Blood and Marrow TRANSPLANTATION

REVIEWS

A Publication of the American Society for Blood and Marrow Transplantation

Issues in Hematology, Oncology, and Immunology

VOLUME 21 NO 1 2011

RELEASE DATE JUNE 20, 2011

IN THIS ISSUE

This publication is supported by an educational grant from

Otsuka

When Less Is More: A Tough Way of Finding a Good Fit

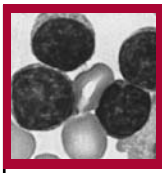
Maxim Norkin, John R. Wingard

Originally, myeloablative conditioning was considered as an essential part of hematopoietic cell transplantation (HCT) to ensure maximum anti-tumor effect. Its greater anti-tumor activity is usually offset by an increased risk of non-relapse mortality, making older patients and those with comorbid conditions poor candidates for such an effective but intense treatment. Obviously, alternatives to myeloablative conditioning are needed to allow greater proportions of patients to be considered for allogeneic HCT.

Observations of durable responses following donor lymphocyte infusions due to graft-versus-tumor (GVT) effect led to the development of reduced intensity conditioning (RIC) regimens. The rationale for RIC is to provide reliable donor stem cell engraftment with subsequent disease control via GVT. Introduction of RIC significantly expanded transplantation options to older patients, virtually eliminating age as a barrier for allogeneic transplantation and extending options to patients with comorbidities and nonmalignant conditions. Unfortunately, RIC provides less anti-tumor activity and increases the possibility for posttransplantation relapse and graft failure.

Many of these issues were addressed in the satellite symposium held in February 2011 at the Tandem BMT meeting in Honolulu, HI. Dr. Shaw focused on RIC regimens in pediatric transplant recipients, who differ from adults given a significantly higher prevalence of primary nonmalignant diseases, fewer comorbid conditions, and the desirability of fertility preservation. Prolonged posttransplantation survival is anticipated, so long-term adverse effects caused by the conditioning regimen should be strongly considered in the selection of the preparative regimen, particularly if total body irradiation (TBI) is considered. Dr. Forman reviewed radiation-based transplantation regimens for hematologic malignances and described options for decreasing their toxicities. TBI has high anti-tumor potency, but its benefit is frequently offset by increased toxicity. Novel approaches using non-myeloblative doses of TBI, radioimmunotherapy, or total marrow radiation instead of TBI have the potential of reducing side effects without a significant compromise of posttransplantation outcomes. Dr. Andersson described alternatives to radiation-based conditioning with a focus on the toxicity and efficacy of commonly used chemotherapy agents.

Despite its excellent anti-tumor activity, myeloablative conditioning does not necessarily translate into better posttransplantation outcomes than less intense conditioning because of its higher risk of complications. No prospective studies have ever compared myeloablative and RIC, and such studies will be difficult but important. Retrospective studies addressing this issue are subject to significant selection bias because RIC is typically offered to older and less fit patients, and questions remain about the utility of RIC in patients at high risk for relapse or in patients who are not in remission. In addition, the efficacy of RIC for malignancies that may be less susceptible to GVT effect requires further confirmation. Therefore, appropriate selection of the intensity of preparative regimen is not trivial and, for now, should be individualized based on patient and disease characteristics. Further study is needed to determine the optimal RIC regimen(s) and when RIC should be chosen over ablative regimens.



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Publishing and Production Services
CJP Medical Communications, a division of Carden Jennings Publishing Co., Ltd.

Blood and Marrow Transplantation Reviews is published by CJP Medical Communications.
375 Greenbrier Dr., Suite 100, Charlottesville, VA 22901
phone (434) 817-2000; fax (434) 817-2020

This publication is supported by an educational grant from Otsuka America Pharmaceutical, Inc.

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Symposium Report

Impact of Conditioning Regimens on Transplantation Outcomes

Adapted from a continuing medical education symposium presented at the 2011 BMT Tandem Meetings on February 18, 2011, in Honolulu, Hawaii.
This program is supported by an educational grant from Otsuka America Pharmaceutical, Inc.

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Statement of Need

Through society recommendations and a review of ongoing clinical research, the American Society for Blood and Marrow Transplantation (ASBMT) and the Medical College of Wisconsin have determined that a need exists to educate physicians on learning gaps involving the role of conditioning regimens in transplantation outcomes. This educational activity will address the identified learning gaps and meet stated learning objectives.

Target Audience

The program will be oriented to a targeted audience of physicians and medical care professionals specializing in oncology, hematology, immunology, and microbiology.

Learning Objectives

After completing this activity, participants should be able to:

- Compare the benefits and toxicities of total body irradiation–based and chemotherapy-only conditioning regimens.
- Assess the specific needs of the pediatric patient when selecting conditioning regimens.
- Evaluate the impact of these modalities in myeloablative and reduced-intensity conditioning regimens.

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Jeffrey Szer, MD, discloses that he is a member of the advisory board for Celgene, Pfizer, Genzyme, and Novartis; and he has received a travel grant from Genzyme.

Borje S. Andersson, MD, PhD, discloses that he receives consulting fees from Otsuka America, Inc.; he is a clinical investigator for Genzyme, Inc.; and he is a member of the safety data committee for AMGEN.

Stephen J. Forman, MD, does not have any relevant financial relationships with any commercial interests.

Peter Shaw, MD, discloses that he is a member of Busulfex Steering Committee for Otsuka Pharmaceuitcals; and he refers to off label use of Buslphan and fludarbine in his presentation.

Introduction

Despite 30 years of widespread clinical practice, infection, relapse, GvHD and organ-toxicity remain major hurdles that limit application and success of hematopoietic stem cell transplantation (HSCT). Transplantation-related mortality (TRM) has been the price for aggressive therapy that improved relapse-free survival for many patients undergoing HSCT. Even in the best hands, 100day TRM remains at 5% in autografts, 10% with matched sibling donors and 20% with unrelated donors, and results primarily from organ damage from conditioning, leading to sinusoidal obstructive syndrome (SOS) or multi-organ failure.

The introduction of reduced-intensity conditioning (RIC) was a major shift in the field of HSCT. The rationale for using RIC was to harness the graft-versus-tumor (GVT) effect to increase the likelihood of cure without the toxicity associated with traditional myeloablative therapy. With RIC, patients who were considered unsuitable for standard myeloablative conditioning preceding allogeneic HSCT—older patients and those with comorbidities or nonmalignant conditions—can now access potentially curative therapy. The number of transplants using RIC has steadily increased in the last decade, mainly for patients 50 and older.

Despite these advances in HSCT, many challenges remain, including high relapse rates in certain malignancies, high risk of graft-versus-host disease (GVHD), and stubborn risk of non-relapse mortality. For pediatric patients with hematological malignancies or nonmalignant indications for HSCT, the risk of very long-term complications is a major limitation of current conditioning regimens, in particular those based on total-body irradiation (TBI). New research has focused on identifying novel combination regimens that provide effective disease control while decreasing the risks of both acute and chronic treatment-related toxicity.

Conditioning Regimens in Pediatric Transplantation

Peter J. Shaw, MD

Pediatric patients are managed with a unique set of treatment considerations and therapeutic goals in mind. Unlike adult patients with cancer, children rarely have medical comorbidities such as diabetes and cardiovascular disease that must be managed alongside the hematologic malignancy. Thus, up to now, myeloablative therapy has been tolerated and so used in the majority of cases, but RIC is starting to be used more in pediatric HSCT. Treatment in children requires us to optimize both the quality as well as quantity of survival. This includes limiting restrictions on growth and future fertility, as well as preventing the potential neuropsychological consequences of childhood cancer and its treatment and over a very long time period. Use of RIC allows us to hope that not only will TRM be lower at 100days and 1 year, but allow us to look at the most significant, truly long-term events. For example, the Australian government's life-events website lists milestones such as marriage, buying a house, reaching financial independence, and developing a serious illness or second cancer. For pediatric patients with long life expectancies, all of these events should be included within the scope of possible transplantation outcomes and we should do our best to optimize these life-long outcome measures.

Neuroblastoma Experience

15 years ago, survival in children with neuroblastoma was dismal. Among children aged 12 months and older with newly diagnosed disseminated neuroblastoma, the 5-year survival was 12% to 14%, and treatment was associated with significant toxicity [1]. The era of high-dose myeloablative chemotherapy and HSCT improved prognosis in children with advanced neuroblastoma significantly. In an early randomized study from the European Neuroblastoma Study Group, one-third of children with stage III and IV neuroblastoma were still alive with a median follow-up of 14.3 years. For those who were aged >1 year at diagnosis (n = 48), the 5-year EFS was significantly better with high-dose melphalan and HSCT compared with no further treatment beyond induction chemotherapy and surgery (33% versus 17%; $P = .01$) [2].

Neuroblastoma treatment continues to evolve with ongoing studies of multi-agent chemotherapy, involved-field irradiation, and other modalities. The phase III High-Risk Neuroblastoma-1 (HR-NBL1) trial from the International Society of Paediatric Oncology European Neuroblastoma Group (SIOPEN) is currently evaluating induction therapy with rapid-schedule cisplatin, vincristine, carboplatin, etoposide, and cyclophosphamide (COJEC) followed by transplantation randomization to carboplatin, etoposide, melphalan (CEM) or busulfan/melphalan (BuMel) in patients aged 1 to 20 years at the time of diagnosis with neuroblastoma (NCT00030719). Progressive reduction in TRM has allowed patients to benefit from the increasing number of post-BMT treatments that are provided for Neuroblastoma.

The Need for Long-Term Follow Up

In the pediatric population, we expect prolonged survival and so long-term, life-long, follow up is vital. We may often have to wait many years before these effects become apparent. For example, in a study of long-term visual complications after childhood HSCT, all patients who were conditioned with single-dose TBI developed cataracts and 82% required surgery. In addition, 83% of those who received fractionated TBI developed cataracts, and 28% of these patients had surgery. In this group, the median time to cataract surgery was 3.0 years after HSCT (range, 2.2 to 5.3 years). By comparison, only 36% of children who were conditioned with oral busulfan-based conditioning therapy developed cataracts after a median follow-up of 6 years (range, 2.0 to 17.3 years), and none required surgery. But in the busulfan group, cataracts were beginning to emerge in those patients who had the longest follow up [5]. Hence regular ocular examinations in children treated with HSCT should be performed, and not restricted to those who underwent conditioning with TBI.

Since 1969, researchers at the Fred Hutchinson Cancer Research Center have been monitoring thyroid function in patients who were younger than 18 years at the time of HSCT. In this cohort (n = 791), thyroid dysfunction, including hypothyroidism and thyroiditis, continued to increase for 28 years after HSCT. Patients who were <10 years old were significantly more likely to develop thyroid dysfunction than patients who were 10 to 18 years old at the time of HSCT ($P < .001$). Among those who developed thyroid tumors (n = 17), the tumors were diagnosed a median of 9.9 years after HSCT (range, 4.5 to 22.3 years) [6]. Given the long-term risk of thyroid dysfunction, children who

receive HSCT should be monitored for thyroid abnormalities throughout life.

Secondary Malignancies

Beyond TRM, other life-threatening toxicities related to HSCT may develop over time. Friedman and colleagues reported a large increase in breast cancer among female patients who survived for at least 5 years following allogeneic HSCT (n = 3337). The 25-year cumulative incidence of breast cancer was 11%, and the median time to breast cancer diagnosis was 12.5 years following HSCT (range, 5.7 to 24.8 years). In multivariate analysis, the risk of breast cancer was especially pronounced in patients with 20 or more years since HSCT (hazard ratio [HR], 10.8), in those who were younger than age 18 at the time of HSCT (HR, 9.5), and in those who received TBI (HR, 4.0) [7].

The risk of secondary malignancies is not limited to breast cancer. In a study from the Center for International Blood and Marrow Transplant Research (CIMBTR), 28,874 individuals who underwent allogeneic HSCT developed new solid invasive cancers at twice the expected rate. The relative risks of some secondary cancers were especially high. For instance, patients who received HSCT had a 5-fold risk of melanoma, nearly 6-fold risk of thyroid cancer, and 7-fold risk of lung cancer. Secondary cancers of the oral cavity, though rare, occurred at rates much higher than expected: 13-fold higher for tongue cancer, 14-fold higher for salivary gland cancer, and nearly 28-fold higher for lip cancer [8].

In the CIMBTR study, the overall risk of developing a secondary malignancy increased over time, reaching 3-fold higher than expected among those who survive for more than 15 years following HSCT (Figure 1). The risk of invasive solid cancer was also related to age at HSCT and exposure to radiation as part of the conditioning regimen. For a pediatrician, it was most worrying that children irradiated at ages <10 years had a 55-fold increased risk of developing secondary cancers relative to expected rates in the general population [8]. Changing trends in transplantation, such as decreased use and dosing of radiation and increased use of RIC regimens, may modify the overall risk of secondary cancers among HSCT survivors.

Cardiovascular Morbidity and Mortality

Treatment with HSCT is also associated with late adverse cardiovascular and metabolic effects. In 2010, Hoffmeister examined the risk of hypertension in a retrospective study of 689 pediatric patients who survived for at least 5 years after HSCT. With up to 36 years of follow-up, hypertension developed in 17% of HSCT survivors, including nonobese, adolescent, and young adult patients. Compared with hypertension risk in the general population, the risk of hypertension was twice that among HSCT survivors aged 18 to 39 years and 3-fold higher for patients aged 11 to 17 years. The type of preconditioning regimen was a factor in long-term hypertension risk. Use of TBI, a known risk factor for radiation nephropathy, doubled the risk of hypertension among long-term HSCT survivors (HR, 2.1; P = .01) [9].

An analysis from the Bone Marrow Transplantation Survivor Study also found excess rates of hypertension among long-term survivors of allogeneic or autologous HSCT, as well as increased risk of diabetes and cardiovascular events. The study included 1089 patients who survived at least 2 years after HSCT and 383 sibling controls. After a mean follow-up of 8.6 years, transplantation survivors had a 3.65-fold higher risk of diabetes and a 2-fold higher risk of hypertension compared with sibling controls matched for age and body mass index (BMI). In this cohort, TBI more than tripled the risk of diabetes [10].

Hypertension, diabetes, and associated cardiovascular risk factors associated with HSCT may contribute to excess cardiovascular mortality following radiation therapy. In a study of 4122 survivors of childhood cancer, radiation therapy was identified as a significant risk factor for long-term cardiovascular morbidity and mortality. After a median follow-up of 27 years, survivors of childhood cancer had a 5-fold higher risk of dying from cardiovascular disease than expected. There was a direct relationship between total radiation dose delivered to the heart and lifetime risk of cardiac mortality, beginning with a 60% excess risk at 1 Gy. From there, the risk of cardiac mortality increased dramatically, to 12.5-hold higher after a radiation dose of 5 to 14.9 Gy and 25-fold higher after radiation doses exceeding 15 Gy [11]. With excess mortality risk beginning at radiation exposure levels of 1 Gy, there appears to be no truly safe threshold dose of cardiac irradiation when given to a pediatric patient.

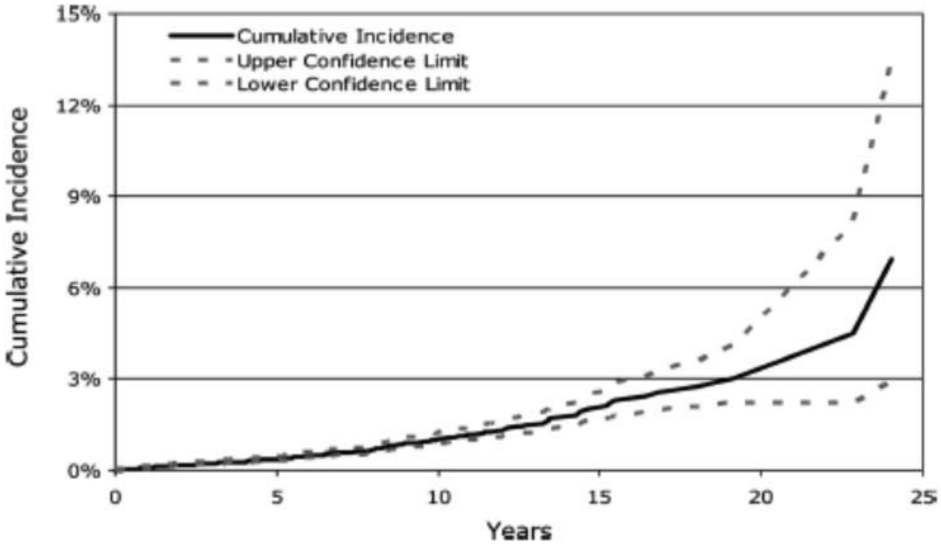

Figure 1. Cumulative incidence of secondary malignancies following childhood allogeneic hematopoietic stem cell transplantation (HSCT) [8]. Copyright 2009. Reproduced with permission of American Society of Hematology (ASH) in the format Journal via Copyright Clearance Center.

Alternatives to TBI

Pretransplantation conditioning with busulfan and cyclophosphamide (Bu/CY) has been evaluated as a strategy for avoiding the adverse effects of TBI in children with hematologic malignancies. In a retrospective analysis of CIBMTR data, Davies compared outcomes in 627 children who received cyclophosphamide plus TBI (n = 451) or oral Bu/CY (n = 176) in preparation for HLA-matched

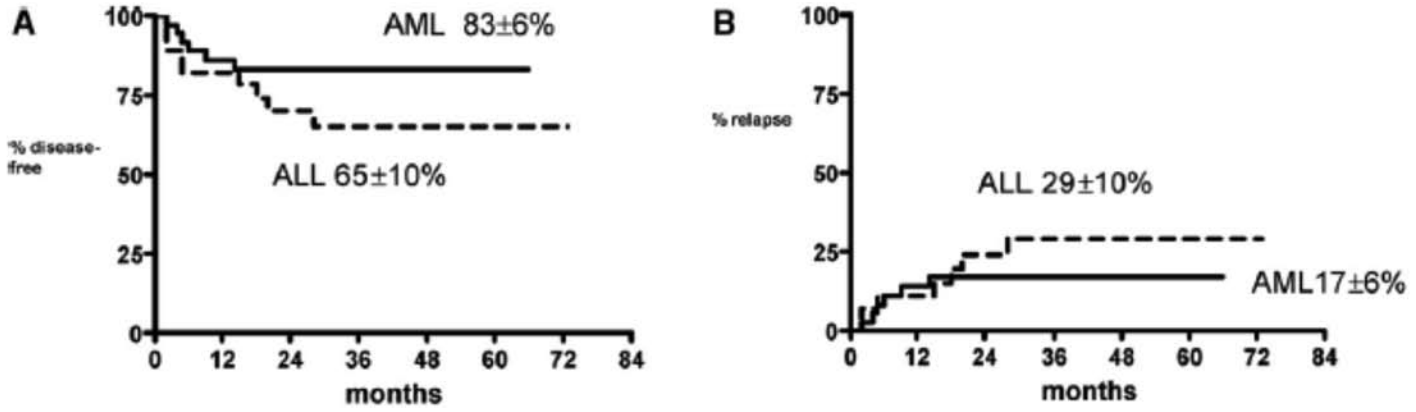

Figure 2. Busulfan, fludarabine, and total body irradiation in patients with acute leukemia. A. Disease-free survival. B. Risk of relapse. AML indicates acute myeloid leukemia; ALL, acute lymphoblastic leukemia. Reprinted from [16] with permission from Elsevier.

sibling transplantations for the treatment of acute lymphoblastic leukemia (ALL). After a median follow-up of 37 months, the estimated 3-year overall survival rate was better following TBI compared with oral Bu/CY (55% versus 40%; P = .003). However, the survival advantage in the TBI group may have resulted primarily from better safety rather than better disease control. Treatment with oral Bu/CY significantly increased the risk of TRM (HR, 1.68; P = .012). By comparison, the risk of relapse was not significantly elevated in the oral Bu/CY group (HR, 1.30; P = .117) [12].

As a follow-up to the retrospective CIBMTR analysis, the Pediatric Blood and Marrow Transplant Consortium (PBMTC) prospectively compared the safety and efficacy of busulfan-based conditioning regimens with TBI. The phase II trial included 43 children with ALL who were randomly assigned to treatment with oral busulfan (n = 21) or TBI (n = 22), in addition to cyclophosphamide 120 mg/kg to ensure engraftment. Patients who received busulfan received etoposide 40 mg/kg, to enhance the antileukemic effect of therapy and avoid concerns of rejection, and all the patients who received busulfan and unrelated donor grafts also received antithymocyte globulin for increased immunosuppression. After a median follow-up of 6.4 months, relapses were similar in the TBI group (n = 7) and in the busulfan group (n = 9). The 3-year EFS rate was significantly better following TBI compared with busulfan (58% versus 29%, respectively; P = .03). Overall survival at 3 years also favored TBI over oral busulfan (67% versus 47%, respectively; P = .009) [13].

Together, findings from the IBMTR and PBMTC studies demonstrate the superiority of TBI compared with busulfan, but do not necessarily support its continued routine use in children undergoing HSCT for hematologic malignancies. Rather, the authors of both studies reiterated concerns related to the late effects of TBI and called for continued exploration of alternative preconditioning regimens with a better long-term safety profiles [12,13].

Next Steps in Pediatric HSCT

Several advances in the treatment of adult hematologic malignancies are now being evaluated and adopted in the pediatric setting. Borrowed best practices include the preferential use of intravenous (IV) versus oral busulfan; the use of pharmacokinetic monitoring to target area under the curve (AUC) as a surrogate estimate for total systemic exposure; bypassing the adverse interactions between busulfan and cyclophosphamide metabolism; and transitioning from cyclophosphamide to a nucleoside analog, most commonly fludarabine as the preferred immunosuppressive partner with busulfan.

New evidence is available to support some of these approaches in management of children undergoing HSCT. In 2009, Bartelink evaluated the link between busulfan exposure and clinical outcomes in 102 children who received IV busulfan in preparation for HSCT. The EFS outcomes were optimal when the exposure to busulfan, as measured by AUC, was 78 mg × h/L (95% confidence interval [CI], 74 to 82 mg × h/L). Furthermore, targeting the dose of busulfan to a narrow therapeutic range increased EFS [14].

In 2011, Bartelink described outcomes after replacing the Bu/CY regimen with busulfan and fludarabine (BuFlu) for myeloid malignancies and all nonmalignant indications at the University Medical Center, Utrecht, the Netherlands. The study included 28 pediatric patients treated with BuFlu with a targeted dose of busulfan (AUC > 74 mg × h/L) and 44 historical controls with comparable age, gender, HSCT indication, and match-grade who were treated with Bu/CY between 2005 and 2009. Compared with the Bu/CY group, patients treated with BuFlu had a shorter neutropenic period (21 days versus 10 days; P = .02), required fewer platelet transfusions (median 10 versus 3; P = .04), and required fewer red blood cell transfusions (median 5 versus 1; P = .02). The risk of SOS was also significantly lower with BuFlu (3%) compared with Bu/CY (18%; P = .02) [15].

In adult patients with acute leukemia, the BuFlu regimen has also been used in combination with low dose TBI. The regimen included fludarabine 50 mg/m^2 on days –6 to –2, IV busulfan 3.2 mg/kg daily on days –5 to –2, and 400 cGy TBI on day –1 or 0, followed by allogeneic HSCT. Patients also received prophylaxis against GVHD with methotrexate, cyclosporine , and antithymocyte globulin. One-year treatment-related mortality with this approach was reported to be less than 10%, and at 4 years, the cumulative risk of relapse was 17% for patients with AML and 29% for patients with ALL. The estimated 3-year disease-free survival was 86% for patients with AML and 65% for those with ALL (Figure 2) [16]. This regimen has also been used in the pediatric setting (Russell J, personal communication, 2010), with an expectation that the combination offers less long-term toxicity when compared to TBI-based conditioning therapy.

Identifying patients with a high risk of failure prior to RIC transplantation is an important component of treatment planning. In patients with ALL, pretransplantation minimal residual disease (MRD) is a highly sensitive marker for disease burden that significantly predicts posttransplantation relapse-free survival [17]. Patients who are MRD-negative at the time of HSCT have an excellent prognosis and may benefit from a less toxic regimen. By comparison, patients who are MRD-positive generally have poor outcomes and may be appropriate candidates for investigational preconditioning regimens to support HSCT.

What we have learnt in conditioning for pediatric HSCT will also be of value as the gene therapy field continues to expand. Patients with primary immunodeficiency disorders such as Wiskott–Aldrich syndrome are frequently treated by allogeneic HSCT. In a recent report, hematopoietic stem cell gene transfer was used to achieve complete immunologic correction of the disease, using a retrovirus-based stem cell gene transfer – after busulfan-based conditioning [19]. Type I mucopolysaccharidosis is another common indication for pediatric HSCT. Experimental gene therapy has been used to correct multiple defects associated with this disease, and to a degree not achievable by HSCT, but these mice were treated with total body irradiation. [20]. The same advances we make in optimization of transplantation conditioning regimens for HSCT will likely be transferable to the gene therapy field in the future.

Conclusions

Pediatric HSCT places a special emphasis on the potential long-term adverse effects of therapy. New regimens are intended to be safer for longer, with a lower risk of GVHD, less morbidity, and fewer secondary malignancies. Advances in transplantation regimens for nonmalignant indications will be applicable to gene therapy, and vice versa.

Radiation-Based Transplantation Regimens for Hematologic Malignancy

Stephen J. Forman, MD

The rationale for radiation-based regimens originates from the work of E. Donnall Thomas, MD, more than half a century ago in Cooperstown, New York, and later at the University of Washington in Seattle. Thomas and colleagues were among the first to demonstrate the dose–response relationship that allows total body irradiation radiation therapy to control leukemia [21]. Most of the TBI regimens used for hematologic malignancies around the world today use hyperfractionated radiation, which mimics the fractionated approach that radiation oncologists use for solid tumors. Hyperfractionated radiation uses doses that are highly tolerable (1200 to 1320 rads). Higher doses are difficult to achieve in the context of a standard TBI approach due to organ toxcitity.

In addition to killing malignant cells, the primary goal of radiation therapy is to facilitate the engraftment of donor stem cells. In 1998, Clift and colleagues reported long-term follow-up findings demonstrating the relationship between radiation dose and disease control in 71 patients receiving allogeneic HSCT during first remission of acute myeloid leukemia (AML) [21,22]. All patients received 120 mg/kg cyclophosphamide followed by 12.0 Gy or 15.75 Gy of TBI and marrow from HLA-identical siblings. Radiation was delivered in 6 daily exposures each of 200 cGy of TBI (n = 34) or 7 daily exposures each of 225 cGy of TBI (n = 37).

After 11 years, the higher dose of TBI reduced the cumulative risk of relapse compared with the lower dose regimen (12.0 Gy) (14% versus 39%; $P = .06$). The trade-off for improved leukemia control, however, was an increase in the risk of non-relapse mortality. The probability of overall survival at 11 years was equivalent in both treatment groups (51%), with a greater risk of non-relapse mortality in the 15.75-Gy group compared with the 12.0-Gy group (38% versus 19%; $P = .05$) (Figure 3) [21]. Although high-dose TBI failed to improve overall survival relative to low-dose treatment, it significantly improved disease control. As more patients with advanced disease are now being considered for allogeneic HSCT, disease control is an especially important consideration for therapy.

Since this landmark study, researchers have explored various options for decreasing relapse by combining TBI with other chemotherapy agents. In 2008, Laport described long-term outcomes in 79 patients with Philadelphia chromosome (Ph)-positive ALL who received fractionated TBI and high-dose etoposide (n = 67), etoposide/cyclophosphamide (n = 11), or etoposide/busulfan (n = 1) prior to undergoing HLA-matched allogeneic HSCT. The preparatory regimen of fractionated TBI of 1320 cGy was delivered in 11 fractions over 4 days followed by etoposide at 60 mg/kg administered as a single infusion over 4 hours. With this approach to preconditioning, the 10-year overall survival was 54% for patients who were in first remission at the time of HSCT and 29% for those beyond first remission ($P = .011$). For patients in first remission and beyond first remission, the cumulative relapse rates were 28% and 41%, respectively [23]. Therefore, even with 1320 cGy delivered during first remission, there is a substantial risk of relapse that needs to be addressed.

Limitations of TBI

Planning radiation treatment fields that are safe and therapeutically appropriate is a complex and time-consuming process that requires collaboration with a radiation oncologist. Radiation therapy is associated with acute toxicity, including radiation mucositis, enteritis, dermatitis, and pneumonitis. The effective management of acute radiation toxicity requires experienced nursing care, particularly in the first 3 weeks following treatment. Prophylactic regimens may lessen the burden of these toxicities. For instance, treatment with the recombinant keratinocyte growth factor palifermin, reduces the risk of oral mucosal injury following radiation therapy for hematologic malignancies and is now incorporated into many radiation therapy algorithms [24].

After TBI, patients are also vulnerable to long-term adverse effects on the heart, lungs, thyroid, and eyes caused by damage to parenchymal cells and the underlying vasculature. Accordingly, patients require lifelong monitoring for the early detection and appropriate management of late effects such as cardiovascular disease, lung disease, thyroid dysfunction, and cataracts. Radiation therapy increases the long-term risk of developing second malignancies.

Age and comorbidity restrictions also limit the wider use of conventional transplantations with myeloablative conditioning. Traditionally, TBI has been an option for relatively young

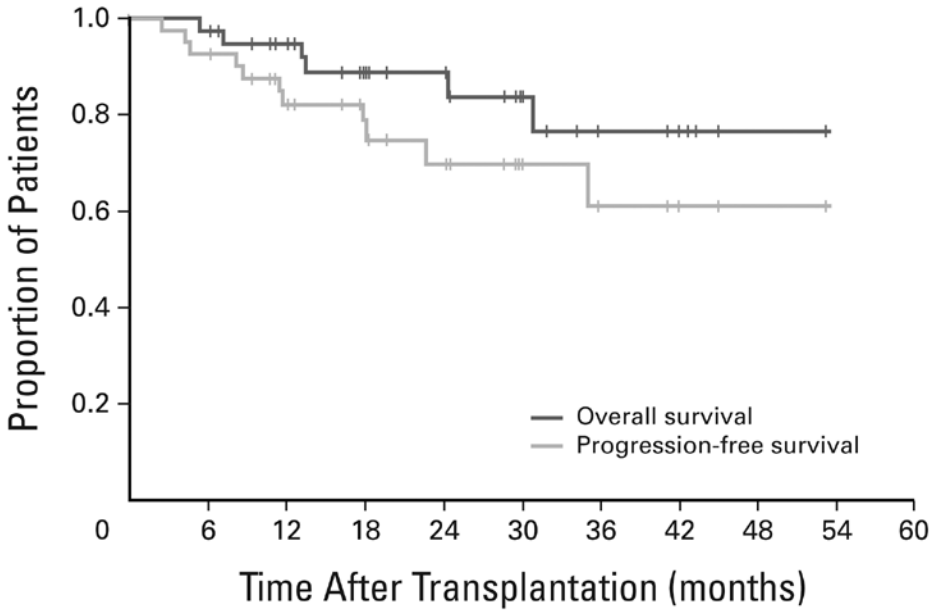

Figure 3. Overall survival and progression-free survival following standard-dose 90-Y ibritumomab tiuxetan added to high-dose etoposide, arabinoside, cytarabine, and melphalan (Z-BEAM) in patients with refractory, relapsed, or poor-risk non-Hodgkin lymphoma (NHL) [30]. Reprinted with permission. © 2008 American Society of Clinical Oncology. All rights reserved.

patients with no significant comorbidities, and is rarely offered to patients older than age 50 [25]. TBI is most useful as an antitumor agent in patients with advanced disease, for whom RIC allogeneic HSCT is less effective. For these patients, the graft-versus-leukemia (GVL) effect typically is not strong enough to induce durable disease remission.

Novel Approaches to Radiation Therapy

With advances in technology, novel approaches to radiation therapy have expanded treatment options for patients with hematologic malignancies. Radiation therapy has been used to facilitate immunosuppression, and in combination with immunotherapy, to target malignancy. More recently, total marrow irradiation has been used to enhance the antileukemic effect of conditioning regimens, including RIC and conventional myeloablative conditioning.

Nonmyeloablative Conditioning

Ideally, non-myeloablative conditioning regimens should be minimally toxic, should provide reliable engraftment, and should achieve a high degree of donor hematopoietic chimerism to achieve anti-tumor immunity following HSCT. In 2003, Maris and colleagues described a condition regimen that included a single fraction of radiation (200 cGy) followed by post-transplantation cyclosporin and mycophenolate mofetil (MMF). With this initial conditioning regimen, all patients who received HLA-matched sibling allografts achieved mixed donor–host chimerism. Furthermore, 83% of patients achieved engraftment. After the addition of fludarabine 30 mg/m^2 per day on days –4, –3, and –2, the risk of graft rejection fell from 17% to 3%. Thus, treatment with fludarabine for 3 days combined with a single dose of radiation led to full engraftment in nearly all patients undergoing transplantations with HLA-matched related and HLA-matched unrelated donors [26].

The advent of non-myeloablative conditioning extended the age of transplantation eligibility for most patients with hematologic malignancy, such that age is no longer considered a barrier to treatment. This is an important advance, given that the median age of patients with most diseases that can be cured with HSCT, including acute and chronic leukemias, myelodysplasia, myeloproliferative diseases, myeloma, and non-Hodgkin's lymphoma, is older than 60 years [25]. Yet conventional HSCT has only been a therapeutic option for patients younger than age 50 [25].

Non-myeloablative conditioning regimens have produced durable remissions across the spectrum of lymphoid and myeloid malignancies, including AML, chronic myeloid leukemia (CML), chronic lymphocytic leukemia (CLL), myeloma, and lymphoma. Thus, non-myeloablative HSCT is a reasonable option for achieving disease control in most hematologic malignancies especially for patients in remission.

Radiation Therapy and Immunosuppression

Even with RIC, transplantation outcomes are limited by risks associated with acute GVHD and non-relapse mortality. To reduce the risk of acute GVHD without compromising the efficacy of HSCT, Kohrt and colleagues developed a novel RIC regimen that altered the host immune profile to favor T/NK cells. The regimen incorporated fractionated total lymphoid irradiation (80 cGy daily for 10 days) and antithymocyte globulin, followed by granulocyte colony-stimulating factor (G-CSF)-mobilized grafts and cyclosporin/MMF. Among 111 patients with lymphoid (n = 64) and myeloid (n = 47) malignancies, 97% achieved durable chimerism by day 56. Acute GVHD occurred with very low frequency, irrespective of whether the graft was from a related (2%) or unrelated (10%) donor. At 36 months, overall survival and EFS were 60% and 40%, respectively. Thus, an approach to RIC conditioning that combined low-dose total lymphoid irradiation and antithymocyte globulin to facilitate engraftment ("GVHD prevention approach") was well tolerated and yielded a high incidence of sustained remission after allogeneic HSCT from related and unrelated donors [27].

Radioimmunotherapy

As an alternative to exposing the whole body to radiation, radioimmunotherapy delivers radiation directly to tumor cells by coupling a radionuclide with a monoclonal antibody that targets a tumor-specific cell-surface antigen. Once bound to target antigens, radioimmunoconjugates provide radiation exposure to malignant cells. Depending on the isotope, however, there may be radiation exposure to adjacent untargeted cells.

In addition to guiding the radionuclide to target cells, the monoclonal antibody conjugate may exert its own anti-tumor activity. Many well-characterized cell-surface antigens have been incorporated into radioimmunotherapies, particularly CD20. These antigens target malignant cells that are highly sensitive to radiation therapy. Given that its major

toxicity is myelosuppression, radioimmunotherapy is ideally used in the peripheral stem cell transplantation (PSCT) setting.

Radioimmunotherapy has been successfully integrated with autologous transplantation regimens. Vose and colleagues piloted the addition of iodine-131 (131-I) tositumomab to high-dose carmustine, etoposide, cytarabine, and melphalan (BEAM) followed by autuologous stem cell transplantation (ASCT) for the treatment of chemotherapy-resistant relapsed or refractory B-cell non-Hodgkin's lymphoma (NHL). In a phase I study, 131-I tositumomab/BEAM was associated with a 5-year overall survival rate of 55% in this population of poor-prognosis patients [28]. In a phase II study, the 131-I tositumomab/BEAM preparative regimen was evaluated in 40 patients with relapsed or high-risk chemosensitive diffuse large B-cell lymphoma (DLBCL). Following stem cell collection, all patients received 75 cGy total body dose of 131-I tositumomab (dosimetric dose on day –19 and therapeutic dose on day –12) followed by a standard BEAM transplantation regimen (day –6 to –1) and PSCT (day 0). After a median follow-up of 28 months, the estimated 3-year progression-free survival rate was 70%. Patients achieved a 3-year overall survival rate of 81%, without excess toxicity [29].

Building on these findings, the Blood and Marrow Transplant Clinical Trials Network (BMT CTN 0401) initiated a multicenter phase III trial to compare the standard transplantation regimen of rituximab plus BEAM with a regimen that added 131-I tositumomab to BEAM in patients with persistent or relapsed chemotherapy-sensitive DLBCL. Progression-free survival results from the BMT CTN 0401 trial, which accrued rapidly and completed in 2010, are expected later this year. Findings from the trial may change the standard of care for patients with DLBCL who are undergoing autologous HSCT.

The radioimmunoconjugate yttrium-90 (90-Y) ibritumomab tiuxetan delivers low-dose beta radiation to tumor cells bearing the cell surface antigen CD20. In a phase II trial, Krishnan evaluated the safety and efficacy of adding standard-dose 90-Y ibritumomab tiuxetan to high-dose BEAM (Z-BEAM) in patients with refractory, relapsed, or poor-risk NHL who were ineligible for fractionated TBI due to older age or prior radiation therapy. Treatment included rituximab and indium-111 ibritumomab tiuxetan 185 MBq on day –21; rituximab and 90-Y ibritumomab tiuxetan 14.8 Mbq/kg on day –14; high-dose BEAM on days –7 to –1; and autologous HSCT on day 0. After a median follow-up of 18.4 months, the estimated 2-year overall survival and progression-free survival were 88.9% and 69.8%, respectively (Figure 3) [30].

The use of novel radioimmunoconjugates to deliver supplemental radiation to targeted sites is not limited to the autologous transplantation setting. The 131-I–labed anti-CD45 antibody delivers targeted hematopoietic radiation that is 3-fold stronger to the bone marrow and spleen compared with TBI, and 10-fold stronger to the lymph nodes. In 2009, Pagel evaluated the feasibility of preconditioning with a 131-I–labeled anti-CD45 antibody plus fludarabine and low-dose TBI (2 Gy) in elderly patients with advanced AML or high-risk myelodysplastic syndrome (MDS). At the time of transplantation, 86% of patients had AML or MDS with >5% marrow blasts. Following treatment, all patients had a complete remission, and all patients had 100% donor-derived CD3+ and CD33+ cells in the peripheral blood by day 28 after allogeneic HSCT. The estimated probabilities of relapse and nonrelapse mortality at 1 year were 40% and 22%, respectively [31].

Certain patients with hematologic malignancies are not going to benefit from standard nonmyeloablative regimens, including those with multiple disease relapses and high disease burdens. Incorporating radioimmunotherapy is an opportunity to preserve the tolerability of reduced-intensity preparative regimens while delivering more radiation to malignant cells, thereby increasing the efficacy of treatment. Ongoing trials of novel radioimmunoconjugates in autologous and allogeneic HSCT are underway.

Total Marrow Irradiation

Reduced intensity regimens are used predominately in older patients with hematologic malignancies. These regimens depend on immunosuppressive therapies to promote the GVT effect and facilitate donor–cell engraftment. Compared with classical pretransplantation conditioning regimens, reduced-intensity preparative regimens are less effective for patients with advanced disease.

In order to improve disease control, some investigators have begun to add radiation therapy to augment the antitumor effect of reduced intensity regimen while avoiding the high toxicity profile of TBI. A new approach involves the use of helical tomotherapy to deliver

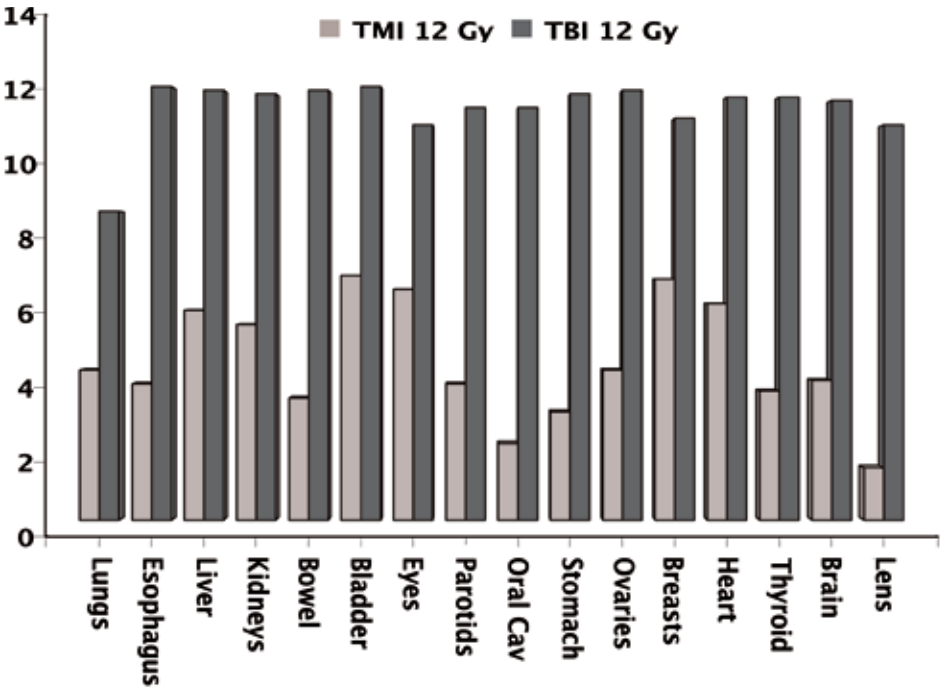

Figure 4. Median radiation dose to non-marrow structures with total body irradiation (TBI) and total marrow irradiation (TMI). Reprinted from [32] with permission from Elsevier.

radiation to the bone marrow via computed tomography (CT)-guided intensity-modulated radiation therapy (IMRT). This amplifies the anti-leukemia activity of the RIC regimen and allows patients with higher burden of disease to undergo transplantation. Specifically, helical tomotherapy enhances the anti-leukemia component of the RIC regimen by targeting the marrow. Compared with TBI, helical tomotherapy reduces the median radiation dose to surrounding organs by 30% to 70% (Figure 4) [32]. Thus, helical tomotherapy may increase the efficacy of RIC regimens while decreasing the late effects of radiation by minimizing the total dose delivered to critical structures.

In 2011, Rosenthal evaluated the feasibility of augmenting RIC with total marrow and lymph node irradiation (TMLI) in a phase I/II trial of 33 patients with advanced hematologic malignancies. All patients had factors that precluded full transplantation, including older age (median age, 55.2 years) and/or compromised organ function. The conditioning regimen included fludarabine 25 mg/m^2 for 5 days, melphalan 140 mg/m^2 for 1 day, and TMLI delivered at 150 cGy in 8 fractions over 4 days. All patients achieved engraftment at a median of 14 days after HSCT. The 1-year overall survival, EFS, and nonrelapse mortality were 75%, 65%, and 19%, respectively [33]. These findings suggest that the use of RIC with TMLI allows patients with advanced hematologic malignancies, who might not otherwise be candidates for RIC, to undergo transplantation with a low risk of toxicity.

At the 2011 BMT Tandem Meeting, Stein presented preliminary findings from an ongoing phase I/II trial of TMLI added to full-intensity transplantation in patients with advanced hematologic malignancies. Patients were treated with an escalating dose of total marrow irradiation (TMI) in combination with high-dose etoposide (60 mg/kg) and cyclophosphamide (100 mg/kg). The first cohort of patients received 1200 cGy delivered at 150 cGy in twice-daily fractions, with dose escalation to 1500 cGy without any dose-limiting toxicities. All patients were in relapse or induction failure at the time of HSCT. The regimen was associated with a low risk of nonrelapse mortality at 30 days (0%) and 100 days (8%). After a median follow-up of 9.2 months, overall survival is 63%, and 17% of patients have relapsed [34]. Additional follow-up data will provide more information about the safety and efficacy of this fully ablative regimen in patients with advanced hematologic malignancies.

For patients undergoing autologous HSCT, total marrow irradiation can be delivered in a fully ablative dose via helical tomotherapy. In 2010, Somlo demonstrated the feasibility of ablative-dose total marrow irradiation as part of consolidation/tandem autologous HSCT in 22 patients with multiple myeloma. Following the first cycle of autologous HSCT with high-dose melphalan (200 mg/m^2), responding patients received TMI as the sole ablative modality for the second transplantation at a median of 63.5 days later (range, 44 to 119 days). Radiation doses were escalated from 1000 cGy by increments of 200 cGy, reaching dose-limiting toxicities at 1800 cGy. After a median of 35 months of follow-up, overall survival and progression-free survival were 82% and 49%, respectively. Based on this experience, the phase II component of the trial is ongoing with total marrow irradiation of 1600 cGy as part of tandem autologous HSCT [34].

Conclusions

Radiation therapy remains an important component of RIC transplantation regimens, especially for patients with advanced hematological malignancies. Delivering targeted radiation therapy for lymphoma, leukemia, and myeloma may offer a more effective approach to transplantation while reducing the risk of acute and late toxicity. Total marrow irradiation may provide the opportunity to increase the dose of therapeutic radiation, thus taking advantage of the dose–response relationship in hematologic malignancy.

Alternatives to Radiation-Based Conditioning Therapy

Borje S. Andersson, MD, PhD

Myeloablative conditioning followed by allogeneic transplantation is commonly associated with 100-day treatment-related mortality rates of >20%, with even higher rates in patients with older age, advanced disease, and/or unrelated donors. Conditioning regimens that incorporate TBI are also limited by potential late effects such as stunted growth, neuropsychologic dysfunction, and secondary malignancies.

Cyclophosphamide plus TBI (CY/TBI) and oral Bu/CY are 2 of the most commonly used myeloablative conditioning regimens in patients undergoing allogeneic HSCT. In the meta-analysis of 7 randomized clinical trials comparing these regimens in patients with CML, preconditioning with CY/TBI reduced the risk of transplantation-related mortality by 47% compared with oral Bu/CY (P = .02). Despite this advantage, CY/TBI did not significantly improve survival, but showed a nonsignificant trend toward reduced all-cause mortality (P = .12), and there was no difference in disease relapse rate (P = .28) compared with oral Bu/CY [35].

Although the cumulative risk of major complications did not differ between groups, the types of complications in each group were distinct. In particular, regimens that incorporated TBI were associated with more severe late effects on growth and development in children [35]. Concerns about treatment toxicity and late effects of radiation therapy have prompted the exploration of alternative conditioning regimens that might yield improved safety with acceptable antitumor activity without the use of TBI (Table 1).

Mechanisms of Cyclophosphamide Toxicity

Dose-limiting toxicities of CY are well established in patients undergoing treatment for hematologic malignancies and include cardiotoxicity, hemorrhagic cystitis, and venoocclusive disease of the liver. In 1996, McDonald described the mechanism by which busulfan can potentiate cyclophosphamide-mediated hepatic injury in patients receiving Bu/CY. Thus, the hepatic metabolism of both BU and CY is mediated primarily by glutathione S-transferase (GST). Within the liver, busulfan is conjugated to glutathione, GSH, by GST and it forms a lipophilic intermediary compound, tetrahydrothiophene, which is toxic to hepatocytes and sinusoidal endothelial cells. When busulfan is administered before cyclophosphamide, the process of busulfan metabolism depletes hepatic cytosolic GSH, which is necessary for the detoxification of activated cyclophosphamide. Thus, pretreatment with busulfan can lead to cyclophosphamide-mediated hepatic injury [36].

Exposure to 4-hydroxycyclophosphamide (HCY) (This is factually wrong and comes from Hassan et al,; HCY is a chemically

unstable intermediary which in itself is nontoxic, the further spontaneous breakdown of HCY actually form the truly toxic metabolites, acrolein and phosphorodiamidic mustard, both of which are conjugated to GSH, and without the GSH these two final metabolites will cause the toxic effects in both the liver and other organs), the toxic metabolite of cyclophosphamide, is modulated by other drugs in the Bu/CY conditioning regimen. In a study of 14 patients being prepared for HSCT, Bu/CY was associated with greater cyclophosphamide clearance (P = .0014), shorter half-life (P = .0027), greater peak plasma concentration of HCY (P = .006), and greater ratio of area under the plasma concentration/time curve (AUCs) of HCY to cyclophosphamide (P = .0116) compared with cyclophosphamide and TBI. Overall, preconditioning with Bu/CY resulted in greater exposure to the toxic HCY metabolite (HCY is a surrogate marker for the ultimate toxic metabolites, see above) per cyclophosphamide dose when compared with the cyclophosphamide/TBI regimen [36].

Table 1. Comparison of Alternatives to Radiation-Based Conditioning Therapy*

Regimen	Secure Engraftment	Acute Toxicity	Anti-Leukemic Activity	GVHD
Myeloablative Regimens				
CY/TBI	+++	++ - ++++	+ - +++	++++
BU/CY2 (oral/IV)	++ / +++	+++ / ++	+ / ++	+++ / ++
Bu4Flu	+++	+	(+ -) ++	+
Reduced-Intensity Regimens				
IV BU/CY2	+++	++	++	++
Bu4Flu	+++	+	(+ -) ++	+
CloBu4 variants	+++	+ (- ++?)	++++	+ (- ++?)

*GVHD indicates graft-versus-host disease; CY, cyclophosphamide; TBI, total body irradiation; BU, busufan; IV, intravenous; Flu, fludarabine; Clo, clofarabine.

IV Busulfan and Nucleoside Analogs

With this insight, researchers explored options for optimizing systemic drug exposure and lessening the toxicity of myeloablative preparative regimens. For instance, new approaches to busulfan dosing evaded first-pass hepatic metabolism and opened options for safer cyclophosphamide use. Treatment with intravenous (IV) busulfan provided a standardized approach to drug delivery with 100% bioavailability and absolute dose assurance, coupled with an avoidance of the hepatic first-pass extraction. The use of nucleoside analogs, which do not rely on glutathione for their metabolism, was also explored, leading to a new standard for preconditioning with IV busulfan and fludarabine (BuFlu).

Myeloid Leukemia

For patients with myeloid leukemia, IV BuFlu is associated with >80% long-term survival due to increased safety and decreased TRM. In 2007, Russell described a variant of the BuFlu regimen in a study of 64 adult patients with acute leukemia in first or second remission. Patients were treated with fludarabine 50 mg/m^2 on days –6 to –2 (again, it is from day minus 6 to day minus two), IV busulfan 3.2 mg/kg daily on days –5 to –2, and 400 cGy TBI on day –1 or 0, followed by allogeneic HSCT. Patients also received prophylaxis against GVHD with methotrexate, cyclosporine A, and antithymocyte globulin. The risk of transplantation-related mortality in the first 100 days was only 3%. For patients with AML, the estimated 3-year rates of overall survival and EFS were both 83% (Figure 2) [16].

More recently, Alatrash reported encouraging survival outcomes following treatment with IV BuFlu among patients age 55 and older with AML or MDS. The analysis included 79 patients (median age, 58 years, range 56 to 76) who had been treated with IV BuFlu conditioning regimen variants at the M.D. Anderson Cancer Center in Houston and the Albert Einstein Hospital in Sao Paolo in Brasil between 2001 and 2009. The probability of survival at 2 years was highest for patients in first complete remission at the time of HSCT (71%), followed by those in second complete remission (44%) and those with refractory disease (32%). Corresponding 2-year EFS rates were also associated with disease status (68%, 42%, and 30%, respectively) [37]. These findings suggest that age alone should not be a decisive adverse factor when assessing the feasibility of allogeneic HSCT for patients with AML or MDS.

Despite encouraging safety and efficacy data supporting the use of IV BuFlu, there have been some concerns about the possibility of a suboptimal antileukemic effect with fludarabine compared with cyclophosphamide, particularly in patients with active leukemia at the time of HSCT. To date, no randomized phase III trials have prospectively compared the IV BuFlu preconditioning regimen with Bu/CY in patients undergoing allogeneic HSCT. Recent nonrandomized data from the M.D. Anderson Cancer Center, however, support the use of BuFlu as a preferred preconditioning regimen over Bu/CY in patients with AML/MDS [38].

The M.D. Anderson analysis included sequential cohorts of 67 patients treated with Bu/CY2 between 1997 and 2001 and 148 patients who received BuFlu between 2001 and 2005. Among patients who received transplantations during first remission, 3-year overall survival was significantly greater with BuFlu compared with Bu/CY2 (78% versus 42%, respectively; P = .03), as was 3-year EFS (74% versus 42%, respectively; P = .011). Patients younger than age 40 had impressive 3-year EFS rates in both treatment groups, although outcomes strongly favored BuFlu compared with Bu/CY2 (94% versus 83%, respectively; P = .001). The BuFlu regimen also demonstrated superior safety, with a significantly lower TRM rate compared with Bu/CY2 at 1 year (6% versus 21%; P = .012). The overall incidence of acute grade II-IV GVHD was 16% after BuFlu and 33.3% after Bu/CY2, and the corresponding incidence of extensive chronic GVHD was 34.1% and 36.1%, respectively. Thus, replacing cyclophosphamide with fludarabine appeared to reduce the toxicity of treatment in combination with busulfan without sacrificing antileukemic activity [38].

Treatment with IV BuFlu is now considered a "platform technology" on which additional research can be built, allowing for the testing of new concepts such as the addition of mobilizing agents to isolate leukemic cells from the marrow stroma and the use of posttransplantation maintenance immunotherapy to reduce the incidence of GVHD (Figure 5) [39].

One promising line of research involves the use of clofarabine (Clo), a new-generation nucleoside analog, to enhance the antileukemic activity of pretransplantation conditioning therapy for allogeneic HSCT. In 2011, Valdez

demonstrated the synergistic cytotoxicity of clofarabine, fludarabine, and busulfan in several AML cell lines. In particular, combination therapy with clofarabine, fludarabine, and busulfan showed pronounced synergy at very low drug concentrations (IC5-10), suggesting increased antileukemic efficacy with very low risk of toxicity [40]. To evaluate the clinical activity of clofarabine, 51 patients with advanced AML or MDS were randomly assigned to 1 of 4 treatment groups:

- Clofarabine 10 mg/m^2 and fludarabine 30 mg/m^2
- Clofarabine 20 mg/m^2 and fludarabine 20 mg/m^2
- Clofarabine 30 mg/m^2 and fludarabine 10 mg/m^2
- Clofarabine 40 mg/m^2

Patients received clofarabine/fludarabine or single-agent clofarabine infused over 1 hour once daily for 4 days, followed each day by busulfan infused over 3 hours to a targeted daily AUC of 6000 µMol-minutes. All evaluable patients were engrafted after allogeneic HSCT, with marrow and T-cell chimerism studies showing 100% donor DNA in all treatment groups. The projected median overall survival was 23 months, with a trend toward better survival in the higher-dose clofarabine groups (clofarabine 30 to 40 mg/m^2). These early results suggest that clofarabine with or without fludarabine in combination with IV busulfan is sufficiently immunosuppressive to support allogeneic HSCT in patients with myeloid leukemia [41]. Additional follow-up data will clarify whether clofarabine improves disease control in this high-risk population, which has historical 3-year survival rates of approximately 20% with busulfan and fludarabine-based preconditioning.

Lymphoid Malignancies

Preconditioning with IV BuFlu and low-dose TBI (4 Gy) is an established strategy for patients with lymphoid malignancies prior to allogeneic HSCT. In the 2007 study from Russell, the 3-year overall survival and EFS rates were 78% and 65%, respectively, for patients with ALL following IV BuFlu, TBI, and allogeneic HSCT (Figure 2) [16].

In 2010, Kebriaei evaluated a novel clofarabine-based preconditioning regimen in 14 patients with high-risk ALL. All patients received clofarabine 40 mg/m^2 and busulfan 130 mg/m^2 infused daily for 4 days followed by allogeneic HSCT 2 days later. After 30 days, all patients achieved complete remission with no detectable minimal residual disease. With a median follow-up of 3 months, the overall survival and disease-free survival rates are both 92% [42]. With additional follow-up data, the clofarabine/busulfan regimen may prove to be as effective as conventional conditioning therapy, but without the use and potential complications of TBI. Additional phase II trials are currently evaluating variants of the clofarabine/busulfan preconditioning regimen in patients with both ALL and AML.

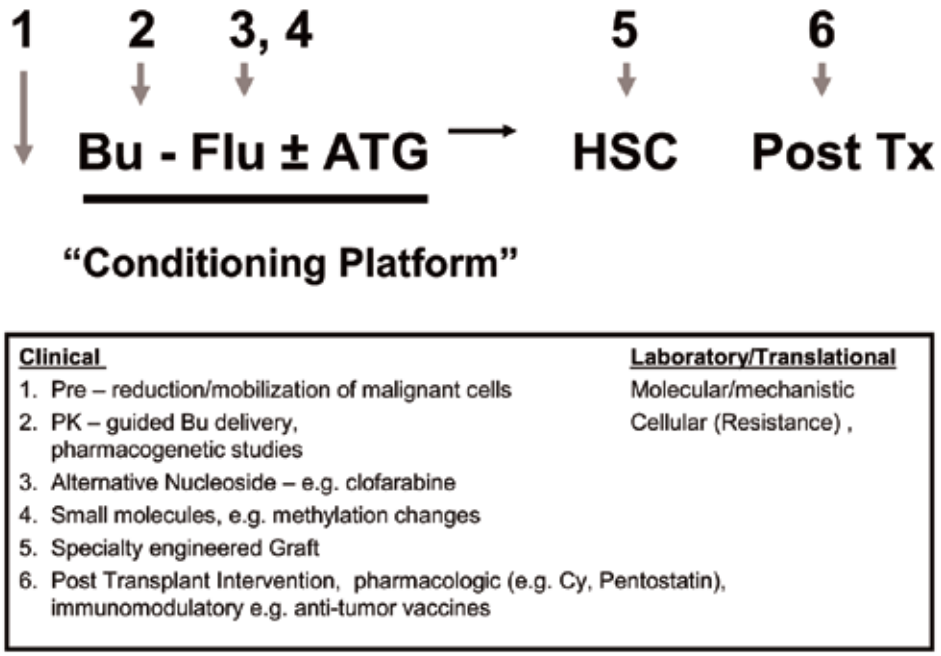

Figure 5. Investigational approaches added to the "conditioning platform" of intravenous busulfan/fludarabine in patients undergoing hematopoietic stem cell transplantation (HSCT) [39].

Conclusions

Conditioning regimens for HSCT have advanced over the past 30 years, expanding options for patients who were not traditionally considered candidates for potentially curative therapy. New and emerging options challenge the notion that more intense antileukemic activity is necessarily accompanied by increasing toxicity and transplantation-related mortality. The use of nucleoside analogs in combination with IV busulfan provides safe access to ablative conditioning with low toxicity and effective disease control. These advances are especially pronounced in patients with myeloid malignancies, who have long-term survival rates exceeding 80% when treated with BuFlu and allogeneic HSCT during first complete remission. Moving forward, the IV BuFlu regimen provides a well-defined platform for the continuing exploration of treatment refinements (Figure 5).

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Impact of Conditioning Regimens on Transplantation Outcomes

CME Assessment Test

1. **Which of the following has NOT been shown to increase the risk of late complications associated with childhood hematopoietic stem cell transplantation (HSCT)?**
   A. Younger age at time of transplantation
   B. Use of total body irradiation in the conditioning regimen
   C. Longer duration of follow-up after transplantation
   D. Use of busulfan in the conditioning regimen

2. **Compared with busulfan and cyclophosphamide (Bu/CY) as a pretransplantation conditioning regimen, total body radiation is associated with which of the following outcomes?**
   A. Lower risk of relapse
   B. Lower risk of transplantation-related mortality
   C. Worse overall survival
   D. Worse event-free survival

3. **Which agent is known to potentiate cyclophosphamide-related hepatic injury?**
   A. Melphalan
   B. Fludarabine
   C. Busulfan
   D. Clofarabine

4. **In patients treated an intravenous (IV) busulfan-based conditioning regimen, replacing cyclophosphamide (Bu/CY) with fludarabine (BuFlu) reduces the toxicity of treatment without sacrificing antileukemic activity.**
   A. True
   B. False

5. **The combination of iodine-131 (131-I) tositumomab and high-dose carmustine, etoposide, cytarabine, and melphalan (BEAM) followed by autologous stem cell transplantation (ASCT) may change the standard of care for which hematologic malignancy?**
   A. Diffuse large B-cell lymphoma
   B. Mantle cell lymphoma
   C. Follicular lymphoma
   D. Multiple myeloma

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