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Allogeneic hematopoietic cell transplantation with non-myeloablative conditioning for patients with hematologic malignancies: improved outcomes over two decades

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ABSTRACT

We have used a non-myeloablative conditioning regimen for allogeneic hematopoietic cell transplantation for the past 20 years. During that period, changes in clinical practice have been aimed at reducing morbidity and mortality from infections, organ toxicity, and graft-*versus*-host disease. We hypothesized that improvements in clinical practice led to better transplantation outcomes over time. From 1997–2017, 1,720 patients with hematologic malignancies received low-dose total body irradiation ± fludarabine or clofarabine before transplantation from HLA-matched sibling or unrelated donors, followed by mycophenolate mofetil and a calcineurin inhibitor ± sirolimus. We compared outcomes in three cohorts by year of transplantation: 1997–2003 (n=562), 2004–2009 (n =594), and 2010–2017 (n=564). The proportion of patients ≥60 years old increased from 27% in 1997–2003 to 56% in 2010–2017, and with scores from the Hematopoietic Cell Transplantation Comorbidity Index of ≥3 increased from 25% in 1997–2003 to 45% in 2010–2017. Use of unrelated donors increased from 34% in 1997–2003 to 65% in 2010–2017. When outcomes from 2004–2009 and 2010–2017 were compared to 1997–2003, improvements were noted in overall survival (*P*=0.0001 for 2004–2009 and *P*≤0.0001 for 2010–2017), progression-free survival (*P*=0.002 for 2004–2009 and *P*<0.0001 for 2010–2017), non-relapse mortality (*P*<0.0001 for 2004–2009 and *P*<0.0001 for 2010–2017), and in rates of grades 2–4 acute and chronic graft-*versus*-host disease. For patients with hematologic malignancies who underwent transplantation with non-myeloablative conditioning, outcomes have improved during the past two decades. Trials reported are registered under clinicaltrials.gov. Identifiers: *NCT00003145,*

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Introduction

In 1997 we introduced a minimally intensive conditioning regimen for allogeneic hematopoietic cell transplantation (HCT) that enabled treating elderly and medically infirm younger patients with advanced hematologic malignancies in the outpatient setting. We previously reported outcomes for patients who underwent HCT from 1997–2009 using this regimen, which included low-dose total body irradiation (TBI) with or without fludarabine.[1] Five-year overall survival ranged from 25% to 60% (depending on disease type, comorbidities, and graft-*versus*-host disease [GvHD]), non-relapse mortality (NRM) was 24%, and relapse-related mortality was 35%. The most significant contributor to NRM was GvHD.

Between 1997 and 2017 a number of changes in clinical practice were introduced that were aimed at improving HCT outcomes including reductions in the use of systemic glucocorticoids as treatment for acute GvHD,[2,3] use of ursodiol to reduce hepatic complications,[4,5] addition of sirolimus for control of GvHD,[6-8] use of fluoroquinolones for antibacterial prophylaxis during periods of neutropenia,[9-11] use of more mold-active azoles for antifungal prophylaxis,[12,13] and initiation of pre-emptive antiviral therapy based on more sensitive polymerase chain reaction (PCR)-based cytomegalovirus (CMV) diagnostic testing.[14-16]

During the same time period, patient and donor characteristics as well as indications for HCT also changed. The proportion of patients older than 60 years increased from 27% to 56%, proportion of patients with hematopoietic cell transplantation-comorbidity index (HCT-CI)[17] scores ≥3 increased from 25% to 45%, use of unrelated donors increased from 35% to 65%, and increasing numbers of patients underwent HCT for acute myeloid leukemia (AML) while decreasing numbers of patients underwent HCT for multiple myeloma and chronic myelogenous leukemia (CML). The current study analyzed whether benefits associated with the changes in clinical care for patients undergoing HCT outweighed the adverse outcomes expected from older patient age, increased comorbidities, and greater use of unrelated donors. To that end, we compared outcomes in three cohorts of patients by year of HCT: 1997–2003, 2004– 2009, and 2010–2017.

Methods

Patients

Between December 16, 1997 and June 30, 2017, 1,720 consecutive patients with hematologic malignancies underwent HCT at the Fred Hutchinson Cancer Research Center (FHCRC) or collaborating centers. We included patients who were entered onto prospective clinical trials registered with clinicaltrials.gov at both FHCRC and collaborating centers (Online Supplementary Table S1), and patients transplanted outside of prospective trials at FHCRC. This study was approved by the Institutional Review Board (IRB) at FHCRC and all patients signed IRB-approved consents.

Graft source, conditioning, and post-engraftment immunosuppression

All patients received unmodified grafts consisting of granulocyte colony-stimulating factor (GCSF)-mobilized peripheral blood stem cells (PBSC). Donors and recipients were matched at HLA-A, -B, -C, -DRB1, and -DQB1 by high-resolution typing except for 104 unrelated donor-recipient pairs who were mismatched at the level of one HLA class I allele. Conditioning regimens and immunosuppression to aid engraftment and control GvHD are summarized in the *Online Supplementary Table S1*.

Clinical endpoints

Patients had bone marrow aspirations to assess disease status on days 28, 84, and 365 after HCT, and otherwise as clinically indicated. Acute and chronic GvHD were diagnosed and graded as previously described.[18,19] Relapse was defined as recurrence of malignancy based on imaging, marrow morphology, flow cytometry, cytogenetics, and/or disease-specific molecular markers. Progression was defined as ≥50% increase in disease burden.[20] Relapse-related mortality included deaths after relapse or progression of disease present before HCT, regardless of other events. NRM included deaths in the absence of relapse or progression.

Clinical assessment of organ complications and infections through day 100

Liver injury was assessed according to peak bilirubin concentration.[19] Acute kidney injury was defined as a serum creatinine concentration that was at least 2-fold higher than the baseline value.[21] CMV infection was defined as the presence of pp65 antigen or DNA in plasma,[22] and CMV disease was defined as dysfunction of an organ infected by CMV.[23] One or more positive blood cultures for gram-negative bacteria were defined as gram-negative bacteremia; gram-negative organisms tend to cause the most serious infections in patients who are neutropenic after HCT.[24] Invasive fungal infections were defined according to consensus criteria and included cases deemed proven or probable.[25]

Statistical analysis

Overall survival (OS) and progression-free survival (PFS) were estimated using the Kaplan-Meier method. Rates of acute and chronic GvHD, relapse or progression, and NRM were estimated according to standard methods.[26] Death was treated as a competing risk factor for all other time-to-event endpoints. Relapse was treated as a competing risk for NRM. Relapse-related mortality refers to survival after relapse among patients that relapsed. Multivariate Cox regression analysis of cause-specific hazards, stratified by center, was used for adjusted comparisons between groups defined by year of HCT. Adjusted estimates of survival and cumulative incidence were based on methods previously described.[27] Briefly, the adjusted curves represent the hypothetical outcomes for the three transplant eras if each era had the patient characteristics of the first era, based on results from the adjusted Cox regression models. Multivariate logistic regression was used for adjusted comparisons of rates of elevated bilirubin and creatinine. The adjusted models included the following variables that varied over era of HCT and were potentially related to one or more endpoints: treatment type (on-protocol, off-protocol); age (≤49, 50–59, or ≥60 years); disease relapse risk (low, standard,

high); multiple myeloma diagnosis; AML diagnosis; CMV serostatus (recipient and donor negative, recipient and/or donor positive); donor relation (related, unrelated); sex mismatch (female to male, others); prior HCT (no, yes); HLA-allele mismatch (no, yes); HCT-CI (0, 1–2, 3, ≥4, or missing). All *P*-values were two-sided and are unadjusted for multiple comparisons.

Results

Study population

Our study population of 1,720 total patients in three tertiles is summarized in Table 1 and consisted of 562 patients who underwent HCT during the period from

Table 1. Transplant and patient characteristics by transplant era.

	1997–2003 (n = 562)		2004–2009 (n = 594)		2010–2017 (n = 564)		P
	n	%	n	%	n	%	
Transplant characteristics							
Conditioning regimen[a]							
TBI 2 Gy	127	33	99	17	53	9	
TBI 2 Gy + fludarabine	435	77	454	76	283	50	
TBI 3 Gy + fludarabine			37	6	173	31	
TBI 4 − 4.5 Gy + fludarabine			4	1	16	3	
TBI 2 Gy + clofarabine					40	7	
GVHD prophylaxis[b]							
CI + MMF	562	100	529	89	451	80	
CI + MMF + sirolimus			65	11	113	20	
Patient characteristics							
Center / treatment[c]							
Non-FH on-protocol	287	51	208	35	130	23	< 0.0001
FH on-protocol	264	47	327	55	333	59	
FH off-protocol	11	2	59	10	101	18	
Diagnosis							
ALL	17	3	30	5	45	8	< 0.0001
AML	90	16	184	31	167	30	
CLL	62	11	65	11	73	13	
CML	39	7	12	2	6	1	
HL	34	6	30	5	17	3	
MDS / MPN	84	15	71	12	90	16	
MM	129	22	95	16	56	10	
NHL	101	18	101	17	107	19	
WM	6	1	6	1	2	< 1	
Donor[d]							
Related	371	66	249	42	197	35	< 0.0001
Unrelated	191	34	345	58	367	65	
Disease risk group[e]							
Low	129	23	131	22	169	30	0.002
Standard	270	48	297	50	276	49	
High	163	29	166	28	118	21	
Age, years							
≤ 49	174	31	131	22	102	18	< 0.0001
50 − 59	236	42	208	35	147	26	
≥ 60	152	27	255	43	316	56	
Sex match							
Female to male	169	30	137	23	141	25	0.04
Others	393	70	457	77	423	75	
CMV serostatus							
R− / D−	129	23	172	29	169	30	0.02
R+ / D−	141	25	172	29	158	28	
R+ / D+	84	15	73	12	68	12	
R− / D+	208	37	177	30	169	30	
HCT-CI[f]							
0	118	21	89	15	56	10	< 0.0001
1,2	129	23	137	23	152	27	
3	73	13	131	22	113	20	
≥ 4	67	12	125	21	141	25	
Missing	174	31	113	19	102	18	
HLA-allele mismatch[g]							
No	534	95	552	93	530	94	0.72
Yes	28	5	42	7	34	6	
Prior HCT[h]							
No	427	76	416	70	433	77	0.008
Yes	135	24	179	30	131	23	

1997–2003, 594 from 2004–2009, and 564 from 2010–2017. An increasing number and proportion of patients underwent HCT at FHCRC over time (*P*<0.0001) on protocols considered standard-of-care. The proportion of patients age ≥60 years at the time of HCT increased, as did the proportion of patients with an HCT-CI score ≥3. Unrelated donors were utilized more frequently over time. The distribution of diagnoses changed over time (*P*<0.0001), most notably with an increase in the proportion of patients with AML (16% during 1997–2003 and 30% during 2010–2017) and decreases in CML and multiple myeloma.

The distribution of disease relapse risk groups 20 changed over time (*P*<0.0001), with a decreasing proportion of patients with high-risk disease and an increasing proportion with low-risk disease.

A minority of patients had a prior HCT, but the types of prior HCT changed over time. The number of patients who had a prior planned autologous HCT decreased from 107 (19%) during 1997–2003 to one during 2010–2017, while the number of patients who had unsuccessful prior autologous or allogeneic HCT increased (Table 1). Planned autologous HCT were typically performed in conjunction with a tandem allogeneic HCT for patients with multiple myeloma.

Major endpoints by era of transplant

Associations of major endpoints with the time period of HCT are summarized in Table 2 and Figures 1 and 2. As described in the Methods section, results were adjusted for risk factors that varied over the three time periods and were potentially related to one or more endpoints of interest. OS, PFS, and NRM all had a significant association with the era of HCT. When compared to 1997–2003, significant improvements were noted during 2004–2009 and 2010–2017 for OS (hazard ratio [HR] 0.72, *P*=0.0001 and HR 0.60, *P*<0.0001), PFS (HR 0.78, *P*=0.002 and HR 0.63, *P*<0.0001), and NRM (HR 0.58, *P*<0.0001 and HR 0.52, *P*<0.0001). The risk of relapse or progression was lower during 2010–2017 when compared to 1997–2003 (HR 0.71, *P*=0.006). The incidence of relapse-related mortality trended toward improvement in later time periods, but the differences from 1997–003 were not statistically significant.

Rates of grades 2–4 acute GvHD, grades 3–4 acute GvHD, and chronic GvHD were all significantly associated with time period of HCT and, when compared to 1997–2003, all improved significantly during 2004–2009 and 2010–2017. Given we increasingly used 'triple immunosuppression' consisting of MMF, a calcineurin inhibitor, and sirolimus for patients undergoing HCT from unrelated donors,[6,8] we evaluated its impact on acute GvHD separately. Adding sirolimus reduced the rates of grades 2–4 and 3–4 acute GvHD when compared to patients who received MMF and a calcineurin inhibitor without sirolimus (HR 0.52, 95% CI: 0.40–0.67, *P*<0.0001 and HR 0.54, 95% CI: 0.29–1.01, *P*=0.05, respectively).

Association of relapse or progression with era of transplant in patients with acute myeloid leukemia in remission

Since the overall rate of relapse or progression was significantly decreased in the most recent time period, we evaluated whether this was also true for the 351 patients with AML in first or second complete remission (CR1/CR2): 64 during 1997–2003, 147 during 2004–2009, and 140 during 2010–2017. Among these patients, measurable (minimal) residual disease (MRD) at the time of HCT as determined by flow cytometry, cytogenetics, or molecular analysis was detected in 16 patients (11%) during 2004–2009 and 32 patients (23%) during 2010–2017. Data were unavailable prior to 2004. We found no statistically significant change in the rate of relapse for patients with AML in CR1/CR2 over time. When compared to 1997–2003, the adjusted HR for relapse during 2004–2009 was 0.84 (95% CI: 0.4–1.6, *P*=0.61) and during 2010–2017 was 0.89 (95% CI: 0.4–1.8, *P*=0.75).

Associations of organ complications and infections with era of transplant

Associations of liver and kidney injuries, gram-negative bacteremia, invasive fungal infections, and CMV infection with era of HCT are shown in Table 3. Compared to 1997–2003, the incidences of patients having liver or kidney complications, gram-negative bacteremia, or an invasive fungal infection through day 100 were significantly lower in the two most recent time periods of HCT.

Serologic CMV infection was evaluated in recipients who were seropositive at the time of HCT as these patients were at the highest risk for development of CMV disease and CMV-associated mortality.[28] CMV diagnostic testing evolved during the three time periods, and we included measurement via both the pp65 antigen detection assay and PCR-based detection of CMV DNA in plasma in our analysis. While the incidence of CMV reactivation did not significantly change over time, the incidence of CMV disease was significantly decreased in the most recent era (*P*=0.001).
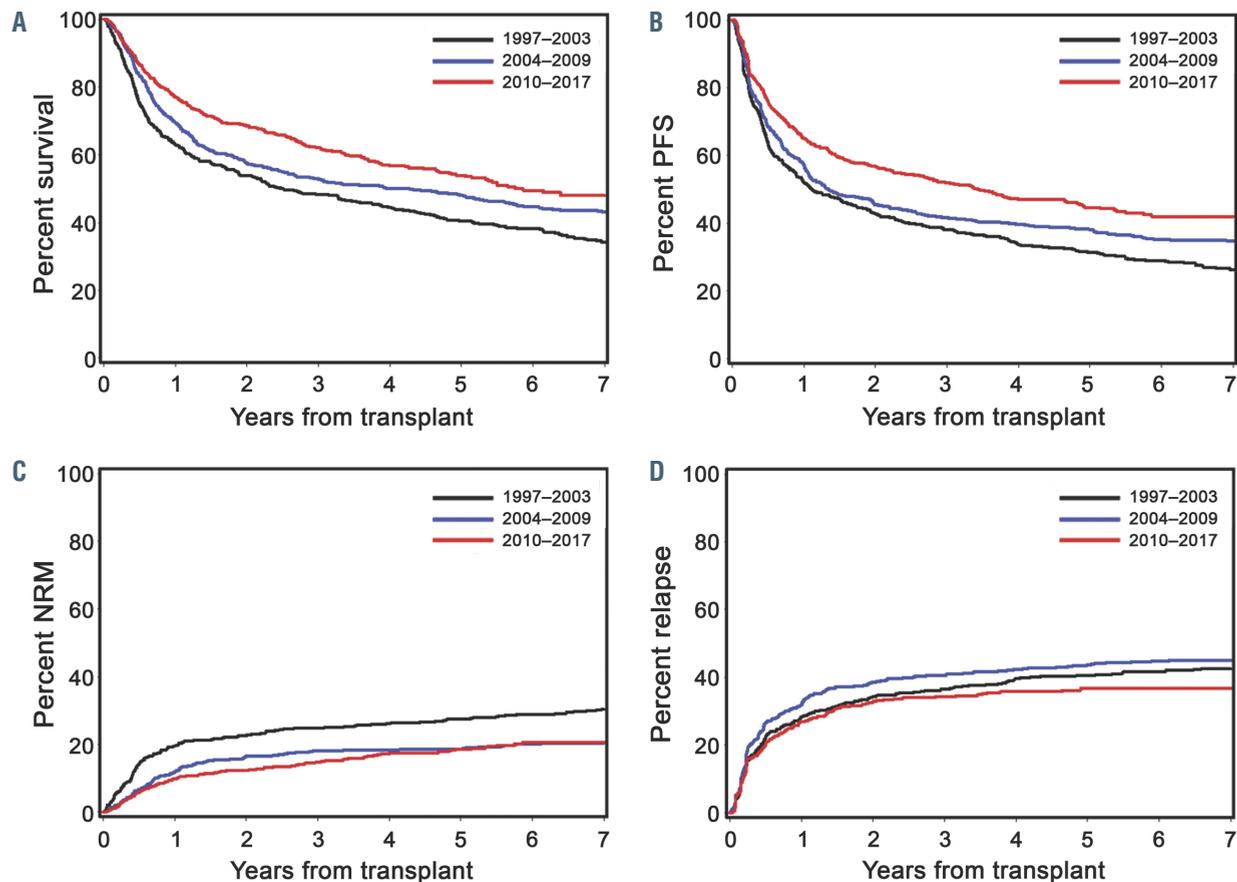
Figure 1. Adjusted cumulative incidence rates of major clinical endpoints by era of transplant. (A) Overall survival, (B) progression-free survival (PFS), (C) non-relapse mortality (NRM), and (D) relapse. Era of transplant: 1997–2003 (black line), 2004–2009 (blue line), and 2010–2017 (red line).

Comparison of endpoints in two most recent transplant eras

Comparisons of clinical endpoints and in the incidences of organ complications and infections in the most recent transplant era (2010–2017) to those in 2004–2009 are shown in the *Online Supplementary Tables S2* and *S3*. OS, PFS, relapse rate, and the rate of grades 2–4 acute GvHD significantly improved in the most recent era as compared to 2004–2009. Over this same time period, significant improvements were also noted in the incidences of gram-negative bacteremias, invasive fungal infections, and in CMV antigenemia and disease.

Discussion

Over the period from 1997–2017, we found marked improvements in OS, PFS, NRM, and in the rates of acute and chronic GvHD after HCT with non-myeloablative conditioning. We also noted a trend toward reduced relapse-related mortality. During this same time period, patient age and burden of comorbidity at the time of HCT increased, higher proportions received grafts from unrelated donors, and AML became the leading indication for HCT, while the numbers of patients with multiple myeloma and CML declined. Consistent with these shifts in diagnoses, there was a decrease in patients who underwent HCT after a prior planned autologous transplanta-

tion, which is likely related to the decrease in multiple myeloma as an indication (decreased use of planned tandem autologous-allogeneic transplantation),[29] and increases in prior unsuccessful autologous HCT for non-Hodgkin lymphoma and prior unsuccessful allogeneic HCT for AML. These data underscore the fact that non-myeloablative conditioning has been increasingly recognized as an option for patients with recurrent hematologic malignancies after prior HCT with high-intensity conditioning regimens.[30] Interestingly we did not see an increase in the number of patients with MDS who underwent HCT, despite the increasing recognition of MDS-associated mortality risk and life expectancy benefit of HCT for patients with high-risk MDS.[31,32] We suspect that this lack of increase in MDS patients may be due to referral patterns from providers who are unaware that HCT is a therapeutic option for their MDS patients who are older or medically infirm.

The substantial reduction in NRM, lower risk of relapse seen primarily in the most recent cohort, and modest improvement in relapse-related mortality all contributed to increased overall survival. Contributors to the improvement in NRM over time are summarized in Table 4 and include the reductions seen in liver and kidney complications; decreases in incidences of gram-negative bacteremia, invasive fungal infections, and CMV disease; and reduced rates of acute and chronic GvHD. We speculate that several changes in clinical practice contributed to the

Table 2. Association of transplant era with major endpoints.

	Adjusted[a] (n=1,668) HR (95% CI)	P
Overall survival		
1997 – 2003	1.0	
2004 – 2009	0.72 (0.6 – 0.9)	0.0001
2010 – 2017	0.60 (0.5 – 0.7)	<0.0001
Progression-free survival		
1997 – 2003	1.0	
2004 – 2009	0.78 (0.7 – 0.9)	0.002
2010 – 2017	0.63 (0.5 – 0.8)	< 0.0001
Relapse/progression		
1997 – 2003	1.0	
2004 – 2009	0.93 (0.8 – 1.1)	0.52
2010 – 2017	0.71 (0.6 – 0.9)	0.006
Non-relapse mortality		
1997 – 2003	1.0	
2004 – 2009	0.58 (0.5 – 0.7)	< 0.0001
2010 – 2017	0.52 (0.4 – 0.7)	< 0.0001
Relapse-related mortality[b]		
1997 – 2003	1.0	
2004 – 2009	0.85 (0.7 – 1.1)	0.15
2010 – 2017	0.80 (0.6 – 1.0)	0.10
Acute GvHD grade 2–4		
1997 – 2003	1.0	
2004 – 2009	0.81 (0.7 – 1.0)	0.03
2010 – 2017	0.64 (0.5 – 0.8)	< 0.0001
Acute GvHD grade 3–4		
1997 – 2003	1.0	
2004 – 2009	0.67 (0.5 – 1.0)	0.03
2010 – 2017	0.54 (0.4 – 0.8)	0.004
Chronic GvHD		
1997 – 2003	1.0	
2004 – 2009	0.59 (0.5 – 0.7)	< 0.0001
2010 – 2017	0.57 (0.5 – 0.7)	< 0.0001

HR: hazard ratio; CI: confidence interval; GvHD: graft-*versus*-host disease; MM: multiple myeloma; AML: acute myeloid leukemia; CMV: cytomegalovirus; R: recipient; D: donor; HCT: hematopoietic cell transplantation; HCT-CI: hematopoietic cell transplantation comorbidity index. [a]Adjusted for transplant center (stratification); treatment type (on-protocol, off-protocol), age (≤49, 50–59, ≥60 years); disease risk group (low, standard, high); MM diagnosis; AML diagnosis; CMV (R– and D–, R+ or D+); donor relation (related, unrelated); sex mismatch (female to male, others); prior HCT (no, yes); allele mismatch (no, yes); HCT-CI (0, 1–2, 3, ≥4, missing). Kahl disease risk groups and HCT-CI assigned as described.[17,20] [b]Relapse-related mortality refers to survival after relapse among patients that relapsed.

Table 3. Association of transplant era with incidences of organ complications and infections.

Organ Toxicity (n=1,548)	Adjusted[a] OR (95% CI)	P
Bilirubin > 4 mg/dL		
1997 – 2003	1.0	
2004 – 2009	0.28 (0.18 – 0.42)	< 0.0001
2010 – 2017	0.22 (0.14 – 0.35)	< 0.0001
Bilirubin > 10 mg/dL		
1997 – 2003	1.0	
2004 – 2009	0.18 (0.08 – 0.43)	0.0001
2010 – 2017	0.21 (0.09 – 0.50)	0.0005
Creatinine > 2x baseline		
1997 – 2003	1.0	
2004 – 2009	0.63 (0.47 – 0.84)	0.002
2010 – 2017	0.71 (0.52 – 0.97)	0.03
Infections (n=1,502)	**HR (95% CI)**	**P**
Gram-negative bacteremia		
1997 – 2003	1.0	
2004 – 2009	0.82 (0.70 – 0.97)	0.02
2010 – 2017	0.68 (0.56 – 0.82)	< 0.0001
Invasive fungal infection		
1997 – 2003	1.0	
2004 – 2009	0.75 (0.63 – 0.89)	0.0008
2010 – 2017	0.63 (0.52 – 0.76)	< 0.0001
CMV antigenemia[b]		
1997 – 2003	1.0	
2004 – 2009	1.15 (0.94 – 1.40)	0.18
2010 – 2017	0.86 (0.69 – 1.08)	0.19
CMV disease[b]		
1997 – 2003	1.0	
2004 – 2009	0.86 (0.70 – 1.06)	0.16
2010 – 2017	0.66 (0.52 – 0.85)	0.001

OR: odds ratio; HR: hazard ratio; CI: confidence interval; MM: multiple myeloma; AML: acute myeloid leukemia; R: recipient; D: donor; CMV: cytomegalovirus; HCT: hematopoietic cell transplantation; HCT-CI: hematopoietic cell transplantation comorbidity index. [a]Adjusted for transplantation center; treatment type (protocol, treatment plan); age (≤49, 50–59, 60+ years); disease risk group (low, standard, high); MM diagnosis; AML diagnosis; CMV (R– and D–, R+ or D+); donor relation (related, unrelated); sex mismatch (female to male, others); prior HCT (no, yes); allele mismatch (no, yes); and HCT-CI (0, 1–2, 3, ≥4, missing). [b]CMV endpoints evaluated only among seropositive recipients at HCT (n = 911 for multivariate analysis).

reduction in NRM. Topically-active glucocorticoids such as beclomethasone dipropionate were introduced which, when given with prednisone to patients with gastrointestinal GvHD in a randomized, placebo-controlled trial, allowed for a rapid taper of prednisone dosing starting 10 days after initiation, significantly reduced the risk of GvHD-treatment failure, and reduced the risk of mortality by 37% after a median follow-up of 3.6 years.[3] Decreases in the use of systemic glucocorticoids for treatment of acute GvHD, along with the addition of sirolimus to MMF with a calcineurin inhibitor as GvHD prophylaxis have also resulted in lower rates of fungal infections[2] and CMV reactivation.[6,8] Changes in infection prophylaxis also likely contributed to reductions in NRM. This included a shift in antibacterial prophylaxis from cephalosporins to fluoroquinolones during periods of neutropenia,[9-11] a shift in antifungal prophylaxis from fluconazole to extended-spectrum triazoles (itraconazole, voriconazole, posaconazole) with greater activity against mold,[12,13] empirical anti-

fungal therapy for patients with pulmonary nodules,[33] and the adoption of pre-emptive antiviral therapy driven by highly sensitive PCR-based CMV DNA diagnostic testing of blood samples.[14-16]

Reduction in the incidences of acute GvHD in our later patient cohorts were due, in part, to the increasing use of sirolimus as a component of GvHD prophylaxis for unrelated recipients[6,8] and the adoption of ursodiol to prevent cholestasis and hyperbilirubinemia.[4] In addition to our data here documenting the beneficial effect of sirolimus, we recently reported the results of a randomized phase III trial showing that the addition of sirolimus to the standard prophylactic regimen of MMF and cyclosporine in unrelated recipients reduced the day 100 cumulative incidence of grades 2–4 acute GvHD (26% *vs.* 52%), resulting in significantly reduced use of steroids and markedly improved 1-year NRM, PFS, and OS.[8] While there was no difference in chronic GvHD between the two groups, patients affected by chronic GvHD in the triple-drug group had a trend towards lower NRM after 1 year compared with those in the standard group (4% in triple-drug group *vs.* 15% in the standard group). Ursodiol use, at a dose of 12 mg/kg/day
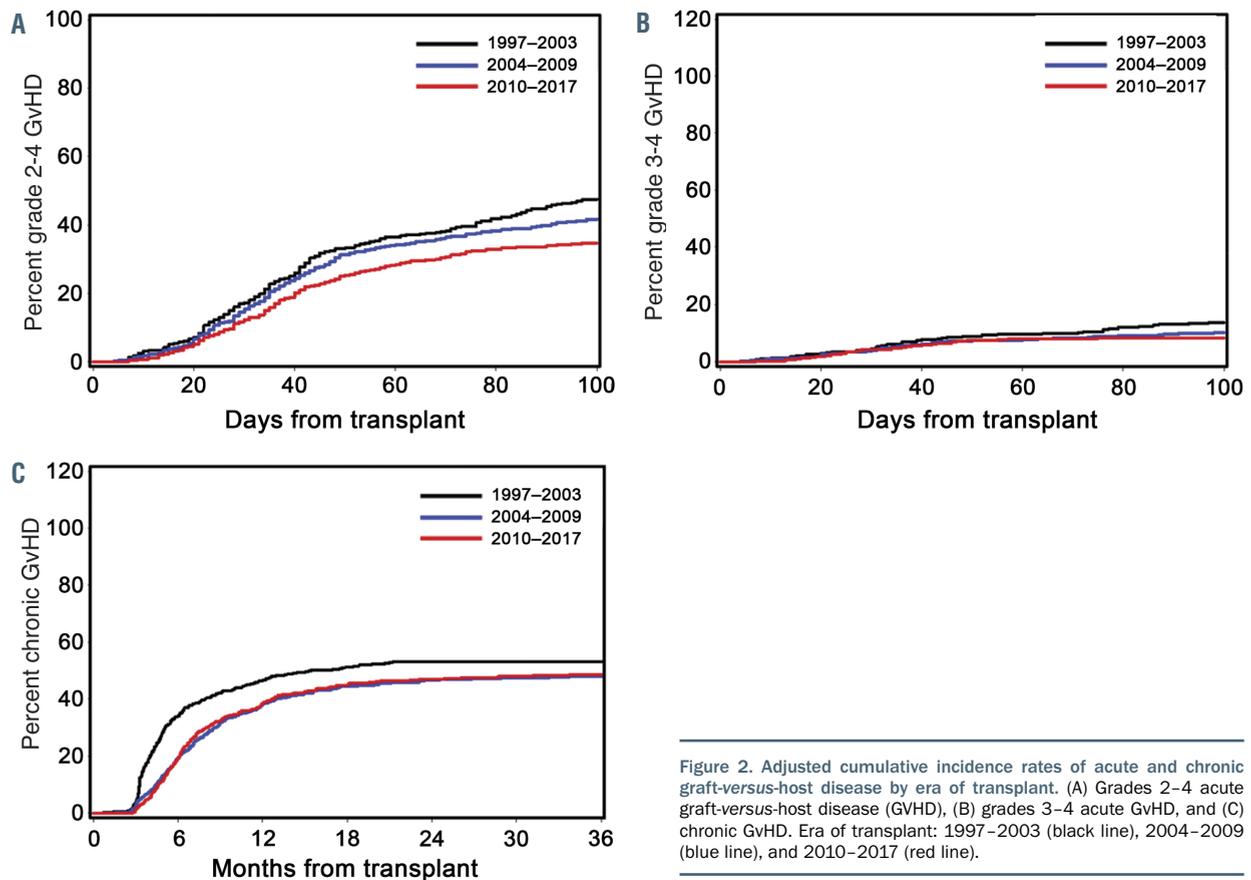
Figure 2. Adjusted cumulative incidence rates of acute and chronic graft-*versus*-host disease by era of transplant. (A) Grades 2–4 acute graft-*versus*-host disease (GVHD), (B) grades 3–4 acute GvHD, and (C) chronic GvHD. Era of transplant: 1997–2003 (black line), 2004–2009 (blue line), and 2010–2017 (red line).

starting on the day before HCT, significantly reduced the incidences of stages 2–4 liver and intestinal acute GvHD and grades 3–4 acute GvHD, and improved both 1-year OS and NRM.[4]

The overall incidence of relapse was significantly lower during 2010–2017 compared to earlier time periods. This corresponded both with an increasing use of higher TBI doses (3–4.5 Gy) for patients who did not receive preceding myelosuppressive chemotherapy, and with an increasing proportion of patients who underwent HCT with diseases at a lower risk of relapse. The majority of our patients who received increased TBI dosing carried a diagnosis of AML, MDS, or a myeloproliferative neoplasm based on a prospective TBI dose escalation study showing a reduction in relapse with higher TBI dosing in MDS/MPN patients who had not had previous myelosuppressive (induction-type) chemotherapy.[34] The lower grade MDS/MPN patients benefitted by increasing the TBI dose to 3 Gy whereas the patients with excess blasts or a history of CMML benefitted by increasing the TBI dose to 4.5 Gy. Based on the findings in the low grade MDS patients, the TBI dose was increased to 3 Gy in AML patients who had not received induction chemotherapy in the preceding 3–6 months or had a previous allogeneic transplant from a different donor. In a sub-analysis, we found no significant change in the rate of relapse in the subset of AML patients who underwent HCT in CR1/CR2, a finding that has also been noted in AML patients who predominantly received high-intensity conditioning prior to HCT.[35] While we also noted an increase in the number of AML patients in CR1/CR2 with MRD at the time of non-myeloablative HCT, we recognize that

the detection of MRD and its association with disease risk-stratification relative to HCT evolved over our time period of analysis and may have affected the selection of patients in our cohort who underwent HCT after non-myeloablative conditioning *versus* receiving other therapies.

The overall incidence of relapse-related mortality trended toward improvement for our 2004–2009 and 2010–2017 patient cohorts. Hypomethylating agents, in particular, gained increasing use for the treatment of relapsed myeloid malignancies during our later time periods,[36,37] and are likely a contributor to the observed modest attenuation of relapse-related mortality. More recently, there has been an expansion in the treatment armamentarium for relapsed disease including ibrutinib, venetoclax, and enasidinib,[38-40] checkpoint inhibitors,[41,42] tyrosine kinase inhibitors,[43] and monoclonal antibodies.[44,45] Many of these agents were used too infrequently to have influenced the outcomes reported here, but we anticipate that their use will increase and may lead to further reduction in mortality after relapse.

Interestingly, we found that the significant improvements in OS, PFS, rate of grades 2–4 acute GvHD, and in the incidence of infectious complications continued to occur over our total period of analysis. However, the statistically significant improvements in other clinical outcomes were limited to particular eras. For example, NRM, relapse-related mortality, rate of grades 3–4 acute GvHD, rate of chronic GvHD, and the incidences of liver and kidney injury improved significantly only when 2004–2009 was compared to 1997–2003; the rate of relapse or progression improved significantly only in the most recent

Table 4. Summary of changes in clinical practice that affected non-relapse mortality over time.

Increased use of topically-active GI glucocorticoids for patient with GI acute GvHD
Decreased use of systemic glucocorticoids for patients with acute GvHD
Addition of sirolimus to CI and MMF for GvHD prophylaxis
Increased use of ursodiol to prevent cholestasis and hyperbilirubinemia
Increased use of fluoroquinolones as antibacterial prophylaxis in neutropenic patients
Increased use of mold-active triazoles as antifungal prophylaxis
Increased use of empiric antifungal therapy for patients with pulmonary nodules
Increasing use of pre-emptive antiviral therapy for CMV viremia

NRM: non-relapse mortality; GI: gastrointestinal; GvHD: graft-*versus*-host disease; CI: calcineurin inhibitor; MMF: mycophenolate mofetil; CMV: cytomegalovirus.

era. While those outcomes which did not show statistical improvements in the most recent era had hazard ratios that trended toward improvement, it is likely that the continual improvement in OS and PFS had different predominant components at different times: improvements in NRM, relapse-related mortality, and organ toxicity contributing earlier (between 1997–2003 and 2004–2009) and improvement in the rate of relapse contributing later (between 2004–2009 and 2010 –2017).

The present findings are similar to those of prior analyses which predominantly included patients who received high-intensity conditioning.[35,46,47] It is notable, however, that in these prior studies the increased use of lower-intensity conditioning regimens and granulocyte colony-stimulating factor-mobilized peripheral blood stem cell (PBSC) grafts were cited as prominent reasons for the improved outcomes – particularly reductions in NRM. The homogeneous nature of the current patient population, with all patients receiving lower-intensity conditioning regimens and HLA-matched PBSC grafts, allowed us to more independently appraise the influence of changes in supportive care that are critical for successful outcomes after HCT.

In conclusion, we show that improvements in supportive care after HCT with non-myeloablative conditioning for patients with advanced hematologic malignancies during the past two decades have yielded higher rates of overall survival and PFS and lower risks of NRM, grades 2–4 acute GvHD, and chronic GvHD. During this period, the age of patients and burden of comorbidity at the time of HCT has increased, and use of unrelated donors has also increased, thereby making allogeneic HCT more widely available for patients with otherwise incurable hematologic malignancies. These results should encourage the referral of elderly and medically infirm younger patients with hematologic malignancies for evaluation at a transplant center.

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