirect evidence suggesting azacitidine may mediate enhanced GVT effects and modulate GVHD by increasing T cell tumor antigen responsiveness and numbers of circulating regulatory T cells [22,23].

Pre-emptive treatment strategies are being investigated that, by using monitoring, initiate therapy upon detection of MRD or other biologic surrogate of impending relapse. In the RELAZA trial [24], azacitidine was used to treat patients with imminent relapse as defined by decreasing CD34$^+$ cell donor chimerism ("CD34 chimerism") after AlloSCT. Twenty patients with decreasing CD34 chimerism while in complete hematologic remission received 4 cycles of standard-dose azacitidine (75 mg/m$^2$/day for 7 days). Responses were observed in 16 patients during treatment, with CD34 chimerism either increasing (50%) or stabilizing (30%) without signs of hematologic relapse. Additional cycles were given to 11 patients. Although 13 of 20 patients ultimately relapsed, the time to relapse was longer than expected in this very-high-risk cohort, suggesting that a pre-emptive strategy may be effective, although alternative monitoring approaches and/or use of more intensive pre-emptive therapy may be necessary.

## II. STRATEGIES TO IMPROVE CELL THERAPY FOR RELAPSE

Donor cell therapy remains the foundation of most approaches to induce remission for AlloSCT relapse, attempting to restore or kindle a potentially curative GVT effect. However, except for chronic myelogenous leukemia and, to some extent, other indolent malignancies, responses to unmodified DLI or second transplantation in overt relapse after AlloSCT are disappointing. Although data are limited, adjunctive therapies are now routinely used in conjunction with donor cells for their direct cytotoxic and/or immunomodulatory effects.

### Remission Induction before Cellular Therapy

In acute leukemias and other aggressive malignancies, rapid tumor growth kinetics, a high tumor burden at the time of relapse detection, and use of immune escape mechanisms limit the clinical efficacy of DLI alone. Consistent with this, DLI [25] and second transplantation [26] result in better outcomes if complete remission (CR) can be induced before cell therapy, affording time to establish a robust GVT effect [27] and, perhaps, increase tumor cell immunogenicity as well [28].

There are no standard cytotoxic regimens for any cancer relapse after AlloSCT, and an individualized approach to agents, doses, and schedules is often driven by such factors as prior chemosensitivity, interval from AlloSCT to relapse, age, and comorbidities, including GVHD. In AML, approximately 45% of patients achieve CR following standard anthracycline/cytarabine–based combinations [29,30]. Importantly, remission induction alone is not sufficient for long-term disease control in most patients. In a recent European Group for Blood and Marrow Transplantation analysis of patients with AML relapse after reduced-intensity conditioning AlloSCT, durable remissions were observed almost exclusively in patients whose chemotherapy-induced CR was consolidated with either DLI or a second SCT [30] (Figure 1).
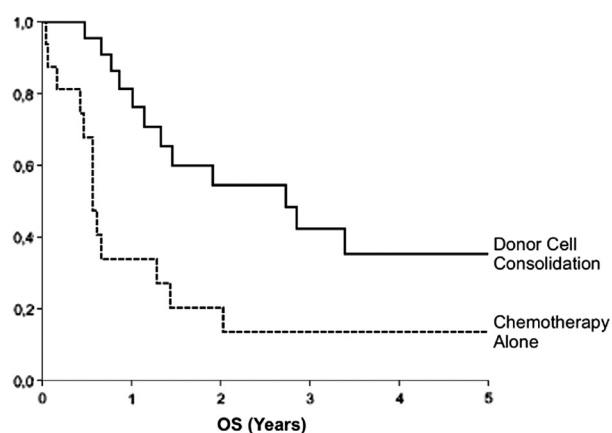


**Figure 1.** Donor–cell consolidation of remission for AML relapse after AlloSCT. Analysis of European Bone Marrow Transplantation Registry data from 38 patients in CR after first-line cytoreductive therapy for relapsed AML after AlloSCT demonstrated improved OS with use of donor cells for consolidation: 55% ± 11% vs. 20% ± 10% (P = .038). DLI and second AlloSCT were considered as time-dependent variables. Adapted from [30].

In addition to cytoreduction, chemotherapy given before donor cell infusion might have immunomodulatory effects that promote GVT activity, for example, by lymphodepletion, suppression of regulatory T cells, and/or release of activating cytokines [31].

### Use of Novel Agents

Therapeutic agents with novel mechanisms of action are under investigation for their ability to control cancer cell proliferation, including progression after AlloSCT (Table 2). As compared with conventional chemotherapy, these drugs often have less systemic toxicity and might therefore be used in patients with a recent history of intensive treatment, including conditioning for SCT, with active GVHD or other comorbidities. In addition to direct cytotoxic activity, some drugs (eg, hypomethylating agents, thalidomide and its derivatives, etc.) reportedly increase antitumor immune responsiveness by increased tumor immunogenicity [32] and enhanced activation of T cells and NK cells [33]. These and other immunomodulatory agents (eg, bortezomib [34] and rituximab [35]) may have prophylactic or therapeutic benefit in GVHD and hence may be useful adjuncts to reduce the risk of GVHD with DLI.

Although the literature on the use of novel agents for relapse after AlloSCT is predominantly retrospective and/or anecdotal, monotherapy for overt relapse generally appears to yield modest responses of limited duration (Table 2). Further, immunomodulatory effects of even highly targeted agents can be heterogeneous, yielding unanticipated negative effects on the immune response (ie, exacerbation of GVHD and/or interference with GVT). As an example of interference with GVT, the multi–tyrosine kinase inhibitor dasatinib has been shown to potently inhibit effector T cell function in vivo [36] and could theoretically blunt any GVT response. Such unexpected "targets" of novel agents highlight the need for their evaluation in clinical trials, including assessment of their immunomodulatory properties in the allogeneic setting.

### Cytokines to Augment the Efficacy of Donor Immune Cells

Various cytokines have been investigated for their capacity to improve the efficacy of donor cells. An older approach

**Table 2**
New Drugs in Treatment of Relapse after Allogeneic Stem Cell Transplantation

| References | Study Design | Diagnosis | Number Treated | Dosage | Responses | DLI | Outcome | Remarks |
|---|---|---|---|---|---|---|---|---|
| **Hypomethylating Agents** | | | | | | | | |
| Azacytidine | | | | | | | | |
| Jabbour, Cancer 2009 | Retrospective, single-center | AML | 4/9/4 (17 total) | 1,62,440 mg/m² d1-5, median 8 courses | ORR: 56% (33% CR, 22% PR) | No | Not reported | Patients with "indolent" disease reccurrence |
| Lübbert, BMT 2010 | Prospective pilot | AML/CMML | 26 | 100 mg within 3 d | CR: 16%, temporary disease control: 50% | Day 10 of each course in 73% of patients | 2-yr OS 16% | |
| Czibere, BMT 2010 | Retrospective, multicenter | AML/MDS | 22 | 100 mg/m² d1-5, q 4w, up to 8 courses | ORR 72% (23% CR) | In 82% of patients | Median OS: 144 d, 2-yr OS: 23% | |
| Bolanos-Meade, BBMT 2011 | Retrospective, single-center | Myeloid malignancies | 10 | Various | CR: 60%, SD: 10% | No | Median OS: 422.5 d | Patients without circulating blasts only |
| Schröder, ASH (#656) 2011; ASH (#1964) 2012 | Propspective, single-arm, multicenter, phase 2 | AML/MDS | 30 | 100 mg/m² d1-5, q4w, up to 8 courses | ORR: 47% (CR: 23%, PR: 7%, SD: 17%) | Increasing doses after alternating courses | Median OS: 117 d | Up-regulation of regulatory T cells following 5-azacytidine |
| **Tyrosine Kinase Inhibitors (Exclusive of CML)** | | | | | | | | |
| Imatinib | | | | | | | | |
| Wassmann, Blood 2005 | Prospective, single-arm, multicenter | Ph+ ALL | 27 | 400 mg/d-800 mg/d | Molecular CR: 54% | No | 2-yr DFS: early responders 54%; nonresponders 8% | |
| Nilotinib | | | | | | | | |
| Tiribelli, Leuk Res 2009 | Case report | Imatinib-resistant Ph+ ALL | 1 | 400 mg b.i.d | Molecular CR | Monthly DLI | CCR | |
| Dasatinib | | | | | | | | |
| Ishida, Int J Hematol 2010 | Case report | Ph+ ALL | 1 | Not reported | Molecular CR | No | CCR following SCT2 | Particular side effects after SCT2 |
| Czyz, Med Oncol. 2010 | Case report | Ph+ ALL, persistent MRD | 1 | Not reported | Molecular CR | No | CCR | |
| Conchon, BMT 2010 | Case report | Imatinib-resistant Ph+ ALL | 1 | 140 mg/d, initially combined with VCR and dexamethasone | Molecular CR | No | CMR after 8 mo of dasatinib maintenance | |
| Tchibana, Leuk Lymphoma 2011 | Case report | Imatinib-resistant Ph+ ALL | 1 | 140 mg/d (salvage), 80 mg/d (maintenance) | Minor hematological response as salvage, molecular CR as maintenance after SCT2 | No | CMR after SCT2 and short dasatinib maintenance | |
| Sorafenib | | | | | | | | |
| Metzeler, Blood 2009 | Compassionate-use results | FLT3-ITD + AML | 5 | 2 × 400 mg/d | CCR in 3/5 patients | No | 2 × durable remission | |
| Sharma BBMT 2011 | Retrospective, single-center | AML | 16 | 2 × 400 mg/d -600 mg/d +/− chemotherapy | No CR, reduction of circulating blasts in 80% | No; 3 pts. received SCT2 | Median OS 83 d | Sorafenib regarded as not effective in early relapse |
| **Immunomodulators** | | | | | | | | |
| Thalidomide | | | | | | | | |
| Kröger, Blood 2004 | Prospective, single-center, phase 1/2 | Multiple myeloma | 18 | 100 mg/d, escalation to 300 mg in case of no response | ORR: 67%, CR: 22 | Escalating dosages, Day 14 of every cycle | 2-yr OS: 100%, DFS: 84% | Only patients refractory to or relapsing after prior DLI |

(*Continued*)

**Table 2**
(*Continued*)

| References | Study Design | Diagnosis | Number Treated | Dosage | Responses | DLI | Outcome | Remarks |
|---|---|---|---|---|---|---|---|---|
| **Lenalidomide** | | | | | | | | |
| Minemma, Leukemia 2011 | Retrospective, single-center | Multiple myeloma | 16 | 25 mg/d, + dexamethasone with progression | ORR lenalidomide only: 46%, + Dex: 87.5% | No (some with prior DLI) | Not reported | Severe aGvHD after lenalidomide alone |
| Lioznov, BMT 2010 | Retrospective, single-center | Multiple myeloma | 24 | 15 mg/d-25 mg/d, +/− dexamethasone | ORR: 66%, (CR: 8%, VGPR: 8%, PR: 50%, SD: 13%) | No | Median OS: 19.9 mo, median TTP: 9.7 mo | Less GvHD, increase of activated NK and T cells |
| Spina, Leuk Lymphoma 2011 | Retrospective, multicenter | Multiple myeloma | 13 | 10 mg/d-25 mg/d + dexamethasone | ORR: 100%, 69% long-term responders | No | Not reported | Increases the frequency of CD4 + Foxp3 + T cells |
| **Bortezomib** | | | | | | | | |
| Kröger, Exp Hematol 2006 | Prospective pilot | Multiple myeloma | 11 | 1.3 mg/m m$^2$ days 1,4,8 and 11 | CR: 30%, PR: 50%, MR: 20% | No | Not reported | Patients with measurable disease only, progressive disease excluded |
| El-Ceikh, Hematologica 2008 | Retrospective, multicenter | Multiple myeloma | 37 | 1.0 mg/m$^2$-1,3 mg/m$^2$ days 1,4,8,11 +/− dexamethasone | ORR: 73% (CR: 19%, VGPR: 19%, PR: 36%) | No | 18 mo OS 65% | |
| **Monoclonal Antibodies** | | | | | | | | |
| **Rituximab** | | | | | | | | |
| Wudhikarn, BBMT 2011 | Retrospective | NHL | 27 | Not reported; additional chemotherapy in 7 pts. | ORR 44% | Not reported | Median response duration: 23 mo (1-68) | Response even in patients pretreated with rituximab |
| **Bi 20 (FBTA05)** | | | | | | | | |
| Buhmann, BMT 2009 | Compassionate-use results | CLL/NHL | 6 | Escalating doses (10 μg-2000 μg) | CLL: 3 × transient response, NHL: 1 SD | DLI in escalating doses or SCT2 | | |
| **Blinatumumab** | | | | | | | | |
| Handgretinger, Leukemia 2011 | Retrospective, single-center | Pediatric ALL | 3 (2 MRD) | | 3 × CR | | 2 of 3 relapsed quickly after stopping drug; 1 CCR after haplo SCT2 | Expansion of donor-derived T cells in all cases |
| **Brentuximab Vedotin** | | | | | | | | |
| Gopal, Blood 2012 | Prospective, pooled data (3 studies) | Hodgkin's lymphoma | 25 | 1.2 mg/kg-1.8 mg/kg, 1-16 (median: 8) cycles | ORR: 50%, CR: 38% | No | Median PFS; 7,8 mo, median OS: not reached | |

CMML indicates chronic myelomonosytic leukemia; MDS, mydelodysplastic syndrome; ORR, overall response rate; CCR, complete cytogenetic response; CMR, complete molecular response.

with new interest is the combination of granulocyte macrophage colony-stimulating factor and/or IFN-α and DLI. Both cytokines have been shown to increase the capacity of dendritic cells and leukemia cells to present target antigens and to provide costimulatory signals and adhesion molecules for improved donor T cell stimulation. In murine models, CD8-dependent GVHD and GVT effects are enhanced by IFN signaling through its ability to both sensitize the leukemia cells to killing and to augment donor cytotoxic T lymphocyte (CTL) function [37]. Several reports have successfully used DLI plus IFN-α in diseases with historically poor responses to DLI alone; however, small, heterogeneous cohorts make it difficult to determine the real contribution of IFN-α [38,39].

Tang et al. [40] reported on 16 patients with relapsed acute leukemia (AML, 7; ALL, 9) treated with IFN-α plus granulocyte colony-stimulating factor mobilized donor leukocytes. IFN-α (3 MU/day) was given from day −5 before DLI until CR, toxicity, or relapse (median 17 days, range 5 to 50). Twelve of 16 patients achieved CR, including 6 of 9 patients who received no additional cytotoxic therapy. At last follow-up, seven patients were alive in CR. Compared with 14 similar patients treated with DLI alone, IFN-α/DLI resulted in a higher CR rate (75% versus 14%, $P = .001$) and improved leukemia-free survival (50% versus 7%, $P = .05$), albeit with increased acute GVHD (56% versus 27%, $P = .05$).

Another approach to increase the potency of DLI is to interrupt the counter-regulatory effect of CTLA-4 on T cell activation through administration of ipilimumab, a neutralizing monoclonal antibody against CTLA-4. In a phase I trial for post-AlloSCT relapse, some immune-mediated adverse events were observed, although there was no significant GVHD, even in patients subsequently receiving DLI for disease progression [41]. Ipilimumab showed modest activity in lymphoid malignancies, particularly Hodgkin's lymphoma, with 2 prolonged CRs in patients treated at the highest dose level. Consistent with expected biologic effects, a dose-dependent, T cell activation and expansion was observed in vivo.

Further, immune-mediated systemic ("abscopal") antitumor effects of targeted radiation, mediated through novel tumor antigen expression and inflammation-induced recruitment of antigen-presenting cells, are well described, and synergy with cellular immunotherapy, including CTLA-4 blockade, has been demonstrated in murine models [42]. In an ongoing clinical phase I/II trial, the National Cancer Institute is studying radiation-targeted DLI for relapse after AlloSCT, looking at systemic effects of single-fraction radiation to isolated tumors, including safety, clinical responses outside the radiation field, and effects on allogeneic lymphocyte populations (NCT00984165).

### Second Allogeneic Transplantation

Although there are no prospective trials, second AlloSCT is frequently used for relapse after AlloSCT, particularly in acute leukemia. Available evidence is based on retrospective registry data, mainly with second AlloSCT from the same HLA-identical related donor. With the important caveat that the recipients of second AlloSCT represented a highly selected minority of individuals with relapse [43], reported long-term OS was between 20% and 30%, with a respective cumulative incidence of relapse and nonrelapse mortality (NRM) of around 40% each. Duration of remission after initial AlloSCT, disease status at initial and second AlloSCT, and age were the most important factors for OS.

Second AlloSCT after unrelated-donor transplantation, donor selection for second AlloSCT, and optimal second-transplantation conditioning regimens remain open questions. National registry studies in Italy and Germany have examined these issues in relapsed acute leukemia [44,45]. Independent of donor selection at first AlloSCT, both groups found a trend for increased NRM after unrelated-donor second AlloSCT as compared with second transplantation with a related donor. However, long-term survivors were identified even after 2 unrelated SCTs. The intensity of second-transplantation conditioning did not appear to influence OS, although NRM was lower after reduced-intensity conditioning.

Use of a different donor for second AlloSCT was generally not associated with better OS, either in the related or unrelated setting. However, after relapse after unrelated-donor AlloSCT, the German study found a trend for improved OS after changing to a different unrelated donor in patients without a history of acute or chronic GVHD after first AlloSCT. This suggests distinct subgroups of patients for whom increased GVT effects with a different donor may offset the risk of NRM. Prospective studies are needed to determine optimal patient selection, donor selection, and conditioning regimens for second AlloSCT treatment of relapse.

## III. STRATEGIES TO ENHANCE T CELL FUNCTION
### Novel Cellular Therapies

Improving antitumor potency and specificity of donor cellular therapy for relapse after AlloSCT would optimize GVT and GVHD reactivity and likely improve efficacy. Minor histocompatibility antigens are important targets for T cell–mediated GVHD and GVT reactivity [46]. It may be possible to isolate and expand T cells that recognize minor histocompatibility antigen selectively expressed on hematopoietic cells to induce GVT without GVHD [47]. Alternatively, tumor-associated, overexpressed self-antigens such as WT-1, proteinase-3, or PRAME are promising targets for T cell–directed GVT responses.

Although feasible, the generation of tumor-specific CTL has proven time-consuming and often difficult. An alternative strategy to enhance GVT activity is donor T cell activation and expansion with CD3/CD28 costimulation ex vivo. In preliminary studies, ex vivo activated DLI yielded several responses, notably even in patients with typically DLI-refractory tumors, such as AML, ALL, and non-Hodgkin lymphomas, suggesting that activated DLI may offer greater GVT potency [48]. This also provides a possible strategy to obtain cells for adoptive immunotherapy for relapse prevention or treatment after umbilical cord blood transplant when it is not possible to recontact the donor. Based on a hypothesis that tumor-infiltrating lymphocytes found after AlloSCT relapse are donor cells and an enriched source of tumor-specific lymphocytes, a phase I trial demonstrated donor origin, feasibility, and safety of administering ex vivo CD3/CD28 costimulated and expanded tumor-derived donor lymphocytes. It also provided evidence that tumor-antigen reactive donor T cells were expanded, plausibly yielding cell products enriched for tumor-specific CTLs [49]. Although responses to tumor-derived DLI were of short duration in the DLI-refractory patients treated, this approach could provide DLI therapy for patients without another source of donor cells.

It is possible to activate and expand donor cells with other biologic activities. For instance, cytokine-induced killer (CIK) cells can be generated in vitro and expanded for clinical use. These unique cells are derived from cytotoxic T cells that express CD3 and CD56 and recognize targets through the
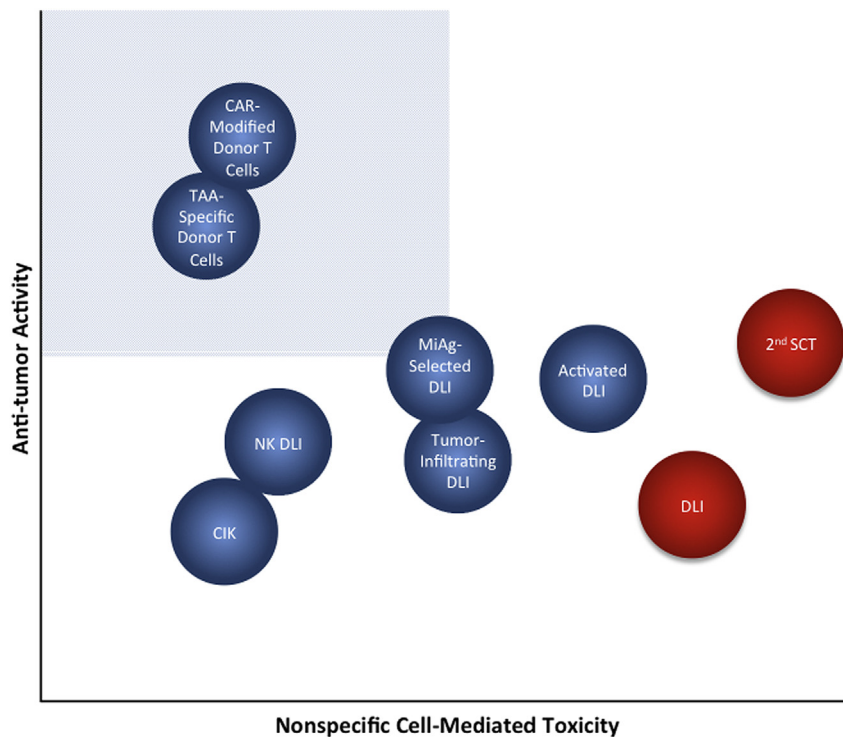
**Figure 2.** Theoretical relative therapeutic potential of cellular therapies for relapse. The shaded quadrant represents the zone of optimal specificity with respect to tumor versus off-target cytotoxic tissue damage, which maximizes antitumor potency and minimizes cell-mediated morbidity. Conventional DLI and second AlloSCT (depicted in red) are the currently available cell-based treatments for relapse, against which novel therapies (blue) will be judged. TAA, tumor-associated antigen; MiAg, minor histocompatibility antigen.

NKG2D activating receptor. Importantly, cell killing is HLA unrestricted and TCR independent, can kill leukemia cells in vitro, and can induce minimal GVHD in animal models. Expanded donor CIK cells were given to a small number of patients with relapsed malignancy after AlloSCT [50]. Although there was minimal GVHD, only 1 of 18 patients had a sustained remission; however, a number of prolonged remissions, several in patients who did not respond to conventional DLI, suggest that CIK cells may contribute to GVT activity without GVHD. For maximum effect, CIK cells will likely have to be used before overt hematologic relapse in high-risk patients (ie, in a preventive or pre-emptive approach) or in combination with other relapse therapies.

In addition to the critical role of T cells, NK cells are increasingly implicated as important mediators of GVT activity, particularly in myeloid diseases and in the setting of haploidentical transplant [51]; NK cell biology and implications for graft selection and ways to exploit their therapeutic potential were discussed in detail during the workshop [20,52]. Plausibly, it might be possible to augment NK-mediated GVL through increasing availability of endogenous IL-15, a key cytokine for NK cell development, expansion, and function in vivo. This theory can now be tested in the clinic, with recombinant human IL-15 now in clinical trials; alternatively, the novel IL-15 super agonist, ALT-803, which has shown promise in early biologic testing [53], is slated to begin clinical testing later this year.

### Targeted Therapies

Targeted therapies hold the promise of antitumor activity without inducing nonspecific GVHD. This is particularly true for targeted antibodies (reviewed in 2009 Workshop Proceedings [1]). A key limitation is that tumor-specific targets are not well defined for most hematologic malignancies. Potential targets have been exploited in previous studies, for example, with the monoclonal antibodies CD52 (alemtuzumab) and CD20 (rituximab) and with antibody–drug conjugates, such as CD33 (gemtuzumab ozogamicin), CD25 (denileukin diftitox), and CD22 (inotuzumab ozogamicin), all small studies demonstrating limited clinical activity in AlloSCT relapse [1]. The limited activity might be related in part to variable or weak target expression or variable or incomplete activity of the targeted agent. In addition, nonspecific toxicities develop when the target is broadly expressed on other cell types and tissues.

CD19 is an ideal tumor target, with expression largely restricted to normal and malignant B cells. The antibody blinatumomab is a bispecific T cell–engaging antibody specific for CD19 and CD3. It serves to direct cytotoxic T cells to CD19-expressing target cells [54] and has activity in both non-Hodgkin lymphoma and ALL. Impressively, in ALL relapse after conventional chemotherapy, blinatumomab induced CR in 68% of patients [55]. Few patients have been treated with blinatumomab for ALL relapse after AlloSCT. A small case series reported hematologic CR in 3 of 3 pediatric patients, although disease rapidly recurred in 2 of those treated [56]. Given its specificity, clinical activity, and toxicity profile, it is reasonable to test blinatumomab in a larger group of patients with post-AlloSCT ALL relapse, alone or in combination with other agents.

Targeted cellular therapy may be even more promising because T cells can both expand, amplifying their effect, and persist in vivo, providing long-term vaccine-like antitumor activity. Efficient gene transfer techniques now permit genetic modification of T cells to confer novel antigen specificity by stably expressing novel T cell receptors, that is, chimeric

antigen receptors (CARs), on their surface. CAR-modified T cells become activated and kill in an antigen-dependent but HLA-independent manner, making this an attractive approach for any tumor with a defined target. It was recently shown that T cells expressing a CAR targeting CD19 linked to a potent signaling domain (CD137/4-1BB) demonstrated massive in vivo expansion, tumor-specific trafficking, and long-term persistence in vivo. CD19-CARs have induced rapid and sustained antitumor activity in chemotherapy-refractory chronic lymphocytic leukemia [57] and B cell lymphomas [58]. These cells have also induced remission for refractory ALL and for relapse of ALL after umbilical cord blood transplantation [59]. It will be of great interest to continue to test this approach in relapse after AlloSCT, particularly for patients with ALL, chronic lymphocytic leukemia, and non-Hodgkin lymphoma; ongoing trials evaluating CD19-CAR transduced donor T cells appear to be promising [59,60].

Any new cellular therapy ultimately has to be judged in relationship to conventional DLI or second allogeneic SCT. For most indications other than chronic myelogenous leukemia, conventional DLI has some, albeit disappointing, activity and is associated with significant GVHD-related toxicity. Second AlloSCT may be curative for a subset of patients with extensive morbidity and mortality. Figure 2 depicts a theoretical assessment of the potential for some investigational cellular therapies to treat relapse, with the ideal cell therapy providing maximal antitumor activity with minimal nonspecific cell-mediated toxicity. It is hypothesized that nonspecific ex vivo activation of donor T cells or generation of minor antigen—directed T cells would enhance activity of DLI, although limited studies suggest toxicity is similar to DLI. We believe that engineered tumor antigen—specific and CAR-modified donor T cells (such as CD19-directed CARs) have the potential to provide potent antitumor activity with limited if any GVHD, a hypothesis supported by early clinical observations [59-61]. Numerous other cell therapy approaches hold promise but as yet have shown limited clinical activity. We recognize there are limited clinical data for any novel cell therapy other than conventional DLI, and so where any cellular therapy might fit into this idealized perspective could be subjected to vigorous debate. It will certainly require constant modification as well, as we believe the next few years will bring development of new therapies or modifications of existing approaches leading to enhanced activity and limited toxicity to treat relapse after AlloSCT.

## FUTURE DIRECTIONS AND RESEARCH PRIORITIES

The Scientific Session on Prevention and Treatment of Relapse highlighted ongoing clinical development of new management approaches and, importantly, identified recent field advances that are ripe for clinical development. We remain optimistic about the future potential to treat relapsed disease. Although no major breakthroughs have occurred in treatment results, major advances have been made in developing potential novel strategies. Several trial concepts were discussed in the Workshop's Protocol Planning Committee meetings. The use of novel agents, immune stimulation and modulation, and enhanced cellular therapies all constitute critical areas for future study. Studying novel immune modulators and combinations of immune modulation with cellular therapy are particularly relevant and appropriate for initiation of multicenter prospective trials to treat relapse. A major achievement of the second workshop was participant commitment to the establishment of an international transplant relapse consortium and development of a platform for conducting such multi-institutional trials to speed clinical investigation of relapse prevention and treatment strategies—of both novel approaches as well as those more commonly used, albeit understudied.

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