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# Impact of clinical, cytogenetic, and molecular profiles on long-term survival after transplantation in patients with chronic myelomonocytic leukemia

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## ABSTRACT

Chronic myelomonocytic leukemia (CMML) is a heterogeneous group of clonal hematopoietic malignancies with variable clinical and molecular features. We analyzed long-term results of allogeneic hematopoietic cell transplantation in patients with CMML and determined clinical and molecular risk factors associated with outcomes. Data from 129 patients, aged 7-74 (median 55) years, at various stages of the disease and transplanted from related or unrelated donors were analyzed. Using a panel of 75 genes somatic mutations present before hematopoietic cell transplantation were identified in 52 patients. The progression-free survival rate at 10 years was 29%. The major cause of death was relapse (32%), which was significantly associated with adverse cytogenetics (hazard ratio, 3.77;  $P=0.0002$ ), CMML Prognostic Scoring System (hazard ratio, 14.3,  $P=0.01$ ), and MD Anderson prognostic scores (hazard ratio, 9.4;  $P=0.005$ ). Mortality was associated with high-risk cytogenetics (hazard ratio, 1.88;  $P=0.01$ ) and high Hematopoietic Cell Transplantation Comorbidity Index (score  $\geq 4$ : hazard ratio, 1.99;  $P=0.01$ ). High overall mutation burden ( $\geq 10$  mutations: hazard ratio, 3.4;  $P=0.02$ ), and  $\geq 4$  mutated epigenetic regulatory genes (hazard ratio 5.4;  $P=0.003$ ) were linked to relapse. Unsupervised clustering of the correlation matrix revealed distinct high-risk groups with unique associations of mutations and clinical features. CMML with a high mutation burden appeared to be distinct from high-risk groups defined by complex cytogenetics. New transplant strategies must be developed to target specific disease subgroups, stratified by molecular profiling and clinical risk factors.

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## Introduction

Chronic myelomonocytic leukemia (CMML) can present either as a myeloproliferative type (MP-CMML) or a myelodysplastic type (MD-CMML), with leukocyte counts of  $\geq 13 \times 10^9/L$  and  $< 13 \times 10^9/L$ , respectively, and is therefore classified by the World Health Organization (WHO) as a myelodysplastic/myeloproliferative neoplasm (MDS/MPN).<sup>1</sup> The clinical course in terms of survival and risk of evolution into acute myeloid leukemia (AML) varies.<sup>2</sup> Prognosis tends to be worse with MP-CMML than with MD-CMML.<sup>3</sup> The blast percentage (in blood and bone marrow) has clear prognostic implications.<sup>4,5</sup> CMML-0 allows for  $< 2\%$  blasts in peripheral blood or  $< 5\%$  blasts in the bone marrow or both; CMML-1 refers to cases with 2% to 4% blasts in the peripheral blood or 5% to 9% in bone marrow or both; and CMML-2 refers to cases with 5% to 19% blasts in peripheral blood, 10% to 19% in bone marrow, or the presence of Auer rods.

The highly heterogeneous nature of CMML has led to the development of various prognostic scoring systems in an attempt to assign individual patient risk.<sup>6-9</sup> These systems have incorporated hematologic indices, cytogenetic abnormalities, and transfusion dependency. More recently, recurrent somatic mutations have been identified in genes associated with signaling pathways (*RAS*, *CBL*, *CSF3R*, and

*JAK2*), DNA methylation (*DNMT3A*, *IDH1*, *IDH2*, and *TET2*), transcription (*RUNX1*), epigenetic regulation (*ASXL1*, *EZH2*, and *SETBP1*), and splicing (*SF3B1*, *SRSF2*, *U2AF1*, and *ZRSR2*).<sup>9-11</sup> *TET2* and *SRSF2* mutations are the most prevalent in CMML.<sup>12,13</sup> Patients with MP-CMML have a higher propensity for alterations in signaling pathways,<sup>14</sup> whereas patients with MD-CMML predominantly have mutations associated with epigenetics.<sup>15</sup> Recognition of associations between somatic mutations and clinical features has improved the risk stratification of CMML (molecular CMML Prognostic Scoring System, m-CPSS).<sup>16</sup> Mutations in *RUNX1*, *NRAS*, *SETBP1*, and *ASXL1* appear to be associated with unfavorable outcomes.<sup>16,17</sup>

Treatment of CMML with chemotherapy alone only infrequently results in prolonged remission. A randomized trial comparing oral etoposide and hydroxyurea showed superior survival with hydroxyurea.<sup>18</sup> Treatment with hypomethylating agents results in less toxicity than associated with conventional chemotherapy; but again, remissions tend to be of short duration.<sup>19,20</sup> The only therapeutic modality with proven curative potential is allogeneic hematopoietic cell transplantation (HCT).<sup>21-27</sup> Published data indicate that the major factors determining long-term relapse-free survival and overall survival are cytogenetic risk category, comorbidities, patient's age and achievement of complete remission.<sup>21,25-27</sup> In the present study, we analyzed long-term outcomes after allogeneic HCT for patients with CMML and, in a subcohort, carried out a comprehensive mutation analysis of 75 genes implicated in myeloid malignancies to define the relationship between somatic mutations and previously established risk factors.

## Methods

### Patients

Between May 1986 and September 2017, 129 patients with CMML underwent HCT at the Fred Hutchinson Cancer Research Center. All provided informed consent for enrollment in investigational protocols and for long-term follow-up as required by the institutional review board of the Center. The characteristics of the patients and their diseases are summarized in Table 1. Patients were 7-74 (median, 55) years of age. The diagnosis and stratification of CMML, and determination of AML transformation were based on WHO 2016 criteria for all cases.<sup>1</sup> The disease was also risk-categorized by cytogenetics,<sup>28</sup> the MD Anderson Prognostic Score (MDAPS),<sup>6</sup> the CMML-specific Prognostic Scoring System (CPSS),<sup>8</sup> and the revised International Prognostic Scoring System (IPSS-R).<sup>29</sup> The HCT Comorbidity Index (HCT-CI) scores were 0-1 in 35 patients, 2-3 in 49 patients, and 4-11 in 45 patients.<sup>30</sup>

### Donor and transplant characteristics

Donor and transplant characteristics are summarized in Table 2. All patients (and donors) were HLA genotyped, following institutional standards. Genotyping was carried out retrospectively in patients transplanted before the routine use of molecular typing. Donors for 42 patients (33%) were related (38 HLA-identical siblings, 4 HLA-mismatched family members), whereas 87 patients (67%) had unrelated donors (68 HLA-matched, 19 HLA mismatched, including 2 cord blood transplants). The stem cell source was bone marrow in 34 (26%) patients, peripheral blood stem cells in 93 (72%) and cord blood in two. Reduced-intensity conditioning regimens were used in 19% of patients and high-intensity (myeloablative) regimens in 81% of patients. Graft-versus-host dis-

ease (GvHD) prophylaxis consisted of a calcineurin inhibitor-based regimen in all patients. The severity of any acute and chronic GvHD was assessed and the conditions were treated as described previously.<sup>31,32</sup>

### Mutation analysis

Mutation analysis was performed on DNA from bone marrow mononuclear cells collected prior to transplantation in 52 patients. DNA was extracted using a DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA, USA), following the manufacturer's protocol. Next-generation sequencing libraries were prepared from 400 ng genomic DNA using the Archer VariantPlex Myeloid 75 gene

**Table 1. Patient and disease characteristics.**

Variable	N. of patients	%
Patients	129	
Age (years)	7-74 (median, 55)	
Gender		
Male	85	65.9
Female	44	34.1
Diagnosis		
WHO		
CMML-0	21	16.3
CMML-1	31	24.0
CMML-2	38	29.5
CMML-T*	37	28.7
CMML-nonspecified	2	1.6
Subgroup		
Myelodysplastic (MD)	88	68.2
Myeloproliferative (MP)	40	31.0
Indeterminate	1	0.8
Cytogenetic risk†		
Low	72	55.8
Intermediate	22	17.1
High	30	23.3
Not available‡	5	3.9
CPSS		
Low	1	0.8
Intermediate-1	16	12.4
Intermediate-2	74	57.4
High	30	23.3
Not determined‡	8	6.2
MDAPS		
Low	29	22.5
Intermediate-1	34	26.4
Intermediate-2	46	35.7
High	15	11.6
Not determined‡	5	3.9
IPSS-R for myelodysplastic-CMML		
Very low	8	9.1
Low	10	11.4
Intermediate	26	29.5
High	20	22.7
Very high	20	22.7
Not determined‡	4	4.5
HCT-CI		
0-1	35	27.1
2-3	49	38.0
≥4	45	34.9

\*Progressed to blasts ≥20% with a previous history of CMML. †According to Such E, *et al.*<sup>28</sup> ‡Due to failed cell growth in cytogenetic study. WHO: World Health Organization; CMML: chronic myelomonocytic leukemia; CPSS: CMML Prognostic Scoring System; MDAPS: MD Anderson Prognostic Score; IPSS-R: Revised International Prognostic Scoring System; HCT-CI: Hematopoietic Cell Transplantation Comorbidity Index.

panel (ArcherDX, Boulder, CO, USA) following the manufacturer's instructions. We employed highly sensitive and specific targeted sequencing methods, exploiting unique molecular barcodes by anchored multiplex polymerase chain reaction (PCR) chemistry to tag the starting DNA material before PCR amplification.<sup>33</sup> These methods significantly enhance the sensitivity and specificity of variant detection by filtering out duplicate reads and PCR errors. We followed variant calling procedures as previously reported.<sup>34</sup> Pairwise associations between mutations and clinical parameters were evaluated by the Fisher exact test and Pearson correlation, corrected for the testing of multiple hypotheses. Hierarchical clustering of associations was performed by in-house scripts in R/Bioconductor. The Euclidean distance between variables was calculated, and the final partition of variables was obtained by the `hclust` function in the R package 'stat' with complete linkage as the argument. Details are provided in the *Online Supplementary Methods*.

### Statistical analysis

Survival was defined as the time from transplantation to death or date of last contact. Relapse-free survival was defined as the time from transplantation to relapse or death from causes other than relapse. Non-relapse mortality (NRM) was defined as death without prior relapse. Estimates of the probability of overall and relapse-free survival were obtained by the Kaplan-Meier method,

and estimates of the probability of relapse, NRM, and GvHD were summarized using cumulative incidence estimates. NRM was considered a competing risk for relapse, and death without chronic GvHD a competing risk for chronic GvHD. Associations of various factors with the cause-specific hazards of failure for each of these endpoints were assessed using Cox regression. All *P*-values are two-sided and do not incorporate adjustment for multiple comparisons; however, because of the large number of individual mutations considered, we report only associations significant at the 0.01 level of significance, unless the mutation is considered of interest based on prior studies.

## Results

### Conventional risk classification

As shown in Table 1, according to WHO criteria, 21 patients (16%) had CMML-0, 31 (24%) had CMML-1, 38 (30%) had CMML-2, and 37 patients (29%) had CMML-T (progressed to a blast count  $\geq 20\%$  with previous history of CMML). In two patients, the staging was inconclusive. In 88 patients (68%), the white blood cell count was  $< 13 \times 10^9/L$ , thus qualifying as MD-CMML, and 40 patients (31%) had MP-CMML with a white cell count  $\geq 13 \times 10^9/L$ . In one patient, the white blood cell count from the pre-HCT period was not available. Among 124 patients with cytogenetic data, 72 (56%) were considered low risk, 22 (17%) intermediate risk, and 30 (23%) high risk according to the CMML-specific cytogenetic classification.<sup>28</sup> More than 80% of patients were "higher" risk according to the CPSS classification (i.e., 30 were high risk and 74 intermediate-2 risk), while 50% of patients were "higher" risk according to the MDAPS (i.e., 15 were high risk and 46 intermediate-2 risk).

### Engraftment and graft-versus-host disease

One hundred twenty patients (93%) achieved sustained engraftment, as defined by absolute neutrophil counts of  $\geq 0.5 \times 10^5/\mu L$  for three or more consecutive days. Seven of the remaining nine patients had donor cell engraftment, as determined by chimerism analysis, but died before day 100 without achieving an absolute neutrophil count  $\geq 0.5 \times 10^5/\mu L$ , while two patients died with recurrent CMML. Of the 126 patients evaluable for GvHD (surviving beyond day 28 with donor cell engraftment), acute GvHD of grades II-IV developed in 93 (74%) and grades III-IV in 32 (25%). Chronic GvHD occurred in 57 patients within 2 years, for a cumulative incidence of 45%.

### Relapse

Relapse or disease progression occurred in 40 patients between 7 and 2,490 (median 154) days after transplantation. The estimated probability of relapse or disease progression was 28% at 3 years, and 32% at 10 years (Figure 1A).

### Survival

With a median follow-up of 9.3 (range 0.4-25.2) years, 39 patients are alive while 90 patients have died. The overall survival rates at 3 and 10 years were 38% and 28%, respectively, while the relapse-free survival rates at the corresponding times were 37% and 29% (Figure 1A).

### Clinical determinants of post-transplant outcomes

The results of the univariate analyses are summarized in Table 3. Relapse incidence was significantly increased

**Table 2. Donor and transplant characteristics.**

Variable	N. of patients	%
<b>Donor</b>		
Related	42	32.6
HLA-matched	38	29.5
HLA-mismatched	4	3.1
Unrelated	87	67.4
HLA-matched	68	52.7
HLA-mismatched*	19	14.7
<b>Cell source</b>		
Bone marrow	34	26.4
Peripheral blood	93	72.1
Cord blood	2	1.6
<b>Conditioning regimen</b>		
Myeloablative conditioning		
BU (16 mg/kg) / CY (120 mg/kg) $\pm$ ATG	41	31.8
FLU (120 mg/m <sup>2</sup> ) / BU (16 mg/kg)	13	10.1
TBI (2 Gy) / I-131-antiCD45	12	9.3
BU (7 mg/kg) / TBI (12 Gy)	11	8.5
BU (7 mg/kg) / CY (50 mg/kg) / TBI (12Gy)	10	7.8
CY (120 mg/kg) / TBI (14.4 or 13.2 Gy)	10	7.8
TREG (42 g/m <sup>2</sup> ) / FLU (150 mg/m <sup>2</sup> ) / TBI (2 Gy)	9	7.0
Reduced-intensity conditioning		
FLU (90 mg/m <sup>2</sup> ) / TBI (2-3 Gy)	21	16.3
Others <sup>†</sup>	2	1.6
<b>GvHD prophylaxis</b>		
CSP/MTX	43	33.3
CSP/MMF	36	27.9
TAC/MTX	35	27.1
TAC/MMF	6	4.7
CSP/MMF/MTX	2	1.6
CSP/others	7	5.4

\*Includes two cord blood transplants. †FLU (175 mg/m<sup>2</sup>) / CY (50 mg/kg) / TBI (3 Gy), FLU (125 mg/m<sup>2</sup>) / melphalan (140 mg/m<sup>2</sup>). HLA: human leukocyte antigen; BU: busulfan; CY: cyclophosphamide; ATG: antithymocyte globulin; FLU: fludarabine; TBI: total-body irradiation; TREG: treosulfan; I-131-antiCD45, iodine-131 monoclonal antibody BC8; GvHD, graft-versus-host disease; CSP, cyclosporine; MMF, mycophenolate mofetil; TAC, tacrolimus; MTX, methotrexate.

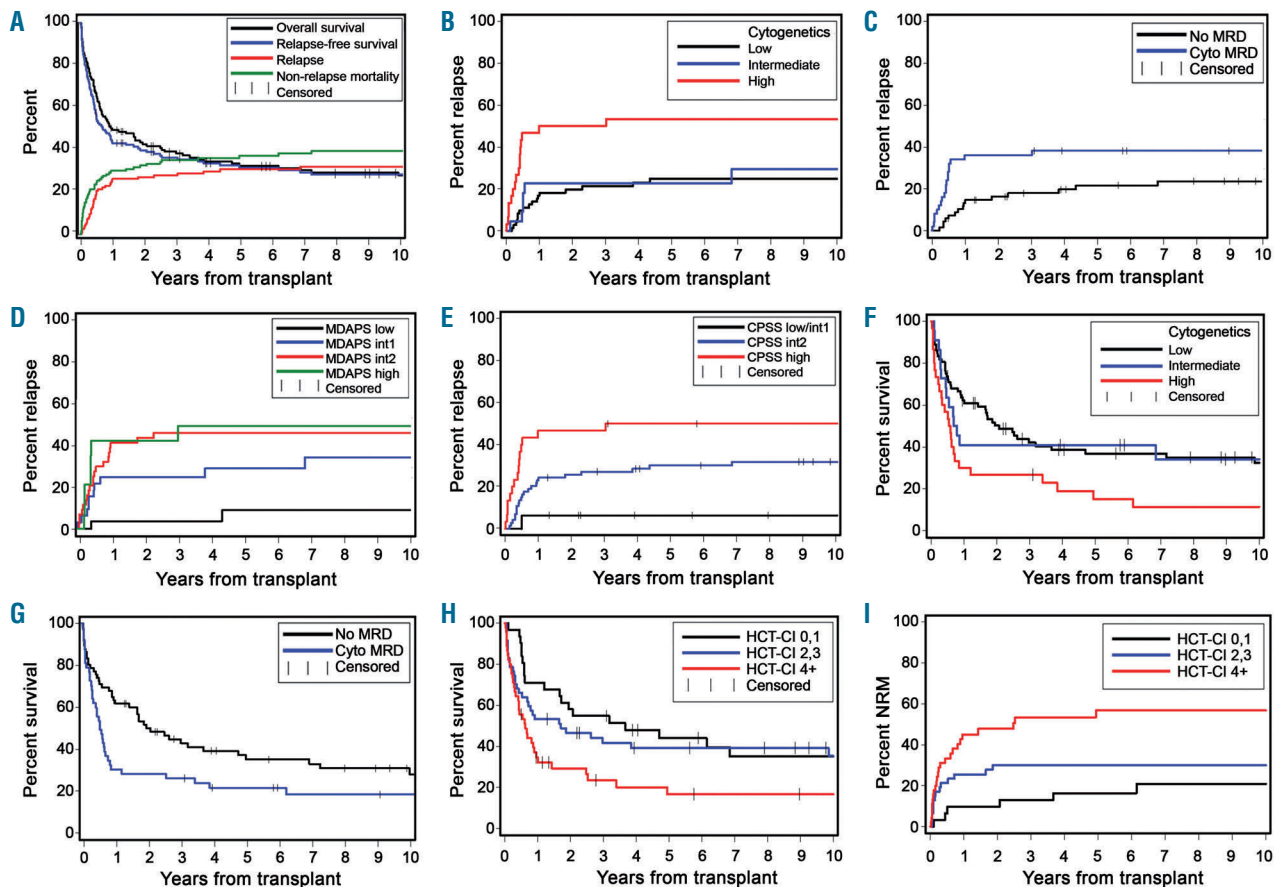


with higher-risk cytogenetics [hazard ratio (HR), 3.77; 95% confidence interval (95% CI): 1.9-7.5;  $P=0.0002$ ] (Figure 1B) and the presence of measurable residual disease by cytogenetics at HCT (HR, 2.55; 95% CI: 1.3-5.0;  $P=0.007$ ) (Figure 1C). Classification of CMML by WHO criteria was associated with relapse only with progression to  $\geq 20\%$  blasts (HR, 3.61; 95% CI: 1.2-10.5;  $P=0.02$ ) (Online Supplementary Figure S1A). Stratification for MDAPS and CPSS showed statistically significant associations with relapse. Specifically, high risk by MDAPS criteria was associated with relapse incidence [HR, 5.24 ( $P=0.03$ ), 7.15 ( $P=0.008$ ) and 9.41 ( $P=0.005$ ) for intermediate-1 ( $n=34$ ), intermediate-2 ( $n=46$ ) and high-risk patients ( $n=15$ ), respectively] (Figure 1D). Similarly, patients who were classified as high risk by CPSS ( $n=30$ ; 25%) had a incidence of relapse than that of lower-risk patients (HR, 14.3; 95% CI: 1.9-108;  $P=0.01$ ) (Figure 1E). Among patients with leukocyte counts  $<13 \times 10^9/L$  (MD-CMML), the very high-risk group, as determined by the IPSS-R ( $n=20$ ), was associated with a higher relapse incidence (HR, 7.82; 95% CI: 1.7-35;  $P=0.007$ ) (Online Supplementary Figure S1B).

In multivariate analysis the two strongest factors for

relapse determined in univariate analysis, cytogenetic risk and MDAPS classification, remained statistically significant (Table 4).

Overall mortality increased with high-risk cytogenetics (HR, 1.88; 95% CI: 1.2-3.0;  $P=0.01$ ) (Figure 1F), the presence of measurable residual disease as determined by cytogenetics (HR, 1.65; 95% CI: 1.1-2.6;  $P=0.02$ ) (Figure 1G), and a high HCT-CI [HCT-CI  $\geq 4$  ( $n=45$ ): HR, 1.99; 95% CI: 1.2-3.4;  $P=0.01$ ] (Figure 1H), primarily due to increased NRM (HCT-CI  $\geq 4$ : HR, 3.39; 95% CI: 1.5-7.5;  $P=0.003$ ) (Figure 1I). Bone marrow blast counts  $\geq 20\%$  at HCT also had a negative impact on survival, although the effect did not reach statistical significance (HR, 1.67; 95% CI: 0.9-3.2;  $P=0.13$ ) (Online Supplementary Figure S1C). Risk stratification by the prognostic scoring systems was generally less predictive of survival, likely due to a high incidence of NRM (Online Supplementary Figure S1D-F). Survival after reduced intensity conditioning was not different from that after myeloablative conditioning (HR, 1.1; 95% CI: 0.7-1.9;  $P=0.71$ ) (Online Supplementary Figure S1G, H). Pre-transplant therapy and disease status at transplant (complete remission vs. non-complete remission) did not significantly affect overall survival. However, there was a



**Figure 1. Clinical risk factors associated with relapse and overall mortality/survival in patients with chronic myelomonocytic leukemia following hematopoietic cell transplantation.** Overall and relapse-free survival (RFS) and the probabilities of relapse and non-relapse mortality (NRM) are shown for all 129 patients. Tick marks indicate censored patients. (A) Survival, RFS, relapse and NRM for all patients. (B) Relapse by cytogenetic risk. (C) Relapse by measurable residual disease (MRD) at transplantation as indicated by cytogenetics. (D) Relapse by MD Anderson Prognostic Score (MDAPS) and (E) by CMML-specific Prognostic Scoring System (CPSS) risk group. (F) Overall survival dependent upon cytogenetic risk and (G) MRD by cytogenetics. (H) NRM and (I) overall survival by Hematopoietic Cell Transplantation-Comorbidity Index (HCT-CI).

trend toward higher relapse incidence in patients given pre-HCT intensive cytotoxic chemotherapy and hypomethylating agents, and toward lower relapse incidence with achievement of complete remission at transplant (Table 3). Year of transplant did not have an influence on relapse incidence, overall survival (Table 3), or NRM (for years 2000-2010: HR, 0.7; 95% CI: 0.38-1.50;  $P=0.57$ ; for years after 2010: HR, 1.05; 95% CI: 0.49-2.25;  $P=0.60$ ).

### Mutational landscape

In a subcohort of 52 patients for whom pre-transplant bone marrow samples were available, we carried out a mutational analysis of 75 genes. At least one somatic mutation was identified in 44 of the 52 patients (85%) (median number/patient = 5; range, 0–21) (*Online Supplementary Figure S2* and *Online Supplementary Tables S1* and *S2*). The most common mutations were those in *ASXL1* (52%), *TET2* (42%), and *SRSF2* (25%), consistent

**Table 3.** Univariate regression analyses.

Parameter	N.	Overall survival (mortality)		Relapse/progression	
		HR (95% CI)	P	HR (95% CI)	P
<b>Conditioning by intensity</b>					
Myeloablative conditioning	103	1			
Reduced-intensity conditioning	23	1.10 (0.7–1.9)	0.71	1.32 (0.6–2.8)	0.46
<b>Age at transplant</b>					
<55 years	62	1		1	
55+ years	67	1.13 (0.7–1.7)	0.57	0.95 (0.5–1.8)	0.88
<b>CMV serostatus</b>					
Recipient –ve and Donor –ve	32	1		1	
Recipient +ve or Donor +ve	95	1.11 (0.7–1.8)	0.67	0.92 (0.5–1.8)	0.81
<b>Sex match</b>					
Others	90	1		1	
Female to male	37	0.94 (0.6–1.5)	0.78	0.48 (0.2–1.1)	0.07
<b>HCT-CI</b>					
0, 1	31	1		1	
2, 3	47	1.10 (0.6–1.9)	0.73	0.94 (0.4–2.0)	0.87
4+	45	1.99 (1.2–3.4)	0.01	1.08 (0.5–2.4)	0.84
<b>Interval (diagnosis to transplant)</b>					
< 6 months	29	1		1	
6–18 months	66	1.02 (0.6–1.7)	0.95	0.97 (0.4–2.2)	0.93
> 18 months	34	1.34 (0.7–2.4)	0.33	1.72 (0.7–4.1)	0.22
<b>Pre-transplant therapy†</b>					
Supportive care	32	1		1	
Low intensity	34	1.01 (0.6–1.8)	0.99	1.87 (0.7–5.1)	0.22
Induction chemo ± HMA	38	1.31 (0.7–2.3)	0.36	2.23 (0.9–5.9)	0.09
HMA	25	1.37 (0.7–2.6)	0.32	2.28 (0.8–6.4)	0.12
<b>Status at transplant</b>					
Non-complete remission	64	1		1	
Complete remission	64	1.00 (0.7–1.5)	0.99	0.58 (0.3–1.1)	0.1
<b>Individual mutations</b>					
<i>ASXL1</i>	27	1.4 (0.7–2.8)	0.33	1.1 (0.4–3.0)	0.89
<i>TET2</i>	22	1.8 (0.9–3.6)	0.10	1.6 (0.6–4.6)	0.40
<i>RUNX1</i>	9	1.1 (0.4–2.6)	0.90	1.9 (0.6–6.1)	0.29
<i>SETBP1</i>	5	0.8 (0.2–2.6)	0.67	0.6 (0.1–4.3)	0.54
<i>NRAS</i>	6	1.9 (0.7–5.1)	0.22	4.7 (1.4–16)	0.03
<i>WT1</i>	10	4.3 (1.7–11)	0.004	6.3 (1.6–24)	0.01
<i>ATRX</i>	6	4.9 (1.8–13)	0.005	17.3 (4.1–73)	0.0005
<b>Total number of mutations</b>					
≥10	15	1.5 (0.7–3.2)	0.30	3.4 (1.2–9.6)	0.02
<b>Functional groups‡</b>					
<i>Epigenetic</i>					
Trend over 0, 1, 2, 3, 4, 5+ mutations	16, 11, 12, 3, 4, 6	1.2 (1.0–1.5)	0.09	1.5 (1.1–2.0)	0.02
≥ 4	10	1.5 (0.7–3.4)	0.33	5.4 (1.9–16)	0.003
<i>Tumor suppressor</i>					
Presence	7	2.3 (0.9–5.9)	0.09	3.1 (0.8–11)	0.13
<i>Signaling</i>					
Trend over 0, 1, 2, 3, 4, 5+ mutations	16, 11, 10, 4, 4, 7	1.1 (0.9–1.3)	0.58	1.1 (0.8–1.5)	0.57

\*According to Such E, *et al.*<sup>28</sup> †Supportive care includes transfusion, treatment with granulocyte colony-stimulating factor and erythropoiesis-stimulating agents. Low intensity treatments include hydroxyurea, lenalidomide, steroids, azathioprine, imatinib, and ruxolitinib. Hypomethylating agents include azacitidine and decitabine. ‡According to *Online Supplementary Table S1*. HR: hazard ratio; 95% CI: 95% confidence interval; CMV: cytomegalovirus; HCTCI: Hematopoietic Cell Transplantation - Comorbidity Index; HMA: hypomethylating agents.

with previous reports on mutation profiles in patients with CMML.<sup>16,17</sup> Among the mutations in *ASXL1*, 67% were nonsense mutations, while the remainder were either frameshift or stop/gain mutations. Mutations were also frequent in *WT1* (27%), *RUNX1* (17%), *DNMT3A* (17%), *SMC1A* (17%), *EZH2* (12%), and *ATRX* (12%), the high frequency presumably being related to the fact that more than 85% of our cohort (104 of 129 patients) had intermediate-2 or high-risk disease according to the CPSS (Table 1).

### Incorporation of mutations into the overall analysis

Among mutations with prognostic weight reported in previous studies,<sup>16</sup> such as *ASXL1*, *NRAS*, *RUNX1*, and *SETBP1*, only mutations in *NRAS* (n=6) were significantly associated with relapse (HR, 4.7; 95% CI: 1.4-16; *P*=0.03). In addition, mutations in *ATRX* (n=6) and in the *WT1* gene (n=10) were significantly associated with relapse (HR, 17.3; 95% CI: 4.1-73; *P*=0.0005; and HR, 6.3; 95% CI: 1.6-24; *P*=0.01, respectively) and inferior survival (HR, 4.9; 95% CI: 1.8-13; *P*=0.005; and HR, 4.3; 95% CI: 1.7-11; *P*=0.004, respectively).

The impact of mutations was not affected by adjustment in the multivariate analysis, and results remained unchanged.

We then grouped mutations and clinical parameters that co-occurred by unbiased clustering of a correlation matrix across individual mutations and clinical features, including prognostic scoring systems (MDAPS, CPSS, and the CMML-specific cytogenetic classification), leukocyte counts (MP-CMML), and blast counts (Figure 2 and *Online Supplementary Figure S3*). This approach identified two groups: group 1 mainly included mutations in signaling pathways (e.g. *RAS* or *JAK2*) and *TP53*, associated with high-risk cytogenetics and blasts (Figure 2A and *Online Supplementary Figure S3A*), while group 2 included mutations in epigenetic regulatory genes and splicing factors (Figure 2A). Within this group, mutations in *DNMT3A*, *SETBP1*, and *EZH2* tended to co-occur, and mutations in *TET2* and *WT1* were associated with mutations in *ATRX*. The total number of mutations [ $\geq 10$  mutations (n=15): HR, 3.4; 95% CI: 1.2-9.6; *P*=0.02] and greater number of mutations in genes regulating epigenetic processes [ $\geq 4$  mutations (n=10): HR, 5.4; 95% CI: 1.9-16; *P*=0.003], but not mutations in signaling pathways and tumor suppressor genes (*TP53* and *PPM1D*), were associated with relapse (Figure 2B, C, Table 3, and *Online Supplementary Table S1*). When cytogenetics, total number of mutations, established prognostic scoring systems and mutations in epigenetic processes and signaling pathways were considered for their impact on relapse (Figure 2D and *Online Supplementary Figure S3B*), mutations in epigenetic processes [ $\geq 4$  mutations; odds ratio (OR)=8.8], high blast count ( $\geq 20\%$ ; OR=4.7), high-risk MDAPS (OR=4.0) and high-risk cytogenetics (OR=3.2) were the major factors contributing to the probability of relapse (details in *Online Supplementary Table S3*). Pairwise association analysis showed that high blast counts, high-risk by CPSS, and high-risk cytogenetics were closely associated, and mutations in epigenetic regulators tended to occur predominantly in disease classified as high-risk by the MDAPS (Figure 2D and *Online Supplementary Table S3*). The m-CPSS risk model did not allow for further differentiation of risk groups as virtually all patients became classified as intermediate-2 or high risk. All patients were upgraded to

higher risk groups due to their prevalent high-risk mutations. (*Online Supplementary Figure S3B*). As illustrated in Figure 2E, the delay between diagnosis and HCT was longer (median, 536 days) among patients with higher numbers of mutations than among those with fewer mutations (median delay, 309 days), including epigenetic regulators (522 vs. 344 days), but not mutations in signaling pathways (475 vs. 366 days). These observations were consistent with a previous report on non-transplanted patients showing increasing mutations with longer disease duration.<sup>15</sup> Overall, the data indicate that molecular annotation uncovered distinct subgroups of CMML that were not distinguished by conventional risk classification. Specifically, a very high-risk group (independent of high-risk cytogenetics and high blast counts) with a long delay to HCT was characterized by a higher number of mutations in epigenetic regulators.

### Discussion

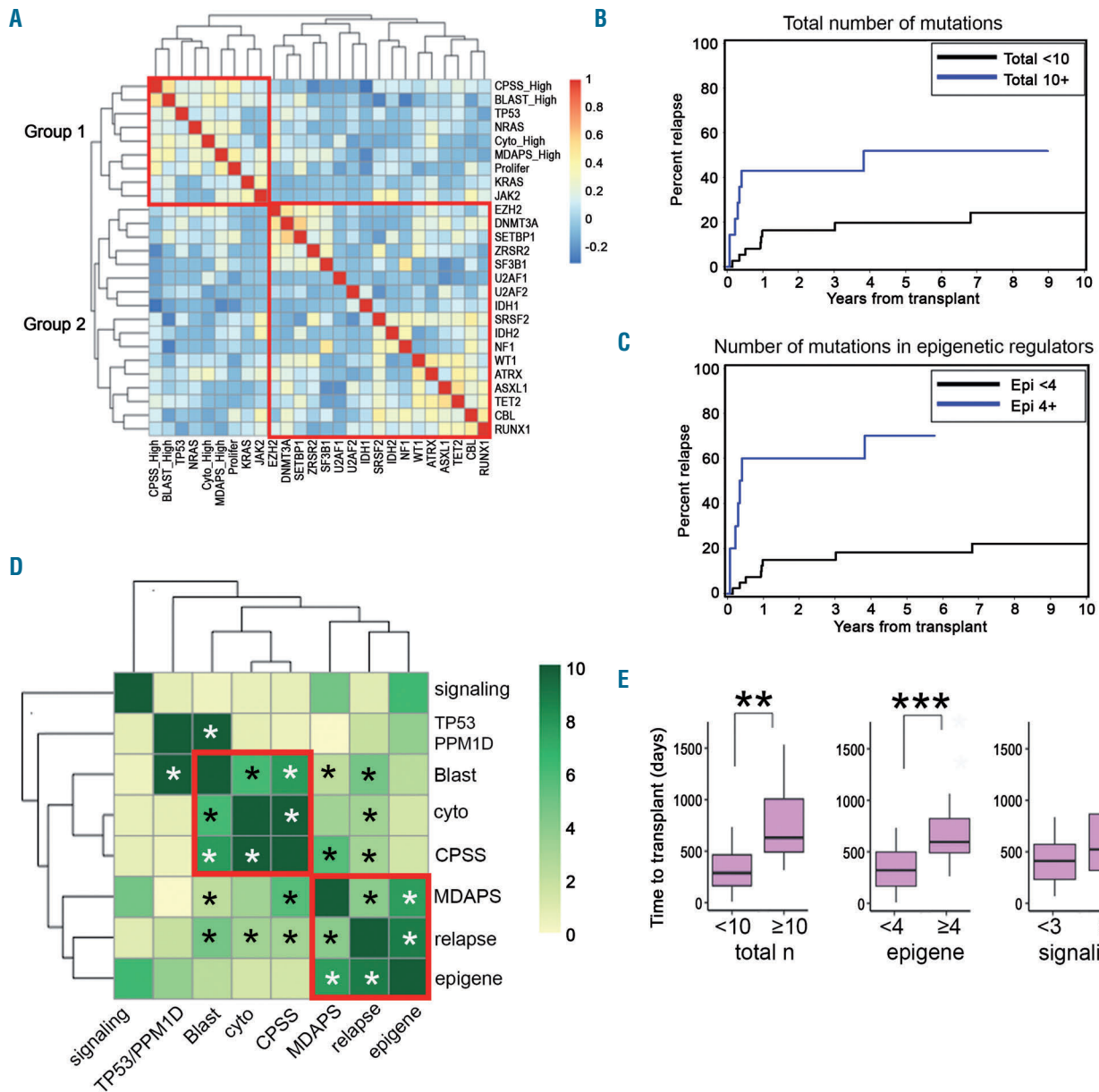
We analyzed the long-term outcomes of allogeneic HCT in 129 patients with CMML in relation to clinical, pathological, and molecular characteristics. The results confirm the curative potential of HCT, since transplanted patients had 3- and 10-year relapse-free survival rates of 37%, and 29%, respectively. The outcomes were superior in lower-risk patients, who had survival probabilities of 40% to 50% at 10 years. However, the relapse incidence and NRM rates were high. As in our previous studies<sup>21</sup> and in reports from other centers,<sup>25,26,35</sup> high-risk cytogenetics, disease transformation (to CMML-T), and risk classification in scoring systems such as IPSS-R, CPSS and MDAPS, were strongly correlated with post-transplant relapse. As in earlier analyses,<sup>36</sup> there was a suggestion that intensive cytotoxic therapy prior to HCT was associated with inferior outcome, presumably related to the fact that patients considered to be at high risk were more likely to receive induction-type chemotherapy. Nevertheless, it was of note that the intensity of conditioning did not significantly affect overall transplant outcome, although it must be noted that a broad spectrum of conditioning regimens was given to this cohort. Furthermore, as in a previous analysis<sup>21</sup> and in other trials in patients with myeloid malignancies,<sup>37</sup> patients with high HCT-CI scores had inferior survival, although the probability of relapse was not altered. The source of stem cells did not affect outcome significantly, and results with related and unrelated donors were similar.

The present results indicate that providing HCT at an earlier disease stage and in patients with a low comorbidity burden who have not received cytotoxic therapy results in superior transplant outcomes, as also suggested by others.<sup>5</sup> These three parameters are likely interrelated as prolonged pre-HCT observation and therapy tend to be associated with the acquisition of new co-morbidities, and likely new mutations, a concept supported by results in our subcohort of patients with mutational data. However, disease characteristics such as high-risk cytogenetics and evidence of measurable residual disease by cytogenetics, still had a profound impact on relapse and survival, even with high-intensity conditioning regimens.

We hypothesize that mutational profiling assists in defining prognosis more narrowly. The analysis of mutations in 75 genes uncovered distinct risk groups defined by

mutations and disease phenotype which differed for HCT outcomes. In particular, the total number of mutations and more mutations in epigenetic regulators were significantly associated with relapse and with inferior survival. Reminiscent of observations in patients with MPN or MDS,<sup>15,88</sup> patients with more mutations experienced a higher incidence of relapse, especially when the mutations occurred in genes involved in epigenetic regulation. Those

mutations occurred predominantly in patients with dysplastic CMML, as also observed by others.<sup>15</sup> Of note, however, our unsupervised clustering analysis uncovered a previously unrecognized group of high-risk patients with a higher mutation burden involving epigenetic regulators. This group of patients was not closely associated with high-risk disease as determined by cytogenetics, suggesting independent mechanisms and heterogeneity in the dis-



**Figure 2. Molecular profiling and risk factors associated with hematopoietic cell transplantation outcomes in patients with chronic myelomonocytic leukemia.** (A) Unsupervised hierarchical clustering of a correlation matrix between mutations and clinical parameters. Group 1 consists of mutations in mitotic signaling pathways, myeloproliferative type of chronic myelomonocytic leukemia (CMML) (Prolifer), mutations in *TP53*, and high-risk disease by cytogenetics and prognostic scoring systems. Group 2 consists of mutations in epigenetic pathways, methylation, and splicing regulation. (B) Increased relapse following hematopoietic cell transplantation in patients with high mutation burden, stratified by total number of mutations ( $\geq 10$  vs.  $< 10$ ), and (C) stratified by number of mutations in epigenetic regulators ( $\geq 4$  vs.  $< 4$ ; classified in *Online Supplementary Table S1*). (D) Unsupervised clustering of association (odds ratio, corresponding to the scaling bar) between groups of mutations (signaling pathways, epigenetic regulators, and tumor suppressors), high-risk cytogenetics (cyto), risk stratification systems (CPSS, MDAPS) and relapse. CMML with a high mutation burden in epigenetic regulators (epigene) was distinct from previously recognized high-risk diseases by cytogenetic abnormalities, blast count, and mutations in tumor suppressors (red boxes). (E) Time from diagnosis to transplant. High mutation burden ( $\geq 10$ ), in particular, in epigenetic processes ( $\geq 4$ ), was associated with a longer interval from diagnosis to transplant, consistent with the concept of disease evolution and acquisition of more mutations (in epigenetic but less so in signaling pathways) with disease duration \* $P < 0.05$  (individual values in *Online Supplementary Table S3*). \*\* $P = 0.01$ , \*\*\* $P = 0.03$ .



ease process. Mutations in *TP53* were less frequent in patients with CMML (<10%) than previously reported for MDS<sup>39</sup> and were not strongly correlated with unfavorable outcomes, underscoring the biological differences between MDS and CMML. The difference may be related to the fact that CMML is infrequently a "secondary disease" (1 of 129 patients in the present series), whereas about 30% of MDS patients transplanted at our Center present with treatment-related or secondary disease.<sup>40</sup> The data need to be interpreted with caution, in view of the limited sample size.

The present mutational data suggest a significant association of *ATRX* and *WT1* with post-transplant relapse and overall mortality in patients with CMML. Although the numbers of cases with these mutations were limited, most mutations occurred in important functional protein domains, suggesting that the mutations would be of functional relevance.<sup>41</sup> Somatic mutations in *ATRX* are also seen in up to 43% of patients with MDS with unexplained microcytosis<sup>42</sup> and in a rare subtype of MDS associated with thalassemia (ATMDS).<sup>43</sup> Previous studies in CMML have not included *ATRX* in their mutation panels,<sup>16,17</sup> and additional investigations are warranted to confirm this association of *ATRX* mutations with CMML. *WT1* mutations are common in patients with high-risk MDS and AML<sup>38</sup> where they are associated with relapse.<sup>39,44</sup> *WT1* mutations in the present study occurred in or adjacent to loci that have been shown to be mutated in AML.<sup>44</sup> Our CMML cohort was predominantly composed of high-risk patients as determined by CPSS and m-CPSS criteria, which, in turn, might be responsible for the prevalence of *WT1* mutations and unfavorable transplant outcomes. Although there was a suggestive association of each individual mutation with relapse and survival, the biological impact of these mutations in a limited cohort of CMML patients must be assessed cautiously. Mutations co-occur with other mutations and rarely work as a single dominant factor. Cooperation with other mutations is likely.

Functional data in the right context (e.g., the exact identical mutation at the endogenous locus in hematopoietic cells) are often lacking. The functional consequences of the frequent mutations in *ATRX* or *WT1* will need to be tested in hematopoietic cells to confirm the proposed biological impact of these mutations.

In conclusion, this analysis adds mutational risk factors to previously identified clinical risk factors for post-HCT outcome in patients with CMML, such as comorbidity, cytogenetic risk, and high-risk disease according to the CPSS and MDAPS. Molecular profiling identified distinct high-risk disease groups with high mutation burden, particularly in epigenetic processes that characterized disease entities distinct from the conventional high-risk groups defined by cytogenetics. Of note, the data also show that these high-risk features are only incompletely overcome by HCT, and relapse and NRM rates remain high. This study confirms the clinical and molecular heterogeneity of CMML which significantly affects the outcome following HCT. New transplant strategies that target specific disease subgroups must be developed. Furthermore, early transplantation should be considered for patients with intermediate-risk disease and lower HCT-CI. Vigilant surveillance and early enrollment in clinical trials for post-transplant relapse must be planned for patients with high-risk disease as defined by complex cytogenetics and high mutation burden.

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