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ORIGINAL REPORT

Relation of Clinical Response and Minimal Residual Disease and Their Prognostic Impact on Outcome in Acute Myeloid Leukemia

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ABSTRACT

Purpose
Both presence of minimal residual disease (MRD) and achievement of complete remission (CR) with incomplete platelet recovery (CRp) rather than CR after induction therapy predict relapse in acute myeloid leukemia (AML). These results suggest a correlation between response (peripheral count recovery) and MRD at the time of morphologic remission. Here we examine this hypothesis and whether MRD and response provide independent prognostic information after accounting for other relevant covariates.

Patients and Methods
We retrospectively analyzed data from 245 adults with AML who achieved CR, CRp, or CR with incomplete blood count recovery (CRi) after induction therapy. Bone marrow samples were collected on or closest to the first date of blood count recovery, and MRD was determined by 10-color multiparameter flow cytometry.

Results
The 71.0% of patients who achieved CR had MRD less frequently and had lower levels of MRD than the 19.6% of patients achieving CRp and 9.4% achieving CRi. Although pretreatment covariates such as cytogenetics, monosomal karyotype, relapsed or refractory rather than newly diagnosed AML, and *FLT3* internal tandem duplication were associated with relapse, their prognostic effect was much lower once MRD and response were taken into account, the univariable statistical effect of which was not materially affected by inclusion of pretreatment covariates.

Conclusion
Our data indicate that post-therapy parameters including MRD status and response are important independent prognostic factors for outcome in patients with AML achieving remission. MRD status and type of response (CR *v* CRp or CRi) should play important, and perhaps dominant, roles in planning postinduction therapy.

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INTRODUCTION

Acute myeloid leukemia (AML) is a clinically, morphologically, and genetically heterogeneous disease with a variable response to therapy. With standard chemotherapy, complete remission (CR) rates range from 20% to 90%, and relapse, the most common event ultimately leading to death, occurs in 10% to 95%.[1-3] Currently, risk stratification is determined by several patient- and disease-related factors assessed at diagnosis. However, commonly assessed covariates such as age, de novo versus secondary AML, cytogenetics, and aberrations in the *FLT3* and *NPM1* genes are of limited predictive value. For example, Walter et al,[4] using area under the receiver operating characteristic curves (AUCs), found that inclusion of these covariates produced predictive ability for treatment resistance (defined in several ways) that was only intermediate between a coin flip (AUC = 0.5) and certainty (AUC = 1.0). One way to improve predictive accuracy is by including more molecular data as measured pretreatment (eg, mutation in *DNMT3a*, *IDH1*, or *IDH2* gene[5]). Not mutually exclusive, an alternative means is to incorporate information gained only after treatment has begun, such as type of response to therapy (ie, CR *v* response less than

CR) and minimal residual disease (MRD) as measured by multiparameter flow cytometry (MFC).

There is increasing evidence that MRD level after induction therapy is independently associated with risk of relapse and survival.[6-14] The prognostic significance of response to induction therapy has also been recognized, with a recent study showing that achievement of CR, rather than CR with incomplete platelet recovery (CRp; ie, CR with platelet count < 100,000/μL), is independently associated with longer relapse-free survival (RFS).[15] These data suggest a potential correlation between MRD and peripheral count recovery, but this topic has not been explored. The work described here had two goals: to study the relationship between MRD and clinical response (CR, CRp, and CR with incomplete blood count recovery [CRi]) and to examine whether these post-therapy parameters had independent effects on subsequent outcome in patients with AML who achieved CR, CRp, or CRi at our institution from 2008 to 2012.

PATIENTS AND METHODS

Study Population

Our study included 245 adults with newly diagnosed (n = 165) or relapsed or refractory AML (n = 80) who achieved CR, CRp, or CRi after induction therapy at the University of Washington/Fred Hutchinson Cancer Research Center from 2008 to 2012. Patients with acute promyelocytic leukemia were excluded. Cytogenetic risk group was assigned based on Southwest Oncology Group criteria.[16-19] All patients were treated according to institutional review board–approved protocols. Standard-dose induction regimens included 7 + 3 (cytarabine plus daunorubicin) or mitoxantrone plus etoposide; high-dose induction regimens contained cytarabine at individual doses $\geq$ 1 g/m^2 with or without other drugs; low-dose regimens were less intense than 7 + 3 (eg, azacitidine, decitabine, or low-dose cytarabine with or without other drugs). Response was determined after one cycle of high- or standard-intensity therapy and after two cycles of low-intensity therapy. Typically, bone marrow was collected 21 to 28 days after an induction cycle. If the peripheral count had recovered to the defined level, MRD determination was based on that marrow. If the count had not recovered to this level, another marrow was obtained 1 to 2 weeks later, and the process was repeated. A third marrow was requested the next week if the peripheral count still had not recovered, and MRD was based on that marrow.

Response definition followed the criteria proposed by the International Working Group.[20] CR required < 5% blasts by morphologic evaluation (based on $\geq$ 200-cell count), neutrophil count $\geq$ 1,000/μL, and platelet count $\geq$ 100,000/μL. Criteria for CRp were identical but with platelet count < 100,000/μL. CRi was said to be present if morphologic blast count was < 5% and absolute neutrophil count < 1,000/μL. Response was taken as the best of these, with CR better than CRp and CRp better than CRi. MRD was assessed by 10-color MFC. Relapse was defined by marrow with $\geq$ 5% blasts by morphology unrelated to blood count recovery. Follow-up data were current as of October 1, 2013.

Flow Cytometry Detection of MRD

Ten-color flow cytometry analysis was performed as previously described at University of Washington Medical Center Hematopathology Laboratory.[21,22] The panel included three tubes as follows: (1) HLA-DR PB, CD15 FITC, CD33 PE, CD19 PE-TR, CD117 PE-Cy5, CD13 PE-Cy7, CD38 A594, CD34 APC, CD71 APC-A700, and CD45 APC-H7; (2) HLA-DR PB, CD64 FITC, CD123 PE, CD4 PE-TR, CD14 PE Cy5.5, CD13 PE-Cy7, CD38 A594, CD34 APC, CD16 APC-A700, and CD45 APC-H7; and (3) CD56 Alexa488, CD7 PE, CD5 PE-Cy5, CD33 PE-Cy7, CD38 A594, CD34 APC, and CD45 APC-H7. All antibodies were obtained from Beckman Coulter (Fullerton, CA) and Becton Dickinson (San Jose, CA). Up to 1 million events were acquired, and data were analyzed using software developed in our laboratory. MRD was defined as a neoplastic blast population with an abnormal pattern of antigen expression deviating from normal or regenerating myeloid progenitors.[23] The abnormal blast population was qualified as a percentage of total white cells. Any level of abnormal blast population detected by flow cytometry was considered MRD positive.

Statistical Analysis

Overall survival (OS) and RFS were defined from the date of treatment start and estimated by the Kaplan-Meier method, with patients still alive and/or in continued response censored at date of last contact. Probabilities of relapse and nonrelapse mortality (NRM) were summarized using cumulative incidence estimates,[24,25] with death in CR, CRp, or CRi as a competing risk for relapse and relapse as a competing risk for NRM. Log-rank tests were used to assess differences between time-to-event curves. Cox regression for multivariable analyses was performed to assess the independent effects of the following factors: age (numeric), type of AML (newly diagnosed *v* relapsed or refractory), de novo versus secondary AML (antecedent hematologic disorder or therapy related) within the newly diagnosed group, cytogenetic risk group (favorable *v* intermediate *v* unfavorable *v* miscellaneous and of unknown prognostic significance as per Southwest Oncology Group criteria), monosomal karyotypes (yes *v* no),[16,26] *FLT3* internal tandem duplication (ITD; yes *v* no), *NPM1* mutation status (*NPM1* mutated and *FLT3* wild type *v* other), type of induction chemotherapy (low *v* standard *v* high intensity), response (CR *v* CRp or CRi), MRD status at response (yes *v* no), MRD level at response, and hematopoietic cell transplantation (HCT) during response (yes *v* no). Effect sizes (hazard ratios [HRs]) are provided with 95% CIs, with two-sided $P < .05$ considered statistically significant. The proportional hazards assumption underlying the Cox model was tested. Statistical analyses were performed using STATA software (STATA, College Station, TX).

RESULTS

Patient Characteristics

A total of 245 patients achieved CR, CRp, or CRi, including 165 with newly diagnosed AML (by 2008 WHO criteria)[27] and 80 with relapsed or refractory AML; of these 80 patients, 16 had relapsed AML, and 64 were refractory to one (n = 59) or two courses (n = 5) of induction therapy ($\geq$ 5% blasts by morphology unrelated to blood count recovery). Table 1 lists the detailed characteristics of the 245 patients. Best response achieved was CR in 174 (71.0%), CRp in 48 (19.6%), and CRi in 23 patients (9.4%); 73 patients (29.8%) had flow cytometric evidence of MRD (median level, 1.0% of total white cells; range, 0.004% to 7.6%); 136 patients (55.5%) underwent HCT during remission, including 103 in first CR; 100 of the 245 patients experienced relapse, and 115 died. Median follow-up was 28 months (range, 2 to 68 months) in the 130 patients still alive; 107 of these patients remained in remission.

Relationship Between MRD Status and Response

The frequency of MRD assessed by MFC was lowest in patients achieving CR (19.0%), higher in the CRp group (54.2%), and highest in the CRi group (60.9%; $P < .01$; Table 2). The tendency for CRp or CRi to be associated with MRD was true regardless of treatment intensity or whether patients had newly diagnosed or relapsed or refractory AML (Appendix Table A1, online only). Similarly, MRD level was lowest with CR (median, 0.5%; range, 0.004% to 3.9%), intermediate with CRp (median, 1.1%; range, 0.1% to 4.0%), and highest with CRi (median, 2.7%; range, 0.1% to 7.6%).

MRD Status and Response As Independent Prognostic Factors for Relapse, Overall Mortality, and Nonachievement of RFS

Their strong inter-relationship suggested that response and MRD might provide similar, overlapping information regarding risks

Table 1. Demographic and Clinical Characteristics of Study Population (N = 245)

Characteristic	No.	%
Age, years		
Median	54	
Range	18 to 80	
Sex		
Female	104	42.4
Male	141	57.6
AML status		
Newly diagnosed	165	67.3
De novo	97	59
Secondary	68	41
Refractory or relapsed	80	32.7
Chemotherapy regimen		
High intensity	132	53.9
Low intensity	40	16.3
Standard	73	29.8
Response		
CR	174	71.0
CRp	48	19.6
CRi	23	9.4
MRD status		
Negative	172	70.2
Positive	73	29.8
MRD level, %*		
Median	1.0	
Range	0.004 to 7.6	
No. of patients		
1	< 0.01 to 0.001	
10	< 0.1 to 0.01	
24	< 1 to 0.1	
38	≥ 1	
Cytogenetic risk group		
Favorable	26	10.7
Intermediate	121	49.6
Miscellaneous or unknown	21	8.6
Unfavorable	76	31.1
Monosomy karyotype		
No	214	88.1
Yes	31	11.9
HCT		
No	109	44.5
In CR1	103	42.0
Not in CR1	33	13.5

Abbreviations: AML, acute myeloid leukemia; CR, complete remission; CR1, first complete remission; CRi, complete remission with absolute neutrophil count < 1,000/μL; CRp, complete remission with platelet < 100,000/μL; HCT, hematopoietic cell transplantation; MRD, minimal residual disease.
*Among 73 patients with MRD.

Table 2. Correlation of MRD With Response

	All Patients		CR		CRp		CRi	
MRD Status	No.	%	No.	%	No.	%	No.	%
Total	245	100.0	174	71.0	48	19.6	23	9.4
Positive	73	29.8	33	19.0	26	54.2	14	60.9
Negative	172	70.2	141	81.0	22	45.8	9	39.1
Level, %								
Median	1.0		0.5		1.1		2.7	
Range			0.004 to 3.9		0.1 to 4.0		0.1 to 7.6	

Abbreviations: CR, complete remission; CRi, complete remission with absolute neutrophil count < 1,000/μL; CRp, complete remission with platelet < 100,000/μL; MRD, minimal residual disease.

of relapse, NRM, RFS, and OS. We examined this possibility through multivariable analyses including the covariates described in Patients and Methods. In general, univariable analyses showed expected results (Table 3 summarizes results for relapse; Table 4 summarizes results for relapse or death). The unadjusted risk of MRD positivity versus negativity was 3.81 (95% CI, 2.56 to 5.66; $P < .001$) for relapse, 2.51 (95% CI, 1.73 to 3.63; $P < .001$) for death (Appendix Table A2, online only), and 2.79 (95% CI, 1.92 to 4.04; $P <. 001$) for relapse or death (complement of RFS). Likewise, as MRD increased (in 1% or 0.1% increments), so did risks of these outcomes (Tables 3 and 4). Achievement of CRp or CRi rather than CR conferred an unadjusted risk of 6.31 (95% CI, 4.19 to 9.51; $P < .001$) for relapse, 2.96 (95% CI, 2.04 to 4.30; $P < .001$) for death, and 3.33 (95% CI, 2.30 to 4.83; $P < .001$) for relapse or death. Although both MRD and CRp or CRi increased risks for relapse, death or relapse, and death, neither affected NRM (Figs 1 and 2). Intermediate and unfavorable cytogenetics as compared with favorable cytogenetics were associated with increased risks for relapse, death, and relapse or death. Although there was no statistically significant effect of intermediate versus unfavorable cytogenetics on these outcomes, a monosomal karyotype conveyed increased risk for each (Tables 3 and 4). Considered on their own, HCT in CR and newly diagnosed AML were associated with decreased risks for relapse, death, and relapse or death, whereas the opposite was true for presence of *FLT3* ITD.

Multivariable analyses adjusting for these covariates as well as induction treatment intensity indicated that despite the strong correlation between response and MRD (Table 2), each provided independent prognostic information for relapse (MRD positivity *v* negativity: HR, 3.28; 95% CI, 1.87 to 5.75; $P < .001$; CRp or CRi *v* CR: HR, 3.72; 95% CI, 2.13 to 6.51; $P < .001$; Table 3), death (MRD positivity *v* negativity: HR, 2.50; 95% CI, 1.47 to 4.24; $P < .001$; CRp or CRi *v* CR: HR, 2.26; 95% CI, 1.30 to 3.92; $P < .001$; Appendix Table A2, online only), and either relapse or death (MRD positivity *v* negativity: HR, 3.12; 95% CI, 1.80 to 5.43; $P < .001$; CRp or CRi *v* CR: HR, 2.56; 95% CI, 1.49 to 4.40; $P < .001$; Table 4).

After accounting for MRD and response, the statistical associations between pretreatment cytogenetics (other than monosomal karyotype), *FLT3* ITD mutation status, disease status (relapsed or refractory *v* newly diagnosed), and treatment intensity were much weaker with regard to relapse (Table 3), death (Appendix Table A2, online only), and relapse and death (Table 4) than had been the case when these covariates were considered individually; in contrast, accounting for these pretreatment covariates had no effect on the statistical impact of response and MRD (Tables 3 and 4). HCT in CR remained an independent prognostic factor; patients who underwent HCT were less likely to experience relapse and had better OS and RFS. The proportional hazards assumption was verified for all covariates except AML status and monosomal karyotype for relapse, age and treatment status for RFS, and age and *FLT3*/*NPM1* mutation status for OS. However, it should be noted that these were not the most important covariates in the multivariable analyses.

We also examined various statistical interactions (data not shown). A significant interaction ($P = .009$) was identified between HCT and response such that the negative effect of CRp or CRi on

Table 3. Univariable and Multivariable Cox Regression Analyses for Relapse

Covariate	Univariable Analysis			Multivariable Analysis		
	HR	95% CI	*P*	HR	95% CI	*P*
Age (continuous)	1.01	0.99 to 1.02	.391	0.99	0.97 to 1.01	.45
MRD (positive *v* negative)	3.81	2.56 to 5.66	< .001	3.28	1.87 to 5.75	< .001
MRD (continuous by 1-unit increase)	1.29	1.16 to 1.43	< .001			
MRD (continuous by 0.1-unit increase)	1.03	1.01 to 1.04	< .001			
Response (CRp or CRi *v* CR)	6.31	4.19 to 9.51	< .001	3.72	2.13 to 6.51	< .001
Cytogenetic risk group						
Intermediate *v* favorable	4.38	1.37 to 14.0	.013	2.36	0.66 to 8.51	.188
Miscellaneous *v* favorable	2.45	0.59 to 10.3	.22	3.78	0.69 to 20.8	.126
Unfavorable *v* favorable	4.96	1.53 to 16.0	.008	2.26	0.61 to 8.38	.221
Treatment regimen						
High intensity *v* standard	1.96	1.16 to 3.33	.012	1.19	0.60 to 2.38	.623
Low intensity *v* standard	2.48	1.32 to 4.65	.005	0.80	0.34 to 1.92	.624
AML status						
Newly diagnosed *v* relapsed or refractory	0.56	0.37 to 0.83	.004	0.64	0.36 to 1.14	.132
De novo *v* secondary	1.01	0.60 to 1.72	.964			
FLT3/*NPM1* status						
FLT3 ITD positive *v* other	1.8	1.08 to 3.01	.025	1.72	0.91 to 3.24	.093
NPM1 positive/*FLT3* ITD negative *v* other	0.72	0.32 to 1.58	.41	0.64	0.27 to 1.52	.309
Monosomy karyotype (yes *v* no)	2.19	1.30 to 3.71	.003	2.42	1.03 to 5.66	.042
HCT (yes *v* no)	0.47	0.32 to 0.71	< .001	0.21	0.12 to 0.40	< .001

Abbreviations: AML, acute myeloid leukemia; CR, complete remission; CRi, complete remission with absolute neutrophil count < 1,000/μL; CRp, complete remission with platelet < 100,000/μL; HCT, hematopoietic cell transplantation; HR, hazard ratio; ITD, internal tandem duplication; MRD, minimal residual disease.

death or relapse was greatly reduced in patients who underwent HCT compared with those who did not (HCT: HR, 1.36; 95% CI, 0.67 to 2.79; $P = .397$ *v* no HCT: HR, 5.16; 95% CI, 2.43 to 11.0; $P < .001$). There was no interaction between MRD and HCT for any outcome. A significant interaction ($P = .012$) was identified between MRD and response for relapse; the effect of CRp or CRi was more obvious in patients without MRD than in those with MRD. Specifically, HR for relapse was 1.94 (95% CI, 0.95 to 3.99; $P = .071$) or CRp or CRi versus CR in patients with MRD; it was 7.31 (95% CI, 3.43 to 15.6; $P < .001$) for CRp or CRi versus CR in patients without MRD. In contrast, there were no significant

Table 4. Univariable and Multivariable Cox Regression Analyses for RFS

Covariate	Univariable Analysis			Multivariable Analysis		
	HR	95% CI	*P*	HR	95% CI	*P*
Age (continuous)	1.02	1.00 to 1.03	.029	1.01	0.99 to 1.03	.464
MRD (positive *v* negative)	2.79	1.92 to 4.04	< .001	3.12	1.80 to 5.43	< .001
MRD (continuous by 1-unit increase)	1.19	1.06 to 1.34	.003			
MRD (continuous by 0.1-unit increase)	1.02	1.01 to 1.03	.003			
Response (CRp or CRi *v* CR)	3.33	2.30 to 4.83	< .001	2.56	1.49 to 4.40	< .001
Cytogenetic risk group						
Intermediate *v* favorable	3.37	1.22 to 9.29	.019	1.76	0.48 to 6.46	.397
Miscellaneous *v* favorable	4.08	1.30 to 12.8	.016	1.77	0.50 to 6.25	.377
Unfavorable *v* favorable	3.91	1.40 to 10.9	.009	4.14	0.91 to 18.9	.067
Treatment regimen						
High intensity *v* standard	1.34	0.85 to 2.10	.209	0.81	0.42 to 1.56	.524
Low intensity *v* standard	1.84	1.06 to 3.22	.031	0.53	0.22 to 1.28	.157
AML status						
Newly diagnosed *v* relapsed or refractory	0.51	0.35 to 0.74	< .001	0.45	0.26 to 0.78	.004
De novo *v* secondary	0.73	0.44 to 1.20	.217			
FLT3/*NPM1* status						
FLT3 ITD positive *v* other	1.81	1.10 to 2.99	.02	1.87	1.02 to 3.40	.052
NPM1 positive/*FLT3* ITD negative *v* other	0.61	0.26 to 1.41	.244	0.62	0.25 to 1.55	.307
Monosomy karyotype (yes *v* no)	2.13	1.29 to 3.50	.003	2.52	1.09 to 5.81	.03
HCT (yes *v* no)	0.63	0.43 to 0.91	.014	0.29	0.16 to 0.53	< .001

Abbreviations: AML, acute myeloid leukemia; CR, complete remission; CRi, complete remission with absolute neutrophil count < 1,000/μL; CRp, complete remission with platelet < 100,000/μL; HCT, hematopoietic cell transplantation; HR, hazard ratio; ITD, internal tandem duplication; MRD, minimal residual disease; RFS, relapse-free survival.

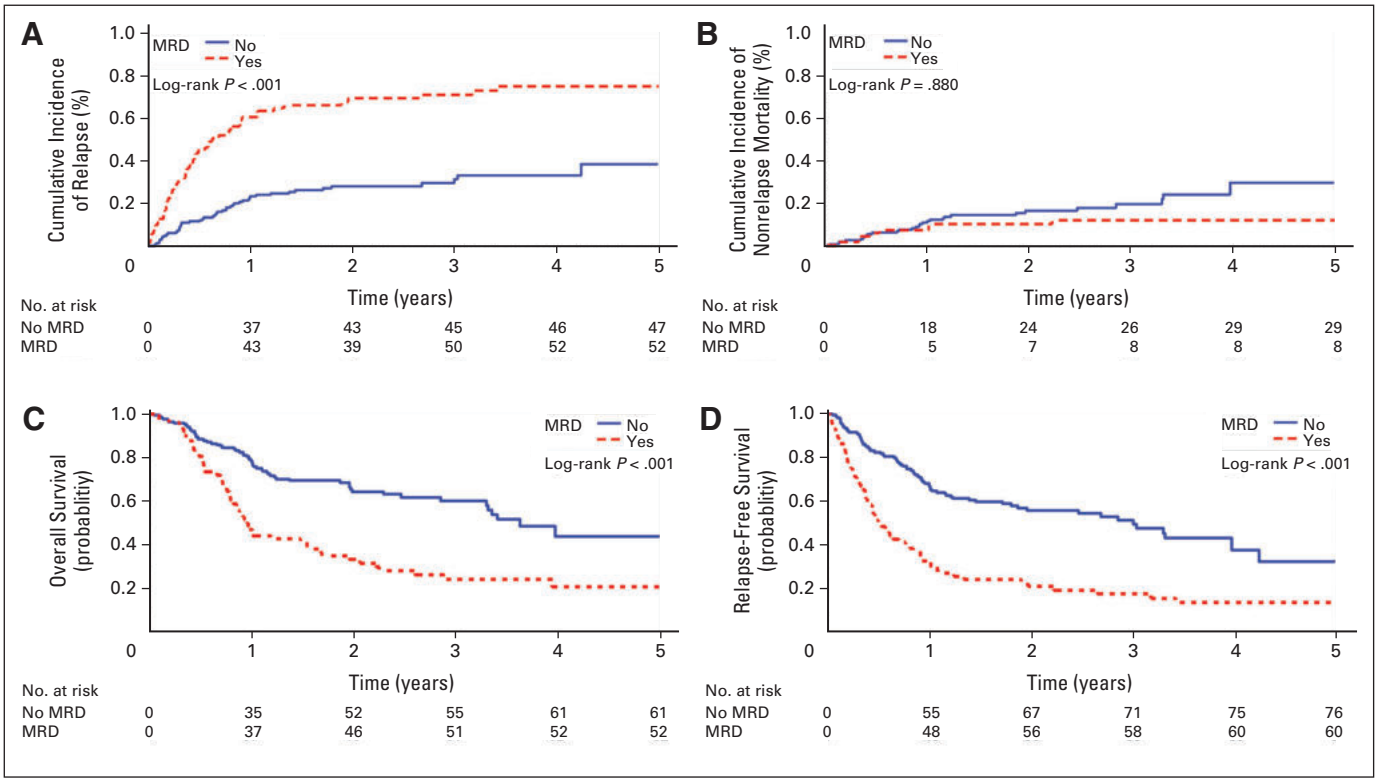

Fig 1. Impact of minimal residual disease (MRD) after induction therapy on outcomes in patients with acute myeloid leukemia. Cumulative incidence of (A) relapse and (B) nonrelapse mortality. Kaplan-Meier survival analysis of probability of (C) overall and (D) relapse-free survival.

interactions between response and MRD with regard to death or to relapse or death. Considering CRp or CRi as a so-called positive test and CR as a negative test, the sensitivity of CRp or CRi was 60%, 50%, and 50% for predicting relapse, RFS, and OS (at 3 years), respectively; corresponding specificities were 91%, 95%, and 87%, respectively. Likewise, MRD was more specific than sensitive; sensitivities of 54%, 46%, and 47% for predicting relapse, RFS, and OS (at 3 years), respectively, with corresponding specificities of 86%, 89%, and 84%, respectively.

DISCUSSION

It is a truism that in medicine, the choice of therapy often depends on prognosis. For example, in AML, as patients' likely outcome with standard therapy worsens, their reluctance to receive such therapy in lieu of a clinical trial grows. However, our ability to deliver accurate prognoses, at least on the basis of commonly measured pretreatment covariates, is limited.[4] Although inclusion of more pretreatment genetic and pharmacogenetic, epigenetic, or proteomic data may improve prognostic ability, Bayes law implies the potential of post-treatment data to do the same. A simple example is that the rate of peripheral blood blast clearance during induction therapy predicts CR and RFS.[28,29] However, many patients do not have circulating blasts. In the last decade, MRD detected by flow cytometry or molecular methods has proven to be predictive of relapse and survival,[6-14] and risk-directed therapy based on MRD assessment may improve outcome in high-risk patients,[30-34] although this remains untested in a randomized trial. Another post-treatment parameter—response as defined by blood count recovery (CR *v* CRp or CRi)—has also been recognized as prognostically significant,[15] suggesting a potential, but yet to be proven, relationship between MRD and response.

Our study seems to be the first to demonstrate a significant correlation between MRD and response (Table 2); patients who achieved CR with incomplete blood count recovery (CRp or CRi) more frequently had MRD and higher levels of MRD than patients achieving CR. This finding suggests that failure of blood count recovery may result from inadequate treatment of AML, as well as the more commonly assumed toxicity to normal progenitors. It has been suggested that persistent leukemic blasts disrupt the marrow microenvironment and act as cytotoxic agents.[35] In any event, delaying therapy in patients with only partial blood count recovery despite marrow with < 5% blasts may be unlikely to improve blood counts.

Perhaps our most noteworthy findings are that despite their strong inter-relationship, MRD and response convey independent prognostic information and that accounting for these post-treatment factors renders some traditional pretreatment prognostic factors less relevant. In particular, although monosomal karyotype, *FLT3* ITD mutation, and relapsed or refractory AML are assumed to be associated with greater risk of relapse, whereas favorable cytogenetics are assumed to be associated with a reduced risk of relapse,[10,14,15,36] and were found to be so in our univariable analyses, these factors lost much of their significance once MRD and response were taken into account. Clearly, risk assessment at diagnosis should be adjusted in light of post-therapy data. For example, the unfavorable effect of achieving only a CRp or CRi was much more obvious in the absence of HCT. Hence, our study provides empiric support for the currently only intuitive view that patients with favorable cytogenetics or

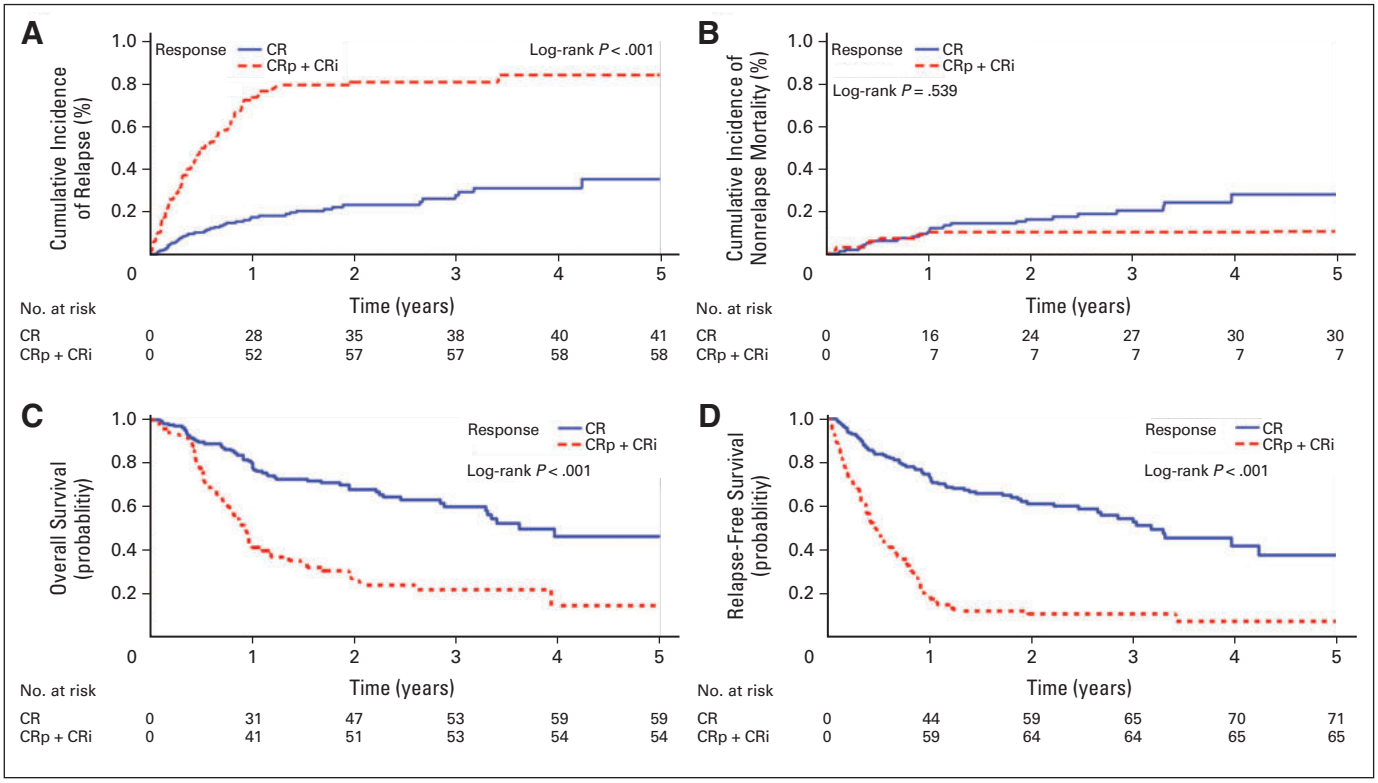

Fig 2. Impact of response after induction therapy on outcome in patients with acute myeloid leukemia. Cumulative incidence of (A) relapse and (B) nonrelapse mortality. Kaplan-Meier survival analysis of probability of (C) overall and (D) relapse-free survival. CR, complete remission; CRi, complete remission with incomplete blood count recovery; CRp, complete remission with incomplete platelet recovery.

with *NPM1* mutation–positive/*FLT3* ITD–negative status pretreatment who attain only one of these lesser responses should receive HCT. In contrast, patients who enter a second CR and are MRD negative may not necessarily require HCT in second CR, because whether the CR is the first or second becomes less relevant in this case. However, it should be noted that for most of our patients with refractory disease, only one course of therapy failed.

Our study has several limitations. First, there are a considerable number of patients who remained in CR, CRp, or CRi, and addition of more relapses might alter our conclusions. Second, although our conclusions were not affected by intensity of induction therapy, relatively few patients received nonintense therapies such as azacitidine or decitabine, and information about therapy after CR, CRp, or CRi (other than receipt of HCT) was often missing, because patients frequently returned to their local communities to receive subsequent therapy. Third, although in principle MRD should be measured at a fixed time, preferably when absolute neutrophil and platelet counts are highest, this was not logistically feasible. Fourth, the predictive value of MRD may have been affected by interlaboratory variations in testing and lack of consensus on the timing and type of technique used. Even with our relatively sophisticated MRD protocols, 20% of patients who had no MRD before HCT experienced relapse after HCT.[37] With less sensitive MRD measurements, the relative prognostic importance of response may increase; in any event, monitoring of blood count recovery is straightforward and consistent among laboratories.

In addition, we did not use Mantel-Byar methodology,[38] in which patients would enter the HCT group only after undergoing HCT, and this may have contributed to an overestimation of the effect of HCT. However, if anything, this seems likely to have increased the effect of response and MRD. Finally, our population was obviously heterogeneous; however, this reflects the disease itself, which is treated with various regimens and includes newly diagnosed and relapsed cases. Although several regimens were included in each of our high-, standard-, and low-intensity cohorts, treatment intensity had no effect on outcome after response and MRD were taken into account. More importantly, including patients with both newly diagnosed and relapsed or refractory disease enabled us to observe that response and MRD were more important than pretreatment disease status, a finding with clinical implications as noted.

In conclusion, our study suggests that although peripheral count recovery (CR *v* CRp or CRi) and MRD level are linked, each is an independent prognostic factor for relapse, OS, and RFS in AML. Information about these post-treatment factors is likely more important than information about several traditional pretreatment prognostic factors and should play a major—and perhaps the dominant—role in planning postinduction therapy. Testing of this hypothesis will be greatly helped by standardization of methods for MRD determination and for timing of assessment of MRD and response.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at www.jco.org.

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REFERENCES

1. Burnett AK, Goldstone AH, Stevens RM, et al: Randomised comparison of addition of autologous bone-marrow transplantation to intensive chemotherapy for acute myeloid leukaemia in first remission: Results of MRC AML 10 trial—UK Medical Research Council Adult and Children's Leukaemia Working Parties. Lancet 351:700-708, 1998

2. Ferrara F, Schiffer CA: Acute myeloid leukaemia in adults. Lancet 381:484-495, 2013

3. Lowenberg B: Strategies in the treatment of acute myeloid leukemia. Haematologica 89:1029-1032, 2004

4. Walter RB, Othus M, Ho PA, et al: Prediction of therapeutic resistance in adult acute myeloid leukemia: Analysis of 4,550 newly diagnosed patients from MRC/NCRI, HOVON/SAKK, SWOG, and MD Anderson Cancer Center. Blood 122, 2013 (abstr 64)

5. Im AP, Sehgal AR, Carroll MP, et al: DNMT3A and IDH mutations in acute myeloid leukemia and other myeloid malignancies: Associations with prognosis and potential treatment strategies. Leukemia 28:1774-1783, 2014

6. Venditti A, Buccisano F, Del Poeta G, et al: Level of minimal residual disease after consolidation therapy predicts outcome in acute myeloid leukemia. Blood 96:3948-3952, 2000

7. Kern W, Voskova D, Schoch C, et al: Determination of relapse risk based on assessment of minimal residual disease during complete remission by multiparameter flow cytometry in unselected patients with acute myeloid leukemia. Blood 104:3078-3085, 2004

8. Buccisano F, Maurillo L, Gattei V, et al: The kinetics of reduction of minimal residual disease impacts on duration of response and survival of patients with acute myeloid leukemia. Leukemia 20:1783-1789, 2006

9. San Miguel JF, Vidriales MB, López-Berges C, et al: Early immunophenotypical evaluation of minimal residual disease in acute myeloid leukemia identifies different patient risk groups and may contribute to postinduction treatment stratification. Blood 98:1746-1751, 2001

10. Terwijn M, van Putten WL, Kelder A, et al: High prognostic impact of flow cytometric minimal residual disease detection in acute myeloid leukemia: Data from the HOVON/SAKK AML 42A study. J Clin Oncol 31:3889-3897, 2013

11. Yin JA, O'Brien MA, Hills RK, et al: Minimal residual disease monitoring by quantitative RT-PCR in core binding factor AML allows risk stratification and predicts relapse: Results of the United Kingdom MRC AML-15 trial. Blood 120:2826-2835, 2012

12. Krönke J, Schlenk RF, Jensen KO, et al: Monitoring of minimal residual disease in NPM1-mutated acute myeloid leukemia: A study from the German-Austrian Acute Myeloid Leukemia Study Group. J Clin Oncol 29:2709-2716, 2011

13. Jourdan E, Boissel N, Chevret S, et al: Prospective evaluation of gene mutations and minimal residual disease in patients with core binding factor acute myeloid leukemia. Blood 121:2213-2223, 2013

14. Freeman SD, Virgo P, Couzens S, et al: Prognostic relevance of treatment response measured by flow cytometric residual disease detection in older patients with acute myeloid leukemia. J Clin Oncol 31:4123-4131, 2013

15. Walter RB, Kantarjian HM, Huang X, et al: Effect of complete remission and responses less than complete remission on survival in acute myeloid leukemia: A combined Eastern Cooperative Oncology Group, Southwest Oncology Group, and M. D. Anderson Cancer Center study. J Clin Oncol 28:1766-1771, 2010

16. Medeiros BC, Othus M, Fang M, et al: Prognostic impact of monosomal karyotype in young adult and elderly acute myeloid leukemia: The Southwest Oncology Group (SWOG) experience. Blood 116:2224-2228, 2010

17. Slovak ML, Kopecky KJ, Wolman SR, et al: Cytogenetic correlation with disease status and treatment outcome in advanced stage leukemia post bone marrow transplantation: A Southwest Oncology Group study (SWOG-8612). Leuk Res 19:381-388, 1995

18. Mrózek K, Heerema NA, Bloomfield CD: Cytogenetics in acute leukemia. Blood Rev 18:115-136, 2004

19. Grimwade D, Hills RK, Moorman AV, et al: Refinement of cytogenetic classification in acute myeloid leukemia: Determination of prognostic significance of rare recurring chromosomal abnormalities among 5876 younger adult patients treated in the United Kingdom Medical Research Council trials. Blood 116:354-365, 2010

20. Cheson BD, Bennett JM, Kopecky KJ, et al: Revised recommendations of the International Working Group for Diagnosis, Standardization of Response Criteria, Treatment Outcomes, and Reporting Standards for Therapeutic Trials in Acute Myeloid Leukemia. J Clin Oncol 21:4642-4649, 2003

21. Wood B: 9-color and 10-color flow cytometry in the clinical laboratory. Arch Pathol Lab Med 130:680-690, 2006

22. Kussick SJ, Fromm JR, Rossini A, et al: Four-color flow cytometry shows strong concordance with bone marrow morphology and cytogenetics in the evaluation for myelodysplasia. Am J Clin Pathol 124:170-181, 2005

23. Wood BL: Myeloid malignancies: Myelodysplastic syndromes, myeloproliferative disorders, and acute myeloid leukemia. Clin Lab Med 27:551-575, 2007

24. Kaplan EL, Meier P: Nonparametric estimation from incomplete observations. J Am Stat Assoc 53:457-481, 1958

25. Gooley TA, Leisenring W, Crowley J, et al: Estimation of failure probabilities in the presence of competing risks: New representations of old estimators. Stat Med 18:695-706, 1999

26. Breems DA, Van Putten WL, De Greef GE, et al: Monosomal karyotype in acute myeloid leukemia: A better indicator of poor prognosis than a complex karyotype. J Clin Oncol 26:4791-4797, 2008

27. Swerdlow SH, Campo E, Harris NL, et al: (eds): WHO Classification of Tumours of Haematopoietic and Lymphoid Tissue (ed 4). Geneva, Switzerland, WHO Press, 2008

28. Elliott MA, Litzow MR, Letendre LL, et al: Early peripheral blood blast clearance during induction chemotherapy for acute myeloid leukemia predicts superior relapse-free survival. Blood 110:4172-4174, 2007

29. Vainstein V, Buckley SA, Shukron O, et al: Rapid rate of peripheral blood blast clearance accurately predicts complete remission in acute myeloid leukemia. Leukemia 28:713-716, 2014

30. Yan CH, Liu DH, Liu KY, et al: Risk stratification-directed donor lymphocyte infusion could reduce relapse of standard-risk acute leukemia patients after allogeneic hematopoietic stem cell transplantation. Blood 119:3256-3262, 2012

31. Rubnitz JE, Inaba H, Dahl G, et al: Minimal residual disease-directed therapy for childhood acute myeloid leukaemia: Results of the AML02 multicentre trial. Lancet Oncol 11:543-552, 2010

32. Grimwade D, Jovanovic JV, Hills RK, et al: Prospective minimal residual disease monitoring to predict relapse of acute promyelocytic leukemia and to direct pre-emptive arsenic trioxide therapy. J Clin Oncol 27:3650-3658, 2009

33. Zhu HH, Zhang XH, Qin YZ, et al: MRD-directed risk stratification treatment may improve outcomes of t(8;21) AML in the first complete remission: Results from the AML05 multicenter trial. Blood 121:4056-4062, 2013

34. Platzbecker U, Wermke M, Radke J, et al: Azacitidine for treatment of imminent relapse in MDS or AML patients after allogeneic HSCT: Results of the RELAZA trial. Leukemia 26:381-389, 2012

35. Dvořáková M, Karafiát V, Pajer P, et al: DNA released by leukemic cells contributes to the disruption of the bone marrow microenvironment. Oncogene 32:5201-5209, 2013

36. Walter RB, Buckley SA, Pagel JM, et al: Significance of minimal residual disease before myeloablative allogeneic hematopoietic cell transplantation for AML in first and second complete remission. Blood 122:1813-1821, 2013

37. Walter RB, Gooley TA, Wood BL, et al: Impact of pretransplantation minimal residual disease, as detected by multiparametric flow cytometry, on outcome of myeloablative hematopoietic cell transplantation for acute myeloid leukemia. J Clin Oncol 29:1190-1197, 2011

38. Mantel N, Byar DP: Evaluation of response-time data involving transient states: An illustration using heart-transplant data. J Am Stat Assoc 69:81-86, 1974

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Relation of Clinical Response and Minimal Residual Disease and Their Prognostic Impact on Outcome in Acute Myeloid Leukemia

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Appendix

Table A1. Correlation of MRD With Response in Different Patient Groups

	All Patients		CR		CRp		CRi	
MRD Status	No.	%	No.	%	No.	%	No.	%
High intensity regimen	132		94	71.2	30	22.7	8	6.1
Positive	41	31.1	21	22.3	15	50	5	60
Negative	91	68.9	73	77.7	15	50	3	40
Standard intensity	73		64	87.7	4	5.5	5	6.8
Positive	9	12.3	6	9.4	2	50	1	20
Negative	64	87.7	58	90.6	2	50	4	80
Low intensity regimen	40		16	40	14	35	10	25
Positive	23	57.5	6	37.5	9	64.3	8	80
Negative	17	42.5	10	62.5	5	35.7	2	20
Newly diagnosed AML	165		133	80.6	18	10.9	14	8.5
Positive	38	23	21	15.8	9	50	8	57.1
Negative	127	77	112	84.2	9	50	6	42.9
Relapsed or refractory AML	80		41	51.3	30	37.5	9	11.2
Positive	35	43.8	12	29.3	17	56.7	6	66.7
Negative	45	56.2	29	70.7	13	43.3	3	33.3

Abbreviations: AML, acute myeloid leukemia; CR, complete remission; CRi, complete remission with absolute neutrophil count $< 1{,}000/\mu L$; CRp, complete remission with platelet $< 100{,}000/\mu L$; MRD, minimal residual disease.

Table A2. Univariable and Multivariable Cox Regression Analyses for Overall Mortality

	Univariable Analysis			Multivariable Analysis		
Covariate	HR	95% CI	*P*	HR	95% CI	*P*
Age (continuous)	1.02	1.00 to 1.03	.026	1.01	0.98 to 1.03	.55
MRD (positive *v* negative)	2.51	1.73 to 3.63	< .001	2.50	1.47 to 4.24	< .001
MRD (continuous by 1-unit increase)	1.17	1.05 to 1.32	.007			
MRD (continuous by 0.1-unit increase)	1.02	1.01 to 1.03	.007			
Response (CRp or CRi *v* CR)	2.96	2.04 to 4.30	< .001	2.26	1.30 to 3.92	.004
Cytogenetic risk group						
Intermediate *v* favorable	3.15	1.14 to 8.70	.027	2.07	0.58 to 7.39	.264
Miscellaneous *v* favorable	4.26	1.36 to 13.4	.013	1.78	0.51 to 6.26	.368
Unfavorable *v* favorable	3.64	1.31 to 10.2	.014	3.92	0.88 to 17.5	.073
Treatment regimen						
High intensity *v* standard	1.31	0.83 to 2.06	.243			
Low intensity *v* standard	1.77	1.02 to 3.09	.044			
AML status						
Newly diagnosed *v* relapsed or refractory	0.52	0.36 to 0.76	< .001	0.49	0.30 to 0.82	.007
De novo *v* secondary	0.72	0.44 to 1.19	.202			
FLT3/*NPM1* status						
FLT3 ITD positive *v* other	1.75	1.06 to 2.89	.028	1.71	0.95 to 3.09	.074
NPM1 positive/*FLT3* ITD negative *v* other	0.62	0.27 to 1.45	.272	0.63	0.26 to 1.56	.32
Monosomy karyotype (yes *v* no)	1.91	1.16 to 3.14	.011	1.62	0.72 to 3.64	.246
HCT (yes *v* no)	0.63	0.43 to 0.91	.015	0.32	0.18 to 0.55	< .001

Abbreviations: AML, acute myeloid leukemia; CR, complete remission; CRi, complete remission with absolute neutrophil count $< 1{,}000/\mu L$; CRp, complete remission with platelet $< 100{,}000/\mu L$; HCT, hematopoietic cell transplantation; HR, hazard ratio; ITD, internal tandem duplication; MRD, minimal residual disease.