Cytogenetic clonal heterogeneity is not an independent prognosis factor in 15-60-year-old AML patients: results on 1291 patients included in the EORTC/GIMEMA AML-10 and AML-12 trials

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ORIGINAL ARTICLE

Cytogenetic clonal heterogeneity is not an independent prognosis factor in 15–60-year-old AML patients: results on 1291 patients included in the EORTC/GIMEMA AML-10 and AML-12 trials

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Abstract

The presence of cytogenetic clonal heterogeneity has been associated with poor prognosis in patients with acute myeloid leukemia (AML). Here, we reassessed this association. The study cohort consisted of all patients with an abnormal karyotype randomized in the EORTC/GIMEMA AML-10 and AML-12 trials. Abnormal karyotypes were classified as no subclones present (cytogenetic abnormality in a single clone), defined subclones present (presence of one to three subclones), and composite karyotypes (CP) (clonal heterogeneity not allowing enumeration of individual subclones). The main endpoints were overall survival (OS) and disease-free survival (DFS). Among 1291 patients with an abnormal karyotype, 1026 had no subclones, 226 at least 1 subclone, and 39 a CP. Patients with defined subclones had an OS similar to those with no subclones (hazard ratio (HR) 1.05, 95% confidence interval (CI) 0.88–1.26), but CP patients had a shorter OS (HR = 1.58, 95% CI 1.11–2.26). However, in a multivariate Cox model stratified by protocol and adjusted for age, cytogenetic risk group, secondary versus primary AML, and performance status, clonal heterogeneity lost its prognostic importance (HR = 1.10, 95% CI 0.91–1.32 for defined subclones versus no subclones; HR = 0.96, 95% CI 0.67–1.38 for CP versus no subclones). Also, the impact of having a donor on DFS was similar in the three clonal subgroups. In summary, in patients with cytogenetic abnormality, presence of subclones had no impact on OS. The dismal outcome in patients with a CP was explained by the known predictors of poor prognosis.

Trial registration: AML-10: ClinicalTrials.gov identifier: NCT00002549, retrospectively registered July 19, 2004; AML12: ClinicalTrials.gov identifier: NCT00004128, registered January 27, 2003.

Frédéric Baron and Marian Stevens-Kroef contributed equally to this work.

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Keywords AML · Cytogenetic · Clonal heterogeneity · Clonal evolution · Transplantation

Background

Although the overall survival (OS) in younger acute myeloid leukemia (AML) patients has improved in the recent decades, disease relapse remains the first cause of death in younger patients with AML [1–6]. Approximately 60% of younger patients diagnosed with AML have chromosomal aberrations in their leukemic cells [1]. Despite the impressive recent progress in the identification of genetic alterations associated with AML [7, 8], chromosomal aberrations still belong to the strongest predictive factors in younger AML patients and remain mandatory for prognostic classification [1, 2, 9]. Specifically, patients can be classified in favorable, intermediate, or adverse cytogenetic risk groups [9, 10]. This classification not only provides prognostic information but also influences the choice of post-remission treatment [11, 12], since patients without favorable cytogenetic features most likely benefit from allogeneic stem cell transplantation (allo-HSCT) [12, 13]. Even after allo-HSCT cytogenetic risk group, classification remains a strong prognostic factor [14–16].

In patients with acute lymphoblastic leukemia, several studies have provided evidence for a complex and multiclonal evolution of the leukemia [17, 18]. In addition, a higher clonal heterogeneity has been associated with poorer outcomes, which is explained by the increased probability that a specific subclone becomes chemotherapy-resistant [17]. In the AML setting, karyotype evolution has been demonstrated at the time of AML relapse [19]. More recent studies using whole-genome sequencing confirmed the observation that AML relapse is associated with the addition of new mutations and clonal evolution [20–22].

A large (n = 1274) retrospective study assessing data from two prospective trials carried out by the Study Alliance Leukemia (SAL) group reported in 2013 that the presence of cytogenetic clonal heterogeneity, as detected by metaphase karyotyping, was associated with poor prognosis in patients with cytogenetically intermediate and adverse-risk AML [23]. This led us to assess the prognostic importance of cytogenetic clonal heterogeneity in a large number of AML patients included in the large EORTC/GIMEMA AML-10 and AML-12 prospective trials. In addition, we evaluated the effect of different types of remission-induction chemotherapies and of the availability of a donor in this subset of patients.

Methods

Study design

In the EORTC/GIMEMA AML-10 trial [1], patients were randomized to receive either daunorubicin (DNR; 50 mg/m^2), mitoxantrone (MTZ; 12 mg/m^2), or idarubicin (IDA; 10 mg/m^2) on days 1, 3, and 5 in addition to standard-dose cytarabine (SDAC; 25-mg/m^2 bolus followed by 100 mg/m^2 given as a continuous infusion daily for 10 days) and etoposide (100 mg/m^2 on days 1–5) for induction chemotherapy.

In the EORTC/GIMEMA AML-12 trial [2], patients were randomized between induction with SDAC or high-dose cytarabine (HiDAC; 3 g/m^2 every 12 h as a 3-h IV infusion on days 1, 3, 5, and 7), in addition to DNR (50 mg/m^2 per day on days 1, 3, and 5) and etoposide (50 mg/m^2 per day on days 1–5).

In both trials, a second cycle of induction was administered in patients who achieved a partial response (PR). Patients who achieved a complete remission (CR) or a CR with incomplete count recovery (CRi) after one or two courses of induction chemotherapy received a consolidation chemotherapy with the same anthracycline as in the induction course plus intermediate dose cytarabine (500 mg/m^2 every 12 h as a 2-h IV infusion on days 1–6). Patients ≤ 45 years in the AML-10 trial and ≤ 50–60 years in the AML-12 trial, respectively, were then scheduled to undergo an allo-HSCT in first CR/CRi if they had an HLA-identical sibling donor (in both trials) or, in AML-12 trial only, if they had an unrelated donor and required two induction courses or had AML with chromosome abnormalities involving 3q, 5, 7, t(6;9), t(9;22), 11q23, or complex abnormalities [2]. Patients without a donor were scheduled to undergo an autologous HSCT (auto-HSCT) in first CR/CRi.

Criteria for response and relapse followed the Report of the National Cancer Institute-sponsored workshop [24].

Cytogenetic assessment

For both trials, cytogenetic examinations were performed at diagnosis. Cytogenetic data were centrally collected, reviewed, and classified using the EORTC risk classification [25]. For the current analysis, all cytogenetic data were centrally re-reviewed. Karyotypes were described following the International System for Human Cytogenetic Nomenclature [26]. Chromosomal gains or structural aberrations had to be detected in at least two metaphases and chromosomal losses in three metaphases to be categorized as clonal [26]. These thresholds were applied to the karyotypes as a whole, but not to single unequivocally related subclones. For the current analysis, clonal heterogeneity was classified similar as in the SAL study [23], as either no subclones (cytogenetic abnormality present in a single clone), presence of defined subclones, or composite karyotypes (CP) when karyotypic heterogeneity was too complex to

Table 1 Patient characteristics according to cytogenetic clonal heterogeneity

	No subclones ($N = 1026$), N (%)	Defined subclones ($N = 226$), N (%)	Composite karyotype ($N = 39$), N (%)	Chi-squared test: P value
Study and randomized arm				0.064
AML-10	556 (54.2)	109 (48.2)	20 (51.3)	
DNR	196 (19.1)	33 (14.6)	4 (10.3)	
MTZ	184 (17.9)	26 (11.5)	6 (15.4)	
IDA	176 (17.2)	50 (22.1)	10 (25.6)	
AML-12	470 (45.9)	117 (51.8)	19 (48.7)	
SDAC	235 (22.9)	56 (24.8)	12 (30.8)	
HiDAC	235 (22.9)	61 (27.0)	7 (17.9)	
Gender				0.76
Male	563 (54.9)	130 (57.5)	22 (56.4)	
Female	463 (45.1)	96 (42.5)	17 (43.6)	
Age (years)				0.026
15–25	128 (12.5)	40 (17.7)	0 (0.0)	
26–35	196 (19.1)	48 (21.2)	6 (15.4)	
36–45	261 (25.4)	55 (24.3)	10 (25.6)	
46–60	441 (43.0)	83 (36.7)	23 (59.0)	
Disease				0.18
De novo AML	979 (95.4)	218 (96.5)	35 (89.7)	
Antecedent MDS	21 (2.0)	4 (1.8)	3 (7.7)	
t-AML	26 (2.5)	4 (1.8)	1 (2.6)	
Cytogenetic risk group (MRC)				< 0.001
Not assessable	2 (0.2)	1 (0.4)	0 (0.0)	
Favorable	317 (30.9)	71 (31.4)	0 (0.0)	
Intermediate	434 (42.3)	60 (26.5)	6 (15.4)	
Adverse	273 (26.6)	94 (41.6)	33 (84.6)	
Monosomal karyotype (MK)				< 0.001
Not assessable	1 (0.1)	0 (0.0)	0 (0.0)	
MK-	919 (89.6)	169 (74.8)	12 (30.8)	
MK1	31 (3.0)	16 (7.1)	1 (2.6)	
MK2	14 (1.4)	7 (3.1)	1 (2.6)	
MK3	60 (5.8)	34 (15.0)	25 (64.1)	
Missing	1 (0.1)	0 (0.0)	0 (0.0)	
Number of subclones				
1		193 (85.4)		
2		25 (11.1)		
3		8 (3.5)		
WHO performance status				0.80
0	408 (39.8)	96 (42.5)	16 (41.0)	
1	461 (44.9)	103 (45.6)	17 (43.6)	
2–4	155 (15.1)	27 (11.9)	6 (15.4)	
Missing	2 (0.2)	0 (0.0)	0 (0.0)	
WBC (10^9/L)				0.16
< 25	612 (59.6)	150 (66.4)	29 (74.4)	
≥ 25 and < 100	308 (30.0)	55 (24.3)	8 (20.5)	
≥ 100	105 (10.2)	21 (9.3)	2 (5.1)	
Missing	1 (0.1)	0 (0.0)	0 (0.0)	
Donor availability in patients who reached a CR/CRi ($N = 919$)				0.70
No	449 (61.0)	97 (62.2)	16 (59.3)	
Yes	251 (34.1)	48 (30.8)	11 (40.7)	
Missing	36 (4.9)	11 (7.1)	0 (0.0)	

DNR daunorubicin, *MTZ* mitoxantrone, *IDA* idarubicin, *SDAC* standard-dose cytarabine, *HiDAC* high-dose cytarabine, *WBC* white blood cells, *NA* not applicable

[a] No molecular data were available for patients included in the AML-10 trial

allow enumeration of individual subclones. In addition, cytogenetic risk groups were classified using the refined UK Medical Research Council (MRC) classification [10]. Monosomal karyotype (MK) was defined as the presence of two or more monosomies, or a single monosomy in the presence of structural abnormalities as previously reported [27, 28]. MK were further subclassified between one monosomy (MK1), or two (MK2) or three (MK3) monosomies as previously described [28].

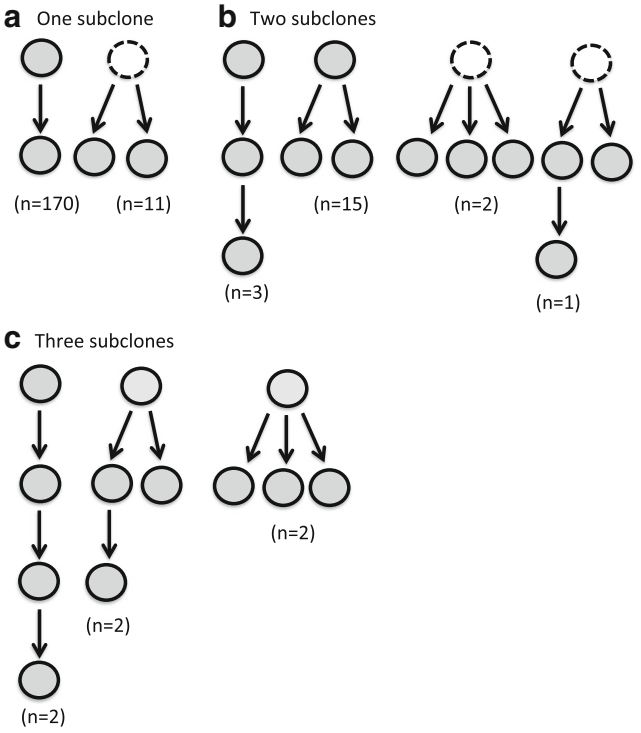

Fig. 1 Presumed ancestral trees of *related* subclones (see text for more information). **a** Pattern with one subclone; the left pattern is linear and the right is branched. **b** Pattern with two subclones; the left pattern is linear and the other three patterns are branched. **c** Pattern with three subclones; the left pattern is linear and the other two patterns are branched

Statistical analyses

The duration of OS was calculated from the date of randomization until death. Disease-free survival (DFS) was calculated as the time from CR/CRi until the first relapse or death, whichever occurred first or as the time from allo-HSCT until the first relapse or death, whichever occurred first. The follow-up of patients still alive and in first CR/CRi, was censored at the last date to be alive.

The Kaplan-Meier method was used to estimate OS and DFS rates [29]. Confidence intervals for the 5-year OS and DFS rates were obtained using the normal approximation of the distribution of log(− log(survival)) and the Greenwood variance formula [30]. The confidence interval of the median OS and DFS was estimated using the Brookmeyer and Crowley method [31]. Cox model stratified by protocol was used to compare OS and DFS between groups [32]. In order to investigate whether clonal heterogeneity provided additional prognostic information when taking known prognostic factors into account, a Cox model stratified by protocol and adjusted for known prognostic factors was used. The predictive value of clonal heterogeneity for OS and DFS was tested based on the interaction term in a Cox model.

Cumulative incidence of relapse and of death without relapse from the date of CR/CRi was estimated using the Aalen-Johansen estimator [33]. Confidence intervals of the 5-year cumulative incidence rates were estimated using a Greenwood-like variance estimator [34]. A proportional subdistribution hazard Fine-Gray model stratified by protocol was used to compare the incidence of relapse and death without relapse between groups [35].

All tests were performed at a two-sided significance level of 0.05. SAS 9.4 (SAS Institute Inc., Cary, NC) was used for all statistical analyses.

Results

Patients

In the AML-10 trial, 2157 patients were randomized to receive DNR, MTX, or IDA between November 1993 and December 1999. The current analyses were performed in a subgroup of 685 patients with an abnormal karyotype (see supplemental Fig. A1 for a detailed flow chart): 556 of them had a single cytogenetically abnormal clone (no subclones), 109 at least one defined subclone, and 20 a CP (Table 1). In the AML-12 trial, 1942 patients were randomized between HiDAC and SDAC from September 1999 to January 2008. The current analyses were performed in a subgroup of 606 patients with an abnormal karyotype: 470 of them had no subclones, 117 at least 1 defined subclone, and 19 a CP. Interestingly, patients with successful cytogenetic assessment had better OS than those without (supplemental Table A2). Median follow-up for the 1291 patients included in the current analyses was 10.7 years (95% CI 10.1–11.4 years).

The median number of analyzed metaphases was 20 both among patients with subclones (interquartile range (IQR) 15–22, range 2–76) as well as those without subclones (IQR 17–26, range 3–89). All patients with a composite karyotype (n = 39) were older than 25 years, and 84.6% had adverse MRC cytogenetic features. Patients with defined subclone(s) had an adverse cytogenetic profile more often than those without defined subclones (42 versus 27%, $P < 0.001$). Among patients with defined subclone(s) (n = 226), 193 had 1 subclone, 25 had 2 subclones, and 8 had 3 subclones. Ancestral patterns of clonal evolution among patients with only related clones are depicted in Fig. 1. Briefly, among patients with 1 subclone, the linear (a sideline with cytogenetic abnormalities present in the stemline and additional cytogenetic abnormalities) pattern was more frequently observed as compared to the branched pattern (two or more sidelines with some of the cytogenetic abnormalities in common, but different additional cytogenetic abnormalities) (170 linear versus 11 branched), while in patients with 2 or 3 subclones, the branched pattern was more frequently observed (5 linear versus 22 branched). In 12 patients with 1 subclone, 2 unrelated clones were observed. In 5 patients with 2 or 3 subclones, both related and unrelated clones were present. For 1 patient with 4 subclones, the pattern could not be defined based on the available information.

Table 2 Outcomes by cytogenetic clonal heterogeneity

	No subclones	Subclones	Composite karyotype	*P* value
Number of patients	1026	226	39	
Median OS, years (95% CI)	1.73 (1.49, 2.05)	1.46 (1.19, 2.17)	1.16 (0.78, 1.69)	
5-year OS, % (95% CI)	38.0 (35.0, 41.1)	35.9 (29.6, 42.2)	16.7 (6.8, 30.3)	
HR for OS	1.00	1.05 (0.88, 1.26)	1.58 (1.11, 2.26)	0.036[a]
Number of patients with response data	1020			
CR/CRi after one/two inductions, number (%) of patients	736 (72.2)	156 (69.3)	27 (69.2)	0.64[b]
In patients with CR/CRi				
Number of patients	736	156	27	
Number (%) of patients given auto-HSCT in CR1	274 (37.2)	57 (36.5)	7 (25.9)	
Number (%) of patients given allo-HSCT in CR1	203 (27.6)	42 (26.9)	9 (33.3)	
5-year relapse incidence, % (95% CI)	45.8 (42.2, 49.4)	48.0 (40.0, 55.7)	66.7 (44.5, 81.6)	
HR for relapse incidence	1.00	1.09 (0.84, 1.41)	1.93 (1.17, 3.19)	0.033[c]
5-year incidence of NRM, % (95% CI)	12.5 (10.2, 15.1)	7.1 (3.8, 12.0)	18.5 (6.3, 35.8)	
HR for NRM incidence	1.00	0.47 (0.25, 0.88)	1.56 (0.71, 3.46)	0.028[c]
Median DFS from CR/CRi, years (95% CI)	1.59 (1.34, 2.12)	2.24 (1.24, NR)	0.73 (0.38, 1.47)	
5-year DFS from CR/CRi, % (95% CI)	41.7 (38.0, 45.2)	44.9 (36.9, 52.5)	14.8 (4.7, 30.5)	
HR for DFS	1.00	0.89 (0.71, 1.13)	2.09 (1.38, 3.15)	0.001[a]

OS overall survival, *CI* confidence interval, *HR* hazard ratio, *CR* complete remission, *CRi* complete remission with incomplete blood recovery, *CR1* first complete remission or complete remission with incomplete blood recovery, *auto-HSCT* autologous hematopoietic stem cell transplantation, *allo-HSCT* allogeneic hematopoietic stem cell transplantation, *NRM* non-relapse mortality, *DFS* disease-free survival, *NR* not reached

[a] Cox model stratified by protocol was used

[b] Logistic regression model adjusted for protocol was used

[c] Proportional subdistribution hazard Fine-Gray model stratified by protocol was used

Impact of clonal heterogeneity on patients' outcomes

In the present analysis of cytogenetic abnormal AML patients, those with no subclones and those with defined subclones had a similar OS from randomization, a similar probability of achieving a CR/CRi, and a similar relapse incidence and DFS from CR (Table 2). Further, in comparison to patients with no subclones, those with a CP had a similar probability of achieving a CR/CRi but a higher incidence of relapse (HR = 1.93, 95% CI 1.17–3.19), shorter DFS from CR/CRi (HR = 2.09, 95% CI 1.38–3.15), and shorter OS (HR = 1.58, 95% CI 1.11–2.26) (Table 2 and Fig. 2). Importantly, the impact of harboring defined subclones or a CP on OS was similar in all MRC cytogenetic groups (test for interaction in the Cox model $P = 0.28$).

In the multivariate analysis, the relative prognostic importance of cytogenetic clonal heterogeneity was no longer significant, neither for relapse incidence ($P = 0.78$), DFS from CR ($P = 0.62$), or OS ($P = 0.58$) (Table 3). This was mainly due to the inclusion of the cytogenetic risk group in the models, which was strongly associated with clonal heterogeneity status, and had a strong prognostic importance.

In a sensitivity analysis, using the same population of patients with adverse/intermediate cytogenetic features only and including the same covariates [age modeled as a continuous variable using one linear term, disease (de novo AML versus antecedent MDS versus t-AML), cytogenetic risk group, and clonal heterogeneity] as in the analysis of the SAL group [23], the relative prognostic importance of clonal heterogeneity status regarding OS was not significant (overall comparison $P = 0.37$, HR = 1.15, 95% CI 0.94–1.40 for the presence of defined subclones versus no subclones; HR = 0.97, 95% CI 0.68–1.40 for CP versus no subclones).

Impact of clonal heterogeneity on the effects of the type of remission-induction chemotherapy

Consistent with the original results of the AML-10 trial including all patients [1], the effect of the type of anthracycline used (IDA versus MTZ versus DNR) on the OS was not significant among patients with an abnormal karyotype ($P = 0.58$). The magnitude of the effect was similar according to the clonal heterogeneity status (test for interaction $P = 0.18$). Similarly, in patients who reached CR/CRi, the type of anthracycline did not impact the DFS from CR/CRi ($P = 0.57$), and there was no evidence of an impact of clonal heterogeneity status on the magnitude of the treatment difference regarding DFS (test for interaction $P = 0.20$).

Among patients from the AML-12 trial, the estimate of the difference in OS between HiDAC and SDAC was similar as in the original analysis including all patients

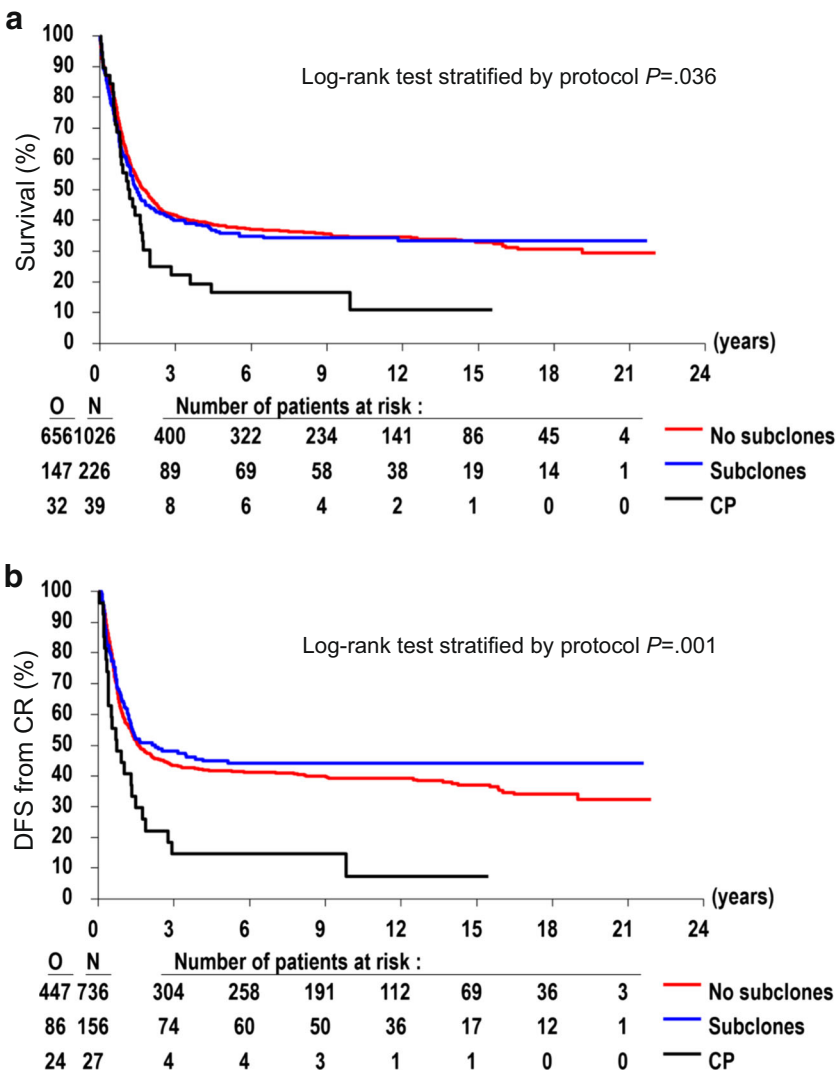

Fig. 2 Overall survival (**a**) and disease-free survival (DFS) from complete remission (CR) (**b**) according to cytogenetic clonal heterogeneity. *CP* composite karyotype

[2] (HR = 0.95, 95% CI 0.77–1.17), and its magnitude was not impacted by the clonal heterogeneity status (test for interaction $P = 0.51$).

Impact of clonal heterogeneity on the effect of having a donor on AML outcomes

Consistent with the analysis including all patients from the two trials [1, 2], in the current analysis of patients with an abnormal karyotype DFS from CR/CRi was significantly longer in patients with a donor ($n = 310$, 254 of them received an allo-HSCT in CR1) than in those without a donor ($n = 562$) (HR = 0.76, 95% CI 0.64–0.92, $P = 0.004$) (Fig. 3a). This was due to a significantly lower incidence of relapse in patients with a donor (HR = 0.59, 95% CI 0.47–0.73, $P < 0.001$) and despite the higher incidence of non-relapse mortality in patients with a donor (HR = 1.75, 95% CI 1.22–2.52, $P = 0.002$) compared to those without. The beneficial effect of having a donor on DFS from CR/CRi was not observed in patients with favorable risk cytogenetics (HR = 0.85, 95% CI 0.58–1.25).

Two-hundred-fifty-four patients received an allo-HSCT in first CR. This included 203 patients with no subclones, 42 patients with defined subclones, and 9 patients with a CP. Interestingly, the impact on DFS of having a donor versus no donor was comparable in the 3 clonal heterogeneity groups (test for interaction $P = 0.28$) (Fig. 3). This remained true even after excluding the AML patients with favorable cytogenetic features from the analyses (test for interaction $P = 0.19$).

In multivariate analysis adjusted for age and cytogenetic risk group and stratified by protocol, clonal heterogeneity showed no significant association ($P = 0.41$) with the incidence of relapse after allo-HSCT (HR = 1.39, 95% CI 0.79–2.45, for subclones versus no subclones; HR = 1.57, 95% CI 0.54–4.51, for CP versus no subclones). In contrast, in this multivariate model, MRC cytogenetic risk group was, overall, strongly associated ($P < 0.001$) with the incidence of relapse

Table 3 Multivariate analyses

Parameter	Levels	Hazard ratio (95% CI)	*P* value
Relapse incidence[a]			
Clonal heterogeneity	No subclones	1.00	0.78
	Subclones	1.10 (0.84, 1.44)	
	Composite karyotype	0.99 (0.59, 1.67)	
Age (years)	15–45	1.00	0.19
	46–60	1.15 (0.94, 1.40)	
Cytogenetic risk group (MRC)	Favorable	1.00	< 0.001
	Intermediate	2.35 (1.81, 3.05)	
	Adverse	4.84 (3.67, 6.38)	
Donor	No	1.00	< 0.001
	Yes	0.53 (0.42, 0.67)	
DFS from CR/CRi[b]			
Clonal heterogeneity	No subclones	1.00	0.62
	Subclones	0.94 (0.74, 1.20)	
	Composite karyotype	1.19 (0.78, 1.80)	
Age (years)	15–45	1.00	0.004
	46–60	1.30 (1.09, 1.54)	
Cytogenetic risk group (MRC)	Favorable	1.00	< 0.001
	Intermediate	2.16 (1.73, 2.70)	
	Adverse	4.13 (3.25, 5.25)	
Donor	No	1.00	< 0.001
	Yes	0.71 (0.59, 0.85)	
Overall survival[b]			
Clonal heterogeneity	No subclones	1.00	0.58
	Subclones	1.10 (0.91, 1.32)	
	Composite karyotype	0.96 (0.67, 1.38)	
Age (years)	15–45	1.00	< 0.001
	46–60	1.42 (1.23, 1.63)	
Cytogenetic risk group (MRC)	Favorable	1.00	< 0.001
	Intermediate	2.30 (1.89, 2.80)	
	Adverse	3.78 (3.09, 4.63)	
Disease	De novo AML	1.00	0.11
	sAML	1.28 (0.95, 1.73)	
WHO performance status	0	1.00	< 0.001
	1	1.13 (0.97, 1.32)	
	2–4	1.66 (1.35, 2.03)	

CI confidence interval, *CR* complete remission, *CRi* complete remission with incomplete blood recovery

[a] Fine and Gray model including all covariates presented in the table and stratified by protocol was used

[b] A Cox model including all covariates presented in the table and stratified by protocol was used

after allo-HSCT (HR = 1.61, 95% CI 0.68–3.80 for intermediate versus favorable risk group; HR = 6.20, 95% CI 2.86–13.43 for adverse versus favorable risk group).

Discussion

In a large study by the SAL group, the presence of cytogenetic clonal heterogeneity, as detected by metaphase karyotyping, has been shown to be a frequent phenomenon and to be associated with poor prognosis among cytogenetically non-favorable risk group AML patients [23]. That study also observed that, adjusting for other known prognostic factors (including age, disease type (de novo AML versus antecedent MDS versus t-AML), and cytogenetic risk group (intermediate versus adverse risk)), the presence of CP (HR = 1.70, 95% CI 1.18–2.43 for CP) but not defined subclones (HR = 1.06, 95% CI 0.74–1.53) was associated with shorter OS compared

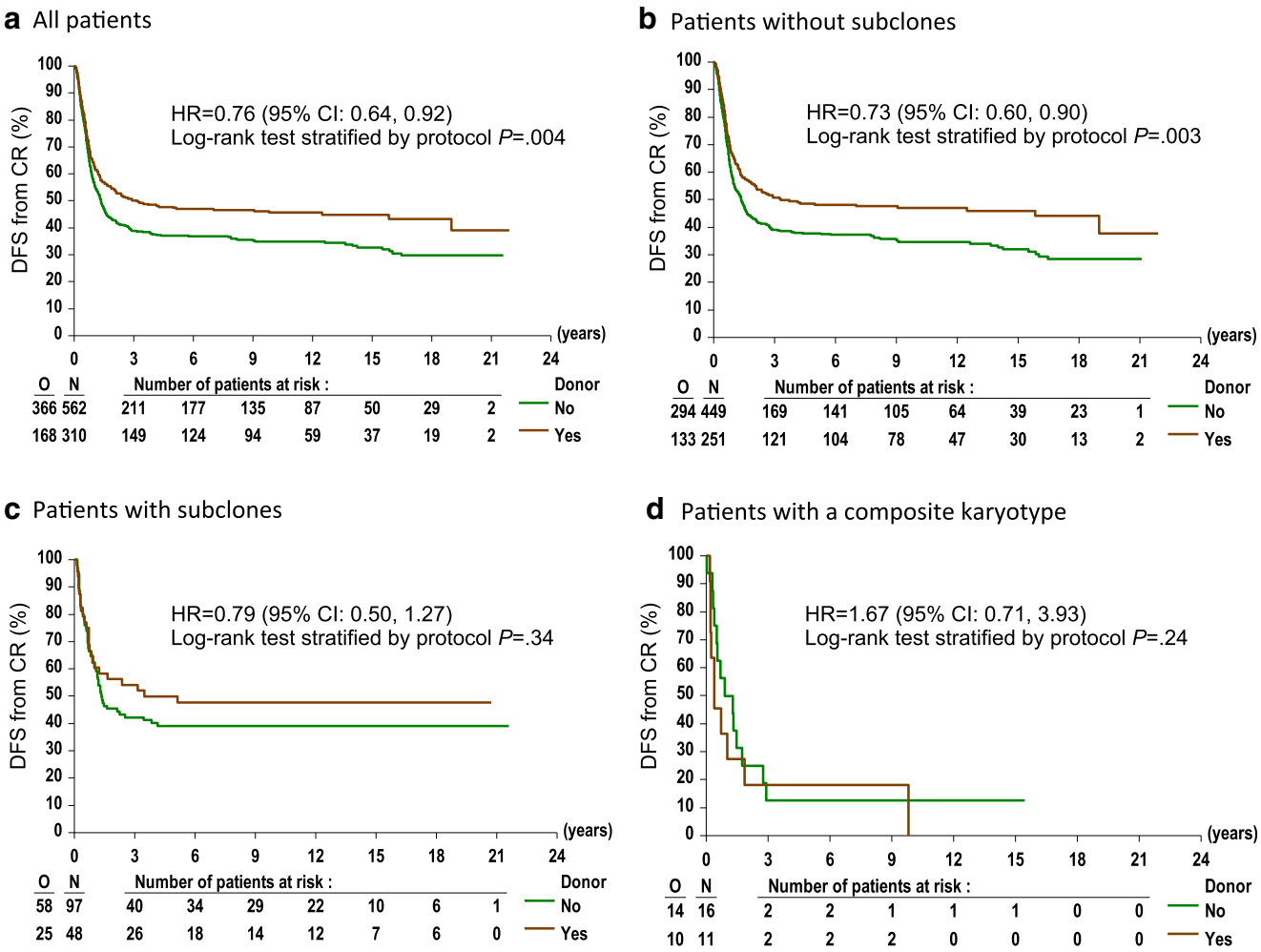

Fig. 3 Disease-free survival (DFS) from complete remission (CR) in patients with or without a donor in all patients with an abnormal karyotype (**a**), in those without (**b**) or with (**c**) defined subclones, and in those with a composite karyotype (**d**)

to detecting no subclones. The study also reported that subclone formation was of greatest importance among patients with adverse risk karyotypes [23]. Here, we reanalyzed the impact of cytogenetic clonal heterogeneity on outcomes in an independent large sample of younger AML patients with an abnormal karyotype. Further, we assessed how clonal heterogeneity modified the effects of the type of remission-induction chemotherapy and of having a donor on AML outcomes. Several observations have been made.

The present study in 1291 younger AML patients with an abnormal karyotype evidenced cytogenetic clonal heterogeneity in 20.5% of the patients (17.5% of patients with defined subclones and 3.0% with CP). This percentage of patients with clonal heterogeneity is somewhat lower compared to what has been observed in the SAL study, where 32.8% (19.7% had defined subclones ($n = 252$) and 13.1% with CP ($n = 166$)) of patients with an abnormal karyotype had subclones [23]. An explanation may be that only patients ≤ 60 years of age were included in the present study. As also observed by the SAL group, a linear pattern prevailed in the majority of patients with one defined subclone [23]. However, in cases with two or three defined subclones, the branched pattern was more common (Fig. 1).

Patients with leukemia with defined subclones and particularly those with a CP had adverse cytogenetic features more often than those without subclones. Further, patients with a CP were older than patients with defined subclones and those without subclones. With regard to outcome, CP was associated with a shorter OS and DFS from CR. This was not the case for the presence of defined subclones. However, importantly, in contrast to what was observed by the SAL group [23], CP was not an independent prognostic factor in our study. The dismal outcome of patients with a CP was explained by the known predictors of poor prognosis including adverse risk cytogenetic features and age. The reasons of the discrepancies between the present and the SAL studies remain unclear.

We previously reported that in the EORTC/GIMEMA AML-10 trial, the type of anthracycline showed no impact

on OS or DFS from CR/CRi among all randomized patients [1]. Consistent with the previous analysis, in the present study including only patients with cytogenetic abnormality, the type of anthracycline did not show an effect on OS or DFS from CR. This lack of a treatment difference was not impacted by the clonal heterogeneity status.

In the EORTC/GIMEMA AML-12 trial, we demonstrated better OS in patients younger than 46 years of age having received HiDAC in comparison to those having received SDAC in induction treatment [2]. This was not observed in older patients, 46–60 years of age. In the current study, including only data for patients with cytogenetic abnormality at diagnosis, the estimate of treatment effect was similar, as in the original trial analysis. Furthermore, as in AML-10, this lack of treatment difference was not impacted by the clonal heterogeneity status.

Previous studies have demonstrated better DFS from CR/CRi in AML patients with a donor in comparison to those without a donor [12, 36, 37]. This is particularly the case for younger patients and those with intermediate or adverse risk cytogenetic features [12, 36]. In the current study, we confirmed a significantly longer DFS from CR/CRi (due to lower risk of relapse) in patients with a donor in comparison to those without. Interestingly, the impact of having a donor on the outcome was quite consistent according to different clonal heterogeneity status. Furthermore, adjusting for cytogenetic risk group, clonal heterogeneity status had no impact on the incidence of relapse after allo-HSCT. These data are different from those of the SAL group [23], who demonstrated that allo-HSCT was able to overcome the adverse prognosis in AML patients with subclones, but not in patients with abnormal karyotypes without subclones. However, it should be emphasized than only 9 of 39 CP patients received an allo-HSCT in the current study (somewhat limiting our ability to detect a possible benefit of allo-HSCT in this group of patients).

There are some limitations in our study including the relatively small number of patients with a composite karyotype ($n = 39$) and the fact that clonality was defined on the basis of cytogenetic data only (and not also by molecular methods).

Conclusions

In conclusion, in the present study, clonal heterogeneity as defined by the presence of subclones as compared to cytogenetic abnormalities without clonal subclones did not show an effect on patient's outcomes. The dismal outcome in the patients with a CP was explained by the known predictors of poor prognosis including adverse-risk cytogenetic features and age.

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Authors' contributions *Conception and design*: All authors.

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Collection and assembly of data: All authors.

Data analysis and interpretation: All authors.

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Compliance with ethical standards

Ethics approval and consent to participate The current retrospective analysis includes only data from patients included in phase III multicenter prospective trials (either the EORTC/GIMEMA AML-10 or the EORTC/GIMEMA AML-12). Both prospective phase III trials were approved by the internal review boards of EORTC and GIMEMA and the ethical committee of each participating institution, and were conducted in accordance with the Declaration of Helsinki. All patients signed the respective informed consent form.

Consent for publication Not applicable.

Competing interests Petra Muus has consulting or advisory role for Alexion, Opsona, Akari, and Ra Pharma and has a speaker's bureau at Alexion. Xavier Thomas has consulting or advisory role for Celgene, Pfizer, Amgen, and Sunesis. Adriano Venditti has consulting or advisory role for Sandoz, Novartis, Janssen, and Jazz Pharmaceuticals and has received research grant from Sandoz. Heiko Becker has received honoraria from Bristol-Myers Squibb. Sergio Amadori has consulting or advisory role for Novartis, Celgene, and Abbvie. The other authors have nothing to disclose with respect to this manuscript.

Abbreviations Allo-HSCT, Allogeneic stem cell transplantation; AML, Acute myeloid leukemia; CR, Complete response; CRi, CR with incomplete counts recovery; CP, Composite karyotypes; DFS, Disease-free survival; IDA, Idarubicin; DNR, Daunorubicin; HiDAC, High-dose cytarabine; HR, Hazard ratio; MDS, Myelodysplastic syndrome; MRC, UK Medical Research Council; MK, Monosomal karyotype; MTZ, Mitoxantrone; OS, Overall survival; PR, Partial response; SAL, Study Alliance Leukemia group; SDAC, Standard-dose cytarabine; tAML, Therapy-related AML; WBC, White blood cell

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