Reduced incidence of relapse and graft-*versus*-host disease in acute myeloid leukemia after allogeneic hematopoietic cell transplantation

Haesook T. Kim,[1] Robert J. Soiffer,[2] Corey Cutler,[2] John Koreth,[2] Sarah Nikiforow,[2] Roman M. Shapiro,[2] Amar Kelkar,[2] Mahasweta Gooptu,[2] Rizwan Romee,[2] Catherine J. Wu,[2] Joseph H. Antin,[2] Jerome Ritz[2] and Vincent T Ho[2]

[1]Department of Data Science, Dana-Farber Cancer Institute, Harvard School of Public Health, and [2]Department of Medical Oncology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA, USA

Correspondence: H. T. Kim
htkimc@jimmy.harvard.edu

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Abstract

Advances in HLA typing, conditioning regimens, graft-*versus*-host disease (GVHD) prophylaxis/treatment, and supportive care have led to significant improvement in survival after allogeneic hematopoietic cell transplantation (alloHCT). Despite this progress, disease relapse after transplantation remains a daunting challenge and continues to be a major driver of mortality in patients with acute myeloid leukemia (AML). To assess whether progress has been made in relapse, we investigated our institution's experience with all AML patients who underwent their first allogeneic transplants from 2010 through 2022. A total of 1,169 patients were identified. The year of transplant was divided into two groups: 2010-2016 and 2017-2022. Several shifts in baseline clinical characteristics were noted during these periods. Patients who underwent transplantation in 2017-2022 were older (P<0.0001), had higher HCT-comorbidity index scores (P<0.0001), and were more likely to be transplanted in first complete remission (P<0.0001). Over these periods, we observed significant reductions in relapse (cumulative incidence at 3 years: 36% and 48%, respectively; P<0.0001) and severe acute GVHD (cumulative incidence at 6 months: 5% and 11%, respectively; P=0.0012), which resulted in significant improvement in progression-free (3-year estimates: 53% and 41%, respectively; P<0.0001) and overall survival (3-year estimates: 62% and 47%, respectively; P<0.0001). We also observed significant improvement in post-relapse survival over time for the relapse patients (2-year estimates: 23% and 12.6%, respectively; P<0.0001). Our results show that there has been an encouraging reduction in AML relapse, GVHD and mortality after alloHCT over the past decade.

Introduction

Acute myeloid leukemia (AML) is a heterogeneous disease characterized by the abnormal accumulation of myeloblasts in the bone marrow and blood that interfere with normal blood cell production.[1] For patients with intermediate or adverse risk AML, or those who are refractory or relapsed after conventional chemotherapy, allogeneic hematopoietic cell transplantation (alloHCT) is considered standard of care and offers the best chance of long-term leukemia-free survival. As such, AML is the most common disease indication for alloHCT, comprising 45-50% of all allogeneic transplants performed worldwide.[2-4]

Since the first allogeneic stem cell transplant performed for treatment of AML five decades ago,[5-7] the field has witnessed continuous progress through the decades with major advances in HLA tying, conditioning regimens, graft-*versus*-host disease (GVHD) prophylaxis, donor selection and supportive care.[4,8-10] Indeed, in a retrospective study comparing outcomes between the period 1993-1997 and 2003-2007, Gooley *et al*. reported significant decreases in non-relapse death by 52% and thereby overall death by 41% during this period. Significant decreases were also noted in the risk of severe GVHD, bacterial, viral and fungal infections and organ damage.[10] More recently, Carpenter *et al*. reported decreasing chronic GVHD over the past 15 years.[11] However, despite these improvements, disease recurrence after alloHCT remains a major challenge and has been the primary cause of treatment failure, especially for aggressive malignancies such as AML.[12] To assess whether

we have made any progress in reducing relapse over the last decade, we investigated relapse and transplant outcomes for all AML patients who were transplanted at our institution from 2010 through 2022.

Methods

Patients

The blood and marrow transplant data repository of the Dana-Farber Cancer Institute was queried to identify patients with AML who received their first allogeneic transplant between 2010 and 2022 and 1,169 patients were identified. For patients who relapsed, electronic medical records were reviewed by the last author (VTH) to confirm and document type and site(s) of relapse. The study protocol was approved by the institutional review board of the Dana-Farber/Harvard Cancer Center.

Transplantation techniques

Reduced-intensity conditioning (RIC) regimens in this study included fludarabine (120 mg/m^2) with intravenous busulfan (3.2 mg/kg [Flu/Bu1] or 6.4 mg/kg [Flu/Bu2]); fludarabine (120 mg/m^2) with melphalan (100-140 mg/m^2) (Flu/Mel); or fludarabine with cyclophosphamide (14.5 mg/kg for 2 days) and low-dose total-body irradiation (TBI) 200 cGy (Flu/Cy/TBI). Myeloablative conditioning (MAC) generally consisted of high-dose busulfan (12.8 mg/kg) with fludarabine 120 mg/m^2 (Flu/Bu4), high-dose busulfan with cyclophosphamide 60 mg/kg for 2 days (Bu/Cy) or high-dose cyclophosphamide (1,200 mg/m^2 or 60 mg/kg for 2 days) with 1,200 cGy fractionated TBI (Cy/TBI). GVHD prophylaxis included tacrolimus with methotrexate (TAC/MTX), tacrolimus with methotrexate and sirolimus (TAC/Sir/MTX), tacrolimus with sirolimus (TAC/Sir), or post-transplant cyclophosphamide with tacrolimus and mycophenolate mofetil (PTCY) (Table 1). Infection prevention strategy included a minimum of 1 year of acyclovir for HSV/VZV and atovaquone or sulfamethoxazole/trimethoprim for *Pneumocystis jirovecii*. Use of fluconazole or other triazoles for fungal prophylaxis was at the discretion of the transplant physician. Cytomegalovirus (CMV) was managed with a pre-emptive strategy with weekly polymerase chain reaction monitoring in the first 100 days after transplant. After its Food and Drug Adminstration approval, letermovir was prescribed for the first 100 days to patients who were CMV seropositive and undergoing transplantation with PTCY or other *in vivo* or *ex vivo* T-cell depletion.

Study endpoints and statistical analysis

Endpoints in this study included morphologic relapse (defined as ≥5% blasts in bone marrow [BM] or blood, or presence of extramedullary disease), progression-free survival (PFS), overall survival (OS), non-relapse mortality (NRM), acute GVHD, and chronic GVHD with the primary interest in relapse. Acute GVHD was graded using the modified Glucksberg criteria,[13] while chronic GVHD was graded according to the National Institute of Health consensus criteria.[14,15] The Kaplan-Meier method was used to estimate PFS and OS whereas cumulative incidence of NRM, relapse, and GVHD were estimated in the context of a competing risks framework. The log-rank test and Gray test[16] were used for group comparison. Multivariable regression analysis was performed for OS and PFS using Cox model and Fine and Gray regression analysis[17] for cumulative incidence of NRM, relapse and GVHD. Definitions of endpoints and detailed statistical analysis are provided in the *Online Supplementary Appendix*.

Results

Year of transplant

Prior to conducting the analysis, we first investigated changes in the risk of morphologic relapse over time by assessing the linearity of year of transplant using the method of restricted cubic spline function on log relative hazard and a potential cutoff year using the classification and regression tree for survival data.[18-20] We found that the relative risk associated with the year of transplant was non-linear overall, remaining stable initially before decreasing linearly after 2016. A similar pattern was observed in PFS (*Online Supplementary Figure S1*). Based on this result, year of transplant was dichotomized into 2010-2016 and 2017-2022 for comparison of baseline characteristics and multivariable regression analysis.

Patients

The baseline characteristics of all patients are summarized in Table 1. As shown in Table 1, baseline characteristics were well balanced between 2010-2016 and 2017-2022 with respect to sex, diagnosis, time from diagnosis to transplant and cytogenetic risk at transplant. Differences in baseline characteristics include age, disease status at transplant, HCT-comorbidity index (HCT-CI), Karnofsky performance score, donor type, cell source, conditioning regimen, and GVHD prophylaxis. These differences largely reflect changes in our practice in recent years. Notably, we transplanted more older patients (median age: 62 in 2017-2022 *vs*. 58 in 2010-206; age ≥70 years: 18% in 2017-2022 *vs*. 8.8% in 2010-2016; $P<0.0001$) and more patients in first complete remission (CR1) ($P<0.0001$) with higher comorbidity scores (HCT-CI ≥2: 70% in 2017-2022 *vs*. 57.5% in 2010-2016; $P<0.0001$) in recent years. There was also a notable increase in haploidentical and unrelated donor transplants in recent years, with concurrent reduction in umbilical cord blood transplants since 2017. There have also been shifts in GVHD prophylaxis toward more PTCY-based regimens after 2017;[21] conditioning regimens with less Cy/TBI in the MAC setting, and less Flu/BU1 or ATG regimens in the RIC setting.

Table 1. Baseline characteristics.

Variables	All		2010-2016		2017-2022		*P*
	N	%	N	%	N	%	
Total	1,169	100	612	100	557	100	
Age, years							<0.0001
<50	319	27.3	189	30.9	130	23.3	
50-59	267	22.8	163	26.6	104	18.7	
60-69	429	36.7	206	33.7	223	40	
>=70	154	13.2	54	8.8	100	18	
Median (range)	60 (18-79)	-	58 (18-77)	-	62 (20-79)	-	
Patient sex							0.52
Female	528	45.2	282	46.1	246	44.2	
Male	641	54.8	330	53.9	311	55.8	
Pt-dnr sex mismatch							0.64
Male pt & female dnr	203	17.4	103	16.8	100	18	
Diagnosis							0.89
AML	894	76.5	467	76.3	427	76.7	
s-AML	275	23.5	145	23.7	130	23.3	
Time from DX to transplant, months							0.67
<=6	772	66	408	66.7	364	65.4	
>6	397	34	204	33.3	193	34.6	
Disease status							<0.0001
CR1	814	69.6	389	63.6	425	76.3	
CR>=2	162	13.9	101	16.5	61	11	
Relapse/IF	193	16.5	122	19.9	71	12.7	
Cytogenetic risk							0.58*
Low	54	5	25	4.6	29	5.5	
Int	704	65.6	351	64.8	353	66.5	
High	315	29.4	166	30.6	149	28.1	
UNK	96	-	70	-	26	-	
KPS							0.002*
<90	781	69.5	428	73.5	353	65.1	
90-100	343	30.5	154	26.5	189	34.9	
UNK	45	-	30	-	15	-	
HCT-CI							<0.0001
0-1	427	36.5	260	42.5	167	30	
2-4	515	44.1	253	41.3	262	47	
>=5	227	19.4	99	16.2	128	23	
Median (range)	2 (0-11)	-	2 (0-11)	-	3 (0-10)	-	
Recipient CMV sero status							0.053
Positive	620	53.1	341	55.9	279	50.1	
Donor CMV sero status							
Positive	396	34.4	211	34.6	185	34.1	0.85
UNK	17	-	3	-	14	-	
HLA type (A, B, C, DRB1)							<0.0001
8/8 MRD	268	22.9	176	28.8	92	16.5	
8/8 MUD	620	53	303	49.5	317	56.9	
Haplo	93	8	18	2.9	75	13.5	
MMRD	8	0.7	4	0.7	4	0.7	
MMUD	180	15.4	111	18.1	69	12.4	
Cell source							0.0005
PBSC	968	82.8	492	80.4	476	85.5	
BM	168	14.4	92	15	76	13.6	
Cord	33	2.8	28	4.6	5	0.9	

Continued on following page.

Variables	All		2010-2016		2017-2022		*P*
	N	%	N	%	N	%	
Conditioning regimen							0.09**
RIC	609	52.1	304	49.7	305	54.8	
Flu/Bu1	78	-	73	-	5	-	
Flu/Bu2	385	-	159	-	226	-	
Flu/Mel	38	-	13	-	25	-	
Flu/Mel+ATG	23	-	21	-	2	-	
Flu/Cy/TBI	64	-	19	-	45	-	
Other	21	-	19	-	2	-	
MAC	560	47.9	308	50.3	252	45.2	
Cy/TBI	114	-	110	-	4	-	
Flu/Bu4	396	-	163	-	233	-	
Bu/Cy	30	-	30	-	0	-	
Other	20	-	5	-	15	-	
GVHD prophylaxis							<0.0001
Tac/MTX	590	50.5	296	48.4	294	52.8	
Tac/MTX/Sir	189	16.2	119	19.4	70	12.6	
Tac/Sir	155	13.3	122	19.9	33	5.9	
Tac/MMF/Cy	164	14	26	4.2	138	24.8	
Other	71	6.1	49	8	22	3.9	

*UNK (unknown) was not counted in *P* value calculation. **Comparison for conditioning intensities. Pt-dnr: patient and donor; Pt: patient; Dnr: donor; s-AML: secondary or therapy-related acute myeloid leukemia; CR: complete remission; IF: induction failure; KPS: Karnofsky performance score; HCT-CI: hematopoietic cell transplantation comorbidity index; CMV: Cytomegalovirus; DX: diagnosis; 8/8 MRD: 8/8 matched related donor; 8/8 MUD: 8/8 matched unrelated donor; Haplo: haploidentical transplant; MMRD: mismatched related donor; MMUD: mismatched unrelated donor; PBSC: peripheral blood stem cell; BM: bone marrow; RIC: reduced-intensity conditioning; MAC: myeloablative conditioning; Flu: fludarabine; Bu1: busulfan with 3.2 mg/kg; Bu2: busulfan with 6.4 mg/kg; Bu4: busulfan with 12.8 mg/kg; Mel: melphalan; Cy: cyclophosphamide; TBI: total-body irradiation; ATG: antithymocyte globulin; GVHD: graft-*versus*-host disease; Tac: tacrolimus; MTX: methotrexate; Sir: sirolimus; MMF: mycophenolate mofetil.

Clinical outcomes

We observed substantial improvement in outcomes since 2010-2016. With the median follow-up among survivors of 60 months (range, 5-170) for the entire cohort, cumulative incidence of relapse was 36% at 3 years in the period of 2017-2022 compared to 48% in 2010-2016 (*P*<0.0001) while NRM rates were relatively stable (3-year estimates: 11% and 11%, respectively; *P*=0.48) (Figure 1A, B; *Online Supplementary Table S1A*). Due largely to the reduction in relapse, PFS (3-year estimates: 53% and 41%, respectively; *P*<0.0001) and OS (3-year estimates: 62% and 47%, respectively; *P*<0.0001) were also significantly improved in 2017-2022 (Figure 1C, D; *Online Supplementary Table S1A*). When we restricted the analysis to patients transplanted in CR1, we observed a similar reduction over time in relapse (3-year estimates: 35% *vs*. 45%, respectively; *P*=0.0019) and improved PFS (3-year estimates: 55% *vs*. 45%, respectively; *P*=0.009) and OS (3-year estimates: 64% *vs*. 51%, respectively; *P*=0.0014) without substantial change in NRM (*P*=0.24) (*Online Supplementary Table S1B*).

There has also been a statistically significant decrease in the incidence of acute GVHD in 2017-2022. Cumulative incidence of grade 2-4 acute GVHD at 6 months was 16% in 2017-2022 compared to 25% in 2010-2016 (*P*=0.0007) (Figure 1E; *Online Supplementary Table S1A*). Likewise, the cumulative incidence of grade 3-4 acute GVHD has declined significantly in recent years, (6-months estimates: 5% *vs*. 11%, respectively; *P*=0.0012) (Figure 1F; *Online Supplementary Table S1A*). However, the incidence of chronic GVHD has not changed much (all chronic GVHD: 41% *vs*. 43% at 2 years, respectively; *P*=0.88; moderate-severe chronic GVHD 25% *vs*. 28% at 2 years, respectively; *P*=0.48) (*Online Supplementary Table S1A*). When we further subdivided 2017-2022 into 2017-2019 and 2020-2022, the incidence of chronic GVHD at 2-years was 38% in 2020-2022 *versus* 45% in 2017-2019; *P*=0.01; the incidence of moderate-severe chronic GVHD at 2 years was 21% in 2020-2022 *versus* 30% in 2017-2020 *P*=0.0028 indicating the improvement in chronic GVHD is more recent. These outcomes are summarized and presented as a stack plot in Figure 2A.

Adjustment for risk factors

In multivariable analysis using Cox model for OS and PFS and the Fine and Gray competing risks regression model for NRM, relapse and GVHD, consistent with results from the univariable analysis, there was a significant reduction in relapse risk for patients transplanted since 2017 compared to prior to 2017 (subdistribution hazard ratio [sHR] =0.63; *P*<0.0001) but not in NRM (sHR=0.85; *P*=0.29). (Figure 2B; *Online Supplementary Table S2A*) There was also a significant improvement in PFS (HR=0.69; *P*<0.0001) and OS (HR=0.64; *P*<0.0001). (Figure 2B; *Online Supplementary Table S2A*). In regard with other factors associated with relapse, use of haploidentical transplant (sHR=0.51 com-

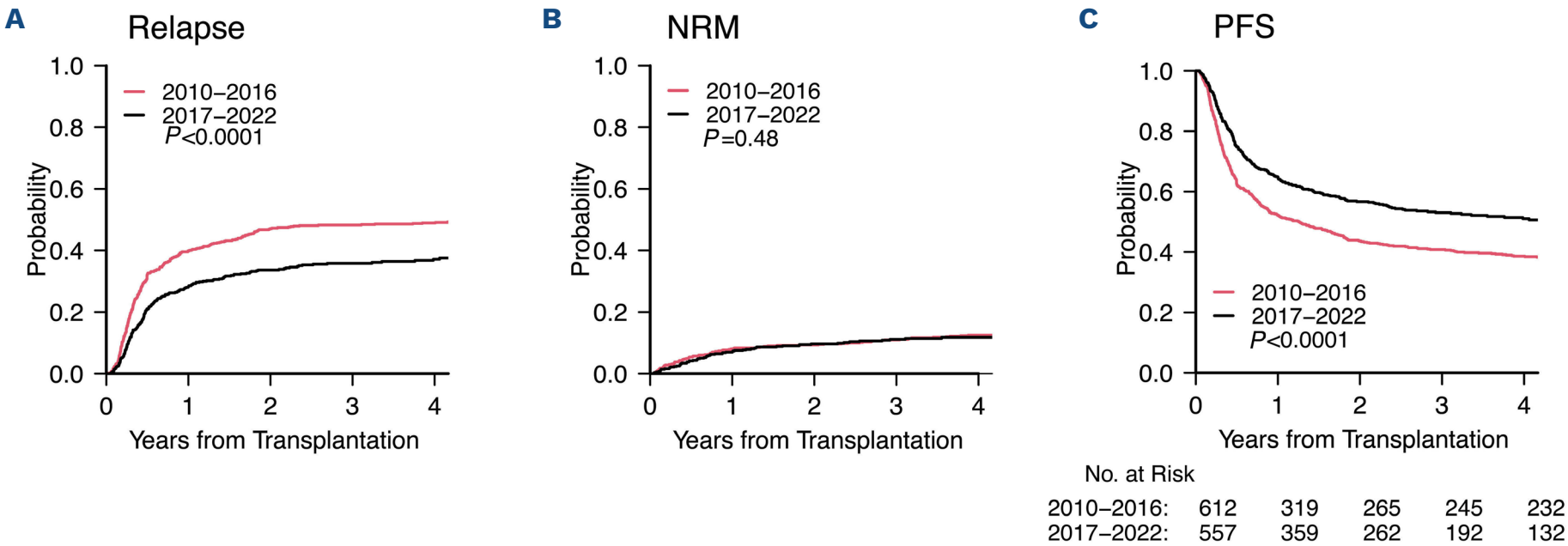

Figure 1. Clinical outcomes according to year of transplant. (A, B) Cumulative incidence of relapse and non-relapse mortality (NRM), respectively. (C) Progression-free survival (PFS). (D) Overall survival (OS). (E, F) Cumulative incidence of grade 2-4 acute graft-*versus*-host disease (GVHD) and grade 3-4 acute GVHD, respectively. Gray test was used for group comparison of relapse, NRM and acute GVHD. Log-rank test was used for group comparison of PFS and OS. Number at risk for relapse and NRM are the same as those in PFS.

pared to 8/8 matched related donor; *P*=0.0017), use of PTCY prophylaxis (sHR=0.68; *P*=0.013) and MAC (sHR=0.69; *P*=0.0017) were associated with a lower risk of relapse. MAC, however, was also associated with a higher risk of NRM compared to RIC (sHR=1.89; *P*=0.001). On the other hand, absence of complete remission at time of transplant (sHR=1.41; *P*=0.0047) and high cytogenetic risk (sHR=1.99; *P*=0.01) were associated with a higher risk of relapse (*Online Supplementary Table S2A*).

Consistent with the univariable analysis, grade 2-4 acute GVHD (sHR=0.66; *P*=0.0012) and grade 3-4 acute GVHD (sHR=0.51; *P*=0.0025) were significantly improved in ≥2017 compared to <2017. (Figure 2B; *Online Supplementary Table S2B*). Mismatched unrelated donor transplant was associated with a higher risk of acute GVHD (sHR=1.8; *P*=0.0044 for grade 2-4 acute GVHD) although the association for grade III-IV acute GVHD was not statistically significant (sHR=1.45; *P*=0.24). Chronic GVHD risk was not different for ≥2017 compared to <2017 (sHR=0.94; *P*=0.53 for all chronic GVHD; sHR=0.85; *P*=0.16 for moderate-severe chronic GVHD) (Figure 2B; *Online Supplementary Table S2B*). Notably, GVHD prophylaxis with PTCY was associated with significant reduction in all chronic GVHD (sHR=0.46; *P*<0.0001) and moderate-severe chronic GVHD (sHR=0.37; *P*<0.0001), but not with acute GVHD (*Online Supplementary Table S2B*). Indeed, the cumulative incidence of chronic GVHD was 25% at 2 years for PTCY compared with 45% for no PTCY (*P*<0.0001); moderate-severe chronic GVHD was 11% at 2 years for PTCY compared with 29% for no PTCY (*P*<0.0001) and severe chronic GVHD was 1.8% at 2 years for PTCY compared with 12% for no PTCY (*P*<0.0001) (*Online Supplementary Figure S2*). When we further explored if the occurrence of acute GVHD was a risk factor for development of chronic GVHD in multivariable models for chronic GVHD treating acute GVHD

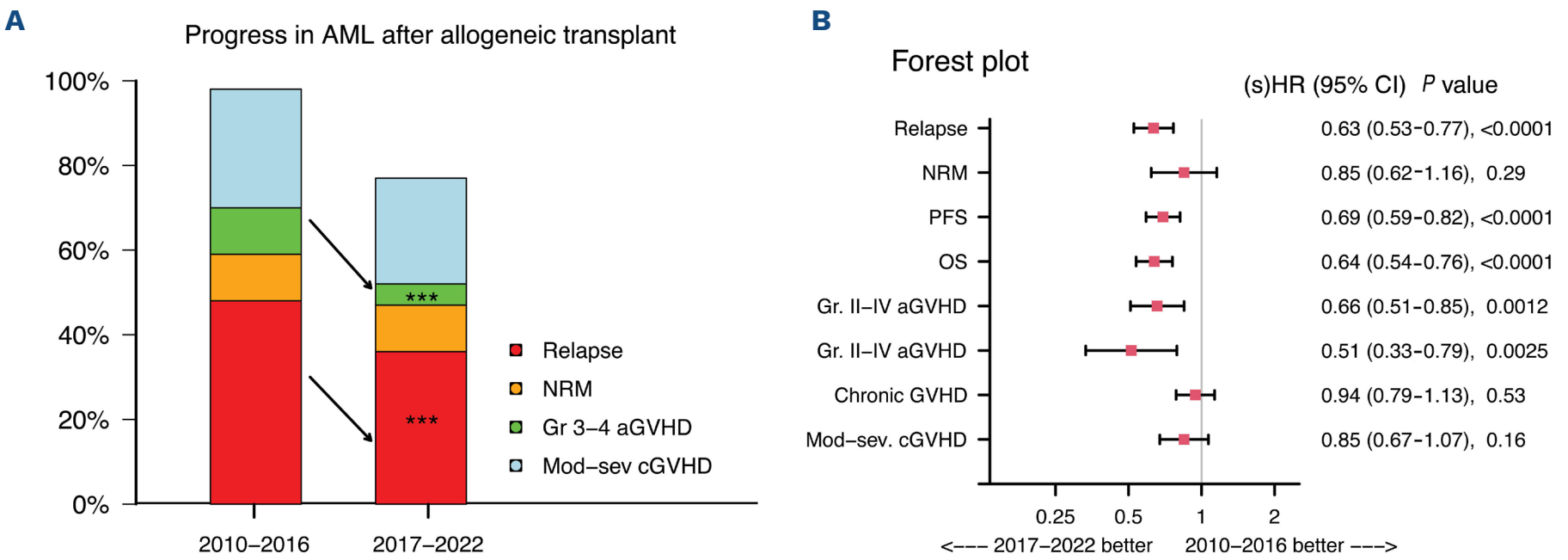

Figure 2. Summary outcomes. (A) Stack plot of outcomes from univariable analysis. Three-year estimates of relapse and non-relapse mortality, 6-months estimate of grade 3-4 acute graft-*versus*-host disease (GVHS) and 2-year estimate of moderate-severe chronic GVHD were plotted. (B) Forest plot of subdistribition hazard ratio (sHR) for relapse, NRM, acute GVHD and chronic GVHD and hazard ratio (HR) for progression-free survival and overall survival from multivariable analysis. Gr: grade; Mod-sev: moderate-severe.

as a time dependent variable, the HR was 1.12 for grade 2-4 acute GVHD (P=0.32) and 0.84 (P=0.32) for grade 3-4 acute GVHD indicating that acute GVHD is not a precursor to the development of chronic GVHD (*data not shown*). This is not surprising since the incidence rate of acute GVHD is much lower compared to the incidence of chronic GVHD. Of the 501 cases of chronic GVHD, 16.8% (N=89) had a prior history of grade 2 acute GVHD and 5.8% (N=31) had a prior history of grade 3-4 acute GVHD. The remaining 411 developed *de novo* chronic GVHD.

Conditioning regimen and disease status

Due to changes of conditioning regimens in recent years, we investigated the interaction between conditioning intensity and year of transplant on relapse and the interaction was not significant (P=0.49 from multivariable model). The incidence of relapse after RIC was significantly reduced in ≥2017 compared to <2017 (39% *vs*. 53% at 3-years, respectively; P<0.0001) without significant change in NRM (11%% *vs*. 8.9% at 3 years, respectively; P=0.13). Likewise, the incidence of relapse in MAC was significantly reduced in ≥2017 compared to <2017 (32% *vs*. 44% at 3-years, respectively; P=0.01) without change in NRM (11% *vs*. 13% at 3 years, respectively; P=0.72). Consequently, PFS and OS were significantly improved in ≥2017 (*Online Supplementary Table S3A*). Since some changes in practice occurred in the conditioning regimen around 2017, we further investigated conditioning regimens in RIC and MAC separately and noted reduction in relapse in RIC over time. In multivariable analysis, RIC Flu/Bu2 was associated with a lower risk of relapse (sHR=0.74 compared to Flu/Bu1; P=0.11) although this association did not reach statistical significance. Likewise, other RIC regimens (sHR=0.30; P<0.0001 for Flu/Mel±ATG; sHR=0.52; P=0.014 for Flu/Cy/TBI compared to Flu/Bu1) were significantly associated with a lower risk of relapse. Importantly, although the intensity was increased, Flu/Bu2 was not associated with a higher risk of NRM (sHR=1.11; P=0.77) but the risk of NRM was compromised in Flu/Mel±ATG (sHR=2.30; P=0.07) (*Online Supplementary Table S3B*). In the MAC setting, Flu/Bu4 and Bu/Cy were not associated with a lower risk of relapse compared to Cy/TBI but both regimens were associated with a lower risk of NRM compared to Cy/TBI (sHR=0.44; P=0.0019 for Flu/Bu4; sHR=0.25; P=0.035 for Bu/Cy).

We next investigated the interaction between disease status and year of transplant on relapse. Because all outcomes were very similar between CR1 and subsequent complete remission (CR2+), we combined CR1 with CR2+ as CR in this analysis. When we assessed disease status by year of transplant, the incidence of relapse was significantly lower in ≥2017 compared to <2017 in both CR (35% for ≥2017 *vs*. 45% for <2017; P=0.001) and no CR (43% for ≥2017 *vs*. 61% for <2017; P=0.01) without difference in NRM (*Online Supplementary Table S3C*).

Effect of graft-*versus*-host disease on relapse

Since both relapse and acute GVHD have decreased in recent years, we investigated the association between relapse and GVHD further in the entire cohort. In multivariable analysis treating GVHD as a time-dependent variable, we found that the occurrence of acute GVHD was not associated with relapse but associated with an increased risk of NRM (HR=1.62, HR=4.9, HR=5.99 with P=0.03; P<0.0001; P<0.0001 for grade 2, 3, 4, respectively, compared to no or grade 1 acute GVHD). This increased risk of NRM did not affect PFS (HR=1.12; P=0.32) or OS (HR=1.16; P=0.21) in patients with grade 2 acute GVHD but it affected them in patients with grade 3-4 acute GVHD

(*Online Supplementary Table S4A*). Unlike acute GVHD, the occurrence of chronic GVHD was associated with a lower risk of relapse (HR=0.59, HR=0.43, HR=0.26 with *P*=0.014; *P*=0.0002; *P*<0.0001 for mild, moderate and severe, respectively, compared to no chronic GVHD). In addition, mild and moderate chronic GVHD were not associated with NRM and only severe chronic GVHD was associated with an increased risk of NRM (HR=2.45; *P*=0.0001 compared to no chronic GVHD). Overall, the occurrence of chronic GVHD was associated with improved OS (HR=0.28, HR=0.21, HR=0.35 with *P*<0.0001 for mild, moderate, severe compared to no chronic GVHD) (*Online Supplementary Table S4A*). This result was largely consistent when restricted to ≥2017 only (*Online Supplementary Table S4B*) and restricted to patients who received PTCY only (*Online Supplementary Table S4C*). Lastly, for long-term survivors who were alive and leukemia-free at 2 years after transplant (N=527), mild or moderate chronic GVHD did not adversely affect relapse, PFS and OS but severe chronic GVHD had a higher risk of NRM (sHR=6.73; *P*<0.0001) and worse PFS (HR=2.51; *P*=0.0004) and OS (HR=2.89; *P*=0.0002) compared to no chronic GVHD (*Online Supplementary Table S5*).

Post-relapse survival

We next investigated outcomes of patients at the time of their first relapse after alloHCT to determine the site(s) of relapse and factors associated with post relapse survival. For the entire relapsed cohort (N=516), the 2-year post-relapse survival was 16% (95% confidence interval [CI]: 13-20%). Univariable and multivariable analyses identified year of transplant (2-year post-relapse survival: 23% for ≥2017 *vs*. 12.6% for <2017, HR=0.61; *P*<0.0001), time from transplant to relapse (2-year post-relapse survival: 5%, 11%, 24%, 34% for ≤3 months, 3-<6 months, 6-≤12 months *vs*. >12 month from HCT to relapse; HR=3.04, HR=2.06, HR=1.4; *P*<0.0001; *P*<0.0001; *P*=0.045, respectively), and absence of 8/8 matched related donor (2-year post-relapse survival: 12.7% *vs*. 29%; HR=1.78; *P*<0.0001) as risk factors for inferior post-relapse survival (Figure 3A-C; *Online Supplementary Table S6*). When DLI and subsequent alloHCT were included as time dependent variables, both cellular therapies were favorable for post-relapse survival (HR=0.76; *P*=0.046; HR=0.78; *P*=0.16, respectively) although subsequent alloHCT did not reach statistical significance. We also investigated an interaction between year of transplant and conditioning

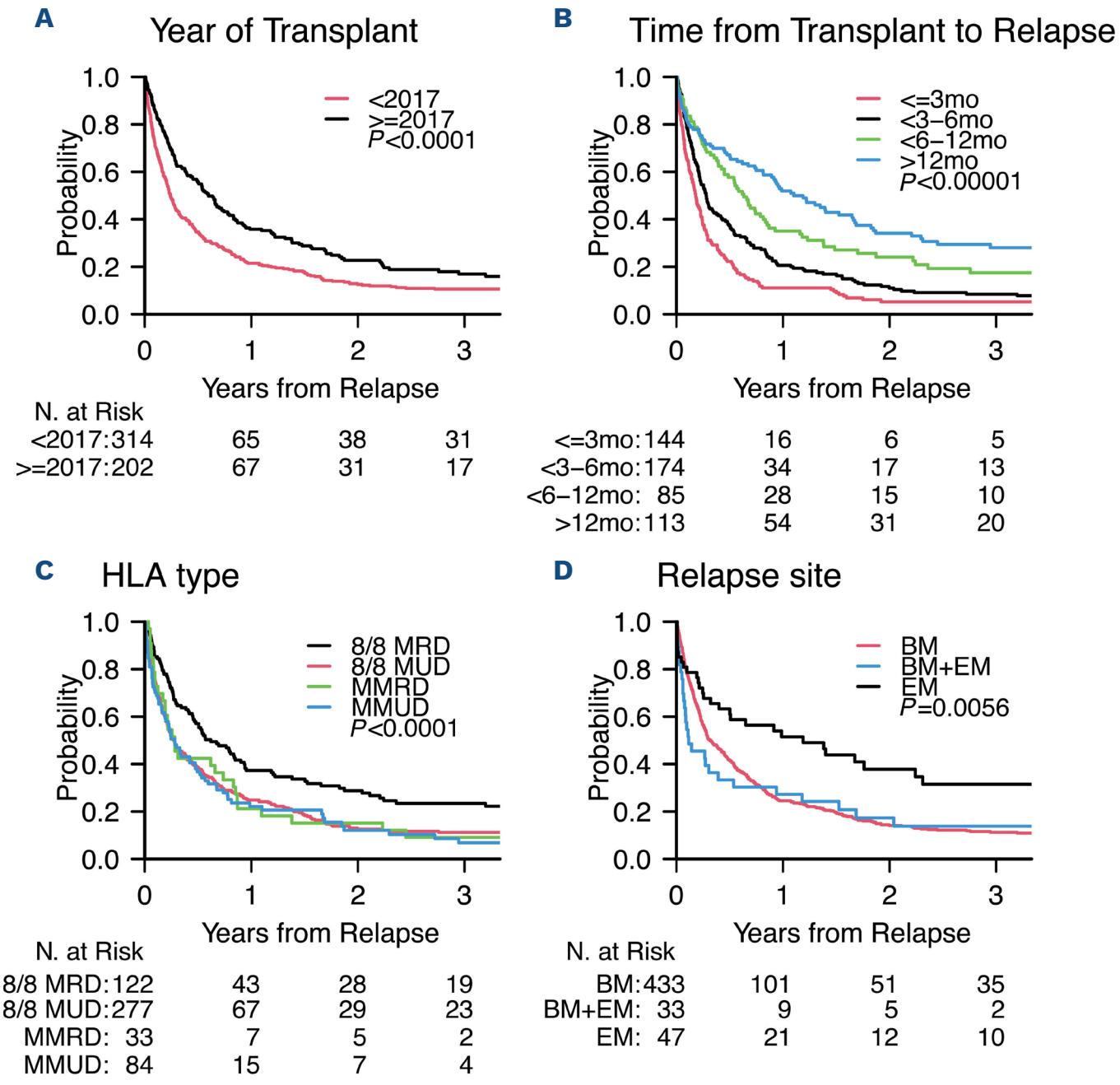

Figure 3. Post-relapse survival. According to (A) year of transplant, (B) time from transplant to relapse, (C) HLA type and (D) relapse site. Twelve patients with missing relapse site were excluded. Log-rank test was used for group comparison. 8/8 MRD: 8/8 matched related donor; 8/8 MUD: 8/8 matched unrelated donor; MMRD: mismatched related donor including haploidentical transplant; MMUD: mismatched (≤7/8) unrelated donor; BM: bone marrow; EM: extramedullary disease; mo: months.

intensity and the interaction from the multivariable analysis was significant (*P*<0.0001). We found that post-relapse survival was similar between two periods in RIC (14% ≥2017 *vs*. 15% for <2017 at 2-year post relapse; *P*=0.4), but survival of patients who relapsed after MAC transplants (mostly Bu4/flu) since 2017 was much better than those who relapsed after MAC transplants (mostly Cy/TBI, Bu/Cy) before 2017 (35% for ≥2017 *vs*. 9% for <2017; *P*<0.00001) (*Online Supplementary Figure S2*).

We also investigated relapse site. Over 80% (85.4%) had marrow (BM) only involvement after first relapse, 8.7% extramedullary (EM) only involvement and the remaining 6.5% had both BM and EM (BM+EM) involvement at time of their first relapse. Median time from transplant to relapse was 4.6, 3.9, and 15.1 months for BM, BM+EM and EM involvement, respectively (*P*<0.0001), suggesting that isolated EM relapse is more common later after alloHCT (*Online Supplementary Table S6*). Also, post-relapse survival was significantly higher for patients who had isolated EM disease at time of relapse: 14% for BM, 17% for BM+EM and 38% for EM only at 2-years post relapse (*P*=0.0056). HR from multivariable analysis was 0.43 (*P*<0.0001) for EM compared to BM (Figure 4D; *Online Supplementary Table S6*).

Discussion

While transplant-related mortality has steadily declined in recent decades, disease relapse has emerged as the most prominent cause of treatment failure after alloHCT in the current era.[4,12] The concern for higher relapse after alloHCT is even more relevant in the era of better molecular prognostication tools in AML, where patients with high-risk mutations/genetics are more frequently referred for alloHCT. As such, one might hypothesize that relapse incidence would be worse in recent years as our transplanted AML population is higher risk and older. Nevertheless, this large retrospective study of patients with AML who underwent their first allogeneic transplant from 2010 through 2022 at our institution demonstrated a significant and steady decrease in the incidence rates of morphologic relapse since 2017. Notably, the relapse rate has decreased by 25% from 48% in the period 2010-2016 to 36% in the period 2017-2022 resulting in significant improvements in OS from 47% to 62% and PFS from 41% to 53% in the period from 2010-2016 to 2017-2022. Importantly, the incidence of severe acute GVHD also decreased significantly over this period, while the decline in chronic GVHD incidence is more recent. In 2023, the 1-year rate of moderate-severe chronic GVHD was 7.7%, with the projected 2-year rate expected to be around 10%, indicating that it is likely to continue decreasing in the future. These results are remarkable in that both relapse and acute GVHD rates have significantly decreased together, likely reflecting major collective advances that have been made in the field in the last decade. In regard with the stable NRM rate over the decade spanning 2010-2022, the 3-year NRM in the period from 2010-2016 was already very low at around 10-11%. While the acute GVHD rate did decrease significantly, this might not have translated into a further decline in NRM because patients transplanted in the more recent era had significantly higher HCT-CI score (*P*<0.0001) and older (*P*<0.0001) who may have other age-related competing causes of death. These two factors were significant risk factors for increased risk of NRM (*Online Supplementary Table S2A*).

The observation of reduced relapse in the face of reduction in GVHD might appear contrary to the dogma linking GVHD to graft-*versus*-leukemia (GVL). However, when we investigated GVHD as time-dependent variables on relapse and NRM, acute GVHD was not associated with relapse but was associated with NRM. Particularly, severe acute GVHD, albeit a low incidence rate in recent years, is a significant risk factor for NRM. In contrast, chronic GVHD was associated with a reduced risk of relapse and an improved OS, consistent with previous reports.[22,23] Thus, even though both relapse and acute GVHD rates have decreased in recent years, patients with chronic GVHD are still two to four times less likely to relapse, thus still maintaining the competing risks relationship between these two events. Importantly, patients with chronic GVHD are three to four times more likely to survive longer. As most relapses and chronic GVHD occur within 2 years of transplant, we further investigated the impact of chronic GVHD on long-term survivors who were alive and leukemia-free at 2 years. In that landmark analysis, we found that only those patients who had severe chronic GVHD had a high risk of NRM compared to those with no chronic GVHD. Taken together, chronic GVHD is initially protective against relapse but for long-term survivors, severe chronic GVHD is detrimental.[24]

We also found that patients who were transplanted in recent years had longer post-relapse survival. This likely reflects the increasing availability and effectiveness of salvage and targeted therapies in this disease. Indeed, as more patients could be salvaged effectively with therapies after relapse, we observed a higher number of relapsed patients in the recent era who proceeded to donor lymphocyte infusion or second alloHCT (27% in <2017 *vs*. 42% >=2017; *P*=0.001). More detailed investigation on post relapse treatment is underway. In our investigation on relapse site, we found that patients with isolated extramedullary (EM) relapse at their first relapse had much better post-relapse survival compared to patients who relapsed with BM or BM+EM involvements (2-year post-relapse survival: 40% *vs*. 14% *vs*. 17%, respectively). In the case of BM+EM, the outcome is as poor as BM alone, suggesting BM involvement dominates the outcome. The reason for why isolated EM relapses have better survival is not entirely clear, but it may be related to longer time from HCT to EM relapse and the fact that EM relapse reflects tumor immune escape in sanctuary sites, while the GVL effect is preserved to some extent in the blood and marrow. The question of whether the biology and mechanism

of immune-escape between BM and EM relapse is different warrants further research.[25]

In the past 10 years, our center has made a number of changes in practice that could have accounted, at least in part, for the improvements we observed in this study. First, we stopped using sirolimus as part of GVHD prophylaxis in MAC after observing a high incidence of VOD/SOS[26] but kept sirolimus in many of our RIC regimens until recent years.[27] Another important practice change since about 2017 has been the shift away from umbilical cord blood transplantation or standard tac/mtx/sirolimus-based GVHD prophylaxis in favor of PTCY-based transplantation in all patients with HLA-mismatched unrelated or haploidentical related donors. This shift is also likely responsible for the favorable outcomes we observed in recent years. Indeed, the positive impact of PTCY on GVHD was highlighted in the BMT CTN 1703 study,[28] as well as in our recent report.[21] After 2017, our center also shifted towards using a higher dose of busulfan in our Flu/Bu RIC transplants, with busulfan IV dose of 6.4 mg/kg instead of 3.2 mg/kg to give some added dose intensity. In the MAC setting. there was also a shift away from high-dose Cy/TBI and Bu/Cy in favor of the reduced-toxicity MAC regimen of Flu/Bu4, following the randomized phase III trial between MAC and RIC in AML/MDS[28,29] and our own studies.[31,32] These changes in conditioning regimens could also have contributed to the lower relapse and improved outcomes we observed in recent years. In addition, other practice changes such as use of post transplant maintenance therapies with *FLT3*-internal tandem duplication inhibitors for patients with the mutation could also have led to the reduction in relapse in the recent era.[33-35] A number of patients with high-risk AML were treated on recent clinical trials adding venetoclax to the conditioning regimen and HMA/venetoclax maintenance therapy post-transplant;[36-37] although the benefit of adding venetoclax to the conditioning regimen and/or maintenance therapy is unclear and requires further randomized trials. Evolution of pre-transplant induction regimens coupled with the advent of measurable residual disease (MRD) essays could also have led to improved transplant outcomes as more patients are now receiving HMA based regimens, or liposomal daunorubicin/cytarabine (Vyxeos®) that allow AML patients to achieve deeper CR with less toxicity, so they are in better condition upon admission for transplant. We also now routinely use molecular testing results to guide pre-transplant therapy as well as to guide selection of transplant conditioning intensity/regimen.

Our study is subject to the inherent limitations of a single-center retrospective study. We used the standard definition of morphologic relapse, so patients who had MRD relapse who were salvaged and never had frank relapse were not captured. Another important limitation is that MRD status at transplant was not available for most patients who were transplanted before 2018, as high resolution flow cytometry MRD testing was not available or part of standard clinical practice in the earlier years. Our study is also limited by the fact that molecular mutation status for most of our patients in the earlier years are not available. Molecular testing based on next-generation sequencing was started only after 2015. Therefore, we cannot ascertain the molecular risk profile of most patients in our <2017 cohort, and assess what proportion of these patients, for example, had *TP53* or other very high-risk molecular mutations. Our recent study on the effect of molecular genetics using next-generation sequencing on transplant outcomes, however, showed that 11% of our transplanted patients had *TP53* mutation at diagnosis.[38] It is important to note that at our center, patients with *TP53* mutations and/or other very high-risk features are not steered away from transplant, but are strongly encouraged to be transplanted on clinical trials.

In conclusion, our large single transplant center experience shows clear and encouraging progress with allogeneic transplantation for patients with AML in recent years, with steady decline in incidence of relapse and GVHD that have translated into significant improvements in PFS and OS. Further studies are warranted to assess the reasons for these improvements, and if similar encouraging results are seen at other transplant centers and in analysis of registry data.

Disclosures

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Contributions

HTK conceived the project idea, designed the study, performed statistical analysis, interpreted the results and wrote the manuscript. VTH collected and procured relapse data and edited the manuscript. RS and JR also edited the manuscript. All authors reviewed the manuscript and approved the final version for submission.

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Data-sharing statement

De-identified individual participant data that underlie the reported results will be made available 6 months after publication for a period of 1 year after the publication date to researchers who provide a sound proposal. The data will be provided in compliance with applicable privacy laws, data protection, and requirements for consent and anonymization. Proposals for access should be sent to htkimc@jimmy.harvard.edu.

References

1. Döhner H, Weisdorf DJ, Bloomfield CD. Acute myeloid leukemia. N Engl J Med. 2015;17;373(12):1136-1152.
2. D'Souza A, Fretham C, Lee SJ, et al. Current use of and trends in hematopoietic cell transplantation in the United States. Biol Blood Marrow Transplant. 2020;26(8):e177-e182.
3. Phelan R, Chen M, Bupp C, et al. Updated trends in hematopoietic cell transplantation in the United States with an additional focus on adolescent and young adult transplantation activity and outcomes. Transplant Cell Ther. 2022;28(7):409.e1-409.e10.
4. Horowitz M, Schreiber H, Elder A, et al. Epidemiology and biology of relapse after stem cell transplantation. Bone Marrow Transplantation. 2018;53(11):1379-1389.
5. Thomas ED, Lochte HL Jr, Lu WC, Ferrebee JW. Intravenous infusion of bone marrow in patients receiving radiation and chemotherapy. N Engl J Med.1957;257(11):491-496.
6. Thomas ED, Storb R, Clift RA, et al. Bone marrow transplantation. N Engl J Med. 1975;292(17):895-902.
7. Thomas ED, Buckner CD, Clift RA, et al. Marrow transplantation for acute non-lymphoblastic leukemia in first remission. N Engl J Med. 1979;301(11):597-599.
8. Singh AK, McGuirk JP. Allogeneic stem cell transplantation: a historical and scientific overview. Cancer Res. 2016;76(22):6445-6451.
9. Henig I, Zuckerman T. Hematopoietic stem cell transplantation - 50 years of evolution and future perspectives. Rambam Maimonides Med J. 2014;5(4):e0028.
10. Gooley TA, Chien JW, Pergam SA, et al. Reduced mortality after allogeneic hematopoietic-cell transplantation. N Engl J Med. 2010;363(22):2091-2101.
11. Carpenter PA, Gooley TA, Boiko JR, et al. Decreasing chronic graft-versus-host disease rates in all populations. Blood Adv. 2024;26;8(22):5829-5837.
12. Dohner H, Wei AH, Appelbaum FR, et al. Diagnosis and management of AML in adults: 2022 recommendations from an international expert panel on behalf of the ELN. Blood. 2022; 140(12):1345-1377.
13. Przepiorka D, Weisdorf D, Martin P, et al. 1994 Consensus conference on acute GVHD grading. Bone Marrow Transplant. 1995;15(6):825-828.
14. Filopovich AH, Weisdorf D, Pavletic S, et al. National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. Diagnosis and Staging Working Group report. Biol Blood Marrow Transplant. 2005;11(12):945-956.
15. Jagasia MH, Greinix HT, Arora M, et al. National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. The 2014 Diagnosis and Staging Working Group report. Biol Blood Marrow Transplant. 2015;21(3):389-401.e1.
16. Gray RJ. A Class of K-Sample tests for comparing the cumulative incidence of a competing risk. Ann Stat. 1988;16(3):1141-1154.
17. Fine JP, Gray RJ. A Proportional hazards model for the subdistribution of a competing risk. J Am Stat Assoc. 1999:94(446):496-509.
18. Harrell FE. Regression modeling strategies: with applications to linear models, logistic regression, and survival analysis. Springer New York, NY; 2001.
19. Breiman L., Friedman JH, Olshen RA, Stone CJ. Classification and regression trees. Taylor & Francis. 1984.
20. T.M Therneau and E.J Atkinson. An introduction to recursive partitioning using the rpart routines. https://www.stat.auckland.ac.nz/~yee/784/files/techrep.pdf. Accessed April 1, 2025.
21. Maurer K, Ho, VT, Inyang E, et al. Posttransplant cyclophosphamide vs tacrolimus-based GVHD prophylaxis: lower incidence of relapse and chronic GVHD. Blood Adv. 2023;7(15):3903-3915.
22. Weiden PL, Flournoy N, Thomas ED, et al. Anti leukemic effects of graft versus host disease in human recipients of allogeneic marrow grafts. N Engl J Med. 1979;300(19):1068-1073.
23. Weiden PL, Sullivan KM, Flournoy N, Storb R, Thomas ED, Seattle Marrow Transplant Team. Anti leukemic effect of chronic graft-versus-host disease: contribution to improved survival after allo geneic marrow transplantation. N Engl J Med. 1981;304(25):1529-1533.
24. Pidala JA, Gooley TA, Luznik L, Blazar BR. Chronic graft-versus-host disease: unresolved complication or ancient history? Blood. 2024;144(13):1363-1373.
25. Zeiser, R and Vago L. Mechanisms of immune escape after allogeneic hematopoietic cell transplantation. Blood. 2019;133(12):1290-1297.
26. Roeker LE, Kim HT, Glotzbecker B, et al. Early clinical predictors of hepatic veno-occlusive disease/sinusoidal obstruction syndrome after myeloablative stem cell transplantation. Biol Blood Marrow Transplant. 2019;25(1):137-144.
27. Lewis C, Kim HT, Roeker LE, et al. Incidence, Predictors, and outcomes of veno-occlusive disease/sinusoidal obstruction syndrome after reduced-intensity allogeneic hematopoietic cell transplantation. Biol Blood Marrow Transplant. 2020;26(3):529-539.
28. Bolaños-Meade J, Hamadani M, Wu J, et al. Post-transplantation cyclophosphamide-based graft-versus-host disease prophylaxis. N Engl J Med. 2023;388(25):2338-2348.
29. Scott BL, Pasquini MC, Logan BR, et al. Myeloablative versus reduced-intensity hematopoietic cell transplantation for acute

myeloid leukemia and myelodysplastic syndromes. J Clin Oncol. 2017;35(11):1154-1161.
30. Scott BL, Pasquini MC, Fei M, et al. Myeloablative versus reduced-intensity conditioning for hematopoietic cell transplantation in acute myelogenous leukemia and myelodysplastic syndromes-long-term follow-up of the BMT CTN 0901 clinical trial. Transplant Cell Ther. 2021;27(6):483.e1-483.e6.
31. Chen YB, Coughlin E, Kennedy KF, et al. Busulfan dose intensity and outcomes in reduced-intensity allogeneic peripheral blood stem cell transplantation for myelodysplastic syndrome or acute myeloid leukemia. Biol Blood Marrow Transplant. 2013;19(6):981-987.
32. Gooptu M, Kim HT, Ho VT, et al. A comparison of the myeloablative conditioning regimen fludarabine/busulfan with cyclophosphamide/total body irradiation, for allogeneic stem cell transplantation in the modern era: a cohort analysis. Biol Blood Marrow Transplant. 2018;24(8):1733-1740.
33. Chen Y-B, Li S, Lane AA, et al. Phase I trial of maintenance sorafenib after allogeneic hematopoietic stem cell transplantation for FMS-like tyrosine kinase 3 internal tandem duplication acute myeloid leukemia. Biol Blood Marrow Transplant. 2014;20(12):2042-2048.
34. Stone RM, Mandrekar SJ, Sanford BL, et al. Midostaurin plus chemotherapy for acute myeloid leukemia with a FLT3 mutation. N Engl J Med. 2017;377:454-464.
35. Burchert A, Bug G, Fritz LV, et al. Sorafenib maintenance after allogeneic hematopoietic stem cell transplantation for acute myeloid leukemia with FLT3-internal tandem duplication mutation (SORMAIN). J Clin Oncol. 2020;38(26):2993-3002.
36. Garcia JS, Kim HT, Murdock HM, et al. Adding venetoclax to fludarabine/busulfan RIC transplant for high-risk MDS and AML is feasible, safe, and active. Blood Adv. 2021;5(24):5536-5545.
37. Garcia JS, Kim HT, Murdock HM, et al. Prophylactic maintenance with venetoclax/azacitidine after reduced-intensity conditioning allogeneic transplant for high-risk MDS and AML. Blood Adv. 2024;8(4):978-990.
38. Murdock HM, Kim HT, Denlinger N, et al. Impact of diagnostic genetics on remission MRD and transplantation outcomes in older AML patients. Blood. 2022;139(24):3546-3557.