original reports abstract

Donor Clonal Hematopoiesis and Recipient Outcomes After Transplantation

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PURPOSE Clonal hematopoiesis (CH) can be transmitted from a donor to a recipient during allogeneic hematopoietic cell transplantation. Exclusion of candidate donors with CH is controversial since its impact on recipient outcomes and graft alloimmune function is uncertain.

PATIENTS AND METHODS We performed targeted error-corrected sequencing on samples from 1,727 donors age 40 years or older and assessed the effect of donor CH on recipient clinical outcomes. We measured long-term engraftment of 102 donor clones and cytokine levels in 256 recipients at 3 and 12 months after transplant.

RESULTS CH was present in 22.5% of donors, with *DNMT3A* (14.6%) and *TET2* (5.2%) mutations being most common; 85% of donor clones showed long-term engraftment in recipients after transplantation, including clones with a variant allele fraction < 0.01. *DNMT3A*-CH with a variant allele fraction ≥ 0.01, but not smaller clones, was associated with improved recipient overall (hazard ratio [HR], 0.79; $P = .042$) and progression-free survival (HR, 0.72; $P = .003$) after adjustment for significant clinical variables. In patients who received calcineurin-based graft-versus-host disease prophylaxis, donor *DNMT3A*-CH was associated with reduced relapse (subdistribution HR, 0.59; $P = .014$), increased chronic graft-versus-host disease (subdistribution HR, 1.36; $P = .042$), and higher interleukin-12p70 levels in recipients. No recipient of sole *DNMT3A* or *TET2*-CH developed donor cell leukemia (DCL). In seven of eight cases, DCL evolved from donor CH with rare *TP53* or splicing factor mutations or from donors carrying germline *DDX41* mutations.

CONCLUSION Donor CH is closely associated with clinical outcomes in transplant recipients, with differential impact on graft alloimmune function and potential for leukemic transformation related to mutated gene and somatic clonal abundance. Donor *DNMT3A*-CH is associated with improved recipient survival because of reduced relapse risk and with an augmented network of inflammatory cytokines in recipients. Risk of DCL in allogeneic hematopoietic cell transplantation is driven by somatic myelodysplastic syndrome–associated mutations or germline predisposition in donors.

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INTRODUCTION

Allogeneic hematopoietic cell transplantation is the only curative treatment for most high-risk hematologic malignancies. Patients with an available donor have improved overall survival (OS) compared with patients who do not have a donor.[1,2] The National Marrow Donor Program (NMDP) prioritizes donors under age 45[3] years because younger donor age has been associated with improved recipient survival,[4] but suitable younger donors are not uniformly available to all transplant candidates. The likelihood of finding a matching donor in the NMDP Be The Match Registry varies widely depending on ethnic background. Although HLA-matched adult donors can be identified in the NMDP registry for 75% of White patients, far fewer patients of color will find matched donors (16%-19% of African Americans, 33%-42% of Asian Americans, and 34%-40% of Hispanic Americans).[5] The best available graft for many patients may thus be from an older donor such as an HLA-identical sibling or haploidentical relative,[6,7] highlighting the importance of understanding characteristics of older donors that influence recipient outcomes.

Clonal hematopoiesis (CH) is an age-related, asymptomatic condition in which leukemia-associated somatic mutations are detected in the blood of individuals without a hematologic malignancy. In the nontransplant setting, CH is uniformly associated with adverse outcomes, including an elevated risk of developing hematologic malignancies[8,9] and an increased risk of nonhematologic outcomes because of altered inflammatory signaling.[10] These clinical effects of native CH are apparent with large clones,[8,10] but CH at very low clonal abundance with an uncertain biologic effect has been reported to be ubiquitous in adults over age 40 years.[11]

ASSOCIATED CONTENT

Data Supplement

Author affiliations and support information (if applicable) appear at the end of this article.

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CONTEXT

Key Objective

Clonal hematopoiesis (CH) can be transmitted from a donor to a recipient during allogeneic hematopoietic cell transplantation, but its impact on recipient outcomes is unclear. This study assessed the frequency of CH in 1,727 transplant donors age 40 years and older and its effect on survival, relapse, and graft-versus-host disease (GVHD) in recipients.

Knowledge Generated

CH with mutations in *DNMT3A* was associated with improved overall survival, reduced risk of relapse, and elevated risk of chronic GVHD in patients who received traditional calcineurin inhibitor–based GVHD prophylaxis. Rare cases of donor cell leukemia evolved from CH with less common mutations in *TP53* and splicing factor genes.

Relevance

Older individuals with the most common forms of CH need not be excluded from stem-cell donation. More broadly, inhibiting de novo DNA methylation in donor immune cells could reduce risk of relapse in transplant recipients.

Exploratory studies have found that CH in transplant donors can engraft in recipients[12-15] but have reported conflicting results on the impact of donor CH on transplant-specific clinical outcomes, graft immunologic function, and risk of donor cell leukemia (DCL).[12,13,16,17] These studies have been limited by modest sample sizes that affected outcomes analysis, cohort characteristics that restricted generalizability, and a lack of mechanistic rationale. Furthermore, these cohorts have not parsed the clinical impact of different CH mutations or used sequencing technologies that support evaluation of low-abundance clones, which could have unique dynamics in the context of allogeneic transplantation. Current evidence has thus been insufficient to resolve disagreement about whether to screen older candidate donors for CH,[18,19] and some transplant centers have begun excluding donors found to have CH on the basis of the assumption that the adverse associations of native CH also apply in the context of transplant.[20] In this study, we performed a comprehensive analysis of samples from donors age 40 years or older to determine the impact of CH on overall recipient outcomes, risk of DCL, and measures of graft alloimmune activity.

PATIENTS AND METHODS

Patients

All patients who underwent transplantation with donor age 40 years or older at Dana-Farber Cancer Institute (Cohort 1) or Johns Hopkins University (Cohort 2) between 2000 and 2016 were considered for study inclusion, and 1,727 samples met technical requirements for analysis (Data Supplement, online only). The study was conducted with approval and waivers of consent from both institutional review boards. Additional details are provided in the Data Supplement (online only).

Genetic Studies

We sequenced 46 genes mutated recurrently in CH and myeloid malignancies (Data Supplement).[5,6,19-21] We included samples with average unique molecular identifier–deduplicated consensus coverage of at least 1,000× and considered all variants with a variant allele fraction (VAF) ≥ 0.005. We classified variants as pathogenic on the basis of published genetic criteria.[20,22] Genetic analysis was blinded to clinical characteristics and locked before merging with clinical data.

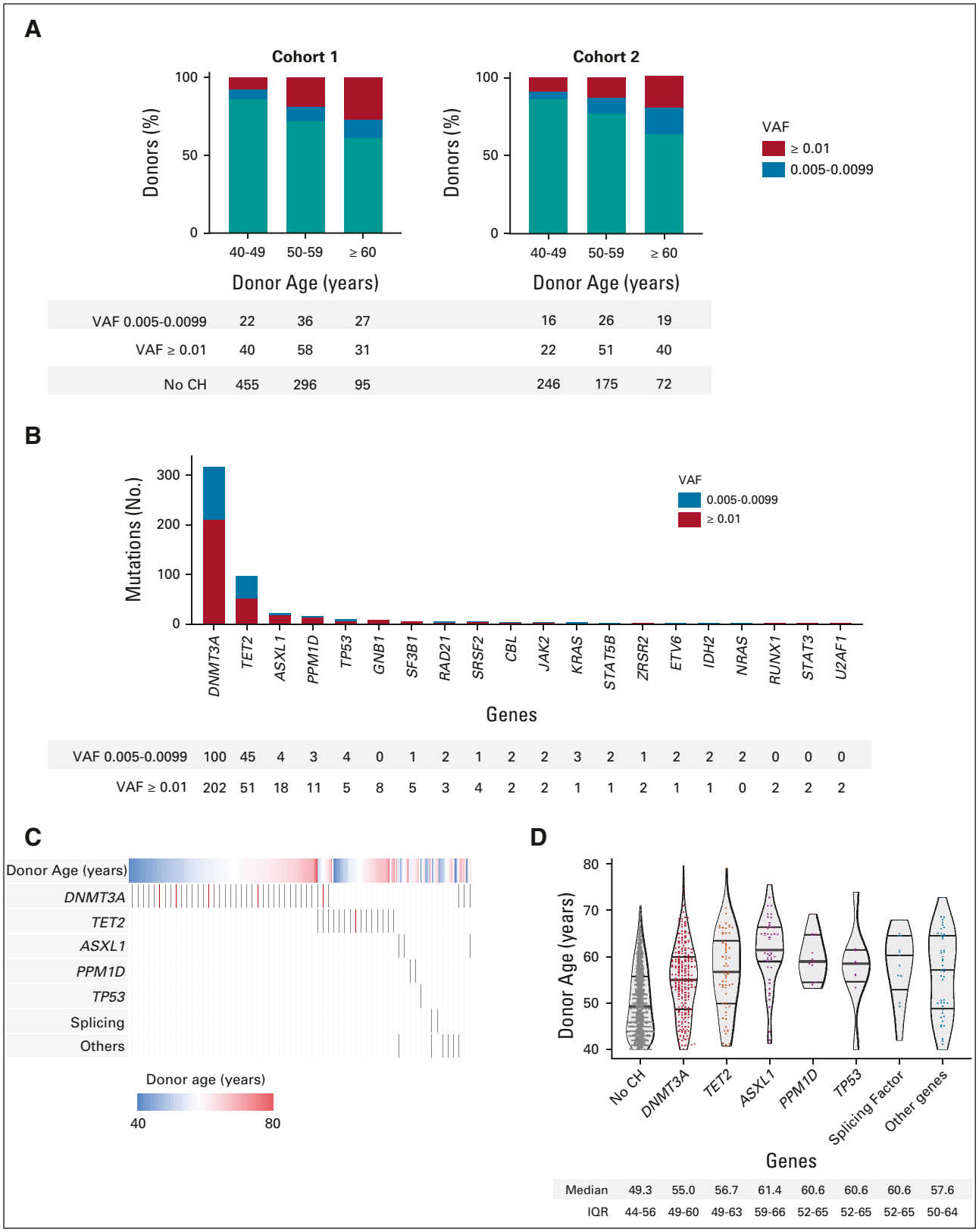
Statistical Analysis

The primary end point was progression-free survival (PFS), defined as the time from transplantation until death or relapse of the original disease, whichever occurred first. OS was defined as the time from transplantation until death from any cause or until censoring at the time last known to be alive. OS and PFS were estimated using the Kaplan-Meier method, and the difference was tested using log-rank tests. Cumulative incidences of nonrelapse mortality (NRM), relapse, and graft-versus-host disease (GVHD) were estimated in competing risk frameworks that treated relapse, NRM, and relapse or death without GVHD as competing events, respectively. Cumulative incidences were compared using the Gray test.[23] Multivariable analysis was performed using Cox models for OS and PFS and Fine and Gray models for NRM, relapse, and GVHD.[23,24] Additional details are provided in the Data Supplement.

RESULTS

Clinical and Genetic Characteristics of Donor CH

We identified CH with VAF ≥ 0.005 in 388 of 1,727 donors (22.5%, Data Supplement). Its prevalence increased with advancing age: CH was present in 12.6% of donors age 40-49 years, 26.6% of donors age 50-59 years, and 41.2% of donors age 60 years or older. The proportion of donors with CH in each age decade was similar in both cohorts (Fig 1A). In multivariable analysis stratified by cohort and considering donor, recipient, and transplant variables, only older donor age was independently associated with the presence



VAF 0.005-0.0099	22	36	27		16	26	19	
VAF ≥ 0.01	40	58	31		22	51	40	
No CH	455	296	95		246	175	72	

VAF 0.005-0.0099	100	45	4	3	4	0	1	2	1	2	2	3	2	1	2	2	2	0	0	0
VAF ≥ 0.01	202	51	18	11	5	8	5	3	4	2	2	1	1	2	1	1	0	2	2	2

Median	49.3	55.0	56.7	61.4	60.6	60.6	60.6	57.6
IQR	44-56	49-60	49-63	59-66	52-65	52-65	52-65	50-64

FIG 1. Characteristics of CH in transplant donors age 40 years and older. (A) The proportion of donors with and without CH in each cohort, subdivided by donor age decade. CH with VAF 0.005-0.0099 is in blue, and CH with VAF ≥ 0.01 is in red. (B) The number of mutations in each gene mutated in two or more donors, with variants at VAF 0.005-0.0099 again in blue and variants at VAF ≥ 0.01 in red. (C) The patterns of comutation among donors with CH. Each column represents a donor, with rows for donor age and the most commonly mutated genes or gene groups. Donors were hierarchically grouped on the basis of the presence of mutations in genes other than *DNMT3A* or *TET2*, *DNMT3A*, and then *TET2*. Genes mutated more than once in the same donor are in red. (D) The distribution of donor age on the basis of CH status and mutations in individual genes or groups of genes. Medians and IQRs are reported below each column. CH, clonal hematopoiesis; IQR, interquartile range; VAF, variant allele fraction.

TABLE 1. Characteristics of Donors and Recipients of Donors With and Without CH

Characteristic	All	No CH	CH	UV *P*	MV *P*
Full cohort	**1,727 (100)**	**1,339 (77.5)**	**388 (22.5)**		
DFCI	1,060 (61.3)	846 (63.2)	214 (55.2)	.005	NA
JHU	667 (38.7)	493 (36.8)	174 (44.8)		
Recipient age, years, median (range)	55 (0.5-78)	54 (0.5-78)	56 (6-76)	.45	—
Recipient sex				.64	—
Female	700 (40.5)	547 (40.8)	153 (39.4)		
Male	1,027 (59.5)	792 (59.2)	235 (60.6)		
Donor sex				.13	—
Female	813 (47.1)	617 (46.1)	196 (50.5)		
Male	908 (52.6)	717 (53.5)	191 (49.2)		
Unknown	6 (0.3)	5 (0.4)	1 (0.3)		
Donor age, years, median (range)	51 (40-80)	49 (40-71)	56 (40-80)	< .001	< .001
Related donors	53 (40-80)	52 (40-71)	57 (40-80)		
Unrelated donors	46 (40-60)	45 (40-60)	46 (40-59)		
DRI				.84	—
Low	291 (16.9)	229 (17.1)	62 (15.9)		
Intermediate	1,020 (59.1)	781 (58.3)	239 (61.6)		
High	292 (16.9)	229 (17.1)	63 (16.3)		
Very high	61 (3.5)	47 (3.5)	14 (3.6)		
NA	63 (3.6)	53 (6.2)	10 (2.6)		
Disease category				.37	—
Disease				.33	
Lymphoid	718 (41.6)	501 (41.4)	151 (42.3)		
NHL	319 (18.5)	243 (18.1)	76 (19.6)		
ALL	149 (8.6)	116 (8.6)	33 (8.5)		
Chronic lymphocytic leukemia	116 (6.7)	92 (6.9)	24 (6.2)		
Hodgkin lymphoma	68 (3.9)	50 (3.7)	18 (4.7)		
Multiple myeloma	66 (3.8)	53 (4)	13 (3.4)		
Myeloid	929 (53.8)	718 (53.6)	211 (54.3)		
AML	609 (35.3)	455 (34.1)	154 (39.7)		
MDS	189 (10.9)	154 (11.5)	35 (9)		
Chronic myeloid leukemia	63 (3.6)	55 (4.1)	8 (2.1)		
MPN	36 (2.1)	26 (1.9)	10 (2.6)		
MDS/MPN overlap	32 (1.9)	28 (2.1)	4 (1)		
Others	80 (4.6)	67 (5)	13 (3.4)		
RBC disorder	48 (2.8)	40 (3)	8 (2.1)		
Other disease	19 (1.1)	16 (1.2)	3 (0.8)		
Other leukemia	13 (0.8)	11 (0.8)	2 (0.5)		
HCT-CI				.59	—
0	576 (33.7)	450 (33.6)	126 (32.4)		
1-2	548 (31.7)	415 (31)	133 (34.3)		
≥ 3	585 (34.2)	458 (34.2)	127 (32.7)		

(continued on following page)

TABLE 1. Characteristics of Donors and Recipients of Donors With and Without CH (continued)

Characteristic	All	No CH	CH	UV *P*	MV *P*
Unknown	18 (1)	16 (1.2)	2 (0.5)		
Median (range)	1 (0-12)	1 (0-10)	1 (0-12)		
Graft source				.06	.98
BM	703 (40.7)	526 (39.3)	177 (45.6)		
BM and PBSC	2 (0.1)	2 (0.1)	0		
PBSC	1,022 (59.2)	811 (60.6)	211 (54.4)		
Conditioning intensity				.23	—
Myeloablative	632 (36.6)	500 (37.3)	132 (34)		
TBI-based	323 (51.1)	275 (55)	48 (36.4)		
Nonmyeloablative	1,094 (63.3)	838 (62.3)	256 (66)		
TBI-based	482 (44.1)	356 (42.5)	126 (49.2)		
Unknown	1 (0.1)	1			
Donor type				< .0001	.51
Haploidentical	454 (26.3)	333 (24.8)	121 (31.2)		
Mismatched, related	38 (2.2)	30 (2.2)	8 (2.1)		
Mismatched, unrelated	71 (4.1)	58 (4.3)	13 (3.3)		
Matched, related	889 (51.5)	674 (50.3)	215 (55.4)		
Matched, unrelated	273 (15.8)	242 (18.1)	31 (8)		
Syngeneic	2 (0.1)	2			
GVHD prophylaxis				.003	.66
PTCy	671 (38.9)	495 (37)	176 (45.4)		
Other regimens	1,056 (61.1)	844 (63)	212 (54.6)		

NOTE. Data are No. (%) unless otherwise specified. The top row in bold reports the proportion of donors with and without CH and sums to 100. Otherwise, the distribution within each category sums to 100 within columns. UV comparisons between those with and without CH were performed with chi-square or Fisher's exact test for categorical variables and the Wilcoxon rank-sum test for continuous variables. All variables with UV *P* values < 0.2 were tested for association with the presence of CH in a MV model stratified by the center.

Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; BM, bone marrow; CH, clonal hematopoiesis; DFCI, Dana-Farber Cancer Institute; DRI, disease risk index; GVHD, graft-versus-host disease; HCT-CI, hematopoietic cell transplant comorbidity index; JHU, Johns Hopkins University; MV, multivariable; NA, not available; NHL, Non-Hodgkin lymphoma; PBSC, peripheral blood stem cell; PTCy, post-transplant cyclophosphamide; TBI, total body irradiation; UV, univariable.

of CH (median 56 *v* 49 years with and without CH, respectively, $P < .001$, Table 1).

Among 501 total mutations, 324 had VAF ≥ 0.01 and 177 had VAF of 0.005-0.0099. The most frequently mutated genes were *DNMT3A* (302 mutations in 253 donors), *TET2* (96 mutations in 89 donors), *ASXL1* (22 mutations in 22 donors), and *PPM1D* (14 mutations in 14 donors, Fig 1B). No other gene was mutated in more than 10 donors. Most donors with CH (n = 301, 77.5%) had only one mutation. Donors with mutations other than *DNMT3A* or *TET2* were more likely to have mutations in more than one gene (compared with donors who had *DNMT3A* or *TET2* mutations; odds ratio 3.5; 95% CI, 2.0 to 6.1; $P < .0001$; Fig 1C). Donors with CH were older than those without CH irrespective of the gene mutated (Fig 1D). Using an orthogonal duplex unique molecular identifier–based sequencing platform, we validated 100% (28 of 28) of variants from 20 donors (VAF range 0.0056-0.2159; Data Supplement).

Donor CH and Recipient Outcomes

In native CH, larger clones have a greater effect on clinical outcomes than smaller clones,[8,10] but no evidence-based VAF cutoff for defining CH has been established. To define a clinically relevant VAF threshold for CH in the setting of transplantation, we examined the relative hazards of PFS, relapse, and NRM across the full range of donor clone sizes and found that the effect of CH was greatest with VAF ≥ 0.01 (Data Supplement). Recipients of donor CH with VAF ≥ 0.01 (n = 241) had improved PFS compared with recipients whose donors did not have CH in a multivariable model that included recipient and donor age, donor-recipient sex mismatch, hematopoietic cell transplantation–specific comorbidity index score,[21] conditioning intensity, donor

type, disease category, and Disease Risk Index classification[22] (hazard ratio [HR], 0.79; 95% CI, 0.66 to 0.95; $P = .011$; Fig 2A). CH with only smaller donor clones (VAF 0.005-0.0099, n = 147) was not significantly associated with any outcome.

Individual gene mutations in CH have distinct associations with clinical outcomes. In nontransplant settings, *DNMT3A* and *TET2* mutations have driven the bulk of association with inflammatory outcomes like cardiovascular disease,[10] whereas mutations other than *DNMT3A* or *TET2* have been associated with a higher risk of progression to hematologic malignancy.[10,23,24] We therefore tested the effects of three prespecified, hierarchically defined groups at VAF ≥ 0.01: (1) donors with mutations in any gene other than *DNMT3A* or *TET2* (Other CH, n = 49), (2) remaining donors with *DNMT3A* mutations (*DNMT3A*-CH, n = 157), and (3) remaining donors with only *TET2* mutations (*TET2*-CH, n = 35).

Only donor *DNMT3A*-CH was significantly associated with recipient outcomes. Recipients whose donors had *DNMT3A*-CH had improved OS and PFS and reduced risk of relapse in the same multivariable model (HR for death 0.78; 95% CI, 0.62 to 0.98; $P = .037$; HR for death or relapse 0.72; 95% CI, 0.58 to 0.89; $P = .003$; subdistribution hazard ratio [sHR] for relapse 0.74; 95% CI, 0.57 to 0.96; $P = .022$, Fig 2B and Data Supplement), when compared with recipients whose donors did not have CH with VAF ≥ 0.01. *DNMT3A*-CH was not associated with differences in NRM, and causes of death without relapse were similar irrespective of the presence of *DNMT3A*-CH (Data Supplement). Neither *TET2*-CH nor Other CH had significant impacts on outcomes.

Alloreactive donor immune cells not only reduce relapse via graft-versus-leukemia (GVL) activity but also mediate a complementary risk of GVHD. Conventional GVHD prophylaxis with calcineurin-based regimens suppresses global T cell function, whereas post-transplant cyclophosphamide (PTCy) is thought to prevent GVHD by selective suppression or elimination of pathogenic alloreactive T cells.[25,26] To evaluate the interactions between *DNMT3A*-CH and immune-modulating therapy, we analyzed GVHD outcomes in patients who did or did not receive PTCy. In multivariable analysis, *DNMT3A*-CH with VAF ≥ 0.01 was independently associated with an increased risk of chronic GVHD in recipients who did not receive PTCy (Fig 2C; sHR 1.37; 95% CI, 1.02 to 1.84; $P = .04$ in multivariable analysis). By contrast, we observed no effect of *DNMT3A*-CH on chronic GVHD in recipients who received PTCy (compared with PTCy and no *DNMT3A*-CH; sHR 1.15; 95% CI, 0.82 to 1.6; $P = .88$). The improvements in relapse, PFS, and OS associated with *DNMT3A*-CH were also confined to recipients who did not receive PTCy (Figs 2D-2F and Data Supplement). Among these patients, donor *DNMT3A*-CH was associated with reduced risk of relapse (sHR from multivariable model 0.59; 95% CI, 0.39 to 0.9; $P = .014$) and reduced risk of death (HR 0.65; 95% CI, 0.47 to 0.9; $P = .01$). This difference was evident for both myeloid and lymphoid diseases (Data Supplement), related and unrelated donors (Data Supplement), and was not evidently influenced by either bone marrow graft source (Data Supplement) or haploidentical donors (Data Supplement), both of which are closely associated with PTCy use. There was no association between *DNMT3A*-CH and acute GVHD (Data Supplement).

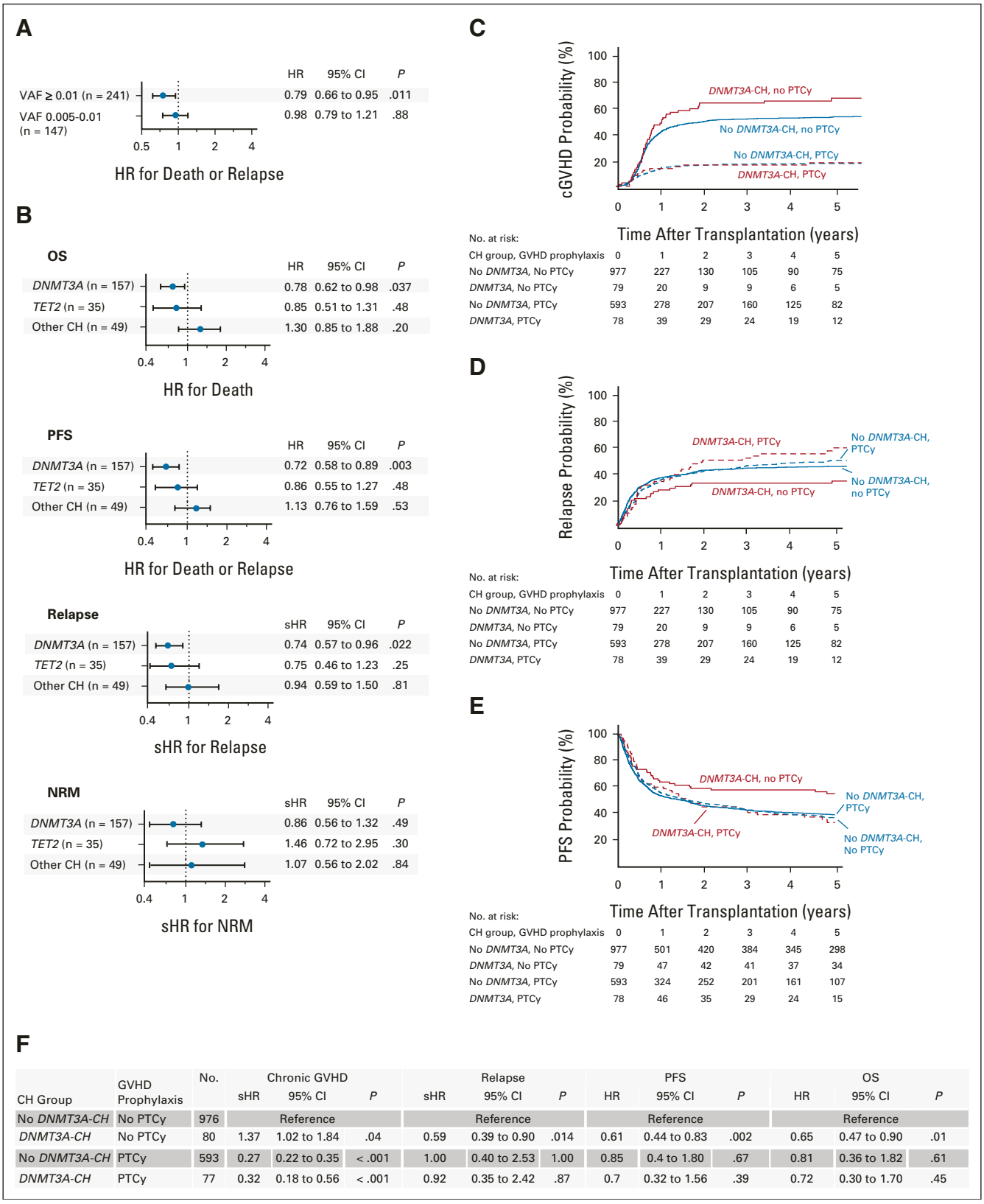
Engraftment and Biologic Characteristics of Donor CH in Recipients

To define the capacity of donor clones to contribute to long-term recipient hematopoiesis, we sequenced samples collected 3 and 12 months after transplant from 69 recipients whose donors had CH and who survived without relapse for at least one year (Data Supplement). In total, 86 of 102 donor mutations were detectable in paired recipients at 12 months (Fig 3A), including 84.9% of *DNMT3A* mutations, 94.4% of *TET2* mutations, and 70.6% of other mutations (Fig 3B). Mutations with VAF ≥ 0.01 engrafted more frequently than mutations with VAF < 0.01 (91.7% *v* 76.2%, $P = .045$, Data Supplement). No other donor, recipient, or transplant variables were associated with 12-month engraftment of the clone (Data Supplement).

DNMT3A encodes the methyltransferase responsible for de novo DNA methylation in hematopoietic stem cells,[27] and a broad spectrum of mutations that variably reduce DNMT3A enzymatic function are frequently identified as early events in myeloid malignancies.[28-30] Since *DNMT3A* R882 hotspot mutations have distinct biochemical function and higher risk of progression to acute myeloid leukemia compared with non-R882 *DNMT3A* mutations,[31-34] we analyzed the long-term engraftment and expansion of 10 R882 and 54 non-R882 clones (median baseline VAF 0.017 *v* 0.012, $P = .20$). All 10 donor R882 mutations were detectable in recipients at 12 months, compared with 46 of 54 non-R882 mutations (85.2%, $P = .34$, Fig 3C). At 12 months, the VAF of R882 mutations was significantly higher than that of non-R882 mutations (median 0.051 *v* 0.021, $P = .004$, Fig 3D, Data Supplement).

In native CH, *DNMT3A*-mutated stem-cell clones invariably contribute to myeloid differentiation and have also been reported to have lymphoid potential.[11,35] The lineage potential of allogeneic donor-engrafted clones, however, has not been assessed. To determine whether *DNMT3A* clones from older donors contribute to the T cell lineage after transplantation, we quantified the representation of 19 *DNMT3A* mutations from 14 donors in purified recipient CD3-positive blood cells one year after transplantation. We found that 18 of 19 (94.7%) donor *DNMT3A* mutations were detectable, including 14 of 15 (93.3%) clones from patients treated with PTCy (Data Supplement).

Native CH has been associated with alterations of inflammatory cytokines,[10] which could modulate graft



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CH Group	GVHD Prophylaxis	No.	Chronic GVHD			Relapse			PFS			OS		
			sHR	95% CI	P	sHR	95% CI	P	HR	95% CI	P	HR	95% CI	P
No *DNMT3A-CH*	No PTCy	976	Reference			Reference			Reference			Reference		
DNMT3A-CH	No PTCy	80	1.37	1.02 to 1.84	.04	0.59	0.39 to 0.90	.014	0.61	0.44 to 0.83	.002	0.65	0.47 to 0.90	.01
No *DNMT3A-CH*	PTCy	593	0.27	0.22 to 0.35	< .001	1.00	0.40 to 2.53	1.00	0.85	0.4 to 1.80	.67	0.81	0.36 to 1.82	.61
DNMT3A-CH	PTCy	77	0.32	0.18 to 0.56	< .001	0.92	0.35 to 2.42	.87	0.7	0.32 to 1.56	.39	0.72	0.30 to 1.70	.45

FIG 2. Association of donor CH with recipient clinical outcomes. (A) The association between donor CH at either VAF 0.005-0.0099 or VAF ≥ 0.01 and recipient PFS in multivariable Cox proportional hazards models. (B) The association between donor CH at VAF ≥ 0.01 and recipient OS, PFS, relapse, and NRM in multivariable models divided by gene group. The groups consist of CH with any mutation other than *DNMT3A* or *TET2* (Other CH), remaining *DNMT3A* mutations, and then remaining *TET2* mutations. (C) The 5-year cumulative incidence of chronic GVHD split by (continued on following page)

FIG 2. (Continued). *DNMT3A*-CH status (VAF ≥ 0.01) and receipt of PTCy for GVHD prophylaxis. (D) The 5-year cumulative incidence of relapse split by *DNMT3A*-CH status (VAF ≥ 0.01) and receipt of PTCy. (E) The 5-year PFS split by *DNMT3A*-CH status (VAF ≥ 0.01) and receipt of PTCy. (F) The associations between *DNMT3A*-CH and outcomes among recipients who did or did not receive PTCy in multivariable Fine-Gray competing risk regressions (chronic GVHD, relapse) and Cox proportional hazards models (PFS and OS). CH, clonal hematopoiesis; GVHD, graft-versus-host disease; HR, hazard ratio; NRM, nonrelapse mortality; OS, overall survival; PFS, progression-free survival; PTCy, post-transplant cyclophosphamide; sHR, subdistribution hazard ratio; VAF, variant allele fraction.

immunologic function[36,37] or accelerate age-associated phenotypes of hematopoietic stem cells from older donors by altering the bone marrow microenvironment.[38] We assessed the association of donor CH with levels of 10 cytokines in 256 recipients with (n = 54) or without (n = 202) donor CH who were alive without relapse at 12 months after transplant. Recipients of donor *DNMT3A*-CH at VAF ≥ 0.01 (n = 21) had higher median interleukin (IL)-12p70 levels (0.37 pg/mL) than other recipients (n = 241, 0.16 pg/mL, Fig 3E, $P = .002$). In *DNMT3A*-CH recipients, IL-12p70 levels were positively correlated with levels of IL-1B, IL-4, IL-5, and interferon gamma and negatively correlated with IL-8, IL-22, tumor necrosis factor alpha, and IL-10 (Fig 3F, Data Supplement).

Evolution to DCL

The evolution of donor CH to DCL has been described in individual cases,[39-41] but the leukemic risk of donor CH has not been evaluated in a single cohort. We identified eight cases of DCL, for a 10-year cumulative incidence of 0.7% (Fig 4A). The median latency between transplantation and DCL diagnosis was 5.2 years (range 0.3-10.3 years). Donors for recipients who developed DCL were significantly older than donors for recipients who did not develop DCL (median 57.3 *v* 50.9 years, $P = .037$, Fig 4B).

To determine the proportion of DCLs that evolved directly from donor CH, we sequenced six DCL samples and analyzed the correlation between DCL mutations and the mutations present in the donor. In five of six cases, DCL mutations were detectable in the donor sample. In both DCLs without available samples, the donors had detectable CH. Donor clones that progressed to leukemia were genetically distinct from more common *DNMT3A*- or *TET2*-mutated CH: three had mutations in myelodysplastic syndrome (MDS)–associated splicing factors (*SF3B1*, *SRSF2*, and *U2AF1*),[42] two had *TP53* mutations, and in two cases, we identified germline mutations in the leukemia predisposition gene *DDX41* that were shared between the recipient and their sibling donors (Data Supplement).[43] Although donor CH with splicing factor or *TP53* mutations was infrequent overall, relatively large proportions developed DCL (Fig 4C).

DISCUSSION

CH in older stem-cell donors may influence recipient outcomes after allogeneic transplantation,[13] but previous studies have not shown an association between donor CH and overall recipient survival, examined its genetic heterogeneity and interaction with immune-modulating therapies, or evaluated the impact of low-abundance clones. Here, we paired sensitive detection of CH in 1,727 healthy older transplant donors with comprehensive clinical annotation of recipient outcomes. Our findings directly inform screening and selection of older donors and have implications for our fundamental understanding of GVL. We find that the presence of *DNMT3A-CH* or *TET2-CH* in stem-cell donors does not adversely affect recipient outcomes and that *DNMT3A*-CH is independently associated with improved survival in recipients as a consequence of reduced relapse. By contrast, rare mutations in MDS-associated genes pose a high risk of leukemic transformation after transplant.

Concerns about the risks of donor CH have stemmed largely from extrapolating results of nontransplant studies linking CH to increased risks of hematologic malignancies[8] and nonhematologic diseases.[10] These effects are reported to be caused by pathologic dysregulation of inflammatory cytokine signaling from differentiated clonal myeloid cells. In this study, we found that recipients of donor CH had evidence of altered inflammatory cytokine signaling, but paradoxically had improved survival mediated by a lower risk of disease relapse, consistent with the critical importance of graft-versus-tumor immunity in allogeneic transplant efficacy. Increased inflammatory signaling from *DNMT3A*-mutant myeloid cells could augment graft alloimmune activity by effects on either T-cell function[44] or malignant cells, where inflammatory signaling positively regulates major histocompatibility complex Class II expression that is required for maintaining tumor immunogenicity after transplantation.[45] IL-12p70, which was significantly higher in recipients of *DNMT3A*-CH than others and was correlated with other proinflammatory cytokines, has been implicated in the development of GVHD and GVL through its positive effects on Th1 polarization and interferon gamma production in $CD4^+$ T cells.[44,46-48] Cross talk with donor-engrafted myeloid cells might explain why *DNMT3A*-CH is associated with an increased risk of chronic GVHD, but not acute GVHD, which is mediated by alloreactive T cells present in the graft at the time of transplantation.[49] Loss-of-function *DNMT3A* mutations in donor-engrafted T cells could also potentiate alloimmune activity in a cell autonomous manner by limiting exhaustion programs[50] or augmenting development of memory $CD8^+$ T-cell populations,[51] both of which are mediated by de novo DNA methylation in T cells. Together, our results provide a mechanistic rationale for exploring therapeutic modulation of DNA methyltransferase activity to augment efficacy of cell-based immune therapies.

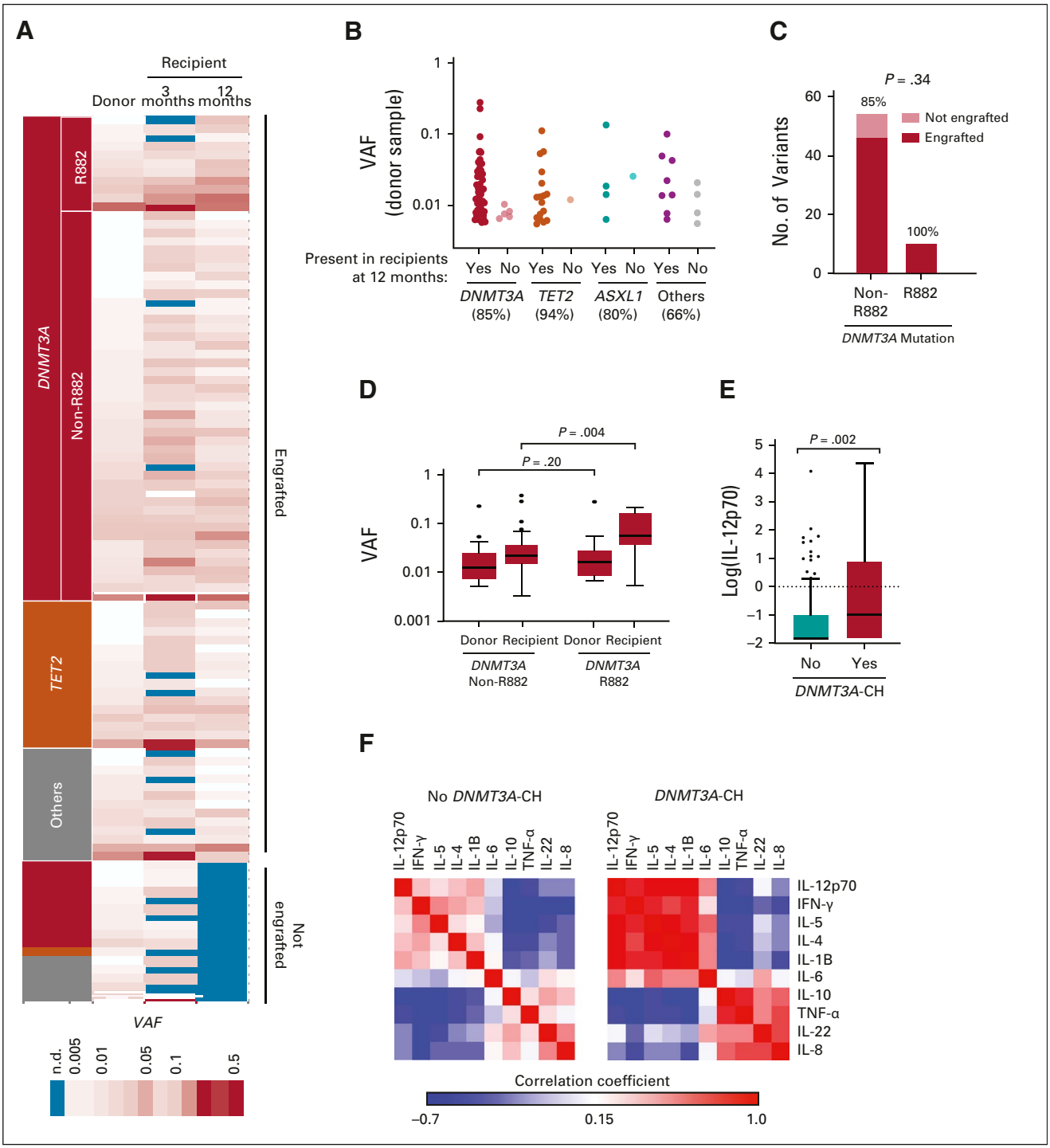

FIG 3. Engraftment of donor CH in recipients after transplantation. (A) The VAFs of 102 donor mutations assessed in 69 recipients after transplantation. Each row is an individual mutation, with columns representing the VAFs in the donors at the time of transplant and in the recipients at 3 and 12 months after transplant. VAFs are color-coded from white (lower VAF) to red (higher VAF), with blue indicating that the mutation was not detected. Mutations that were detectable in recipients at 12 months (engrafted) are on top and those that were not engrafted are on bottom. (B) The donor VAFs of *DNMT3A*, *TET2*, *ASXL1*, and other mutations that did or did not engraft in recipients. (C) The proportion of R882 and non-R882 *DNMT3A* mutations assessed post-transplant (n = 10 and 54, respectively) that did or did not engraft in recipients at 12 months. (D) The VAFs for expansion of non-R882 (left) and R882 *DNMT3A* mutations (right) in donors and corresponding 12-month samples from recipients. (E) The plasma levels of IL-12p70 at 12 months after transplant in recipients of *DNMT3A*-CH (n = 21, red) compared with other recipients (n = 241, teal). (F) Correlations between 10 plasma cytokines measured at 12 months after transplant in the same recipients with and without *DNMT3A*-CH. Correlations are color-coded from blue (negative correlation) to red (positive correlation). CH, clonal hematopoiesis; IFN-γ, interferon gamma; IL, interleukin; n.d., not detected; TNF-α, tumor necrosis factor alpha; VAF, variant allele fraction.

A

DCL Subgroup	Donor Characteristics: Recipient Disease	Donor Type	Donor Age (decade)	Germline Mutations	Somatic Mutations	Max Somatic VAF	DCL Characteristics: Latency (years)	DCL Type	DCL Mutations
Germline predisposition	AML	MRD	50s	*DDX41*	*DNMT3A*	0.16	6.3	AML	*DDX41* (germline), *DDX41* (somatic), *DNMT3A*
	AML	MRD	50s	*DDX41*	None	—	1.9	AML	*DDX41* (germline), *DDX41* (somatic)
MDS-associated	NHL	Haplo	60s	—	*DNMT3A, TET2, SF3B1, SRSF2*	0.278	4.1	AML	*DNMT3A, TET2, RUNX1, NRAS*
	NHL	Haplo	40s	—	*DNMT3A, SF3B1*	0.002	1.3	AML	No sample
	MDS	MRD	50s	—	*U2AF1*	0.0009	9.8	MDS/MPN	*U2AF1, DNMT3A*
TP53	AML	MRD	60s	—	*TP53*	0.32	0.3	MDS	No sample
	ALL	MRD	50s	—	*TP53, DNMT3A*	0.036	10.3	MDS	*TP53*
No variants	NHL	Haplo	60s	—	None	—	6.4	AML	*ASXL1, SETBP1, CBL*

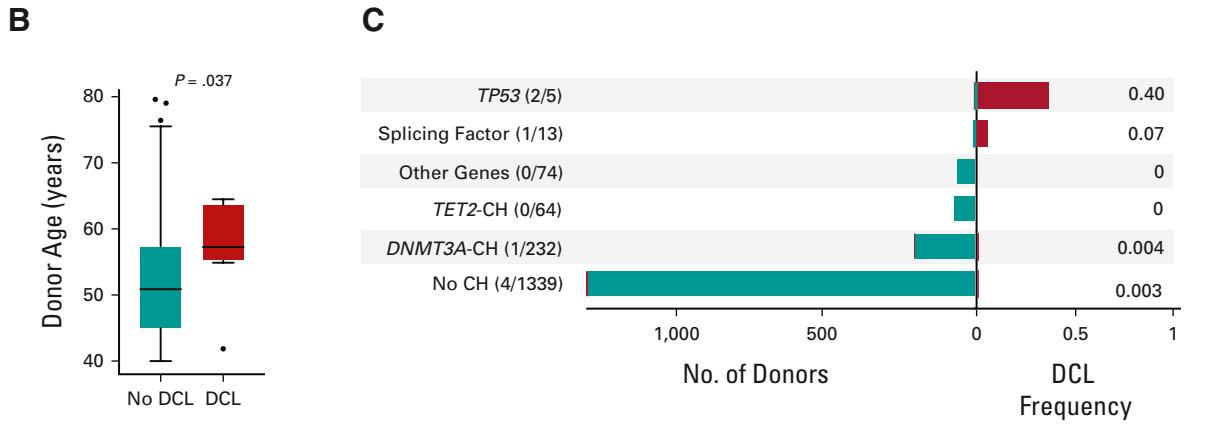

FIG 4. Clinical and genomic features of DCLs. (A) The clinical and genomic features of eight DCLs that developed in the cohort during the study period. Recipient diseases: AML, NHL, MDS, ALL. DCL type: AML, MDS, or MDS/MPN overlap syndrome. Somatic mutations reported include both those meeting criteria for CH and lower abundance mutations identified by sequencing the donor cell leukemia sample. For donor samples with multiple somatic mutations, the maximum VAF is reported. Mutations present in the donor cell leukemia are reported for the six of eight DCLs with available samples. (B) The distribution of donor age for recipients who did or did not develop DCL. (C) The total number of DCLs in each genetic subgroup of CH on the left and the corresponding proportion of recipients in each group who developed DCL on the right. Note that CH is defined here as VAF $\geq$ 0.005, meaning that two of the donors with low-abundance splicing factor mutations reported in (A) are classified as no CH. ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CH, clonal hematopoiesis; DCL, donor cell leukemia; MDS, myelodysplastic syndrome; MPN, myeloproliferative neoplasm; NHL, non-Hodgkin lymphoma; VAF, variant allele fraction.

We empirically defined a VAF cutoff for CH clinical significance. We found that even small clones engraft reliably in recipients, but that the biologic and clinical consequences of donor *DNMT3A*-CH are only meaningful above VAF 0.01. Notably, 246 of 388 donors with CH had VAF $\leq$ 0.02 and thus would not have been evaluated in studies relying on the provisional definition of CH of indeterminate potential.[13] This is particularly important for clinical applications, as small clones are expected to be identified more frequently as ever more sensitive sequencing technologies are deployed in the clinical setting.

Administration of PTCy is an alternative to traditional calcineurin inhibitor (CNI)–based GVHD prophylaxis because it potently reduces the risk of chronic GVHD.[52,53] In our study, the type of GVHD prophylaxis regimen had no overall impact on survival or relapse, but the effects of DNMT3A-CH were only observed in those who received CNI-based regimens. If confirmed by additional studies, this observation may have immediate clinical implications in cases where the best available donor has *DNMT3A*-CH. In such cases, recipients with high-risk hematologic malignancies who could benefit from enhanced GVL might opt for CNI-based regimens, whereas recipients with nonmalignant hematologic disease in whom minimizing GVHD is a priority might opt for PTCy-based prophylaxis.

The potential for leukemic evolution of pre-existing donor clones has been proposed as a basis for excluding candidate donors with CH,[18,20,39] but the magnitude of risk on the basis

of the CH genotype has not been defined. In our study, no recipients of *DNMT3A*- or *TET2*-mutated donor CH developed DCL without concurrent MDS-associated mutations or germline risk alleles. Instead, DCLs arose from donor gene mutations that were rare in the cohort overall, such as *TP53* and MDS-associated splicing factors, supporting findings of published case reports.[39,54,55] High-risk mutations were associated with older donor age, raising the possibility that age alone also contributes to risk of transformation. The frequency of such high-risk gene mutations was lower in this healthy donor cohort than in cross-sectional studies,[8,9] which could either reflect their association with clinical abnormalities that would lead to exclusion from the donor pool or a difference in the age structure of donors compared with population-based studies of CH.[24]

By exonerating donors with the most common form of CH, this study expands the donor pool for patients unable to find matched younger donors in unrelated registries or in whom sibling donors are preferred. By contrast, our results do not engage the question of whether an older sibling donor with CH is equivalent to a younger matched unrelated donor when both choices are available. This question is of interest given studies suggesting that, for both unrelated and haploidentical donors, younger donor age is associated with improved recipient outcomes.[4,56] Although 80% of transplants in our study were from related donors, we nevertheless observed improved outcomes among recipients of *DNMT3A*-CH in the unrelated donor subset. Dedicated analysis of donor registries may definitively determine the extent to which donor CH modulates relapse and survival in recipients of grafts from unrelated donors.

A recommendation to incorporate screening for CH into the standard evaluation of transplant donor candidates would require synthesis of scientific evidence, technical feasibility, cost-effectiveness, and ethical considerations.[18,19] Our results provide scientific evidence for such a policy discussion by showing that individuals age 40 years or older should not be excluded from stem-cell donation on the basis of identification of CH involving sole *DNMT3A* or *TET2* mutations. By contrast, clinicians may consider excluding individuals with splicing factor or *TP53*-mutated CH, or with germline predisposition alleles, from donation because of an apparently elevated risk of DCL.

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DISCLAIMER

The authors attest that all clinical and genetic data required for replication are contained in the article and Data Supplement.

EQUAL CONTRIBUTION

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Donor Clonal Hematopoiesis and Recipient Outcomes After Transplantation

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