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Chromosome abnormalities at onset of complete remission are associated with worse outcome in patients with acute myeloid leukemia and an abnormal karyotype at diagnosis: CALGB 8461 (Alliance)

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ABSTRACT

Achievement of complete remission is essential for long-term survival of acute myeloid leukemia patients. We evaluated the prognostic significance of cytogenetics at complete remission in 258 adults with *de novo* acute myeloid leukemia and abnormal pre-treatment karyotypes, treated on Cancer and Leukemia Group B front-line studies, with cytogenetic data at onset of morphological complete remission. Thirty-two patients had abnormal karyotypes at time of initial complete remission. Of these, 28 had at least 1 abnormality identified pre-treatment, and 4 acute myeloid leukemia-related abnormalities not detected pre-treatment. Two hundred and twenty-six patients had normal remission karyotypes. Patients with abnormal remission karyotypes were older (P<0.001), had lower pre-treatment white blood counts (P=0.002) and blood blast percentages (P=0.004), were less often classified as Favorable and more often as Adverse among European LeukemiaNet Genetic Groups (P<0.001), and had shorter disease-free survival (median 0.6 *vs.* 0.9 years; P<0.001) and overall survival (median 1.2 *vs.* 2.2 years; P<0.001) than patients with normal remission karyotypes. Sixteen patients with normal remission karyotypes also harbored non-clonal abnormalities unrelated to pre-treatment karyotypes. They had shorter overall survival than 210 patients with only normal metaphases (P=0.04). Forty-eight patients with any clonal or non-clonal chromosome abnormality at complete remission had worse disease-free survival (median 0.6 *vs.* 1.0 years; P<0.001) and overall survival (median 1.2 *vs.* 2.5 years; P<0.001) than 210 patients with exclusively normal metaphases. In multivariable analyses, after adjustment for age, the presence of any remission abnormality was associated with shorter disease-free survival (P=0.03) and overall survival (P=0.01). We conclude that detection of any abnormality at complete remission is an adverse prognostic factor. (*clinicaltrials.gov identifier: 00048958*)

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Introduction

Acute myeloid leukemia (AML) is a result of acquisition of somatic genetic alterations, both submicroscopic and those detectable microscopically as numerical or structural chromosome abnormalities, in the leukemic blasts.[1,2] One or more chromosome abnormalities are detected in 55%-60% of adults with AML at diagnosis,[3] and pre-treatment cytogenetic findings are among the most important prognostic factors in AML.[4-9]

Occasionally, chromosome abnormalities are detected in patients who are considered to have achieved a complete remission (CR) based on morphological assessment of their bone marrow. A few studies have assessed the prognostic significance of persistence of an abnormal karyotype at the time of morphological CR,[10-14] and AML patients with chromosome abnormalities in CR samples were found to have worse disease-free survival (DFS),[11,12] relapse-free survival,[14] cumulative incidence of relapse,[12] an increased rate of relapse,[10] and worse overall survival (OS)[12-14] compared with patients who had a normal karyotype at CR. However, some of the previous studies were relatively small,[10,13] and included patients with acute promyelocytic leukemia (APL),[11-13] high-risk myelodysplastic syndrome (MDS),[13] secondary AML evolving from antecedent MDS,[13,14] therapy-related AML,[12,14] or patients who underwent allogeneic hematopoietic stem-cell transplantation (HSCT) in first CR in addition to those treated with chemotherapy post remisson.[11,13,14]

Importantly, cytogenetic remission (CRc), defined as "reversion to a normal karyotype at CR", has been proposed to constitute a separate category of CR.[15] However, because of insufficient data from prospective trials, it has been suggested that this should primarily be used in clinical research studies.[15] The prognostic significance of chromosome abnormalities at CR that differ from the ones found at diagnosis is not clear.

Therefore, we analyzed the clinical outcomes of a relatively large cohort of AML patients with a long follow up who were enrolled onto Cancer and Leukemia Group B (CALGB)/Alliance for Clinical Trials in Oncology (Alliance) front-line treatment studies. Bone marrow (BM) and/or blood samples of all patients successfully underwent cytogenetic analysis both at diagnosis and at onset of CR. To avoid the confounding effects of AML type (*de novo vs.* secondary) and the kind of post-remission therapy (chemotherapy *vs.* allogeneic HSCT), we only included patients with *de novo* AML who had not received allogeneic HSCT in first CR. This means that, to our knowledge, this study is the first to be performed on a patient population of this kind.

Methods

Patients and cytogenetic analysis

We reviewed the cytogenetics database containing AML patients enrolled onto the prospective companion study 8461 carried out by CALGB (now part of the Alliance for Clinical Trials in Oncology group, National Clinical Trials Network) since 1984. Among 2837 newly diagnosed patients with *de novo* AML (excluding APL) enrolled between 1987 and 2013, 396 patients achieved a morphological CR and had successful cytogenetic analyses performed both pre-treatment and no later than 30 days after achieving morphological CR. Two hundred and fifty-eight patients carried at least one clonal chromosome abnormality at diagnosis and thus their pre-treatment karyotype was abnormal, whereas pre-treatment karyotypes of 138 patients were normal (i.e. did not contain any clonal chromosome abnormality) (Figure 1). Only patients with *de novo* AML as defined by World Health Organization (WHO) criteria,[16] who did not undergo allogeneic HSCT in first CR, are included in this study. Details of CALGB treatment protocols are provided in the *Online Supplementary Appendix*. All protocols were approved by the institutional review boards of participating institutions, and all patients provided written informed consent before enrollment in accordance with the Declaration of Helsinki.

Cytogenetic analyses were performed on BM and/or blood samples using unstimulated short-term [24- to 48-hours (h)] cultures in the institutional, CALGB-designated cytogenetics laboratories, and the results were reviewed centrally.[17] Determination of a normal karyotype required analysis of at least 20 metaphases from cultured BM specimens. The clonality criteria and the karyotype interpretation procedure followed the recommendations of the International System for Human Cytogenetic Nomenclature.[18] Since in some cases abnormalities detected at CR occurred in a single cell, descriptions of the patients' karyotypes reported in *Online Supplementary Tables S1* and *S2* contain both the clonal and non-clonal aberrations.

All patients were classified according to their cytogenetic findings at the time of CR into either a cytogenetically abnormal or cytogenetically normal CR group. However, since only 2 of 138 patients with a normal pre-treatment karyotype acquired chromosome abnormalities at CR, all analyses were performed only on 258 patients who harbored a clonal chromosome abnormality or abnormalities at diagnosis.

Patients with a single cell at CR with the same abnormality(ies) as those detected at diagnosis (thereafter referred to as "pre-treatment-related abnormalities") were considered cytogenetically abnormal at CR, as were patients harboring clonal abnormalities at CR. These abnormalities at CR included both pre-treatment-related abnormalities and clonal abnormalities that differed from the pre-treatment abnormalities but are known to be recurrent in AML. In contrast, patients with a non-clonal abnormality(ies) at CR that were not found in the pre-treatment sample (thereafter referred to as "non-clonal pre-treatment-unrelated abnormalities") were considered to have a normal CR karyotype for the initial analyses. Subsequently, outcomes of these patients with a normal CR karyotype who nevertheless harbored non-clonal pre-treatment-unrelated abnormalities were compared with outcomes of patients with the entirely normal CR karyotype that consisted of 100% of normal metaphase cells.

Statistical analysis

Baseline characteristics were compared between CR cytogenetic patient groups using the Wilcoxon rank-sum and Fisher's exact tests for continuous and categorical variables, respectively.[19] For time-to-event analyses, survival estimates were calculated using the Kaplan-Meier method,[20] and the CR cytogenetic patient groups were compared using the log-rank test. The Cox proportional hazards model was used to calculate hazard ratios (HR) for DFS and OS.[19] Multivariable proportional hazards models were constructed for DFS and OS using a forward selection procedure.[19] Variables significant at α=0.20 from the univariable analyses were considered for multivariable analyses. For time-to-event end points, the proportional hazards assumption was checked for each variable individually. All statistical analyses were performed by the Alliance Statistics and Data Center on a database locked

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on September 21, 2015, using SAS 9.4 and TIBCO Spotfire S+ 8.2 software.

Results

Pre-treatment cytogenetic and clinical characteristics, and clinical outcome of patients based on initial CR karyotypes

Among 258 AML patients with an abnormal karyotype at diagnosis, 32 (12%) patients had an abnormal karyotype at CR. They included 28 patients with at least one pre-treatment-related abnormality identical to those observed at diagnosis, 18 of whom had an abnormal clone and 10 a single abnormal cell at CR, and 4 patients with AML-related clonal abnormalities that differed from those present at diagnosis (*Online Supplementary Table S1*). The CR karyotype was considered to be normal in 226 patients. Two-hundred and ten of these patients had only normal metaphase cells, whereas 16 patients with a normal CR karyotype had 1 (n=15) or 2 (n=1) metaphase cells with non-clonal pre-treatment-unrelated abnormalities in addition to the remaining metaphase cells that were entirely normal (*Online Supplementary Table S2*).

The distribution of specific chromosome abnormalities found at diagnosis differed between 32 patients who had an abnormal and 226 patients who had a normal karyotype at CR (P<0.001) (Table 1). Both inv(16)(p13.1q22) or t(16;16)(p13.1;q22) and t(8;21)(q22;q22) [abnormalities present in patients with core-binding factor AML who constitute a Favorable Genetic Group in the European LeukemiaNet (ELN) classification[9,21]] were almost four times less common in patients with an abnormal CR karyotype compared with those with a normal CR karyotype. Conversely, abnormalities defining the ELN Adverse Genetic Group, predominantly a complex karyotype, were twice as frequent among patients with an abnormal CR karyotype compared with those with a normal CR karyotype. Abnormalities denoting the ELN Intermediate-II Genetic Group were more evenly represented among patients with an abnormal and those with a normal CR karyotype (44% *vs.* 31%), although no patient with t(9;11)(p22;q23) at diagnosis had chromosome abnormalities at CR (Table 1).

Compared with 226 patients with a normal CR karyotype, 32 patients who had an abnormal CR karyotype were older (median 63 *vs.* 48 years; P<0.001), had lower white blood cell (WBC) counts (median 4.3 *vs.* 13.5; P=0.002) and a lower percentage of blood blasts (median 25% *vs.* 44%; P=0.004) (Table 2). There were no significant differences in the remaining pre-treatment characteristics between the CR cytogenetic groups.

Median follow up for 89 patients alive was 8.3 years (range 3.1-16.0 years). DFS of 32 patients with an abnormal CR karyotype was shorter than that of 226 patients with a normal CR karyotype (median 0.6 *vs.* 0.9 years; P<0.001), with the 3-year DFS rates of 6% *versus* 33% (Table 3 and Figure 2A). Similarly, patients with an abnormal CR karyotype had a shorter OS (median 1.2 *vs.* 2.2 years; P<0.001), with 3-year OS rates of 19% *versus* 46% (Table 3 and Figure 2B).

The presence of non-clonal pre-treatment-unrelated chromosome abnormalities at CR influences the patients' prognosis

Among 226 patients considered to have a normal karyotype at CR, 16 had either a single metaphase cell (n=15) or 2 metaphase cells (n=1) with non-clonal pre-treatment-unrelated abnormality(ies) that were completely different from those detected at diagnosis (*Online Supplementary Table S2*). These non-clonal pre-treatment-unrelated abnormalities were detected with a similar frequency among patients under 60 years of age (7%, 12 of 179

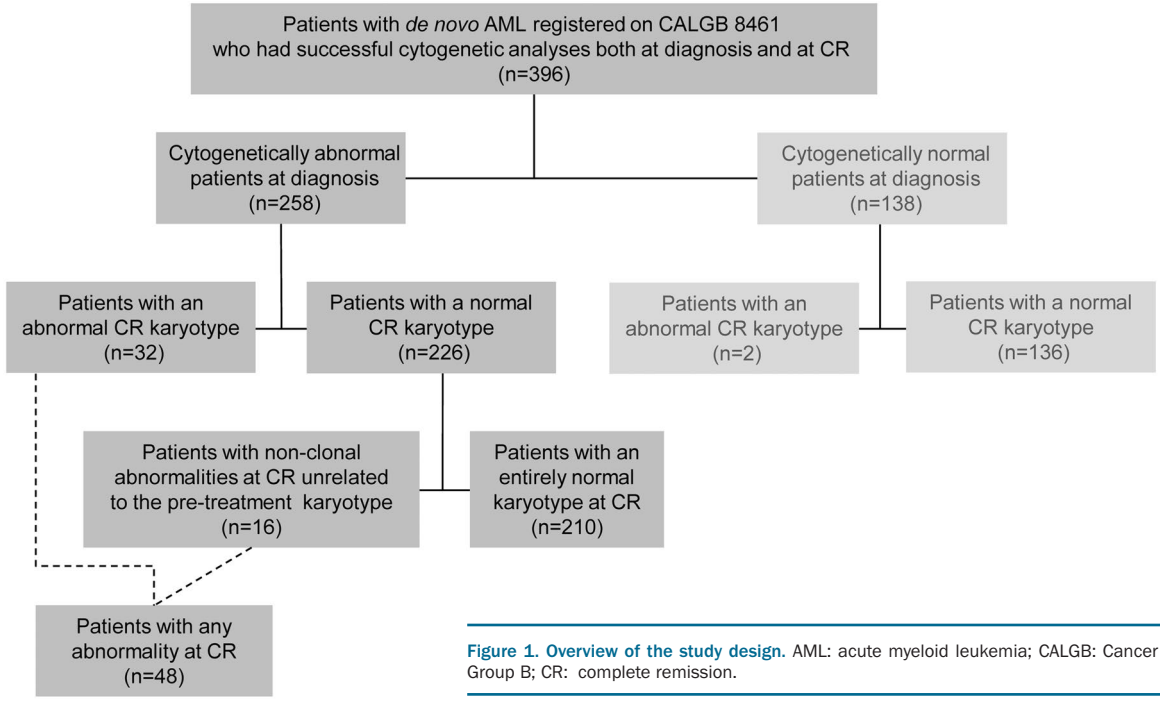

Figure 1. Overview of the study design. AML: acute myeloid leukemia; CALGB: Cancer and Leukemia Group B; CR: complete remission.

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patients) and those aged 60 years or over (9%, 4 of 47 patients; *P*=0.75), and occurred with a similar frequency among the ELN Genetic Groups (*P*=0.39) (*Online Supplementary Table S3*).

Non-clonal pre-treatment-unrelated abnormalities included: reciprocal translocations (n=7), deletions (n=5), exclusively numerical abnormalities (n=3), and a supernumerary marker chromosome (n=1). A search of the Mitelman Database of Chromosome Aberrations and Gene Fusions in Cancer[22] revealed that none of the 7 non-clonal pre-treatment-unrelated reciprocal translocations detected at CR in our patients has been reported as a clonal abnormality in AML patients in the literature. Moreover, the incidental nature of these non-clonal pre-treatment-unrelated abnormalities is emphasized by the fact that none of them was present at the time of relapse in 10 patients for whom relapse cytogenetic data are available (*Online Supplementary Table S2*). This also includes non-clonal deletions of 6q, 11q and 13q, which are known to be recurrent chromosome abnormalities in AML.[3-8,22]

We then tested whether the presence of these non-clonal pre-treatment-unrelated abnormalities found in CR samples may have influenced clinical outcome. Whereas no significant difference in DFS was observed between patients with and without non-clonal pre-treatment-unrelated abnormalities at CR (*P*=0.18), OS of patients with non-clonal pre-treatment-unrelated abnormalities at CR was shorter than that of patients with an entirely normal CR karyotype (median 1.4 *vs.* 2.5 years; 3-year rates 25% *vs.* 48%; *P*=0.04) (*Online Supplementary Table S4 and Figure S1*).

Prognostic significance of any chromosome abnormality at CR

Since we found that the presence of non-clonal pre-treatment-unrelated chromosome abnormalities at CR is associated with adverse outcome, we combined 16 patients with these non-clonal pre-treatment-unrelated abnormalities with 32 patients who had an abnormal CR karyotype, and compared their outcome with that of 210 patients who at CR had only normal metaphase cells.

Disease-free survival of 48 patients with any abnormality at CR, either pre-treatment-related or pre-treatment-unrelated, was shorter than the DFS of 210 patients with an entirely normal CR karyotype (median 0.6 *vs.* 1.0 years; *P*<0.001), with 3-year DFS rates of 10% *versus* 35%. Similarly, OS of patients harboring any chromosome aberration at the time of morphological CR was shorter than that of patients with only normal metaphase cells at CR

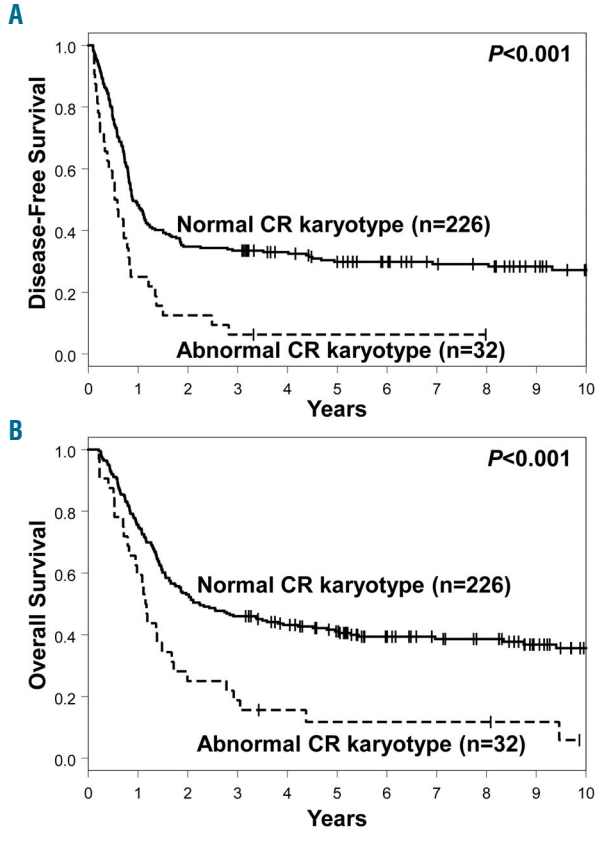

Figure 2. Disease-free survival (A) and overall survival (B) of patients with *de novo* acute myeloid leukemia and abnormal pre-treatment karyotypes according to the presence or absence of chromosome abnormalities at the time of complete remission (CR). In these analyses, patients with non-clonal abnormalities unrelated to abnormalities detected at diagnosis are considered to have a normal karyotype at complete remission.

Table 1. Frequencies of specific clonal pre-treatment cytogenetic abnormalities in acute myeloid leukemia patients whose karyotype was abnormal or normal at complete remission.

Cytogenetic abnormalities at diagnosis	Abnormal CR karyotype (n=32) n. (%)	Normal CR karyotype (n=226) n. (%)
ELN Favorable*	4 (12)	106 (47)
t(8;21)(q22;q22)	2 (6)	42 (19)
inv(16)(p13q22)/t(16;16)(p13;q22)	2 (6)	64 (28)
ELN Intermediate-II*	14 (44)	69 (31)
t(9;11)(p22;q23)	0	8 (4)
Other abnormalities, including	14 (44)	61 (27)
Sole +8	4 (12)	13 (6)
Other sole trisomy	0	10 (4)
Sole chromosome loss other than –5 or –7	0	5 (2)
Sole del(7q) or add(7q)	2 (6)	6 (3)
Sole del(9q)	0	5 (2)
Other sole unbalanced abnormalities	2 (6)	5 (2)
Sole reciprocal translocation or inversion	2 (6)	7 (3)
Two abnormalities	4 (12)	10 (4)
ELN Adverse*	14 (44)	51 (23)
inv(3)(q21q26)/t(3;3)(q21;q26)	0	2 (1)
t(6;9)(p23;q34)	1 (3)	1 (<1)
t(v;11)(v;q23)	1 (3)	8 (4)
–5 or del(5q)	0	2 (1)
–7	2 (6)	2 (1)
Complex karyotype	10 (31)	36 (16)

*CR: complete remission; ELN: European LeukemiaNet Genetic Groups. *The ELN Favorable Genetic Group comprises cytogenetically abnormal-AML patients with t(8;21)(q22;q22)/RUNX1-RUNX1T1 or inv(16)(p13.1q22) or t(16;16)(p13.1;q22)/CBFB-MYH11). Intermediate–II and ELN Adverse Genetic Groups contain the remaining cytogenetically abnormal patients. The ELN Intermediate-II Genetic Group consists of patients with t(9;11)(p22;q23)/MLLT3-KMT2A or those with chromosome abnormalities not classified in the Favorable or Adverse Genetic Group. The ELN Adverse Genetic Group is defined by patients with inv(3)(q21q26.2) or t(3;3)(q21;q26.2)/GATA2–MECOM(EVI1); t(6;9)(p23;q34)/DEK-NUP214; t(v;11)(v;q23)/KMT2A rearranged; –5 or del(5q); –7; abnormalities of 17p; and a complex karyotype containing ≥3 cytogenetic abnormalities in the absence of one of the World Health Organization–designated recurring translocations or inversions: t(8;21), inv(16) or t(16;16), t(9;11), t(v;11)(v;q23), t(6;9), and inv(3)/t(3;3).[21]*

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(median 1.2 *vs.* 2.5 years; *P*<0.001), with 3-year OS rates of 21% *versus* 48% (Table 4 and Figure 3).

To determine whether the presence of any chromosome abnormality at CR remained associated with outcome when controlling for other clinical prognostic factors, we constructed multivariable models for DFS and OS. For DFS, patients with any cytogenetic abnormality at CR, pre-treatment-related or pre-treatment-unrelated, had an approximately 50% higher risk of relapse or death (*P*=0.03), after adjustment for age (*P*<0.001). Similarly, the risk of death was 59% higher for patients who harbored any chromosome abnormality at CR than for those with an entirely normal CR karyotype (*P*=0.01), (after adjustment for age *P*<0.001) (Table 5).

Table 2. Pre-treatment clinical characteristics of cytogenetically abnormal acute myeloid leukemia patients whose karyotype was abnormal or normal at complete remission.

Characteristic	Abnormal CR karyotype (n=32)	Normal CR karyotype (n=226)	*P*
Age, years			<0.001
Median	63	48	
Range	25-84	17-84	
Age group, n. (%)			<0.001
<60 years	14 (44)	179 (79)	
≥60 years	18 (56)	47 (21)	
Sex, n. (%)			0.08
Male	24 (75)	130 (58)	
Female	8 (25)	96 (42)	
Race, n. (%)			0.28
White	30 (94)	185 (84)	
Non-white	2 (6)	34 (16)	
Hemoglobin (g/dL)			0.50
Median	9.3	9.1	
Range	6.6-13.9	2.3-14.7	
Platelet count (x10^9/L)			0.31
Median	62	49	
Range	9-177	5-387	
WBC count (x10^9/L)			0.002
Median	4.3	13.5	
Range	0.7-68.9	0.6-276.8	
Blood blasts, %			0.004
Median	25	44	
Range	0-83	0-98	
Bone marrow blasts, %			0.28
Median	50	60	
Range	5-90	1-96	
Extramedullary involvement, n. (%)	5 (18)	44 (22)	0.81
ELN Genetic Group,* n. (%)			<0.001
Favorable	4 (13)	106 (47)	
Intermediate-II	14 (44)	69 (31)	
Adverse	14 (44)	51 (23)	

*AML: acute myeloid leukemia; ELN: European LeukemiaNet; WBC: white blood cell. *The ELN Favorable Genetic Group comprises cytogenetically abnormal-AML patients with t(8;21)(q22;q22)/RUNX1-RUNX1T1 or inv(16)(p13.1q22) or t(16;16)(p13.1;q22)/CBFB-MYH11). Intermediate–II and ELN Adverse Genetic Groups contain the remaining cytogenetically abnormal patients. The ELN Intermediate-II Genetic Group consists of patients with t(9;11)(p22;q23)/MLLT3-KMT2A or those with chromosome abnormalities not classified in the Favorable or Adverse Genetic Group. The ELN Adverse Genetic Group is defined by patients with inv(3)(q21q26.2) or t(3;3)(q21;q26.2)/GATA2–MECOM(EVI1); t(6;9)(p23;q34)/DEK-NUP214; t(v;11)(v;q23)/KMT2A rearranged; –5 or del(5q); –7; abnormalities of 17p; and a complex karyotype containing ≥3 cytogenetic abnormalities in the absence of one of the World Health Organization–designated recurring translocations or inversions: t(8;21), inv(16) or t(16;16), t(9;11), t(v;11)(v;q23), t(6;9), and inv(3)/t(3;3).[21]*

Discussion

Our cytogenetic analyses performed at diagnosis and at the time of first morphological CR on a relatively large series of *de novo* AML patients receiving induction treatment with cytarabine and an anthracycline (7+3) or similar regimens with long follow up demonstrated that the presence of an abnormal karyotype at CR is associated with adverse prognosis. Both DFS and OS of patients who had at least one cytogenetic abnormality at CR that was identical to abnormalities found in pre-treatment samples or had clonal abnormalities different from those found at diagnosis but known to be recurrent in AML were significantly shorter than DFS and OS of patients with a normal CR karyotype. These results are in line with those of earlier studies, both smaller[10-13] and similar in size[14] to our current series, demonstrating the adverse prognostic significance of persistence of an abnormal karyotype following achievement of morphological CR after completion of induction chemotherapy. However, to our knowledge, only our study included exclusively *de novo* AML patients who did not undergo allogeneic HSCT in first CR.

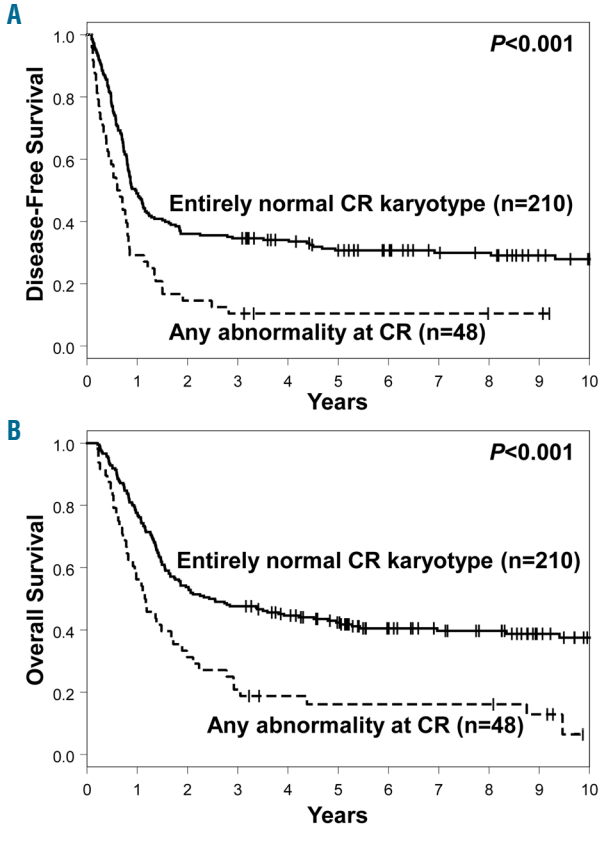

Figure 3. Disease-free survival (A) and overall survival (B) of patients with *de novo* acute myeloid leukemia and abnormal pre-treatment karyotypes according to the presence or absence of any chromosome abnormality or abnormalities, both clonal and non-clonal, pre-treatment-related and pre-treatment-unrelated, at the time of complete remission (CR).

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In contrast to all[10,12-14] but one[11] of the previous studies, we have also analyzed outcome of patients who at the time of CR acquired non-clonal chromosome abnormalities that were unrelated to the clonal chromosome abnormalities detected at diagnosis. Such non-clonal pre-treatment-unrelated abnormalities are usually not considered to be important and the karyotype in such cases is determined to be normal. However, in our study, the presence of non-clonal pre-treatment-unrelated abnormalities was found to portend a significantly shorter OS of patients who harbored them compared with OS of patients whose CR specimens contained 100% of normal metaphase cells. This finding differs from the results of Grimwade *et al.*[11] who identified 11 patients with cytogenetic abnormalities in their CR specimens that were not detected prior to induction treatment. DFS of these patients was virtually identical to that of patients with an abnormal karyotype at diagnosis who had an entirely normal karyotype at CR, although OS of these patients was not assessed. However, our study is not directly comparable to that of Grimwade *et al.*[11] because their cytogenetically analyzed CR samples were obtained not at the onset of CR but in CR at the time of bone marrow harvest for autologous HSCT, which took place between the third and fourth cycle of consolidation therapy. Furthermore, 7 of 11 patients analyzed in their study had a normal karyotype at diagnosis and 3 of the 11 patients had clonal, not non-clonal, aberrations at CR.[11]

The biological significance of cells with non-clonal pre-treatment-unrelated abnormalities at CR is unclear. One possibility is that they might represent small cell populations undetected at diagnosis that were more resistant to chemotherapy, survived induction treatment and subse-

Table 3. Treatment outcomes of cytogenetically abnormal acute myeloid leukemia patients whose karyotype was abnormal or normal at complete remission.

Outcome end point	Abnormal CR karyotype (n=32)	Normal CR karyotype (n=226)	P	HR (95% CI)
Disease-free survival			<0.001	2.17 (1.46-3.21)
Median, years	0.6	0.9		
Disease-free at 3 years, % (95% CI)	6 (1-18)	33 (27-40)		
Disease-free at 5 years, % (95% CI)	6 (1-18)	30 (24-36)		
Overall survival			<0.001	2.18 (1.46-3.26)
Median, years	1.2	2.2		
Alive at 3 years, % (95% CI)	19 (8-34)	46 (39-52)		
Alive at 5 years, % (95% CI)	12 (3-26)	41 (35-48)		

CI: confidence interval; CR: complete remission; HR: hazard ratio.

Table 4. Treatment outcomes of cytogenetically abnormal acute myeloid leukemia patients according to the presence or absence of any clonal or non-clonal, pre-treatment-related or pre-treatment-unrelated chromosome abnormality or abnormalities at complete remission.

Outcome end point	Any abnormality present at CR (n=48)	Entirely normal CR karyotype (n=210)	P	HR (95% CI)
Disease-free survival			<0.001	1.92 (1.36-2.71)
Median, years	0.6	1.0		
Disease-free at 3 years, % (95% CI)	10 (4-21)	35 (28-41)		
Disease-free at 5 years, % (95% CI)	10 (4-21)	31 (25-37)		
Overall survival			<0.001	2.12 (1.49-3.01)
Median, years	1.2	2.5		
Alive at 3 years, % (95% CI)	21 (11-33)	48 (41-54)		
Alive at 5 years, % (95% CI)	16 (7-28)	42 (36-49)		

CI: confidence interval; CR: complete remission; HR: hazard ratio.

Table 5. Multivariable analyses of outcome in cytogenetically abnormal acute myeloid leukemia patients according to the presence or absence of any clonal or non-clonal, pre-treatment-related or pre-treatment-unrelated chromosome abnormality or abnormalities at complete remission.

End point/variables in final models	Hazard ratio*	95% CI	P
Disease-free survival†			
Any abnormality at CR *versus* an entirely normal karyotype at CR	1.49	1.04-2.15	0.03
Age, continuous, 10-year increase	2.19	1.58-3.04	<0.001
Overall survival†			
Any abnormality at CR *versus* an entirely normal karyotype at CR	1.59	1.10-2.30	0.01
Age, continuous, 10-year increase	2.62	1.86-3.68	<0.001

*CI: confidence interval; CR: complete remission. *A hazard ratio greater than 1 corresponds to a higher risk for the first category listed of dichotomous variables and higher values of continuous variables. †Variables considered for model inclusion and evaluated in univariable models were: CR cytogenetic group (any abnormality at CR versus an entirely normal karyotype at CR), age (as a continuous variable, in 10-year increments), sex (male vs. female), ethnic group (white vs. non-white), white blood cell (WBC) count (as a continuous variable, in 50-unit increments), hemoglobin (as a continuous variable, in 1-unit increments), platelet count (as a continuous variable, in 50-unit increments) and extramedullary involvement (present vs. absent).*

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quently gave rise to an abnormal clone or clones responsible for the disease relapse. However, this scenario is highly unlikely since we did not detect any of these non-clonal pre-treatment-unrelated abnormalities again in samples of 10 patients who relapsed and had a successful cytogenetic analysis at that time. Another potential mechanism is that the occurrence of non-clonal pre-treatment-unrelated abnormalities could indicate increased predisposition to genomic instability in leukemic blasts,[23] which might increase the likelihood of generation of therapy-resistant clones that lead to relapse and poor outcome. Regardless of the exact mechanism, our findings, if corroborated, suggest that detection of any chromosome abnormality at CR, whether related to the pre-treatment karyotype or not, should be regarded as a sign of increased risk of relapse or death, and that the patients with such cytogenetically abnormal CR samples should be considered as candidates for more intensive therapy, including allogeneic HSCT[14,24] and/or alternative treatment regimens.

Whereas both our study and previous ones[10-14] show that cytogenetic analysis of CR samples can identify patients who have an increased risk of relapse or death, the resolution of cytogenetic analysis is relatively low. Other, more sensitive methodologies, such as mutation detection using DNA- or RNA-based real-time quantitative polymerase chain reaction (RQ-PCR) and/or next-generation sequencing,[25,26] or multiparameter flow cytometry immunophenotyping,[27-29] have become available. However, these techniques also have limitations. In the case of molecular analyses by RQ-PCR and/or next-generation sequencing, optimal sensitivity thresholds for mutation clearance at CR still need to be established,[26] especially because these sensitive techniques are capable of detecting low levels of fusion transcripts, such as *RUNX1-RUNX1T1* or *CBFB-MYH11*, or mutations in the *DNMT3A* gene, which are known to persist in patients remaining in durable CR.[26,30] Immunophenotyping using multiparameter flow cytometry can be technically challenging and is relatively expensive, with varying threshold levels proposed for risk stratification.[28] One of the advantages of cytogenetics, despite its limited sensitivity, is that this assay is widely available, also in less developed countries.

In summary, our cytogenetic study performed on a relatively large cohort of patients with *de novo* AML, none of whom had received allogeneic HSCT in first CR, with a long follow up has shown that persistence of at least one cell with an abnormality or abnormalities seen in the pre-treatment sample at the time of morphological CR is associated with poor outcome independently of other clinical prognosticators. Moreover, we found that acquisition of non-clonal abnormalities, seemingly unrelated to the diagnostic karyotype, may also have adverse prognostic consequences. Obviously, this new finding should be prospectively validated in future, large studies. If confirmed, detection of any chromosome abnormality at CR should be considered as a factor in clinical decision making.

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