TP53 alterations in acute myeloid leukemia with complex karyotype correlate with specific copy number alterations, monosomal karyotype, and dismal outcome

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To assess the frequency of *TP53* alterations and their correlation with other genetic changes and outcome in acute myeloid leukemia with complex karyotype (CK-AML), we performed integrative analysis using *TP53* mutational screening and array-based genomic profiling in 234 CK-AMLs. *TP53* mutations were found in 141 of 234 (60%) and *TP53* losses were identified in 94 of 234 (40%) CK-AMLs; in total, 164 of 234 (70%) cases had *TP53* alterations. *TP53*-altered CK-AML were characterized by a higher degree of genomic complexity (aberrations per case, 14.30 vs 6.16; $P < .0001$) and by a higher frequency of specific copy number alterations, such as −5/5q−, −7/7q−, −16/16q−, −18/18q−, +1/+1p, and +11/+11q/amp11q13∼25; among CK-AMLs, *TP53*-altered more frequently exhibited a monosomal karyotype (MK). Patients with *TP53* alterations were older and had significantly lower complete remission rates, inferior event-free, relapse-free, and overall survival. In multivariable analysis for overall survival, *TP53* alterations, white blood cell counts, and age were the only significant factors. In conclusion, *TP53* is the most frequently known altered gene in CK-AML. *TP53* alterations are associated with older age, genomic complexity, specific DNA copy number alterations, MK, and dismal outcome. In multivariable analysis, *TP53* alteration is the most important prognostic factor in CK-AML, outweighing all other variables, including the MK category. (*Blood*. 2012;119(9):2114-2121)

Introduction

Chromosomal abnormalities are found in approximately 55% of adult patients with acute myeloid leukemia (AML) and are among the most important independent prognostic factors.[1-3] AMLs exhibiting 3 or more acquired chromosome aberrations in the absence of chromosomal rearrangements listed in the World Health Organization (WHO) 2008 category "AML with recurrent genetic abnormalities" are now defined as AML with complex karyotype (CK-AML).[1,2] CK-AMLs account for 10% to 15% of adult AMLs, and the frequency increases with age. CK-AMLs belong to the cytogenetic adverse-risk group because they are associated with very poor outcome when treated with intensive or nonintensive conventional chemotherapy.[1,3,4] Recently, a new cytogenetic category was introduced, that is, the monosomal karyotype (MK) defined by the presence of one single autosomal monosomy in association with at least one additional autosomal monosomy or one structural chromosomal abnormality (in the absence of core binding factor AML and acute promyelocytic leukemia).[5] This MK category was reported to be associated with a dismal prognosis and to add prognostic information, even in CK-AML.

Complex karyotypes often contain numerous chromosome aberrations that can only be partially or not at all interpreted using standard cytogenetic techniques. Such aberrations include unbalanced translocations with chromosomal material of unknown origin, marker or ring chromosomes, homogeneously staining regions, or double minutes, the latter representing cytogenetic equivalents of high-level DNA amplifications. In general, CK-AMLs are characterized by chromosomal gains and losses, rather than balanced translocations, suggesting distinct mechanisms in leukemogenesis.[6]

In recent years, molecular cytogenetic and array-based techniques have enabled a more precise characterization of these complex genetic changes. The imbalances most frequently found are losses affecting chromosome 5 or 5q (−5/5q−), −17/17p−, −7/7q−, −18/18q−, −16/16q−, −12/12p−, and gains affecting chromosome 8 or 8q (+8/+8q), +11/+11q, +21/+21q, +22/+22q, and +1/+1p.[7] Furthermore, novel potential target genes have been delineated based on the observation that they are located in critical regions of deletions[8] or contained in amplicons, such as *MYC* in

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8q24, *ETS1* and *FLI1* in 11q24, *CDX2* in 13q12, and *ETS2* and *ERG* in 21q22.[7,9-12]

One target located in the commonly deleted region of 17p13 is the tumor suppressor gene *TP53*. In AML, *TP53* alterations (mutations and/or losses, *TP53*[altered]) are rare and have been closely associated with CK-AML.[13-16] Clinically, *TP53* alterations appear to be associated with inferior outcome.[15-17] However, these data are based on a small number of studies, and only one of these addressed both *TP53* losses and mutations but not the prognostic significance.[15]

The objectives of our study were: (1) to study a large cohort of CK-AMLs (n = 234) for *TP53* mutations; (2) to analyze these cases for DNA copy number alterations (CNAs) using array-based techniques; and (3) to correlate *TP53* alterations with specific chromosome abnormalities, CNAs, MK, and clinical outcome.

Methods

Patients

Peripheral blood (PB) and/or bone marrow (BM) samples from 234 adult patients with CK-AML were analyzed. The definition of CK-AML followed recommended criteria.[1,2] The diagnosis of AML was based on French-American-British Cooperative Group criteria,[18] and after 2004 on WHO criteria.[19] A total of 133 patients had de novo AML, 31 secondary AML (s-AML) after myelodysplastic syndrome or myeloproliferative neoplasms, 30 therapy-related (t-AML), and in 40 patients it was unknown. Of these 234 patients, 155 (66%) were treated on consecutive multicenter treatment trials of the German-Austrian AML Study Group (AMLSG) applying age-adjusted intensive chemotherapy: AML HD93 (n = 1),[20] AML HD98A (n = 30),[21] and AMLSG 07-04 (n = 54; NCT00151242) for younger patients (16-60 years); and AML HD98B (n = 27)[22] and AMLSG 06-04 (n = 43; NCT00151255) for elderly patients (> 60 years). All trials were approved by the local ethics committees of all participating institutions; all patients gave informed consent for treatment, cryopreservation of samples, and molecular analyses according to the Declaration of Helsinki. Samples were primarily selected based on availability of sufficient material for genomic profiling and mutational analysis.

Cytogenetic and molecular genetic analysis

For cytogenetic classification, metaphases of sufficient quality could be studied by chromosome banding analysis in 219 patients; karyotypes were described according to the International System for Human Cytogenetic Nomenclature.[23]

Because of evolving technology that occurred in the course of the study, we switched from array comparative genomic hybridization (CGH) to single nucleotide polymorphism (SNP) array-based genomic profiling. Array CGH (n = 131) using the 2.8k platform and/or the 8.0k platform and unpaired SNP analyses using Affymetrix GeneChip Human Mapping 250K Array (n = 61) were performed as previously described[7,24]; Genome-Wide Human SNP Array 6.0 profiling (n = 42) was performed according to the manufacturer's protocols (Affymetrix). Genotyping Version 2.0 console (Affymetrix) was used for analysis of 6.0 arrays. Microarray data will be available at gene expression omnibus at http://www.ncbi.nlm.nih.gov/geo/ (GEO accession number GSE34542).

TP53 sequence analysis

To identify mutations in exons 4 to 10 of *TP53*, denaturating high-performance liquid chromatography was performed as previously described.[25] Aberrant profiles were verified by bidirectional sequencing and compared with wild-type sequence (GenBank; X54156). Mutations were described using 2 different databases (IARC TP53 Database; www.p53.iarc.fr and The TP53 Web site; www.p53.free.fr).[26,27] Subcloning analyses using the TOPO TA Cloning Kit and resequencing were performed according to manufacturer's protocols (Invitrogen).

Statistical analyses

The section on statistical analyses is provided in supplemental Methods (available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article).

Results

TP53 mutation analysis

In 141 of the 234 (60%) patients with CK-AML, a total of 168 mutations were identified; 161 (96%) were located in the sequence-specific DNA-binding domain of p53 (residues 102-292). The majority were missense mutations (n = 130), followed by deletions/insertions (n = 21); 17 resulted in premature stop; 4 preserved the open reading frame (g.13149del6, g.13149del9, g.14001del21, and g.13162ins3), nonsense mutations (n = 9), and splice site mutations (n = 8; Figure 1). Seventeen of 25 patients harboring 2 or more *TP53* mutations exhibited no *TP53* loss; 5 of these showed a homozygous *TP53* mutation; and in all remaining patients with available DNA for subcloning (n = 8), compound heterozygous mutations were confirmed. Hemizygous mutations (loss of 1 allele and at least 1 mutation in the remaining allele; 47% [79 of 168]) were more frequent than homozygous mutations (26% [43 of 168]), heterozygous (18% [30 of 168]), including possible compound heterozygous mutations among the 4 patients with more than 1 heterozygous mutation), and compound heterozygous mutations (10% [16 of 168]); 65 (39%) mutations affected common hot spots, such as codons 175, 245, 248, 273, and 275. Mutations affecting codons 175, 248, and 273 were associated with biallelic *TP53* alteration compared with all other *TP53* mutations (100% [27 of 27] vs 79% [90 of 114], $P = .008$).

CNAs and copy number neutral loss of heterozygosity UPD

In the entire cohort of 234 CK-AML, genomic losses (n = 1.845) were more frequent than gains (n = 778) or high-level DNA amplifications (n = 153). The median number of aberrations per case was 10 (range, 0-51); median numbers of losses, gains, and amplifications per case were 6 (range, 0-43), 2 (range, 0-28), and 0 (range, 0-7), respectively.

Recurrent losses were identified for the following chromosomes: monosomy 5 or losses of 5q (−5/5q−) (n = 147; 63%), −7/7q− (n = 123; 53%), −17/17p− (n = 106; 45%), −16/16q− (n = 66; 28%), −18/18q−, −12/12p− (n = 65 each; 28%), −20/20q− (n = 55; 24%), −3/3p− (n = 54; 23%), and −11/11q− (n = 35; 15%). Most frequent gains were +8/+8q (n = 67; 29%), +11/+11q (n = 61; 26%), +21/+21q (n = 39; 17%), +1/+1p (n = 37; 16%), +22/+22q (n = 33; 14%), +13/+13q (n = 29; 12%), +9/+9p (n = 28; 12%), and +19/+19p (n = 25; 11%). Most frequent high-level DNA amplifications mapped to 21q22, 11q13∼25 (n = 22 each; 9%), and 8q24 (n = 8; 4%).

Usually large, but also submicroscopic, losses (down to 800 kb in size) affecting the *TP53* locus on 17p13 were identified in 94 of 234 (40%) cases. Uniparental disomy of 17p [UPD(17p); 8.14-22.50 Mb in size] encompassing the *TP53* locus was detected in 15 of 103 (15%) cases analyzed by SNP arrays.

Biallelic *TP53* alteration

Combining mutational and microarray findings, 164 of 234 (70%) CK-AMLs exhibited *TP53* alteration (mutation and/or loss of *TP53*); 71 of 164 (43%) cases had biallelic *TP53* alteration by hemizygous mutation pattern, and 38 of 70 (54%) cases without

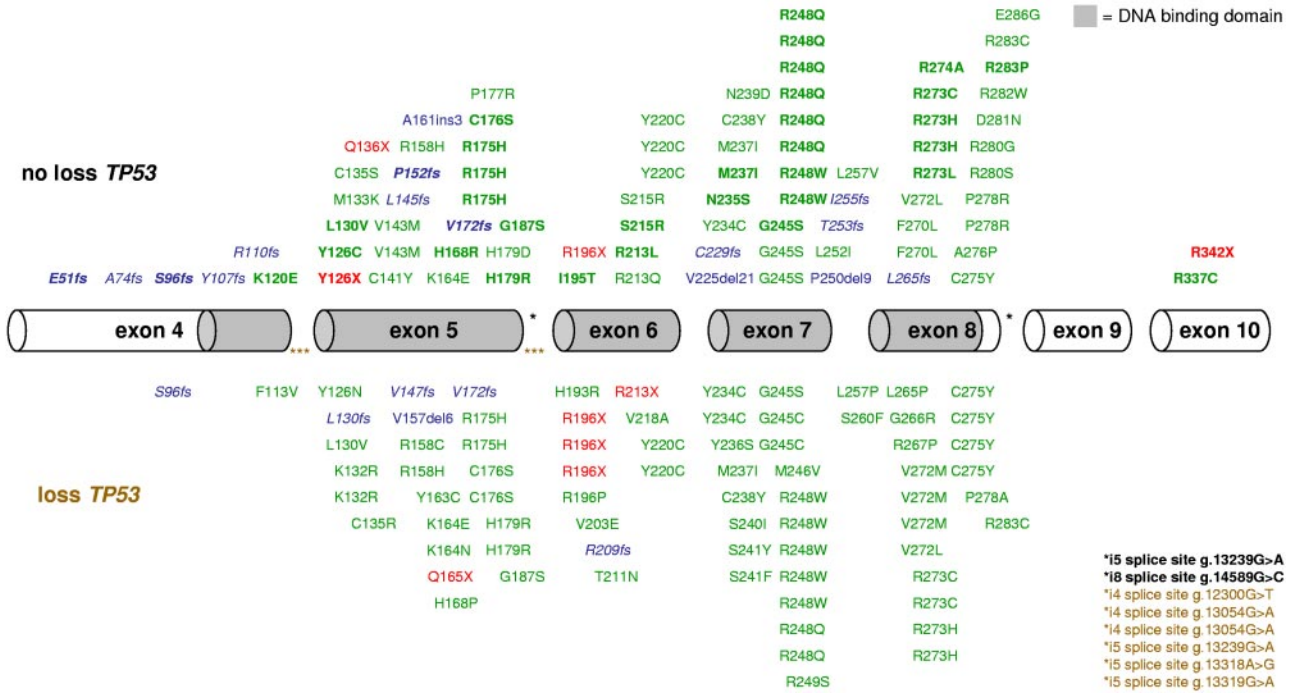

Figure 1. Mapping of 168 *TP53* mutations in 141 CK-AMLs. Hemizygous mutations are indicated in the bottom panel, heterozygous, and/or homozygous mutations are marked in the top panel. Exons 4 to 10 are drawn to relative scale; missense mutations (green), nonsense mutations (red), and insertion/deletion mutations (blue) are shown at their approximate location along the exons. Bold represents homozygous mutations, and blue italics, frameshift mutations leading to a premature stop codon.

TP53 loss exhibited homozygous *TP53* mutation caused by UPD(17p) in 15 of 19 SNP-analyzed cases. In the 4 cases lacking evidence for UPD(17p) in SNP profiling, homozygous *TP53* mutations possibly resulted from intragenic loss of heterozygosity. Furthermore, 19 of 131 (15%) cases analyzed by array CGH on DNA sequence analysis exhibited homozygous *TP53* mutation that are probably caused by UPD(17p) considering the frequency of UPD(17p) found by SNP array analysis. In addition, subcloning analysis confirmed biallelic *TP53* alteration by compound heterozygous mutations in 8 patients. Together, at least 117 of 164 (71%) *TP53*^{altered} CK-AMLs had biallelic *TP53* inactivation (not taken into account the 4 patients with potentially compound heterozygous *TP53* mutations). Patient 96 exhibited a homozygous missense mutation in exon 6 (p.R213L) and an additional heterozygous frameshift mutation in exon 4 (p.A74fs), suggesting that these had occurred sequentially, with p.R213L being the primary event followed by UPD(17p) resulting in the homozygous mutation pattern, whereas the p.A74fs mutation followed the recombination event (supplemental Figure 1).

Correlation of *TP53* alteration with pattern of chromosome abnormalities and CNAs

We correlated *TP53* alterations, as assessed by DNA sequence analysis and array profiling, with the pattern of chromosome abnormalities identified by conventional cytogenetics and with the pattern of CNAs detected by array-based analyses (Table 1).

Correlation with chromosome abnormalities. *TP53* alterations were identified in 157 of 219 (72%) CK-AMLs that could be analyzed by conventional cytogenetics. *TP53*^{altered} CK-AMLs had a higher degree of genomic complexity as measured by total number of aberrations ($\geq$ 5 aberrations, $P < .0001$) and the presence of marker chromosomes ($P = .0005$). *TP53* alterations were correlated with the presence of specific cytogenetic abnormalities, such as −5/5q− ($P < .0001$), concomitant −5/5q− and −7/7q− ($P = .0006$), and 20q− ($P = .02$); we found no correlation with −7/7q− ($P = .14$; Table 1).

Correlation with CNAs. *TP53* alterations were correlated with the total number of losses (mean ± SD; 9.54 ± 7.49 vs 4.00 ± 4.88, $P < .0001$), gains (3.91 ± 3.80 vs 1.94 ± 1.92, $P < .0001$), high-level DNA amplifications (0.84 ± 1.31 vs 0.21 ± 0.83, $P = .0002$), and genomic complexity as measured by total number of aberrations per case (14.30 ± 9.41 vs 6.16 ± 5.53, $P < .0001$). Moreover, *TP53* alterations were positively correlated with specific genomic aberrations, such as −5/5q− ($P < .0001$), −7/7q− ($P = .003$), concomitant −5/5q− and −7/7q− ($P < .0001$), and also −3/3p− ($P = .002$), −16/16q− ($P < .0001$), −18/18q− ($P = .0008$), and −20/20q− ($P = .004$); further correlations were identified for +1/+1p ($P = .001$), +11/+11q ($P = .0002$), +13/+13q ($P = .02$), +19/+19p ($P = .04$), and amplifications in 11q13∼25 [amp(11)(q13∼25)] ($P = .0004$; Table 1; Figure 2).

Correlation of *TP53* alterations with MK

By conventional cytogenetics, 171 of 219 (78%) CK-AMLs fulfilled the MK criteria (CK$^+$/MK$^+$ AML) as previously defined. *TP53* alterations were found in 137 of 171 (80%) CK$^+$/MK$^+$ AMLs and in only 20 of 48 (42%) CK$^+$/MK$^-$ AMLs ($P < .0001$; Table 1). Compared with CK$^+$/MK$^-$ AMLs, CK$^+$/MK$^+$ AMLs were characterized by a higher degree of genomic complexity determined by cytogenetics: more than or equal to 5 aberrations, 88% (151 of 171) versus 54% (26 of 48), $P < .0001$; and by genomic profiling as measured by total number of losses (mean ± SD; 9.29 ± 7.40 vs 3.67 ± 5.72; $P < .0001$) and aberrations per case (13.59 ± 9.61 vs 6.81 ± 6.11, $P < .0001$).

We subsequently determined MK$^+$ AML based on array data (molMK). The frequency of CK$^+$/molMK$^+$ AML was much lower

Table 1. Genetic and clinical characteristics according to *TP53* alteration

	TP53^{unaltered}	*TP53*^{altered}	*P*
Cytogenetics	n = 62	n = 157	
≥ 5 aberrations	38 (61%)	139 (89%)	< .0001
Marker chromosomes	29 (47%)	114 (73%)	.0005
−5/5q−	20 (32%)	124 (79%)	< .0001
−7/7q−	25 (40%)	81 (52%)	.14
−5/5q− and −7/7q−	12 (19%)	70 (45%)	.0006
−20/20q−	8 (13%)	44 (28%)	.02
MK	34 (55%)	137 (87%)	< .0001
Array-based genomics	n = 70	n = 164	
Total no. of losses (mean ± SD)	4.00 ± 4.88	9.54 ± 7.49	< .0001
Total no. of gains (mean ± SD)	1.94 ± 1.92	3.91 ± 3.80	< .0001
Total no. of amplifications (mean ± SD)	0.21 ± 0.83	0.84 ± 1.31	.0002
Total no. of genomic aberrations (mean ± SD)	6.16 ± 5.53	14.30 ± 9.41	< .0001
−3/3p−	7 (10%)	47 (29%)	.002
−5/5q−	20 (29%)	127 (77%)	< .0001
−7/7q−	26 (37%)	97 (59%)	.003
−5/5q− and −7/7q−	13 (19%)	87 (53%)	< .0001
−11/11q−	13 (19%)	22 (13%)	.32
−12/12p−	13 (19%)	52 (32%)	.06
−16/16q−	5 (7%)	61 (37%)	< .0001
−18/18q−	9 (13%)	56 (34%)	.0008
−20/20q−	8 (11%)	47 (29%)	.004
+1/+1p	3 (4%)	34 (21%)	.001
+8/+8q	21 (30%)	46 (28%)	.75
+9/+9p	11 (16%)	17 (10%)	.27
+11/+11q	7 (10%)	54 (33%)	.0002
+13/+13q	3 (4%)	26 (16%)	.02
+19/+19p	3 (4%)	22 (13%)	.04
+21/+21q	7 (10%)	32 (20%)	.09
+22/+22q	6 (9%)	27 (16%)	.15
amp(8)(q24)	4 (6%)	4 (2%)	.24
amp(11)(q13∼25)	0 (0%)	22 (13%)	.0004
amp(21)(q22)	3 (4%)	19 (12%)	.09
Molecular MK	16 (23%)	59 (36%)	.07
Molecular genetics	n = 50	n = 99	
FLT3-ITD positive	3 (6%)	1 (1%)	.11
FLT3-TKD mutation	3 (6%)	1 (1%)	.11
NPM1 mutation	3 (6%)	0 (0%)	.04
Clinical data	n = 52	n = 103	
Sex (male/female)	26 (50%)/26 (50%)	54 (52%)/49 (48%)	.87
Median age, y	54	61	.002
AML history			
De novo	36 (69%)	76 (74%)	.57
Secondary	6 (12%)	9 (9%)	.58
Therapy-related	9 (17%)	16 (16%)	.82
Median WBC, × 10^6/L	12.9	6.5	.18
Median platelet count, × 10^6/L	48	41	.46
Median hemoglobin, g/dL	9.1	8.9	.38
Median BM blast count, %	78	65	.04
Median PB blast count, %	45	30	.18
Median LDH serum level, U/L	391	438	.25
Response	n = 52	n = 103	
CR after induction therapy	26 (50%)	29 (28%)	.01
RD after induction therapy	18 (35%)	53 (51%)	.06
Outcome	n = 52	n = 103	
OS			
Median, mo	10.97	4.14	< .0001
3-y survival rate, %	28	3	
EFS			
Median, mo	1.94	1.12	.0007
3-y survival rate, %	13	1	
RFS			
Median, mo	12.16	6.51	.01
3-y survival rate, %	30	7	

ITD indicates internal tandem duplication; and TKD, tyrosine kinase domain.

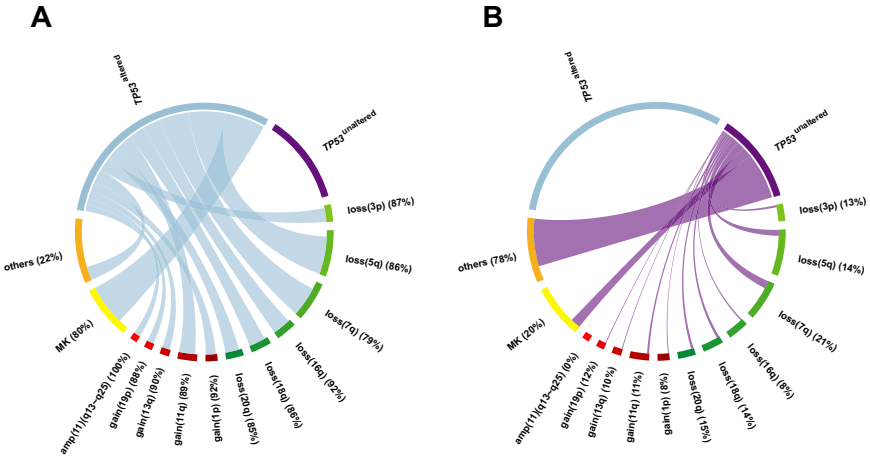

Figure 2. Relative frequencies and pairwise co-occurrences of *TP53* alteration-associated genomic aberrations illustrated using Circos Table Viewer Version 0.52.[28] The percentages indicate the proportion of each aberration associated with (A) *TP53*^{altered} CK-AMLs and (B) *TP53*^{unaltered} CK-AMLs. Unaltered *TP53* and amp(11)(q13~q25) were mutually exclusive. MK is based on cytogenetics analysis.

(75 of 234; 32%) because many monosomies described in chromosome banding analysis were not real monosomies but part of chromosomal material hidden in unbalanced translocation or marker chromosomes. *TP53* alterations were found in 59 of 75 (79%) CK$^+$/molMK$^+$ AMLs and in 105 of 159 (66%) CK$^+$/molMK$^-$ AMLs ($P = .07$; Table 1).

Correlation of *TP53* alterations with clinical characteristics, response to therapy, and survival

Analyses were restricted to patients enrolled into AMLSG multicenter treatment trials applying age-adjusted intensive chemotherapy (n = 155, median age, 59 years; range, 18-81 years). Because there were no significant differences regarding clinical characteristics, response to therapy, and survival for *TP53*$^{monoallelic\ altered}$ and *TP53*$^{biallelic\ altered}$ CK-AMLs (supplemental Table 1; supplemental Figure 2), these genotypes were grouped as *TP53*^{altered} CK-AML for further analyses.

Clinical characteristics. *TP53*^{altered} CK-AML patients were older (median 61 vs 54 years, $P = .002$) and had lower BM blast counts (median 65% vs 78%, $P = .04$; Table 1).

Response to therapy. *TP53* alterations were associated with resistance to chemotherapy. Response to induction therapy was as follows: complete remission (CR) 28% and 50% ($P = .01$), refractory disease (RD) 51% and 35% ($P = .06$), and early/hypoplastic death 21% and 15% ($P = .52$) for CK$^+$/*TP53*^{altered} and CK$^+$/*TP53*^{unaltered} AML, respectively (Table 1). Other variables predicting for poor response to induction therapy were age ($P < .0001$) and genomic losses affecting 5q ($P = .02$), 7q ($P = .03$), and 16q ($P = .04$). Lactate dehydrogenase (LDH) serum levels, white blood cell count (WBC), s/t-AML, and cytogenetic MK did not impact CR achievement.

For multivariable analysis, a conditional model was used with an age cut-point at 60 years to address the different treatment intensities applied in the different age cohorts. This model revealed as significant factors *TP53*^{altered} (odds ratio [OR] = 0.55; 95% confidence interval [CI], 0.30-1.00; $P = .05$) and age (OR for a 10-year difference, 0.67; 95% CI, 0.52-0.87; $P = .003$). No significant impact on CR achievement was found for the variables WBC, platelet counts, cytogenetic MK, and s/t-AML (Table 2).

Survival analysis. The median follow-up time for survival in the 155 CK-AML was 36.6 months (95% CI, 29.9-51.4 months); the estimated 3-year event-free survival (EFS), relapse-free survival (RFS), and overall survival (OS) of the entire cohort were 5% (95% CI, 2%-10%), 17% (95% CI, 9%-31%), and 12% (95% CI, 7%-19%), respectively.

TP53 alterations were associated with inferior survival; the 3-year estimated survival rates for CK$^+$/*TP53*^{altered} and CK$^+$/*TP53*^{unaltered} patients were as follows: EFS, 1% versus 13% (log-rank, $P = .0007$); RFS, 7% versus 30% ($P = .01$); and OS, 3% versus 28% ($P < .0001$), respectively (Table 1; Figure 3). Other variables predicting for inferior OS in univariable analysis were age ($P < .0001$), cytogenetic MK ($P = .03$), and genomic losses of 5q ($P = .03$), 7q ($P = .003$), 16q ($P = .0004$), and gains of 1p ($P = .04$), and amp(11)(q13~25; $P = .05$). LDH and WBC did not impact OS. Among CK$^+$/MK$^+$ AMLs, those with *TP53* alterations had significantly worse OS ($P = .0004$; Figure 4).

Multivariable analysis stratified again for age at a cut-point of 60 years revealed *TP53*^{altered} (hazard ratio [HR] = 2.43; 95% CI, 1.56-3.77; $P = .0001$), logarithm of WBC (HR = 1.62; 95% CI, 1.17-2.26; $P = .004$), and age (HR for 10-year difference, 1.26; 95% CI, 1.01-1.56, $P = .04$) as significant variables; not significant for OS were platelet counts, cytogenetic MK, and s/t-AML (Table 2).

Allogeneic hematopoietic stem cell transplantation in first CR was performed in 30 CK-AML patients. Of those, 14 of 15 *TP53*^{altered} CK-AML relapsed and died, whereas in *TP53*^{unaltered} CK-AML 9 of 15 relapsed and died ($P = .04$). This translated into significantly worse OS for *TP53*^{altered} CK-AML ($P = .04$; supplemental Figure 3).

Table 2. Multivariate analyses of outcome

	Response		OS	
CK-AML	OR	*P*	HR	*P*
TP53 alteration	0.55	.05	2.43	.0001
Age (difference of 10 y)	0.67	.003	1.26	.04
s/t-AML	0.67	.24	1.05	.81
Logarithm of WBC	0.74	.19	1.62	.004
Logarithm of platelets	0.76	.39	1.13	.62
MK*	0.75	.43	0.87	.57

*Determined by chromosome banding analysis.

Discussion

In our series of 234 CK-AMLs, *TP53* was deleted and/or mutated in 70% of cases, thus representing the most frequently known altered gene in this AML subgroup. *TP53* alterations were associated with older age, genomic complexity, specific chromosome abnormalities, monosomal karyotype, specific CNAs, and predicted for dismal outcome.

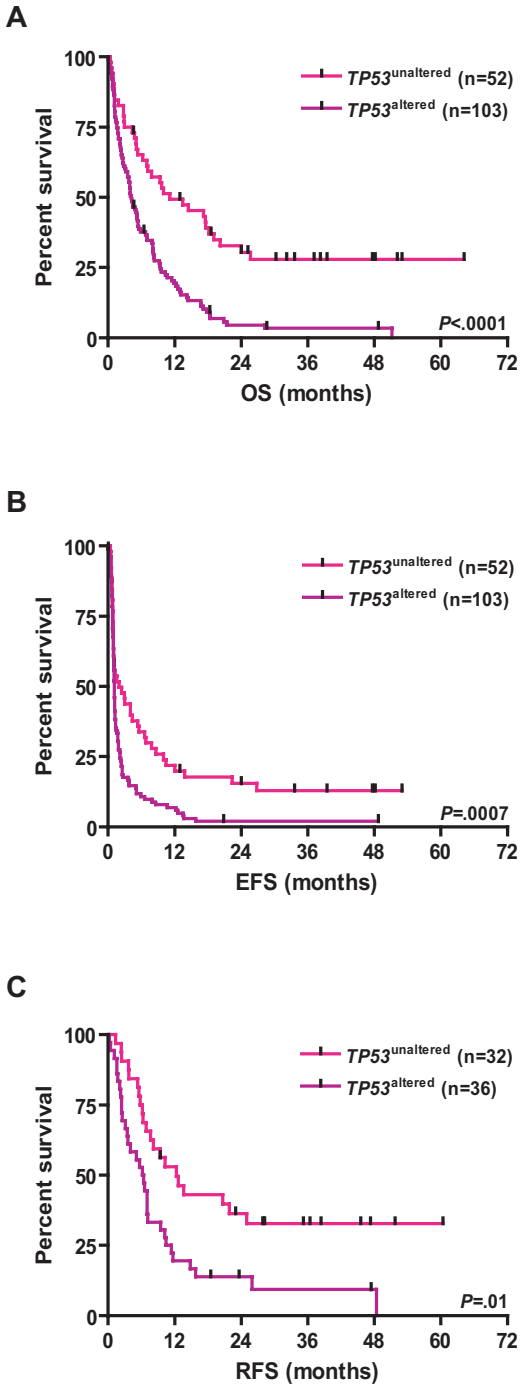

Figure 3. Kaplan-Meier survival estimates according to the *TP53* status. Data are shown for *TP53*^{unaltered} CK-AMLs and *TP53*^{altered} CK-AMLs. (A) OS. (B) EFS. (C) RFS.

Loss of *TP53* was found in approximately 40% of CK-AMLs by array-based techniques, a value that corresponded well with that of 17p abnormalities found on chromosome banding analysis. By DNA sequence analysis, 60% of cases exhibited *TP53* mutations, consistent with previous reports.[6,15,16] Of note, at least two-thirds of mutated cases had biallelic *TP53* alteration resulting from hemizygous, compound heterozygous, and homozygous mutations commonly as a result of homologous recombination leading to UPD. Thus, when assessing for *TP53* mutational status in CK-AML, it will be necessary to include DNA sequence analysis.

TP53^{altered} CK-AMLs were characterized by a significantly higher degree of genomic complexity, as assessed by total number of genomic losses and gains, as well as the frequency of high-level DNA amplifications. This observation fits well into the p53 pathomechanism of genomic instability.[29-35] *TP53*^{altered} CK-AMLs were also associated with specific abnormalities. As previously reported,[15,16,36,37] −5/5q− and/or −7/7q− were significantly more frequent among *TP53*^{altered} CK-AMLs. Because we also applied array-based techniques, we identified additional CNAs associated with *TP53*^{altered} CK-AML, that is, −3/3p−, −16/16q−, −18/18q−, −20/20q−, and gains or amplifications of 1p, 11q, 13q, and 19p. Such genomic pattern associated with *TP53* alterations may pinpoint to candidates cooperating in p53-dependent leukemogenesis.

Recently, the cytogenetic category of "monosomal karyotype" was described, allowing further risk stratification of CK-AML patients.[5] Of note, in our study, CK$^+$/MK$^+$ AMLs were significantly associated with *TP53* alterations, found in 80% of CK$^+$/MK$^+$ AML compared with only 42% in CK$^+$/MK$^-$ AML. Thus, *TP53* alterations appear to be one molecular basis for this purely descriptive cytogenetic subset. The association of *TP53* alterations with CK$^+$/MK$^+$ AML was lost when *TP53* alterations were correlated with CNAs identified by array-based assays. Not unexpectedly, many monosomies described in chromosome banding analysis were not real monosomies but were part of chromosomal material hidden in unbalanced translocations or marker chromosomes.

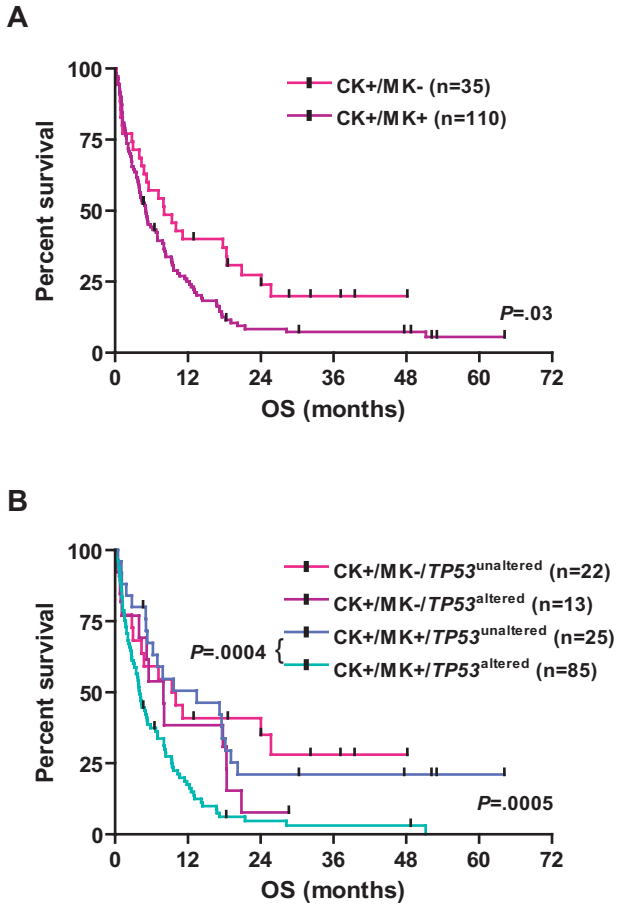

Figure 4. Kaplan-Meier survival estimates according to the cytogenetic status. (A) Data are shown for OS for CK$^+$/MK$^-$ AML and CK$^+$/MK$^+$ AML. (B) Data are shown for OS for the subgroups CK$^+$/MK$^-$/*TP53*^{unaltered}, CK$^+$/MK$^-$/*TP53*^{altered}, CK$^+$/MK$^+$/*TP53*^{unaltered}, and CK$^+$/MK$^+$/*TP53*^{altered}.

Little is known about the pathogenesis of CK-AML, but the high frequency of *TP53* alteration, and in particular biallelic alteration, suggests an important role of p53 in leukemogenesis. Evidence for this hypothesis comes from several observations in mice and human disease: (1) mouse studies requiring biallelic *TP53* inactivation and a concomitant "second hit" for myeloid leukemogenesis[38,39] demonstrated that $p53^{lost}$ myeloid progenitors exhibit aberrant self-renewal, thereby promoting AML[40]; (2) in high-risk MDS and/or AML evolving from a 5q− syndrome, the expansion of preexisting *TP53* mutated subclones was observed[41,42]; and (3) recently, next-generation sequencing of a therapy-related CK-AML genome identified several acquired genetic lesions and a heterozygous intragenic germline *TP53* deletion, becoming homozygous in AML as a result of acquired UPD(17p),[43] a mechanism possibly underlying the sequential *TP53* inactivation in patient 96 (supplemental Figure 1).

Besides being older and having lower BM blasts, $TP53^{altered}$ CK-AML had no distinct clinical phenotype, possibly because of the complexity of concurrent genetic events and different consequences of *TP53* alterations. *TP53* losses or mutations entail various tumor phenotypes,[44] and mouse models investigating *TP53* inactivation identified gain of function for hot spot mutations, such as R175H, R248W, and R273H, as well as increased proliferation related to accelerated tumorigenesis and leukemogenesis, resulting in a more aggressive AML.[35,44-46]

p53 loss of function has been shown to be related to resistance to chemotherapy, also to cytarabine.[46,47] Consistent with this finding, *TP53* alterations in our study were associated with resistance to "3 + 7"-based induction chemotherapy (Tables 1 and 2). Refractory disease was observed in 51% of $CK^+/TP53^{altered}$ compared with 35% of $CK^+/TP53^{unaltered}$ AMLs. In univariable analysis, *TP53* alteration also predicted for inferior OS; median survival times for $CK^+/TP53^{altered}$ and $CK^+/TP53^{unaltered}$ patients were 4.14 and 10.97 months, respectively. In multivariable analysis, *TP53* alteration was by far the strongest prognostic factor for OS, followed by logarithm of WBC and age; of note, the cytogenetic category MK completely lost its prognostic impact. Explorative subset analysis suggested that allogeneic hematopoietic stem cell transplantation had no favorable impact on outcome in $TP53^{altered}$ CK-AML.

TP53 alterations are the most common molecular lesions in CK-AML and predict for resistance to conventional chemotherapy and dismal outcome. *TP53* alterations correlate with specific CNAs and with the MK category. In CK-AML, *TP53* alteration represents the most important prognostic marker, even outweighing the MK category in multivariable analysis. Therefore, *TP53* mutational status should be assessed in clinical trials investigating novel agents to identify compounds that may be effective in this subset of patients.

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Authorship

Contribution: F.G.R. designed and performed research, analyzed and interpreted data, performed statistical analysis, and wrote the manuscript; R.F.S. provided study materials or patients, collected data, and analyzed and interpreted data; L.B. collected, analyzed, and interpreted data; S.K., V.T., V.I.G., P.P., G.H., M.v.L.-T., M.L., J.K., B.S., and A.G. provided study materials or patients and collected data; H.K., M.H., and C.-M.K. performed research and collected data; K.H. designed research and collected data; S.F., T.Z., and P.L. analyzed and interpreted data; and

K.D. and H.D. designed research, provided study materials or patients, collected data, analyzed and interpreted data, and wrote the manuscript.

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