ASBMT™
American Society for Blood and Marrow Transplantation

Transplantation in Remission Improves the Disease-Free Survival of Patients with Advanced Myelodysplastic Syndromes Treated with Myeloablative T Cell-Depleted Stem Cell Transplants from HLA-Identical Siblings

Hugo Castro-Malaspina,[1] Ann A. Jabubowski,[1] Esperanza B. Papadopoulos,[1] Farid Boulad,[2] James W. Young,[1] Nancy A. Kernan,[2] Miguel A. Perales,[1] Trudy N. Small,[2] Katharine Hsu,[1] Michelle Chiu,[2] Glenn Heller,[3] Nancy H. Collins,[2] Suresh C. Jhanwar,[4] Marcel van den Brink,[1] Stephen D. Nimer,[5] and Richard J. O'Reilly[2]

[1] The Allogeneic Bone Marrow Transplantation Service, Department of Medicine; [2] Bone Marrow Transplantation Service, Department of Pediatrics; [3] Department of Epidemiology and Statistics; [4] Cytogenetics Laboratory, Department of Pathology; [5] Hematology Service, Department of Medicine, Memorial Sloan-Kettering Cancer Center, New York, NY.

Correspondence and reprint requests: Hugo Castro-Malaspina, MD, Bone Marrow Transplantation Service, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10021. Phone: (212) 639-8197. Fax: (212) 717-3371 (e-mail: castro-h@mskcc.org).

ABSTRACT

From 1985 to 2004, 49 patients with advanced myelodysplastic syndromes (MDS) (≥5% blasts) or acute myeloid leukemia (AML) transformed from MDS underwent T cell depleted bone marrow or peripheral blood hematopoietic stem cell transplantation (HSCT) from HLA-identical siblings following conditioning with a myeloablative regimen that included total body irradiation (44 patients) or busulfan (5 patients). Thirty-six patients received chemotherapy (3 low dose and 33 induction doses) before conditioning, and 13 patients did not receive any chemotherapy. Prior to transplantation, 22 of the 36 treated patients were in hematologic remission; 4 were in a second refractory cytopenia phase (26 responders); 8 had failed to achieve remission; and 2 of the responders had progression or relapse of their MDS (10 failures). No post-transplantation pharmacologic prophylaxis for graft-versus-host disease (GVHD) was given. The median age was 48 yrs (range 13-61). Forty-five of the 49 patients engrafted; 2 had primary graft failure; and 2 died before engraftment. Only 3 patients developed acute GVHD (aGVHD) (grades I and III) and 1 chronic GVHD (cGVHD). At 3 yrs post-transplantation, the overall survival (OS) was 54% in the responders; 31% in the untreated group; and 0% in the failure group (P=.0004). The disease free survival (DFS) was 50%, 15% and 0% in each group respectively (P=.0008). In multivariate analysis, disease status before cytoreduction remained highly correlated with DFS (P<.001). The cumulative incidence (CI) of relapse at 2-yrs post-transplantation for the responders was 23%; for the untreated group was 38%; and for the failures was 50%. The CI of non-relapse mortality at 2-yrs post-transplantation, for the responders was 23%; for the untreated group was 38%; and for the failures was 40%. All survivors achieved a Karnofsky Performance Status (KPS) of ≥90. These results indicate that patients with advanced MDS who achieve and remain in remission or a second refractory cytopenia phase with chemotherapy before conditioning can achieve successful long-term remissions following a myeloablative T cell depleted allogeneic HSCT.
© 2008 American Society for Blood and Marrow Transplantation

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) is currently the only curative treatment available for patients with myelodysplastic syndromes (MDS) [1-4]. However, the high rate of post-transplantation relapse compromises the success rate in advanced forms of MDS (≥5% blasts in the bone marrow or >1% blasts in the peripheral blood) and acute myeloid

leukemia (AML) transformed from MDS [1-7]. This is in contrast to the lower relapse rate and better disease free survival (DFS) in patients with refractory anemia or refractory cytopenia with multilineage dysplasia with or without ringed sideroblasts [1-8]. Various approaches have been tried to reduce the incidence of post-transplantation relapse in advanced MDS. The use of more intensive preparative regimens combining total body irradiation and busulfan has reduced the incidence of relapse, but has resulted in increased regimen-related morbidity and mortality [9, 10].

The use of remission induction chemotherapy to achieve remission before the administration of cytoreductive therapies for allogeneic hematopoietic stem cell transplantation (HSCT) remains controversial. Data from the European [11-13] and French registries [14] showed improved DFS following allogeneic bone marrow transplantation (BMT) from HLA-matched siblings in MDS patients who were transplanted in hematologic remission compared with patients transplanted with refractory disease (DFS at 3 yrs of 25% versus 15%). However, induction chemotherapy was associated with a morbidity and mortality of 5 to 15% [14]. More recently, the Fred Hutchinson Cancer Center reported their experience in 125 patients with advanced MDS and AML evolved from MDS who received transplants from HLA-identical siblings or unrelated donors after myeloablative conditioning regimens [15]. The relapse-free survival [RFS] at 3 yrs was similar in patients, regardless of whether or not they underwent induction chemotherapy pre-transplantation. However, relapse-free survival (RFS) by response to induction chemotherapy was not given, so any beneficial effect of pre-cytoreduction induction chemotherapy could not be judged.

Although the early results of T cell depleted marrow transplantation in patients with AML and chronic myelogenous leukemia clearly demonstrated that acute and chronic graft-versus-host disease (aGVHD and cGVHD) could be abrogated, the beneficial effect on survival was compromised by the higher incidence of graft rejection [16, 17] and post-transplantation relapse [18]. Stepwise modifications of the myeloablative preparative regimen have provided more intensive immunosuppression and have reduced the incidence of graft rejection and post-transplantation relapse in patients with de novo AML who underwent transplantation in remission to levels comparable to those seen in unmodified myeloablative BMT [16, 19, 20]. The 4-yr DFS and relapse rate in patients with AML in first remission have been 77% and 3.2% respectively, and in patients with AML in second remission, 50% and 12.5% respectively.

We now report the results of T cell-depleted transplantation in 49 patients with advanced MDS, emphasizing their transplantation outcomes with regard to pre-transplantation treatment and response to chemotherapy.

MATERIALS AND METHODS

Patients

From January 1985 to December 2004, 49 patients with advanced MDS (≥5% blasts in the bone marrow or >1% blasts in the peripheral blood) or AML evolved from MDS (≥20% blasts in the peripheral blood or bone marrow) underwent T cell-depleted bone marrow or peripheral blood HSCT from HLA-identical siblings after preparation with a myeloablative regimen at Memorial Sloan Kettering Cancer Center. Analysis of our early experience with T cell depletion in MDS in 1998 showed a higher incidence of graft failure and relapse in untreated patients with refractory anemia with excess blasts (RAEB) 1 and RAEB 2, and in patients with AML evolved from MDS who had failed induction chemotherapy. Thereafter, patients with RAEB 1 and RAEB 2 were given chemotherapy before undergoing TCD HSCT, and if remission or a second refractory anemia phase was not achieved, a non-T cell-depleted (non-TCD) HSCT was offered. Also, patients with AML who failed chemotherapy were offered a non-TCD HSCT.

The patient and disease characteristics are summarized in Table 1. All patients had been diagnosed as having MDS at presentation and were at an advanced phase or had transformed to AML when they were referred for allogenic transplantation. The median age was 47.8 yrs, with 4 patients <20; 24 patients 20-50 yrs old; and 21 patients >50. Forty-one patients had de novo MDS, and only 8 patients had therapy-related MDS.

The MDS subtype and prognostic classification at diagnosis and before transplantation were determined according to the WHO (World Health Organization) and IPSS (International Prognostic Scoring System) criteria [21, 22]. Two patients had no chromosome studies at diagnosis and the IPSS prognostic category could not be determined. The patients who responded to chemotherapy were divided into 2 groups: complete remission and second refractory cytopenia phase. Complete hematologic remission was defined as a cellular marrow with <5% blasts; no overt dysplasia, neutrophils of >1,000/uL; platelets of ≥ 100,000/uL; red cell transfusion independent; and no circulating blasts [23, 24]. Second refractory cytopenia phase was defined as a marrow with <5% blasts, with or without dysplasia, and with persistent pancytopenia with no circulating blasts. Patients who failed to respond to chemotherapy were classified for this analysis in their original WHO subtype or IPSS score before administration of chemotherapy.

All patients were fully informed of the risks of the treatment and their written consent was obtained according to the guidelines approved by our Institutional Review Board.

Table 1. *Patient and Disease Characteristics*

Number of patients	49
Median age in yrs (range)	47.8 (13.3-60.6)
Recipient age distribution	
≤20 yrs	4
21-50	24
≥51	21
Median time from diagnosis to transplant, mos (range)	6.2 (1.8-53.2)
Etiology	
Idiopathic	41
Post radiation and/or chemotherapy	8
Cytogenetics at diagnosis	
Good	24
Intermediate	10
Poor	13
Failure	2
WHO MDS subtype at diagnosis	
5 q- syndrome	1
Refractory cytopenia with multi-lineage dysplasia	7
Refractory anemia with ringed sideroblasts	1
Refractory anemia with excess blasts 1	12
Refractory anemia with excess blasts 2	25
Chronic myelomonocytic leukemia 1	2
Chronic myelomonocytic leukemia 2	1
IPSS at presentation	
Low risk	2
Intermediate risk I	14
Intermediate risk II	19
High risk	12
Unknown (no chromosome studies)	2
WHO MDS status before conditioning	
Refractory anemia with excess blasts 1	6
Refractory anemia with excess blasts 2	8
AML	9
Complete remission	22
Refractory cytopenia in second phase	4

Table 2. *Patient and Donor Characteristics*

Preparative regimen	
TBI/CY	2
TBI/CY and carboplatin	8
TBI/CY and diaziquone	4
TBI/thiotepa/fludarabine	7
TBI/thiotepa/CY	23
Busulfan/melphalan/fludarabine	4
Busulfan/fludarabine	1
Rejection prophylaxis with	
Anti-thymocyte globulin	35
None	14
Source of stem cell	
Bone marrow	32
Peripheral blood	12
Bone marrow and peripheral blood	5
Median CD34 cell dose $\times 10^6$/kg (range)	1.17 (0.12-20.5)
CD34 (x10^8/kg)	0.8 (0.02-3.5)
Donor	
Median age in yrs (range)	45 (11-65)
Number of males	27
Gender (donor:recipient)	
M:F	9
M:M	17
F:M	10
F:F	13
Donor/Recipient CMV serology	
Positive-positive	8
Positive-negative	8
Negative-positive	6
Negative-negative	10
Unavailable	17

TBI indicates total body irradiation; BU, busulfan; CY, cyclophosphamide.

Induction Chemotherapy

Thirty-six of the 49 patients received chemotherapy before conditioning. Three patients were treated with low dose chemotherapy and 33 with full induction chemotherapy. The most common induction chemotherapy regimen was a combination of standard dose cytarabine given for 7 days and an anthracycline given for 3 days.

Preparative Regimen

Forty-four patients were treated with a total body irradiation (TBI)-based regimen and 5 patients with a busulfan-based preparative regimen (Table 2). TBI with lung shielding and testicular boost was given in a hyperfractionated manner (1375-1550 cGy) (18, 19, 25). Cyclophosphamide (CY) was given at the dose of 60 mg/kg/day for 2 days after TBI. Carboplatin was administered before TBI as a continuous i.v. infusion over 5 days, at the doses of 200, 250 or 300 mg/m2/day. Diaziquone was also given before TBI as a continuous infusion over 7 days, at doses of 8 or 12

mg/kg/day. Thiotepa was given after TBI and before CY at a dose of 5 mg/kg/day for 2 days. Fludarabine was given after TBI at a dose of 25 mg/m2/day for 5 days. These regimens were part of phase 2 studies conducted during the time period the patients in these series underwent transplantation.

The use of preparative regimens containing solely chemotherapy started only in 2001. Busulfan 0.8 mg/kg was given by i.v. every 6 hours for 10 doses on days -9 to -7; melphalan 70 mg/m2/day on days -7 and -6; and fludarabine 25 mg/m2/day on days -6 through day -2. Blood samples for pharmacokinetics were collected at 0, 1, 2, 4 and 6 hrs after the first dose of busulfan. Dose adjustments were made from the 7th to the 10th doses to reach a steady-state level of 600-900 ng/mL, with the desired level closer to 900 ng/mL.

Source of Hematopoietic Stem Cells

Thirty-two patients received a marrow graft; 12 a peripheral blood stem cell graft; and 5 a marrow graft followed by a peripheral blood stem cell (PBSC) graft because of low marrow cell dose. All donors were genotypically matched siblings. Donor peripheral blood stem cells were collected after mobilization with

subcutaneous administration of rhG-CSF 8 mcg/kg subcutaneously every 12 hrs for a total of 11 doses. Two 3-4 hr apheresis were performed after the 9th and 11th doses [20].

T Cell Depletion

The marrow grafts were depleted of T-cells by soybean lectin agglutination and sheep red cell rosette depletion [26]. The peripheral blood stem cells were depleted of T cells first by positive CD34 selection using Cell Pro columns or Isolex 300i magnetic cell separator followed by sheep red cell rosette depletion [27].

Graft-versus-Host Disease Prophylaxis

Patients did not receive any pharmacologic post-transplantation prophylaxis for GVHD.

Rejection Prophylaxis

Anti-thymocyte globulin (ATG) and methylprednisolone were given to 35 patients to prevent graft rejection. ATG of equine origin (15-30 mg/kg/dose) was given to 26 patients and of rabbit origin (2.5 mg/kg/day) to 9 patients. Methylprednisolone was given on the days ATG was administered at the dose of 2 mg/kg/day. Of the 26 patients receiving equine ATG, 4 received it pre-transplantation on days -4 and -5; and 22 received it post-transplantation every other day from day +5 to + 13. All 5 patients prepared with the busulfan-containing regimen and 4 patients prepared with the TBI-based regimen received rabbit ATG pre-transplantation on days -3 and -2. The administration of ATG was switched from post-transplantation to pre-transplantation to assess the impact of time of administration on T cell function recovery.

Supportive Care

The patients included in this series underwent transplantation over a period of 19 years during which time supportive care measures used in allogeneic HSCT improved [20]. In general, patients were hospitalized in single rooms on reverse isolation. They received acyclovir prophylaxis against Herpes simplex and zoster infections; fluconazole for fungal prophylaxis (or low dose AmBisome or voriconazole if history of aspergillosis); and atovaquone for toxoplasmosis prophylaxis in patients at risk. Prophylactic anti-bacterials were not given in the peri-transplantation period. The patients conditioned with busulfan received seizure prophylaxis with phenytoin. Recombinant human granulocyte colony stimulating factor (G-CSF) was given in the early post-transplantation period when the cell dose was low or patients had fever or infection despite appropriate antibacterials. No other cytokines were administered. Patients with CMV viremia were treated with ganciclovir or Foscarnet, and those diag-

nosed more recently with EBV viremia received Rituximab therapy.

Older recipients of T cell-depleted transplants have delayed recovery of immune function [28]. Immune function recovery was monitored by flow cytometric analysis of T cell subpopulations and response to the mitogen phytohemagglutinin (PHA) [28]. Infection prophylaxis for Pneumocystis jiroveci, and DNA Herpes viruses was continued until patients achieved a CD4 count of >200/mL and a PHA within 75% of lower limit of normal.

Donor Leukocyte Infusions (DLI)

Nine patients received donor leukocyte infusions (DLI): 4 for relapse and 1 for increasing mixed chimerism with no evidence of hematologic relapse. The remaining 4 patients received DLI as treatment for an opportunistic infection: one Epstein-Barr virus associated lymphoproliferative disorder; one JC virus associated multifocal leukoencephalopathy; one acyclovir-resistant Herpes simplex virus infection; and one persistent Mycobacterium haemophilum cutaneous infection.

Outcome Definition

Neutrophil engraftment was defined as the first of 3 consecutive days with an absolute neutrophil count (ANC) ≥500/µl. Engraftment was confirmed by documentation of chimerism in bone marrow cells using karyotype or fluorescent in situ hybridization (FISH) of the X and Y chromosome in sex mismatched donor-recipient pairs and by measurement of DNA restriction fragment length polymorphisms or short tandem repeats in sex-matched pairs.

Primary graft failure was defined as the absence of neutrophil recovery (≥500/µl) by day 28 and bone marrow biopsy with ≤5% cellularity. Secondary graft failure was defined as loss of ANC to <500/mm3 after primary engraftment with bone marrow biopsy showing ≤5% cellularity.

Acute and chronic GVHD were evaluated according to established criteria [29-31] in patients who survived ≥21 and ≥100 days with engraftment, respectively.

Hematologic relapse was defined as the recurrence of cytopenias associated with marrow morphologic changes diagnostic of MDS. Cytogenetic relapse was defined as the recurrence of pre-transplantation chromosome abnormalities.

For patients who relapsed and died, relapse was the primary cause of death even if the patient died of other events. Causes of death other than relapse were considered to represent competing risks. GVHD was the primary cause of death if the patient developed a fatal complication such as an infection while receiving systemic therapy for GVHD. Infection was the primary cause of death if it occurred in the absence of graft

Table 3. *Chemotherapy Before Conditioning in 36 Patients*

Low dose chemotherapy (azacitidine and cytarabine)	3 (8%)
High dose chemotherapy	33 (92%)
Anthracycline and standard dose Cytarabine	19 (53%)
Anthracycline and high dose Cytarabine	7 (19%)
Anthracycline and etoposide	1 (3%)
Etoposide and high dose Cytarabine	4 (11%)
Etoposide, anthracycline and Cytarabine	1 (3%)
FLAG (fludarabine, Cytarabine and G-CSF)	1 (3%)

failure, GVHD, and relapse. Non-relapse mortality (NRM) was defined as death without evidence of hematologic relapse. In this case, relapse was considered a competing risk, and patients who were alive without relapse were censored at last follow-up.

Statistical Analysis

The primary endpoints of this study were DFS, RFS, and NRM. The Kaplan-Meier estimate was used to compute DFS probability over time [32], whereas the cumulative incidence function was used to estimate the probabilities for the time to relapse and non-relapse mortality [33]. The prognostic factors considered in this analysis were: age at diagnosis ($\leq$50 vs >50); etiology (primary versus treatment related); WHO at diagnosis; blasts at diagnosis; cytogenetic risk at diagnosis; IPSS at diagnosis; WHO at progression before chemotherapy; number of courses of induction; administration of consolidation chemotherapy; disease status before conditioning; preparative regimen; and CD34 cell dose. The log-rank statistics evaluated the marginal effect of the prognostic factors on DFS [34, 35]. Gray's statistics evaluated the individual effects of these factors on the time to relapse and non-relapse mortality [36]. A proportional hazards model was used to determine the joint prognostic factors that predicted DFS.

RESULTS

Disease Status Before Conditioning

Thirteen patients did not receive any chemotherapy before conditioning and 36 patients received chemotherapy, 33 a full course of induction chemotherapy, and 3 low dose chemotherapy (Table 3). Of the 33 patients receiving induction chemotherapy, 22 were in hematologic remission (19 after one course and 3 after 2 courses); 3 in a second refractory cytopenia phase; and 8 failed to respond (5 patients received 2 courses of induction chemotherapy). Of the 3 patients receiving low dose chemotherapy, 2 receiving azacitidine achieved a second refractory cytopenia phase; and one patient receiving cytarabine achieved hematologic and cytogenetic remission.

Only 3 (13.6%) of the 22 complete responders to induction chemotherapy had high-risk cytogenetics,

Table 4. *Transplant Outcomes in 49 High Risk MDS Patients Receiving a T Cell Depleted HSCT From an HLA-Matched Sibling*

	Untreated n=13	Failures n=10	Responders n=26
Engraftment*	12	8*	25*
Time to engraftment (days range)	9-22	8-32	9-21
Graft Failure	3	1	0
Regimen-related toxicity	0	0	1
EBV-related LPD	2	0	0
Acute GvHD	1	1	1
Chronic GvHD	0	0	1
Relapse	5	5	6
Causes of death			
Graft failure	3	1	0
Regimen-related (VOD)	0	0	1
Graft-versus-host disease	0	1	1
Infections			
Bacterial	0	2	4
HZV encephalitis	0	0	1
PML	0	0	1
EBV	2	0	0
Other	1	1**	0
Relapse	4	5	5
Alive in remission	2	0	13
Median follow-up (mo)	9.3	5.8	36
Range	0.8-68.6	0.2-27.9	0.6-213.9

*2 patients non-evaluable for engraftment and 2 had primary graft failure.

**this patient died of metastatic renal cell carcinoma in remission of her MDS.

whereas 4 (50%) of the 8 failures had high-risk cytogenetics. Ten of these 22 patients received a course of consolidation chemotherapy before conditioning for transplantation.

Of the 23 patients achieving complete remission with induction or low dose chemotherapy, only one relapsed before transplantation. One of the 4 patients achieving a second refractory cytopenia phase had disease progression before transplantation. Thus, there were 26 patients who remained in remission or in a second refractory cytopenia phase before conditioning. The 2 patients whose disease relapsed or progressed before pre-transplantation cytoreduction were analyzed together with the 8 patients who failed to achieve remission (10 failures to chemotherapy).

Engraftment

Of the 49 patients, 2 died before engraftment, 2 had primary graft failure, and 45 engrafted. Two patients subsequently developed secondary graft failure (Table 4). The median time to neutrophil engraftment was 13 days (range 8-32). All 4 patients who developed primary or secondary graft failure had active disease pre-transplantation, and 3 had not received any chemotherapy before conditioning. All but one of the graft failure patients received ATG in the peri-transplantation period. Chimerism studies revealed a predominance

of host cells in each of the 3 cases that were adequately evaluated. Persistent or relapsed disease was thought to be the cause of graft failure in all 4 cases. One patient died before a second graft could be obtained. The other 3 patients received a second bone marrow (2 patients) or a peripheral blood stem cell(PBSC) transplantation (1 patient) after secondary conditioning with cyclophosphamide and ATG. However, all died of complications associated with pancytopenia within 44-68 days of documented graft failure.

Graft-versus-Host Disease

Of the 45 patients who engrafted and survived at least 28 days, 2 developed aGVHD (grades I and III, respectively) (Table 4). Another patient who received DLI (10^5 CD3 cells/kg) for acyclovir resistant Herpes simplex virus at 11 mos post-transplantation also developed acute GVHD (grade I). This is the only patient of 43 surviving at least 100 days who developed extensive cGVHD.

Relapse

Sixteen of the 49 patients had documented relapse of their MDS after transplantation. Five of these relapses occurred in 13 patients who did not receive any chemotherapy before conditioning. Relapses were also seen in 5 of the 10 patients who failed to respond to induction chemotherapy (8 patients) or had relapsed with MDS before conditioning (2 patients) (Table 4). Six of the 26 patients who either remained in complete remission (22 patients) or who had attained a second refractory cytopenia phase before conditioning (4 patients) also relapsed (Table 4). The cumulative incidence of relapse at 2 yrs for all patients was 31.6% (Figure 1). The cumulative incidence of relapse with respect to response to pre-conditioning chemotherapy was 38% among those not treated, 50% of the failures, and 23% among the responders to chemotherapy ($P=.248$)(Figure 1). All relapses occurred within 2 yrs of transplantation with a median time to relapse of 9.6 mos.

Relapse was treated with DLI in 4 patients; a second transplant in 3 patients; and supportive therapy in 9 patients. Two patients with a high blast count (>20%), and one patient with 10% blasts did not respond to DLI. One patient who had a cytogenetic relapse with no excess of blasts achieved full donor chimerism after DLI and remains in remission. Three patients underwent a second transplantation, 2 from the same donor and one from a second donor. Two of these transplants were T cell replete and one T cell-depleted. One patient undergoing a second transplantation died of complications associated with GVHD, and the 2 other patients died of relapsed MDS. Of the 16 patients with relapsed disease 2 are alive, one in remission and another one with disease.
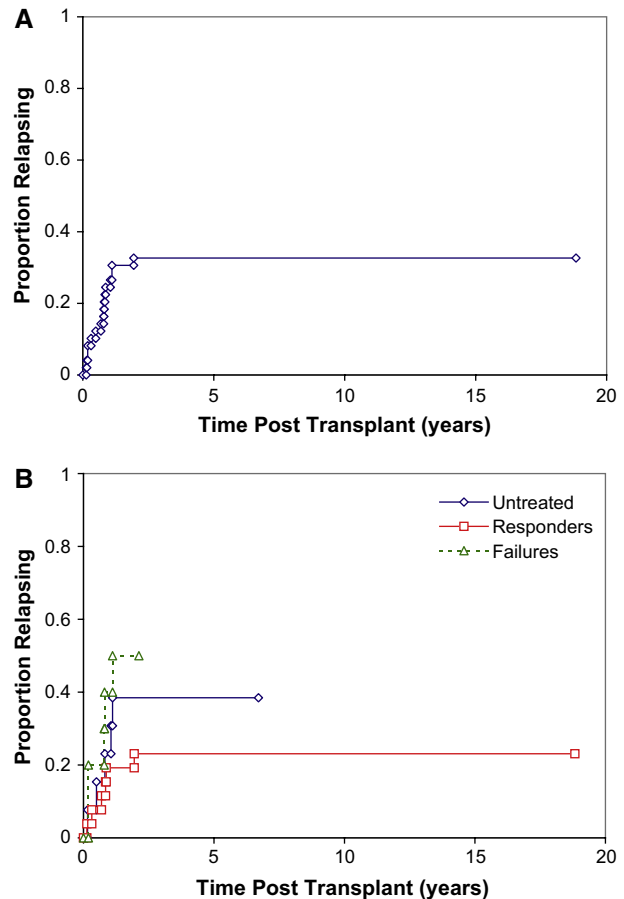

Figure 1. Cumulative incidence of relapse. A, all patients. B, by response to chemotherapy before conditioning.

In the 22 patients who were in complete remission prior to conditioning, cytogenetic risk category was correlated with relapse ($P<.001$). In contrast, WHO classification at diagnosis or at progression before chemotherapy, IPSS at diagnosis, number of induction courses, administration of consolidation, preparative regimen (TBI, thiotepa and cyclophosphamide versus TBI, thiotepa, and fludarabine), and CD34 cell dose did not correlate with relapse.

Survival, Disease-Free Survival, and Functional Status

Of the 49 patients, 16 are alive, 15 in remission, and 1 with relapsed MDS. The median follow-up for all patients is 22 mos (range 0.26-213) and for the surviving patients is 77 mos. The OS and DFS for all patients are shown in Figure 2. The OS and DFS at 3 yrs were 36.7% and 30.6%, respectively. All 15 surviving patients surviving more than 2 yrs have achieved a Karnofsky Performance Status (KPS) of ≥90.

Of the 13 patients not treated before conditioning, 3 are alive, one with relapsed MDS, 2 with no evidence of MDS, but one has recurrence of her Ewing Sarcoma. All of the 10 failures to chemotherapy have died. Of the 22 complete responders, 10 are alive
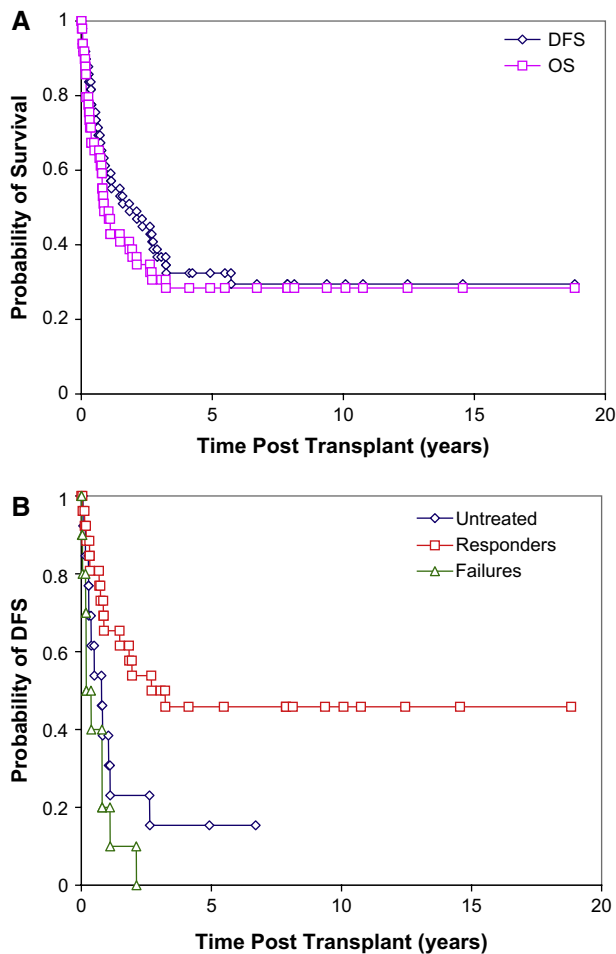
Figure 2. Probability of survival. A, Overall survival and disease-free survival (DFS) for all patients. B, DFS by response to chemotherapy before conditioning.

with no evidence of disease. Among the 4 patients who achieved a second refractory cytopenia phase, 3 are alive in remission, including one after DLI. The OS at 3 years was 54% for the responders, 31% for the untreated, and 0% for the failures ($P=.0004$). The DFS at 3 yrs was 45%, 15% and 0% respectively ($P=.0008$) (Figure 2). In multivariate analysis, disease status prior to conditioning remained highly correlated with DFS ($P<.001$) and there were no other factors that independently predicted DFS.

Comparison of the untreated patients versus the patients treated with chemotherapy before conditioning irrespective of response showed a DFS at 3 yrs of 15.4% for the 13 patients who did not receive chemotherapy and 33% for the 36 patients who received low or induction dose chemotherapy ($P=.18$).

Causes of Death and Non-Relapsed Related Mortality

Nineteen patients died of non-relapse causes and 14 of disease relapse (Table 4). Infectious complications were the main cause of NRM. Bacteria were
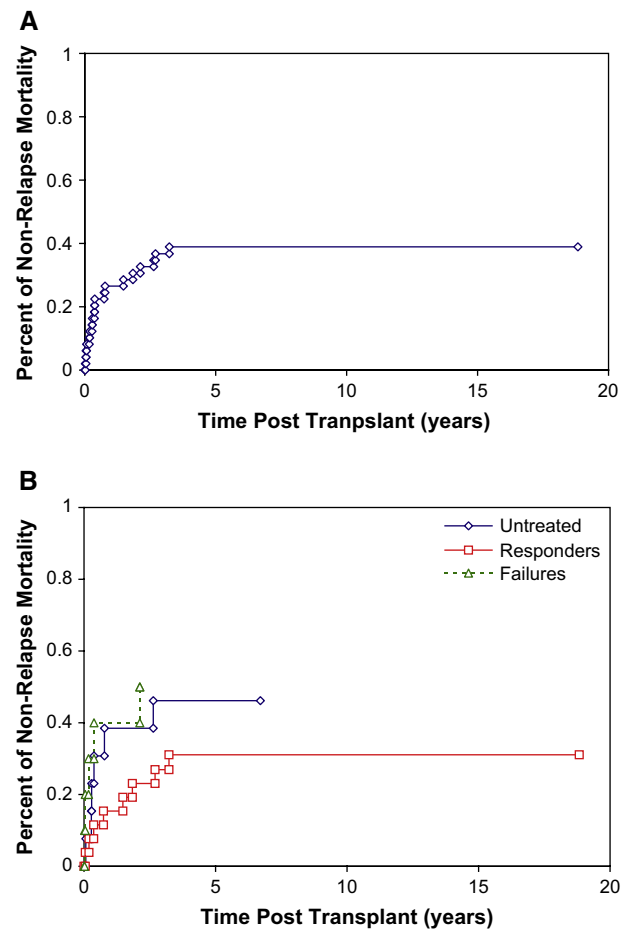
Figure 3. Cumulative incidence of non-relapse mortality. A, all patients. B, by response to chemotherapy before conditioning.

the most common cause of infections occurring before day 100 post-transplantation, whereas viral infections predominated thereafter. All patients who died of infections in the late post-transplantation period had poor immune function as defined by a low CD4 count and poor response to mitogens. Graft failure was only seen in the untreated patients who had active disease before transplantation. Graft-versus-host disease was the cause of death in only one patient who received DLI. Regimen-related deaths were rare, as only one patient developed hepatic veno-occlusive disease.

The cumulative incidence of NRM at 2 yrs for the whole group was 30%, with the lowest relapse rate in the group of patients in remission before cytoreduction. The cumulative incidence of NRM for the responders was 23%; for the untreated group was 38%; and for the failures to chemotherapy was 40% ($P=.252$) (Figure 3). None of the parameters examined was associated with an increased NRM.

DISCUSSION

The use of pre-transplantion chemotherapy and its impact on transplantation outcome is controversial in

unmodified myeloablative HCST for advanced MDS. Data from 2 multi-center European studies including patients with de novo and therapy-related MDS [13, 14] showed a benefit of achieving complete remission before transplantation, when compared with patients who had failed induction chemotherapy. In contrast, data from the Fred Hutchinson Cancer Center in Seattle [15] showed no benefit of induction chemotherapy when comparing the survival between treated (responders and failures together) and untreated patients. The RFS at 3 yrs in these groups was only 13% and 26% respectively. Relapse was nevertheless the main cause of death in the untreated group, 64% versus 21% in the treated group. The value of induction chemotherapy before unmodified myeloablative HSCT thus remains unclear. An ongoing multi-center prospective trial [38] will hopefully clarify the role of induction chemotherapy in this setting.

This relatively large, single-center series, with a median follow-up of 6 yrs for survivors shows that patients with advanced MDS can achieve a significant long-term DFS after a T cell-depleted myeloablative allogeneic HSCT. This success depends on the achievement of sustained remission or a second refractory cytopenia phase after receiving induction or low dose chemotherapy before transplantation conditioning. The survival benefit is largely due to a decrease in post-transplantation relapse as well as GVHD and graft failure. Similar observations were made in a small series of 15 patients with advanced MDS and AML post-MDS who received myeloablative T cell-depleted bone marrow grafts from HLA-matched siblings in remission after induction chemotherapy [37]. There are no other publications specifically reporting the results of myeloablative T cell-depleted transplantation in advanced MDS.

The relapse rate after T cell-depleted myeloablative transplantation in our MDS patients in remission or second refractory cytopenia phase is similar to that seen in recipients of unmodified myeloablative transplantation [4, 13, 14, 15, 39-43]. This suggests that myeloablative conditioning regimens may help eliminate residual MDS/leukemic clones. Also, donor-derived effector cells other than mature T cells contributing to GVHD may redevelop from precursors present in a T cell-depleted graft to provide enhanced resistance to the patient's disease. Candidates include donor NK cells that emerge early after T cell-depleted grafts [44, 45]. Similar observations and conclusions apply to patients with de novo AML in first and second remission treated with T cell-depleted transplants at our institution, who had relapse rates comparable to or better than those seen after unmodified myeloablative transplantation [19, 20]. On the other hand, the higher incidence of relapse in our untreated and induction failure patients suggests that the preparative regimen alone can not compensate for optimal disease control pre-

transplantation. A graft-versus-MDS effect is also operative in recipients of unmodified allografts, but there is much greater overlap with GVHD. The graft-versus-MDS effect has been well documented in large retrospective studies that showed a lower relapse rate in patients with GVHD following myeloablative [46] or non-myeloablative transplants [47-51]. It has been confirmed by the observation that patients treated with DLI for post-transplantation relapse can achieve remission again [52-55]. Our data suggest that T cell-depleted transplants should be limited to patients with advanced MDS whose disease is in remission or who have a low blast count, unless specific alloreactive donor cytotoxic cells are available to be given in the post-transplantation period. Leukemia associated antigens like WT1, which are highly expressed in MDS patients [56-59], are currently being used to generate leukemia specific T cells of donor origin [60-61]. These T cells may decrease the risk of relapse without increasing the risk of GVHD.

The most common causes of NRM in this series were infections and graft failure. The frequency of these complications was lower in the responder group. However, infectious complications occurring early (1 case) and particularly late (>3 mos) post-transplantation (5 cases) were the main cause of NRM in the responders and had a significant impact on survival. All patients who died of infections in the late post-transplantation period had quantitative and qualitative T cell deficiencies. Slow or poor T cell reconstitution following T cell-depleted transplantation is due in part to the administration of ATG for rejection prophylaxis and to the recipient's age, as described by Small et al [28]. All 5 patients who died of late infection were ≥50 yrs old. Elimination of ATG in our most recent trial has resulted in substantial improvement of immune recovery post-transplantation [62]. However, for patients ≥50 yrs, numerical and functional T cell recovery is still delayed, likely reflecting age-associated impairments to the recovery of thymopoiesis. Newer approaches such as administration of interleukin 7 (IL-7) or keratinocyte growth factor (KGF), which stimulate thymopoiesis and T cell reconstitution in experimental animals [63-70], are currently being tested for their capacity to accelerate immune recovery in older patients who have undergone allogeneic HSCT.

Graft failure was a notable non-relapse complication in the untreated group despite prophylaxis with ATG. Most of these patients had a high disease burden with high blast counts before transplantation. The most likely mechanism was persistent disease, but this could not be proved in the absence of autopsies. Graft failure attributed to persistent disease has also been noted after unmodified myeloablative HSCT for advanced MDS [4].

In contrast to unmodified HSCT, the incidence of acute and chronic GVHD in this series was

extremely low. Three patients developed aGVHD, and only one patient developed chronic GVHD after DLI. Moreover, GVHD was the cause of non-relapse death in only 2 patients. This is very similar to our experience in patients with de novo AML in first and second remission [20]. Acute and chronic GvHD remain the main cause of morbidity and mortality after unmodified transplantation following myeloablative [3, 4, 6, 7, 9-15] or reduced-intensity conditioning [43, 47-51], particularly in older patients, who constitute the majority of patients with MDS. There has been a great deal of interest in the use of non-myeloablative or reduced intensity transplantation in older patients with MDS, because of the reduction in aGVHD and early NRM[49-51]. The incidence of cGVHD and late transplantation-related mortality, however, has been comparable to that seen in myeloablative transplantation. Moreover, disease relapse in patients either in remission or with increased blasts has also been higher than that observed after myeloablative conditioning and either T cell replete transplantation [43, 48, 49, 51] or as shown in this series, T cell-depleted grafts. While the addition of antithymocyte globulin [71, 72] or alemtuzumab [73-75] to reduced-intensity preparative regimens has decreased the incidence of GVHD, the rate of post-transplantation relapse in patients with advanced MDS has remained high. This suggests that the intensity of the preparative regimen is important in preventing post-transplantation relapse in these patients. Our T cell-depleted myeloablative transplantation approach offers to patients with advanced MDS in remission and without prohibitive co-morbidities the possibility of durable remission with minimal risk of GVHD. The use of newer approaches to improve immune reconstitution in these patients should further improve the overall and disease-free survival.

In summary, we show that patients with advanced MDS can achieve a significant long- term DFS after T cell-depleted allogeneic HSCT. Such success depends on successful chemotherapy achieving either sustained remission or a second refractory cytopenia phase before undergoing cytoreduction for transplantation. This approach markedly reduces 2 major obstacles limiting the success of allogenic transplantation, disease relapse and GVHD. Infections were the most common cause of non-relapse mortality especially in older patients. Thus, improved prophylaxis, or new agents that enhance recovery of immune function may improve the survival of patients with advanced MDS after T cell-depleted HSCT. Finally, demonstration of the superiority of this approach over other types of transplantation will require prospective trials comparing this type of transplantation to unmodified myeloablative or non-myeloablative allogeneic HSCT.

REFERENCES

1. Appelbaum FR, Storb R, Ramberg RE, et al. Allogeneic marrow transplantation in the treatment of preleukemia. *Ann Intern Medic*. 1984;100:689-693.

2. Appelbaum FR, Storb R, Ramberg RE, et al. Treatment of pre-leukemic syndromes with marrow transplantation. *Blood*. 1987; 69:92-96.

3. Anderson JE, Appelbaum FR, Fisher LD, et al. Allogeneic bone marrow transplantation for 93 patients with myelodysplastic syndrome. *Blood*. 1993;82:677-681.

4. Sierra J, Perez WS, Rozman C, et al. Bone marrow transplantation from HLA-identical siblings as treatment for myelodysplasia. *Blood*. 2002;100:1997-2004.

5. Appelbaum FR, Anderson J. Allogeneic bone marrow transplantation for myelodysplastic syndrome: outcome analysis according to IPSS score. *Leukemia*. 1998;(Suppl. 1):S25-S29.

6. Sutton L, Chastang C, Ribaud P, et al. Factors influencing outcome in *de novo* myelodysplastic syndromes treated by allogeneic bone marrow transplantation: a long term study of 71 patients. *Blood*. 1996;88:358-365.

7. Runde V, De Witte T, Gratwohl A, et al. Bone marrow transplantation from HLA-identical siblings as first line treatment in patients with myelodysplastic syndromes: early transplantation is associated with improved outcome. *Bone Marrow Transplant*. 1998;21:255-261.

8. Anderson JE, Appelbaum FR, Schoch G, et al. Allogeneic marrow transplantation for refractory anemia: a comparison of two preparative regimen and analysis of prognostic factors. *Blood*. 1996;87:51-58.

9. Anderson JE, Appelbaum FR, Schoch G, et al. Allogeneic marrow transplantation for myelodysplastic syndromes with advanced morphology: a phase II study of busulfan, cyclophosphamide and total body irradiation and analysis of prognostic factors. *J Clin Oncol*. 1996;14:220-226.

10. Jurado M, Deeg HJ, Storer B, et al. Hematopoietic stem cell transplantation for advanced myelodysplastic syndrome after conditioning with busulfan and total body irradiation is associated with low relapse mortality but considerable non-relapse mortality. *Biol Blood Marrow Transplant*. 2002;8:161-169.

11. Demuynck H, Verhoef GEG, Zachee P, et al. Treatment of patients with myelodysplastic syndromes with allogeneic bone marrow transplantation from genotypically HLA identical siblings and alternative donors. *Bone Marrow Transplant*. 1996;17: 745-751.

12. de Witte T, Hermans J, Vossen J, et al. Haematopoietic stem cell transplantation for patients with myelodysplastic syndromes and secondary acute myeloid leukemias: a report on behalf of the Chronic Leukemia Working Party of the European Group for Blood and Marrow Transplantation (EBMT). *Br J Haematol*. 2000;110:620-630.

13. de Witte T, Suciu S, Verhoef G. Intensive chemotherapy followed by allogeneic or autologous stem cell transplantation for patients with myelodysplastic syndromes (MDS) and acute leukemia following MDS. *Blood*. 2001;98:2326-2331.

14. Yakoub-Agha I, De la Salmoniere P, Ribaud P, et al. Allogeneic bone marrow transplantation for therapy-related myelodysplastic syndrome and acute leukemia: a long-term study of 70 patients. Report of the French Society of Bone Marrow Transplantation. *J Clin Oncol*. 2000;18:963-971.

15. Scott BL, Storer B, Loken MR, et al. Pretransplantation induction chemotherapy and posttransplantation relapse in patients

with advanced myelodysplastic syndrome. *Biol Blood Marrow Transplant*. 2005;11:65-73.

16. Kernan NA, Bordignon C, Heller G, et al. Graft failure after T cell-depleted human leukocyte antigen identical marrow transplants for leukemia. I. Analysis of factors and results of secondary transplants. *Blood*. 1989;74:2227-2236.

17. Bordignon C, Keever CA, Small TN, et al. Graft failure after T cell depleted human leukocyte antigen identical marrow transplants for leukemia. II. In vitro analysis of host effector mechanisms. *Blood*. 1989;74:2237-2243.

18. Mackinnon S, Papadopoulos EB, Carabasi MH, et al. Adoptive immunotherapy evaluating escalating doses of donor leukocytes for relapse of chronic myeloid leukemia after bone marrow transplantation: separation of graft-vs-leukemia from graft-vs-host disease. *Blood*. 1995;86:1261-1268.

19. Young JW, Papadopoulos EB, Cunningham I, et al. T cell depleted allogeneic bone marrow transplantation in adults with acute nonlymphocytic leukemia in first remission. *Blood*. 1992;91:1083-1090.

20. Papadopoulos EB, Carabasi MH, Castro-Malaspina H, et al. T cell depleted allogeneic bone marrow transplantation as postremission therapy for acute myelogenous leukemia: freedom from relapse in the absence of graft-versus-host disease. *Blood*. 1998;91:1083-1090.

21. Benneth JM. World Health Organization classification of acute leukemias and myelodysplastic syndromes. *Int J Hematol*. 2000;72:131-133.

22. Greenberg P, Cox C, LeBeau MM, et al. International scoring system for evaluating prognosis in myelodysplastic syndromes. *Blood*. 1997;89:2079-2088.

23. Cheson BD, Bennett JM, Kopecky KJ, et al. Revised recommendations of the international working group for diagnosis, standardization of response criteria, treatment outcome, and reporting standards for therapeutic trials in acute myeloid leukemia. *J Clin Oncol*. 2003;21:4642-4649.

24. Cheson BD, Greenberg PL, Bennett JM, et al. Clinical application and proposal for modification of the International Working Group (IWG) response criteria in myelodysplasia. *Blood*. 2006;108:419-425.

25. Shank B, Chu FCH, Dinsmore R, et al. Hyperfractionated total body irradiation for bone marrow transplantation. Results in seventy leukemia patients with allogeneic transplants. *Int J Radiat Oncol Biol Phys*. 1983;9:1607-1611.

26. Collins NH, Bleau SA, Kernan NA, O'Reilly RJ. T cell depletion of bone marrow by treatment with soybean agglutinin and sheep red blood cell rossetting. In: Areman HJ, Deeg HJ, Sacher RA, editors. Bone marrow and stem cell processing: a manual of current techniques. Philadelphia, PA: FA Davis; 1992. p. 171.

27. Collins NH, Fernandez JM, Bleau S, et al. Comparison of bone marrow and G-CSF mobilized peripheral blood progenitors from single normal donors before and after T cell depletion. *Cytotherapy*. 1999;1:223.

28. Small TN, Avigan D, Dupont B, et al. Immune reconstitution following T cell depleted bone marrow transplantation: effect of age and posttransplant graft rejection prophylaxis. *Biol Blood Marrow Transplant*. 1997;3:65-75.

29. Rowlings PA, Przepiorka D, Klein JP, et al. IBMTR severity index for grading acute graft-versus-host disease: retrospective comparison with Glucksberg grade. *Br J Haematol*. 1997;97:855-864.

30. MacMillan ML, Weisdorf DJ, Wagner JE, et al. Response of 443 patients to steroids as primary therapy for acute graft-

versus-host disease: comparison of grading systems. *Biol Blood Marrow Transplant*. 2002;8:387-394.

31. Shulman HM, Sullivan KM, Weiden PL, et al. Chronic graft-versus-host disease syndrome in man. A long-term clinico-pathologic study of 20 Seattle patients. *Am J Med*. 1980;69:204-217.

32. Kaplan EL, Meier P. Non-parametric estimation from incomplete observations. *J Am Stat Assoc*. 1958;53:457-481.

33. Gooley TA, Leisenring W, Crowley J, et al. Estimation of failure probabilities in the presence of competing risks: new representations of old estimators. *Stat Med*. 1999;18:695-706.

34. Mantel N. Evaluation of survival data and two new rank order statistics arising in its consideration. *Cancer Chemother Rep*. 1966;50:163-170.

35. Cox DR. Regression models and life table analysis (with discussions), Series B. *J Royal Stat Soc B*. 1972;34:187-220.

36. Gray RJ. A class of k-sample tests for comparing the cumulative incidence of a competing risk. *The Annals of Statistics*. 1988;16:1141-1154.

37. Mattijssen V, Schattenberg A, Schaap N, et al. Outcome of allogeneic bone marrow transplantation with lymphocyte depleted marrow grafts in adult patients with myelodysplastic syndromes. *Bone Marrow Transplant*. 1997;19:791-794.

38. de Witte T, Oosterveld M, Muus P. Autologous and allogeneic stem cell transplantation for myelodysplastic syndromes. *Blood Reviews*. 2006;21:49-59.

39. Deeg HJ, Shulman M, Anderson JE, et al. allogenic and syngeneic marrow transplantation for myelodysplastic syndromes in patients 55 to 66 years old. *Blood*. 2000;95:1188-1194.

40. Guardiola P, Runde V, Bacigalupo A, et al. Retrospective comparison of bone marrow and granulocyte colony stimulating factor mobilized peripheral blood progenitor cells for allogeneic stem cell transplantation using HLA-identical sibling donors in myelodysplastic syndromes. *Blood*. 2002;99:4370-4378.

41. de Lima M, Couriel D, Thall PF, et al. Once-daily intravenous busulfan and fludarabine: clinical and pharmacokinetic results of a myeloablative, reduced toxicity conditioning regimen for allogeneic stem cell transplantation in AML and MDS. *Blood*. 2004;104:857-864.

42. Solomon SR, Savani BN, Childs R, et al. Improved outcome for peripheral blood stem cell transplantation for advanced primary myelodysplastic syndrome. *Biol Blood Marrow Transplant*. 2005;11:619-626.

43. Alyea EP, kim HT, Ho V, et al. Impact of conditioning regimen intensity on outcome of allogeneic hematopoietic cell transplantation for advanced acute myelogenous leukemia and myelodysplastic syndrome. *Biol Blood Marrow Transplant*. 2006;12:1047-1055.

44. Ruggeri L, Capanni M, Casucci M, et al. Role of natural killer cell alloreactivity in HLA-mismatched hematopoietic stem cell transplantation. *Blood*. 1999;94:333-339.

45. Hsu KC, Keever-Taylor CA, Wilson A, et al. Improved outcome in HLA-identical sibling hematopoietic stem cell transplantation for acute myelogenous leukemia predicted by KIR and HLA genotypes. *Blood*. 2005;105:4878-4884.

46. Castro-Malaspina H, Harris RH, Gajewski J. Unrelated donor marrow transplantation for myelodysplastic syndromes: outcome analysis of 510 transplants facilitated by the National Marrow Donor Program. *Blood*. 2002;99:1943-1951.

47. Martino R, Caballero MD, Perez Simon JA, et al. Evidence for a graft-versus-leukemia effect after allogeneic peripheral blood stem cell transplantation with reduced intensity conditioning

in acute myelogenous leukemia and myelodysplastic syndromes. *Blood*. 2002;100:2243-2245.

48. de Lima M, Anagnostopoulos A, Munsell M, et al. Non-ablative versus reduced-intensity conditioning regimens in the treatment of acute myeloid leukemia and high risk myelodysplastic syndrome: dose is relevant for long-term disease control after allogeneic hematopoietic stem cell transplantation. *Blood*. 2004;104: 865-872.

49. Shimoni A, Hardan I, Shem-Tov N, et al. Allogeneic hematopoietic stem transplantation in AML and MDS using myeloablative versus reduced intensity conditioning: the role of dose intensity. *Leukemia*. 2006;20:322-328.

50. Scott BL, Sandmaier BM, Storer B, et al. Myeloablative vs non-myeloablative allogeneic transplantation for patients with myelodysplastic syndrome and acute myelogenous leukemia with multilineage dysplasia: a retrospective analysis. *Leukemia*. 2006;20:128-135.

51. Martino R, Iacobelli S, Brand R, et al. Retrospective comparison of reduced-intensity conditioning and conventional high-dose conditioning for allogeneic hematopoietic stem cell transplantation using HLA-identical sibling donors in myelodysplastic syndromes. *Blood*. 2006;108:836-846.

52. Kolb HJ, Schattenberg A, Goldman JM, et al. Graft-versus-leukemia effect of donor lymphocyte transfusions in marrow grafted patients. *Blood*. 1995;86:2041-2050.

53. Porter DL, Roth MS, Lee SJ. Adoptive immunotherapy with donor mononuclear cells infusions to treat relapse of acute leukemia or myelodysplasia after allogeneic bone marrow transplantation. *Bone Marrow Transplant*. 1996;18:975-980.

54. Collins RH, Shpilberg O, Drobyski WR, et al. Donor leukocyte infusions in 140 patients with relapsed malignancy after allogeneic bone marrow transplantation. *J Clin Oncol*. 1997;15: 433-444.

55. Depil S, Deconinck E, Milpied N, et al. Donor lymphocyte infusion to treat relapse after allogeneic bone marrow transplantation for myelodysplastic syndrome. *Bone Marrow Transplant*. 2004;33:531-534.

56. Inoue K, Sugiyama H, Ogawa H, et al. WT1 as a new prognostic factor and a new marker for the detection of minimal residual disease in acute leukemia. *Blood*. 1994;84:3071-3079.

57. Bergmann L, Miething C, Maurer U, et al. High levels of Wilms' tumor gene (WT1) mRNA in acute myeloid leukemias are associated with a worse long term treatment. *Blood*. 1997; 90:1217-1225.

58. Patmasirivat P, Frazier G, Kantarjian H, et al. WT1 and GATA1 expression in myelodysplastic syndrome and acute leukemia. *Leukemia*. 1999;891-900.

59. van Dyjk JP, Knops GHJ, van de Locht LTF, et al. Abnormal WT1 expression in the CD-34 negative compartment in myelodysplastic bone marrow. *Brit J Haematol*. 2002;118:1027-1033.

60. Oka Y, Elisseeva OA, Tsboi A, et al. Human cytotoxic T-lymphocyte responses specific for peptides of the wild-type Wilms tumor gene (WT1) product. *Immunogenetics*. 2000;51:99-107.

61. Oka Y, Tsboi A, Taguchi T, et al. Induction of WT1 (Wilms tumor gene)-specific cytotoxic T lymphocytes by WT1 peptide vaccine and the resulting cancer regression. *Proc Nat Acad Sci*. 2004;101:13885-13890.

62. Jakubowski AA, Small TN, Young J, et al. Sustained engraftment and improved reconstitution: results of HLA-matched related, T cell depleted peripheral blood stem cell transplantation for adults with hematologic malignancies without the use of anti-thymocyte globulin. *Blood*. 2007;110:4552-4559.

63. Bolotin E, Smogorzewska M, Smith S, et al. Enhancement of thymopoiesis after bone marrow transplant by in vivo interleukin-7. *Blood*. 1999;88:1887-1894.

64. Fry TJ, Christensen BL, Komschlks KL, et al. Interleukin 7 restores immunity in athymic T cell depleted hosts. *Blood*. 2001;97: 1525-1533.

65. Mackall CL, Fry TJ, Bare G, et al. IL-& increases both thymic-dependent and thymic-independent T cell regeneration after bone marrow transplantation. *Blood*. 2001;97:1491-1497.

66. Alpdogan O, Schamaltaz C, Muriglan SJ, et al. Administration of interleukin-7 after allogeneic bone marrow transplantation improves immune reconstitution without aggravating graft-versus-host disease. *Blood*. 2001;98:2256-2265.

67. Alpdogan O, Muriglan SJ, Eng JM, et al. IL-7 enhances peripheral T cell reconstitution after allogeneic hematopoietic stem cell transplantation. *J Clin Invest*. 2003;112:1095-1107.

68. Min D, Taylor PA, Panoskaltsis-Mortari A, et al. Protection from thymic epithelial cell injury by keratinocyte growth factor: a new approach to improve thymic and peripheral T cell reconstitution after bone marrow transplantation. *Blood*. 2002;99: 4592-4600.

69. Rossi S, Blazar BR, Farrell CL, et al. Keratonocyte growth factor preserves normal thymopoieis and thymic microenvironment during experimental graft-verus-host disease. *Blood*. 2002;100: 682-691.

70. Alpdogan O, Hubbard VM, Smith OM, et al. Keratinocyte growth factor (KGF) is required for postnatal thymic regeneration. *Blood*. 2006;107:2453-2460.

71. Kroger N, Bornhauser M, Ehninger G, et al. Allogeneic stem cell transplantation after fludarabine/busulfan based reduced intensity conditioning in patients with myelodysplastic syndrome or secondary acute myeloid leukemia. *Ann Hematol*. 2003;82: 336-342.

72. Kroger N, Shimoni A, Zabelina T, et al. Reduced-toxicity conditioning with treosulfan, fludarabine and ATG as preparative regimen for allogenic stem cel transplantation in elderly patients with secondary acute leukemia or myelodysplastic syndrome. *Bone Marrow Transplant*. 2006;37:339-344.

73. Ho AY, Pagliuca A, Kenyon M, et al. Reduced-intensity allogeneic hematopoietic stem cell transplantation for myelodysplastic syndrome and acute myeloid leukemia with multilineage dysplasia using fludarabine, busulfan and alemtuzumab conditioning. *Blood*. 2004;104:1616-1623.

74. van Biesen K, Artz A, Smith S, et al. Fludarabine, melphalan and alemtuzumab conditioning in adults with standard-risk advanced myeloid leukemia and myelodysplastic syndrome. *J Clin Oncol*. 2005;23:5728-5738.

75. Tauro S, Craddock C, Peggs K, et al. Allogeneic stem cell transplantation using a reduced intensity conditioning regimen that has the capacity to produce durable remission and long term disease free survival in patients with high risk acute myeloid leukemia and myelodysplasia. *J Clin Oncol*. 2005;23:9387-9393.