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REVIEWS

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Defining the Niche for Reduced-Intensity Conditioning in Adult and Pediatric Stem Cell Transplantation

Jan S. Moreb, MD, John R. Wingard, MD

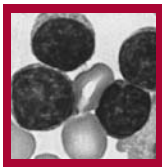
In the late 1990s, the use of reduced-intensity conditioning regimen (RIC) was a major paradigm shift in the field of stem cell transplantation. The main idea was to harness the graft-versus-tumor (GVT) effect to achieve the ultimate goal of cure without the need for toxic myeloablative therapy, allowing older patients and patients with comorbidities to undergo allogeneic transplantation. In addition, patients with non-malignant diseases, mainly children, also were candidates for this approach. It became clear that engraftment after RIC can result in mixed donor chimerism adequate to correct the medical problem without subjecting the patient to short- and long-term toxicities associated with myeloablative transplantation regimens.

The RIC approach has been applied widely and quickly even before assessment of long-term outcomes, such as treatment-related mortality and disease-free and overall survival. Different RIC regimens containing different combinations of chemotherapy, radiation, and immunosuppressive drugs were used depending on disease type or status. Because there has been no clear and set definition of what constitutes a RIC regimen, the results have been difficult to interpret and apply widely. Multiple prospective and retrospective studies have been published that now allow us to look back and assess achievements.

Many of these studies and the lessons learned were discussed at the BMT Tandem Meeting held in February 2009 in Tampa, Florida. The proceedings of that symposium are included in this issue of Reviews. The three speakers eloquently summarized data on the use of RIC allogeneic transplantation in myeloid diseases, lymphomas, and pediatric diseases. The experience shows that certain patients and certain diseases benefit better from RIC transplants. Histology, chemosensitivity, and comorbidity index can influence outcome.

Although advances have been made, many challenges remain, including high relapse rates in certain diseases, high incidence of chronic graft-versus-host disease (cGVHD), and whether certain RIC regimens are better than others. Furthermore, despite the decrease in regimen related toxicity with RIC, the average non-relapse mortality has remained around 15%. This lower non-relapse mortality can be acceptable for high risk patients but not for indolent malignancies and non-malignant pediatric diseases.

The speakers address these challenges and outline future directions that can potentially lead to reduced toxicity and improved long-term outcomes. Augmented RIC regimens, better control of cGVHD without losing GVT effect, and incorporation of new targeted therapies are among the proposed future enhancements. Future prospective randomized clinical trials will need our collective effort to better define the niche for these RIC transplants in the next decade.



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Blood and Marrow TRANSPLANTATION REVIEWS

Symposium Report

Emerging Options in Reduced-Intensity Stem Cell Transplantation: Who, How, and When?

Adapted from a continuing medical education symposium presented at the 2009 BMT Tandem Meetings on February 13, 2009, in Tampa, Florida.
This program is supported by an educational grant from Otsuka America Pharmaceuticals, Inc.

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Program Overview

Reduced-intensity conditioning (RIC) has emerged as a stem cell transplantation option for clinical situations in which an anti-tumor effect beyond that achieved by traditional chemotherapy is desired, yet there is an elevated concern for treatment-related toxicity. In adult populations, RIC compared with traditional myeloablative approaches is most commonly performed in those who are older and have more comorbidities. However, as clinical experience grows with RIC, additional patient populations, including pediatric patients with malignant and non-malignant diseases, are considered candidates for this therapeutic modality.

The increased interest in RIC stem cell transplantation for diverse patient populations raises the following questions: who is the optimal candidate for RIC, based on age, comorbidities, and disease characteristics? What preparative regimens should be used and why? And when in the course of their illness should RIC be considered? In this symposium, three leading experts on stem cell transplantation will review the clinical indications for myeloablative versus reduced-intensity stem cell transplantation; discuss recent research in the application of reduced-intensity conditioning for the treatment of myeloid malignancies, lymphoid malignancies, and hemoglobinopathies; describe available and emerging conditioning regimens for RISCT; and discuss the long-term sequelae of survivorship in patients who have undergone a myeloablative transplant.

Target Audience

This continuing education activity is targeted to clinicians caring for patients undergoing bone marrow and stem cell transplantation.

Learning Objectives

- Describe clinical indications for myeloablative versus reduced-intensity stem cell transplantation
- Discuss recent research in the application of reduced-intensity conditioning for the treatment of myeloid malignancies, lymphoid malignancies, and hemoglobinopathies
- Identify available and emerging conditioning regimens for RISCT
- Discuss the long-term sequelae of survivorship in pediatric patients who have undergone a myeloablative transplant

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This educational activity may contain discussion of published and/or investigational uses of agents that are not approved by the US Food and Drug Administration. For additional information about approved uses, including approved indications, contraindications, and warnings, please refer to the prescribing information for each product, or consult the Physician's Desk Reference.

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Consistent with the current Accreditation Council for Continuing Medical Education policy, the CME Provider must be able to show that everyone who is in a position to control the content of an individual educational activity has disclosed all relevant financial relationships. The CME Provider has a mechanism in place to identify and resolve any conflicts of interest discovered in the disclosure process. The presenting faculty members have all made the proper disclosures, and the following relationships are relevant:

Steven M. Devine, MD, discloses that he is a speaker for Genzyme Inc.

Morris Kletzel, MD, FAAP, MBA, does not have any relevant financial disclosures.

Robert J. Soiffer, MD, discloses that he is an advisor/consultant for Genzyme Inc. and Bristol-Myers Squibb; and is a speaker for Amgen.

Introduction

Reduced-intensity conditioning (RIC) has emerged as an option for hematopoietic stem cell transplantation (HCT) in clinical situations in which an anti-tumor effect beyond that achieved by traditional chemotherapy is desired, yet there is an elevated concern for treatment-related toxicity. RIC regimens are designed to be less myelosuppressive than traditional preparative regimens, yet they still must exert adequate immunosuppressive effects to allow for successful engraftment. In patients who are treated with RIC, the graft-versus-leukemia effect—in which allogeneic T-cells provide an immunological insult against residual leukemia cells that survived the conditioning regimen—is essential for fighting the malignancy. As an alternative to traditional myeloablative conditioning regimens, RIC regimens are commonly used in the treatment of disorders in which traditional myeloablative conditioning regimens are associated with high rates of non-relapse mortality, including the spectrum of lymphoid and myeloid malignancies.

Although RIC regimens are associated with lower rates of severe toxicity and non-relapse mortality, the issues of infection, graft-versus-host disease (GVHD), and relapse of primary disease remain important obstacles to achieving optimal patient outcomes. Compared with traditional myeloablative approaches, RIC is most commonly performed in adult patients who are older and have more comorbidities; however, as clinical experience grows with RIC, additional patient populations, including pediatric patients with malignant and nonmalignant diseases, are considered candidates for this therapeutic modality.

Reduced-Intensity Allogeneic Transplantation in Patients with Myeloid Diseases: Are We Making Progress?

Steven M. Devine, MD

Within the past 15 years, research in the area of RIC allografts for myeloid malignancies has yielded a range of compelling evidence. Single-institution and intergroup studies have shown that RIC transplant is feasible in this population, with reliable engraftment, manageable GVHD risk, and utility in older and sicker patients. Although relapse rates appear higher following RIC transplantations compared with myeloablative allografts, this trend may not be consistent across all patient subgroups. New research has focused on identifying the patient subgroups that are most likely to benefit from RIC allogeneic transplantation.

Definitions of low-, moderate-, and high-intensity conditioning regimens are somewhat arbitrary and, as a result, differ across studies. The Center for International Blood and Marrow Transplant Research (CIBMTR) defines myeloablative regimens as follows:

- Total body irradiation doses of 500 cGy in a single dose or 800 cGy fractionated
- Busulfan >9 mg/kg or melphalan >150 mg/m^2 administered as single agents or in combination with other drugs

In the CIBMTR registry of allogeneic transplantations, the prevalence of RIC has steadily increased since 1998 [1]. Based on 2006 data, approximately 40% of allogeneic transplantations now use reduced-intensity regimens. RIC regimens have contributed to the increased use of allogeneic transplantation in older (and sicker) patients. Currently, more than 50% of patients receiving reduced-intensity conditioning for acute myeloid leukemia (AML) are older than age 50, compared to fewer than 25% of those receiving standard high-intensity regimens (Figure 1) [1].

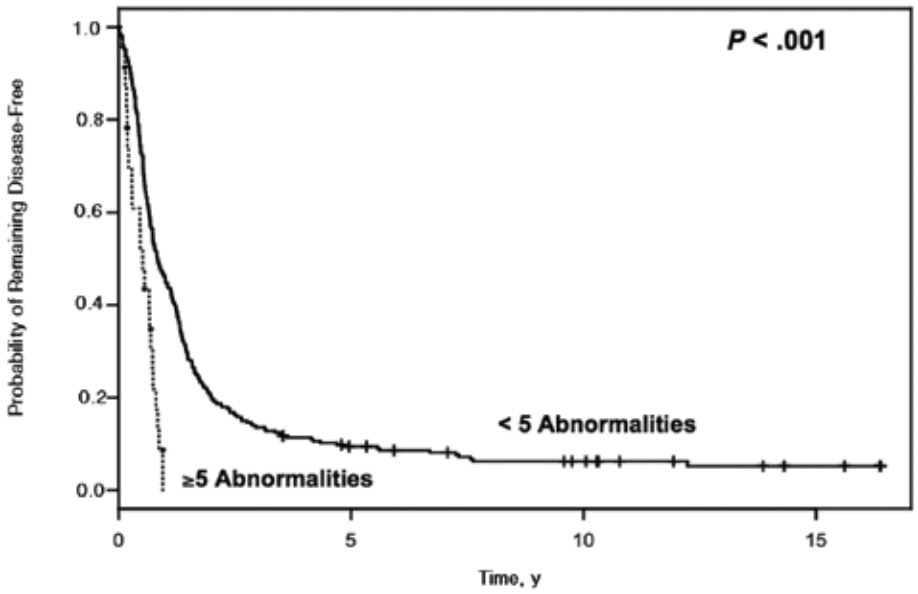

Figure 1. Allogeneic transplantations for acute myeloid leukemia (AML) by conditioning regimen intensity and age.

RIC in Older Patients with AML

The discovery of recurring chromosome aberrations in patients with acute leukemia has aided the identification of clinical and prognostic patient subgroups. The Cancer and Leukemia Group B (CALGB) 8461 trial highlighted the importance of pretreatment cytogenetics in estimating disease-free survival in older patients (≥60 years) with AML [2]. In the study of 635 older patients, complex karyotypes with at least 5 abnormalities were associated with a significantly lower disease-free survival compared with karyotypes with <5 abnormalities (P < .001) (Figure 2). The 2-year overall survival for these poor-prognosis patients was 0%. Given that patients with complex karyotypes appear to gain minimal benefit from current first-line treatment options, these patients may be better suited for clinical trial participation or supportive care [2].

Findings from the CALGB 8461 trial are indicative of the poor prognosis of most older patients with AML. In the extensive CALGB

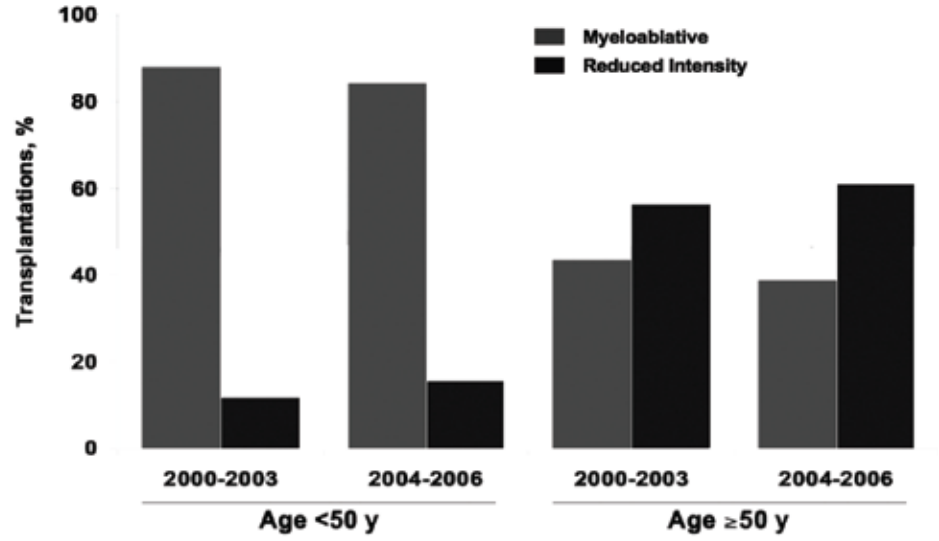

Figure 2. Disease-free survival by number of cytogenetic abnormalities for acute myeloid leukemia (AML) patients ≥60 years old [2].

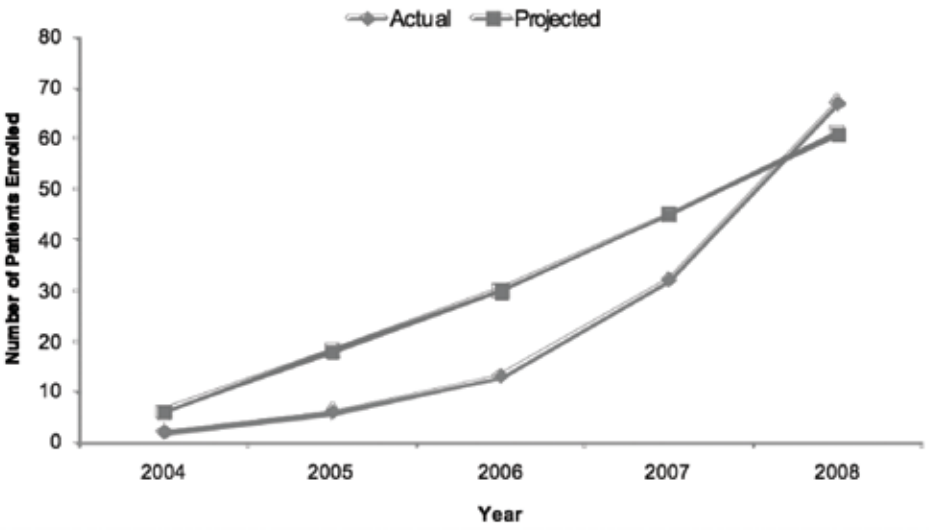

Figure 3. Accrual to the CALGB 100103/BMT CTN 0502 trial through 2008.

database, even the patients with the most favorable prognosis will have long-term survival rates of <10%. In a subset of patients between age 60 and 75 years who received their first post-remission treatment in CALGB chemotherapy studies, the 2-year disease-free survival was 24%, and the 3-year disease-free survival was 17% [3].

To improve upon these outcomes, the ongoing CALGB 100103 trial has been examining RIC allogeneic transplantation as the initial post-remission therapy for older patients (>60 years) with AML. In particular, the trial is designed to determine whether allogeneic HCT from a human leukocyte antigen (HLA)-matched sibling or unrelated donor following nonmyeloablative conditioning with fludarabine and busulfan can achieve a 2-year disease-free survival of up to 40%, which is better than results to date. The fludarabine/busulfan regimen was chosen in 2003 based on the limited data available at the time. The target enrollment is 61 older patients with AML who are in their first morphologic complete remission.

The CALGB 100103 protocol has been modified since the trial started. When the study opened in 2004, only patients with matched sibling donors were eligible to enroll. With this criterion, investigators estimated that 36 patients would be required to demonstrate a 2-year disease-free survival of 40%, and that the accrual should require approximately 36 months to complete enrollment. The initial accrual rate fell dramatically short of projections, however, with only 5 patients enrolled in the first year. In 2006, the CALGB investigators joined with the Blood and Marrow Transplant Clinical Trials Network (BMT CTN) to adjust the protocol of the study, which is now called the CALGB 100103/CTN 0502 trial.

Together, the CALGB and BMT CTN investigators modified the protocol to allow patients with unrelated donors, to include antithymocyte globulin (ATG) in the preparative regimen for all patients, and to remove donor leukocyte infusions. Investigators also lowered the disease-free survival goal to 35% at 2 years. As a result of these modifications, the target enrollment increased to 61 patients. With these adjustments, accrual has increased dramatically. By the end of 2008, 55 patients have been enrolled in the trial (Figure 3).

According to preliminary data from the CALGB 100103/CTN 0502 trial, overall treatment related mortality has been low (<10%), despite expanding the patient population to include those with unrelated donors. Although graft rejection and acute GVHD have not been major issues, chronic GVHD-related complications appear to be a problem. Relapse still appears to be the greatest cause of treatment failure.

With the design and launch of this trial, the CALGB and BMT CTN investigators learned that performing multicenter trials in this disease setting is difficult and requires truly collaborative efforts. The CALGB 100103/CTN 0502 trial is currently suspended, but will reopen in late winter 2009 to increase the number of unrelated-donor recipients.

Future trials of RIC regimens for older AML patients may focus on issues related to dose intensity, mitigating relapse and chronic GVD, and augmenting conditioning with other agents such as clofarabine or treosulfan without increasing toxicity. For example, the CALGB 100801 phase IIa trial will examine the addition 5-azacytidine to reduced-intensity

allogeneic transplantation for myelodysplastic syndrome (MDS) and older patients with AML. The Eastern Cooperative Oncology Group (ECOG) E2906 phase III trial will compare clofarabine as induction and post-remission therapy with standard daunorubicin and cytarabine induction and intermediate-dose cytarabine post-remission therapy, followed by becitabine maintenance versus observation in older patients with newly diagnosed AML.

In summary, initial trials of reduced-intensity allografts reveal that at least a subset of older patients with AML are uniquely sensitive to a graft versus tumor effect. Yet, too many patients relapse after this procedure. Improvements will require better risk assessment, identifying those most likely to respond to immunotherapy, as well as better and earlier interventions with cellular therapies or immunomodulatory drugs designed to mitigate relapse. This will be accelerated through cooperative efforts among BMT groups.

RIC in Younger Patients with AML

Myeloablative conditioning regimens have been the standard approach to allogeneic HCT in younger patients with AML, yet dose intensity modifications may be required to avoid early toxicity and preserve fertility in younger patients with a longer life expectancy. Over the past 2 decades, a handful of studies have addressed the role of conditioning regimen dose intensity in younger patients with AML who are in their first confirmed remission. In 1990, Clift and colleagues showed that an increased dose of total body irradiation (15.75 Gy) reduced the probability of relapse compared with a lower dose (12.0 Gy), but did not improve survival due to an increase in the risk of non-relapse mortality [4]. Later, in 1999, Aversa demonstrated that adding antithymocyte globulin (ATG) and thiotepa to the conditioning regimen prevented rejection of extensively T-cell–depleted bone marrow in patients with acute leukemia who received transplants from matched sibling donors [5].

More recent data continue to underscore the complexity of transplantation dose intensity without identifying an optimal approach. In 2006, Shimoni showed that reduced-intensity conditioning with fludarabine and busulfan 6.4 mg/kg was as effective as myeloablative conditioning with cyclophosphamide or fludarabine and busulfan 12.8 mg/kg in AML patients when disease was in remission at the time of allogeneic HCT, but that patients with active disease could only be salvaged with myeloablative transplantation [6].

At the 2008 American Society of Hematology (ASH) annual meeting, Luger and colleagues reported findings from a large multicenter trial of 3731 myeloablative and 1500 nonmyeloablative/RIC procedures performed between 1997 and 2004. In this nonrandomized study, patients in the myeloablative group were younger (median age, 42 years), more frequently in first remission, and more likely to have received matched sibling-donor grafts. By comparison, patients in the nonmyeloablative/RIC group were older (median age, 55 years), less often transplanted with >10% blasts, and had a lower performance scores. In a multivariate analysis, nonmyeloablative conditioning increased the risk of relapse by 64% ($P < .001$) and the risk of treatment-related mortality by 55% ($P < .001$) compared with myeloablative conditioning. A late increase in treatment-related mortality negated early advantages offered by nonmyeloablative/RIC regimens, resulting in slightly better 5-year leukemia-free survival in the myeloablative group [7].

Given findings from observational studies to date, there is a clear need for prospective trials comparing nonmyeloablative and myeloablative conditioning intensities in AML patients eligible for either approach. Additional research questions may explore whether some RIC regimens are more effective in AML than others; which agents provide the best therapeutic index in this population; whether the specific GVHD prophylaxis regimen influences longer-term outcomes; and whether addition of monoclonal or polyclonal antibodies can improve efficacy results without compromising safety.

Interpreting results of trials in this setting poses an additional challenge. Most trials suggest a very high risk of relapse in patients with AML, and long-term benefits may be related to the development of chronic GVHD. In a recent prospective trial of 93 patients with AML and MDS (median age, 53 years), patients received conditioning with fludarabine 150 mg/m^2 and oral busulfan 8 to 10 mg/kg prior to allogeneic HCT, as well as GVHD prophylaxis with cyclosporine and methotrexate or mycophenolate mofetil. Non-relapse mortality was 8% at 100 days, 16% at 1 year, and 21% at 4 years. Leukemia relapse was the major cause of death, and the cumulative relapse rates at 1 and 4 years were 23% and 37%, respectively. At 4 years, disease-free survival was 43%, and overall survival was 45%. The development of chronic GVHD (53% at 4 years, including 45% extensive GVHD) appeared to be a major factor associated with the sustained remission and improved survival rates observed in this trial [8].

Reduced-intensity transplantation is also feasible in younger patients treated with unrelated donor transplantation in primary refractory AML. In a trial of 186 adults, 136 patients received transplantations using myeloablative conditioning, while 50 received RIC prior to transplantation. In a multivariate analysis, the use of an RIC regimen was associated with improved survival ($P = .036$) [9].

In patients with AML, the benefits of RIC may be particularly striking when combined with adjunctive therapy. Dozens of phase I and II trials are currently evaluating the incorporation of agents such as clofarabine, treosulfan, melphalan, targeted busulfan, carmustine, gemtuzumab ozogamicin, and marrow-targeted anti-CD33, anti-CD45, and anti-CD66 radioimmunotherapy into RIC regimens. Many of these trials are designed to demonstrate that augmented RIC regimens can improve disease control without significantly increasing toxicity, including non-relapse mortality. As an alternative to augmentation, some early-phase studies are evaluating other preemptive therapies initiated post-transplantation. These include the use of azacytidine, lenalidomide, or cellular therapies such as unmanipulated donor lymphocyte infusions (DLI), antigen-specific DLI (eg, anti-WT1), and other approaches.

Lastly, investigators are exploring the use of RIC allografting in other myeloid diseases, including chronic myeloid lymphoma (CML), MDS, and idiopathic myelofibrosis. Preliminary data suggest that RIC with post-transplantation imatinib delays relapse and postpones the need for DLIs in patients with CML [10]. Fludarabine-based RIC regimens also appear to provide adequate disease control in chronic-phase CML patients who are not eligible for myeloablative preparative regimens due to older age or comorbid conditions [11]. Research questions in MDS include the optimal management of patients with elevated blast counts. For patients with idiopathic myelofibrosis, future studies may demonstrate that RIC is as effective as and less toxic than myeloablative transplantation. Current studies in this setting include the use of fludarabine and melphalan conditioning prior to allogeneic transplantation.

Conclusions

Despite a recent surge in early-phase data, significant questions remain regarding the most appropriate clinical situations to apply reduced-intensity conditioning and allogeneic HCT in patients with myeloid diseases. The greatest potential benefit appears to be in older patients with AML in first remission and in those with MDS, yet this still awaits confirmation in prospective trials. Several combination RIC regimens with and without total body irradiation appear effective, and to date there is no way to clearly discern which is best for a particular patient. Collaborative multicenter efforts provide the best opportunity for rapidly evaluating promising treatment approaches.

Reduced-Intensity Allogeneic Hematopoietic Cell Transplantation for Lymphoid Malignancies

Robert J. Soiffer, MD

RIC regimens have emerged as a therapeutic option for patients with lymphoid malignancies who require aggressive tumor control, but do not meet criteria for myeloablative transplant. Ideally, non-myeloablative conditioning regimens should be minimally toxic, should provide reliable and reproducible levels of active agent, and should facilitate a high degree of donor hematopoietic chimerism to potentiate graft-versus-lymphoma (GVL) effects. Particularly in patients with follicular lymphoma, nonmyeloablative stem cell transplantation also exploits GVL immunity to prolong treatment response.

RIC regimens typically include low-dose total body irradiation (200 cGy) with or without the addition of fludarabine. Regimens may also include alkylating agents such as cyclophosphamide, busulfan, and melphalan, as well as ATG and alemtuzumab to facilitate engraftment. To date, no studies have been able to determine the optimal RIC regimen for prolonging survival.

Durable Responses Following RIC

In 2008, Khouri and colleagues reported 8-year efficacy and safety findings from a prospective trial of nonmyeloablative HCT in patients with relapsed follicular lymphoma [12]. In total, 47 patients received a conditioning regimen of fludarabine (30 mg/m^2 daily for 3 days), cyclophosphamide (750 mg/m^2 daily for 3 days), and rituximab (375 mg/m^2 for 1 day plus 1000 mg/m^2 for 3 days). The majority of patients (n = 45) received HLA-matched cells from related donors, whereas 2 patients received cells from unrelated donors. Patients were also given GVHD prophylaxis with tacrolimus and methotrexate. With this regimen, all patients experienced a complete remission, and only 2 patients relapsed during the median follow-up of 60 months. The 5-year overall survival was 85%, and the 5-year progression free survival was 83% (Figure 4) [12]. According to the study authors, these findings represent significant progress toward developing a curative strategy for patients with recurrent follicular lymphoma.

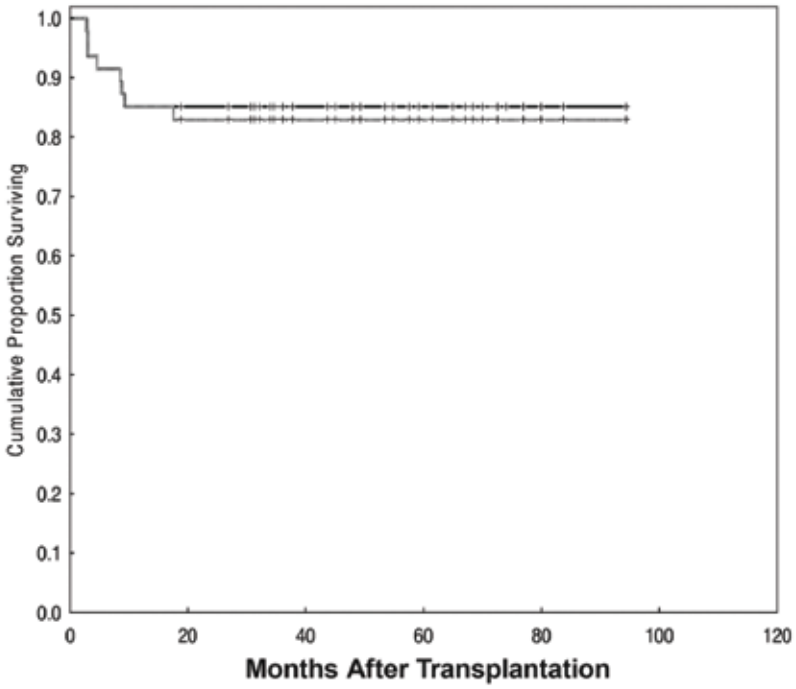

Figure 4. Overall and survival and progression-free survival following reduced-intensity conditioning (RIC) allograft in follicular lymphoma [12].

Nonmyeloablative allogeneic HCT provides durable disease control even among heavily pretreated patients. In another prospective study, 62 patients with indolent or transformed non-Hodgkin's Lymphoma (NHL) underwent RIC with 2 Gy of total body irradiation with or without fludarabine followed by allogeneic HCT from related (n = 34) or unrelated (n = 28) donors. In this heavily pretreated group, patients had received a median of 6 lines of treatment before HCT, and 44% of patients received previous high-dose therapy with autologous HCT. The 3-year overall survival among patients with indolent and transformed disease was 42% and 18%, respectively. Compared with other subgroups, patients with indolent disease and related donors had the highest 3-year survival rate (67%) [13]. These findings demonstrate the utility of nonmyeloablative HCT in chemotherapy-refractory NHL, though patients with untransformed disease appear to have a more favorable prognosis.

Other conditioning regimens also yield durable remissions in patients with follicular lymphoma. In a retrospective analysis of 87

patients with advanced lymphoma, allogeneic HCT followed a conditioning regimen that consisted of fludarabine and low-dose busulfan. Within the subgroup of patients with indolent lymphoma, the 3-year overall survival was 81% [14]. In 2002, the European Group for Blood and Bone Marrow Transplantation (EBMT) evaluated whether the type of conditioning regimen influenced clinical outcomes. The majority of patients (84%) received conditioning with fludarabine-based regimens, including fludarabine plus melphalan (n = 63), cyclophosphamide (n = 41), busulphan (n = 31), or another alkylating agent (n = 13). Among remaining patients, 18 (10%) received conditioning with carmustine, etoposide, cytosine arabinoside, melphalan (BEAM), and 9 patients received fludarabine, AraC, and idarubicin (FLAG-IDA). After a median follow-up of 2 years, the overall survival was 63%. A multivariate analysis identified chemosensitivity, but not conditioning regimen, as a predictor of outcome [15].

Patient Selection Criteria

Patients with relapsed lymphoid malignancies have a variety of histologies, tend to be older, have more comorbidities, and have a history of autologous HCT. Several trials have examined whether these factors influence outcome, and whether certain patient subgroups are more likely to benefit from RIC allogeneic HCT. In 2008, Sorror and colleagues reported outcomes from 220 patients treated at Fred Hutchinson Cancer Research Center in Seattle, WA. In this study, patients received either nonmyeloablative (n = 152) or myeloablative (n = 68) conditioning and were stratified according to the HCT-specific comorbidity index (HCT-CI). For patients with no comorbidities, overall survival was similar in the nonmyeloablative and myeloablative conditioning regimen groups. By comparison, patients with at least 1 comorbidity had significantly better outcomes following nonmyeloablative conditioning. In patients with comorbidities, nonmyeloablative conditioning significantly lowered the risk of non-relapse mortality (P = .009) and increased overall survival (P = .04) compared with myeloablative conditioning (Figure 5) [16].

Histology is also an independent predictor of outcome following RIC allogeneic transplantation for relapsed lymphomas [13,14]. This was shown definitively in a trial of 170 patients with indolent NHL (n = 63), aggressive NHL (n = 61), mantle cell lymphoma (MCL; n = 14), and Hodgkin's disease (n = 32). The 3-year overall survival in these patient groups was 69%, 69%, 45%, and 32%, respectively. Compared with indolent lymphoma, the risk of reaching the overall survival endpoint was significantly increased in the aggressive NHL (hazard ratio [HR], 1.57), MCL (HR, 2.56), and Hodgkin's lymphoma (HR, 3.57) groups (P = .031). Reaching the progression-free survival endpoint was 1.5-fold more likely in the aggressive NHL group than in the indolent NHL group, 2-fold more likely in the MCL group, and more than 4-fold more likely in the Hodgkin's disease group (P < .001) [17].

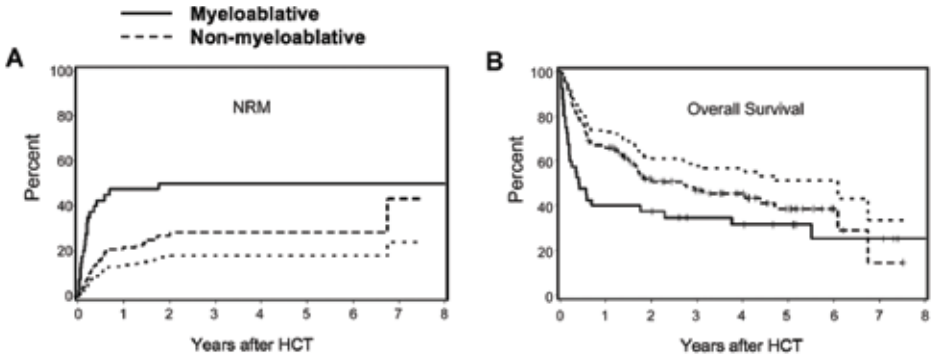

Figure 5. Non-relapse mortality and overall survival in chronic lymphocytic leukemia (CLL)/non-Hodgkin's Lymphoma (NHL) patients with ≥1 comorbidity [16].

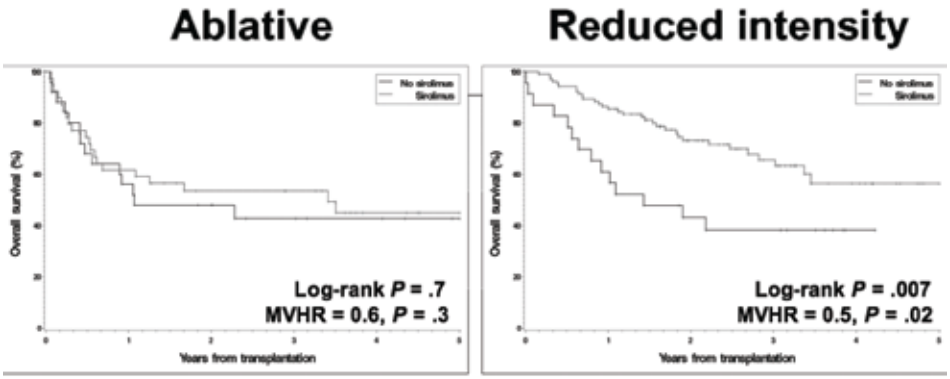

Figure 6. Overall survival with and without sirolimus following myeloablative and reduced-intesity conditioning (RIC) allograft [18].

Future Options for RIC

Chemosensitivity, comorbidity index, and histology—but not specific RIC regimen—appear to influence outcome after RIC allogeneic transplant for lymphoid malignancies. Research aimed at improving outcomes continues and has recently focused on adjuvant therapies that can provide an additional incremental benefit in relapse and survival findings. Potential strategies include the addition of novel agents with anti-lymphoma activity; immune manipulation with selected infusions of cytotoxic effectors engineered to recognize lymphoma cells; and post-transplantation vaccination.

In 2008, Armand and colleagues examined the role of sirolimus on GVHD and survival rates in patients treated with nonmyeloablative or myeloablative allogeneic transplantation. Sirolimus is an inhibitor of the mammalian target of rapamycin (mTOR) kinase, which has shown anti-GVHD activity in several lymphoma subtypes. The retrospective analysis included 190 allogeneic transplantation patients who received GVHD prophylaxis with sirolimus or a combination of a calcineurin inhibitor and methotrexate without sirolimus.

In a multivariate analysis, sirolimus improved 3-year overall survival by 40% compared with the non-sirolimus group (P = .045). When patients were stratified according to conditioning intensity, the benefit of sirolimus was maintained only in the RIC group. In patients receiving RIC, 3-year overall survival was 66% in the sirolimus group and 38% in the non-sirolimus group (P = .02). By comparison, sirolimus did not influence overall survival in patients who received myeloablative conditioning (P = .3) (Figure 6). Within the RIC group, sirolimus also reduced the risk of relapse by 60% (P = .002), but did not alter non-relapse mortality (P = 1.0) [18].

These promising sirolimus data, which demonstrate that additional interventions can decrease the risk of lymphoma progression following RIC allogeneic HCT, bring the hematology community closer to a curative therapy for relapsed and refractory follicular lymphoma.

Reduced-Intensity Transplants in Pediatrics

Morris Kletzel, MD

Traditional allogeneic transplantation has been performed in pediatric patients for 50 years, yet results in this population remain suboptimal. For example, for patients with acute lymphoblastic leukemia (ALL) in their second remission can expect 3-year event-free survival rates of 30% to 40%. For pediatricians, the goals of therapy are to reduce adverse long-term effects, reduce short-term transplant-related mortality, and induce the graft-versus-tumor effect with the ultimate goal of prolonging survival.

Current transplantation strategies include condition regimens consisting of high-dose chemotherapy with or without total body irradiation. This approach is limited to younger patients (<50 years), and associated with high treatment-related morbidity and mortality rates. Moreover, traditional allogeneic transplantation is prohibitive in patients with comorbid illness or organ dysfunction.

Reduced-intensity transplantation provides an important alternative to myeloablative transplantation, and the rationale for this approach is strong. The anti-tumor effects of donor immune cells, particularly the cytotoxic natural killer (NK) cells, are thought to drive the curative potential of allogeneic transplantation. Engraftment of donor cells can occur following conditioning regimens consisting of lower, immune-suppressive doses of chemotherapy. RIC regimens are associated with less toxicity than standard myeloablative regimens. Several other differences between traditional and RIC regimens are summarized in Table 1.

No standard optimal regimen has been defined, and most regimens fall on a continuum from totally nonmyeloablative to fully myeloablative. In general, RIC allogeneic transplantation begins with a conditioning regimen that consists of immune-suppressive doses of chemotherapy and/or total body irradiation, followed by infusion of allogeneic stem cells. RIC allogeneic transplantation is also referred to as:

- Mini-transplantation
- Non-myeloablative transplantation
- Sub-myeloablative transplantation
- Immune ablative transplantation
- Chimeric transplantation
- Transplantation "light"
- Reduced-toxicity transplantation
- Reduced-intensity transplantation

Pre-clinical and early-phase studies also support the use of RIC regimens. For example, early animal data demonstrated that chimerical engraftment could be achieved following a single dose of total body irradiation (2 Gy) with infusion of matched stem cells and GVHD prophylaxis with cyclosporine and mycophenolate mofetil [19]. In a trial of 13 patients with AML who were treated with fludarabine, idarubicin, and ara-C or melphalan conditioning followed by allogeneic peripheral blood stem cells, all patients showed engraftment of ≥90% of donor cells between 14 and 30 days post-infusion. One toxic death from multiorgan failure before receiving stem cells was observed, suggesting that the RIC regimen did not have the toxicity of myeloablative therapy [20].

In another trial of 26 patients with hematological malignancies, the treatment regimen included nonmyeloablative conditioning with fludarabine, anti-T–lymphocyte globulin, and low-dose busulfan, infusion of allogeneic peripheral blood stem cells, and GVHD prophylaxis with cyclosporin A. All patients showed evidence of engraftment, with partial chimerism in 9 patients. No toxic deaths occurred, and severe GVHD was the single major complication and cause of death in 4 patients. At 14 months, the estimated disease-free survival was 77% [21]. Data from these early trials suggest that graft cells may inhibit rejection in patients treated with RIC allogeneic transplantation.

Table 1. Differences between Reduced-Intensity Conditioning (RIC) and Traditional Allogeneic Transplantations*

Traditional Allogeneic Transplantations	RIC Allogeneic Transplantations
Higher doses of chemotherapy and TBI	Lower doses of chemotherapy and TBI
Lower numbers of infused stem cells	Higher numbers of infused stem cells
Intense GVHD prophylaxis	Minimal GVHD prophylaxis
Inpatient hospital care	Outpatient care
High transplant-related toxicity	Minimal transplant-related toxicity
Increased risk of GVHD	Increased risk of chronic GVHD

*TBI indicates total body irradiation; GVHD, graft-versus-host disease.

RIC in Pediatric Leukemia

The Wilms tumor 1 (WT1) gene is a transcription factor located on chromosome 11p13 and is involved in the pathogenesis of Wilms tumor, a pediatric malignancy that occurs in embryonic kidney. In adult patients with acute leukemia, blast cells express high levels of WT1 [22]. In a study of 70 patients with adult leukemia, WT1 was expressed in 7 of 16 cases of acute lymphoblastic leukemia (ALL), 15 of 22 with AML, and 8 of 10 in blast crisis of CML. By comparison, no detectable levels of WT1 RNA were found in chronic leukemias, including chronic lymphocytic leukemia (CLL), plasma cell leukemia, hairy cell leukemia, and CML in chronic phase. These WT1 expression patterns indicate that the WT1 gene is involved in the early stage of hematological cell differentiation [23].

In another study of 150 acute leukemia patients, WT1 RNA was expressed in the

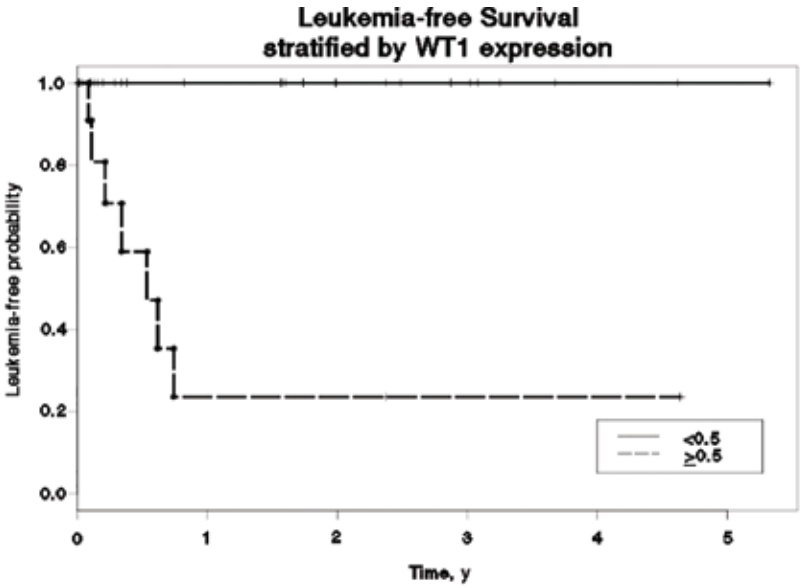

Figure 7. Leukemia-free survival following reduced-intensity conditioning (RIC) allograft stratified by Wilms Tumor 1 (WT1) expression [26].

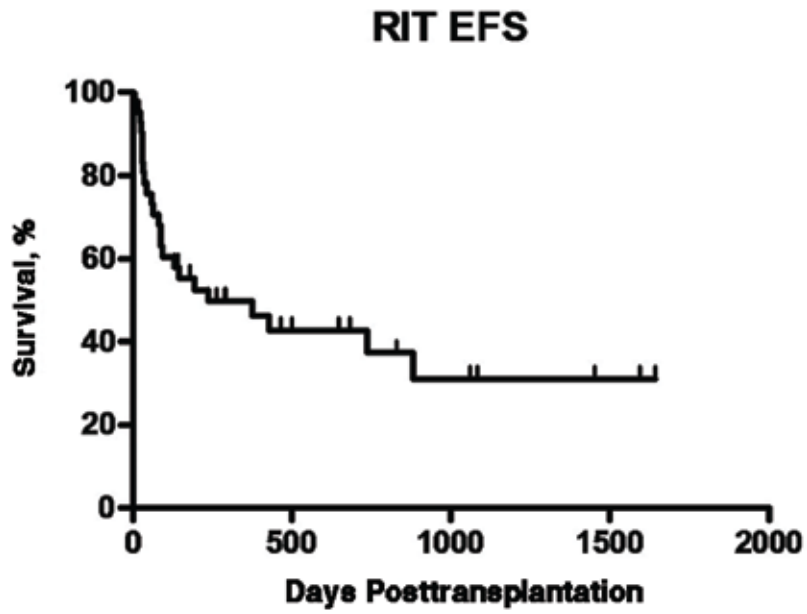

Figure 8. Event-free survival in children with malignant and nonmalignant disease [30].

majority of acute leukemias cells but not in normal mononuclear blood cells and normal CD34+ hematopoietic progenitor cells [24]. These findings suggest that as a "pan-acute leukemic" marker, expression of WT1 may be useful in detecting leukemic blast cells in purged or unpurged hematopoietic stem cell preparations intended for use in autologous bone marrow transplantation. WT1 expression may also be an effective tool for monitoring minimal residual disease (MRD) after chemotherapy in adult patients with acute leukemia [24].

WT1 also appears to be a reliable tool for the detection and monitoring of MRD in children. In 2002, Kletzel and colleagues evaluated the utility of monitoring WT1 expression in pediatric patients with leukemia at diagnosis, during therapy, and following bone marrow transplantation. This prospective study included 204 samples from patients with ALL at diagnosis (n = 45), at relapse (n = 14), and in remission (n = 45); acute non-lymphoblastic leukemia (ANLL) at diagnosis (n = 14), at relapse (n = 5), and in remission (n = 12); and CML in blast crisis (n = 1) and in chronic phase (n = 1). WT1 was expressed in high levels at diagnosis and at relapse in 95% of ALL patients, 100% of ANLL patients, and 1 CML in blast crisis. By comparison, high WT1 expression was observed in only 5 of 90 samples obtained in remission or post-transplantation ($P < .0001$). Importantly, following transplantation, a significant high level of WT1 expression was observed 2 to 6 months prior to clinical relapse [25].

Recent evidence indicates that WT1 gene expression is an important predictor of outcome in pediatric patients undergoing allogeneic HCT for acute leukemia [26]. WT1 expression and outcome were examined in 62 pediatric patients (median age, 4.0 years) with acute leukemia, including AML (n = 33) and ALL (n = 29). The conditioning regimen included:

- Total body irradiation 1200 cGy (8 fractions of 150 cGy on days –8 to –5)
- Etoposide 1000 mg/m^2 as an 8-hour infusion on day –4
- Cyclophosphamide 60 mg/kg per day on days –4 to –2

Patients received allogeneic stem cells from unrelated cord-blood (n = 33), HLA-matched unrelated donors (n = 13), or HLA-matched siblings (n = 16). Patients also received GVHD prophylaxis with cyclosporine A, short-course methotrexate for the sibling-donor recipients, and rabbit ATG for the alternative-donor recipients [26].

Increased expression of WT1 prior to transplantation was associated with a significant increase in the risk of relapse ($P = .003$) (Figure 7). Patients with amplified WT1 expression were 3.3-fold more likely to have a relapse (95% confidence interval [CI], 1.5-7.3). The correlation between WT1 expression and relapse risk was maintained even after controlling for transplantation type, acute GVHD, chronic GVHD, and HLA-antigen mismatch [26]. Additional studies have also found WT1 gene expression to correlate with MRD and other clinical outcomes in both adults and pediatric patients with acute leukemia [27,28].

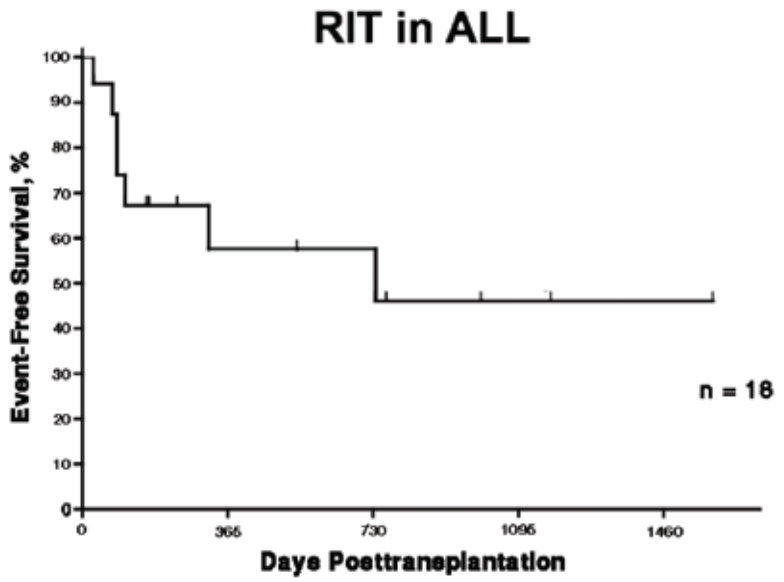

Figure 9. Event-free survival in pediatric patients with acute lymphoblastic leukemia (ALL) [31].

RIC in Nonmalignant Disease

Stem cell transplantation is not limited to malignant diseases in the pediatric setting. Hemoglobinopathies, including thalassemia and sickle cell disease, are challenging diseases for pediatric patients and their caregivers. Supportive care strategies often fail, leading patients to frequent acute exacerbations of their condition. While allogeneic stem cell transplantation has been successful in curing patients of these ailments, treatment-related morbidity and mortality, as well as significant GVHD, complicate the decision to undergo transplantation.

In pediatric patients with nonmalignant disorders, RIC followed by allogeneic HCT provides a promising alternative to conventional transplantation. Along with other institutions, Children's Memorial Hospital in Chicago, IL, has pioneered the use of RIC in this patient population. One recent analysis of the RIC experience at Children's Memorial Hospital included 13 patients (median age, 5.2 years) treated between 2000 and 2004 for a range of nonmalignant disorders including sickle cell anemia (n = 3), inborn errors of metabolism (n = 3), immune deficiencies (n = 2), X-linked hyper immunoglobulin M (IgM) syndrome (n = 2), beta thalassemia (n = 1), chronic granulomatous disease (n = 1), and X-linked lymphoproliferative disease (n = 1). The conditioning regimen consisted of fludarabine, busulfan, and ATG for all patients. Stem-cell sources included unrelated donors (n = 7), matched-sibling peripheral blood stem cells (n = 4), and unrelated cord blood (n = 2) [29].

Even among children with unrelated-donor HCT, engraftment was adequate, with acceptable toxicities. Among 11 evaluable patients, 8 (72%) achieved full donor engraftment, defined as >95% donor cells by polymerase chain reaction (PCR)-variable number tandem repeat (VNTR) screening. Two additional patients achieved partial chimerism, with 29% and 44% engraftment, respectively. The median time to full donor chimerism was 58 days. One patient (8%) developed grade 2 acute GVHD, and 3 patients (37%) developed extensive chronic GVHD. Among the 3 patients with sickle cell anemia, 2 had autologous recovery and 1 developed chronic GVHD. The patient with beta thalassemia experienced late rejection. One patient died from measles encephalitis [29].

Since this initial report of RIC in nonmalignant disease, investigators at Children's Memorial Hospital have evaluated RIC in a total of 55 patients (mean age, 8.1 years), including 38 children with malignant disease (ALL, AML/MDS, CML, NHL, and solid tumors) and 17 with nonmalignant disease (immune deficiencies, metabolic diseases, hemoglobinopathies, and aplastic anemia). The group includes 12 patients with a history of prior HCT, including 7 autologous and 5 allogeneic transplantations. In total, 22 patients received hematopoietic stem cells from matched related donors, and 33 patients received stems cells from alternative sources. Following transplantation, chimerism was evaluated by weekly VNTR assays [30].

Among the 55 patients treated to date, 3 died too early to be evaluable for engraftment. Among the 52 evaluable patients, engraftment failed in 4 of 16 patients with nonmalignant diseases and in 3 of 30 patients with malignant diseases. Compared with patients who achieved engraftment, those who failed to engraft were younger (8.8 years versus 6.3 years), lighter (27 kg versus 18 kg), and received smaller cell doses (5.9 versus 3.9 × 108 mononuclear cells [MNC]/kg; P = .05). In addition, engraftment occurred more quickly in patients who developed significant acute GVHD (grade I-IV) or extensive chronic GVHD. Table 2 summarizes the median number of days to achieve donor-chimerism for various patient subgroups. Event-free survival appears to have stabilized at approximately 30% between days 1000 and >1500 days post-transplantation (Figure 8) [30].

Findings from the 16 patients with recurrent ALL show that the GVL effect is important in this patient subgoup [31]. The 16 ALL patients (median age, 8.9 years) include 8 in second remission, 3 in third remission, and 4 in fourth remission. Seven patients have a history of prior HCT. For the most recent procedure, stem cell sources include peripheral blood in 13 patients and bone marrow for 3 patients. The reasons for undergoing RIC varied among patients, and included prior aspergillosis (n = 1), Philadelphia chromosome (Ph)-positive

Table 2. Days to Achieve Donor Chimerism*

Patient Group	Donor Leukocyte Chimerism		Donor T-Lymphocyte Chimerism	
	50%	>95%	50%	>95%
Evaluable patients (n = 52)	18	26	25	33
Nonmalignant disorders (n = 11)	19	53	26	82
Malignant disease (n = 27)	13	25	21	27
Malignancy and GVHD (n = 7)	13	17	20	19
Malignancy without GVHD (n = 8)	16	32.5	26.5	39

*GVHD indicates graft-versus-host disease.

disease (n = 1), neurological toxicity (n = 2), an infant in second remission (n = 1), relapse post-myeloablative treatment (n = 7), and no reason (n = 6). To date, 5 of the 16 patients have died, due to disease progression (n = 1), infection (n = 2), and neurological complications (n = 2). Acute GVHD was observed in 8 patients, including grade I in 5 patients, grade II in 1 patient, and grade 4 in 2 patients. Two patients developed limited chronic GVHD, and 1 patient developed extensive/severe chronic GVHD. The median event-free survival was 730 days posttransplantation. More than 40% of patients are alive >1460 days posttransplantation (Figure 9) [31].

Conclusions

Overall, the experience at Children's Memorial Hospital has provided important insights regarding the use of RIC allogeneic stem cell transplantation in pediatric patients with malignant and nonmalignant disease. Acute toxicity is minimal, and acute GVHD was not a problem; however, the incidence of chronic GVHD was higher than expected, and may pose a problem for pediatric patients. Those with hemoglobinopathies have difficulty achieving engraftment, and the GVL effect was observed in patients with ALL. For all other patient groups, chimerism was achieved quickly and was sustained. Lastly, the source of stem cells does not appear to influence outcome.

Given the small numbers and the diversity of the patients treated at Children's Memorial Hospital, it is difficult to draw conclusions from these observations. Future prospective randomized trials with a larger patient population will be required to determine the optimal role of RIC in the pediatric population.

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Emerging Options in Reduced-Intensity Stem Cell Transplantation: Who, How, and When?

CME Assessment Test

1. **Which of the following would NOT be a typical component of reduced-intensity conditioning (RIC) regimens?**
   A. Total body irradiation dose of 200 cGy
   B. Fludarabine 125 mg/m^2 as a single agent or in combination with other drugs
   C. Busulfan 8 mg/kg
   D. Melphalan 200 mg/m^2

2. **In the Cancer and Leukemia Group B (CALGB) 8461 trial, complex karyotypes showing 5 or more abnormalities were associated with what type of outcome compared with karyotypes showing <5 abnormalities in older patients (≥60 years) with acute myeloid leukemia (AML)?**
   A. Prolonged disease-free survival
   B. Decreased disease-free survival
   C. Increased treatment-related adverse events
   D. Decreased treatment-related adverse events

3. **The CALGB 100103/Clinical Trials Network (CTN) 0502 trial will evaluate RIC with nonmyeloablative conditioning with fludarabine and busulfan as the initial post-remission therapy for older patients (>60 years) with AML.**
   A. True
   B. False

4. **In patients with AML who are treated with allogeneic hematopoietic stem cell transplantation (HCT), the development of chronic graft-versus-host disease (GVHD) correlates with:**
   A. Sustained remission
   B. Improved survival
   C. Both A and B
   D. None of the above

5. **In patients with follicular lymphoma, which of the following is NOT a predictor of outcome following RIC allogeneic HCT?**
   A. Chemosensisitivy
   B. Type of RIC conditioning regimen
   C. Comorbidities
   D. Histology

6. **Compared with myeloablative conditioning prior to allogeneic HCT, nonmyeloablative conditioning appears to decrease non-relapse mortality and increase overall survival in patients with follicular lymphoma and:**
   A. No comorbidities
   B. At least 1 comorbidity
   C. At least 2 comorbidities
   D. At least 5 comorbidities

7. **Which of the following non-Hodgkin's lymphoma (NHL) histologies is associated with the most favorable progression-free survival and overall survival outcomes?**
   A. Indolent NHL
   B. Aggressive NHL
   C. Mantle cell lymphoma
   D. Hodgkin's lymphoma

8. **In patients treated with nonmyeloablative allogeneic HCT, treatment with adjuvant sirolimus appeared to improve which of the following compared with placebo?**
   A. Overall survival
   B. Relapse risk
   C. Non-relapse mortality
   D. Both A and B

9. **In pediatric patients with acute leukemia, Wilms Tumor 1 (WT1) gene expression is associated with which of the following?**
   A. Increased risk of relapse
   B. Prolonged relapse-free survival
   C. Increased overall survival
   D. Decreased non-relapse mortality

10. **In pediatric patients undergoing allogeneic HCT for the treatment of nonmalignant diseases, which of the following is NOT observed with RIC?**
    A. Adequate engraftment of donor cells in related-donor HCT only
    B. Adequate engraftment of donor cells in related- and unrelated-donor HCT
    C. A high incidence of chronic GVHD
    D. Acceptable toxicity, with a low incidence of acute GVHD

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The CME activity provided a balanced, scientifically rigorous presentation of therapeutic options related to the topic, without commercial bias.	1 2 3 4 5

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1.	A	B	C	D	**5.**	A	B	C	D	**9.**	A	B	C	D
2.	A	B	C	D	**6.**	A	B	C	D	**10.**	A	B	C	D
3.	A	B			**7.**	A	B	C	D					
4.	A	B	C	D	**8.**	A	B	C	D					

Journal Watch

A scan of recent medical literature identified these articles of special importance in the science and clinical application of blood and marrow transplantation.

Arora M, Weisdorf DJ, Spellman SR, et al. HLA-identical sibling compared with 8/8 matched and mismatched unrelated donor bone marrow transplant for chronic phase acute myeloid leukemia. *J Clin Oncol*. 2009;27:1644-1652.

Hematopoietic cell transplantation from an unrelated donor (URD) is an accepted treatment for patients with chronic myeloid leukemia (CML) who lack an HLA-identical sibling donor. Current approaches to selection of URDs focus on matching of HLA alleles based on high-resolution typing methods. The current study compared the outcomes of URD versus matched sibling bone marrow transplantation for patients with chronic phase CML.

The study included 3 groups of patients undergoing bone marrow transplantation for CML in first chronic phase, during which transplantation is expected to have a strong allogeneic effect and a relatively low risk of relapse. There were 3514 recipients of transplants from matched sibling donors; 531 from HLA-, -B, -C, and DRB1 allele-matched (8/8 matched) URDs; and 521 from HLA-mismatched URDs. Overall and leukemia-free survival were compared between groups, along with secondary endpoints.

Five-year overall survival was 63% for recipients of transplants from matched sibling donors, compared to 55% in those with 8/8 matched URDs (relative risk [RR]) 1.35. In the patients with mismatched URDs, survival decreased as the degree of mismatch increased—from 40% to 21% Leukemia-free survival was 55% in the matched sibling donor group versus 50% in the 8/8 matched URD group (RR 1.21). The risk of relapse was similarly low (11% to 14%) across groups. Transplantation-related mortality was 31% in patients with matched sibling donors versus 38% in those with 8/8 matched URDs (RR 1.45).

In a homogeneous population of patients with CML in first chronic phase, outcomes are better in recipients of matched sibling donors, compared to those with 8/8 matched URDs. Overall survival, leukemia-free survival, and transplantation-related mortality are all "modestly though significantly worse" in patients receiving marrow from URDs. Greater degrees of mismatch are associated with poorer outcomes, suggesting that the risks and benefits of using mismatched URD marrow must be weighed carefully.

Bader P, Kreyenberg H, Henze GH, et al. Prognostic value of minimal residual disease quantification before allogeneic stem-cell transplantation in relapsed childhood acute lymphoblastic leukemia: the ALL-REZ BFM Study Group. *J Clin Oncol*. 2009;27:377-384.

Current approaches to salvage therapy are effective in about one-third of children with acute lymphoblastic leukemia (ALL) who experience disease relapse after conventional chemotherapy. Retrospective studies have suggested that minimal residual disease (MRD) may be an important predictor of outcome before allogeneic hematopoietic cell transplantation in children with relapsed ALL.

This hypothesis was tested in a prospective trial including 91 children with relapsed ALL treated at German pediatric centers. All patients were undergoing allogeneic hematopoietic cell transplantation in second or later remission; treatment was according to reported German cooperative trial protocols. Real-time polymerase chain reaction using T-cell receptor and immunoglobulin gene rearrangements was performed to measure MRD, which was evaluated as a predictor of post-transplantation outcomes.

Quantitative MRD was 10^{-4} leukemic cells or greater in 45 patients and below this threshold in 46 patients. Probability of event-free survival (pEFS) was 0.27 in patients with a higher MRD load versus 0.60 in those with lower MRD. Cumulative incidence of relapse (CIR) was 0.57 versus 0.13, respectively. Among "intermediate-risk" patients (strategic group S1), pEFS was 0.20 for those with MRD of 10^{-4} leukemic cells or greater versus 0.68 for those with MRD of less than 10^{-4}. Values for CIR were 0.73 and 0.09, respectively.

A similar pattern was noted for high-risk patients (S3/4 in third complete remission): pEFS was 0.53 for those with higher MRD versus 0.30 for those with lower MRD. Cumulative incidence of relapse was 0.18 versus 0.50. On multivariate analysis, MRD was the only independent predictor of EFS.

The results confirm the predictive value of MRD for children undergoing allogeneic hematopoietic cell transplantation for relapsed ALL. When the pretransplantation MRD load is 10^{-4} leukemic cells or greater, treatment intensification or modification should be considered. The investigators are planning trials of new intervention strategies based on these results.

Bachanova V, McCullar V, Lenvik T, et al. Activated notch supports development of cytokine producing NK cells which are hyporesponsive and fail to acquire NK cell effector functions. *Biol Blood Marrow Transpl*. 2009;15:183-194.

There is a growing body of knowledge on the development of maturation of natural killer (NK) cells, which could have implications for understanding immunity against infections and for the development of new cancer therapies. Notch signaling has been shown to play a critical role in hematopoiesis, but its effects on NK cell maturation and functioning remain unclear. In vitro experiments with umbilical cord blood (UCB) cells were performed to investigate the role of constitutively activated Notch I (ICN) on maturation of NK cells.

Human UCB progenitor cells were cultured on a mouse embryonic liver stroma cell line. Sorting of $CD34^+/ICN^+$ UCB cells produced a population of $CD7^+$ early lymphoid precursor cells. These later developed into committed NK cells, independent of the effects of stroma or human interleukin (IL)-15. These ICN^+ precursor cells expressed L-selectin, providing evidence of homing competence. With further NK lineage commitment, the cells produced IL-13, granulocyte macrophage-colony stimulating factor, and tumor necrosis factor-alpha. They did not show full acquisition of NK inhibitory receptors or cytotoxic effector cell function.

In culture with stroma, the ICN^+ cells also developed into a population with early T-lineage commitment, including IL-2 production and other strong indicators of Th1 commitment. This process occurred only in the presence of EL08-ID2 signals from the stroma.

These in vitro results suggest that, in the absence of stroma, sustained Notch signaling can stimulate UCB 34^+ cells to differentiate into a $CD7^+$ lymphoid precursor cell. From there the cells show NK cell commitment, but with an immature cytokine production profile, low responsiveness, and poor acquisition of NK cell receptors critical for self-tolerance and effector function. Tissues expressing Notch ligand appear to be involved in the differentiation and eventual education of NK cells.

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