Cytogenetic heterogeneity negatively impacts outcomes in patients with acute myeloid leukemia

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ABSTRACT

Clonal heterogeneity is a hallmark of malignant transformation. In acute myeloid leukemia, acquired cytogenetic abnormalities are important independent predictors of initial response to therapy, remission duration, and overall survival. However, whether the presence of multiple cytogenetically characterized clones affects outcomes in acute myeloid leukemia is still not well defined. The aim of this study was to assess the prognostic impact of cytogenetic clonal heterogeneity in acute myeloid leukemia. This analysis included 1403 newly diagnosed acute myeloid leukemia patients fit for intensive chemotherapy, aged between 15 and 88 years, enrolled on Southwest Oncology Group protocols. The presence of multiple cytogenetic clones was found in 164 (24%) patients with abnormal karyotype. The proportion of patients with clonal heterogeneity increased with age, being present in 20% of patients under 40 years of age, but in 30% of those aged over 70 years (P=0.03). Clonal heterogeneity was significantly more common in association with unfavorable karyotype. Clonal heterogeneity was associated with decreased response rates and inferior event-free, relapse-free and overall survival, and was confirmed as an independent predictor of poor prognosis in multivariable analysis. Subgroup analysis showed that clonal heterogeneity adds prognostic information particularly in the unfavorable karyotype group. Our results confirm the negative prognostic impact of clonal heterogeneity in acute myeloid leukemia patients with abnormal karyotype. (*clinicaltrials.gov identifiers: 014343329; 01338974; 00899171; 1059734; 01059734; 00899743; 0143329; 00023777; 00085709; 01360125; 00004217*)

Introduction

Acquired cytogenetic abnormalities can be detected in approximately 50% of patients and are important independent predictors of initial response to therapy, remission duration, and overall survival in acute myeloid leukemia (AML).[1,2] The commonly used cytogenetic risk classifications are in general agreement with each other, and were developed following large co-operative clinical trials. Newly diagnosed AML patients can be grouped into favorable, intermediate and poor risk prognostic categories based on the diagnostic cytogenetic abnormalities. These cytogenetic classifications did not account for the presence or absence of clonal heterogeneity.

Cancer evolution is a complex adaptive process that involves genetic diversification coupled with clonal selection and subclonal expansion.[3] It has been recognized for decades that leukemias are composed of genetically heterogeneous clones, where some genomic alterations are shared by the entire tumor, but not all cancer cells demonstrate an identical genomic and cytogenetic profile.[4,5] Next generation sequencing studies in AML revealed that more than 50% of AML patients have at least one subclone at the time of diagnosis (average 1.5 clones/genome).[6,7] These clonal subpopulations in an individual tumor can be morphologically and functionally distinct with differential sensitivity and/or resistance to therapeutic agents. In fact, karyotypic clonal evolution is frequently encountered at the time of AML relapse.[8]

In AML, the impact of cytogenetic clonal heterogeneity, defined as the presence of two or more distinct cytogenetic clones, either related or unrelated, has not been extensively studied. Previous studies have demonstrated the positive effect of residual normal metaphases in patients with monosomal karyotype AML and the deleterious impact of residual normal metaphases in AML with translocations affecting the genes for the core binding factors.[9,10] More recently, a retrospective analysis on behalf of the Study Alliance Leukemia revealed that approximately one-third of AML patients with abnormal karyotypes present cytogenetic heterogeneity, and that clonal heterogeneity is associated with adverse prognosis.[11] Given that the outcomes of these patients depend not only on the clinical, genetic, and molecular characteristics of AML, but also on the chemotherapeutic regimens used for treatment, we investigated the prognostic impact of clonal heterogeneity in AML patients treated in North America in Southwest Oncology Group (SWOG) clinical trials.

Methods

Study population

Classic metaphase karyotyping was performed according to routine SWOG protocols. A total of 1403 (49.8% of 2816 patients registered) previously untreated adult AML patients with evaluable cytogenetic information at diagnosis enrolled in one of 10 successive prospective SWOG studies1 were included in this analysis. Initial treatment consisted of standard induction therapy with cytarabine and anthracy-

 doi:10.3324/haematol.2014.117267
The online version of this article has a Supplementary Appendix.
Manuscript received on September 16, 2014. Manuscript accepted on December 17, 2014.
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cline in most patients. For patients achieving a complete remission, consolidation therapy varied based on protocol design. In brief, patients received 1-2 cycles of standard dose cytarabine plus anthracycline after enrollment in studies activated for the first 10-15 years of the study period. For more recent studies, the preferred post-remission therapy was four cycles of high-dose cytarabine. Autologous and/or allogeneic transplants were included as post-remission therapies in only a minority of patients included in these analyses. Only patients with complete and centrally reviewed cytogenetic studies were included in analysis. Patients with the t(15;17)(q22;q12) were excluded. All participating patients gave informed consent prior to enrollment. The studies were approved by ethics committees of all participating institutions and were conducted in accordance with the Declaration of Helsinki.

Cytogenetic studies

At diagnosis, samples from bone marrow aspirates were analyzed in SWOG-approved laboratories for cytogenetic abnormalities using standard culturing and banding techniques. Karyotype designation was in accordance with the latest version of the International System for Human Cytogenetic Nomenclature. Abnormalities were considered clonal when at least 2 metaphases had the same aberration in the case of either a structural abnormality or an additional chromosome. If there was loss of a chromosome, it had to be present in at least 3 metaphase cells to be considered a clonal monosomy. Cytogenetic abnormalities were grouped according to published criteria adopted by SWOG as favorable, intermediate, unfavorable, and unknown. The karyotype analysis was based on 20 or more metaphase cells for more than 90% of patients included in this analysis.

Clonal heterogeneity was defined as presence of 2 or more cytogenetically abnormal clonal populations, as previously described.[11] In brief, a defined ancestral clonal evolution followed either mother-daughter and/or branched patterns. In the mother-daughter pattern, a subclone displays all cytogenetic aberrations of the original clone plus additional aberration(s), which define a distinct subclone. In a branched pattern, all subclones harbor common cytogenetic aberrations suggestive of a common ancestry, but also acquire unique additional aberrations that define them as separate subclones. Both patterns of ancestral clonal evolution were often present in the same patient. Finally, composite karyotypes are those where common ancestry cannot be unequivocally determined for the multiple clones sharing some common cytogenetic characteristics. In addition, all karyotypes were individually reviewed by the authors and only cases with complete karyotype information available were included.

Outcome definitions and statistical methods are to be found in the *Online Supplementary Appendix.*

Results

Characteristics of AML patients with subclonal populations

Metaphase analysis at diagnosis identified 683 (48.6%) patients with cytogenetic abnormalities. A single cytogenetically abnormal clone was detected in most of these patients (519 of 683, 76%), while multiple clones (range 2-12) were seen in the remaining 24% of patients. A defined ancestral pattern of subclonal evolution was detected in over 90% of patients (mother-daughter, 61%; branched 15%; both patterns 15%) while the remaining 10% of patients demonstrated composite karyotypes. Base-line characteristics of patients with abnormal karyotypes are summarized in Table 1. The incidence of clonal heterogeneity in AML patients with abnormal karyotype increased with advancing age (P=0.03). When compared to AML patients with a single cytogenetically abnormal clone, patients with subclone formation were older (P=0.025), were more likely to have an unfavorable karyotype (P<0.001), and more frequently had secondary AML (P=0.048).

Table 1. Associations between base-line characteristics of AML patients with clonal heterogeneity.

Characteristics	1 abnormal clone (n=519)	2 or more abnormal clones (n=164)	P
Age (years) (range)	57 (15, 84)	60 (19, 89)	0.025
Age quartiles (N) (%)	0.03		
15-40 years	127 (80)	32 (20)	
40-60 years	180 (78)	50 (22)	
60-70 years	126 (74)	45 (26)	
70-90 years	86 (70)	37 (30)	
WBC at diagnosis ($x10^9$) (range)	10 (0, 545)	8 (1, 137)	0.33
Platelet count ($x10^9$) (range)	48 (3, 8300)	50 (2, 1200)	0.88
Bone Marrow Blasts (%)	67 (0, 100)	62 (0,99)	0.4
Peripheral Blood Blasts (%)	30 (0, 99)	24 (0,97)	0.1
Female Gender (N) (%)	217 (42)	69 (42)	1
ECOG Performance Status (N) (%)		0.43	
0-1	411 (81)	126 (78)	
2+	98 (19)	36 (22)	
Karyotype (N) (%)	<0.001		
Favorable	104 (20)	19 (12)	
Intermediate	70 (13)	7 (4)	
Unfavorable	254 (49)	124 (76)	
Unknown	91 (18)	14 (9)	
Secondary AML (N) (%)	54 (13)	27 (20)	0.048

WBC: White blood cell count; ECOG: Eastern Cooperative Group.

Clinical outcomes associated with cytogenetic subclonal evolution in AML

Initially, we determined whether pattern of ancestral clonal heterogeneity (mother-daughter vs. branched) impacted outcomes in AML patients. Univariate models failed to demonstrate an association between pattern of heterogeneity and complete remission rate [Odds ratio (OR) 1.44; 95% confidence interval (95%CI): 0.56, 3.68; P=0.45)] or overall survival [Hazard ratio (HR) 0.75; 95%CI: 0.47, 1.2; P=0.23]. However, mother-daughter pattern was associated with trend for improvement in event-free survival (EFS) (HR 0.66; 95%CI: 0.42, 1.04; P=0.074) and relapse-free survival (RFS) (HR 0.38; 95%CI: 0.17, 0.84; P=0.017) compared to branched pattern of clonal heterogeneity. Multivariate analyses failed to demonstrate an independent association of pattern of ancestral heterogeneity and response to therapy (OR 0.96; 95%CI: 0.33, 2.8; P=0.95) or survival outcomes [OS- (HR 0.91; 95%CI: 0.54, 1.54; P=0.73)], EFS- (HR 0.8; 95%CI: 0.5, 1.42; P=0.53), RFS- (HR 0.62; 95%CI: 0.22, 1.69; P=0.35). For the remaining of the analyses, both patterns of ancestral clonal heterogeneity were combined as a single cohort.

Effect of clonal heterogeneity on response to therapy

We then evaluated the effect of clonal heterogeneity on initial response to therapy. In univariate analysis, presence of multiple cytogenetic clones, measured quantitatively, was associated with inferior complete remission (CR) rates (OR 0.86; 95%CI: 0.74, 1; *P*=0.043). However, in multivariable analysis adjusting for multiple prognostic cofounders, the number of cytogenetic clones was not independently associated with CR rates (OR 0.96; 95%CI: 0.82, 1.13; *P*=0.66). Prognostic variables independently associated with lower CR rates included advanced age, Eastern Cooperative Group (ECOG) performance status (PS) (2+), and unfavorable karyotype (Table 2).

Similarly, in univariate analysis, an increasing number of cytogenetic clones in AML was associated with inferior EFS (HR 1.12; 95%CI: 1.05, 1.2; *P*<0.001) and RFS (HR 1.2; 95%CI: 1.07, 1.44; *P*=0.0038). In multivariable analysis, presence of two or more clones was not independently associated with EFS (HR 1.05; 95%CI: 0.98, 1.14; *P*=0.17), but it demonstrated a trend towards significance for RFS (HR 1.18; 95%CI: 1, 1.39; *P*=0.054).

Clonal heterogeneity and survival outcomes

Next, we evaluated the impact of the number of cytogenetic clones on OS. In all patients with abnormal karyotype, the presence of at least one additional cytogenetic clone was associated with inferior OS (HR 1.49; 95%CI: 1.23, 1.81; *P*<0.001) (Figure 1A). As previously shown, presence of clonal heterogeneity in patients with CBF-AML did not impact CR rate, EFS, RFS or OS (data not shown). However, when the analysis was limited only to patients with unfavorable risk cytogenetics, presence of one or more additional clone was also associated with worse OS (HR 1.41; 95%CI: 1.12, 1.76; *P*=0.003) (Figure 1B). In univariate analysis, the presence of more than one cytogenetic clone was associated with inferior OS (HR 1.16; 95%CI: 1.08, 1.25; *P*<0.001). A multivariable analysis confirmed that number of abnormal clones was independently associated with worse outcomes in AML (HR 1.0; 95%CI: 1, 1.18; *P*=0.039). Other prognostic variables independently associated with survival outcomes are summarized in Table 2.

Finally, we used interaction models to assess whether the association between presence of clonal heterogeneity and outcome varied by cytogenetic risk group. Clonal heterogeneity continued to be independently associated with worse survival outcomes (OS: *P*=0.0016; RFS: *P*=0.0037; EFS: *P*<0.001), but not associated with CR rates (*P*=0.27). We did not observe any evidence of an interaction between cytogenetic risk group and clinical outcomes (*Online Supplementary Table S1*).

Impact of cytarabine dose on clinical outcomes in patients with clonal heterogeneity

An exploratory analysis was conducted to determine the differential effect of cytarabine dose on response to therapy and survival. In patients with clonal heterogeneity, the use of high doses of cytarabine during induction was not independently associated with higher complete remission rates (OR 1.54; 95%CI: 0.51, 4.64; *P*=0.44) or improved OS (HR 1.36; 95%CI: 0.75, 2.46; *P*=0.31), EFS (HR 1.06; 95%CI: 0.59, 1.88; *P*=0.85) or RFS (HR 1.8; 95%CI: 0.79, 4.11; *P*=0.16).

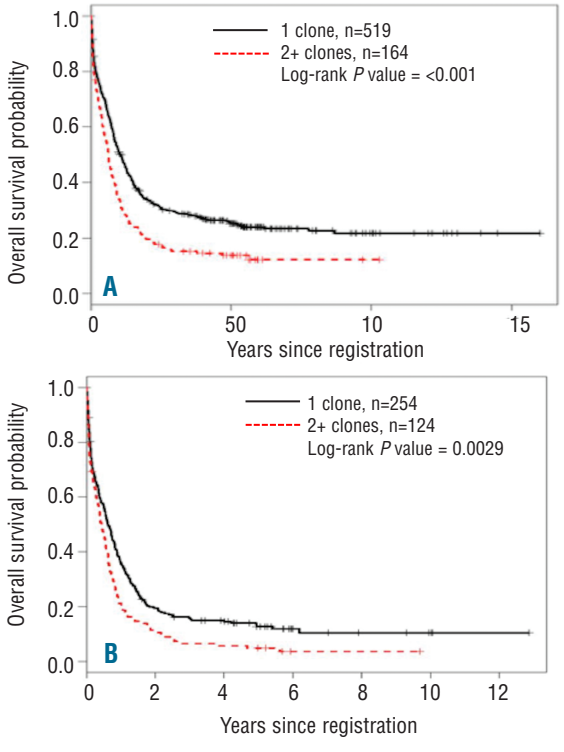

Figure 1. (A) Overall survival for all patients with abnormal karyotype according to clonal heterogeneity status. (B) Overall survival for patients with unfavorable risk cytogenetics according to clonal heterogeneity status.

Table 2. Multivariate analysis of impact of risk factors on response and survival outcomes.

	CR Rate			EFS			RFS			OS		
Factors	OR	95% CI	p	HR	95% CI	p	HR	95% CI	p	HR	95% CI	p
Clonal heterogeneity	0.96	0.82, 1.13	0.66	1.05	0.98, 1.14	0.17	1.18	1, 1.39	0.054	1.09	1, 1.18	0.039
Age (years)	0.98	0.97, 0.99	<0.001	1.02	1.01, 1.02	<0.001	1.02	1.01, 1.03	<0.001	1.03	1.03, 1.04	<0.001
ECOG PS	0.45	0.28, 0.72	<0.001	1.64	1.32, 2.03	<0.001	1.45	0.99, 2.13	0.055	1.65	1.32, 2.06	<0.001
Karyotype (unfavorable risk used as reference)												
Favorable	6.44	3.7, 11.22	<0.001	0.34	0.26, 0.45	<0.001	0.47	0.33, 0.68	<0.001	0.28	0.2, 0.38	<0.001
Intermediate	2.09	1.2, 3.66	0.0096	0.71	0.53, 0.94	0.016	0.8	0.53, 1.23	0.32	0.68	0.5, 0.9	0.0085
Unknown	1.85	1.14, 2.99	0.013	0.59	0.45, 0.76	<0.001	0.58	0.38, 0.86	0.0076	0.49	0.37, 0.64	<0.001

CR: complete remission; EFS: event-free survival; RFS: relapse-free survival; OS: overall survival; OR: Odds ratio; HR: Hazard ratio; CI: confidence interval; ECOG: Eastern Cooperative Group; PS: performance status. - Logistic regression models included for the following prognostic cofounders: clonal heterogeneity, age, white blood cell and platelet counts at presentation, percentage of bone marrow and peripheral blood blasts at diagnosis, sex, performance status (PS) (0-1 vs. 2+) and karyotypic risk group (unfavorable used as reference).

Discussion

The present study was undertaken to better define the effect of cytogenetic clonal heterogeneity in AML patients with abnormal karyotype. Several previous reports have described novel cytogenetic and molecular abnormalities with prognostic importance in AML. Monosomal karyotype, initially described by the HOVON/SAKK co-operative groups, identified AML patients with particularly poor outcomes. Incorporation of mutational abnormalities into a cytogenetic-based classification was proposed on behalf of the European LeukemiaNet and outlined 4 separate prognostic cohorts of AML patients.[15-17] Activating mutations in tyrosine kinases, such as *c-kit* and *FLT3-ITD*, collaborate with the fusion proteins in core-binding factor (CBF) acute myeloid leukemia and defines cohorts of patients with increased incidence of relapse and worse outcomes. Bochtler *et al*. on behalf of the Study Alliance Leukemia has recently demonstrated that presence of cytogenetically defined subclones identifies a cohort of AML patients with resistance to induction therapy and shortened overall survival.[11] Our results confirm these previous observations where the presence of cytogenetic clonal heterogeneity is a frequent and important prognostic factor in AML and is independently associated with decreased response to therapy and worse outcomes, especially among patients with poor-risk cytogenetics, regardless of the chemotherapy regimens used. Also, we demonstrated an association between cytogenetic clonal heterogeneity and poor prognostic features, such as advancing age and secondary AML, further explaining the worse outcomes observed in these patients.

Clonal evolution is not a new concept in cancer and leukemia. In AML, clonal heterogeneity may be a reflection of the genome-wide chromosomal instability that occurs during leukemogenesis. For example, *TP53* alterations have been described in nearly 80% of AML patients with monosomal karyotype and are associated with a higher degree of genomic complexity.[19] Furthermore, inactivation of *TP53* in AML has been linked to the phenomenon known as chromothripsis, which indicates acquisition of numerous rearrangements through a single catastrophic DNA event.[20] The impact of clonal heterogeneity on the outcomes in AML patients may have functional consequences. Selection of chemotherapy resistant subclonal populations following exposure to genotoxic therapies, according to Darwinian principles, represents a plausible explanation for the reduced response rates and decreased survival associated with clonal heterogeneity. In fact, whole genome sequencing studies have demonstrated that AML relapses are commonly driven by subclonal frequency and diversity.[21]

Originally described by Nowell in 1976, the classic model of clonal evolution in cancer follows a sequential acquisition of genomic alterations.[4] Our study confirms prior observations that a significant proportion (24% in this study and 32.8% in Bochtler *et al*.) of AML patients with abnormal karyotype have multiple clones. In our study, 90% of these cases demonstrated a clear ancestral pattern between clones (mother-daughter or branched). This proportion is somewhat higher than previous reports where approximately 60% of cases had such associations.[11] Reasons for this discrepancy could be either due to the more complex nature of cases with composite karyotypes or due to laboratory preference in describing karyotype as distinct clones or as a composite clone for simplicity. For example, SWOG discourages the use of composite karyotype whenever possible, consistent with ISCN guideline that "every effort should be made to describe the subclones so that clonal evolution is made evident".[12] Finally, although the significance of clonal heterogeneity was shown to be more profound in cases where the clones were obviously related than those that were so-called composite in the Bochter study, the small proportion (10%) of patients with composite karyotypes did not allow us to define the prognostic significance of this subtype of clonal evolution in our cohort. Also, we were unable to define the effect of different post-remission therapies on the outcomes of patients with clonal heterogeneity.

Two prior studies have shown that induction regimens containing high doses of cytarabine may improve the outcomes of patients with monosomal karyotype AML, another recently described cytogenetic cohort associated with particular poor outcomes.[22,23] Our exploratory analysis failed to demonstrate any therapeutic benefit of escalation of cytarabine doses during induction in patients with clonal heterogeneity. Post-remission therapies (median 2 cycles, range 1-4) also did not affect the overall findings of the study. It has previously been suggested that the immunological effects of allogeneic stem cell transplantation (alloHSCT) may be more successful than chemotherapy in eradicating all leukemic clones in patients with clonal heterogeneity. Although we were unable to investigate the impact of alloHSCT on the outcomes of our cohort of patients with multiple cytogenetic clones, early transplant evaluation remains indicated for these patients.

In summary, our results demonstrate that cytogenetically defined clonal heterogeneity is an adverse prognostic feature in AML. Patients with non-CBF abnormal karyotype AML and presence of multiple cytogenetically abnormal clones should be considered at high risk for treatment failure. High-dose cytarabine containing regimens cannot improve the poor outcomes associated with cytogenetic clonal heterogeneity.

Acknowledgments

Clinical trials registration numbers: ClinicalTrials.gov Identifier: NCT014343329; NCT01338974; NCT00899171; NCT1059734; NCT01059734; NCT00899743; NCT0143329, NCT00023777; NCT00085709; NCT01360125; NCT00004217.

Funding

This work was supported in part by the following Public Health Service Cooperative Agreement grant numbers awarded by the National Cancer Institute, National Institutes of Health, Department of Health and Human Services: CA32102 and CA38926.

Authorship and Disclosures

Information on authorship, contributions, and financial & other disclosures was provided by the authors and is available with the online version of this article at www.haematologica.org.

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