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ARTICLE

Acute myeloid leukemia

Chromothripsis in acute myeloid leukemia: biological features and impact on survival

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Abstract

Chromothripsis is a one-step genome-shattering catastrophe resulting from disruption of one or few chromosomes in multiple fragments and consequent random rejoining and repair. This study defines incidence of chromothripsis in 395 newly diagnosed adult acute myeloid leukemia (AML) patients from three institutions, its impact on survival and its genomic background. SNP 6.0 or CytoscanHD Array (Affymetrix®) were performed on all samples. We detected chromothripsis with a custom algorithm in 26/395 patients. Patients harboring chromothripsis had higher age ($p = 0.002$), ELN high risk (HR) ($p < 0.001$), lower white blood cell (WBC) count ($p = 0.040$), *TP53* loss, and/or mutations ($p < 0.001$) while *FLT3* ($p = 0.025$), and *NPM1* ($p = 0.032$) mutations were mutually exclusive with chromothripsis. Chromothripsis-positive patients showed a worse overall survival (OS) ($p < 0.001$) compared with HR patients ($p = 0.011$) and a poor prognosis in a COX-HR optimal regression model. Chromothripsis presented the hallmarks of chromosome instability [i.e., *TP53* alteration, 5q deletion, higher mean of copy number alteration (CNA), complex karyotype, alterations in DNA repair, and cell cycle] and focal deletions on chromosomes 4, 7, 12, 16, and 17. CBA. FISH showed that chromothripsis is associated with marker, derivative, and ring chromosomes. In conclusion, chromothripsis frequently occurs in AML (6.6%) and influences patient prognosis and disease biology.

Introduction

Loss of chromosomes and complex karyotype are mechanisms of genomic instability known to be linked to therapy resistance, poor prognosis, and early relapse in AML [1–3]. Nowadays, new high-throughput technologies can discover new alterations responsible for the poor prognosis in subcohorts of AML patients and may reveal

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druggable pathways. There is an urgent need to define genomic phenotypes in AML in a therapeutic perspective.

Chromothripsis is a one-step genomic catastrophe involving one or few chromosomes resulting from chromosome breakage and random DNA rejoining [4]. It has been detected, mainly as occasional finding, in solid tumors [5–13] and hematological malignancies (multiple myeloma [14], AML [15, 16], acute lymphoblastic leukemia [17, 18], and chronic lymphocytic leukemia [19]), as well as in germline cases of congenital disorders [20]. A study on 22,000 cases of primary tumors highlighted an overall incidence of chromothripsis between 2 and 3% [21].

During mitosis, chromothripsis arises from aberrant DNA replication timing. Prolonged arrest of cell cycle and micronuclei formation influence the spatial distribution of damages favoring the acquisition of structural rearrangements [22]. Molecular mechanisms implicated in this event are only partially discovered. Chaotic genomes seemed to form through random non-homologous end joining after DNA damages [23]. An alternative theory presented chromothripsis as a putative incomplete outcome of chromosome fragmentation, the initial event triggering a new form of mitotic cell death [23]. Moreover, recent evidence indicates that chromothripsis preferentially occurs especially in hyperploid cells [24] and in cells with damaged telomeres [25]. Chromothripsis has been associated to *EGFR*, *MDM2*, and *MDM4* amplification, *CDKN2A* and *PTEN* deletion [8], aberrant DNA double-strand break (DSB) response [26], *TP53* mutations [9, 15], complex karyotype [9, 15], and *ATM* mutation [18].

At present, a standardized way to detect chromothripsis does not exist, but few have been proposed. Chromothripsis has been identified with fluorescent in situ hybridization (FISH) [9, 15, 16, 26], or, alternatively, by operator-dependent analyses [14] as well as algorithm-based detection of shattering patterns on SNP array data and eventual integration with DNA sequencing data [21, 27]. Korbel and Campbell defined six different criteria to distinguish chromothripsis from a multi-step process of genomic rearrangement [28]. When analyzed by SNP arrays, chromosomes harboring chromothripsis show a characteristic pattern of alterations: two or three switches of CN state detectable along the chromosome with a clustering of breakpoint locations [29]. These patterns are associated with a high number of chromosomal rearrangements with widespread loss or gain of sequence fragments interspersed in diploid regions [21].

Chromothripsis has been associated with highly aggressive disease in various tumors [8–14] but it did not appear to impact prognosis in prostate cancer [30] and in estrogen receptor-negative breast cancer [12].

Our study describes the incidence of chromothripsis at diagnosis in a large and homogenous AML cohort, its impact on prognosis, and the genetic features associated with this phenomenon.

Subjects and methods

Patients

Samples and data at diagnosis from 395 adult patients affected by de novo or secondary AML according to WHO 2016 criteria [31] were collected from three institutions. The study was approved by the local ethical committees, written permission, and informed consent were obtained from all patients before sample collection according to Helsinki declaration of "Ethical Principles for Medical Research Involving Human Subjects" [32].

Data were collected and managed through custom Electronic Case Report Forms using REDCap electronic data capture tool [33].

SNP microarray analysis

DNA samples were processed by Affymetrix® (Santa Clara, CA, USA) genome-wide human SNP 6.0 ($n = 321$) and CytoscanHD Array ($n = 84$) according to the manufacturer's instructions. Of the 114 SNP 6.0 AML cases obtained from GSE23452, 112 were paired samples, including buccal swab DNA or bone marrow at remission [34]. Array data have been deposited in the NGS-PTL repository (http://www.ngs-ptl.com/documents/documents/3-10-en/media.aspx).

Detection of chromothripsis

Chromothripsis was defined according to Korbel and Campbell's criteria [28], when three out of six criteria were satisfied (the remaining criteria could not be assessed by SNP array analysis).

Chromothripsis was assessed by scanning SNP array segment files using *CTLP*-Scanner (using R v3.3.2 [35] and "*CTLP* scanner" package [21]). The following thresholds were set: Log ratio ≥8, more than 10 breakpoints, minimum segment size of 10 kb, and 0.3 as signal distance between adjacent segments. Events with a prevalent copy number (CN) status and changes involving ≤10% of detected region were excluded.

Microarray statistical analyses

Based on the presence/absence of chromothripsis, AML samples were divided in two groups: a group of cases (chromothripsis-positive) and a group of controls (chromothripsis-negative) and enrichment of CNA events

between the two groups was examined. The data set was stratified for event type and the statistical tests were performed on amplifications of one or more DNA copies and heterozygous or homozygous deletions. In each patient, multiple events of the same type in the same gene were considered as one. Fisher's exact test was used to compare frequencies in genes' event between two groups. All p values were adjusted for multiple testing. For testing at a pathway level, genes were annotated in the Reactome database [36]. Pathway enrichment analysis was performed at patient level by means of an over-representation test. Then, the adjusted p values obtained for a certain pathway across all patients were used as predictor variable in a logistic regression model. The significance level was set at 10^{-4}.

Chromosome banding analysis and FISH

FISH analysis was carried out on previously G-banded metaphases prepared by chromosome banding analysis (CBA) technique according to the manufacturer's instructions.

Clinical statistical analysis

Due to the data-pool feature of our set, missing data will be detailed in the result section. OS was assessed as the time in days from diagnosis to death or last follow-up.

Fisher's exact test and chi-squared test were used for crosstabs and difference between distributions was assessed with median test for independent samples and Mann–Whitney U test. Survival analysis and COX-HR were used as appropriate.

Results

Clinical and molecular patient characteristics

All patients' clinical and molecular characteristics are listed in Table 1, missing data are quantified in Table S1. In our cohort, 26 out of 395 patients (6.6%) showed chromothripsis involving different chromosomes.

Correlation of chromothripsis with clinical and molecular parameters in AML patients

We compared chromothripsis-negative patients with chromothripsis-positive ones (Table 2). Chromothripsis-positive patients were older. They had a higher median age (67 and 60 years, respectively $p = 0.002$, Figure S1C) and a lower WBC count mean at diagnosis (6342/mm^3 vs. 30,059/mm^3, respectively $p = 0.040$, Figure S1A). Chromothripsis-positive patients presented a prevalence of complex karyotype and were classified as HR disease according to ELN [37] definition ($p < 0.001$, Figure S1D).

Based on genetic features, chromothripsis was associated with *TP53* loss ($p < 0.001$) and *TP53* mutations ($p < 0.001$).

Table 1 Clinical and molecular characteristics of the patients enrolled in this study ($N = 395$)

Parameter	Value
Age	Median 59.35 (range, 16–92)
Sex	185/395 patients were female (46.8%)
	210/395 patients were male (53.2%)
De novo AML	307/372 patients (82.5%)
AML secondary to myelodysplastic syndrome	43/372 patients (11.6%)
AML secondary to other myeloid neoplasms	4/372 patients (1.1%)
Therapy-related AML	18/372 patients (4.8%)
WBC (mean)	28.140/mm^3 (100–171.00/mm^3)
WBC count greater than 30,000/mm^3	46/154 patients (29.9%)
WBC count greater than 100,000/mm^3	12/154 patients (7.8%)
European LeukemiaNet (ELN) low risk [37]	35/352 patients (8.9%)
European LeukemiaNet (ELN) intermediate 1 risk [37]	100/352 patients (28.4%)
European LeukemiaNet (ELN) intermediate 2 risk [37]	80/352 patients (22.7%)
European LeukemiaNet (ELN) high risk [37]	137/352 patients (38.9%)
Chemotherapy in induction	251/308 patients (81.5%)
Gemtuzumab Ozagomicin was added	42/251 patients (16.7%)
Complete remission	153/251 patients (60.9%)
Hematopoietic stem cell transplant	85/283 patients (30.0%)
Loss or a mutation of TP53 at diagnosis	63/395 patients (15.9%)
Loss	29/395 patients (7.3%)
Mutation	53/324 patients (16.4%)
Internal tandem duplication in *FLT3*	42/298 patients (14.1%)
Tyrosine kinase domain mutation in *FLT3*	18/298 patients (6.0%)
Mutations in *NPM1* gene	50/286 patients (17.5%)
Mutations in *IDH1* gene	4/121 patients (3.3%)
Mutations in *IDH2* gene	11/135 patients (8.3%)
Mutations in *DNMT3A* gene	7/38 patients (19.4%)
Mutations in *CEBPA* gene	5/106 patients (4.7%)
Mutations in *RUNX1* gene	11/87 patients (12.6%)
Mutations in *CBL* gene	2/91 patients (2.2%)
Mutations in NRAS gene	10/95 patients (10.5%)

Table 2 Correlation of chromothripsis with clinical data in 395 AML patients

Parameters	Patient with chromothripsis	Patient without chromothripsis	Test	P	Significance
Age at diagnosis (median, years)	67 (range, 58–85)	60 (range, 16–92)	Median test	0.002	**
WBC count at diagnosis (mean)	6342/mm^3 (range, 1420–26,700)	30.059/mm^3 (range, 100–171.000)	Mann– Whitney *U*	0.040	*
ELN risk [47]	26/26 patients (100%) HR	35/326 patients (10.7%) LR; 100/326 patients (31.2%) INT-1; 20/326 patients (24.5%) INT-2; 111/326 patients (34.0%) HR	χ^2	<0.001	***
Median absolute pairwise difference (MAPD)	0.19	0.28	Median test	0.270	ns
WT1/10.000 *ABL* (median)	8.400 (range, 20–120.111)	9.200 (range, 6–140.131)	Median test	1.000	ns
FLT3 status	0/21 patients (0%) with *FLT3* alterations	43/277 patients (15.5%) *FLT3* ITD; 18/277 patients (6.5%) *FLT3* TKD	χ^2	0.025	*
TP53 loss	9/26 patients (34.5%)	25/344 patients (6.8%)	Fisher's exact test	<0.001	***
TP53 mut	22/24 patients (84.6 %)	31/300 patients (10.3%)	Fisher's exact test	<0.001	***
Secondary AML (every neoplasm)	6/23 patients (26.1%)	59/349 patients (16.9%)	Fisher's exact test	0.196	ns
NPM1 mut	0/21 patients (0%)	50/263 patients (19%)	Fisher's exact test	0.032	*
IDH1 mut	0/7 patients (0%)	4/109 patients (3.5%)	Fisher's exact test	0.784	ns
IDH2 mut	0/9 patients (0%)	10/125 patients (8.0%)	Fisher's exact test	0.486	ns
DNMT3A mut	1/5 patients (20%)	6/31 patients (19.4%)	Fisher's exact test	0.685	ns
CEBPA mut	0/4 patients (0%)	5/102 patients (1.4%)	Fisher's exact test	0.822	ns
RUNX1 mut	1/3 patients (33.3%)	10/84 patients (11.9%)	Fisher's exact test	0.337	ns
NRAS mut	0/6 patients (0%)	10/89 patients (11.2%)	Fisher's exact test	0.503	ns

mut mutated, *TKD* tyrosine kinase domain, *ITD* internal tandem duplication

Table 3 Induction therapies in patients with and without chromothripsis

Induction therapy	Chromothripsis	
	No (288)	Yes (20)
Chemotherapy—n.	241	10
Hypomethylating agents—n.	23	4
Best supportive therapy—n.	24	6

Only 3/26 patients did not harbor any *TP53* alteration: one patient was *TP53* wild-type and have no karyotype aberration involving 17p, two patients were not tested for *TP53* mutation because of unavailable material at diagnosis. Chromothripsis was mutually exclusive with *FLT3* (Figure S1B, $p = 0.025$) and *NPM1* mutations ($p = 0.032$).

Chromothripsis defined a group of AML patients with poor response to induction therapy

At diagnosis, patients received chemotherapy, hypomethylating agents, or best supportive therapy (Table 3). Patients with chromothripsis were treated with intensive chemotherapy in a smaller proportion ($p = 0.003$). There was no difference in use of Gemtuzumab Ozagomicin during induction between the two group of patients ($p = 1.000$) and there was a similar transplant rate between the two groups [21% of patients with chromothripsis and 31% of patients without chromothripsis received hematopoietic stem cell transplant (HSCT), $p = 0.448$].

Chromothripsis defined a group of patients with poor prognosis. Three out of 10 patients with chromothripsis (30%) responded to induction, a significant lower proportion if compared with 152/229 patients without chromothripsis (66.4%, $p = 0.036$).

Chromothripsis defined a group of AML patients with poor OS

Patients with chromothripsis showed a worse OS (median OS of 120 days compared to 494 days for patients without chromothripsis, $p < 0.001$, Fig. 1a). In patients with available HSCT data, we confirmed the difference by censoring OS at HSCT with a median OS of 120 and 400 days in the two groups ($p < 0.001$, Fig. 1b). Patients with chromothripsis had the worst prognosis among patients with HR features according ELN [37] risk stratification (median OS of 120 and 258 days in the two groups, $p = 0.011$, Fig. 1c). This observation was confirmed when censoring OS at HSCT (median OS of 120 and 211 days in the two groups, $p = 0.022$, Fig. 1d). Moreover, the impact of chromothripsis on OS was evaluated in patients with HR features according ELN [37] risk stratification, who received induction chemotherapy. We report a difference in survival between patients with and without chromothripsis (median OS of 120 and 295 days, respectively, $p = 0.040$, Fig. 1e) and a trend toward statistical significance in HSCT-censored analysis (median OS of 120 and 242 days, respectively, $p = 0.055$, Fig. 1f). Patients with chromothripsis did not show differences in baseline characteristics or in survival compared to patients harboring *TP53* mutation, *TP53* loss, or to the group of patients harboring *TP53* alteration (loss and/or mutation), due to the high co-occurrence of these two phenomena (Figure S2 and Table S2). However, in our set, patients with chromothripsis showed a survival disadvantage near to the statistical significance threshold when compared with patients with *TP53* loss (Fig. S2B, $p = 0.049$).

Chromothripsis was an independent predictor of shorter OS in COX-HR model

We built a prognostic model using COX-HR with forward conditional method, considering chromothripsis event, secondary AML, ELN risk, induction therapy, *FLT3* and *NPM1* mutation as categorical variables and age at diagnosis. *TP53* status was not included in the model for the high co-occurrence of chromothripsis and *TP53* alterations. In the optimal model, chromothripsis (HR: 2.286, 95% CI: 1.327–3.940, $p = 0.003$) and secondary disease was associated with augmented risk of death, while ELN low risk, intermediate 1 and intermediate 2 risk were associated with better outcome (Fig. 2). Chromothripsis was a consistent risk factor in COX-HR model built in ELN HR population considering chromothripsis, secondary AML, induction therapy, *FLT3* mutation, and *NPM1* mutation as categorical variables, and age of diagnosis (HR: 2.070, 95% CI: 1.167–3.672, $p = 0.013$, model not shown). Chromothripsis was also a consistent risk factor in COX-HR model built in ELN HR population treated with intensive therapies considering chromothripsis, secondary AML, *FLT3* mutation, and *NPM1* mutation as categorical variables, and age of diagnosis (HR: 2.227, 95% CI: 1.022–4.850, $p = 0.044$, model not shown).

Genomic characteristics of AML patients with chromothripsis

We detected chromothripsis on chromosome 12 (23% of events), 17 and 5 (17% of events both), chromosomes 6 (10% of events), 3 and 8 (6.6% of events both), 7, 10, 11, 15, and 20 (3.3% of events each) (Fig. 3, and Fig. S3). SNP array analyses showed that chromothripsis-positive patients were characterized by a high grade of genomic aberrations.

We found a minimal common-deleted region in 24/26 patients with chromothripsis (5q31.1–5q33.1). Among

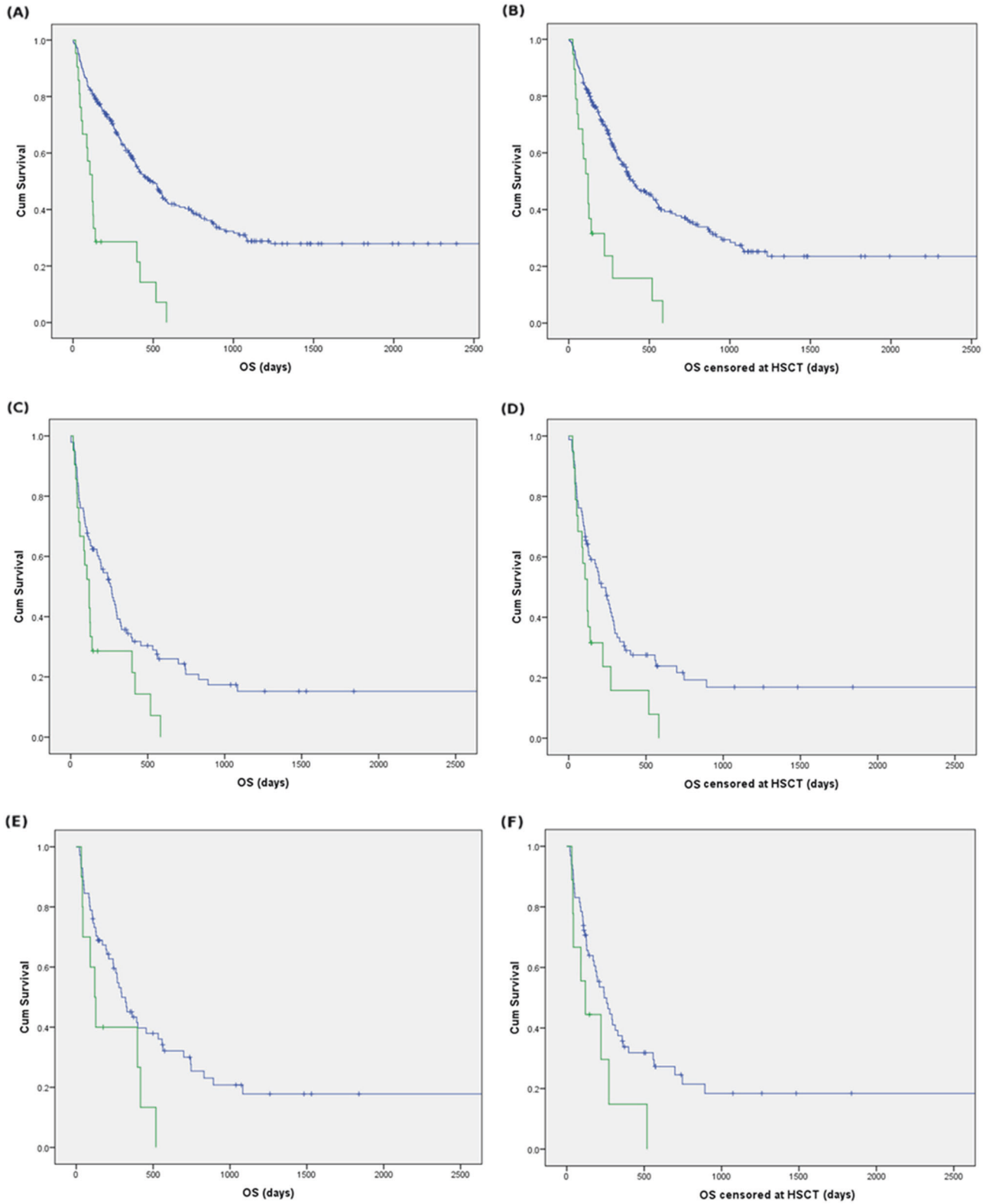

Fig. 1 Association between chromothripsis and OS in AML. OS in patients with (green line) and without chromothripsis (blue line): **a** overall cohort; **b** survival censored at HSCT; **c** patients with ELN [37] HR features; **d** patients with ELN [37] HR features censoring survival at HSCT; **e** patients treated with intensive chemotherapy with ELN 2017h features; **f** patients treated with intensive chemotherapy with ELN [37] HR features censoring survival at HSCT

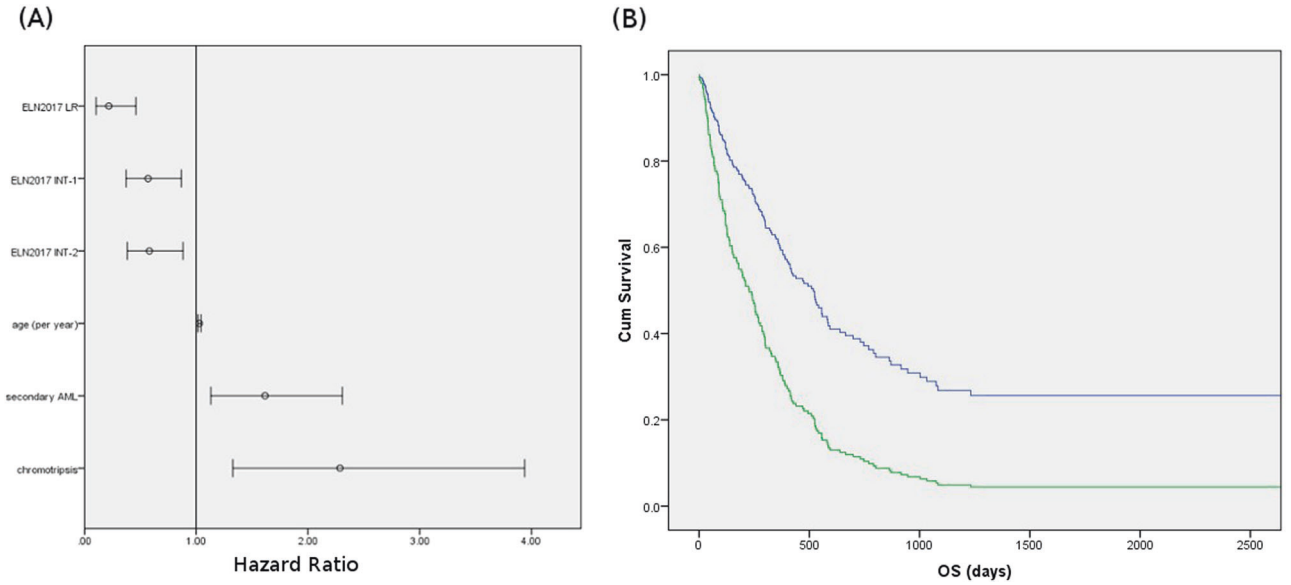

Fig. 2 COX-HR model. COX-HR model in patients' set considering (**a**) forest plot of risk factors in COX-HR optimal model (**b**) in COX-HR model, difference in OS (OS) representing population with (green line) and without (blue line) chromothripsis

chromothripsis-negative patients, 51/369 harbored at least a CNA of 9 Mb in the 5q arm. There was a higher incidence in macroscopic deletions on 5q in patients with chromothripsis ($p < 0.0001$).

Patients with chromothripsis presented higher mean of CNA than patients without chromothripsis (mean of 418 vs. 188 CNA per patient; Fig. 4, CIRCOS [38] external level).

Significative genomic alterations mapped in relatively small chromosomic regions

Fisher's exact test showed that a large group of 1359 genes were significantly altered in deletion (both heterozygous and homozygous) in chromothripsis-positive patients rather than chromothripsis-negative ones (data not shown). These genes map on chromosome 5q, 3q, 12p, 3p, 4q, 7q, 12p, 16q, and 17p. Considering chromosome position of genes associated with chromothripsis, we found that CNA randomly affected the entire 5q and whole chromosome 3. In the other chromosomes, we found that statistically significant CNA mapped in relatively small regions (complete list of genes in Fig. 4, CIRCOS [38] internal level). These regions included deletions of genes involved in Atlas of Genetics and Cytogenetics in Oncology and Haematology, in particular on chromosome 4q28–32 (*SFRP2*), 7q31.1–36.3 (*CAV1, EPHA1*, and *NRF1*), 12p11.21–13.3 (*EPS8*, *RECQL*, and *GUCY2C)*, 16q22–24.3 (transcription factors *CBFA2T3* and *FOXF1*; *CDT1* involved in DNA replication; and the Fanconi Anemia gene *FANCA*), and 17p13–13.1 (*ALOX12* and *CLDN7*) (Fig. 4). Genes were filtered as described in "Methods" and we showed that 95 genes were associated with chromothripsis (complete list of genes in Fig. 4, CIRCOS [38] internal level).

Pathway enrichment in patients with chromothripsis

REACTOME enrichment of pathways is reported in Tables S3 and S4. DNA repair, E2F-mediated regulation of DNA replication, signaling pathways involving PI3K, phospholipid biosynthesis and metabolism, and various growth factors signaling pathways scored in the best 1% of pathways enriched for amplification events in chromothripsis. CTLA4 inhibitory signaling, synthesis of phosphatidylinositol phosphate (PIP) at the late endosome membrane, fanconi anemia pathway, genes regulating G0 and early G1 phase, pre-NOTCH transcription and translation scored in the best 1% of pathways enriched for deletion events in cases with chromothripsis.

In the subcohort of patients with *TP53* alteration, when we compared patients with chromothripsis ($n = 22$) and patients without chromothripsis ($n = 44$), we did not detect any significant difference in terms of differentially altered genes and pathways (with a significance threshold of $p < 10^{-4}$).

Chromothripsis is associated with marker, derivative, and ring chromosomes formation

In order to better characterize the chromosomes affected by chromothripsis, we performed FISH analysis in 7/26 cases with available material. Most cases (5/7) showed the

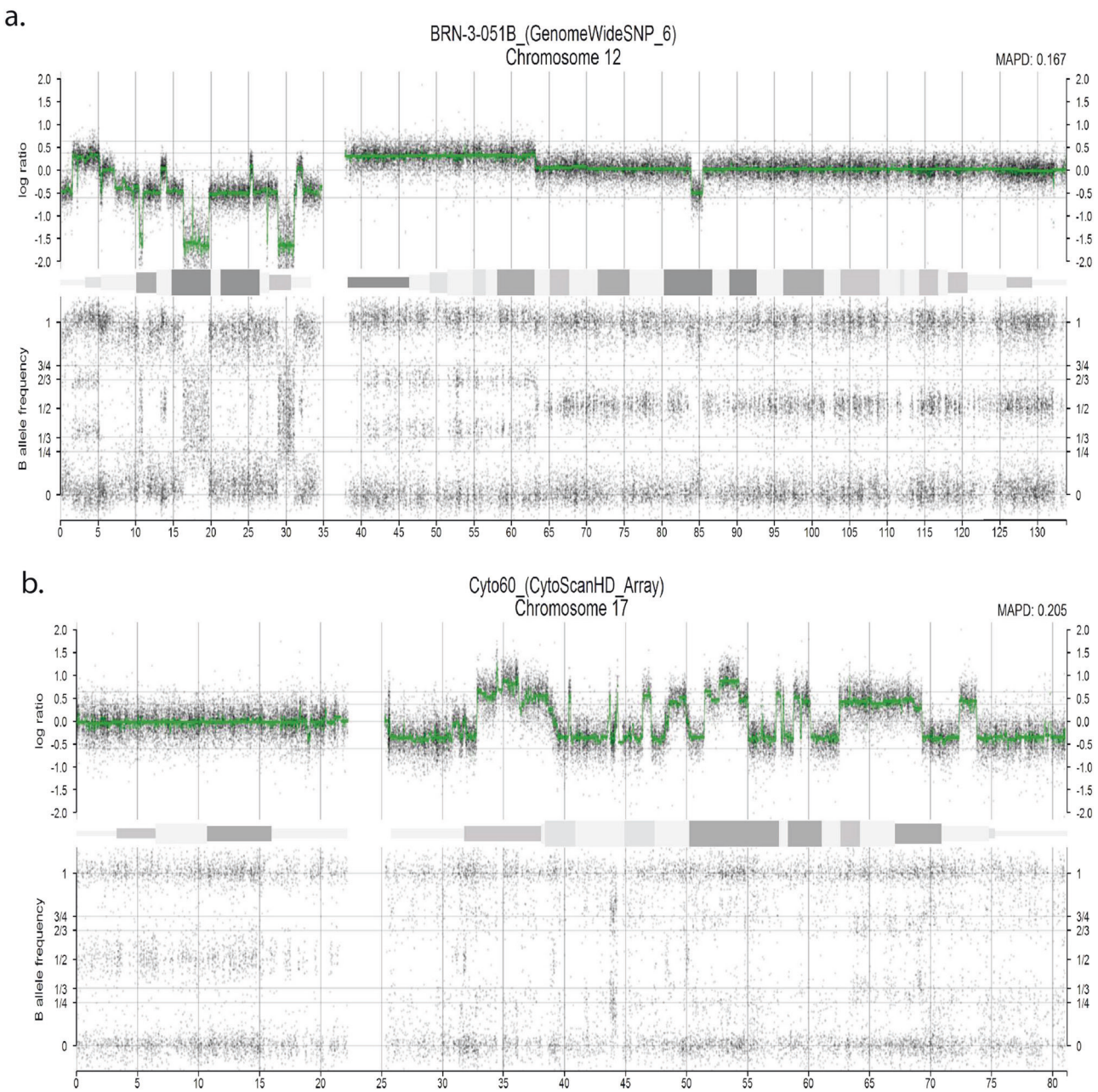

Fig. 3 Representation of two chromosomes affected by chromothripsis in different patients, plotted with Rawcopy v. 1.0. **a** Chromosome 17q with 36 switches and 2–3 changes in CN (involving also homozygous gains). **b** Chromosome 12p with 28 switches and 2–3 changes in CN (involving also homozygous losses)

presence of marker or ring chromosomes. In four cases, the chromosomes affected by chromothripsis (chromosomes 8, 11, 12, and 17) were reported by CBA as monosomic chromosomes, while, by FISH, portions of these chromosomes were identified on marker chromosomes. In cases involving chromosomes 8 and 11, the rearrangement led to amplification of MYC and *KMT2A* (MLL) genes, respectively. In cases involving chromosomes 12 and 17, parts of the chromosomes affected by chromothripsis were identified on markers and on derivative chromosomes resulting from unbalanced translocations. In other two cases, the chromosome involved in chromothripsis was annotated as derivative chromosome. Moreover, FISH highlighted the presence of a homogeneously staining region on the derivative chromosome 3 due to MDS1 and EVI1 complex locus amplification in one case and of a complex translocation involving chromosome 5 in the other case. In the last patient, the chromosome affected by chromothripsis was identified as ring chromosome 17 leading to loss of *TP53*. FISH results are shown in Fig. 5.

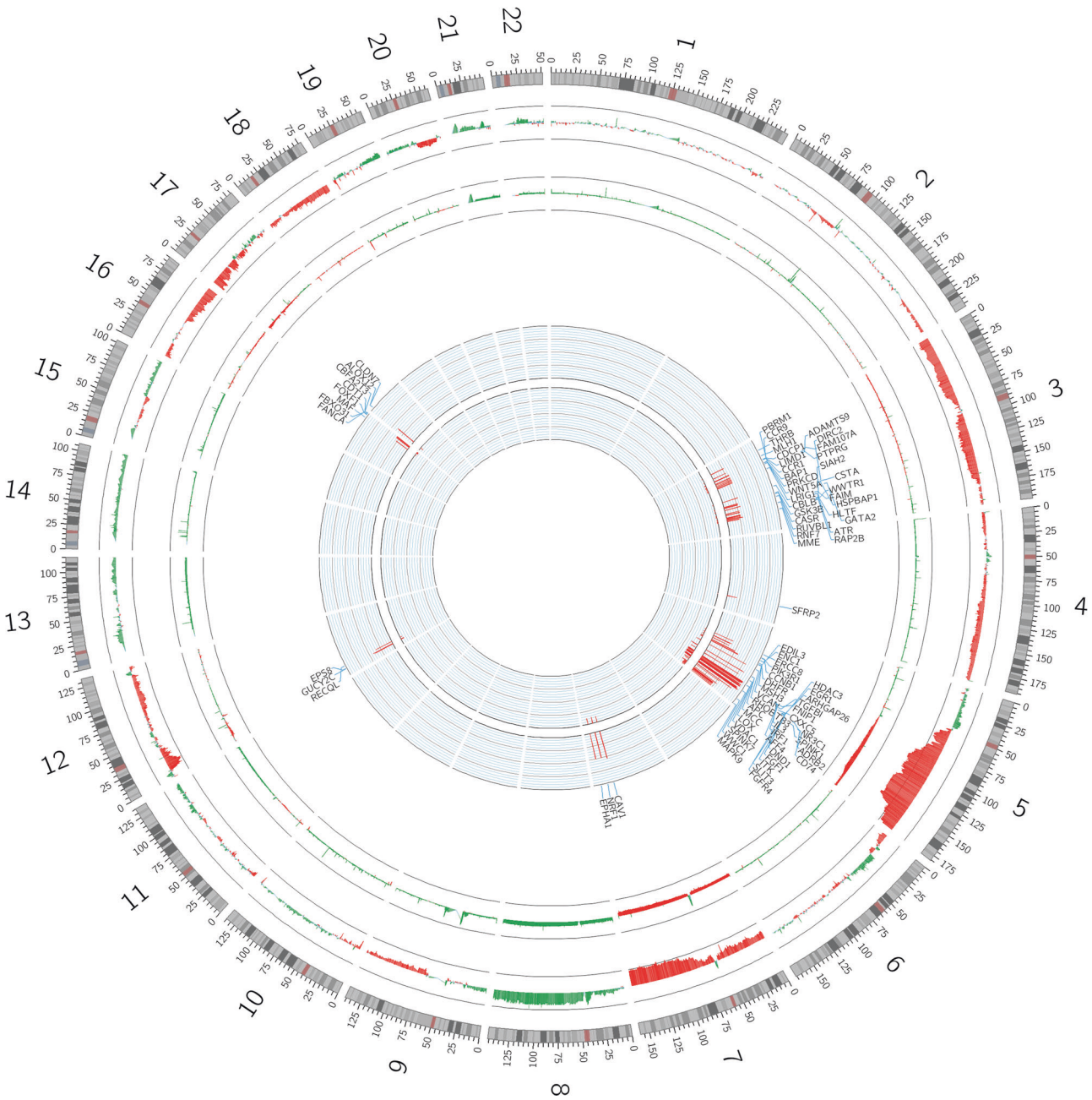

Fig. 4 CIRCOS plot representing the overall CNA and genes altered in the two groups (chromothripsis-positive patients vs. chromothripsis-negative patients). **a** External circular level: genomic landscape in CNA per group of patients (external line represents chromothripsis-positive patients, internal line represents chromothripsis-negative patients). Green lines represent amplifications, red lines represent deletions. **b** Internal list of selected genes (basing on Fisher's exact test and Atlas of Genetics and Cytogenetics in Oncology and Haematology) deleted in heterozygosis and/or homozygosis with relative frequency in CNA displayed with histograms: chromothripsis-positive patients (external level) and chromothripsis-negative patients (internal level)

Discussion

The purposes of this study was to define the incidence of chromothripsis in newly diagnosed adult AML patients, its impact on survival, and its genomic background in AML. To detect chromothripsis, we used a custom algorithm based on *CTLP* scanner [21] and Korbel and Campbell's criteria [28] that detected chromothripsis based on SNP array data.

Our results, obtained in a large set of patients, indicate that chromothripsis is a non-anecdotal finding in AML. The overall incidence of chromothripsis was concordant with studies conducted on fewer patients [15, 16]. Chromothripsis appeared to be associated with higher age and lower

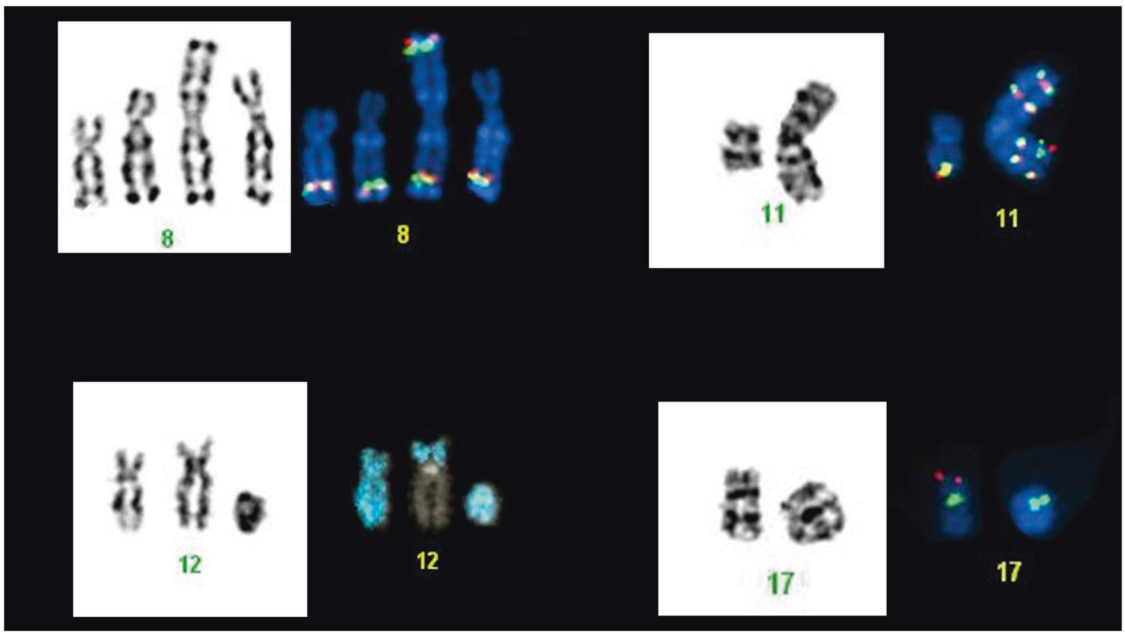

Fig. 5 Karyotype analysis in AML patients harboring chromothripsis. FISH analysis with **a** MYC breakapart probe showing five fusion signals of MYC localized on normal chromosome 8 and on three different marker chromosomes in patient with chromothripsis of chromosome 8. **b** *KMT2A* (MLL) breakapart probe showing four copies of *KMT2A* (four fusion signals) localized on the marker chromosome resulted from chromothripsis of chromosome 11. **c** With whole chromosome paint probe for chromosome 12 marked in blue revealing part of chromosome 12 on the marker chromosome and on the derivative chromosome 3 from translocation t (3;12). **d** Showing deletion of *TP53* gene (red signal) in the case with chromothripsis of chromosome 17 annotated as ring chromosome by CBA

WBC count at diagnosis, and mutually exclusive with *FLT3* and *NPM1* mutations, these data were never reported in acute leukemia. Moreover, we confirmed the strong association between chromothripsis and *TP53* dysregulation [9, 15], thus reinforcing the importance of *TP53* for the maintenance of genomic stability and integrity.

For the first time, we pointed out that the only detection of chromothripsis is sufficient to define a group of patients with poor prognosis in the general AML population and in the ELN [37] high-risk population; chromothripsis was a determinant of poor OS in the COX-HR optimal model. We further performed survival analysis in the subset of patients with *TP53* alterations, as *TP53* alone was reported to define patients with the worst prognosis in AML [39–41]; we did not detect differences in survival defined by to have or not to have chromothripsis, and this was the main limitation of our study. This may be explained by the low number of patients in this subset, the moderate number of patients with missing follow-up information, and by the slightly lower mean OS of patients with *TP53* alteration in our set when compared with literature data [40]. Furthermore, we cannot exclude that chromothripsis may be a phenotypical manifestation, or simply an epiphenomenon, of *TP53* alteration. Further tests are needed in patients' sets enriched to have *TP53* alteration or to have chromothripsis without *TP53* alteration.

Our work describes chromothripsis biological scenario based on SNP high-throughput genomic analyses; even with the limitations due to lack in availability of whole-genome sequencing or gene expression data in our patients' set, we found several events significantly associated with chromothripsis. Patients with chromothripsis presented a high genomic instability, highlighted by the high number of CNA per patient and a high recurrence of losses in chromosome 3 and 5q and high incidence of complex karyotype; this result is consistent with literature [9, 15, 18, 24, 25, 42–44].

Interestingly, we found in most patients with chromothripsis a minimal common-deleted region in 5q31.1–5q33.1, containing key genes involved in RAS, PI3K/AKT, transcriptional factors, DNA damage, histone modification, and SMAD signaling. Moreover, we found heterozygous and homozygous genes' deletions associated with chromothripsis that clustered in relatively small genomic regions. Within altered genes, we hypothesized that *FANCA* could be a candidate to cooperate at a multigenic and multi-step mechanism that initiate and maintain chromothripsis. *FANCA* deletion is found in a relatively small genome region; furthermore, it was found deleted in sporadic AML [45] and it originate a syndrome that predispose to AML. Significantly, when we performed pathway enrichment, we found DNA damage and fanconi anemia pathways scoring within the best 1%, together with

early G0–G1 regulation, cell cycle, and several other pathways that could be possibly related genesis and maintenance of chromothripsis. Tumors characterized by genetic instability and by alterations in DNA damage pathway could be the ideal target of innovative therapeutic approaches like checkpoints inhibitors [46], and combination therapies based on these agents could be an option in chromothripsis patient for patients with poor prognosis. Furthermore, our findings characterize a subset of AML patients with a high burden of alterations and potentially neoantigens that could be the optimal candidates for novel therapies like PD1/PDL1 blocking monoclonal antibodies and immune modulating drugs, maybe in combinations with hypomethylating agents.

Compared to the subset of patients with *TP53* alteration, we did not find significant pathways and genes. This may be due to the low number of patients in this subset, and it requires further studies in enriched population with multi-omics approach.

Finally, although only a subset of cases could be analyzed by CBA and FISH, our results showed that chromothripsis was associated with marker, derivative, and ring chromosomes, suggesting that these complex chromosomal rearrangements can arise from chromothripsis. This finding is in accordance with what Bochtler and colleagues previously reported [15].

In conclusion, chromothripsis is clearly a catastrophic event defining a consistent group of patients with poor prognosis, which could be candidate per se to novel approaches; chromothripsis is associated with losses of 5q31.1–5q33.1 in most patients, and with a complex genomic background in which *FANCA, TP53*, and genes regulating cell cycle seem to be fundamental and demand further preclinical studies.

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Compliance with ethical standards

Conflict of interest GM: ARIAD/INCYTE, Pfizer, Celgene, Amgen, J&J, and Roche as consultant. The remaining authors declare that they have no conflict of interest.

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