National Cancer Institute’s First International Workshop on the Biology, Prevention, and Treatment of Relapse after Allogeneic Hematopoietic Stem Cell Transplantation: Summary and Recommendations from the Organizing Committee

Michael R. Bishop,1,2 Edwin P. Alyea, III,2,3 Mitchell S. Cairo,2,4 J. H. Frederik Falkenburg,2,5 Carl H. June,2,6 Nicolas Kröger,2,7 Richard F. Little,2,8 Jeffrey S. Miller,2,9 Steven Z. Pavletic,1,2 David L. Porter,2,6 Stanley R. Riddell,2,10 Koen van Besien,2,11 Alan S. Wayne,2,12 Daniel J. Weisdorf,2,9 Roy S. Wu,2,8 Sergio Giralt2,13

The National Cancer Institute’s First International Workshop on the Biology, Prevention, and Treatment of Relapse after Allogeneic Hematopoietic Stem Cell Transplantation was organized and convened to identify, prioritize, and coordinate future research activities related to relapse after allogeneic hematopoietic stem cell transplantation (allo-HSCT). Each of the Workshop’s 6 Working Committees has published individual reports of ongoing basic, translational, and clinical research and recommended areas for future research related to the areas of relapse biology, epidemiology, prevention, and treatment. This document summarizes each committee's recommendations and suggests 3 major initiatives for a coordinated research effort to address the problem of relapse after allo-HSCT: (1) to establish multicenter correlative and clinical trial networks for basic/translational, epidemiologic, and clinical research; (2) to establish a network of biorepositories for the collection of samples before and after allo-HSCT to aid in laboratory and clinical studies; and (3) to further refine, implement, and study the Workshop-proposed definitions for disease-specific response and relapse and recommendations for monitoring of minimal residual disease. These recommendations, in coordination with ongoing research initiatives and transplantation organizations, provide a research framework to rapidly and efficiently address the significant problem of relapse after allo-HSCT.

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) can cure a substantial proportion of patients with hematologic malignancies [1-3]. Despite 30 years of research and significant reductions in nonrelapse mortality, the risk of relapse has not...

From the 1Experimental Transplantation and Immunology Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland; 2Organizing Committee, National Cancer Institute’s First International Workshop on the Biology, Prevention, and Treatment of Relapse After Allogeneic Hematopoietic Stem Cell Transplantation; 3Division of Hematologic Malignancies, Dana-Farber Cancer Institute, Boston, Massachusetts; 4Department of Pediatrics, Morgan Stanley Children’s Hospital of New York-Presbyterian, Columbia University, New York, New York; 5Department of Hematology, Leiden University Medical Center, Leiden, the Netherlands; 6Abramson Cancer Center, University of Pennsylvania, Philadelphia, Pennsylvania; 7Center for Stem Cell Transplantation, University Hospital Hamburg-Eppendorf, Hamburg, Germany; 8Cancer Therapy Evaluation Program, National Cancer Institute, National Institutes of Health, Bethesda, Maryland; 9Blood and Marrow Transplant Program, University of Minnesota, Minneapolis, Minnesota; 10Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, Washington; 11Department of Medicine, School of Medicine, University of Chicago, Chicago, Illinois; 12Pediatric Oncology Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland; and 13Department of Stem Cell Transplantation and Cellular Therapies, Memorial Sloan-Kettering Cancer Center, New York, New York.

Financial disclosure: See Acknowledgments on page 453.

Correspondence and reprint requests: Michael R. Bishop, MD, Experimental Transplantation and Immunology Branch, National Cancer Institute, 10 Center Drive, CRC/Room 4-3152, Bethesda, MD 20892 (e-mail: mbishop@mail.nih.gov).

Received December 22, 2010; accepted December 26, 2010

Published by Elsevier Inc. on behalf of American Society for Blood and Marrow Transplantation

1083-8791/$36.00
decreased significantly. Strategies to reduce relapse rates have concentrated predominantly on modifying the conditioning regimen either by adding new agents or increasing doses of available agents. Although dose intensification resulted in lower relapse rates, these attempts were generally counterbalanced by increases in nonrelapse morbidity and mortality [4-6]. The demonstration that donor lymphocyte infusion (DLI) could result in complete remissions in patients with recurrent chronic myelogenous leukemia (CML) [7,8] led to the exploration of less-intensive regimens aimed at reducing treatment-related morbidity and mortality and allowing older and more debilitated patients access to allo-HSCT. These regimens are associated with higher rates of relapse, however [9,10]. Relapse and disease progression are the leading causes of treatment failure for most hematologic malignancies treated with allo-HSCT [11].

The National Cancer Institute’s First International Workshop on the Biology, Prevention, and Treatment of Relapse after Allogeneic Hematopoietic Stem Cell Transplantation was organized to bring together leading experts in the field to comprehensively review the current state of the science regarding the biology and treatment of disease recurrence after allo-HSCT [12]. The Workshop’s organizational structure comprised 6 Working Committees that addressed specific areas considered essential to the problem of relapse after allo-HSCT, including (1) the biology of graft-versus-tumor (GVT) effects, (2) the biology of cancer susceptibility and resistance to treatments other than GVT effects, (3) epidemiology and statistical analysis of relapse, (4) prevention, (5) monitoring, and (6) treatment. After approximately 1 year of planning and preparation, the Workshop was held on November 2-3, 2009, in Bethesda, Maryland. It included more than 200 international participants with expertise both within and outside the transplantation community. The Working Committees reviewed the current state of the science and ongoing basic, translational, and clinical research related to various aspects of their specific area, and then provided recommendations and topics for discussion and debate among all of the Workshop participants.

Over the past several months, reports from all of the 6 Working Committees have been published in Biology of Blood and Marrow Transplantation [13-19]. These reports provide an encyclopedic scientific review of various topics related to relapse after allo-HSCT; describe ongoing basic, translational and clinical research on this subject; and, most importantly, identify specific areas for future research.

This article summarizes the most salient points from the Committees’ reports and provides a framework for a coordinated research effort to address the problem of relapse. The article is divided into two parts. The first part provides a succinct summary of research questions and proposals put forward by each Committee. Although these summaries provide some background information to place these questions and proposals in proper perspective, they do not adequately convey the full complexity and breadth of data on each topic, and the reader is highly encouraged to refer to the specific Committee reports. The second part of the article presents general recommendations for a coordinated effort to address the problem of relapse after allo-HSCT.

COMMITTEE RECOMMENDATIONS

Committee on the Biological Considerations of Hematological Relapse following Allogeneic Stem Cell Transplantation Unrelated to Graft-Versus-Tumor Effects

The Committee identified 3 broad areas that require further study to improve our understanding of the intrinsic factors and extrinsic factors unrelated to GVT effects that permit the persistence of tumor cells beyond the effects of the conditioning regimen (Table 1). These areas include genomic and epigenetic lesions/alterations, cancer stem cells (CSCs), and mechanisms of therapy resistance. Within each of these areas, there is a need to study rare cell populations and genetic and epigenetic events, and then to delineate downstream biochemical pathways that collectively will form a biological “fingerprint” of the events that might predict the potential for disease relapse.

Genomic and epigenetic lesions/alterations

Certain genomic and epigenetic changes are stably transmitted as cells divide and differentiate [20,21]. We must improve our ability to detect and measure rare events, such as leukemic stem cell cells (LSCs) and genetic and epigenetic lesions. Epigenetic information on LSCs and total malignant cells must be acquired and compared with nonmalignant somatic cells. Samples obtained at the time of relapse also could be used to identify selective GVT effects. Comparing preconditioning samples and relapse samples of the

<table>
<thead>
<tr>
<th>Table 1. Recommendations from the Committee on the Biological Considerations of Hematological Relapse following Allogeneic Stem Cell Transplantation Unrelated to Graft-versus-Tumor Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Improve the ability to detect and measure rare events, such as LSCs and genetic and epigenetic lesions. Samples need to obtained pretransplantation and at the time of relapse for comparison of events.</td>
</tr>
<tr>
<td>2. Obtain malignant cells from patients in longitudinal studies (eg, diagnosis, pretransplantation relapse, posttransplantation relapse) to study changes in LSC phenotype and frequency.</td>
</tr>
<tr>
<td>3. Conduct longitudinal studies on LSC and whole-cell populations from patients before and after allo-HSCT relative to the development of resistant mechanisms (eg, growth factors, cell cycle proteins, cell death mechanisms, drug efflux mechanisms, signaling pathways) to radiation and chemotherapy.</td>
</tr>
</tbody>
</table>
malignancy is feasible and has the potential to illuminate the results of both forms of resistance selection. The roles of nucleotide polymorphisms, noncoding genes, and noncoding RNA in leukemogenesis and in the longitudinal progression of LSCs and whole-cell populations requires further exploration, in combination with whole-genome analysis. Novel bioinformatics methods could be used to validate or identify new gene interactions and to identify shared or common nodes in signaling pathways.

**Cancer stem cells**

The hypothesis that only a subpopulation of rare CSCs is responsible for maintenance of a tumor has been supported by studies of acute myelogenous leukemia (AML) [22]. The CSC hypothesis does not exclude the possibility that over time, these cells may undergo changes leading to limited or unlimited self-renewal capacity. This concept has a significant impact in the context of allo-HSCT, because it suggests that long-term disease control can be achieved only through posttransplantation therapies or conditioning regimens that specifically target CSCs. There is a need to obtain malignant cells from patients in longitudinal studies (eg, diagnosis, pretransplantation relapse, posttransplantation relapse), given that stem cell phenotype and frequency can change in the same patient during the course of disease progression. Further advances in this field will require improvements in our ability to identify, enumerate, and expand CSCs, as well as the development of nonhuman primate models that can be used to screen potential strategies for predicting therapeutic efficacy, monitoring of minimal residual disease (MRD), and evaluation of new therapies.

**Mechanisms of therapy resistance**

The development of resistance to drugs, radiation, or both by tumor cells arises from selection of resistant clones that have acquired favorable genetic or epigenetic alterations that increase the cells’ fitness. Growth factors, cell cycle proteins, cell death mechanisms, drug efflux mechanisms, and signaling pathways that are affected by radiation or chemotherapy need to be studied longitudinally in LSCs and whole-cell populations from patients before and after allo-HSCT.

The Committee also concluded that we currently lack the necessary tests, assays, and experimental designs to sufficiently study the foregoing issues. In particular, there is the need to develop new technologies to isolate and study these rare biological events. One of the most glaring deficiencies is the lack of robust preclinical models for evaluation of agents or regimens that may be predictive of therapeutic efficacy or ability to promote resistance. However, given access to cell samples obtained before and after relapse after allo-HSCT, it is likely that currently available technologies could identify critical biomarkers that would permit the identification of patients at risk for relapse. In the future, it may be possible to develop tailored or “personalized” conditioning regimens that might mitigate the evolution of resistance to allogeneic cellular procedures.

**Committee on the Biology Underlying Recurrence of Malignant Disease following Allogeneic HSCT: Graft-versus-Tumor/Leukemia Reaction**

Relapse occurs either because the residual cells that were not eliminated by the conditioning regimen evade the immune response provided by the transplantation of donor cells or because the immune response cannot be sustained. Immune responses after allo-HSCT and DLI illustrate the robust potential of allogeneic T cells, natural killer cell, and/or antibody responses to treat patients with hematologic malignancies. Thus, the central questions are why certain diseases, particularly CML, respond better to the adoptive transfer of allogeneic lymphocytes than other diseases, and why in those diseases that do initially respond, this response is lost, resulting in relapse.

Several issues have limited the successful application of allogeneic cellular therapy, particularly detrimental graft-versus-host disease (GVHD) in patients experiencing clinically significant antitumor responses. A major objective of allo-HSCT research has been to separate GVHD from GVT without incurring a loss of protection against infections [23]. However, the complexity of the interactions between the alloimmune response and tumor biology has made achieving this objective very difficult. The prerequisites for successfully achieving this objective include identifying relevant target structures and the type of effector cells required for optimal and specific responses and gaining a better understanding of the
mechanisms of action in the alloimmune response necessary for tumor elimination [24,25]. There is also the need to better understand the induction, expansion, and trafficking of immune responses, which may allow specific recognition of hematopoietic tissues from host and nontarget organs of GVHD [26]. Knowledge of the tumor biology limiting the effectiveness of alloimmune responses to execute a specific antitumor effect is essential to increase the likelihood of success with no or only limited toxicity. These factors include escape mechanisms of tumor cells to prevent recognition and elimination by alloimmune responses and also may include down-regulation of the target structures to be recognized, suppression of homing or interaction at the tumor site, or development of suppressive factors preventing the development of immune responses in vivo.

Future initiatives to identify and overcome the obstacles preventing the successful application of allogeneic cellular therapy for the treatment of hematologic malignancies should include studies of T cells, natural killer cells, and antibody-mediated mechanisms (Table 2). Specific studies that may be performed in either animals and/or man should include (1) studies to better understand mechanisms of trafficking, target recognition, and in vivo expansion of tumor specific responses; (2) studies of mechanisms that suppress immune responses systemically or locally at the tumor site; (3) studies on the in vitro generation, expansion, and engineering of specific immune cells for adoptive transfer; (4) studies on the susceptibility of CSCs to immune attack; (5) studies on the induction of effective antitumor immunity with vaccines or inhibition of negative regulatory pathways; and (6) high throughput screening for antigens involved in antitumor responses using genome-wide analysis.

Accomplishing any of these studies will require major initiatives to provide the framework and infrastructure for their execution. Specifically, human tissue sampling and correlative studies should be built into all transplantation trials. Related to this, efforts and support should be made to utilize and coordinate existing biorepositories for the collection, storage, and distribution of these samples. Data from these correlative studies and provision of human samples will facilitate the translation of basic research into successful clinical research strategies and will guide the design of clinical trials. However, this will require sufficient financial support for sample collection, storage, and administration, as well as supportive collaboration among institutions and investigators. Additional funding, possibly through specific directed Requests for Applications (RFA), Program Project Grants (P01), or similar funding mechanisms, will be essential to provide the necessary support. This might be secured by promoting interactive program grants and utilizing existing institutional core resources.

Committee on the Epidemiology and Natural History of Relapse following Allogeneic Cell Transplantation

The epidemiology and natural history of relapse after allo-HSCT involve certain factors that can be evaluated across a variety of diseases and those that are disease-specific. Factors that affect the incidence of relapse regardless of disease or disease status include graft manipulation (eg, T cell depleted vs T cell replete), conditioning regimen (myeloablative vs reduced-intensity), donor source (related vs unrelated, HLA disparity), and immunosuppression used for GVHD prevention (agent, dose, duration). After allo-HSCT, additional factors affect relapse, particularly the presence or absence of GVHD and the agents used to treat it. Relapse and survival need to be assessed in relation to newer agents (eg, tyrosine kinase inhibitors in CML, hypomethylating agents in myelodysplastic syndromes), contemporary supportive care, and comparable patient populations. Several factors are disease-specific relative to the risk and subsequent outcome of relapse after allo-HSCT, however. The difficulty lies in the absence of adequate data on the epidemiology and natural history of many diseases, particularly the lymphomas, when taking into account the factors that affect relapse, such as specific histology, cytogenetics, genetic mutations, disease state, and sensitivity to chemotherapy, all of which have been identified as affecting relapse incidence and outcome.

As such, epidemiologic studies need to provide data with details on disease status, previous treatments, biological markers, and posttransplantation events. Analyses of larger cohorts through multicenter collaborations or registries remain essential to probe questions not amenable to or impractical in single-center or prospective studies. Stringent and consistent statistical methods for studying relapse remain an important area of research. The opportunities for improving the prevention and management of post–allo-HSCT relapse are apparent, but clinical data to permit the prompt identification of patients at risk and who would benefit from preemptive therapy are lacking. A better understanding and monitoring of MRD after allo-HSCT could lead to novel preemptive treatments of relapse; however, selection bias necessitates prospective assessment to gauge the real contribution of any new therapies.

Specific areas are considered of high priority relative to the epidemiologic study of relapse (Table 3). First, it is necessary to redefine remission and relapse in each disease using new methodologies (as further defined later), as well as MRD thresholds using standardized morphologic, cytogenetic, flow cytometric, and molecular techniques. Second, data from such studies would be used to develop new or modify current prognostic scoring systems (eg, the International Prognostic Index in non-Hodgkin lymphoma) that
Table 3. Recommendations from the Committee on the Epidemiology and Natural History of Relapse following Allogeneic Cell Transplantation

1. Refine remission and relapse definitions in each disease using remission guidelines and MRD thresholds recommended by the Committee on Disease-Specific Methods and Strategies for Monitoring Relapse following Allogeneic Stem Cell Transplantation.
2. Develop new or modify current prognostic scoring systems (eg, the International Prognostic Index in non-Hodgkin lymphoma) that are specific for relapse.
3. Develop statistical methods for the study of relapse. These would include the use of cumulative incidence function with time-dependent covariates.
4. Develop networks of transplantation centers willing to provide extra data to the various transplantation registries and cooperative groups to define high-risk subsets of patients in each disease and disease category.

are specific for postransplantation relapse. Third, there is the need to develop statistical methods that are specific for the study of relapse. These would include the use of the cumulative incidence function with time-dependent covariates. Finally, the development of networks of transplantation centers willing to provide extra data to the various transplantation registries and cooperative groups is highly encouraged to define high-risk subsets of patients in each disease and disease category. Again, additional funding will be essential to support the increased workload placed on such institutions. The value of these networks would be enhanced if they were to include a central tissue bank as well.

Committee on Prevention of Relapse following Allogeneic Cell Transplantation for Hematological Malignancies

There have been several attempts, some ongoing, to prevent relapse after allo-HSCT, including modification of the conditioning regimen, modification of the allograft, preemptive therapy in the peritransplantation period, and maintenance therapy later postransplantation [27-29]. However, no one strategy or combinations of strategies has been clearly demonstrated to reduce the incidence or the subsequent outcome of relapse after allo-HSCT. There are several unique opportunities for investigation in each of these areas. With regard to conditioning regimens, the ultimate goal is to achieve a state of MRD with a decrease (or, at a minimum, with no increase) in nonrelapse mortality. One strategy might involve the selection of conditioning regimens based on disease and disease state, with higher-risk and aggressive malignancies requiring more intensive conditioning regimens. The biggest question that faces the transplantation community is whether we should abandon high-dose conditioning regimens altogether and focus on developing alternative strategies to achieve MRD with less toxicity through the use of novel and targeted agents (eg, histone deacetylase [HDAC] inhibitors, hypomethylating agents, proteasome inhibitors, monoclonal antibodies) with pretransplantation salvage treatment, low-intensity conditioning regimens, and subsequent therapy in the immediate posttransplantation period. Optimal criteria for agents used in the pretransplantation and peritransplantation periods would include the following: (1) no increased morbidity, (2) either additive or synergistic effects with the agent(s) with which they are combined, and (3) either no effect on or an enhancing effect on GVT.

Several new areas related to graft manipulation need to be tested, including the selective depletion of the donor graft to eliminate cells most likely to cause GVHD while maintaining GVL (eg, alloreactive and naive T cells) and the addition of cells that suppress GVHD (eg, regulatory T cells, mesenchymal stem cells). However, before these new approaches can be tested, several elementary questions remain to be answered, including understanding the critical innate and adaptive immune cells, the mechanisms involved in mediating GVHD and GVL, and the optimal graft composition.

In the postransplantation period, cellular therapy could include prophylactic treatment with T cells that specifically target malignant cells by either selection or genetic modification (eg, chimeric antigen receptors) and vaccines, either alone or in combination with dendritic cells. Essential questions remain related to the timing, dose, and frequency of the prophylactic use of standard DLI.

It is the Committee’s recommendation that future trials should be disease-specific (Table 4). However, before initiating such disease-specific trials, additional disease-specific epidemiologic and biomarker studies are needed. These include the development of validated prognostic scoring systems to identify patients at risk for relapse in the postransplantation period. Critical to this effort is the need to standardize methods that are clinically significant and reproducible to measure MRD, assess the predictive value of MRD, and validate MRD measures. Ultimately, such measurements also may serve as surrogate markers for study endpoints, particularly response, which would enable shorter follow-up times and allow for more rapid clinical trial completion and development of subsequent lines of investigation. Finally, while epidemiologic studies are in progress for specific diseases, efforts should be placed on initiating trials in diseases for which measurement of MRD and biomarkers are relatively well established and novel agents are available (eg, TKI in Philadelphia chromosome-positive acute lymphoblastic leukemia [ALL], bortezomib in multiple myeloma).

Committee on Disease-Specific Methods and Strategies for Monitoring Relapse following Allogeneic Stem Cell Transplantation

There is clinical evidence indicating that intervention before florid relapse after allo-HSCT through
monitoring of MRD improves outcomes in patients with CML undergoing allo-HSCT [30]. Similarly, it has been clearly shown that in pediatric patients with ALL, serial analysis of chimerism and MRD can predict impending relapse, and that early initiation of immunotherapy can prevent relapse in some patients [31]. However, in the majority of hematologic malignancies for which allo-HSCT is used, the clinical relevance of MRD surveillance and the potential impact of specific interventional strategies have not yet been fully elucidated. A wide variety of techniques are available to monitor residual disease after therapy, including in the posttransplantation setting, although applicability varies by specific disease subtype, and the predictive value of each method is currently not well defined for most diseases. Criteria for determining remission status and relapse and detection of MRD have been established for a variety of diseases, and these criteria also could be integrated into the assessment of remission in the posttransplantation period. Whether these techniques and criteria are applicable and have validity in the allo-HSCT setting has not been clearly determined.

The development of standard techniques to monitor MRD and criteria and definitions to define relapse and response to treatment specific for allo-HSCT are essential for the proposed studies by the other Workshop Committees related to the natural history and the epidemiology, prevention, and treatment of relapse. It is with this understanding that the Committee on Disease-Specific Methods and Strategies for Monitoring Relapse undertook the challenge of developing disease-specific recommendations for the assessment and definitions of relapse and response after allo-HSCT, as well as specifying the disease-specific monitoring methods needed to make these assessments [18,19]. These recommendations took into account existing definitions and criteria, practicality, and reproducibility. It is a specific intent of the Workshop that these definitions of remission and relapse be further refined and that methods for monitoring or MRD be incorporated into all future transplantation trials, to provide consistency in reporting and interpretation of data (Table 5). These recommendations (Table 6) are made cautiously with several caveats in mind. First, standardization is critical to the conduct of multicenter studies needed to assess the utility of MRD monitoring in the prediction and possible prevention of overt relapse. Quality control and standardization are essential to ensure comparability of MRD results between different laboratories. Second, various new techniques and markers are emerging, and efforts must be made to incorporate these techniques as they become available. Third, these recommendations are not meant to be definitive. Many of the established definitions of remission and relapse used to evaluate most hematologic malignancies during upfront therapy lack sufficient sensitivity for use after allo-HSCT. As such, standardization and implementation of the different MRD monitoring techniques are critically important. These recommendations are meant to serve as a starting point for systematic use and evaluation among transplantation studies. Studies to assess the utility of these recommendations in each disease entity are needed. In particular, the optimal time points for their use and the predictive value of posttransplantation MRD monitoring need to be prospectively assessed. Subsequent studies must then be performed to evaluate the efficacy of these methods to guide therapeutic interventions designed to prevent overt relapse. Specifically, critical objectives for future studies using the proposed methods and criteria should include the following: (1) to standardize measurement of molecular markers for each hematologic malignancy for which allo-HSCT is used, (2) to determine the optimal frequency for monitoring MRD and chimerism after allo-HSCT, (3) to define kinetic changes in MRD and chimerism that occur after allo-HSCT and establish criteria that incorporate measurement of these molecular markers in the definition of response and remission after allo-HSCT, and (4) to assess the efficacy of interventional strategies based on changes in MRD and/or chimerism to prevent clinical relapse.

Committee on Treatment of Relapse after Allogeneic Hematopoietic Stem Cell Transplantation

The treatment of relapse after allo-HSCT is dependent on many factors, including disease activity, time when the relapse occurred, clinical complications, presence or absence of immunosuppression and/or GVHD, previous therapies, donor availability, susceptibility to GVT induction, and several other logistical and clinical issues. Other than the documented success of using DLI for relapsed CML, there are remarkably limited data on the efficacy of DLI and non-DLI therapies in other clinical situations. Furthermore, the
<table>
<thead>
<tr>
<th>Disease</th>
<th>Definition of Complete Remission</th>
<th>Definition of Relapse</th>
<th>Molecular Markers</th>
<th>Cytogenetics</th>
<th>Chimerism</th>
<th>Imaging</th>
<th>Flow Cytometry</th>
<th>Other Methods</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALL</td>
<td>&lt;5% blasts in bone marrow</td>
<td>&gt;5% blasts in bone marrow</td>
<td>TCR and Ig gene rearrangement</td>
<td>Chromosome banding analysis, FISH</td>
<td>PCR or VNTR/STR</td>
<td>Not applicable</td>
<td>4- to 6-color multiparameter flow cytometry</td>
<td>&gt; 95% of patients</td>
</tr>
<tr>
<td>Applicable Comment</td>
<td>All patients</td>
<td>All patients</td>
<td>90% of all patients</td>
<td>All patients</td>
<td>All patients</td>
<td>All patients</td>
<td>Sensitivity in B-lineage ALL is limited after HSCT due to large numbers of hematogones.</td>
<td></td>
</tr>
<tr>
<td>AML/MDS</td>
<td>IWG</td>
<td>IWG</td>
<td>Molecular mutations</td>
<td>Chromosome banding analysis, FISH</td>
<td>PCR or VNTR/STR</td>
<td>Not applicable</td>
<td>4- to 8-color multiparameter flow cytometry</td>
<td>All patients</td>
</tr>
<tr>
<td>Applicable Comment</td>
<td>All patients</td>
<td>All patients</td>
<td>Subgroups</td>
<td>All patients</td>
<td>All patients</td>
<td>All patients</td>
<td>Few studies</td>
<td></td>
</tr>
<tr>
<td>CLL</td>
<td>IW-CLL/NCI</td>
<td>IW-CLL/NCI</td>
<td>ASO primer (IGH qPCR)</td>
<td>Chromosome banding analysis, FISH</td>
<td>PCR or VNTR/STR</td>
<td>CT</td>
<td>MRD multiparameter flow cytometry</td>
<td>&gt; 95% Predictive for sustained remission if &lt;10&lt;sup&gt;-4&lt;/sup&gt; at 1 year after allo-HSCT.</td>
</tr>
<tr>
<td>Applicable Comment</td>
<td>All patients</td>
<td>All patients</td>
<td>All patients</td>
<td>All patients</td>
<td>All patients</td>
<td>All patients</td>
<td>All patients</td>
<td></td>
</tr>
<tr>
<td>CML</td>
<td>Hematologic; cytogenetic; molecular</td>
<td>Hematologic; cytogenetic; molecular</td>
<td>BCR/ABL1; reverse-transcription PCR</td>
<td>Chromosome banding analysis, FISH</td>
<td>PCR or VNTR/STR</td>
<td>Not applicable</td>
<td>4- to 6-color multiparameter flow cytometry</td>
<td>All patients</td>
</tr>
<tr>
<td>Applicable Comment</td>
<td>All patients</td>
<td>All patients</td>
<td>All patients</td>
<td>All patients</td>
<td>All patients</td>
<td>All patients</td>
<td>Not fully applicable.</td>
<td></td>
</tr>
<tr>
<td>Lymphoma</td>
<td>Cheson criteria</td>
<td>Cheson criteria</td>
<td>ASO primer (igh) for B cell NHL</td>
<td>Chromosome banding analysis, FISH</td>
<td>PCR or VNTR/STR</td>
<td>CT/PET</td>
<td>4- to 6-color multiparameter flow cytometry</td>
<td>Subgroups</td>
</tr>
<tr>
<td>Applicable Comment</td>
<td>All patients</td>
<td>All patients</td>
<td>All patients</td>
<td>All patients</td>
<td>All patients</td>
<td>All patients</td>
<td>Only helpful in identifying aberrant blasts in advanced phase disease.</td>
<td></td>
</tr>
<tr>
<td>Multiple myeloma</td>
<td>EBMT; IMWG</td>
<td>EBMT; IMWG</td>
<td>ASO primer (IGH) for B cell NHL</td>
<td>Chromosome banding analysis, FISH</td>
<td>PCR or VNTR/STR</td>
<td>MRI; PET-CT</td>
<td>4- to 8-color multiparameter flow cytometry</td>
<td>All patients</td>
</tr>
<tr>
<td>Myelofibrosis</td>
<td>IWG-MRT</td>
<td>IWG-MRT</td>
<td>JAK2/MPL</td>
<td>Chromosome banding analysis, FISH</td>
<td>PCR or VNTR/STR</td>
<td>MRI</td>
<td>Flow cytometry</td>
<td>All patients</td>
</tr>
</tbody>
</table>

MDS indicates myelodysplastic syndromes; MRI, magnetic resonance imaging; NCI, National Cancer Institute; NHL, non-Hodgkin lymphoma; PCR, polymerase chain reaction; qPCR, quantitative real-time PCR; STR, short tandem repeat; TCR, T cell receptor; VNTR, variable number tandem repeat; IWG-MRT, International Working Group for Myelofibrosis Research and Treatment; IMWG, International Myeloma Working Group; IW-CLL, International Workshop on Chronic Lymphocytic Leukemia; PET, positron emission tomography; CT, computerized axial tomography.

*Further studies are needed.
Table 6. Recommendations from the Committee on Disease-Specific Methods and Strategies for Monitoring Relapse following Allogeneic Stem Cell Transplantation

1. Specific definitions for posttransplantation remission and relapse using sensitive methods for monitoring or MRD as proposed by the Committee should be incorporated into all future transplantation trials to provide consistency in reporting and interpretation of data.

2. Multicenter studies need to be conducted with specific posttransplantation definitions for remission and relapse using sensitive methods for monitoring or MRD for each disease entity to:
   a. Assess whether the results obtained with methods are reproducible across different institutions and laboratories to ensure that results are comparable.
   b. Assess their utility in the prediction of overt relapse.

3. Once the reproducibility and utility of these definitions and methods are established, subsequent studies should be performed to evaluate the efficacy of these methods to guide therapeutic interventions designed to prevent overt relapse. Specifically, future studies should include the following:
   a. Standardized measurement of molecular markers for each hematologic malignancy for which allo-HSCT is used;
   b. Determination of the optimal frequency for monitoring MRD and chimerism after allo-HSCT;
   c. Definition of kinetic changes in MRD and chimerism that occur after allo-HSCT and modification of criteria for remission and relapse, followed by incorporation of these measures in the definitions of response and remission after allo-HSCT;
   d. Assessment of the efficacy of interventional strategies—based changes in MRD and/or chimerism to prevent clinical relapse.

4. As new techniques and markers emerge, every effort must be made to incorporate these techniques and markers into proposed definitions and methods as they become available.

biology and responsiveness of disease that progresses rapidly after transplantation is likely very different from that of disease that relapses later after transplantation. Thus, treatment options are likely to vary between these different patient groups. As such, there is no single standard approach to treating relapse after allo-HSCT; however, there are general principles that may be considered to guide the treatment of relapse.

Withdrawal of immune suppression and DLI are routinely considered for patients who relapse after allo-HSCT and do not have GVHD. With the possible exception of CML, whether there is a relationship between cell dose and toxicity with DLI remains unclear. Moreover, it is not known whether there is a dose-response effect, or rather a minimal threshold dose that must be achieved before antitumor responses occur. Whether these dose effects might be disease- or disease state–specific is also unknown. There are clinical situations in which responses to DLI have consistently been poor and maneuvers to improve GVT induction need to be tested rapidly and comprehensively. The study and elucidation of the mechanisms underlying relapse should facilitate the development of disease-specific and patient-specific treatment strategies.

Given the multitude of confounding issues and the relatively small numbers of patients, the Committee on Treatment of Relapse for this Workshop unanimously acknowledged the need for well-designed international cooperative trials to rapidly test and disseminate the best strategies for treating relapse after transplantation (Table 7). There is an absolute need for disease-specific clinical trials, particularly in situations where cellular therapies have been ineffective. To facilitate progress in managing disease relapse with cellular, conventional, and biological therapies, it is recommended that such clinical trials and the organizations that execute them contain the following components: (1) creation and maintenance of databases dealing specifically with relapse information, (2) measurement of immunologic effects in addition to disease outcomes, (3) adoption of uniform treatment assessment criteria as proposed by the Workshop, and (4) design of relapse-specific Phase I and II studies. It is essential that pharmaceutical companies and the Food and Drug Administration (or other international regulatory agencies) be actively involved in the design of such trials. To facilitate the rapid dissemination and conduct of such trials, it is recommended that an international collaborative treatment network dedicated to the treatment of postransplantation be established. This network would work in collaboration to bring forward the results of Phase I and II trials for utilization in Phase III randomized trials within larger cooperative groups and networks.

GENERAL RECOMMENDATIONS

The National Cancer Institute’s First International Workshop on the Biology, Prevention, and Treatment of Relapse after Allogeneic Hematopoietic Stem Cell Transplantation closed with an executive summary session chaired by Dr. Sergio Giralt. This summary attempted to place the Workshop presentations into context and to begin to develop overall recommendations to share with the transplantation community. Dr. Giralt titled his session “Where Do

Table 7. Recommendations from the Committee on Treatment of Relapse after Allogeneic Hematopoietic Stem Cell Transplantation

1. Conduct well-designed international cooperative trials that are disease-specific to rapidly test and disseminate the best strategies for relapse treatment after transplantation. Specifically, there is a need for disease-specific clinical trials in the following areas:
   a. In patients eligible to receive DLI, determination of whether there is a dose-response effect or a minimal threshold dose that must be achieved before antitumor responses occur;
   b. Design and implementation of Phase I and II trials for patients who are ineligible to receive DLI (eg, active GVHD) or in whom cellular therapies have been ineffective.

2. All clinical trials designed for the treatment of relapse after allo-HSCT should contain:
   a. Measurement of immunologic effects in addition to disease outcomes;
   b. Treatment response criteria using Workshop-suggested methods.

3. Organizations that execute clinical trials for the treatment of relapse after allo-HSCT should create and maintain databases that deal specifically with relapse information.
Table 8. General Recommendations from the Organizing Committee representing the participants in the National Cancer Institute’s First International Workshop on the Biology, Prevention, and Treatment of Relapse after Allogeneic Hematopoietic Stem Cell Transplantation

1. Establishment of multicenter correlative and clinical trials networks:
   a. Specific networks of researchers and their specific institutions should be established in the following areas:
      (1) Biology (basic and translational)
      (2) Epidemiology
      (3) Treatment.
   b. Networks may be semiautonomous, but should be collaborative relative to specific scientific agendas and sample acquisition.
   c. To most rapidly implement such networks, investigators and institutions with specific research interests in relapse and established core resources should align to address specific research interests.
   d. Using specific and prioritized research agendas, all networks should work in a coordinated and collaborative manner with established organizations.

2. Establishment of biorepositories for pre– and post–allo-HSCT samples.
   a. To aid laboratory and clinical studies of relapse after allo-HSCT, the following should be collected for deposition within the biorepositories:
      (a) Pretransplantation tumor samples to study resistance mechanisms
      (b) An aliquot from the allograft for assessment of graft composition
      (c) Blood samples at set time points posttransplantation and at the time of relapse for analysis of immune functions
      (d) Posttransplantation tumor samples at the time of relapse for comparison to pretransplantation specimens and for mechanisms of resistance to both cytotoxic and immunologic effects of transplantation.
   b. Standards need to be established for sample collection, storage, and administration. It is proposed that investigators and institutions with established repositories set initial standards and to propose specific studies.

3. Refinement, acceptance, and implementation of Workshop response and relapse definitions and monitoring methods
   a. Standard methods, criteria, and definitions that are applicable to the allo-HSCT setting need to be further refined, accepted, and implemented by all transplantation organizations and incorporated into clinical trials to:
      (1) Determine the clinical relevance of MRD surveillance in individual diseases;
      (2) Assess the potential impact of specific interventional strategies after detection of MRD.
   b. Standard methods, criteria, and definitions are meant to serve as the major starting point for universal data collection, reporting, and interpretation in studies related to transplantation after allo-HSCT.

We Go from Here?,” an apt summary of the consensus questions among the participants. This unified query was based on 2 overarching facts that were reconfirmed during the Workshop. First, despite 50 years of animal studies and 30 years of clinical experience with allo-HSCT, the questions as to why patients relapse after allo-HSCT, and what can be done to prevent this treatment failure, remain completely unanswered. Second, outcomes for patients who relapse after allo-HSCT, with the exception of those in chronic-phase CML, are not good. The immediate challenge is to establish research priorities that can rapidly and efficiently result in the greatest scientific and clinical impact, that will produce results most rapidly and efficiently, and that can be implemented practically and relatively economically. Most importantly, such priorities need to be relatively well received by the transplantation community as a whole. It is with these parameters in mind that the following general recommendations (Table 8) are made to address the problem of relapse after allo-HSCT: (1) establishment of multicenter correlative and clinical trials networks; (2) establishment of biorepositories for pre– and post–allo-HSCT samples; and (3) refinement, acceptance, and implementation of workshop response and relapse definitions and monitoring methods.

Establishment of Multicenter Correlative and Clinical Trials Networks

To rapidly implement specific studies and trials dedicated to the treatment of relapse after allo-HSCT, it is recommended that networks of researchers and their respective institutions be established in 3 specific areas: (1) biology of relapse (basic and translational); (2) epidemiology, defining the risk factors and predisposing features; and (3) treatment and prevention. These networks could be semiautonomous, but they need to be collaborative relative to specific scientific agendas and sample acquisition.

Specifically, biology networks, which may be as few as 2 to several institutions working in collaboration on specific projects, would work directly with treatment networks to obtain samples collected on treatment protocols. Similarly, epidemiology networks would receive specific data, whose collection would be incorporated into treatment protocols. Epidemiology networks would comprise institutions willing to provide extra data that are currently required by the Center for International Blood and Marrow Transplant Research, the European Group for Blood and Marrow Transplantation (EBMT), and other international transplantation registries. It is imperative that such epidemiology networks work with, if not directly through, such agencies to ensure that specific data are collected at necessary time points to answer important relapse-specific questions. These would include, but are not be limited to, data related to monitoring of MRD, prevention, and treatment. Planning for the necessary administrative support for the collection of such data is essential. As such, network participation would require institutional commitment to collecting these additional data.

Treatment networks should consist of institutions that have active clinical research programs dedicated to the prevention and treatment of relapse. Similar to the proposed epidemiology networks, treatment networks would work directly with or through established cooperative groups, such as the Blood and Marrow Transplant Clinical Trials Network (BMT CTN), to most rapidly and efficiently facilitate clinical protocols.

The keys to the success of this specific recommendation are that (1) all 3 of the proposed research networks work in a coordinated and collaborative
manner with specific and prioritized research agendas on an international scale; (2) there should be collaboration with or direct use of the resources and infrastructure of such organizations as the Center for International Blood and Marrow Transplant Research, the EBMT, and the Blood and Marrow Transplant Clinical Trials Network; and (3) there must be adequate funding through specific, directed RFAs, PO1s, or similar funding mechanisms.

Establishment of Biorepositories for Pre– and Post–Allo-HSCT Samples

The collection of pre– and post–allo-HSCT samples is needed to aid laboratory and clinical studies of relapse after allo-HSCT. It was strongly suggested that the following be collected for deposition within the biorepositories: (1) pretransplantation tumor samples when available, to study resistance mechanisms before the conditioning regimen; (2) an aliquot from the allograft, for assessment of graft composition and correlation with relapse; (3) blood samples at set time points posttransplantation and at the time of relapse, for analysis of immune functions; and (4) posttransplantation tumor samples at the time of relapse. The posttransplantation tumor samples would be used for comparison with pretransplantation specimens and to probe for mechanisms of resistance to both cytotoxic and immunologic effects of transplantation.

The availability of such samples would enable the further study of several of the key issues that have been identified by specific committees. Relative to the issue of GVT biology, the ability to prospectively study various cell populations from multiple patients under various transplantation conditions would permit identification of key effector populations, determination of the effects of various transplantation variables on cell expansion and contraction, and correlation of these findings with response to or evasion of GVT effects. The availability of pretransplantation and posttransplantation tumor samples provides opportunities to study the effects of conditioning on epigenetic phenomenon, the clinical significance of CSCs to relapse, and, possibly most importantly, the relationship of the resistance to cytotoxic effects and the resistance to GVT effects.

This effort will require biorepositories for the collection, storage, and administration of samples. Standards for sample collection, storage, and administration will need to be established. Given the high costs of the physical infrastructure and for the maintenance, administration, distribution, and prioritization of samples in these biorepositories, it is recommended that investigators and institutions with existing repositories for transplantation samples convene to set standards and to propose specific studies, in accordance with the recommendations of the Workshop Committees. Coordination of efforts among these biorepositories is essential, and an informatics structure will be critical to this effort. To support such repositories, we should promote interactive program grants among institutions to provide synergy, broaden the use of existing institutional core resources, and build on individual strengths to study the biology and systematically address strategies necessary to overcome posttransplantation relapse. It is also strongly suggested that once standards are established, sample collection and defined assays at specific time points also should be built into all clinical transplantation protocols, to facilitate the translation of basic research into future clinical research strategies.

Refinement, Acceptance, and Implementation of Workshop Response and Relapse Definitions and Monitoring Methods

As discussed earlier, standard diagnostic criteria have been established in the definition of relapse for many hematologic malignancies. These criteria are not universally accepted or used, however; definitions are lacking for certain hematologic malignancies for which allo-HSCT is used, and many of the available definitions are not clinically relevant or are insufficient for use in the allo-HSCT setting. In particular, the available definitions do not necessarily use more sensitive methods, such as molecular genetics, tumor-specific molecular primers, fluorescence in situ hybridization (FISH), multiparameter flow cytometry, and chimerism, that are commonly used to monitor patients for relapse after allo-HSCT. One of the most important questions regarding relapse after allo-HSCT is the clinical relevance of MRD surveillance in individual diseases, which in turn must be followed by studies to assess the potential impact of specific interventional strategies. Such assessment will be difficult, if not meaningless, if standard methods, criteria, and definitions that are specifically applicable to the allo-HSCT setting are not used. Standard definitions are also important, because sensitivity will increase as techniques improve. It is the consensus viewpoint of the Workshop that the use of these proposed methods (Table 5) be accepted and implemented as the standard by all transplantation organizations [18,19]. Further refinement of diagnostic criteria using sensitive methods will be essential for the success of initiatives and proposed future studies on the natural history of relapse, therapeutic interventions to prevent clinical relapse, and the treatment of relapse. It is well understood that the proposed methods and definitions have limitations and are dynamic in that they will require constant revision as results become available relative to clinical utility and as new technologies emerge. Nonetheless, they are meant to serve as the major...
starting point for universal data collection, reporting, and interpretation in studies related to relapse after allo-HSCT.

SUMMARY

It has been more than 50 years since Barnes and Loutit [32] described the experiments that gave birth to the field of allo-HSCT, and more than 30 years since E. Donnall Thomas et al. [33] reported the results in the first patients with refractory acute leukemia cured through high-dose therapy and allogeneic bone marrow transplantation. Although significant advances have been made in reducing mortality rates due to toxicity and GVHD, no major advances have been made in relapse-related mortality. This first Workshop demonstrated that there exist the scientific interest and necessary critical mass of investigators to address the essential biological and mechanistic questions and to develop trials to study prevention and treatment of relapse, as well as the need and desire to work together on such projects. Thus, the provision of resources and infrastructure to conduct this work should lead to improved understanding of the causes underlying relapse after allo-HSCT, as well as to the development of potential strategies for prevention and treatment of this complication.

ACKNOWLEDGMENTS

Financial disclosure: This work was supported by the Center for Cancer Research, National Cancer Institute, Intramural Research Program.

REFERENCES


