Chromothripsis is linked to *TP53* alteration, cell cycle impairment, and dismal outcome in acute myeloid leukemia with complex karyotype

The single catastrophic event chromothripsis, initially uncovered in a case of chronic lymphocytic leukemia by whole genome sequencing,[1] is a novel phenomenon of genomic instability. It is characterized by extensive genomic rearrangements reflected by an oscillating pattern of DNA copy-number levels in one or few chromosomes as assessed by e.g., single-nucleotide polymorphism (SNP) microarray analysis or next-generation sequencing (NGS) techniques.[1,2] The chromosome shattering and subsequent erroneous repair mechanisms lead to the formation of highly derivative chromosomes with losses of tumor suppressor genes, activation of oncogenes, and/or generation of novel fusion genes; thus, chromothripsis is thought to promote and perhaps even to cause cancer development. The prevalence of chromothripsis is very heterogeneous across various tumor entities, but on average it can be found in approximately 2-3% of all cancers with up to 25% in bone cancers.[1] While the underlying mechanisms resulting in chromothripsis are still largely unknown, previous reports have associated chromothripsis with *TP53* mutations in subsets of medulloblastoma and acute myeloid leukemia (AML), however, several additional mechanisms have

Table 1. Clinical and genetic characteristics and outcome according to chromothripsis.

Clinical data	Chromothripsis+ n=39	Chromothripsis- n=73	*P*
Sex (male/female)	26 (67%)/13 (33%)	42 (58%)/31 (42%)	0.42
Age [years, median, (range)]	61 (19-82)	56 (21-76)	0.009
WBC count (10^6/l, median)	6	6.1	1.0
Platelet count (10^6/l, median)	43	42	0.39
Hemoglobin (g/dL, median)	9	9	1.0
BM blast count (%, median)	40	80	0.01
PB blast count (%, median)	19	25	0.72
LDH serum level (U/l, median)	445	407	0.38
Total no. of losses (mean±SD)	12.3±8.1	7.0±7.9	<0.001
Total no. of gains (mean±SD)	5.6±4.0	3.2±4.0	0.004
Total no. of amplifications (mean±SD)	1.1±1.7	0.2±0.6	<0.001
Total no. of genomic aberrations (mean±SD)	24.0±10.1	10.5±9.5	<0.001
-5/5q-	32 (82%)	34 (47%)	<0.001
-7/7q-	28 (72%)	34 (47%)	0.02
-12/12p-	19 (49%)	18 (25%)	0.01
-16/16q-	19 (49%)	17 (23%)	0.01
-17/17p-	21 (54%)	22 (30%)	0.02
Monosomal karyotype[§]	39 (100%)	43 (59%)	<0.001
TP53 alteration	33 (85%)	39 (53%)	0.001
Outcome[‡]	**n=16**	**n=46**	
EFS			
(months, median)	0.67	2.3	<0.001
(2-year survival rate, %)	0	13	
RFS			
(months, median)	2.3	10.3	0.008
(2-year survival rate, %)	0	34	
OS			
(months, median)	2.27	8.37	<0.001
(2-year survival rate, %)	0	28	

Multivariable analysis	OS		EFS	
	HR	*P*	HR	*P*
CK-AML (n=61)				
TP53 alteration	1.38	0.42	1.53	0.30
Chromothripsis+	2.47	0.02	2.2	0.04
Monosomal karyotype[§]	2.66	0.06	1.24	0.64
Age (> 60 years)	3.01	0.002	1.84	0.08

[§]Monosomal karyotype determined by chromosome banding analysis. [‡]Outcome analyses are restricted to age-adjusted intensively treated patients; endpoints are defined according to the European LeukemiaNet recommendations.[6] BM: bone marrow; OS: overall survival; EFS: event-free survival; RFS: relapse-free survival; HR: hazard ratio; WBC: white blood cell; PB: peripheral blood: LDH: lactate dehydrogenase; SD: standard deviation; CK-AML: complex karyotype acute myeloid leukemia.

been proposed, including DNA damage in micronuclei, premature chromosome condensation, breakage-fusion-bridge cycles, and telomere dysfunction.[2-4] In AML, *TP53* alterations (loss and/or mutation) are almost exclusively found in cases with complex karyotype (CK) AML and define a subset of patients with distinct genomic alterations and dismal outcome.[5,6]

To gain further insight into the role of chromothripsis in AML, we performed an integrative analysis consisting of genomic profiling (Affymetrix GeneChip Human Mapping 250K Array (n=61), Genome-Wide Human SNP Array 6.0 profiling (n=51) [GEO accession number GSE 34542]) and *TP53* mutation screening in 112 CK-AML patients.[5,7] The findings were correlated with clinical and genetic data of all CK-AML, whereas outcome analysis was restricted to 62 patients treated with intensive chemotherapy. In addition, global gene expression profiling (GEP) was performed in a subset of cases

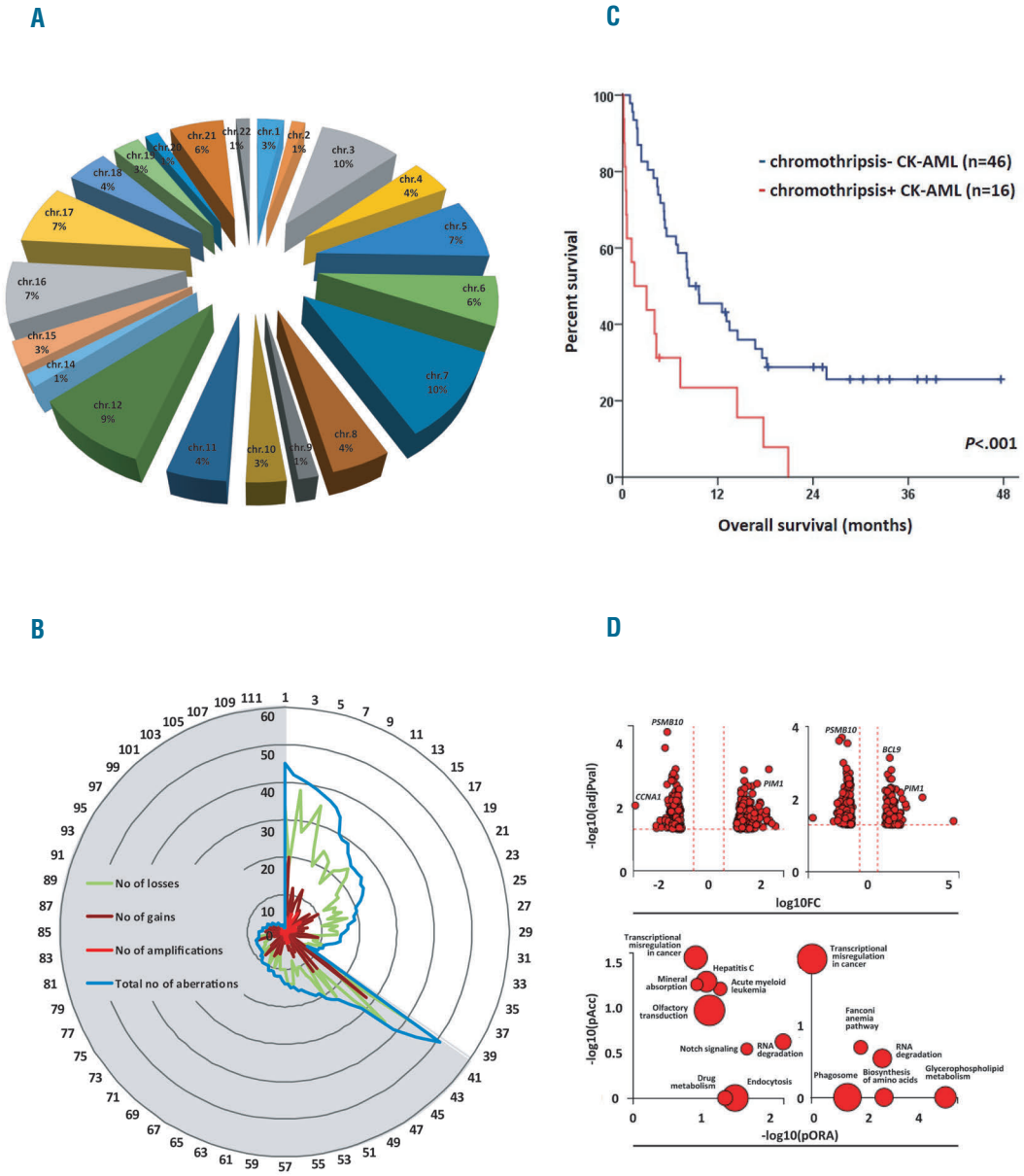

Figure 1. Chromothripsis in CK-AML. (A) Pie chart illustrating the chromosomal distribution of patterns of chromothripsis identified in 39 of 112 CK-AML patients. (B) Network diagram of genomic complexity in 112 CK-AML: cases with chromothripsis are numbered from 1 to 39 (highlighted in white), cases without chromothripsis are numbered from 40 to 112 (highlighted in gray). Aberrations are indicated as the absolute count per each individual case: the green line indicates number of losses, the red line the number of gains, the light red line the number of amplifications, and the blue line the total numbers of aberrations. (C) Kaplan-Meier survival estimate according to chromothripsis in CK-AML. Overall survival is shown for chromothripsis-negative(-) CK-AML (n=46) and chromothripsis-positive(+) CK-AML (n=16) (months; log-rank test). (D) Gene expression data for chromothripsis-positive (n=13) *vs.* –negative (n=14) CK-AML (left panels) and chromothripsis-positive (n=10) *vs.* –negative (n=7) *TP53*^{altered} CK-AML cases (right panels). Upper panel: volcano plots of all 421 (left) and 337 (right) significantly differentially expressed genes in terms of their measured expression change (x-axis) and the significance of their change (y-axis). The most significantly down- and upregulated genes are indicated. Lower panel: the top significantly impacted pathways are plotted in terms of over-representation on the x-axis (pORA) and total pathway accumulation on the y-axis (pAcc). Each pathway is represented by a single dot, and the size of each dot is proportional to the size of the pathway it represents. *P*-values are shown in negative log (base 10) values. CK-AML: complex karyotype acute myeloid leukemia.

(Affymetrix GeneChip Human Exon 1.0 ST Array (n=27) [GEO accession number GSE 21337]).

SNP profiling identified a total of 67 rearrangement patterns in 39 out of 112 (35%) CK-AML patients, consistent with chromothripsis defined by the presence of at least ten switches between two or three copy-number states on an individual chromosome.[2] In comparison to recent reports,[8] the incidence of 35% rearrangement patterns in CK-AML is higher than in other tumor entities. Fifteen of the 39 CK-AML patients exhibited patterns of chromothripsis on up to six different chromosomes (chr; on 2 chr (n=8), on 3 chr (n=4), on 4 to 6 chr [n=1 each]). While chromothripsis was seen in almost all chromosomes, it most frequently affected chr 7 (n=7; 10%) followed by chr 3 and 12 (n=6; 9% each), chr 5, 16, and 17 (n=5; 7% each), chr 6 and 21 (n=4; 6% each), chr 4, 8, 11, and 18 (n=3; 4% each), chr 1, 10, 15, and 19 (n=2; 3% each), and chr 2, 9, 14, 20, 22 (n=1; 2% each) (Figure 1A). CK-AMLs with chromothripsis were characterized by a higher total number of losses (mean±SD; 12.3±8.1 *versus* 7.0±7.9, *P*<0.001), gains (5.6±4.0 *versus* 3.2±4.0, *P*=0.004), high-level DNA amplifications (1.1±1.7 *versus* 0.2±0.6, *P*<0.001), and by a higher general degree of genomic complexity, as measured by total number of copy-number alterations per case (24.0±10.1 versus 10.5±9.5, *P*<0.001) (Table 1 and Figure 1B). Moreover, cases with chromothripsis were associated with specific genomic alterations, i.e., monosomy 5 or losses of 5q (-5/5q-) (*P*<0.001), -7/7q- (*P*=0.02), -12/12p- (*P*=0.01), -16/16q- (*P*=0.01), -17/17p- (*P*=0.02), and "monosomal karyotype" (*P*<0.001). As previously reported, chromothripsis was also correlated with the presence of *TP53* alteration (33/39 (85%) chromothripsis-positive CK-AML *versus* 39/73 (53%) chromothripsis-negative CK-AML, *P*=0.001; Table 1).

Clinically, chromothripsis-positive CK-AML patients were older (median age 61 (range: 19-82) *versus* 56 (range: 21-76) years, *P*=0.009) and showed lower bone marrow blast counts (median 40% *versus* 80%, *P*=0.01; Table 1). Chromothripsis was associated in trend with a lower CR rate (4/16 (25%) chromothripsis-positive versus 21/45 (47%) chromothripsis-negative CK-AML, *P*=0.15), but predicted for inferior survival. The 2-year estimated survival rates for chromothripsis-positive versus chromothripsis-negative patients were as follows: event-free survival (EFS), 0% *versus* 13% (log-rank; *P*<0.001), relapse-free survival (RFS), 0% *versus* 34% (*P*<0.008; see Table 1), and overall survival (OS), 0% *versus* 28% (*P*<0.001), respectively (Table 1 and Figure 1C). Other variables predicting for inferior OS and EFS in univariable analysis were age >60 years (*P*=0.001 and *P*=0.01, respectively), "monosomal karyotype" (*P*=0.004 and *P*=0.03, respectively), and *TP53* alteration (*P*<0.001 and *P*=0.001, respectively). Within chromothripsis-positive CK-AML there was no correlation regarding clinical and genetic characteristics or outcome for any specific chromosome affected by chromothripsis.

Albeit chromothripsis was associated with *TP53* alteration, which has been demonstrated to be the most important prognostic factor in CK-AML thus far, outweighing all other known prognostic variables in multivariable analysis,[5] chromothripsis provides additional independent prognostic information. Multivariable analysis stratified anew for age >60 years revealed chromothripsis as the exclusive significant variable for OS and EFS (OS: hazard ratio (HR), 2.47; 95%- confidence interval (CI), 1.14 to 5.34, *P*=0.02; EFS: HR, 2.20; 95%-CI, 1.04 to 4.64, *P*=0.04). Age retained its significance for OS alone (HR, 3.01; 95%-CI, 1.49 to 6.11, *P*=0.002), whereas *TP53* alteration and "monosomal karyotype" were not significant in the multivariable model in our study (Table 1).

To gain further molecular insight into mechanisms underlying chromothripsis, we performed GEP and analyzed data using iPathwayGuide. Comparing chromothripsis-positive (n=13) and –negative (n=14) CK-AML, GEP disclosed a distinct gene signature of 421 significantly differentially expressed genes at a nominal *P*-value <0.05 (the top 200 differentially expressed genes are listed in *Online Supplementary Table S1*). Among the top most deregulated genes in chromothripsis-positive CK-AML were *CCNA1* (-2.9 log fold change, *P*<0.009; see Online Supplementary Table S1) involved in cell cycle control and proliferation[9] and reflecting our observation of lower bone marrow blast counts in chromothripsis-positive CK-AML, *PSMB10* (-1.7 log fold change, *P*<0.001), known to be critical for immune surveillance,[10] and *PIM1* (1.9 log fold change, *P*<0.002; see Online Supplementary Table S1), encoding a serine threonine kinase involved in FLT3-mediated cell survival,[11] respectively (Figure 1D).

Pathway analysis using "Impact Analysis" revealed ten pathways significantly impacted in chromothripsis-positive CK-AML (*Online Supplementary Table S2*). These pathways are implicated in tumorigenesis, transformation, progression, and/or proliferation (e.g., "transcriptional misregulation in cancer", "RNA degradation", or "Notch signaling") and have been linked to "acute myeloid leukemia". In accordance, a gene set enrichment analysis (GSEA) using the Molecular Signatures Database (MSigDB)[12] revealed similar results, in particular with regard to genes involved in RNA degradation or proliferation. Beyond that, GSEA disclosed significantly deregulated genes involved in genomic stability (e.g., "chromatin remodeling complex" (*ARID1A, ASF1A*), "cell cycle" (*CCNA1, RAD52, AURKA*), "negative regulation of cell cycle", or "cell cycle arrest" [*ZAK, HPGD, KAT25*]) in chromothripsis-positive CK-AML (*Online Supplementary Table S3*).

As chromothripsis was significantly correlated with *TP53* alteration, but not all $TP53^{altered}$ CK-AML exhibited excessive genomic rearrangements, we performed an explorative subset analysis for chromothripsis associated genes within $TP53^{altered}$ CK-AML to gain additional insight into the causal processes of chromothripsis. Comparing chromothripsis-positive (n=10) *versus* –negative (n=7) $TP53^{altered}$ CK-AML, GEP disclosed a distinct chromothripsis-associated gene signature of 337 significantly differentially expressed genes at a nominal *P*-value <0.05 (the top 200 differentially expressed genes are listed in *Online Supplementary Table S4*). The top most deregulated genes in chromothripsis-positive $TP53^{altered}$ CK-AML were, once more, *PSMB10* (-1.8 log fold change, *P*<0.001) and *BCL9* (1.4 log fold change, *P*<0.001), respectively (Figure 1D). *BCL9* encodes the B-cell CLL/lymphoma 9 protein that is involved in signal transduction through the Wnt pathway and plays a role in B-cell malignancies and tumor progression of several human cancers, but has not been associated with myeloid malignancies as of yet. Pathway analysis pointed again to "transcriptional misregulation in cancer" and "RNA degradation" pathways. Moreover, chromothripsis-positive $TP53^{altered}$ CK-AML also showed a significant deregulation of genes associated with genomic instability (e.g., "Fanconi anemia pathway genes" [*ERCC1, FANCA, MLH1*, and *EME1*]) and transcriptional regulation (e.g., *CEBPA*) (Figure 1D, *Online Supplementary Table S5*). Since some of the impacted pathways are linked to

telomere dysfunction (e.g., "Fanconi anemia pathway", "cell cycle", or "RNA metabolic process"; *Online Supplementary Table S3*) which may (i) result in chromothripsis by telomere fusions leading to chromatin bridges[13] and (ii) drive aberrant hematopoietic differentiation and myelodysplastic syndrome (MDS),[14] our data support a role for telomere dysfunction as one of the molecular mechanisms underlying chromothripsis in CK-AML.

Next, the hypothesis that chromothripsis might promote and perhaps even cause oncogenesis was strengthened by sequential genomic profiling of four cases with antecedent MDS. SNP analysis revealed that the patterns of chromothripsis delineated in AML were already present at diagnosis of MDS, thereby supporting chromothripsis as an early event in myeloid leukemogenesis.

In summary, chromothripsis is a frequent genomic alteration in CK-AML and correlates with *TP53* alteration, genomic instability, specific copy-number alterations, and "monosomal karyotype". While further studies confirming these findings with state-of-the-art NGS technology are warranted,[15] our SNP-based analysis nevertheless clearly shows that chromothripsis defines a subset of CK-AML cases with very dismal outcomes, and indeed outweighs *TP53* alteration in multivariable analysis. Detailed molecular profiling links chromothripsis to altered *TP53* in CK-AML and to an additional pathogenic role as, for instance, the deregulation of genes associated with telomere dysfunction, genomic instability (e.g., Fanconi anemia pathway genes), mitotic regulation (e.g., *CCNA1*), or transcriptional regulation (e.g., *CEBPA*). These findings also point to novel potential treatment targets such as *PIM1* in this acutely high-risk AML.

Frank G. Rücker,[1] Anna Dolnik,[1] Tamara J. Blätte,[1] Veronica Teleanu,[1] Aurélie Ernst,[2] Felicitas Thol,[3] Michael Heuser,[3] Arnold Ganser,[3] Hartmut Döhner,[1] Konstanze Döhner[*1] and Lars Bullinger*[*1,4]

[1]Department of Internal Medicine III, University Hospital of Ulm, Albert-Einstein-Allee 23; [2]Division of Molecular Genetics, German Cancer Consortium (DKTK), German Cancer Research Center (DKFZ), Heidelberg; [3]Department of Hematology, Hemostasis, Oncology, and Stem Cell Transplantation, Hannover Medical School and [4]Department of Hematology, Oncology and Tumor Immunology, Charité-University Medicine Berlin, Campus Virchow Klinikum, Berlin, Germany

*KD and LB contributed equally to this work.

Funding: this work was supported by the Austrian Science Fund (FWF), SFB Grants F4705-B20 (TL) and F4704-B20 (PV). The authors declare no conflict of interests.

Correspondence: frank.ruecker@uniklinik-ulm.de

doi:10.3324/haematol.2017.180497

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.

References

1. Stephens PJ, Greenman CD, Fu B, et al. Massive genomic rearrangement acquired in a single catastrophic event during cancer development. Cell. 2011; 144(1): 27-40.
2. Rausch T, Jones DT, Zapatka M, et al. Genome sequencing of pediatric medulloblastoma links catastrophic DNA rearrangements with TP53 mutations. Cell. 2012;148(1-2): 59-71.
3. Rode A, Maass KK, Willmund KV, Lichter P, Ernst A . Chromothripsis in cancer cells: An update. Int J Cancer. 2016;138(10):2322-2333.
4. Ernst A, Jones DT, Maass KK, et al. Telomere dysfunction and chromothripsis. Int J Cancer. 2016;138(12):2905-2914.
5. Rücker FG, Schlenk RF, Bullinger L, et al. TP53 alterations in acute myeloid leukemia with complex karyotype correlate with specific copy number alterations, monosomal karyotype, and dismal outcome. Blood. 2012;119(9):2114-2121.
6. Döhner H, Estey E, Grimwade D, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. Blood. 2017;129(4):424-447.
7. Rücker FG, Russ AC, Cocciardi S, et al. Altered miRNA and gene expression in acute myeloid leukemia with complex karyotype identify networks of prognostic relevance. Leukemia. 2013;27(2):353-361.
8. Kloosterman WP, Koster J, Molenaar JJ. Prevalence and clinical implications of chromothripsis in cancer genomes. Curr Opin Oncol. 2014;26(1):64-72.
9. Ji P, Agrawal S, Diederichs S, et al. Cyclin A1, the alternative A-type cyclin, contributes to G1/S cell cycle progression in somatic cells. Oncogene. 2005;24(16):2739-2744.
10. Yang XW, Wang P, Liu JQ, et al. Coordinated regulation of the immunoproteasome subunits by PML/RARα and PU.1 in acute promyelocytic leukemia. Oncogene. 2014;33(21):2700-2708.
11. Kim KT, Baird K, Ahn JY, et al. Pim-1 is up-regulated by constitutively activated FLT3 and plays a role in FLT3-mediated cell survival. Blood. 2005;105(4):1759-1767.
12. Subramanian A, Tamayo P, Mootha VK, et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci USA. 2005;102(34):15545-15550.
13. Maciejowski J, Li Y, Bosco N, Campbell PJ, de Lange T. Chromothripsis and kataegis induced by telomere crisis. Cell. 2015; 163(7):1641–1654.
14. Colla S, Ong DST, Ogoti Y, et al. Telomere dysfunction drives aberrant hematopoietic differentiation and Myelodysplastic Syndrome. Cancer Cell. 2015;27(5):644–657.
15 Korbel JO, Campbell PJ. Criteria for inference of chromothripsis in cancer genomes. Cell. 2013;152(6):1226-1236.