New Lesions Detected by Single Nucleotide Polymorphism Array–Based Chromosomal Analysis Have Important Clinical Impact in Acute Myeloid Leukemia

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ABSTRACT

Purpose

Cytogenetics is the primary outcome predictor in acute myeloid leukemia (AML). Metaphase cytogenetics (MC) detects an abnormal karyotype in only half of patients with AML, however. Single nucleotide polymorphism arrays (SNP-A) can detect acquired somatic uniparental disomy (UPD) and other cryptic defects, even in samples deemed normal by MC. We hypothesized that SNP-A will improve detection of chromosomal defects in AML and that this would enhance the prognostic value of MC.

Patients and Methods

We performed 250K and 6.0 SNP-A analyses on 140 patients with primary (p) and secondary (s) AML and correlated the results with clinical outcomes and *Flt-3*/nucleophosmin (*NPM-1*) status.

Results

SNP-A is more sensitive than MC in detecting unbalanced lesions (pAML, 65% v 39%, $P = .002$; and sAML, 78% v 51%, $P = .003$). Acquired somatic UPD, not detectable by MC, was common in our AML cohort (29% in pAML and 35% in sAML). Patients with SNP-A lesions including acquired somatic UPD exhibited worse overall survival (OS) and event-free survival (EFS) in pAML with normal MC and in pAML/sAML with abnormal MC. SNP-A improved the predictive value of *Flt-3* internal tandem duplication/*NPM-1* status, with inferior survival seen in patients with additional SNP-A defects. Multivariate analyses confirmed the independent predictive value of SNP-A defects for OS (hazard ratio [HR] = 2.52; 95% CI, 1.29 to 5.22; $P = .006$) and EFS (HR = 1.72; 95% CI, 1.12 to 3.48; $P = .04$).

Conclusion

SNP-A analysis allows enhanced detection of chromosomal abnormalities and provides important prognostic impact in AML.

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INTRODUCTION

Although balanced chromosomal translocations are frequently found in acute myeloid leukemia (AML) in younger adults, older patients tend to have a normal karyotype or unbalanced chromosomal abnormalities on routine metaphase cytogenetics (MC).¹⁻⁴ Despite the established clinical prognostic value of MC, there is wide clinical heterogeneity among patients with similar MC results, especially normal MC, categorized as intermediate risk group.^{1,3,4} Novel markers, such as mutations in *Flt-3* internal tandem duplication (ITD) and *NPM-1*, have improved prognostic stratification in AML,⁶⁻¹² but likely only explain part of this discrepancy. We hypothesize that the clinical variability seen in AML

may be partially explained by additional chromosomal lesions not detected by MC because of technical limitations.^{3,4,13,14}

MC is limited by low resolution, dependence on dividing cells, and an inability to detect copy number–neutral loss of heterozygosity (CN-LOH), exemplified by acquired somatic uniparental disomy (AS-UPD). Whole-genome scanning technologies using single nucleotide polymorphism microarrays (SNP-A) as a karyotyping tool have excellent resolution, use interphase cells, and allow for the precise detection of copy number variants (CNV) and LOH. This technology uses thousands of oligonucleotide probes to identify the genotype at specific SNP loci and quantitates gene dosage at that site. The comparison of copy number and genotype

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Fig 1. Distribution of patients based on acute myeloid leukemia (AML) type and comparison of cytogenetic detection rate including uniparental disomy (UPD) between metaphase cytogenetics (MC) and single nucleotide polymorphism array (SNP-A) karyotyping. (A) Distribution of patients with primary AML (pAML) and secondary AML (sAML); (B) frequency of cytogenetic defects in pAML; (C) frequency of cytogenetic defects in sAML. APL, acute promyelocytic leukemia; MDS, myelodysplastic syndrome; MPD, myeloproliferative disorder.

at sequential SNP loci allows for detection and quantitation of LOH through deletion or AS-UPD. SNP-A analysis has been applied to myelodysplastic syndrome (MDS) and MDS/myeloproliferative disorder (MPD) overlap syndromes, and new cryptic lesions were found in many patients with MDS with a normal or abnormal karyotype by MC.¹⁵⁻¹⁹ These SNP-A defects had prognostic significance, showing negative impact on overall survival (OS).¹⁹ For example, AS-UPD of 7q conveys a poor OS as del7/del7q detected by MC.¹⁹

The first application of whole-genome scanning in AML was a pioneering study using 10K SNP-A. Despite the small cohort size and low array density, homozygous mutations involving genes including *Flt-3*, *CEBPA*, and *RUNX1* were found in seven of 13 patients with AS-UPD encompassing the corresponding chromosomal region.²⁰ This initial study demonstrated the importance of AS-UPD as a molecular lesion in AML. The goal of our study was to apply SNP-A for detection of cryptic unbalanced lesions in patients with normal and abnormal karyotype by MC and characterize the clinical significance of these chromosomal defects.

PATIENTS AND METHODS

Patient Characteristics

A total of 1,344 patients with AML were seen at Cleveland Clinic (CC; $n = 584$) and Johns Hopkins Cancer Center (JHCC; $n = 760$) from 1997 to 2007; DNA samples were available for 111 and 29 patients, respectively.²¹ Informed consent was obtained according to protocols approved by CC and JHCC institutional review boards from 140 patients with primary AML (pAML; $n = 77$) and secondary AML (sAML; $n = 63$) classified by French-American-British or WHO criteria (Fig 1A).^{22,23} Patients had newly diagnosed ($n = 107$), relapsed ($n = 15$), or persistent/refractory AML ($n = 6$), and 12 patients were in remission. Routine MC detected chromosomal abnormalities in 44% of patients with newly diagnosed, relapsed, and persistent AML. All patients with AML in remission had normal MC.

Clinical parameters studied included OS, relapse-free survival (RFS), remission duration (RD), and event-free survival (EFS). Clinical outcomes and response criteria were based on the revised recommendations of the International Working Group for Diagnosis, Standardization of Response Criteria, Treatment Outcomes, and Reporting Standards for Therapeutic Tri-

als in AML.²⁴ All patients were treated either with standard cytarabine-based remission induction chemotherapy or under protocols using experimental regimens (Table 1). Regimens were classified as being intensive chemotherapy (IC) or low-dose chemotherapy on the basis of input from leukemia experts. Only patients receiving IC were included in the outcome analysis.

Cytogenetic and SNP-A Analyses

MC was performed on marrow aspirates and analyzed according to standard methods.²⁵ DNA was extracted using the ArchivePure Kit (5Prime, Gaithersburg, MD). To study the germline genotype, T lymphocytes were isolated using immunomagnetic beads (Miltenyi, Auburn, CA). Affymetrix Human Mapping 250K array and Genome-Wide Human SNP Array 6.0 (Affymetrix, Santa Clara, CA) were used for karyotyping.¹⁹ Fifty-three patients were analyzed simultaneously using 250K and 6.0 arrays, and results were completely congruent in all samples.²⁶ Without prior knowledge of the patient karyotype by MC, patient samples were sent for SNP-A analysis. If an SNP-A lesion was detected and confirmed by MC, then no further analysis was performed. In the case of microdeletions or microduplications, lesions seen in both SNP-A analysis and CNV databases were considered germline and excluded. Conversely, patient samples with new SNP-A lesions not present in CNV databases were analyzed further by CD3 analysis. If CN-LOH or AS-UPD were detected, telomeric lesions or interstitial defects ≥ 25 Mb were considered true somatic abnormalities, whereas interstitial defects less than 25 Mb underwent CD3 analysis. The simultaneous testing of CD3⁺ cells in blood or bone marrow samples allowed for the determination of germline lesions, which were excluded. After the stringent algorithm was applied to all patient samples for the detection of SNP-A defects, only five patients needed CD3 analysis to rule out germline abnormalities (Fig 2). All five patients were confirmed to have somatic lesions. The evaluation of serial samples also provided helpful insights in understanding the biology of disease progression (Data Supplement, online only).

Flt-3 (ITD and Tyrosine Kinase Domain) and Nucleophosmin Mutations

FLT3-ITD mutation was detected using polymerase chain reaction and capillary electrophoresis.

Biostatistical Evaluation of SNP-A Data

Signal intensity was analyzed and SNP calls determined using Gene Chip Genotyping Analysis Software Version 4.0 (GTYPE). Copy number was also determined.

Table 1. Univariate Analysis of Clinical and Biologic Factors

Variable	OS			RFS			RD			EFS		
	P	HR	95% CI	P	HR	95% CI	P	HR	95% CI	P	HR	95% CI
Age, years												
≥ 60	.0005	2.40	1.47 to 3.95	.03	2.04	1.08 to 3.84	.06	1.87	0.96 to 3.62	< .0001	3.11	1.94 to 4.99
< 60		0.41	0.25 to 0.68		0.49	0.26 to 0.93		0.53	0.28 to 1.04		0.32	0.20 to 0.51
WBCs												
≥ 30 × 10 ⁹ /L	.40	1.27	0.47 to 1.39	.41	1.33	0.66 to 2.53	.23	1.56	0.74 to 3.08	.58	1.15	0.68 to 1.87
< 30 × 10 ⁹ /L		0.79	0.71 to 2.13		0.74	0.40 to 1.52		0.64	0.32 to 1.35		0.87	0.53 to 1.46
Type of AML												
sAML	.0008	2.34	1.43 to 3.81	.21	1.55	0.77 to 2.96	.04	2.27	1.02 to 6.04	.0003	2.38	1.5 to 3.73
pAML		0.43	0.26 to 0.70		0.64	0.34 to 1.29		0.44	0.17 to 0.98		0.42	0.27 to 0.67
SNP-A												
With lesions	< .0001	2.96	1.74 to 5.29	.05	1.83	0.99 to 3.48	.31	1.39	0.73 to 2.75	.0027	2.04	1.27 to 3.35
No lesions		0.34	0.19 to 0.58		0.55	0.29 to 1.01		0.72	0.36 to 1.37		0.49	0.30 to 0.78
Metaphase cytogenetics												
Poor	< .0001	4.27	2.29 to 7.61	.48	1.49	0.44 to 3.81	.40	1.62	0.47 to 4.16	.0013	2.77	1.52 to 4.76
Favorable/intermediate		0.23	0.13 to 0.44		0.67	0.26 to 2.28		0.62	0.24 to 2.11		0.36	0.21 to 0.66
<i>Fit-3</i> ITD												
Mutant	.27	1.42	0.39 to 1.36	.36	1.47	0.62 to 3.08	.40	1.46	0.58 to 3.23	.51	1.22	0.65 to 2.14
Wild type		0.70	0.74 to 2.57		0.68	0.32 to 1.60		0.69	0.31 to 1.73		0.81	0.47 to 1.53
<i>Fit-3</i> TKD												
Mutant	.39	0.57	0.09 to 1.83	.74	0.79	0.13 to 2.62	.98	0.98	0.30 to 6.08	.45	0.66	0.16 to 1.77
Wild type		1.75	0.55 to 10.72		1.26	0.36 to 7.79		1.01	0.16 to 3.37		1.52	0.56 to 6.01
<i>NPM-1</i>												
Mutant	.05	0.52	0.24 to 1.01	.31	0.67	0.29 to 1.41	.82	0.91	0.38 to 1.97	.0019	0.37	0.17 to 0.71
Wild type		1.93	0.99 to 4.20		1.48	0.71 to 3.49		1.09	0.51 to 2.63		2.71	1.41 to 5.87

Abbreviations: OS, overall survival; RFS, relapse-free survival; RD, remission duration; EFS, event-free survival; HR, hazard ratio; AML, acute myeloid leukemia; sAML, secondary AML; pAML, primary AML; SNP-A, single nucleotide polymorphism array; ITD, internal tandem duplication; TKD, tyrosine kinase domain; *NPM-1*, nucleophosmin.

Statistical Analysis of Clinical Data

Clinical outcomes (OS, RFS, RD, and EFS) were analyzed separately in clinically defined cohorts of patients. We used the Kaplan-Meier method to analyze OS, RFS, RD, and EFS, with a log-rank *P* value of ≤ .05 considered

significant. Comparison between groups was made using a two-sided Fisher's exact test. The Cox proportional hazards model was used to assess univariate and multivariate analyses for OS, RFS, RD, and EFS, because examination of log (-log) survival plots and Martingale and Schoenfeld

Fig 2. Diagnostic algorithm to identify somatic single nucleotide polymorphism array (SNP-A) defects. AML, acute myeloid leukemia; MC, metaphase cytogenetics.

residuals suggested that the underlying assumption of proportional hazards was not violated. The factors assessed in the univariate analysis for prognostic significance included age (≥ 60 v < 60 years), WBC count (≥ 30 v $< 30 \times 10^9/L$), AML type (sAML v pAML), MC results (good/intermediate v poor), SNP-A results (with lesions v no lesions), mutant versus wild-type *Flt3*-ITD, *Flt3* tyrosine kinase domain (TKD), and nucleophosmin (*NPM-1*). Good- and intermediate-risk MC were analyzed together because of the small number of patients with good-risk cytogenetics by MC ($n = 6$). Univariate variables with $P \leq .1$ were considered significant. Each variable was retained in the multivariate model regardless of its statistical significance or lack thereof. Only variables with $P < .05$ in multivariate analysis were considered significant.

RESULTS

SNP-A-Based Karyotyping

The principle of SNP-A karyotyping has been discussed previously in detail.^{15-17,19,27} Critical to the accurate identification of somatically acquired genomic abnormalities is the elimination of inherited CNVs found in normal control individuals and nonclonal areas of pseudo-UPD or germline-encoded UPD. These CNVs were excluded using direct comparison to DNA from normal T cells from the same patient and/or a stringent algorithm compiled from a large number of normal individuals (Fig 2). After these regions were subtracted from the primary data, somatic defects detected by SNP-A were assembled and mapped on the genome.

Comparison of MC and SNP-A Results

We compared MC and SNP-A karyotyping in primary and secondary AML separately because these likely have components of different underlying biology. SNP-A is superior to MC in the detection of abnormal karyotype including AS-UPD in both types of AML (Figs 1B and 1C). Subset analysis showed that SNP-A detected chromosomal defects in 19 (68%) of 28 patients with pAML and 13 (68%) of 19 patients with sAML having normal MC. SNP-A also facilitated the detection of cryptic defects in patients with non-core-binding factor (CBF)/acute promyelocytic leukemia (APL) primary or sAML with an abnormal MC. In this group, additional aberrations were found in 35 (73%) of 48 patients, including AS-UPD in 15 (31%) of 48 patients. A subset analysis showed new defects in 11 (65%) of 17 non-CBF/APL pAML cases and 24 (77%) of 31 sAML cases with previously abnormal MC result.

We then assessed highly predictive cytogenetic risk groups defined by the Medical Research Council AML 11 criteria.³ Patients with CBF AML/APL belong to the favorable risk group. We detected additional lesions by SNP-A in three of six patients with newly diagnosed CBF AML/APL, including chromosomal gains (three of six) and losses (two of six). AS-UPDs were not detected in CBF AML. Two patients with CBF AML had SNP-A–detected defects (del8 and trisomy 3) and experienced relapse within a year after successful induction/consolidation chemotherapy, but achieved a successful complete remission (CR) after reinduction chemotherapy. Neither patient had a *c-KIT* mutation. No patient with a normal SNP-A result experienced relapse, regardless of the MC result. Similarly, intermediate-risk group patients did not have a higher frequency of SNP-A lesions. An increased frequency of SNP-A abnormalities were detected in patients with poor-risk cytogenetics ($-7/-7q$, $-5/-5q$, and ≥ 3 cytogenetic abnormalities).

In 12 patients (eight with pAML; four with sAML), aspirates obtained after successful induction chemotherapy showed normal MC in all patients, but SNP-A revealed chromosomal aberrations in seven of 12 patients. There was no difference in OS (16 months v not reached; $P = .7$) or rate of second CR between patients with and without new SNP-A lesions.

Clinical Impact of Lesions Identified by SNP-A

To assess the clinical impact of SNP-A analysis in AML, we performed outcome analysis based on the presence or absence of identified genomic aberrations (Fig 3). Only patients who received IC were included in the clinical outcomes analysis. The low-dose chemotherapy treatment group was small and therefore excluded from survival analysis. We excluded patients who received only supportive treatments (transfusions, hydroxyurea), who had inadequate clinical data or who refused further therapies from outcome analysis. First, we studied the impact of SNP-A in all patients with AML not stratified by MC results. OS, RFS, and EFS were inferior in patients with SNP-A defects (Figs 3A, 3D, and 3J). We then analyzed three separate groups: normal MC pAML, normal MC sAML, and abnormal MC pAML/sAML. In pAML with normal MC, patients with SNP-A defects showed worse OS and EFS (Figs 3B and 3K). In sAML with normal MC, there was a trend toward worse OS and EFS; few patients reached CR in this group, precluding analysis of RFS and RD (not shown). Patients with AML who had normal MC and WBC $\geq 30 \times 10^9/L$ were more prone to have SNP-A abnormalities, but similar associations were not observed with regard to age, AML type, and *Flt3* ITD/TKD or *NPM-1* status. In abnormal MC pAML/sAML, OS was inferior in patients with SNP-A defects (Fig 3C), with a trend toward worse EFS (Fig 3L). When the presence of AS-UPD was analyzed separately from other types of SNP-A defects, AS-UPD was predictive of poor OS and EFS (Data Supplement), but not RFS and RD (not shown).

Univariate analysis demonstrated that age, AML type, SNP-A results, MC findings, and *NPM-1* status were significantly associated with OS and EFS, whereas age and SNP-A status were associated with RFS and RD. The results of multivariate analysis showed that SNP-A findings, AML type, and MC results were independent prognostic factors for OS. The AML type and age were independent predictors for RD, and SNP-A status, age, AML type, MC results, and *NPM-1* status were predictive for EFS (Tables 2 and 3).

Combined Analysis of *Flt3*-ITD/TKD and *NPM-1* Mutations and SNP-A Karyotyping

We investigated the impact of SNP-A–detected lesions in the AML cohort, particularly those with normal MC, and stratified them based on *Flt3*-ITD/TKD and *NPM-1* status. A higher frequency of *Flt3*-TKD and *NPM-1* mutations was found in patients with normal SNP-A results compared with those with aberrations (12% v 1%, $P = .02$, and 29% v 15%, $P = .12$; Data Supplement). The occurrence of *Flt3*-TKD and *NPM-1* mutations were more common in patients with pAML compared with sAML (7% v 0%, $P = .07$, and 32% v 4%, $P \leq .0001$; Data Supplement). Using SNP-A, we detected AS-UPD of 13q in nine of 21 patients with *Flt3*-ITD mutations and loss of 5q in one patient with *NPM-1* mutation. The presence of both *Flt3*-ITD and *NPM-1* mutations was found in nine of 119 patients. When all patients with AML having *Flt3*-ITD mutation were studied, survival was not

Fig 3. Comparison of outcomes between patients with and without single nucleotide polymorphism array (SNP-A) lesions. Overall survival, relapse-free survival, remission duration (RD), and event-free survival in (A, D, G, J) all patients with acute myeloid leukemia (AML); (B, E, H, K) patients with primary AML (pAML) and normal metaphase cytogenetics (MC); and (C, F, I, L) patients with pAML and secondary AML and abnormal MC. Les, lesions.

significantly impaired compared with wild-type patients (7 v 13 months; $P = .27$). However, the presence of SNP-A–detected defects worsened the OS and EFS in those with and without *Flt3*-ITD mutation (Data Supplement). There was a trend for worse RFS and RD in

patients with SNP-A defects who are either mutant or wild-type for *Flt3*-ITD. Patients who are *NPM-1* wild-type also showed worse OS and EFS if they had SNP-A defects, with a similar trend in patients with *NPM-1* mutation (Data Supplement).

Table 2. Multivariate Analysis of Clinical and Biologic Factors

Variable	P	HR	95% CI
OS			
Type of AML			
sAML	.01	2.31	1.21 to 4.47
pAML		0.43	0.22 to 0.82
Metaphase cytogenetics			
Poor	.0009	3.72	1.75 to 7.60
Favorable/intermediate		0.27	0.13 to 0.57
SNP-A results			
With lesions	.006	2.52	1.29 to 5.22
No lesions		0.40	0.19 to 0.77
RD			
Type of AML			
sAML	.02	3.60	1.20 to 13.5
pAML		0.28	0.07 to 0.83
Age, years			
≥ 60	.01	2.87	1.20 to 6.92
< 60		0.35	0.14 to 0.84
EFS			
Age, years			
≥ 60	.007	2.26	1.25 to 4.09
< 60		0.44	0.24 to 0.80
Type of AML			
sAML	.01	2.08	1.17 to 3.69
pAML		0.48	0.27 to 0.85
SNP-A results			
With lesions	.04	1.72	1.12 to 3.48
No lesions		0.52	0.32 to 0.80
<i>NPM-1</i>			
Wild type	.007	2.86	1.33 to 6.79
Mutant		0.35	0.14 to 0.75
Metaphase cytogenetics			
Poor	.02	2.30	1.12 to 4.47
Favorable/intermediate		0.44	0.22 to 0.89

Abbreviations: HR, hazard ratio; OS, overall survival; AML, acute myeloid leukemia; sAML, secondary AML; pAML, primary AML; SNP-A, single nucleotide polymorphism array; RD, remission duration; EFS, event-free survival; *NPM-1*, nucleophosmin.

Table 3. Distribution of Treatment Types Received by Patients With AML

Treatment Group	Cleveland Clinic (N = 111)	Johns Hopkins Cancer Center (N = 29)
Intensive chemotherapy		
Standard (n = 89)		
7 + 3 (cytarabine + anthracyclines)		
Cytarabine + mitoxantrone	40	0
Cytarabine + idarubicin	32	1
Cytarabine + daunorubicin	7	0
AcDVP	0	8
High-dose cytarabine	1	0
Investigational (n = 13)		
Cytarabine + gemtuzumab	0	1
Cytarabine + clofarabine*	3	0
FLAM/AcDac	0	2
Arsenic + gemtuzumab†	6	1
Low-dose chemotherapy (n = 16)		
Decitabine/5-azacytidine	8	0
High-dose lenalidomide	3	0
Low-dose cytarabine + hydroxyurea	1	0
Triapine-fludarabine‡	0	3
Chloretazine	1	0
Supportive care only (n = 6)		
Hydroxyurea	2	4
Unavailable clinical data (n = 11)	2	9
Refused further treatment (n = 5)	5	0

Abbreviations: AML, acute myeloid leukemia; IV, intravenous; AcDVP, daunorubicin 45 mg/m² IV push (days 1, 2, 3) + cytarabine 667 mg/m² IV continuous infusion (days 1, 2, 3) + etoposide 400 mg/m² IV over 6 hours (days 8, 9, 10); FLAM, flavopiridol 50 mg/m² (days 1, 2, 3) + cytarabine 667 mg/m² IV continuous infusion (days 1, 2, 3) + mitoxantrone 40 mg/m² IV for one dose (day 9); AcDac, cytarabine 667 mg/m² IV CI (days 1, 2, 3 and days 10, 11, 12) + daunorubicin 45 mg/m² IV push (days 1, 2, 3).

*Phase I/II study.

†Arsenic 0.25 mg/kg/day IV for 5 days (week 1) then 0.25 mg/kg/day IV twice a week (weeks 2-12) + gemtuzumab 3 mg/m² IV (day 8).

‡Triapine 105 mg/m² IV (days 1-5), followed by fludarabine 30 mg/m² IV (days 1-5).

DISCUSSION

In this study, cytogenetic abnormalities were analyzed using high-density SNP-A in a large AML cohort. Previous studies show the superb resolution of SNP-A in detecting novel genomic abnormalities in other myeloid and lymphoid disorders.^{16,18,19,28,29} In AML without balanced chromosomal abnormalities, we demonstrated that SNP-A is superior to MC; new defects in both non-CBF and CBF AML may allow for substratification of these groups.

The higher detection rate of chromosomal defects seen in SNP-A is likely due to its ability to detect lesions even when cells are not actively dividing or its capacity to detect small CN changes and CN-LOH. However, unlike MC, SNP-A cannot detect balanced chromosomal lesions and cannot resolve clonal mosaicism.²⁷ The high-resolution level of SNP-A may lead to false-positive findings related to artifacts, normal CNVs, and germline LOH. Consequently, SNP-A results must be carefully analyzed. We examined a large number of control samples and used internal and public CNV databases to distinguish germline variants from somatic defects, excluding all known

CNVs from our evaluation. We have excluded most small regions of homozygosity, as observations made in controls suggest that they represent autozygosity or germline UPD. Through this method, large regions of germline UPD were found in a small proportion of controls, whereas large, often invariantly occurring areas of UPD were found at much higher frequency in patients, suggesting that these regions are of somatic origin. Whenever possible, we confirmed relevant clonal lesions by parallel analysis of nonclonal cells or serial samples derived from the same patients. In addition, fidelity of 250K SNP-A results was reaffirmed by application of 6.0 arrays in a significant number of cases. The application of a stringent algorithm was useful for excluding lesions that are most likely nonsomatic; however, it does have the limitation of leading to false-negative results (eg, true somatic lesions overlapping with CNVs). Our analyses produced plausible results which, if avoided, may improve the significance level of differences found. AS-UPD is a common and clinically important lesion in AML, accounting for a significant proportion of newly detected SNP-A defects in patients with pAML/sAML. Our results concur with findings of prior studies involving smaller patient numbers studied using 10K SNP-A.^{20,29} Previously, description of AS-UPD9p in MPD paved the way for further studies, leading to the discovery of *JAK2* V617F mutations in MPD, followed shortly by *NF-1* mutation in

UPD17q11.2 in juvenile myelomonocytic leukemia.^{31,32} By analogy, in AML, AS-UPD of other chromosomal regions may indicate the presence of mutations in a homozygous constellation, for example, association of UPD13q with *Flt-3* mutation, UPD19q in *CEBPA*, or biallelic *AML1/RUNX1* in UPD21q.^{20,33,34} Examples of other putative homozygous mutations include UPD1p (*c-Mpl*),³⁵ 4q(*c-KIT*),³⁶ 11p (*WT1*),²⁰ and 11q (*MLL*, *c-CBL*).^{35,37,38}

In AML, SNP-A abnormalities demonstrate prognostic information beyond that of MC or *Flt3/NPM-1* mutational status. Previously, we and others have shown the clinical correlation of SNP-A karyotypic results in MDS.^{18,19} New lesions detected by SNP-A negatively affected OS, RFS, RD, and EFS in our AML cohort including individual subsets, like normal MC pAML and abnormal MC pAML/sAML. The absence of survival difference in the normal MC sAML group may be related to the small number of patients analyzed and a more aggressive disease process. Importantly, our results indicate that CN-LOH is a defect that confers worse outcome similar to that of deletions. The early relapse observed in two patients with CBF AML with SNP-A lesions may be an important observation, because additional lesions detected by MC do not influence relapse in this AML type.³⁹ There is evidence that *c-KIT* mutants in CBF AML predict early relapse, but both patients do not carry this mutation.⁴⁰ This suggests that other genes may be responsible, and it would be reasonable to investigate this issue in future studies.

Molecular diagnostics may supplant the more subjective morphologic prognostic factors. Mutational analysis for *Flt3*-ITD/TKD^{7,11,41,42} and *NPM-1*⁴³ provide important prognostic information in AML. However, the impact of mutational status may depend on coexisting chromosomal lesions (eg, t(8;21)) or other genomic features.^{7,42} In agreement with previous reports, we found that patients with *Flt3*-TKD and *NPM-1* mutations are primarily associated with pAML rather than sAML. The higher frequency of *Flt3*-TKD and *NPM-1* mutations in patients without SNP-A defects compared with those with SNP-A lesions may partially explain the better survival seen in these patients and vice versa. We demonstrated that SNP-A–detected lesions can further risk-stratify patients with and without *Flt3*-ITD/*NPM-1* mutations. This finding is important because in our cohort of patients, univariate (with the exception of *NPM-1* for OS and EFS) and multivariate analysis (with the exception of *NPM-1* for EFS) did not reveal that these mutations have significant effects on outcomes.

Previously validated prognostic markers of unfavorable outcome in AML include older age,^{3,4,44} high WBC count,^{45–48} chemotherapy exposure or MDS,^{3,8,49} presence of certain cytogenetic abnormalities,^{2,4} and mutational status.^{7,41} Univariate analysis confirmed the important role of some of these factors in predicting outcomes. Mul-

tivariate analysis showed that new lesions detected by SNP-A, sAML, and abnormal chromosomal changes detected by MC were predictors of worse OS. Patients with age ≥ 60 years and sAML portend shorter remission duration, and new SNP-A–detected lesions, age ≥ 60 years, sAML, poor-risk karyotype by MC, and *NPM-1* wild-type status predicted for worse EFS in our cohort. The inferior OS in the absence of worse RFS and RD seen in patients with AML with new SNP-A defects suggests a higher rate of resistant disease, a more aggressive clinical course, and higher treatment-related mortality at the time of relapse. A similar mechanism, in addition to higher treatment-related failure, is likely responsible for the worse EFS seen in patients with new SNP-A defects.

In summary, our study demonstrates the diagnostic utility of SNP arrays in AML. New SNP-A–detected defects, including AS-UPD, provide important prognostic information in AML and may reveal new pathogenic mechanisms that may serve as novel targets for new molecular treatments.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

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