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Persistence of Minimal Residual Disease Assessed by Multi-Parameter Flow Cytometry is Highly Prognostic in Younger Patients with Acute Myeloid Leukemia

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Abstract

Background—Predicting outcome in patients with acute myeloid leukemia (AML) using pre-treatment predictors has been the cornerstone of management. Post-treatment prognostic factors are increasingly evaluated.

Methods—Among 280 younger patients treated with intermediate dose cytarabine (total of 5 g/m²) and idarubicin based induction chemotherapy who achieved remission, 186 were assessed for MRD using an 8-color multi-parameter flow cytometry (MFC) panel performed on bone marrow specimens, with a sensitivity of 0.1% or higher.

Results—166 patients had available samples at 1-2 months post induction at the time of achieving complete remission (CR) and 79% became negative for MRD, with MRD-negative status associated with improvement in relapse free survival (RFS) (p= 0.0002) and overall survival (OS) (p= 0.0002). 116 were evaluated for MRD status during consolidation and 86% were negative with a MRD-negative status associated with a significant improvement in RFS (p<0.0001) and OS (p<0.0001). 69 patients were evaluated for MRD status after completion of all

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therapy and 84% were negative with a MRD-negative status associated with improvement in RFS ($P<0.0001$) and OS ($P<0.0001$). On multivariate analysis including age, cytogenetics, achieving CR vs. CRp/CRi, and MRD, achieving MRD-negative status was the most important independent predictor of RFS and OS at response ($p=0.008$ and $p=0.0008$, respectively), during consolidation ($p<0.0001$ for both), or at completion of therapy ($p<0.0001$ and $p=0.002$).

Conclusion—Achieving MRD-negative status by MFC is associated with a highly significant improvement in the outcome of younger patients with AML receiving ara-C plus idarubicin-based induction and consolidation regimens.

Keywords

Acute myeloid leukemia; Minimal residual disease; prognostic; survival; relapse-free survival

Introduction

Predicting the outcome of therapy in patients with acute myeloid leukemia (AML) treated with cytotoxic chemotherapy regimens has been an important aspect of therapeutic decision making particularly related to selection of post-remission therapy.¹ This prediction has been mainly based on pre-treatment factors related to the patients' ability to tolerate intensive therapy as well as disease-related variables such as karyotype, molecular features, as well as other biological features hitherto determined to be associated with resistance to the standard cytarabine plus anthracycline regimens.²⁻⁴

Although response to therapy and particularly achievement of morphologic complete remission (CR) has been clearly associated with better outcomes, few other measures of leukemic cell drug sensitivity such as early blast clearance or early marrow response have been consistently used to determine the likely durability of response.⁵⁻⁷ This is in contrast to studies in pediatric acute lymphoblastic leukemia (ALL) where early measures of drug sensitivity such as early response to chemotherapy have been commonly utilized to determine the need for intensification of therapy.^{8,9} This may be partly due to higher likelihood of inherent leukemia resistance to cytotoxic agents and lower probability that intensification is well-tolerated and improves the outcome in adult AML. On the other hand, with the high response rates achieved after intensive chemotherapy regimens, pediatric investigators have begun to increasingly rely on markers of minimal residual disease (MRD) for selection of patients with ALL and AML for further intensification.^{10,11} In the adult population, it can be argued that MRD assessment is of more value in the younger patients who can tolerate more intensive induction therapy and have a higher likelihood of achieving CR and who are more likely to be candidates for allogeneic stem cell transplant.

Recent availability of sensitive assays that can detect residual, submicroscopic leukemia based on leukemia-specific features such as aberrant immuno-phenotype or abnormal molecular markers, as well as the prospects of future availability of novel agents that are more disease-specific and potent, have re-kindled interest in MRD assessment in adult AML.¹²⁻¹⁵ Although prior studies have established the value of MRD assessment in the adult patients with AML treated with cytotoxic regimens, its value in prognostication and post-remission therapeutic decision making as well as the best assay to detect it continues to

be debated.¹² Herein, we report data on adult patients with AML treated at our institution with intensive chemotherapy regimens and examine the prognostic value of MRD detection using multi-parameter flow cytometry.

Methods

Patients

From April 2007 to June 2015, 318 patients with newly diagnosed AML were treated with one of four regimens containing intermediate dose (5 g/m^2 total dose) of cytarabine in addition to idarubicin for induction and consolidation courses. All patients were younger than 65 years of age, or if older had to have ELN favorable cytogenetics/molecular features and were deemed fit to receive chemotherapy. Among these 280 (88%) achieved CR or CR with incomplete recovery of platelet count (CRp) or peripheral blood counts (CRi) defined by the revised international working group (IWG) criteria.¹⁶ One hundred and eighty six (58%) had available flow MRD analysis and are subject of this study. Ninety four patients including 62 (66%) with core binding factor leukemia (who were typically monitored by molecular testing alone) did not have available flow MRD analysis. Table 1 summarizes the characteristics of these patients. All patients were treated on clinical trials approved by the MD Anderson Cancer Center Institutional Review Board and written informed consent was obtained from all patients before entry into the studies in accordance to the Declaration of Helsinki.

Treatment regimens and sample collection

The details of the various treatment regimens have been previously published.^{17, 18} Briefly, in all regimens, the induction course contained idarubicin (I)(total dose $12 - 30 \text{ mg/m}^2$) as well as intermediate dose cytarabine (A) (total dose $5 - 10 \text{ g/m}^2$). In the FIA regimen, fludarabine 30 mg/m^2 daily $\times 5$ days was given in addition to IA. Similarly, in the CIA and CLIA regimens, clofarabine 15 mg/m^2 daily $\times 5$ days and cladribine 5 mg/m^2 daily $\times 5$ days were administered in addition to IA. In the FLAG-Ida regimen, fludarabine 30 mg/m^2 daily $\times 5$ as well as GCSF $5 \text{ } \mu\text{g/kg}$ were included in addition to IA. Patient outcomes in terms of survival and relapse-free survival were similar for the FIA, CIA, CLIA regimens; the FLAG-Ida regimen was used almost entirely for patients with favorable risk cytogenetics and as such is expected to be associated with better outcomes. In all the above regimens consolidation consisted of up to 6 cycles of attenuated doses of the same agents. A summary of the treatment regimens is shown in Supplemental Table 1.

Response Definitions

Response assessment was based on the revised criteria defined by the International Working Group for AML.¹⁶ To achieve CR, patients needed to have less than 5% blasts by morphological assessment of the bone marrow specimen together with an absolute neutrophil count $> 1,000/\mu\text{L}$ and platelet count $> 100,000/\mu\text{L}$ in the peripheral blood with no evidence of extramedullary disease. Definition of CRp met the above criteria but with platelet count $< 100,000/\mu\text{L}$ whereas the definition of CRi met the above criteria but with absolute neutrophil count $< 1,000/\mu\text{L}$ or platelet count $< 100,000/\mu\text{L}$.¹⁶

MRD analysis by flow cytometry

MRD assessment by multi-parameter flow cytometry (MFC) was performed on whole bone marrow (BM) specimens using a standard stain-lyse-wash procedure with ammonium chloride lysis. 1×10^6 cells were stained per analysis tube, and data were acquired on at least 2×10^5 cells when specimen quality permitted. Data on standardized 7- to 8-color staining combinations were acquired on FACSCanto II cytometers using FACSDiva software (BD Biosciences) and analyzed using FCS Express (De Novo Software). Several different tube configurations were used through the course of the study, all with staining for CD2, CD4, CD5, CD7, CD13, CD14, CD15, CD19, CD33, CD34, CD38, CD45, CD56, CD64, CD117, CD123, and HLA-DR. All tubes included CD34 and CD45. Both CD34+ cells and a broader gate including CD45 dim mononuclear cells and monocytes were analyzed in parallel for all cases.

MRD was identified in comparison with the known patterns of antigen expression by normal maturing myeloid precursors and monocytes as previously described.^{19, 20} Specimens for comparison included normal and regenerating marrows. MRD was quantitated as a percentage of total leukocytes, after exclusion of most RBC precursors by forward scatter. A distinct cluster of at least 20 cells showing altered expression of at least two antigens was regarded as an aberrant population, yielding an optimal sensitivity of 1 in 10^4 cells, or 0.01%. For cases with significant phenotypic overlap between leukemic blasts and normal myeloid precursors or monocytes, the sensitivity was lower, with an average sensitivity for all cases estimated at 0.1%. All specimens with positive results were included for analysis of clinical outcomes. Specimens with negative results but with suboptimal cell counts were excluded. When available, the phenotypic profiles of pre-treatment blasts were compared to specimens submitted for MRD testing.

Statistical analysis

Patient characteristics were summarized using frequency (%) for categorical variables and median (range) for continuous variables. Overall survival (OS) was defined as the time interval between treatment initiation date and the date of death due to any cause. Patients alive were censored at the date of undergoing allogeneic stem cell transplant or last follow-up date. Relapse-free survival (RFS) is defined as the time interval between response date and the date of disease relapse or date of death due to any cause, whichever occurred first. Patients remaining alive and in continued remission were censored at the date of transplant if they underwent an allogeneic stem cell transplant or at the last follow-up date. The probabilities of OS and RFS were estimated using the method of Kaplan and Meier.²¹ Cox proportional hazards regression models were fit to assess the association between OS or RFS and patient characteristics.²² We used the method described by Gooley et al to estimate the cumulative incidence of relapse considering death as a competing event.²³ All statistical analyses were conducted in SAS and Splus.

Results

Patient characteristics and disposition

Table 1 summarizes the patient characteristics. All patients were diagnosed as having AML based on the World Health Organization criteria of having ≥ 20% blasts in the pre-treatment bone marrow or peripheral blood.¹ The median age of the cohort of 186 patients was 51 years (range, 17-77 years); only 6 (3%) patients were older than 65 and all had European LeukemiaNet favorable disease.⁴ The median white blood count at diagnosis was $4.7 \times 10^9/L$ (Range, $0.5-103 \times 10^9/L$). Cytogenetics were favorable risk in 34 (18%), intermediate risk in 115 (62%) and adverse in 27 (15%) and was not available in 10 (5%) patients. 174 (94%) achieved CR with 11 (6%) achieving CRp and 1 (<1%) achieving CRi. Among them 174 (94%) achieved their response after 1 course of induction and 12 (6%) required at least 2 courses. 67 (36%) patients underwent an allogeneic stem cell transplant in first CR. Overall, 47 (25%) patients have relapsed or died with a median follow-up of 17 months (range, 1.2 – 77.4 months) for the survivors. The median relapse free survival (RFS) for the entire population has not been reached (range, 0.4 – 76.5 months) and the median overall survival is 60.8 months (range, 1.2 – 77.4 months)(Supplemental Figure 1). Among the patients who were transplanted in first CR, 13 have relapsed and 45 are surviving in CR (9 died in CR post-transplant). The last MRD evaluation was positive in 10 and negative in 56 (and not available in 1) among the transplanted patients with no difference in survival post-transplant between the two groups ($p=0.8$).

The characteristics of the 94 patients seen and treated during the same period who were not included in the study due to the lack of availability of MRD data are also shown in Table 1. The majority (66%) had favorable risk cytogenetics and as such were monitored by reverse transcription polymerase chain reaction (RT-PCR) analysis of the relevant fusion transcripts and not by flow cytometry. Similarly, 61 (65%) of this cohort were treated with the FLAG-Ida regimen which was specifically designed for this subset.

Among the 186 patients who achieved CR/CRp/CRi and had MRD analysis, 166 patients had available samples at 1-2 months post induction at the time of achieving complete remission (CR) and 79% became MRD negative (Figure 1). 116 were evaluated for MRD status during consolidation (after a median of 2 cycles of consolidation, range 1 to 5) and 86% were negative. 69 patients were evaluated for MRD status after completion of all therapy and 84% were negative (Figure 1).

Predictors of outcome

We evaluated the potential predictors of RFS and OS including the known covariates such as patients' age, white blood cell count (WBC) at presentation, cytogenetics, as well as MRD at various time-points. On univariate analysis, cytogenetics (favorable vs. others), percentage of bone marrow blasts at diagnosis, type of response (CR vs. CRi/CRp) and type of therapy (FLAG-Ida vs. CLIA/CIA/FIA) as well as achievement of negative MRD at all the 3 time points indicated, were factors predictive of a better outcome for relapse-free survival and overall survival (Tables 2a and 2b). The type of therapy was confounded by cytogenetics as

the FLAG-Ida regimen was specifically used in patients with favorable risk cytogenetics (Supplemental Table 2).

Achievement of a MRD-negative state at all the time points investigated [at the time of achieving response (1-2 months after start of therapy), during consolidation (3-7 months after start of therapy), and after completion of all therapy (8+ months after start of therapy)] was associated with a significant improvement in RFS and OS (Figure 2). We also examined the prognostic value of achieving an MRD negative state in the subgroup of patients with intermediate risk cytogenetics. Again, achieving MRD negativity at CR, during consolidation and after completion of therapy was associated with a significantly better RFS and OS (Supplemental Figure 2). We did not perform this analysis in the favorable and adverse cytogenetics subgroups due to the limited numbers of patients in these subsets (N=34 and N=27, respectively).

Cumulative incidence of relapse

The cumulative incidence of relapse was estimated using the method by Gooley et al and is presented in Figure 3.²³ There is a significant difference in the incidence of relapse for the MRD negative and MRD positive cohorts at the recorded time points (at response, during consolidation and at the completion of consolidation)(Figure 3).

When patients in the intermediate risk cytogenetic category (N=105) were considered, there was also a significant difference in the cumulative incidence of relapse between patients who were MRD negative or positive at the time of achieving response as well as during consolidation and after completion of therapy (Supplemental Figure 3). Again, this analysis was not performed for the favorable and poor risk groups given their small sample size (N=34 and N=27, respectively).

Outcome prediction by MRD in relation to other covariates

We then examined the value of MRD status at response, during consolidation and after completion of therapy in predicting RFS and OS in relation to other known prognostic covariates. At the time of achieving response (1-2 months after start of therapy), data was available for 166 patients. Multivariate analysis including age, cytogenetics (favorable vs. others), type of response, and MRD status as covariates demonstrated cytogenetics ($p=0.02$) and MRD positive status ($p=0.008$) as the only significant predictors of RFS (Table 3a). Similar analysis for OS, indicated age ($p=0.05$), cytogenetics ($p=0.01$) and MRD status ($p=0.0008$) as the important predictors of OS (Table 3b). Multivariate analysis performed at other time points (during consolidation and after completion of therapy) showed MRD status as the only statistically significant predictor for RFS ($p<0.0001$ for both time points) and for OS ($p<0.0001$ and $p=0.002$, respectively) (Table 3a and 3b).

Discussion

Despite significant progress in the treatment of patients with acute leukemia, the majority of patients relapse.²⁴ Although a subset of relapsed patients can be rescued with allogeneic stem cell transplant, the vast majority are resistant to their salvage treatments and succumb to their disease.²⁵ Therefore, new agents with different mechanisms of action which can

overcome this resistance to cytotoxic chemotherapy are needed. Clearly, however, the best strategy to manage relapse is to prevent it.

Assessment of minimal residual leukemia persisting after initial therapy has been of significant interest in pediatric patients with ALL (and more recently in adult ALL and AML), although defining the most reliable assay for this purpose remains controversial, except in certain subgroups.¹² The utility of polymerase chain reaction (PCR) based assays for MRD monitoring in acute promyelocytic leukemia (APL) and core binding factor leukemias is well established and more recent reports have demonstrated the potential importance of MRD monitoring in patients with *NPM1* mutated patients.²⁶⁻³⁰ There are fewer large studies examining the benefit of MRD monitoring by flow cytometry in adult AML and concerns regarding standardization and reproducibility of the assays remain.³¹⁻³³ However, the available data suggests that MRD monitoring by any means, be it imperfect, can assist in identifying individuals who are destined to relapse. This is of increasing relevance and importance as the potential for the development of novel, less toxic and more effective agents against residual leukemia populations is ever more realistic.

Our data, in a relatively large population of younger patients with AML who received a more intensive induction and consolidation regimen and achieved a high CR rate, further demonstrates the significant value of MRD assessment in patients with AML. The more intensive regimens with higher doses of cytarabine used in induction and continued use of anthracyclines in consolidation, is close to the limit of tolerance of cytotoxic chemotherapy in this younger population akin to the very intensive regimens used in the pediatric trials. As such, persisting overt leukemia (primary refractory disease) and even persisting MRD after such therapy can be more closely attributed to the inherent biological resistance of the leukemic cells rather than the inadequacy of therapy and clearly selects patients who are no longer benefiting from the cytotoxics.³⁴ We have previously reported that patients who fail to achieve morphological remission after one course of such intensive induction regimens have a dismal prognosis and very few can be salvaged with an allogeneic stem cell transplant.³⁵ The data from this report suggests that this observation can be taken a step further, identifying the patients more likely to relapse after achieving CR and prevent relapse by selecting novel therapeutic strategies to eradicate MRD followed by an allogeneic stem cell transplant.

In pediatric leukemia, where the population is more tolerant of cytotoxic agents, MRD assays have been successfully used to select patients for dose intensification.^{10, 11} Although in the adult population, one can consider persistent MRD as an indication for an allogeneic stem cell transplantation, recent data reporting that detectable MRD prior to an allogeneic stem cell transplant is a reliable predictor of failure, suggests that this strategy may not be the best in dealing with persistent MRD after induction and consolidation.^{36, 37} Alternative strategies such as monoclonal antibody based therapies including antibody-drug-conjugates and T-cell engaging antibodies, as well as in specific cases, use of appropriate small molecule inhibitors such as IDH or FLT3 kinase inhibitors may provide us with more effective and less toxic ways to eradicate MRD. Currently, and based on precedent, allogeneic stem cell transplantation remains the only time-tested, immunologically driven tool to produce long-term cures in less favorable subsets of AML and in relapsed disease. However, with

more sensitive and validated assays for MRD and using novel therapeutic strategies including combinations of antibodies and small molecule inhibitors, one can predict that more and more patients can achieve long term cure without the need for an allogeneic stem cell transplant, as has been the case for patients with APL where sensitive MRD monitoring and availability of effective targeted agents has rendered transplant unnecessary except for select few relapsed patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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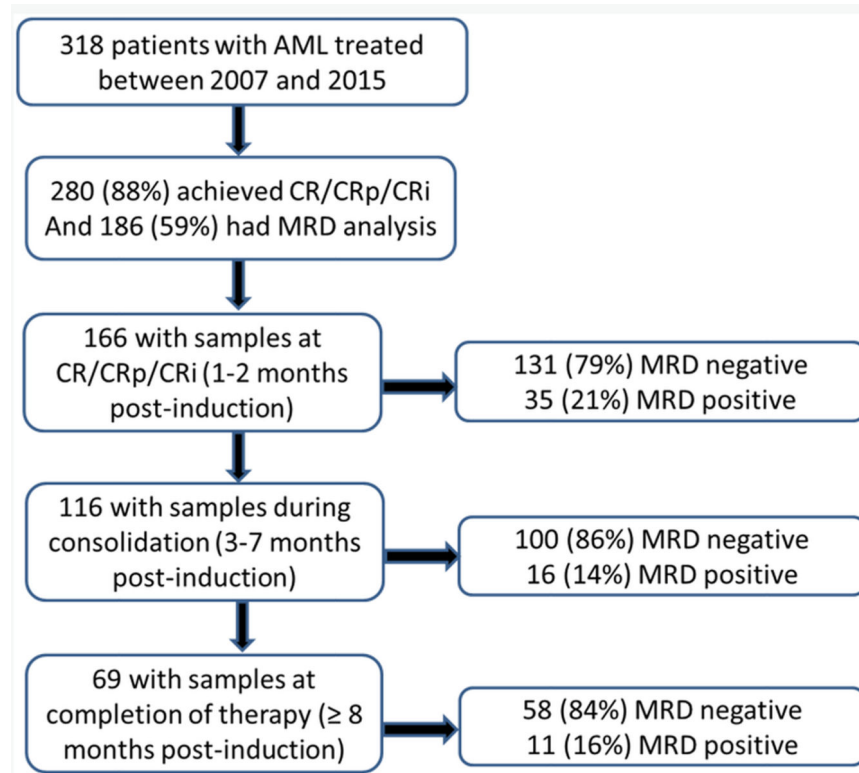
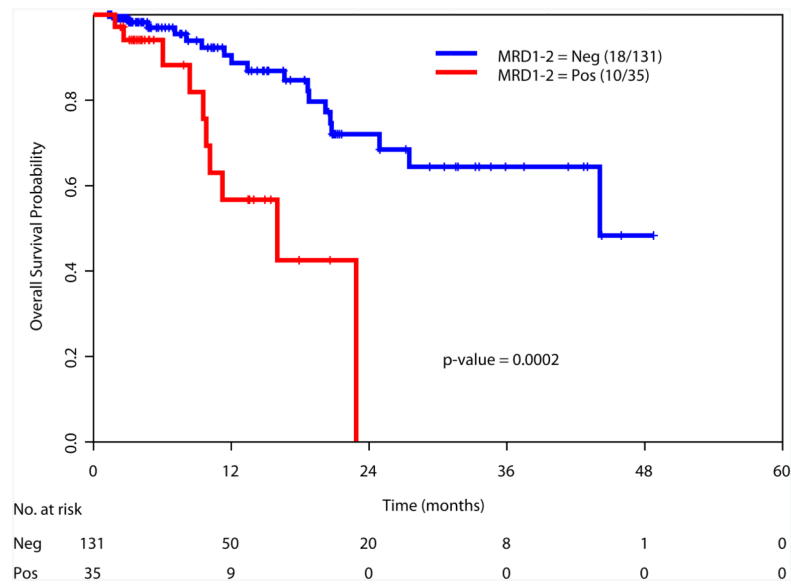
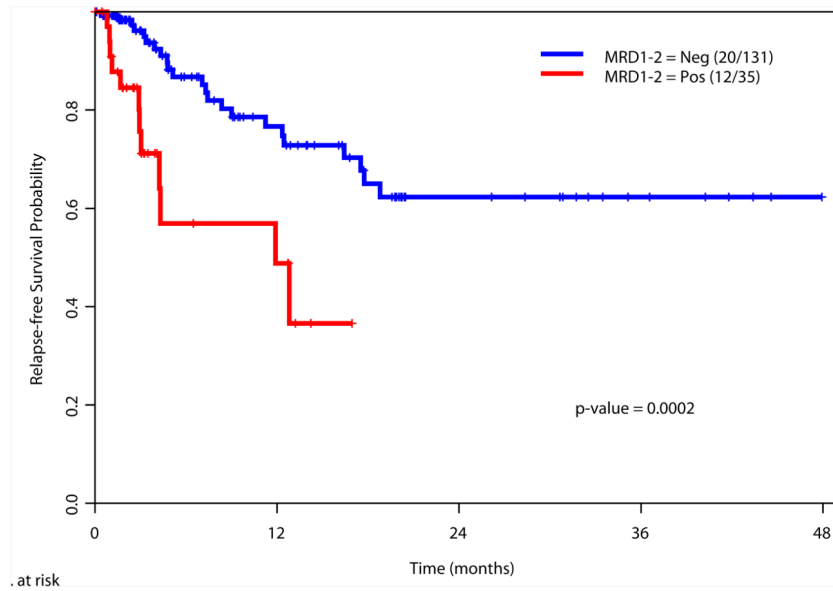
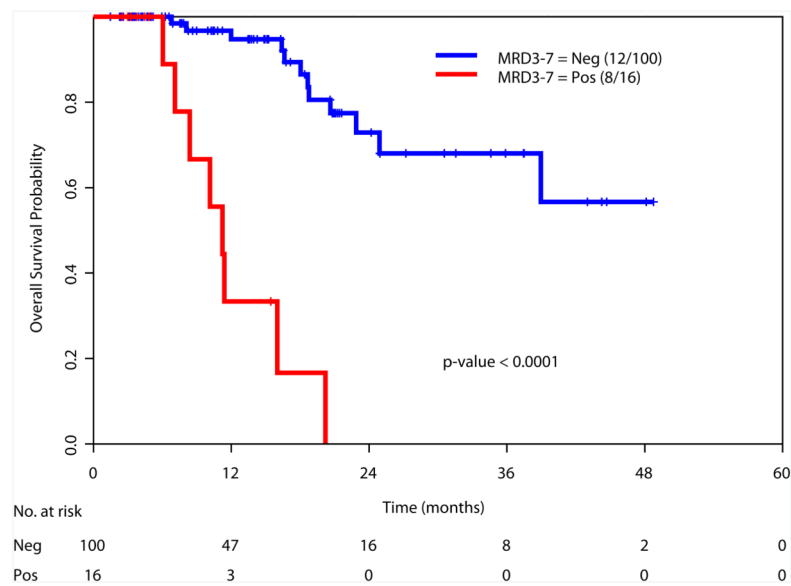
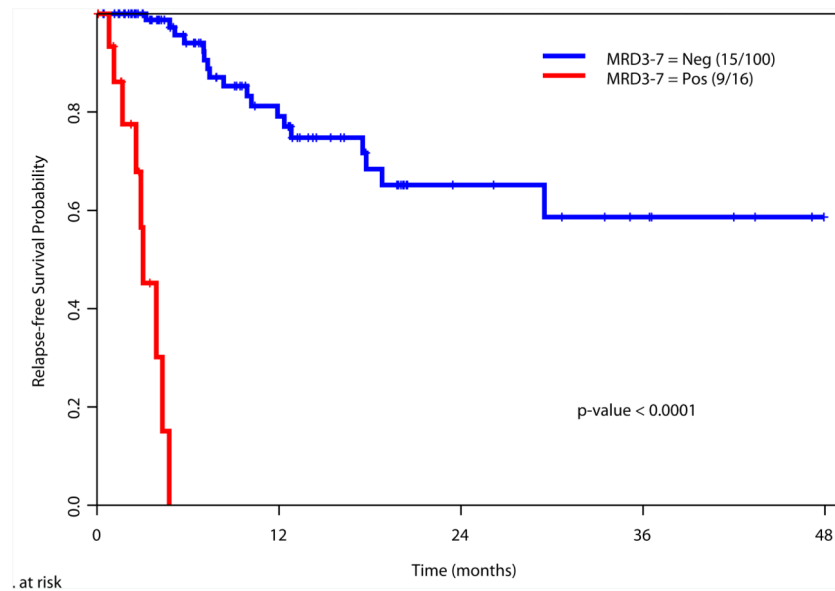
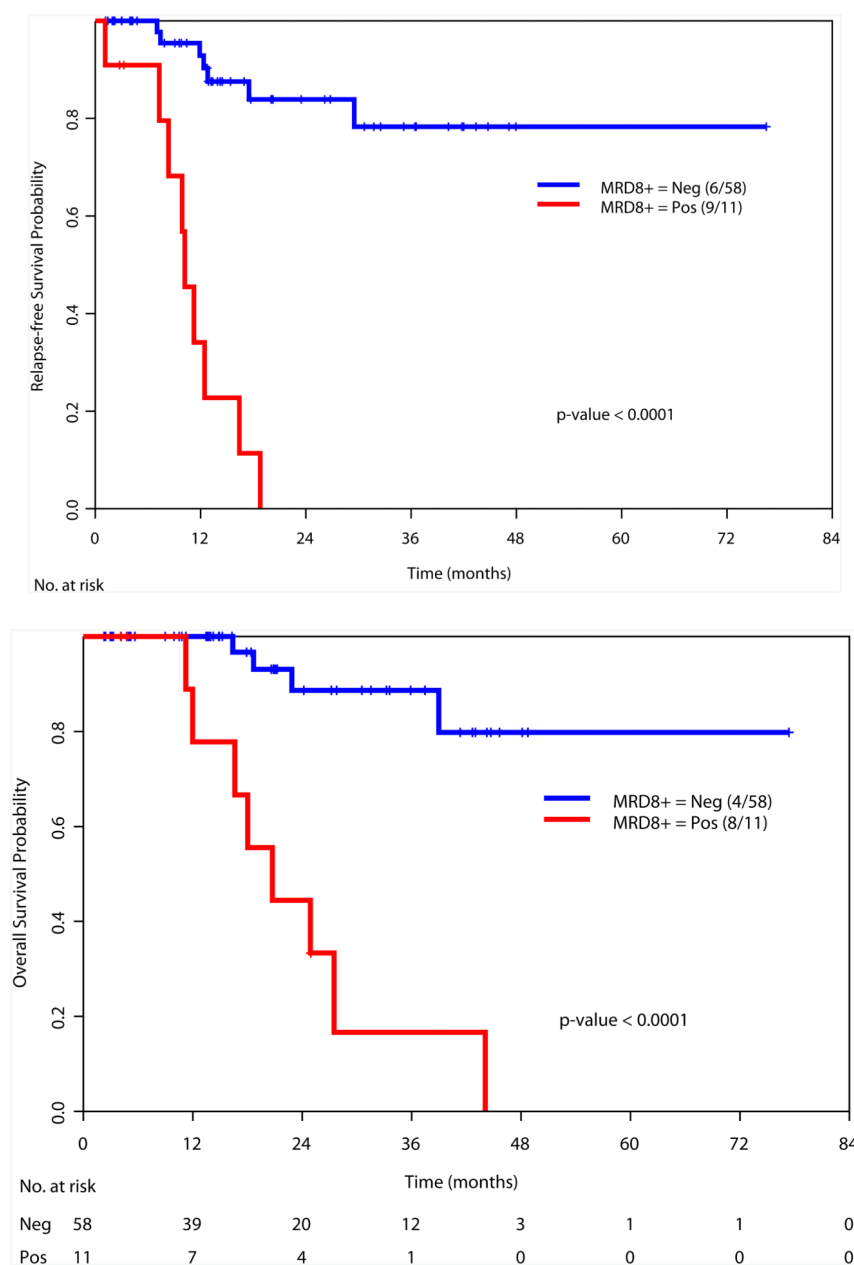


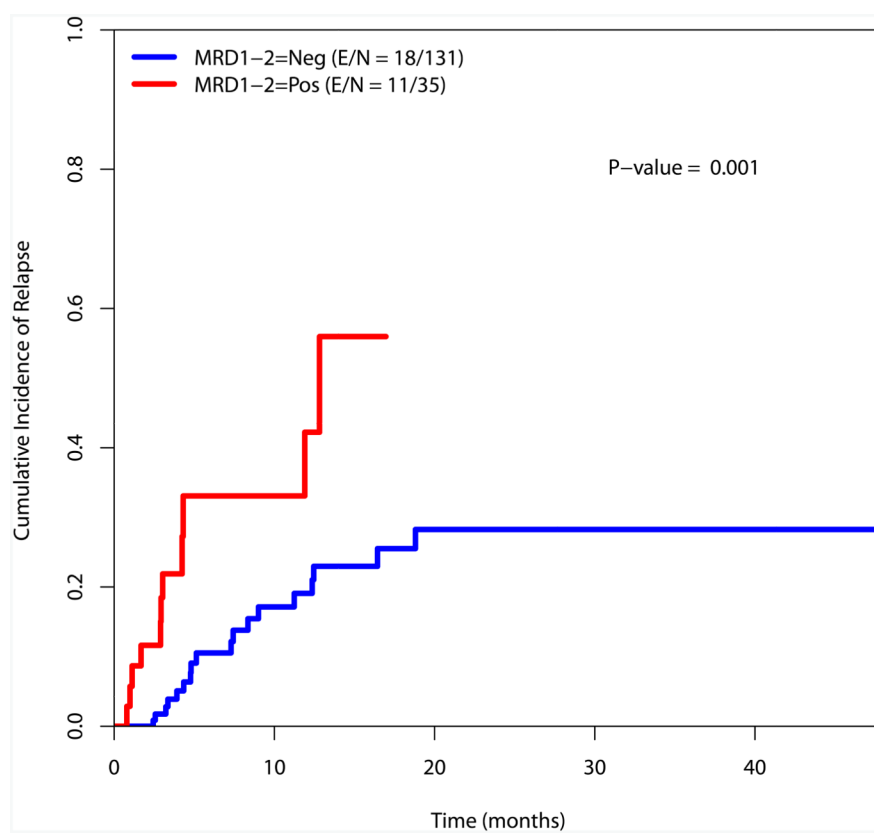
Figure 1. Patient disposition and sample availability

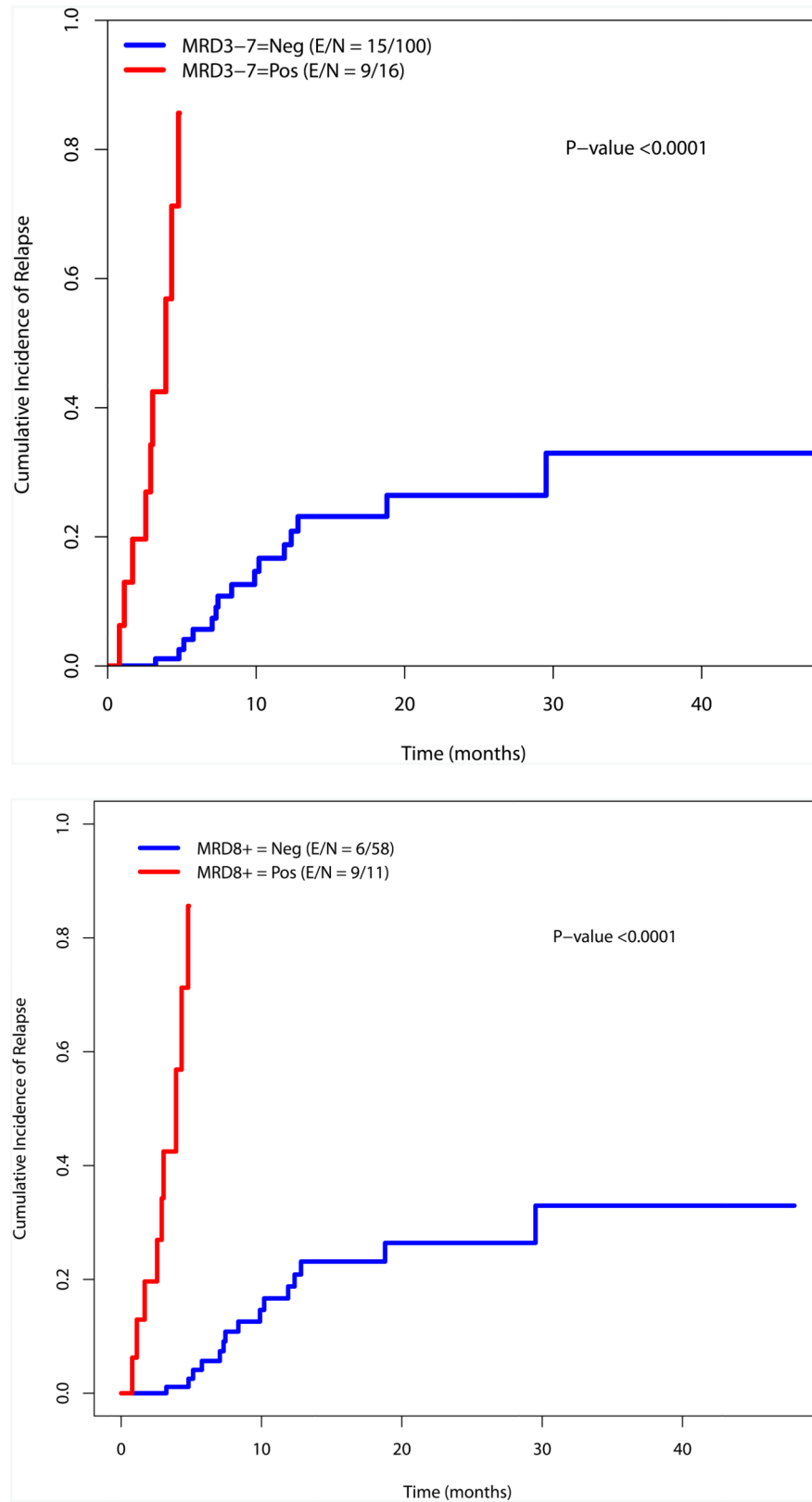




**Figure 2.**

- a.** Relapse-free survival based on MRD status at response (1-2 months after start of therapy)
- b.** Overall survival based on MRD status at response (1-2 months after start of therapy)
- c.** Relapse-free survival based on MRD status during consolidation therapy (3-7 months after start of therapy)
- d.** Overall survival based on MRD status during consolidation (3-7 months after start of therapy)
- e.** Relapse-free survival based on MRD status at completion of therapy (8+ months after start of therapy)
- f.** Overall survival based on MRD status at completion of therapy (8+ months after start of therapy)



**Figure 3.**

- a. Cumulative incidence of relapse by MRD status at response
- b. Cumulative incidence of relapse by MRD status during consolidation
- c. Cumulative incidence of relapse by MRD status at completion of therapy

Table 1
Patient characteristics

Parameter	Overall cohort with CR/CRI N=280 (%)	Study cohort with MRD data N=186 (%)	CR/CRI without MRD data N=94 (%)	P-value
Median age in years [Range]	51 [17 – 79]	51 [17 - 77]	53 [19 - 79]	0.08
Age ≥ 65	18 (6)	6 (3)	12 (13)	
Cytogenetics				
Favorable	96 (34)	34 (18)	62 (66)	<0.0001
Intermediate	137 (49)	115 (62)	22 (23)	
Adverse	36 (13)	27 (15)	9 (10)	
NA	11 (4)	10 (5)	1 (1)	
Median WBC × 10 ⁹ /L [Range]	7.0 [0.5 – 103]	4.7 [0.5 – 103]	9.3 [0.6 – 97]	0.01
Treatment regimen				
CIA	120 (43)	102 (55)	18 (19)	<0.0001
FIA	43 (15)	34 (18)	9 (10)	
FLAG-Ida	95 (34)	34 (18)	61 (65)	
CLIA	22 (8)	16 (9)	6 (6)	
Courses to CR/CRp/Cri				0.91
1	263 (94)	174 (94)	89 (95)	
>1	17 (6)	12 (6)	5 (5)	
Response				0.63
CR	259 (93)	174 (94)	85 (90)	
CRp	19 (7)	11 (6)	8 (8)	
CRi	2 (<1)	1 (<1)	1 (1)	

Table 2a

Univariate Cox regression analysis for relapse-free survival.

Covariate	HR	95% CI		P value
Age	1.02	0.99	1.05	0.128
log(WBC)	0.94	0.72	1.22	0.622
HGB	1.05	0.83	1.34	0.672
log(PLT)	0.94	0.66	1.33	0.712
log(Blast)	0.93	0.75	1.15	0.509
log(mono)	1.02	0.78	1.34	0.863
log(neutrophils)	1.15	0.87	1.53	0.324
promyelocytes	1.09	0.58	2.06	0.781
albumin	0.80	0.43	1.48	0.478
log(LDH)	1.03	0.66	1.60	0.910
bilirubin	1.27	0.59	2.76	0.545
creatinine	0.60	0.15	2.45	0.476
BM Blast	1.00	1.00	1.00	0.036
Cytogenetics = Favorable (vs. Others)	0.24	0.08	0.67	0.007
MR1-2 (Positive vs. Negative)	3.24	1.57	6.68	0.002
MR3-7 (Positive vs. Negative)	75.15	15.02	375.87	<0.001
MR8+ (Positive vs. Negative)	14.58	5.03	42.25	<0.001
Response=CRp or CRi (vs. CR)	5.21	2.15	12.62	0.0003
FLT3 ITD positive (vs. Other)	1.41	0.63	3.17	0.401
FLT3 ITD negative/NPM1 positive (vs. Other)	0.60	0.23	1.60	0.310
Treatment = CIA (vs. FLAG)	3.93	1.38	11.20	0.010
Treatment = CLIA (vs. FLAG) *	<0.001	<0.001	NA	0.991
Treatment = FAI (vs. FLAG)	2.66	0.75	9.50	0.131

* Not estimable due to no events (i.e., relapse or death) in the CLIA group due to short follow-up. The FLAG regimens was used only for patients with CBF leukemia confounding the outcomes.

Table 2b

Univariate Cox regression analysis for overall survival.

Covariate	HR	95% CI		P value
Age	1.02	0.99	1.04	0.253
log(WBC)	0.89	0.66	1.20	0.446
HGB	1.05	0.82	1.35	0.675
log(PLT)	1.07	0.74	1.54	0.737
log(Blast)	0.86	0.68	1.10	0.236
log(monocytes)	0.95	0.71	1.28	0.753
log(neutrophils)	1.14	0.83	1.57	0.431
promyelocytes	1.34	0.75	2.43	0.325
albumin	0.94	0.50	1.76	0.839
log(LDH)	1.20	0.73	1.96	0.474
bilirubin	1.10	0.48	2.54	0.818
creatinine	0.54	0.12	2.49	0.430
BM Blast	1.00	1.00	1.00	0.058
Cytogenetics = Favorable (vs. Others)	0.14	0.03	0.58	0.007
MR1-2 (Positive vs. Negative)	4.28	1.85	9.90	0.001
MR3-7 (Positive vs. Negative)	15.11	5.43	42.05	<0.001
MR8+ (Positive vs. Negative)	10.97	3.27	36.81	<0.001
Response=CRp or CRi (vs. CR)	3.14	1.09	9.04	0.034
FLT3 ITD positive (vs. Other)	1.98	0.82	4.78	0.130
FLT3 ITD negative/NPM1 positive (vs. Other)	0.57	0.19	1.69	0.310
Treatment = CIA (vs. FLAG)	6.62	1.57	27.85	0.010
Treatment = CLIA (vs. FLAG) *	<0.001	<0.001	NA	0.993
Treatment = FAI (vs. FLAG)	4.45	0.81	24.50	0.09

* Not estimable due to no events (i.e., death) in the CLIA group due to short follow-up. The FLAG regimens was used only for patients with CBF leukemia confounding the outcomes.

Table 3a
Multivariate analysis of covariates for relapse-free survival

Parameter	Hazard ratio (95% confidence interval)	P value
At Response (1 – 2 months from Therapy Initiation) (N=166)		
Age	1.03 (1.00, 1.07)	0.08
Cyto (Fav vs. others)	0.24 (0.07, 0.81)	0.02
Response (CRi/CRp vs. CR)	2.39 (0.92, 6.24)	0.07
MRD at CR	2.83 (1.31, 6.09)	0.008
During Consolidation (3 – 7 Months from Therapy Initiation) (N=116)		
Age	1.00 (0.97, 1.04)	0.82
Cyto (Fav vs. others)	0.46 (0.15, 1.42)	0.17
Response (CRi/CRp vs. CR)	1.28 (0.39, 4.19)	0.68
MRD at 3-7 Months	50.38 (9.18, 276.63)	<0.0001
At Completion of Therapy (8 Months from Therapy Initiation) (N=69) *		
Age	1.00 (0.96, 1.05)	0.91
Cyto (Fav vs. others)	0.98 (0.27, 3.62)	0.98
MRD at 8 Months	12.98 (3.82, 44.12)	<0.0001

* The effect of response cannot be estimated because among the 65 evaluable patients, only 1 patient had CRp, the remaining 64 patients all had CR.

Table 3b
Multivariate Cox regression analysis of covariates for overall survival

Parameter	Hazard ratio (95% confidence interval)	P value
At Response (1 – 2 months from Therapy Initiation) (N=166)		
Age	1.05 (1.00, 1.09)	0.05
Cyto (Fav vs. Others)	0.07 (0.01, 0.57)	0.01
Response (CRi/CRp vs. CR)	1.06 (0.34, 3.30)	0.93
MRD at CR	5.17 (1.98, 13.49)	0.0008
During Consolidation (3 – 7 Months from Therapy Initiation) (N=116)		
Age	1.00 (0.96, 1.04)	0.89
Cyto (Fav vs. Others)	0.42 (0.09, 1.97)	0.27
Response (CRi/CRp vs. CR)	0.88 (0.22, 3.46)	0.85
MRD at 3-7 Months	12.57 (3.94, 40.07)	<0.0001
At Completion of Therapy (8 Months from Therapy Initiation) (N=69) *		
Age	1.01 (0.97, 1.06)	0.60
Cyto (Fav vs. Others)	0.91 (0.14, 5.75)	0.92
MRD at 8 Months	10.19 (2.34, 44.34)	0.002

* The effect of response cannot be estimated because among the 65 evaluable patients, only 1 patient had CRp, the remaining 64 patients all had CR.