Relative impact of residual cytogenetic abnormalities and flow cytometric measurable residual disease on outcome after allogeneic hematopoietic cell transplantation in adult acute myeloid leukemia

Corentin Orvain,[1,2,3,4,5] Jacob A. Wilson,[6] Min Fang,[1,7] Brenda M. Sandmaier,[1,2] Eduardo Rodríguez-Arbolí,[1,8] Brent L. Wood,[9] Megan Othus,[10] Frederick R. Appelbaum[1,2] and Roland B. Walter[1,2,7,11]

[1]Clinical Research Division, Fred Hutchinson Cancer Center, Seattle, WA, USA; [2]Department of Medicine, Division of Hematology/Oncology, University of Washington, Seattle, WA, USA; [3]Maladies du Sang, CHU d'Angers, Angers, France; [4]Fédération Hospitalo-Universitaire Grand-Ouest Acute Leukemia, FHU-GOAL, Angers, France; [5]Université d'Angers, INSERM UMR 1307, CNRS UMR 6075, Nantes Université, CRCI2NA, Angers; [6]Cytogenetics Laboratory, Fred Hutchinson Cancer Center, Seattle, WA, USA; [7]Department of Laboratory Medicine and Pathology, University of Washington, Seattle, WA, USA; [8]Department of Hematology, Hospital Universitario Virgen del Rocío, Instituto de Biomedicina de Sevilla (IBIS/CSIC/CIBERONC), University of Seville, Seville, Spain; [9]Department of Pathology and Laboratory Medicine, Children's Hospital, Los Angeles, CA, USA; [10]Public Health Sciences Division, Fred Hutchinson Cancer Center, Seattle, WA, USA and [11]Department of Epidemiology, University of Washington, Seattle, WA, USA.

Correspondence: R.B. Walter
rwalter@fredhutch.org

Received: June 14, 2022.
Accepted: July 25, 2022.
Prepublished: August 4, 2022.

https://doi.org/10.3324/haematol.2022.281585

Abstract

Measurable residual disease (MRD) before hematopoietic cell transplantation (HCT) is an independent established prognostic factor in patients with acute myeloid leukemia (AML). Several methods exist to evaluate the presence of residual leukemia cells, but how these are used best in combination is unclear. In order to examine how residual cytogenetic abnormalities and MRD testing by multiparameter flow cytometry (MFC) may refine risk assessment before HCT, we analyzed 506 adults with cytogenetically abnormal AML who underwent both routine karyotyping and MFC MRD testing before receiving a first allograft while in morphologic remission. Testing for residual cytogenetic abnormalities and MFC MRD identified four groups of patients with differential relapse-free survival (RFS) (hazard ratio [HR]=1.63 for $Cyto^{abnormal}/MFC^{negative}$ [*P*=0.01, n=63], HR=3.24 for $Cyto^{normal}/MFC^{positive}$ [*P*<0.001, n=60], and HR=5.50 for $Cyto^{abnormal}/MFC^{positive}$ [*P*<0.001, n=56] with $Cyto^{normal}/MF^{Cnegative}$ as reference [n=327]) and overall survival (OS) (HR=1.55 for $Cyto^{abnormal}/MFC^{negative}$ [P=0.03], HR=2.69 for $Cyto^{normal}/MFC^{positive}$ [*P*<0.001], and HR=4.15 for $Cyto^{abnormal}/MFC^{positive}$ [*P*<0.001] with $Cyto^{normal}/MFC^{negative}$ as reference). Results were similar for patients who received myeloablative or non-myeloablative conditioning. C-statistic values were higher, indicating higher accuracy, when using pre-HCT cytogenetic and MFC MRD information together for prediction of relapse, RFS, and OS, rather than using either test result alone. This study indicates that residual cytogenetic abnormalities and MFC MRD testing provide complementary prognostic information for post-HCT outcomes in patients with cytogenetically abnormal AML undergoing allogeneic HCT.

Introduction

For many adults with acute myeloid leukemia (AML) who have achieved a morphologic remission, allogeneic hematopoietic cell transplantation (HCT) remains an important part of curative therapy.[1,2] However, the likelihood of post-HCT relapse varies considerably across individual patients. Among established prognostic factors, evidence of residual leukemia cells at the time of HCT plays a key role in informing on relapse risk. Currently of greatest interest are assays to quantify measurable residual disease (MRD) via multiparameter flow cytometry (MFC) or assessment of molecular abnormalities.[3–14] However, less sensitive methods have been used as well for the detection of submicroscopic amounts of AML cells, including routine cytogenetic analysis via chromosome (G-)banding or fluorescence *in situ* hybridization (FISH), with some studies indicating prognostic significance in the context of allogeneic HCT.[15–20]

In a previous analysis that included 286 children and

adults with AML in either remission or relapse transplanted between 2006 and 2009, our group showed that a significant subset of patients (15%) had discordant results from cytogenetic analysis and MFC-based MRD testing. The presence of residual AML before HCT as assessed by either cytogenetics or MFC was associated with increased relapse risk and inferior survival.[20] In this earlier study, there was no difference in survival for those testing positive with only one methodology *versus* both methodologies, but cohort sizes were small and patient characteristics heterogeneous (children and adults; remission and relapse; cytogenetically normal or abnormal at diagnosis), limiting statistical analyses and interpretation.[20]

In order to re-evaluate the relationship between pre-HCT residual cytogenetic abnormalities and MFC-based MRD testing and post-HCT outcomes, we examined a large cohort of adults with cytogenetically abnormal AML who underwent allogeneic HCT in first or second remission at our institution between April 2006 and May 2021.

Methods

Study cohort

We identified all adults ≥18 years of age with AML (2016 World Health Organization criteria)[21] who received a first allograft while in first or second morphologic remission (i.e., <5% blasts in bone marrow) with or without peripheral blood count recovery between 4/2006 and 5/2021. Data from 440 of the 506 patients in the final study cohort have been partially reported.[22–32] The HCT-specific comorbidity index (HCT-CI) was calculated as described.[33] Related or unrelated donors were selected by high resolution HLA-typing. Post-HCT maintenance therapy was not typically done except in a small subset of patients with *FLT3*-mutated AML after midostaurin was approved in 2017. Information on post-HCT outcomes was captured via the Long-Term Follow-Up Program through medical records from our outpatient clinic and local clinics that provided primary care for patients in addition to records obtained on patients in research studies. All patients were treated on Institutional Review Board-approved research protocols (all registered with ClinicalTrials.gov) or standard treatment protocols and gave consent in accordance with the Declaration of Helsinki. Follow-up was current as of February 10, 2022.

Classification of disease risk and treatment response

The refined MRC/NCRI and the ELN 2017 criteria were used to assign cytogenetic risk at diagnosis.[2,34] Cytogenetically normal AML was considered in patients with a normal karyotype regardless of how many metaphases were available for analysis.[30–32,35] Since molecular data at time of diagnosis were lacking in many patients, only cytogenetic risk could be used to classify patients. Secondary AML was defined as disease following an antecedent hematologic disorder or treatment with systemic chemotherapy and/or radiotherapy for a different disorder.[30–32] Treatment responses were categorized as proposed by the European LeukemiaNet[2] except that post-HCT relapse was defined as emergence >5% blasts by morphology or MFC in blood or bone marrow, emergence of cytogenetic abnormalities seen previously, or presence/emergence of any level of disease if leading to a therapeutic intervention.[30–32]

Cytogenetics studies and multiparameter flow cytometry

At the time of diagnosis and before HCT, samples from bone marrow aspirates were tested for cytogenetic abnormalities using standard culturing and G-banding analysis. The karyotype analysis was based on 20 metaphases for most samples as a routine procedure and FISH studies were performed according to standard procedures at the time of diagnosis and pre-HCT assessment in a subset of patients. The presence of any clonal abnormality by either karyotyping or FISH was considered an abnormal cytogenetic result. Ten-color flow cytometry was performed as a routine clinical test on bone marrow aspirates obtained before starting conditioning therapy. The methodology of the MFC MRD assay has remained essentially unchanged throughout the study period.[22–24,26,28,30,36] Any measurable level of MRD was considered positive, consistent with prior analyses.[22–32]

Statistical analysis

Categorical variables were presented as numbers with proportions and compared using the Chi² test or the Fisher's exact test, for small samples (expected values <5). Continuous variables were presented as medians with interquartile range (IQR) and compared using the non-parametric Mann and Whitney test. Unadjusted probabilities of relapse-free survival (RFS) (events: relapse and death) and overall survival (OS) (event: death) were estimated using the Kaplan-Meier method and compared with the log-rank test; associations with RFS and OS were assessed using Cox regression. Probabilities of relapse (with non-relapse mortality [NRM] as a competing event) and NRM (death without prior relapse with relapse as a competing risk) were summarized using cumulative incidence estimates; associations with cumulative incidence of relapse and NRM were assessed using cause-specific regression models. All tests were two-sided with a significant level $P<0.05$. Statistical analyses were performed with R (R Foundation for Statistical Computing, Vienna, Austria; http://www.r-project.org).

Results

Characteristics of study cohort

Of 1,011 adults with AML allografted between 2006 and 2021, 506 patients with cytogenetically abnormal AML at diagnosis and pre-HCT karyotyping and MFC MRD testing available were included in our analysis. In addition to 422 patients with cytogenetically normal AML, we excluded 21 patients because they did not agree to their data being used for research purposes, 40 because they did not have cytogenetic data available at diagnosis, ten because they did not undergo pre-HCT MRD testing, and 12 because they did not have informative pre-HCT cytogenetic studies (not enough metaphases and no FISH study); see Figure 1. Of the 506 patients, cytogenetics studies were abnormal in 119 patients (24%) at the time of HCT (for details on cytogenetic and FISH analyzes, see the *Online Supplementary Table S1*). No cytogenetic abnormal result was based on an abnormal FISH study alone. Discordant results between pre-HCT cytogenetics and MFC MRD testing were observed in 123 patients (24%) with 63 (12%) having pre-HCT cytogenetic abnormalities but negative MRD by MFC and 60 (12%) having normal pre-HCT cytogenetics but positive MFC MRD. Table 1 shows patient characteristics according to pre-HCT cytogenetics and MFC MRD status. Patients with cytogenetic abnormalities were older irrespective of pre-HCT MFC MRD status (P<0.001). Patients with cytogenetically adverse-risk disease at diagnosis were more likely to have persistent abnormal cytogenetics and/or positive MFC MRD pre-HCT (P<0.001). Those with secondary AML were more likely to be MFC MRD-positive, irrespective of the pre-HCT karyotype (P<0.001). *FLT3*, *NPM1*, and *CEBPA* mutational status was infrequently available for patients but those with *FLT3*-ITD mutations at diagnosis were more likely to have pre-HCT normal cytogenetics and negative MFC MRD (P=0.002).

Relationship between pre-hematopoietic cell transplantation cytogenetic abnormalities, multiparameter flow cytometry measurable residual disease, and post-hematopoietic cell transplantation outcome

With a median follow-up of 5.38 years after HCT in survivors (IQR, 2.48-9.18), there were 173 relapses, 239 deaths,

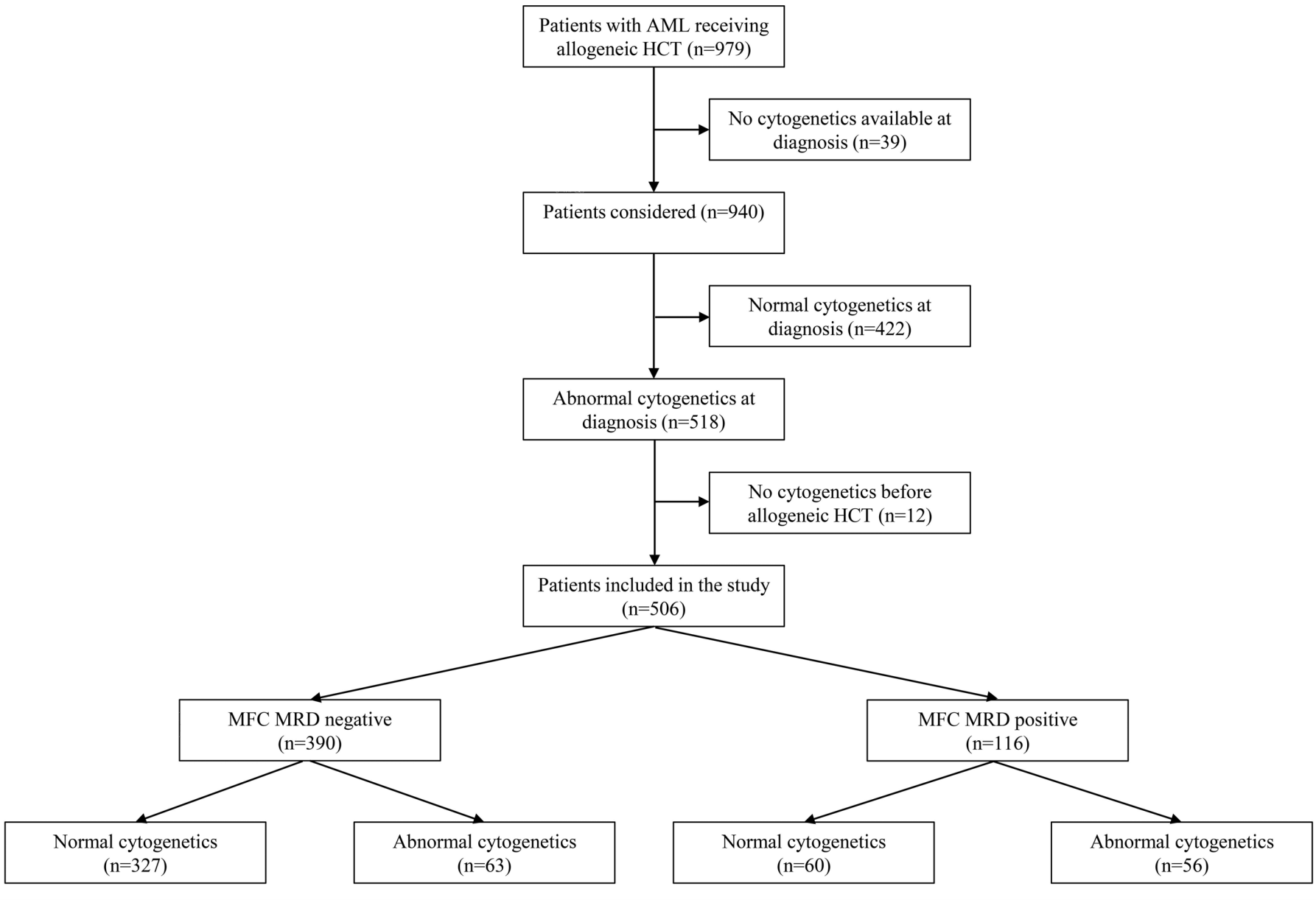

Figure 1. Flowchart describing the selection and distribution of analyzed patients. AML: acute myeloid leukemia; HCT: hematopoietic cell transplantation; MFC: multiparameter flow cytometry; MRD: measurable residual disease.

and 91 NRM events contributing to the probability estimates for relapse, RFS, OS, and NRM. The cumulative incidence of relapse was higher in patients with pre-HCT cytogenetic abnormalities and MFC MRD positivity (3-year cumulative incidence of relapse, 51% *vs*. 27% for those without pre-HCT cytogenetic abnormalities and 67% *vs*. 23% for those without pre-HCT MFC MRD positivity, respectively) whereas NRM was similar in all groups (Table 2; *Online Supplementary Figure S1; Online Supplementary Figure S2*). This translated into lower RFS (median, 0.5 *vs*.

Table 1. Pre-hematopoietic cell transplantation demographic and clinical characteristics of patients with abnormal cytogenetics at diagnosis, stratified by cytogenetics and multiparameter flow cytometry status before hematopoietic cell transplantation.

	Cyto AbN at diagnosis (N=506)	Cyto Normal MFC- (N=327)	Cyto AbN MFC- (N=63)	Cyto Normal MFC+ (N=60)	Cyto AbN MFC+ (N=56)
Median age at HCT in years (IQR)	53 (40-63)	50 (39-60)	63 (55-69)	53 (41-62)	62 (47-67)
Female sex, N (%)	219 (43)	149 (46)	26 (41)	21 (35)	23 (41)
HCT-CI, N (%)					
0	171 (34)	120 (37)	17 (27)	18 (30)	16 (29)
1-2	175 (35)	105 (32)	24 (38)	28 (47)	18 (32)
> 3	160 (32)	102 (31)	22 (35)	14 (23)	22 (39)
WBC count at diagnosis x10^9/L, median (IQR)	6 (2-32)	9 (2-39)	4 (2-24)	3 (1-19)	3 (1-13)
Cytogenetic risk (MRC), N (%)					
Favorable	69 (14)	54 (17)	8 (13)	3 (5)	4 (7)
Intermediate	229 (45)	163 (50)	26 (41)	23 (38)	17 (30)
Adverse	208 (41)	110 (34)	29 (46)	34 (57)	35 (62)
Secondary AML	149 (29)	79 (24)	19 (30)	28 (47)	23 (41)
Median CR duration before HCT in days (IQR)	98 (70-142)	98 (72-143)	97 (72-131)	116 (68-146)	79 (55-130)
Remission status, N (%)					
First remission	421 (83)	274 (84)	51 (81)	50 (83)	46 (82)
Second remission	85 (17)	53 (16)	12 (19)	10 (17)	10 (18)
Recovered peripheral blood counts before HCT*, N (%)	368 (73)	247 (76)	37 (59)	48 (80)	36 (64)
Unrelated donor, N (%)	366 (72)	238 (73)	41 (65)	49 (82)	38 (68)
HLA matching, N (%)					
Identical related donor	112 (22)	76 (23)	15 (24)	10 (17)	11 (20)
Matched unrelated donor	245 (48)	160 (49)	29 (46)	28 (47)	28 (50)
1-2 allele mismatch	53 (10)	24 (7)	11 (17)	11 (18)	7 (12)
Haplo-identical	23 (5)	11 (4)	4 (6)	1 (2%	7 (12)
Cord blood	73 (14)	56 (17)	4 (6)	10 (17)	3 (5)
Stem cell source, N (%)					
PB	389 (77)	246 (75)	57 (90)	39 (65)	47 (84)
BM	44 (9)	25 (8)	2 (3)	11 (18)	6 (11)
Cord blood	73 (14)	56 (17)	4 (6)	10 (17)	3 (5)
Conditioning regimen, N (%)					
MAC	318 (63)	210 (64)	34 (54)	40 (67)	34 (61)
Non-MAC	188 (37)	117 (36)	29 (46)	20 (33)	22 (39)
GvHD prophylaxis, N (%)					
CNI+MMF ± sirolimus	229 (45)	152 (46)	24 (38)	28 (47)	25 (45)
CNI+MTX ± other	202 (40)	132 (40)	25 (40)	25 (42)	20 (36)
PTCy	67 (13)	37 (11)	13 (21)	7 (12)	10 (18)
Other	8 (1.6)	6 (12)	1 (2)	0	1 (2)

*Absolute neutrophil count (ANC) ≥1x10^9/L and platelets ≥100x10^9/L. AbN: abnormal; IQR: interquartile range; AML: acute myeloid leukemia; BM: bone marrow; CNI: calcineurin inhibitor; GvHD: graft-*versus*-host disease; HCT: hematopoietic cell transplantation; HLA: human leukocyte antigen; MAC: myeloablative conditioning; MFC: multiparameter flow cytometry; MMF: mycophenolate mofetil; MRD: measurable residual disease; MTX: methotrexate; NMA: non-myeloablative; PB: peripheral blood; PTCy: post-transplantation cyclophosphamide; UCB: umbilical cord blood; WBC: total white blood cell count.

Table 2. Outcome probabilities (with 95% confidence interval) stratified by pre-hematopoietic cell transplantation measurable residual disease status.

	CI relapse (95% CI)				Relapse-free survival (95% CI)				Overall survival (95% CI)			CI non-relapse mortality (95 % CI)		
	1 year	3 years	5 years	Median	1 year	3 years	5 years	Median	1 year	3 years	5 years	100 days	1 year	3 years
All patients (n=506) N %	27 (23-31)	33 (29-37)	35 (31-40)	4.2 (2.4-6.8)	62 (58-67)	53 (48-57)	48 (43-53)	6.5 (4.7-11.5)	73 (69-77)	59 (55-63)	54 (49-58)	4 (3-6)	10 (8-13)	15 (12-18)
Pre-HCT cytogenetics														
Normal (n=387) N %	22 (18-26)	27 (23-32)	30 (25-34)	8.6 (4.6-NR)	68 (64-73)	59 (55-65)	54 (49-60)	11 (6.8-NR)	77 (73-82)	65 (61-70)	60 (54-65)	4 (2-6)	10 (7-13)	14 (10-17)
Abnormal (n=119) N %	46 (37-55)	51 (42-60)	54 (44-63)	0.5 (0.4-1.1)	43 (35-53)	30 (23-40)	28 (20-38)	1.5 (0.9-2.7)	58 (49-67)	38 (30-48)	34 (26-44)	5 (1-9)	12 (6-18)	19 (11-26)
Pre-HCT MFC														
Negative (n=390) N %	17 (13-21)	23 (18-27)	25 (20-29)	11 (6.5-NR)	73 (69-78)	63 (58-68)	58 (53-64)	NR (9.1-NR)	80 (76-84)	68 (63-73)	63 (58-69)	5 (3-7)	10 (7-13)	15 (11-18)
Positive (n=116) N %	63 (54-72)	67 (58-76)	71 (62-79)	0.4 (0.3-0.5)	25 (19-35)	18 (12-27)	13 (8-22)	0.9 (0.8-1.5)	49 (40-59)	30 (22-40)	22 (16-32)	3 (0-6)	11 (6-17)	15 (8-22)
Pre-HCT cytogenetics/MFC														
Normal/MFC- (n=327) N %	15 (11-19)	21 (17-26)	23 (18-28)	NR (8.8-NR)	75 (71-80)	66 (60-71)	60 (55-66)	NR (11-NR)	81 (77-86)	70 (65-75)	65 (60-71)	5 (2-7)	10 (7-13)	13 (10-17)
Abnormal/MFC- (n=63) N %	26 (15-37)	31 (19-42)	33 (21-45)	2.4 (1.1-NR)	63 (52-76)	49 (37-63)	46 (35-61)	5.4 (2.0-NR)	73 (63-85)	56 (45-71)	54 (42-69)	6 (0-12)	11 (3-19)	21 (10-31)
Normal/MFC+ (n=60) N %	59 (46-71)	60 (48-73)	65 (52-78)	0.6 (0.5-0.9)	31 (21-46)	26 (17-40)	19 (11-33)	1.2 (0.9-4.0)	56 (45-70)	41 (30-55)	31 (21-46)	2 (0-5)	10 (2-18)	14 (5-23)
Abnormal/MFC+ (n=56) N %	68 (56-81)	74 (62-86)	76 (64-88)	0.2 (0.3-0.4)	20 (11-33)	10 (4-22)	7 (3-20)	0.9 (0.6-1.5)	41 (30-56)	18 (10-32)	13 (7-27)	4 (0-9)	13 (4-21)	16 (6-27)

CI: cumulative incidence; HCT: hematopoietic cell transplantation; MFC: multiparameter flow cytometry; MRD: measurable residual disease; NR: not reached.

8.6 years in those without pre-HCT cytogenetic abnormalities and 0.4 *vs*. 11 years in those without pre-HCT MFC MRD positivity, respectively) and OS (median, 1.5 *vs*. 11 years in those without pre-HCT cytogenetic abnormalities and 0.9 years *vs*. not reached in those without pre-HCT MFC MRD positivity, respectively). Considering results from pre-HCT karyotyping and MFC MRD testing separately, four groups of patients were identified with differential risk of relapse (3-year cumulative incidence of relapse, 21% for Cyto^{normal}/MFC^{negative} *vs*. 31% for Cyto^{abnormal}/MFC^{negative} *vs*. 60% for Cyto^{normal}/MFC^{positive} *vs*. 74% for Cyto^{abnormal}/MFC^{positive}) whereas NRM was similar between the four groups (Figure 2; Table 2). Similar results were observed for RFS (median, not reached *vs*. 2.4 years *vs*. 0.6 years *vs*. 0.2 years) and OS (not reached *vs*. 5.4 years *vs*. 1.2 years *vs*. 0.9 years) (Figure 2; Table 2).

When comparing persistent cytogenetic abnormalities according to pre-HCT MFC MRD status, patients with negative MFC MRD test were more likely to have abnormalities not qualifying for adverse-risk (49% *vs*. 31%, *P*<0.001) whereas patients with positive MFC MRD test were likely to have monosomal karyotypes (6% *vs*. 30%, *P*<0.001) with deletions (41% *vs*. 61%, P=0.043) and additional material (19% *vs*. 54%, *P*<0.001) being more frequent (*Online Supplementary Table S2*). Mutational data was only available in a very small fraction of patients before HCT and only three patients had evidence of persistent mutations in our cohort, one with a *FLT3*-ITD mutation and both pre-HCT abnormal cytogenetics and positive MFC MRD, and two patients with a *NPM1* mutation, one with both pre-HCT normal cytogenetics and negative MFC MRD and one with pre-HCT abnormal cytogenetics but negative MFC MRD.

In univariable analysis, both pre-HCT cytogenetic abnormalities and MRD by MFC were individually associated

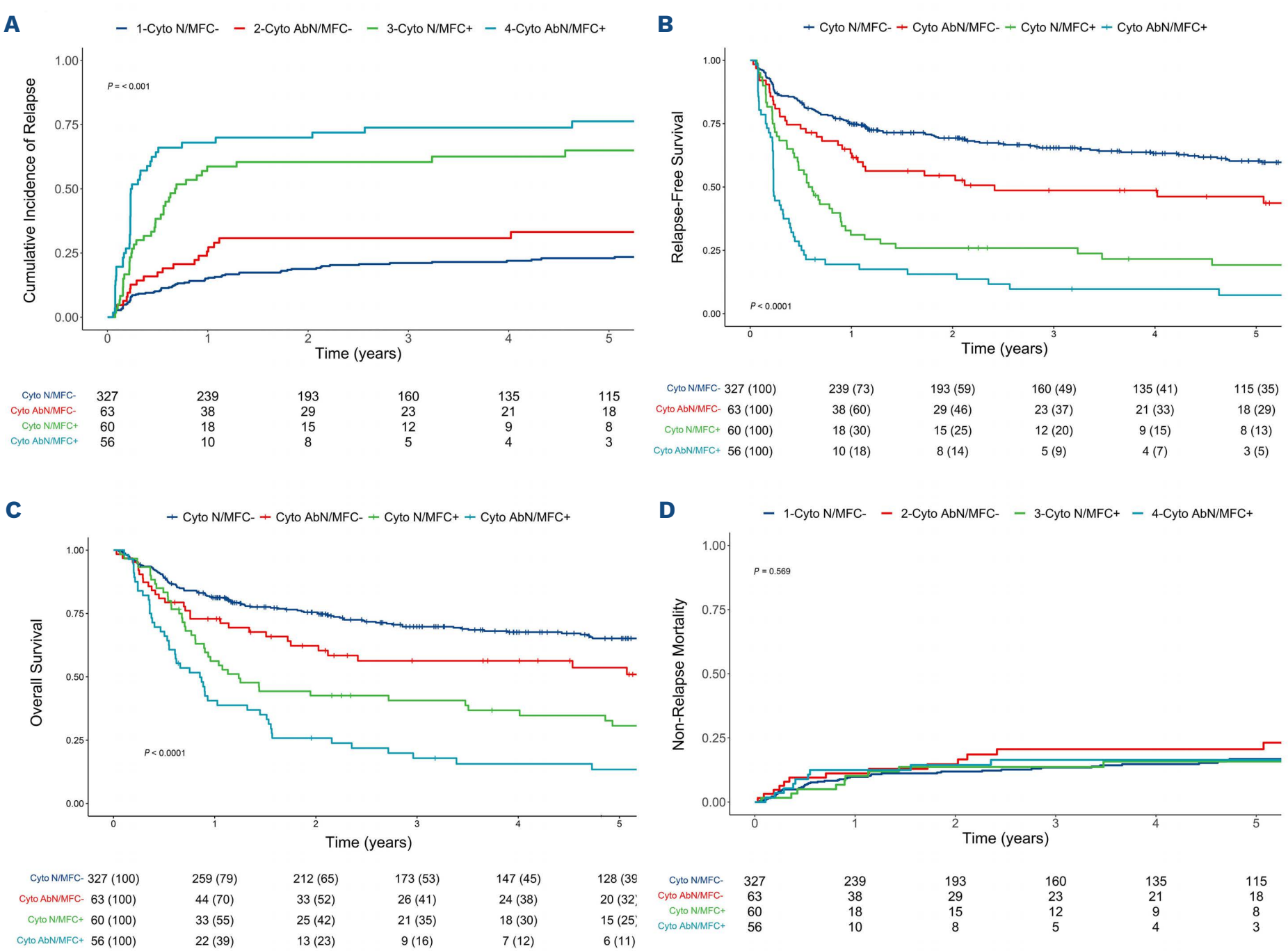

Figure 2. Post-hematopoietic cell transplantation (HCT) outcomes for 506 adults with acute myeloid leukemia and initial abnormal cytogenetics undergoing allogeneic HCT while in first or second morphologic remission, stratified by both pre-HCT cytogenetics and pre-HCT multiparameter flow cytometry. (A) Cumulative incidence of relapse, (B) relapse-free survival, (C) overall survival, and (D) cumulative incidence of non-relapse mortality. Cyto N: normal cytogenetics; Cyto AbN: abnormal cytogenetics; MFC: multiparameter flow cytometry.

with RFS (HR=2.23, 95% CI: 1.72–2.88, *P*<0.001, and HR=3.73, 95% CI: 2.90–4.81, *P*<0.001, respectively) and OS (HR=2.05, 95% CI: 1.56–2.69, *P*<0.001, and HR=3.03, 95% CI: 2.33–3.93, *P*<0.001, respectively) (Table 3). Results from pre-HCT cytogenetic and MFC MRD testing identified four groups of patients with differential RFS (HR=1.63, 95% CI: 1.12–2.37, *P*=0.01, for Cyto^{abnormal}/MFCnegative; HR=3.24, 95% CI: 2.32–4.51, *P*<0.001, for Cyto^{normal}/MFC^{positive}; and HR=5.50, 95% CI: 3.94–7.66, *P*<0.001, for Cyto^{abnormal}/MFC^{positive}, with Cyto^{normal}/MFC^{negative} as reference) and OS (HR=1.55, 95% CI: 1.04–2.32, *P*=0.03, for Cyto^{abnormal}/MFC^{negative}; HR=2.69, 95% CI: 1.91–3.79, *P*<0.001, for Cyto^{normal}/MFC^{positive}; and HR=4.15, 95% CI: 2.94–5.85, *P*<0.001, for Cyto^{abnormal}/MFC^{positive}, with Cyto-^{normal}/MFC^{negative} as reference) (Table 3). In a multivariable Cox regression model, both cytogenetic abnormalities and MFC MRD were independently associated with relapse (HR=1.67, 95% CI: 1.16– 2.41, *P*=0.006 and HR=4.44, 95% CI: 3.11–6.34, *P*<0.001, respectively), RFS (HR=1.53, 95% CI: 1.13– 2.06, *P*=0.006 and HR=3.21, 95% CI: 2.39–4.30, *P*<0.001, respectively), and OS (HR=1.41, 95% CI: 1.03 –1.93, *P*=0.034 and HR=2.43, 95% CI: 1.81–3.27, *P*<0.001, respectively) (Table 4). The performance of pre-HCT cytogenetics and MFC MRD test results, separately or together, as predictors for RFS and OS was evaluated by estimating C-statistics in univariable regression models. Higher C-statistic values (indicating higher predictive accuracy) were observed when considering both the results from cytogenetic and MFC MRD testing rather than karyotyping or MFC MRD testing alone. This was true for the prediction of RFS (0.65 *vs*. 0.58 *vs*. 0.63) as well as the prediction of OS (0.63 *vs*. 0.58 *vs*. 0.61). The C-statistics values were also higher when using the information from both cytogenetic testing and MFC MRD testing separately rather than when assuming residual disease positivity if either or both assays were positive (C-statistic, 0.63 and 0.61 for RFS and OS, respectively).

Relationship between pre-hematopoietic cell transplantation cytogenetics/multiparameter flow cytometry measurable residual disease status, conditioning intensity, and post-hematopoietic cell transplantation outcomes

Overall, similar results were obtained in the subset of patients receiving MAC conditioning compared to the entire study cohort. However, the risk of relapse and OS of patients with or without cytogenetic abnormalities but with negative MFC MRD test were not different (for relapse, HR=1.56, 95% CI: 0.76–3.22, *P*=0.2, for Cyto^{abnormal}/MFC^{negative}; HR=5.88, 95% CI: 3.58–9.65, *P*<0.001, for Cyto^{normal}/MFC^{positive}; and HR=10.2, 95% CI: 6.13–17.0, *P*<0.001, for Cyto^{abnormal}/MFC^{positive}, with Cyto^{normal}/MFC^{negative} as reference; for OS, HR=1.49, 95% CI: 0.83–2.66, *P*=0.2, for Cyto^{abnormal}/MFC^{negative}; HR=3.24 , 95% CI: 2.07–5.07, *P*<0.001, for Cyto^{normal}/MFC^{positive}; and HR=4.38, 95% CI: 2.76–6.96, *P*<0.001, for Cyto^{abnormal}/MFC^{positive}, with Cyto^{normal}/MFC^{negative} as reference) (Figure 3A and B). Similar results were observed for patients receiving non-MAC for the outcome of relapse (HR=1.53, 95% CI: 0.77–3.03, *P*=0.2, for Cyto^{abnormal}/MFC^{negative}; HR=2.87, 95% CI: 1.48–5.56, *P*=0.002, for Cyto^{normal}/MFC^{positive}; and HR=4.83, 95% CI: 2.63–8.86, *P*<0.001, for Cyto^{abnormal}/MFC^{positive}, with Cyto^{normal}/MFC^{negative} as reference) and OS (HR=1.55, 95% CI: 0.88–2.72, *P*=0.13, for Cyto^{abnormal}/MFC^{negative}; HR=2.08, 95% CI: 1.22–3.55, *P*<0.001, for Cyto^{normal}/MFC^{positive}; and HR=3.93, 95% CI: 2.35–6.57, *P*<0.001, for Cyto^{abnormal}/MFC^{positive}, with Cyto^{normal}/MFC^{negative} as reference) (Figure 3C and D).

Discussion

There is increasing interest in using results from MRD testing in patients with AML to inform on prognosis and, perhaps, to guide therapeutic decision making.[37] Our current analyses show that both MFC MRD testing as well as standard cytogenetic analysis, via karyotyping and FISH, provides prognostic information for patients undergoing allografting for AML in morphologic remission. Further, our data indicate that cytogenetic and MFC MRD testing provides complementary rather than identical information. With this, our findings suggest that, for optimal risk assessment and outcome prediction, results from both tests should be considered.

Previous studies that have assessed the prognostic role of abnormal cytogenetics at the time of morphologic response in patients with AML have come to mixed conclusions.[15–20,38,39] Although routine cytogenetic testing has a low sensitivity for the detection of residual leukemia considering only 20-30 metaphases are typically analyzed, malignant myeloid cells divide readily in culture, and our results show that pre-HCT cytogenetic studies are useful to evaluate the outcome of adults with AML after HCT. In our cohort, approximately one quarter of patients with cytogenetically abnormal AML at diagnosis were found to have abnormal cytogenetics during the pre-HCT evaluation, and half of these had no disease detectable by flow cytometry. Relative to patients in whom cytogenetic abnormalities could no longer be detected, patients with abnormal cytogenetic findings at the time of HCT had an increased risk of relapse and shorter RFS and OS. Considering that 506 of 940 patients in our cohort had cytogenetically abnormal AML at diagnosis and informative results from pre-HCT karyotyping studies available, one of the limitations of cytogenetic testing as measure for residual AML is that it is applicable to only 54% to 65% of patients.[17,38,39]

As more MRD assays are becoming available, some patients may be evaluated with more than one assay. A small number of previous studies suggest that the use of more than one of MRD test might improve prognostication.[13,40]

Table 3. Univariable regression models of study cohort (patients with abnormal cytogenetics at diagnosis).

	Relapse		Relapse-free survival		Overall survival		Non-relapse mortality	
	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*
Median age at HCT	1.02 (1.00-1.03)	0.004	1.03 (1.02-1.04)	<0.001	1.02 (1.02-1.03)	<0.001	1.05 (1.03-1.07)	<0.001
Female sex	0.96 (0.71-1.30)	0.8	0.84 (0.66-1.08)	0.2	0.77 (0.59-1.00)	0.047	0.66 (0.42-1.01)	0.056
Secondary AML (n=149)	1.32 (0.96-1.81)	0.082	1.43 (1.11-1.84)	0.006	1.38 (1.06-1.80)	0.018	1.65 (1.08-2.52)	0.020
Cytogenetic risk (MRC)								
Favorable (n=69)	Ref.		Ref.		Ref.		Ref.	
Intermediate (n=229)	1.95 (1.06-3.61)	0.032	1.65 (1.07-2.54)	0.023	1.75 (1.10-2.80)	0.019	1.37 (0.75-2.53)	0.3
Adverse (n=208)	3.27 (1.79-5.97)	<0.001	2.10 (1.36-3.23)	<0.001	2.23 (1.40-3.56)	<0.001	0.98 (0.51-1.89)	>0.9
Remission status at HCT								
First remission (n=421)	Ref.		Ref.		Ref.		Ref.	
Second remission (n=85)	1.14 (0.77-1.68)	0.5	1.14 (0.83-1.56)	0.4	1.24 (0.90-1.72)	0.2	1.13 (0.66-1.93)	0.7
Pre-HCT cytogenetics status								
Negative (n=387)	Ref.		Ref.		Ref.		Ref.	
Positive (n=119)	2.48 (1.81-3.38)	<0.001	2.23 (1.72-2.88)	<0.001	2.05 (1.56-2.69)	<0.001	1.78 (1.11-2.84)	0.016
Pre-HCT MFC status								
Negative (n=390)	Ref.		Ref.		Ref.		Ref.	
Positive (n=116)	5.05 (3.72-6.84)	<0.001	3.73 (2.90-4.81)	<0.001	3.03 (2.33-3.93)	<0.001	1.83 (1.10-3.06)	0.021
Pre-HCT cytogenetics/MFC status								
Normal/MRD- (n=327)	Ref.		Ref.		Ref.		Ref.	
Abnormal/MRD- (n=63)	1.64 (1.00-2.69)	0.051	1.63 (1.12-2.37)	0.011	1.55 (1.04-2.32)	0.032	1.63 (0.93-2.89)	0.091
Normal/MRD+ (n=60)	4.37 (2.95-6.46)	<0.001	3.24 (2.32-4.51)	<0.001	2.69 (1.91-3.79)	<0.001	1.66 (0.84-3.26)	0.14
Abnormal/MRD+ (n=56)	7.43 (5.03-11.0)	<0.001	5.50 (3.94-7.66)	<0.001	4.15 (2.94-5.85)	<0.001	2.60 (1.27-5.32)	0.009
Recovered peripheral blood counts before HCT* (n=368)	1.06 (0.75-1.50)	0.7	0.84 (0.64-1.10)	0.2	0.77 (0.58-1.01)	0.05	0.56 (0.37-0.86)	0.008
Stem cell source								
PB (n=389)	Ref.		Ref.		Ref.		Ref.	
BM (n=44)	1.66 (1.06-2.58)	0.026	1.24 (0.83-1.86)	0.3	1.23 (0.81-1.86)	0.3	0.51 (0.19-1.40)	0.2
UCB (n=73)	0.73 (0.45-1.20)	0.2	0.83 (0.57-1.21)	0.3	0.97 (0.66-1.41)	0.9	1.01 (0.57-1.80)	>0.9
Conditioning regimen								
MAC (n=318)	Ref.		Ref.		Ref.		Ref.	
Non-MAC (n=188)	1.48 (1.09-2.00)	0.012	1.83 (1.43-2.33)	<0.001	1.73 (1.34-2.23)	<0.001	2.74 (1.81-4.15)	<0.001

*Absolute neutrophil count (ANC) ≥1x10^9/L and platelets ≥100x10^9/L. AML: acute myeloid leukemia; BM: bone marrow; HCT: hematopoietic cell transplantation; HLA: human leukocyte antigen; MAC: myeloablative conditioning; MFC: multiparameter flow cytometry; MRD: measurable residual disease; PB: peripheral blood; UCB: umbilical cord blood.

The emerging data for the current and a prior study from our institution indicate that the same principle is true for cytogenetics/MFC MRD testing.[20] Almost a quarter of patients (24% in the present series) have discordant results.[20] Whereas normal cytogenetics with positive MFC can be explained by the low sensitivity of cytogenetics, abnormal cytogenetics with negative MFC might be due to some leukemic cells having normal patterns of antigen expression or changing immunophenotypes and/or to the presence of preleukemic cytogenetic abnormalities.[17,20] In

Table 4. Multivariable regression models of study cohort (patients with abnormal cytogenetics at diagnosis).

	Relapse		RFS		OS		Non-relapse mortality	
	HR (95% CI)	***P***	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***
Median age at HCT	1.00 (0.98-1.01)	0.7	1.01 (1.00-1.02)	0.054	1.01 (1.00-1.02)	0.056	1.04 (1.02-1.07)	<0.001
Female sex	1.15 (0.84-1.57)	0.4	1.00 (0.77-1.29)	>0.9	0.85 (0.65-1.12)	0.2	0.75 (0.48-1.18)	0.2
Secondary AML (n=149)	0.83 (0.59-1.17)	0.3	0.93 (0.70-1.22)	0.6	1.01 (0.76-1.35)	>0.9	1.17 (0.73-1.89)	0.5
Cytogenetic risk (MRC)								
Favorable (n=69)	Ref.		Ref.		Ref.		Ref.	
Intermediate (n=229)	2.69 (1.33-5.42)	0.006	1.78 (1.07-2.94)	0.026	1.99 (1.17-3.38)	0.011	1.13 (0.54-2.33)	0.7
Adverse (n=208)	3.44 (1.68-7.05)	<0.001	1.79 (1.06-3.01)	0.028	2.02 (1.17-3.47)	0.011	0.69 (0.32-1.49)	0.3
Remission status at HCT								
First remission (n=421)	Ref.		Ref.		Ref.		Ref.	
Second remission (n=85)	1.97 (1.24-3.12)	0.004	1.66 (1.13-2.43)	0.010	1.81 (1.24-2.64)	0.002	1.36 (0.69-2.67)	0.4
Pre-HCT cytogenetics status								
Negative	Ref.		Ref.		Ref.		Ref.	
Positive	1.72 (1.21-3.12)	0.002	1.56 (1.17-2.08)	0.002	1.49 (1.11-2.02)	0.009	1.27 (0.76-2.11)	0.4
Pre-HCT MFC status								
Negative	Ref.		Ref.		Ref.		Ref.	
Positive	4.41 (3.12-6.22)	<0.001	3.25 (2.45-4.33)	<0.001	2.42 (1.82-3.23)	<0.001	1.48 (0.85-2.60)	0.2
Recovered peripheral blood counts before HCT (n=368)	1.39 (0.96-2.01)	0.083	1.15 (0.87-1.54)	0.3	1.00 (0.74-1.35)	>0.9	0.81 (0.50-1.30)	0.4
Stem cell source								
PB (n=389)	Ref.		Ref.		Ref.		Ref.	
BM (n=44)	1.78 (1.11-2.86)	0.016	1.50 (0.98-2.30)	0.061	1.35 (0.87-2.10)	0.2	0.79 (0.28-2.26)	0.7
Cord blood (n=73)	0.83 (0.49-1.39)	0.5	1.07 (0.72-1.60)	0.7	1.25 (0.84-1.86)	0.3	1.83 (0.97-3.45)	0.063
Conditioning regimen								
MAC (n=318)	Ref.		Ref.		Ref.		Ref.	
Non-MAC (n=188)	1.75 (1.20-2.55)	0.003	1.66 (1.22-2.25)	0.001	1.47 (1.07-2.03)	0.019	1.42 (0.83-2.43)	0.2

*Absolute neutrophil count (ANC) ≥1x10^9 and platelets ≥100x10^9. AML: acute myeloid leukemia; BM: bone marrow; HCT: hematopoietic cell transplantation; HLA: human leukocyte antigen; MAC: myeloablative conditioning; MFC: multiparameter flow cytometry; MRD: measurable residual disease; MTX: methotrexate; PBSC: peripheral blood; UCB: umbilical cord blood.

A

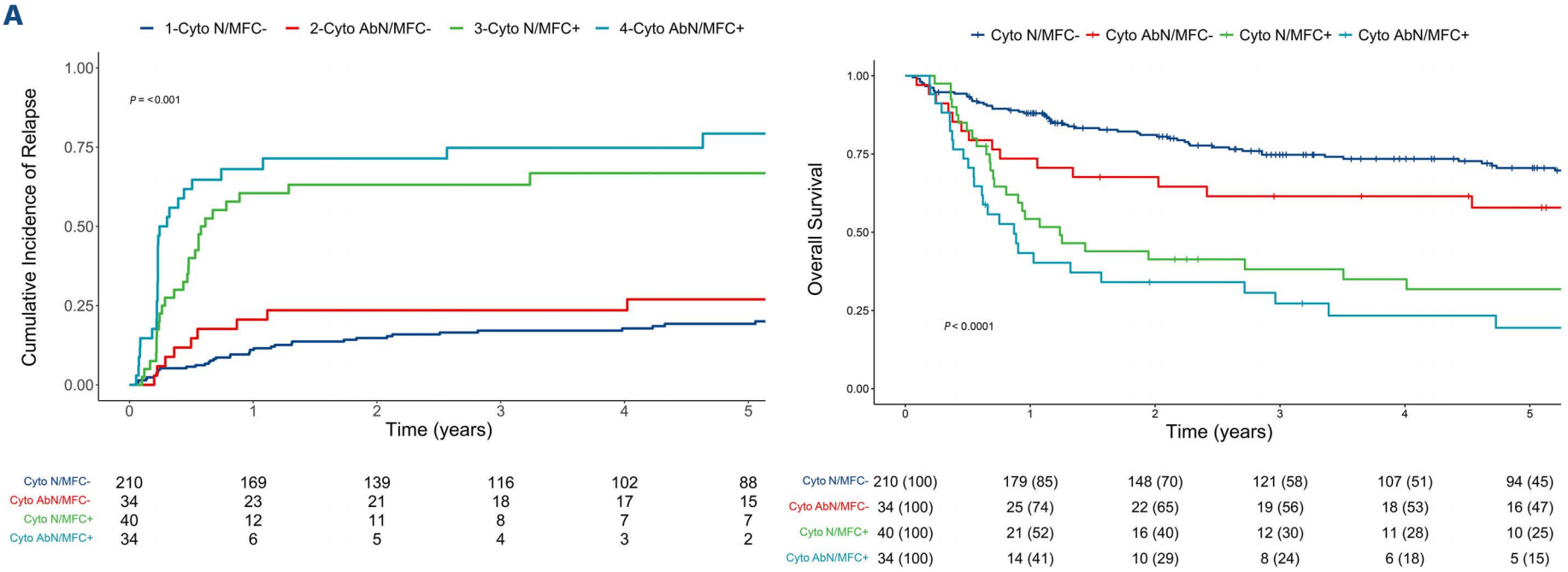

Figure 3. Post-hematopoietic cell transplantation (HCT) outcomes for 506 adults with acute myeloid leukemia and initial abnormal cytogenetics undergoing allogeneic HCT while in first or second morphologic remission, stratified by both pre-HCT cytogenetics and multiparameter flow cytometry and conditioning intensity. (A) Cumulative incidence of relapse and (B) overall survival for patients receiving myeloablative conditioning (MAC) and (C) cumulative incidence of relapse and (D) overall survival for patients receiving non-MAC, respectively. Cyto N: normal cytogenetics; Cyto AbN: abnormal cytogenetics; MFC: multiparameter flow cytometry.

contrast to a previous study including some of the patients used in the current analysis, combining pre-HCT cytogenetics and MFC distinguished four groups of patients with different prognosis whereas the first study showed that patients with either or both markers positivity had similar outcomes.[20] Whereas we have previously shown that MFC MRD identified two groups of patients with different prognosis,[22,26,31,32] we show herein that also considering pre-HCT cytogenetics may further refine the prognostication of patients. This assay could distinguish a group of patients with decreased survival among those with non-measurable residual disease by MFC and a group of patients with very poor outcome among those with MRD by MFC. The predictive value of MFC MRD was also improved when considering pre-HCT cytogenetics with increased C-statistic values from 0.63 with MFC MRD alone to 0.65 with the combined assays for RFS and from 0.61 to 0.63 for OS. The combination of cytogenetics and MFC has also been shown to be associated with post-HCT outcomes in other studies although because of the relative small sample size, the input of each MRD method on outcome prognostication could not be assessed.[41,42] Similar results were observed in other series combining two of the following: MFC, *WT1* quantification, *NPM1* quantification, or next-generation sequencing (NGS).[12,13,40,43–45] How pre-HCT cytogenetics and MFC MRD can be used together with newer methods for MRD detection such as NGS will require further studies.

The retrospective nature of our study analyzing patients nonrandomly assigned to different conditioning regimens limits our ability to draw definitive conclusions on the

management of patients with pre-HCT MRD. Our general preference has been for the use of myeloablative conditioning whenever we felt it could be safely administered based on patient age and comorbidities. MFC-based MRD testing has been routinely performed on bone marrow specimens during the pre-HCT work-up in our institution since 2006 and has always been available to transplantation teams. Despite being recognized as a relevant prognostic marker, the pre-HCT MRD status did not play any major role in the selection of the type of preparative regimen. In addition, we did not preemptively apply other strategies to try and prevent relapse (i.e., withdrawal of immunosuppressive agents, donor lymphocyte infusions, or maintenance chemotherapy) based on pre-HCT MRD status. Because mutational profiles were only available for a small subset of patients, our ability to account for disease risk is limited. Pre-HCT molecular data was also infrequently available and potential associations with pre-HCT cytogenetics and MFC MRD could not be extensively explored in this study.

This study confirms that data from pre-HCT cytogenetic studies are associated with post-HCT outcomes, as are data from MFC MRD testing. The combined use of pre-HCT cytogenetics and MFC MRD data further refines risk assessment. Our findings indicate that more accurate prognostic information can be gained by distinguishing four groups of patients based on data from cytogenetic and MFC MRD testing. The observation that these four patient subsets have different post-HCT outcome expectations could serve as the basis for the refined evaluation of pre-HCT or post-HCT preemptive strategies aimed at reducing relapse risks and improving outcomes in adults with AML undergoing allografting. How additional information from NGS will further impact risk assessment is unknown.

Disclosures

CO has receieved honoraria from Novartis.

Contributions

RBW conceived and designed the study. CO, JAW, MF, BMS, ERA, BLW, FRA and RBW collected and assembled the data. CO, MO and RBW analyzed and interpreted the data. All authors wrote the manuscript and approved the final version of the manuscript.

Acknowledgments

The authors acknowledge the excellent care provided by the physicians and nurses of the HCT teams, the staff in the Long-Term Follow-up office at the Fred Hutchinson Cancer Center, the Hematopathology Laboratory at the University of Washington, and the patients for participating in our research protocols.

Funding

Research reported in this publication was supported by grants P01-CA078902, P01-CA018029, and P30-CA015704 from the National Cancer Institute/National Institutes of Health (NCI/NIH), Bethesda, MD, USA.

Data-sharing statement

The datasets analyzed during the current study are available from the corresponding author upon reasonable request.

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