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

Frank G Rücker1; Richard F Schlenk1; Lars Bullinger1; Sabine Kayser1; Veronica Teleanu1; Helena Kett1; Marianne Habdank1; Carla-Maria Kugler1; Karlheinz Holzmann1; Verena I Gaidzik1; Peter Paschka1; Gerhard Held2; Marie von Lilienfeld-Toal3; Michael Lübbert4; Stefan Fröhling1; Thorsten Zenz1,5,6; Jürgen Krauter7; Brigitte Schlegelberger8; Arnold Ganser7; Peter Lichter9; Konstanze Döhner1; Hartmut Döhner1

1 Department of Internal Medicine III, University Hospital of Ulm, Ulm, Germany
2 Department of Internal Medicine I, University of Saarland, Homburg, Germany
3 Department of Internal Medicine III, University Hospital of Bonn, Bonn, Germany
4 Department of Hematology-Oncology, University of Freiburg Medical Center, Freiburg, Germany
5 Department of Translational Oncology, National Center for Tumor Diseases (NCT) and German Cancer Research Center (DKFZ), Heidelberg, Germany.
6 Department of Medicine V, University of Heidelberg, Heidelberg, Germany.
7 Department of Hematology, Hemostasis, Oncology, and Stem Cell Transplantation, Hannover Medical School, Hannover, Germany
8 Institute of Cell and Molecular Pathology, Hannover Medical School, Hannover, Germany
9 Division of Molecular Genetics, German Cancer Research Center, Heidelberg, Germany

This study was supported in part by the Bundesministerium für Bildung und Forschung (BMBF), Grant No. 01GS0439 NGFN2 and Grant No. 01GS0871 NGFNplus

Corresponding author: Dr. Hartmut Döhner
Department of Internal Medicine III, University Hospital of Ulm
Albert-Einstein-Allee 23, 89081 Ulm, Germany
Phone: 49.731.500.45501; Fax: 49.731.500.45505
E-mail: hartmut.doehner@uniklinik-ulm.de

Running head: TP53 mutation and outcome in complex karyotype AML

The authors declare the originality of this work and that there are no conflicts of interest
ABSTRACT

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-AML. TP53 mutations were found in 141/234 (60%) and TP53 losses were identified in 94/234 (40%) CK-AML; in total, 164/234 (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-AML, 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.
INTRODUCTION

Chromosomal abnormalities are found in about 55% of adult patients with acute myeloid leukemia (AML) and are among the most important independent prognostic factors(1-3). AML exhibiting three 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-AML account for 10% to 15% of adult AML, and the frequency increases with age. CK-AML belong to the cytogenetic adverse-risk group, since they are associated with very poor outcome when treated with intensive or non-intensive 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-AML 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 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-AML (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.
PATIENTS AND 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). One-hundred and thirty-three patients had de novo AML, 31 secondary AML (s-AML) following 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 to 60 years); 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).

Due to evolving technology that occurred in the course of the study, we switched from array-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, Santa Clara, CA). Genotyping console version 2.0 (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*, denaturing high-performance liquid chromatography (DHPLC) was performed as previously described(25). Aberrant profiles were verified by bidirectional sequencing and compared with wildtype sequence (GenBank; X54156). Mutations were described using two different databases (IARC TP53 Database; p53.iarc.fr and The TP53 Web Site; p53.free.fr)(26,27). Subcloning analyses using the TOPO® TA Cloning® Kit and resequencing were performed according to manufacturer’s protocols (Invitrogen, Carlsbad, CA).

**Statistical analyses**

The section on statistical analyses is provided in the Supplementary Information.
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 vast majority were missense mutations (n=130), followed by deletions/insertions (n=21) [17 resulted in premature stop; four 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 two or more **TP53** mutations exhibited no **TP53** loss; five 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 one allele and at least one mutation in the remaining allele; 47% (79/168)] were more frequent than homozygous mutations [26% (43/168)], heterozygous [18% (30/168); include possible compound heterozygous mutations among the 4 patients with more than one heterozygous mutation], and compound heterozygous mutations [10% (16/168)]; 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% (27/27) versus 79% (90/114), *P*=.008].

**Copy number alterations (CNAs) and copy number neutral loss of heterozygosity (uniparental disomy, 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 94/234 (40%) cases. UPD(17p) (8.14 to 22.50 Mb in size) encompassing the TP53 locus was detected in 15/103 (15%) cases analyzed by SNP-arrays.

**Biallelic TP53 alteration**

Combining mutational and microarray findings, 164/234 (70%) CK-AML exhibited TP53 alteration (mutation and/or loss of TP53); 71/164 (43%) cases had biallelic TP53 alteration by hemizygous mutation pattern, and 38/70 (54%) cases without TP53 loss exhibited homozygous TP53 mutation caused by UPD(17p) in 15/19 SNP-analyzed cases. In the four cases lacking evidence for UPD(17p) in SNP-profiling, homozygous TP53 mutations possibly resulted from intragenic loss of heterozygosity. Furthermore, 19/131 (15%) cases analyzed by array-CGH on DNA sequence analysis exhibited homozygous TP53 mutation that are likely 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 eight patients. Together, at least 117/164 (71%) TP53-altered CK-AML 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 frame shift 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 (Supplementary 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 as identified by conventional cytogenetics, and with the pattern of CNAs as detected by array-based analyses (Table 1).

*Correlation with chromosome abnormalities.* TP53 alterations were identified in 157/219 (72%) CK-AML that could be analyzed by conventional cytogenetics. TP53*altered* CK-AML had a higher degree of genomic complexity as measured by total number of aberrations (≥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 versus 4.00±4.88, \(P<.0001\)), gains (3.91±3.80 versus 1.94±1.92, \(P<.0001\)), high-level DNA amplifications (0.84±1.31 versus 0.21±0.83, \(P=.0002\)), and genomic complexity as measured by total number of aberrations per case (14.30±9.41 versus 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 monosomal karyotype (MK)**

By conventional cytogenetics, 171/219 (78%) CK-AML fulfilled the MK criteria (CK+/MK+ AML) as previously defined(5). TP53 alterations were found in 137/171 (80%)
CK+/MK+ AML, and in only 20/48 (42%) CK+/MK- AML (P<.0001) (Table 1). Compared with CK+/MK- AML, CK+/MK+ AML were characterized by a higher degree of genomic complexity determined by cytogenetics [≥5 aberrations, 88% (151/171) versus 54% (26/48), P<.0001] and by genomic profiling as measured by total number of losses (mean±SD) (9.29±7.40 versus 3.67±5.72; P<.0001) and aberrations per case (13.59±9.61 versus 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 [75/234 (32%)], due to the fact that 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 59/75 (79%) CK+/molMK+ AML, and in 105/159 (66%) CK+/molMK- AML (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)]. Since there were no significant differences regarding clinical characteristics, response to therapy, and survival for TP53 monoallelic altered and TP53 biallelic altered CK-AML (see Supplementary Table 1 and Supplementary 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 versus 54 years, P=.002) and had lower BM blast counts (median 65% versus 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), early/hypoplastic death (ED/HD) 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
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 (OR, 0.55; 95%-CI, 0.30 to 1.00; P=.05) and age (OR for a 10 years difference, 0.67; 95%-CI, 0.52 to 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 to 51.4); 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% to 10%), 17% (95%-CI, 9% to 31%) and 12% (95%-CI, 7% to 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), gains of 1p (P=.04), and amp(11)(q13~25) (P=.05). LDH and WBC did not impact OS. Among CK+/MK+ AML, 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 (HR, 2.43; 95%-CI, 1.56 to 3.77; P=.0001), logarithm of WBC (HR, 1.62; 95%-CI 1.17 to 2.26; P=.004), and age (HR for 10 years difference, 1.26; 95%-CI, 1.01 to 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 (HSCT) in first CR was performed in 30 CK-AML patients. Of those, 14/15 TP53 altered CK-AML relapsed and died, whereas in
TP53\textsuperscript{altered} CK-AML 9/15 relapsed and died (\(P=.04\)). This translated into significantly worse OS for TP53\textsuperscript{altered} CK-AML (\(P=.04\)) (Supplementary Figure 3).
DISCUSSION

In our series of 234 CK-AML, 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.

Loss of TP53 was found in about 40% of CK-AML by array-based techniques, a figure that corresponded well to 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 mutations, compound heterozygous, and from 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-AML 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-AML 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-AML. Since 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+ AML 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.

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<sup>lost</sup> 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 pre-existing *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 (Supplementary Figure 1).

Besides being older and having lower BM blasts, *TP53<sup>altered</sup>* CK-AML had no distinct clinical phenotype, possibly due to the complexity of concurrent genetic events and to 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<sup>+</sup>/TP53<sup>altered</sup> compared with 35% of CK<sup>-</sup>/TP53<sup>altered</sup> AML. In univariable analysis, *TP53* alteration also predicted for inferior OS,
median survival times for CK+/TP53\textsuperscript{altered} and CK+/TP53\textsuperscript{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 HSCT had no favorable impact on outcome in TP53\textsuperscript{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.
ACKNOWLEDGMENTS

The authors thank all members of the German-Austrian AML Study Group (AMLSG) for their participation in this study and for providing patient samples. The following AML Study Group institutions and investigators participated in this study:

Daniel Oruzio, MD, Klinikum Augsburg, Augsburg, Germany; Dietmar Reichert, MD, Ubb-Emmius-Klinik Aurich, Aurich, Germany; Jörg Westermann, MD, Charité Berlin, Berlin, Germany; Christian Teschendorf, MD, Klinikum Bochum, Bochum, Germany; Ingo Schmidt-Wolf, MD, Universitätsklinikum Bonn, Bonn, Germany; Florian Lordick, MD, Städtisches Klinikum Braunschweig, Braunschweig, Germany; Bernd Hertenstein, MD, Klinikum Bremen-Mitte, Bremen, Germany; Helga Bernhard, MD, Klinikum Darmstadt, Darmstadt, Germany; Ulrich Germing, MD, Universitätsklinikum Düsseldorf, Düsseldorf, Germany; Carsten Schwänen, MD, Klinikum Esslingen, Esslingen, Germany; Mohammed Wattad, MD, Kliniken Essen Süd, Ev. Krankenhaus Essen-Werden gGmbH, Essen, Germany; Elke Jäger, MD, Krankenhaus Nordwest GmbH, Frankfurt, Germany; Michael Lübbert, MD, Universitätsklinikum Freiburg, Freiburg, Germany; Andrea Distelrath, MD, Klinikum Fulda, Fulda, Germany; Volker Runde, MD, Wilhelm-Anton-Hospital, Goch, Germany; Detlef Haase, MD, Universitätsklinikum Göttingen, Göttingen, Germany; Walter Fiedler, MD, Klinikum Hanau, Hanau, Germany; Hans Salwender, MD, Allgemeines Krankenhaus Altona, Hamburg, Germany; Elisabeth Lange, MD, Klinikum Hamm, Hamm, Germany; Andreas Sendler, MD, Klinikum Hanau, Hanau, Germany; Hartmut Kirchner, MD, Krankenhaus Siloah, Hannover, Germany; Uwe Martens, SLK-Kliniken GmbH Heilbronn, Heilbronn, Germany; Michael Pfreundschuh, MD, Universitätsklinikum Homburg/Saar, Homburg, Germany; David Nachbaur, Universität Innsbruck, Austria; Martin Bentz, MD, Städtisches Klinikum Karlsruhe, Karlsruhe, Germany; Stefan Kremers, MD, Caritas-Krankenhaus Lebach, Lebach, Germany; Frank Hartmann, MD, Klinikum Lemgo, Lemgo, Germany; Andreas Petzer, MD, Krankenhaus der Barmherzigen Schwestern Linz, Linz, Austria; Gerhard Heil, MD, Klinikum Lüdenscheid, Lüdenscheid, Germany; Thomas Fischer, MD, Klinikum Magdeburg, Magdeburg, Germany; Thomas Kindler, MD, Universitätsklinikum Mainz, Mainz, Germany; Martin Grießhammer, MD, Klinikum Minden, Minden, Germany; Katharina Götze, MD, Technische Universität München, München, Germany; Ali-Nuri Hünerlithürkoglu, Lukaskrankenhaus Neuss, Neuss, Germany; Claus-Henning Körne, MD, Klinikum Oldenburg, Oldenburg, Germany; Thomas Südhoff, MD, Klinikum Passau, Passau, Germany; Karin Corduan, MD, Elisabeth Krankenhaus Recklinghausen, Recklinghausen, Germany; Michael Schenk, MD, Krankenhaus der Barmherzigen Brüder, Regensburg, Germany; Artur Wehmeier, MD,
Klinikum Remscheid, Remscheid; Germany; Axel Matzdorff, MD, Caritas-Klinik St Theresia, Saarbrücken, Germany; Richard Greil, MD, Salzburger Landeskliniken, Salzburg, Austria; Wolfgang Grimminger, MD, Klinikum Schwäbisch-Gmünd, Mutlangen, Germany; Hans-Günther Mergenthaler, MD, Klinikum Stuttgart, Stuttgart, Germany; Else Heidemann, MD, Diakonie-Klinikum Stuttgart. Stuttgart, Germany; Heinz Kirchen, MD, Krankenhaus der Barmherzigen Brüder, Trier, Germany; Helmut Salih, MD, Universitätsklinikum Tübingen, Tübingen, Germany; Wolfgang Brugger, MD, Klinikum Villingen-Schwenningen, Villingen-Schwenningen, Germany; Elisabeth Koller, MD, Hanuschkrankenhaus, Vienna, Austria; and Aruna Raghavachar, MD, Helios Klinikum Wuppertal, Wuppertal, Germany.
AUTHORSHIP CONTRIBUTIONS

Frank G. Rücker: designed research, performed research, analyzed and interpreted data, performed statistical analysis, and wrote the manuscript

Richard F. Schlenk: provided study materials or patients, collected data, analyzed and interpreted data

Lars Bullinger: collected data, analyzed and interpreted data

Sabine Kayser: provided study materials or patients, collected data

Veronica Teleanu: provided study materials or patients, collected data

Helena Kett: performed research, collected data

Marianne Habdank: performed research, collected data

Carla-Maria Kugler: performed research, collected data

Karlheinz Holzmann: designed research, collected data

Verena I. Gaidzik: provided study materials or patients, collected data

Peter Paschka: provided study materials or patients, collected data

Gerhard Held: provided study materials or patients, collected data

Marie von Lilienfeld-Toal: provided study materials or patients, collected data

Michael Lübbert: provided study materials or patients, collected data

Stefan Föhling: analyzed and interpreted data

Thorsten Zenz: analyzed and interpreted data

Jürgen Krauter: provided study materials or patients, collected data

Brigitte Schlegelberger: provided study materials or patients, collected data

Arnold Ganser: provided study materials or patients, collected data

Peter Lichter: analyzed and interpreted data

Konstanze Döhner: designed research, provided study materials or patients, collected data, analyzed and interpreted data, and wrote the manuscript

Hartmut Döhner: designed research, provided study materials or patients, collected data, analyzed and interpreted data, and wrote the manuscript

All authors approved the manuscript.

CONFLICT OF INTEREST DISCLOSURES

The authors declare no competing financial or other interests.
CORRESPONDECE

Hartmut Döhner, M.D., Department of Internal Medicine III, University Hospital of Ulm, Albert-Einstein-Allee 23, 89081 Ulm, Germany; phone: 49.731.500.45501; fax: 49.731.500.45505, e-mail: hartmut.doehner@uniklinik-ulm.de
REFERENCES


Table 1: Genetic and clinical characteristics according to TP53 alteration

<table>
<thead>
<tr>
<th></th>
<th>TP53 unaltered</th>
<th>TP53 altered</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytogenetics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥5 aberrations</td>
<td>38 (61%)</td>
<td>139 (69%)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Marker chromosomes</td>
<td>29 (47%)</td>
<td>114 (73%)</td>
<td>.0005</td>
</tr>
<tr>
<td>-5/6q</td>
<td>20 (32%)</td>
<td>124 (79%)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>-7/7q</td>
<td>25 (40%)</td>
<td>81 (52%)</td>
<td>.14</td>
</tr>
<tr>
<td>-5/6q and -7/7q</td>
<td>12 (19%)</td>
<td>70 (45%)</td>
<td>.0008</td>
</tr>
<tr>
<td>-20/20q</td>
<td>8 (13%)</td>
<td>44 (28%)</td>
<td>.02</td>
</tr>
<tr>
<td>Monosomal karyotype</td>
<td>34 (55%)</td>
<td>137 (67%)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Array-based genomics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n=70</td>
<td>n=164</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total number of losses (mean±SD)</td>
<td>4.00±4.88</td>
<td>9.54±7.49</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Total numbers of gains (mean±SD)</td>
<td>1.94±1.92</td>
<td>3.91±3.80</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Total numbers of amplifications (mean±SD)</td>
<td>0.21±0.83</td>
<td>0.84±1.31</td>
<td>.0002</td>
</tr>
<tr>
<td>Total numbers of genomic aberrations (mean±SD)</td>
<td>6.10±5.53</td>
<td>14.30±9.41</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>-3/3p</td>
<td>7 (10%)</td>
<td>47 (29%)</td>
<td>.002</td>
</tr>
<tr>
<td>-5/6q</td>
<td>20 (25%)</td>
<td>127 (77%)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>-7/7q</td>
<td>26 (37%)</td>
<td>97 (59%)</td>
<td>.003</td>
</tr>
<tr>
<td>-5/6q and -7/7q</td>
<td>13 (15%)</td>
<td>87 (53%)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>-11/11q</td>
<td>13 (19%)</td>
<td>22 (13%)</td>
<td>.32</td>
</tr>
<tr>
<td>-12/12p</td>
<td>13 (19%)</td>
<td>52 (32%)</td>
<td>.06</td>
</tr>
<tr>
<td>-16/16q</td>
<td>5 (7%)</td>
<td>61 (37%)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>-18/18q</td>
<td>9 (13%)</td>
<td>56 (34%)</td>
<td>.0008</td>
</tr>
<tr>
<td>-20/20q</td>
<td>8 (11%)</td>
<td>47 (29%)</td>
<td>.004</td>
</tr>
<tr>
<td>+1/1p</td>
<td>3 (4%)</td>
<td>34 (21%)</td>
<td>.001</td>
</tr>
<tr>
<td>+8+8q</td>
<td>21 (30%)</td>
<td>46 (28%)</td>
<td>.75</td>
</tr>
<tr>
<td>+9/9p</td>
<td>11 (16%)</td>
<td>17 (10%)</td>
<td>.27</td>
</tr>
<tr>
<td>+11/11q</td>
<td>7 (10%)</td>
<td>54 (33%)</td>
<td>.0002</td>
</tr>
<tr>
<td>+13/13q</td>
<td>3 (4%)</td>
<td>20 (10%)</td>
<td>.02</td>
</tr>
<tr>
<td>+19/19p</td>
<td>3 (4%)</td>
<td>22 (13%)</td>
<td>.04</td>
</tr>
<tr>
<td>+21/21q</td>
<td>7 (10%)</td>
<td>32 (20%)</td>
<td>.09</td>
</tr>
<tr>
<td>+22/22q</td>
<td>6 (9%)</td>
<td>27 (16%)</td>
<td>.15</td>
</tr>
<tr>
<td>amp(8q24)</td>
<td>4 (6%)</td>
<td>4 (2%)</td>
<td>.24</td>
</tr>
<tr>
<td>amp(11)(q13-25)</td>
<td>0 (0%)</td>
<td>22 (13%)</td>
<td>.0004</td>
</tr>
<tr>
<td>amp(21)(q22)</td>
<td>3 (4%)</td>
<td>19 (12%)</td>
<td>.09</td>
</tr>
<tr>
<td>Molecular monosomal karyotype</td>
<td>16 (23%)</td>
<td>50 (36%)</td>
<td>.07</td>
</tr>
<tr>
<td>Molecular genetics</td>
<td>n=50</td>
<td>n=99</td>
<td></td>
</tr>
<tr>
<td>FLT3-ITD positive</td>
<td>3 (6%)</td>
<td>1 (1%)</td>
<td>.11</td>
</tr>
<tr>
<td>FLT3-TKD mutation</td>
<td>3 (6%)</td>
<td>1 (1%)</td>
<td>.11</td>
</tr>
<tr>
<td>NPM1 mutation</td>
<td>3 (6%)</td>
<td>0 (0%)</td>
<td>.04</td>
</tr>
<tr>
<td>Clinical data</td>
<td>n=52</td>
<td>n=103</td>
<td></td>
</tr>
<tr>
<td>Gender (male/female)</td>
<td>26 (50%)/26 (50%)</td>
<td>54 (52%)/49 (48%)</td>
<td>.87</td>
</tr>
<tr>
<td>Age (years, median)</td>
<td>54</td>
<td>61</td>
<td>.002</td>
</tr>
<tr>
<td>AML history</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>De novo</td>
<td>36 (60%)</td>
<td>76 (74%)</td>
<td>.57</td>
</tr>
<tr>
<td>Secondary</td>
<td>6 (12%)</td>
<td>9 (9%)</td>
<td>.58</td>
</tr>
<tr>
<td>Therapy-related</td>
<td>9 (17%)</td>
<td>16 (10%)</td>
<td>.32</td>
</tr>
<tr>
<td>WBC (10^3/L, median)</td>
<td>12.9</td>
<td>6.5</td>
<td>.18</td>
</tr>
<tr>
<td>Platelet count (10^9/L, median)</td>
<td>48</td>
<td>41</td>
<td>.46</td>
</tr>
<tr>
<td>Hemoglobin (g/dL, median)</td>
<td>9.1</td>
<td>8.9</td>
<td>.38</td>
</tr>
<tr>
<td>BM blast count (%) median)</td>
<td>76</td>
<td>65</td>
<td>.04</td>
</tr>
<tr>
<td>PB blast count (%) median)</td>
<td>45</td>
<td>30</td>
<td>.18</td>
</tr>
<tr>
<td>LDH serum level (U/L, median)</td>
<td>391</td>
<td>488</td>
<td>.25</td>
</tr>
</tbody>
</table>
Table 1 continued

<table>
<thead>
<tr>
<th>Response</th>
<th>TP53 unaltered</th>
<th>TP53 altered</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=52</td>
<td>n=103</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CR after induction therapy</td>
<td>26 (50%)</td>
<td>29 (28%)</td>
<td>.01</td>
</tr>
<tr>
<td>RD after induction therapy</td>
<td>18 (35%)</td>
<td>53 (51%)</td>
<td>.06</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Outcome</th>
<th>n=52</th>
<th>n=103</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>OS</td>
<td>10.97</td>
<td>4.14</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>(months, median)</td>
<td>26</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>(3-year survival rate, %)</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>EFS</td>
<td>1.94</td>
<td>1.12</td>
<td>.0007</td>
</tr>
<tr>
<td>(months, median)</td>
<td>13</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>(3-year survival rate, %)</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>RFS</td>
<td>12.16</td>
<td>6.51</td>
<td>.01</td>
</tr>
<tr>
<td>(months, median)</td>
<td>30</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>(3-year survival rate, %)</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: ITD, internal tandem duplication; TKD, tyrosine kinase domain; WBC, white blood cell count; BM, bone marrow; PB, peripheral blood; LDH, lactate dehydrogenase; CR, complete remission; RD, refractory disease; OS, overall survival; EFS, event-free survival; RFS, relapse-free survival.
Table 2: Multivariable analyses of outcome

<table>
<thead>
<tr>
<th></th>
<th>Response</th>
<th></th>
<th>OS</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR</td>
<td>P value</td>
<td>HR</td>
<td>P value</td>
</tr>
<tr>
<td>CK-AML</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TP53 alteration</td>
<td>0.55</td>
<td>.05</td>
<td>2.43</td>
<td>.0001</td>
</tr>
<tr>
<td>Age (difference of 10 years)</td>
<td>0.67</td>
<td>.003</td>
<td>1.26</td>
<td>.04</td>
</tr>
<tr>
<td>s/t-AML</td>
<td>0.67</td>
<td>.24</td>
<td>1.05</td>
<td>.81</td>
</tr>
<tr>
<td>Logarithm of WBC</td>
<td>0.74</td>
<td>.19</td>
<td>1.62</td>
<td>.004</td>
</tr>
<tr>
<td>Logarithm of platelets</td>
<td>0.76</td>
<td>.39</td>
<td>1.13</td>
<td>.62</td>
</tr>
<tr>
<td>Monosomal karyotype $^6$</td>
<td>0.75</td>
<td>.43</td>
<td>0.87</td>
<td>.57</td>
</tr>
</tbody>
</table>

Abbreviations: Response, CR achievement after induction chemotherapy; OS, overall survival; OR, odds ratio; HR, hazard ratio; s/t-AML, secondary or therapy-related AML; WBC, white blood cell count

$^6$ Monosomal karyotype determined by chromosome banding analysis
Figure legends

**Figure 1. Mapping of 168 TP53 mutations in 141 CK-AML.** Hemizygous mutations are indicated in the lower panel, heterozygous and/or homozygous mutations are marked in the upper 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 indicates homozygous mutations, blue italic indicates frameshift mutations leading to a premature stop codon.

**Figure 2. Relative frequencies and pairwise co-occurrences of TP53 alteration-associated genomic aberrations illustrated using Circos Table Viewer v0.52(28).** The percentages indicate the proportion of each aberration associated with A: TP53\textsubscript{altered} CK-AML and B: TP53\textsubscript{unaltered} CK-AML. Unaltered TP53 and amp(11)(q13~q25) were mutually exclusive. MK indicates monosomal karyotype based on cytogenetics analysis.

**Figure 3. Kaplan-Meier survival estimates according to the TP53 status.** Data are shown for TP53\textsubscript{unaltered} CK-AML and TP53\textsubscript{altered} CK-AML for A: Overall survival (OS), B: Event-free survival (EFS), and C: Relapse-free survival (RFS).

**Figure 4. Kaplan-Meier survival estimates according to the cytogenetic status.** A: Data are shown for overall survival (OS) for CK+/MK- AML and CK+/MK- AML, B: Data are shown for overall survival (OS) for the subgroups CK+/MK- TP53\textsubscript{unaltered}, CK+/MK- TP53\textsubscript{altered}, CK+/MK+/TP53\textsubscript{unaltered}, and CK+/MK+/TP53\textsubscript{altered}. 
Figure 1

For personal use only.on September 14, 2017. By guest www.bloodjournal.org From
Figure 3

A

Percent survival

TP53\textsuperscript{unaltered} (n=52)

TP53\textsuperscript{altered} (n=103)

P<.0001

OS (months)

B

Percent survival

TP53\textsuperscript{unaltered} (n=52)

TP53\textsuperscript{altered} (n=103)

P=.0007

EFS (months)

C

Percent survival

TP53\textsuperscript{unaltered} (n=32)

TP53\textsuperscript{altered} (n=36)

P=.01

RFS (months)
Figure 4

A

- CK+/MK- (n=35)
- CK+/MK+ (n=110)

P = .03

B

- CK+/MK- /TP53\textit{un}altered (n=22)
- CK+/MK+/TP53\textit{al}tered (n=13)
- CK+/MK+ /TP53\textit{un}altered (n=25)
- CK+/MK+/TP53\textit{al}tered (n=85)

P = .0004

P = .0005
TP53 alterations in acute myeloid leukemia with complex karyotype correlate with specific copy number alterations, monosomal karyotype, and dismal outcome

Frank G Rücker, Richard F Schlenk, Lars Bullinger, Sabine Kayser, Veronica Teleanu, Helena Kett, Marianne Habdank, Carla-Maria Kugler, Karlheinz Holzmann, Verena I Gaidzik, Peter Paschka, Gerhard Held, Marie von Lilienfeld-Toal, Michael Lübbert, Stefan Fröhling, Thorsten Zenz, Jürgen Krauter, Brigitte Schlegelberger, Arnold Ganser, Peter Lichter, Konstanze Döhner and Hartmut Döhner