Leukemia Research 39 (2015) 265–272

Contents lists available at ScienceDirect

Leukemia Research

journal homepage: www.elsevier.com/locate/leukres

AML with gain of chromosome 8 as the sole chromosomal abnormality (+8sole) is associated with a specific molecular mutation pattern including *ASXL1* mutations in 46.8% of the patients

Tamara Alpermann, Claudia Haferlach, Christiane Eder, Niroshan Nadarajah, Manja Meggendorfer, Wolfgang Kern, Torsten Haferlach, Susanne Schnittger*

MLL Munich Leukemia Laboratory, Munich, Germany[1]

ARTICLE INFO

Article history:
Received 8 July 2014
Received in revised form
25 November 2014
Accepted 28 November 2014
Available online 16 December 2014

Keywords:
AML
Trisomy 8
Molecular mutations
Genetic stability
Prognosis

ABSTRACT

Trisomy 8 is the most frequent cytogenetically gained aberration in AML. We compared 79 adult de novo AML with trisomy 8 as the sole cytogenetic abnormality (+8sole) to 511 normal karyotype AML patients (NK). +8sole patients were older ($p = 0.013$), presented lower WBC counts ($p = 0.010$), harbored more often *ASXL1* mutations ($p < 0.001$) and *RUNX1* mutations ($p = 0.009$), but less frequent *FLT3*-ITD ($p = 0.038$), *NPM*1 mutations ($p < 0.001$) and double-mutated *CEBPA* ($p = 0.038$) than NK patients. No prognostic difference was found between +8sole and NK. With respect to genetic stability we found +8sole was instable, and molecular markers were either stable or gained in number and diversity.

1. Introduction

A gain of chromosome 8 (+8) belongs to the most frequent cytogenetic aberrations as it is present in about 10% of all AML cases [1]. It presents either as the sole cytogenetic abnormality (+8sole) or in combination with a large variety of different cytogenetic abnormalities and is also frequently present in complex karyotypes. A gain of chromosome 8 can also occur as an obtained aberration during the course of the disease. There is still dissent whether +8 is a primary event or a secondary hit in the pathogenesis of AML and whether it might even be constitutional in some cases [2–4]. Therefore, the genetic stability of +8 has to be evaluated. Furthermore, correlations of +8 with morphological categories as well as a male predominance have been reported [5–7]. Regarding the clinical outcome the effect of +8 was addressed in diverse studies. Some groups did show that the effect was driven by the accompanying cytogenetic abnormalities and not by the +8 itself [8,9], others did show an independent prognostic adverse effect [10–13], and still others were not able to show any adverse effect on survival [1,6]. Unfortunately, the cohorts and their treatments were not comparable in all cases, this might add to the different results, but due to limited data on +8 we included all of these studies. The aim of this study was to further evaluate +8sole with respect to biological features, molecular genetic alterations, and prognostic significance in comparison with normal karyotype AML and its stability at relapse.

2. Patients and methods

2.1. Patients

The cohort comprised of 79 consecutively newly diagnosed adult de novo AML with +8sole. All samples were sent between August 2005 and August 2012 to our laboratory for routine diagnostics and all analyses were performed within the same period. Molecular analysis was performed on *FLT3*-ITD, *FLT3*-TKD, *MLL*-PTD and mutations in *ASXL1*, *CEBPA*, *IDH1*, *IDH2*, *NPM1*, *RUNX1* and *WT1*. Thirty-one (39.2%) patients were female, 48 (60.8%) male. The median age was 67.7 years (range 25.4–89.3 years).

For comparison of the clinical and genetic features of these patients a control cohort of normal karyotype (NK) patients was drawn. We focussed on the same time period and as well included all patients with available material for molecular analysis. In total 511 consecutively newly diagnosed adult de novo AML with normal karyotype for which molecular analysis for the before mentioned molecular markers was performed were included as control cohort. 246 (48.1%) patients were female, 265 (51.9%) male and the median age was 64.4 years (range 17.8–100.4 years).

Within the total cohort of patients ($n = 590$), follow-up data was evaluated only for patients receiving aggressive chemotherapeutic treatment regimes ($n = 472$

* Corresponding author at: MLL Munich Leukemia Laboratory, Max-Lebsche-Platz 31, 81377 Munich, Germany. Tel.: +49 89 990 17 300; fax: +49 89 990 17 309.
E-mail address: susanne.schnittger@mll.com (S. Schnittger).
[1] www.mll.com.

http://dx.doi.org/10.1016/j.leukres.2014.11.026
0145-2126/

Table 1
Clinical, morphological, and molecular characteristics of the total cohort, patients with +8sole, and normal karyotype patients.

	Total cohort ($n=590$)		+8sole ($n=79$)		Normal karyotype ($n=511$)		p
	n	%	n	%	n	%	
Male	313	53.1	48	60.8	265	51.9	n.s.
Female	277	46.9	31	39.2	246	48.1	
Age (median; mean; range)	64.8; 62.5; 17.8–100.4		67.6; 66.2; 25.4–89.3		64.4; 61.9; 17.8–100.4		0.013
WBC (median; mean; range)	24.0; 48.8; 0.5–600.0		14.7; 33.6; 0.6–211.8		25.2; 51.1; 0.5–600.0		0.010
Hb (median; mean; range)	9.2; 9.3; 2.7–17.5		9.3; 9.5; 2.7–17.5		9.1; 9.2; 2.8–16.3		n.s.
PLT (median; mean; range)	64.0; 94.9; 3.0–950.0		62.0; 93.3; 4.0–509.0		66.0; 95.1; 3.0–950.0		n.s.
% BM blasts (median; mean; range)	66.3; 62.0; 3.0[a]–99.0		58.5; 57.8; 4.0[a]–98.0		67.0; 62.7; 3.0[a]–99.0		n.s.
Median follow-up in months	40.8		33.5		41.1		n.a.
FAB classification							
M0	26	4.4	6	7.6	20	3.9	n.s.[b]
M1	189	32.0	19	24.1	170	33.3	n.s.[b]
M2	196	33.2	32	40.5	164	32.1	n.s.[b]
M4	138	23.4	11	13.9	127	24.9	0.032[b]
M5	19	3.2	8	10.1	11	2.2	0.002[b]
M6	20	3.4	3	3.8	17	3.3	n.s.[b]
M7	2	0.3	0	0	2	0.4	n.a.
Molecular mutations							
*ASXL1*mut	97	16.4	37	46.8	60	11.7	<0.001
*CEBPA*mut[c]	63	10.7	5	6.3	58	11.4	n.s.
CEBPA double-mutated	27	4.6	0	0	27	5.3	0.038
FLT3-ITD	185	31.4	15	19.0	170	33.3	0.013
FLT3-TKD	54	9.2	4	5.1	50	9.8	n.s.
*IDH1*mut	76	12.9	14	17.7	62	12.1	n.s.
*IDH2*mut	105	17.8	15	19.0	90	17.6	n.s.
MLL-PTD	53	9.0	3	3.8	50	9.8	n.s.
*NPM1*mut	297	50.3	14	17.7	283	55.4	<0.001
*RUNX1*mut	99	16.8	22	27.8	77	15.1	0.009
*WT1*mut	40	6.8	4	2.5	38	7.4	n.s.

n.s.: not significant; BM: bone marrow; n.a.: not applicable.
[a] All samples presenting with less than 20% of bone marrow blasts, are AML M6.
[b] Compared versus all other FAB subtypes; mut = mutated.
[c] Regardless whether single- or double-mutated.

patients; 50 cases with +8sole and in 422 NK patients) [14]. Median time of follow-up was 40.8 months.

Patients gave informed consent to the genetic analysis and to the use of laboratory results for research. The study was approved by the Internal Review Board and adhered to the Declaration of Helsinki.

2.2. Cytomorphology

Bone marrow and/or peripheral blood smears were investigated in all 590 cases by May Grünwald Giemsa staining, combined with myeloperoxidase, and non-specific esterase [15]. All cases were classified according to the WHO [16] and the FAB [17] classification.

2.3. Cytogenetics and FISH

Chromosome banding analysis was performed in all 590 patients according to standard procedures [18]. Seventy-nine patients carrying a +8sole were included in this study, irrespective whether they carried a trisomy 8 ($n=76$) or tetrasomy 8 ($n=3$).

In addition, fluorescence in situ hybridization (FISH) was done in all 79 patients with a +8sole and was performed with a centromere (alpha satellite) 8 specific probe (Abbott, Wiesbaden, Germany) [18].

2.4. Molecular genetics

All 590 cases were analyzed for *FLT3*-ITD [19], *FLT3*-TKD [20], *MLL*-PTD [21] and mutations in *ASXL1* [22], *CEBPA* [23], *IDH1* [24], *IDH2*, *NPM1* [25], *RUNX1* [26] and *WT1* by a combination of gene scan analysis, melting curve analysis, Sanger sequencing, or next generation sequencing. *CEBPA* mutations were divided into single- or double-mutations. *IDH2*mut indicates mutations of *IDH2*R140 or *IDH2*R172. Furthermore, cases with relapse that were included for the evaluation of genetic stability were investigated at diagnosis and at relapse for mutations in *DNMT3A* [27], *KRAS*, *NRAS* [28], *SETBP1* [29], *SRSF2* [30], *TET2* [31], and *TP53* [32] in addition.

2.5. Statistical analyses

Statistical analyses were performed using SPSS version 19.0.0 (SPSS by IBM, IBM Corporation, Armonk, NY, USA). All p-values reported are two-sided, accepting $p=0.05$ as indicating a statistically significant difference. Dichotomous variables were compared between different groups using the χ^2-test or Fisher's exact test and continuous variables by Student's T-test. Survival curves were calculated for overall survival (OS) and event-free survival (EFS) according to Kaplan–Meier and compared using the two-sided log rank test. To eliminate the effect of allogeneic stem cell transplantation OS was recalculated censoring patients on the day of transplantation (OS^{TXcens}). OS was defined as the time from diagnosis to last follow-up or death. EFS was defined as the time from diagnosis to failure (persistent leukemia, relapse, death) or last follow-up, and OS^{TXcens} was calculates from diagnosis to either day of transplantation, last follow-up or death.

3. Results

3.1. Clinical features

Comparing the clinical features of the two analyzed cohorts +8sole and NK showed that patients with a +8sole were older than NK patients (mean 66.2 vs 61.9; $p=0.013$) and presented with lower WBC counts (mean 33.6 vs 51.1; $p=0.010$). A trend to a prevalence for male sex in cases with +8sole compared to NK patients was seen (60.8% vs 50.9%), but no statistical significance was reached ($p=0.148$).

No difference with respect of hemoglobin level, platelet count, and bone marrow blast percentage was observed. Focussing on the FAB classification, the subcohorts were distributed differently within the +8sole and NK groups. Namely, M4 was less frequent in patients with a +8sole compared to NK patients (13.9% vs 24.9%; $p=0.032$), whereas M5 was more frequent in +8sole (10.0% vs 2.2%; $p=0.002$). No difference was revealed within the subcategories of M0, M1, and M6. M7 was not tested due to the presence of only two patients within this FAB subgroup (for details see Table 1).

3.2. Molecular mutations

Patients with +8sole showed both *ASXL1*mut and *RUNX1*mut more often than those with normal karyotype (46.8% vs 11.7%;