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TP53 mutations and *MDM2*^{SNP309} identify subgroups of AML patients with impaired outcome

Running title: TP53 mutations and MDM2^{SNP309} in AML

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Abstract

Background: *TP53* is commonly mutated in several cancers and confers treatment resistance and poor prognosis. Altered expression of mouse double minute 2 (*MDM2*), a negative regulator of p53, may also attenuate normal p53 signaling, thereby enhancing tumor transformation and resistance to apoptosis. The single nucleotide polymorphism (SNP) 309 has been reported to increase *MDM2* expression and impair normal p53 response. Experimental design: We investigated the frequency and impact of *TP53* mutations (*TP53mut*) and *MDM2*^{SNP309} on treatment outcome and overall survival (OS) in 189 Swedish AML patients. The genetic analyses were performed using SSCA and direct sequencing (for mutations in exon 5-8 of *TP53*) and Pyrosequencing (for the *MDM2*^{SNP309}). Results: We found a high frequency (22%) of *TP53mut* in patients with cytogenetic aberrations, with association to high-risk cytogenetics ($p<0.001$). *TP53mut* patients had lower response rates (22% compared with 76% CR in *TP53* wild-type (wt) patients, $p<0.001$), and reduced OS (2 and 16 months, respectively, $p<0.001$). In *TP53wt* patients with high or intermediate risk cytogenetic aberrations, the *MDM2*^{SNP309} conferred an impaired outcome, with patients carrying the alternative G allele having shorter OS compared with T/T patients (median 9 vs. 50 months, $p=0.020$). Conclusions: Our results show that *TP53mut* analysis and *MDM2*^{SNP309} genotyping may be useful tools for prognostication, risk stratification and selection of patients most likely to benefit from new drugs targeting the p53 signaling pathway.

Key words: AML, *TP53*, *MDM2*, SNP309, prognostic markers

Introduction

TP53 is a common gene to be altered in human cancer; about 50% of solid tumors have altered *TP53* gene. Mutations in the *TP53* gene are infrequent in AML with normal karyotype (1), however, in AML with a complex karyotype, *TP53* is the most frequently altered gene and some studies confirm 70% deletions and/or mutations (1, 2). The p53 protein plays a key role as a tumor suppressor and functions as a transcription factor controlling genes involved in preventing cancer formation (3). In hematologic malignancies, *TP53* alterations cause resistance to chemotherapy and poor survival (4). The oncoprotein MDM2 (mouse double minute 2) is a negative regulator of p53. In unstressed cells, the p53 levels are normally very low and regulated by MDM2 mediated ubiquitination of p53. In the absence of stress, p53 and MDM2 are linked to each other through an autoregulatory negative feedback loop that maintains a low concentration level (5). In stressed cells, p53 is very rapidly activated and accumulates in the nucleus, activating transcription of target genes (5). MDM2 has been reported to be overexpressed in AML and this may attenuate the p53 signaling pathway and enhance tumor transformation and resistance to apoptosis (6, 7). A single nucleotide polymorphism (SNP309, *rs2279744*) located in the promoter region of the *MDM2* gene is shown to contribute in regulation of MDM2 levels and influence cellular p53 levels. The SNP309 is a T to G transversion, where the G allele binds with a higher affinity to the SP1 transcription factor than the wild-type T allele, resulting in increased *MDM2* mRNA and protein levels and subsequently a decreased p53 level affecting the cellular response to DNA damage (8). Therefore, the *MDM2*^{SNP309} has potential as a clinical biomarker in AML, with the aim of future treatment individualization and improvement of patient outcomes.

To evaluate the impact of *TP53* mutations and *MDM2*^{SNP309} for response to chemotherapy and overall survival we performed *TP53* mutation analysis and *MDM2* genotyping for the SNP309 on a study group of 189 unselected AML patients.

Material and Methods

Patients

This retrospective study included 189 Swedish patients diagnosed with AML between 1988 and 2010 (median age at diagnosis 64 years, range 19-88). Patients were diagnosed and treated at Linköping University Hospital (n=102) or Karolinska University Hospital in Huddinge (n=87). The study was conducted in compliance with the Helsinki declaration and approved by the local ethical committees. Patient characteristics are summarized in **Table I**. Bone marrow or peripheral blood samples collected at diagnosis were used to isolate DNA for the genetic analysis. Cytogenetic and molecular genetic findings were used to determine risk group as defined by ELN (European Leukemia Net) (9), taking other prognostic factors into account such as age, performance status and comorbidity, with minor modifications (see Swedish Hematology Association guidelines, <http://www.sfhem.se/upl/files/103791.pdf> accessed 2014-09-05). Thus, cytogenetically normal (CN) AML patients with *FLT3*-ITD negative/*NPM1* mutation positive genotype were classified as low risk, and *FLT3*-ITD positive/*NPM1* mutation negative CN-AML patients or patients with AML secondary to myelodysplastic syndrome (MDS) were classified as high risk. *FLT3*-ITD positive/*NPM1* mutation positive or *FLT3*-ITD negative/*NPM1* mutation negative CN-AML were classified as intermediate risk. From 2005, Swedish AML patients have been treated according to nationwide AML treatment guidelines (<http://www.sfhem.se/upl/files/103791.pdf> accessed 2014-09-05) with the majority of the patients receiving induction treatments including daunorubicin 60mg/m² once a day for three days combined with Cytarabine (AraC) as 1000mg/m² twice a day in 2h i.v infusions for 5 days. Patients diagnosed before 2005 have been treated according to regional guidelines which most commonly included AraC doses of 200 mg/m² as 24 h i.v infusions for 7 days combined with either daunorubicin or idarubicin (10). Other drugs used in combination with AraC and/or

Daunorubicin/Idarubicin included Mitoxantrone, Etoposide, Cladribine and 6-thioguanine. All patients received induction treatment with curative intent. For treatment details, see **Table II**. Treatment response was determined in all but 8 patients, defined as non-complete remission (no CR) or morphologic complete remission (CR) (9).

Genomic DNA extraction and *TP53* mutation detection

Bone marrow or peripheral blood samples were collected at diagnosis from 189 AML patients and mononuclear cells were separated by Ficoll-Paque gradient centrifugation and genomic DNA was extracted. The DNA was amplified by a PCR for the DNA binding domain (exon 5-8) to identify mutations in *TP53*. Five different PCRs were carried out in a volume of 10 µl containing 10-20 ng DNA, 5 µl HotStar Taq Master Mix (Qiagen, Sollentuna, Sweden), 20 µM for each of the primers, forward primer (TTCAACTCTGTCTCCTTCCT) and reverse primer (ACTGCTTG TAGATGGCCATG) for exon 5A and forward primer (GTGCAGCTGTGGGTTGATTC) and reverse primer (CAGCCCTGTCGTCTCTCCAG) for exon 5B and forward primer (TTGCCCAGGGTCCCCAGGCC) and reverse primer (AACCCCTCCTCCCAGAGAC) for exon 6 and forward primer (CCTCCCCTGCTTGCCACAGG) and reverse primer (GGAAGAAATCGGTAAGAGGTGG) for exon 7 and forward primer (CTGCCTCTTGCTTCTCTTTT) and reverse primer (CTCCTCCACCGCTTCTTGTC) for exon 8. The cycling conditions for each of the reactions were an initial denaturation at 95° for 15 min followed by 35 cycles at 94°C for 40s, 55°C for 40s, 72°C for 60s and an end extension at 72° for 5 min.

To detect the *TP53* status, the single strand conformation analysis (SSCA) was performed. 1 µl PCR product was labeled with ³²P-dATP and the same primers as earlier were used in a

secondary PCR reaction for 15-20 cycles. Labeled PCR products were diluted 20 times with loading solution (0.1% SDS, 10 mM EDTA, 50% formamide, xylene-cyanol and bromo-phenol blue). The DNA samples were heat denatured 95° for 3 min and rapidly cooled and the products were subsequently separated on a 6% non-denaturing polyacrylamide gel containing 10% glycerol and the fragments were separated at 6 W for 16 hours at room temperature followed by autoradiography. Samples with mobility shift were sequenced to determine the exact nucleotide sequence and compared to the corresponding *TP53* reference sequence (NM_000546.4).

***MDM2* SNP309 genotyping**

PCR amplification of the *MDM2* gene region covering the promoter SNP309 (*rs2279744*) was performed using the HotStarTaq master mixture (VWR International, Stockholm, Sweden) with primers and MgCl₂ at final concentrations of 0.4 mM and 1.5 mM, respectively, in a total volume of 10 µL. The PCR primers were forward CGGGAGTTCAGGGTAAAGGT and reverse biotin-TCGGAACGTGTCTGAACTTG. The SNP was analyzed using Pyrosequencing on a PyroMark 96MD instrument (Qiagen, Sweden) according to the manufacturers' instructions. Briefly, the biotinylated DNA strand was isolated using the Pyrosequencing Vacuum Prep Work Station (Qiagen, Sweden) and the sequencing primer CAGGGTAAAGGTCACG was annealed to the template at 80°C for 2 minutes. After cooling to room temperature, the sample plate was transferred to the Pyrosequencing instrument and sequencing was performed with the nucleotide dispensing order TGGCGGCTGCGGCGCTGTC. The genotype frequencies found in our patient material was compared to a material of 214 healthy controls.

Statistical analysis

Chi² or Fisher's exact test was used to compare genotype distribution between patients with CR and no CR, between patients and controls (for *MDM2*^{SNP309}), and to compare the distributions of categorical variables between genotype groups. Analysis of the probability of CR in *TP53* mutated and wild-type patients were further investigated using logistic regression analysis, adjusted for age and risk group. Odds ratios with 95%CI are presented. Nonparametric independent samples median tests were used to compare median age between genotype groups. Kaplan Meier survival analysis was used together with the log rank test for significance to investigate the impact of *MDM2* genotype and *TP53* mutation on overall survival times (OS) (calculated as time from diagnosis until death or date of the latest follow-up (for patients still alive)). The univariate analysis of *MDM2*^{SNP309} was done in the entire patient material, as well as in patients with cytogenetic aberrations stratified in risk groups. This was based on the hypothesis that abnormal karyotypes would indicate a disruption in the DNA repair systems, in which normal function of MDM2 is a factor. In the Kaplan Meier analysis of OS differences between *MDM2* genotypes, patients with *TP53* mutations were excluded. Patients treated by allo-SCT (n=59) were censored at the time of transplantation in the Kaplan Meier analysis. Multivariable Cox regression analysis was performed using a forced entry method including age, risk group, treatment (chemotherapy only vs. chemotherapy followed by allo-SCT), *MDM2*^{SNP309} genotype and *TP53* mutation as covariates. Based on Kaplan-Meier results, analysis of interaction was also performed on the combined impact of *MDM2* genotype and cytogenetic group (low-, intermediate- or high risk aberrations, or normal karyotype, together with *MDM2* T/T or T/G+G/G genotype) in a cox regression adjusted for age and treatment. Hazard ratios (HR) and 95% CI are presented. A p-value of 0.05 was considered significant. Analyses were performed using IBM SPSS Statistics v.22 (IBM, Armonk, New York, USA).

Results

TP53 status analysis

TP53 mutations analysis was performed in the DNA binding domain (exon 5-8) in our AML cohort. Among 189 patients, 19 (10.1%) were detected with *TP53* mutations, with 1 out of 108 normal karyotype patients (0.93%) and 18 out of 75 aberrant karyotype patients (24%). For six patients, karyotype data was missing, out of which two patients carried *TP53* mutations. Thus, *TP53* mutations are associated with aberrant karyotype ($p<0.001$). Almost all mutations identified were missense mutations, except one which was a nonsense mutation resulting in a stop codon. Two of the cases had more than one mutation and among the mutations three hotspot mutations were involved (codon 175, 248 and 273). Mutational details are presented in **Table III**. The median age at diagnosis was higher for patients with mutated *TP53* gene compared with patients carrying the wild-type gene (median 71 and 63 years, respectively, $p=0.020$), and there was also a significant correlation between *TP53* mutations and high risk cytogenetic aberrations ($p<0.001$, OR 19.3 (95%CI 5.574-88.61) for high risk vs. low+intermediate risk), with a majority of complex karyotypes including monosomies (karyotype details of *TP53* mutated cases are presented in **Table III**). Aberrations involving chromosome 17p (location of *TP53*) were observed in four patients with *TP53* mutations. No deletions of chromosome 17 were reported in the *TP53* wild type patients (complete cytogenetic details missing in 13 patients). There was no statistically significant association detected between *TP53* mutation and the presence of *FLT3*-ITD, but an indication of an inverse correlation between *TP53* and *NPM1* mutations ($p=0.062$). In total, only one *TP53* mutated patient had known *FLT3*-ITD and none had *NPM1* mutation. However, *FLT3*-ITD and *NPM1* mutations were not routinely analyzed in a large proportion of the aberrant karyotype patients.

TP53 mutation and *MDM2*^{SNP309} (*rs2279744*) genotypes are summarized in **Table I** along with patient characteristics.

Survival and complete remission are associated with *TP53* status

Kaplan-Meier survival analysis showed that patients with mutated *TP53* gene (n=19) had a significantly shorter OS compared with wild-type *TP53* gene (n=170) (log rank, $P < 0.001$), see **Figure 1**. Median OS were 2 months in patients with *TP53* mutation and 16 months in *TP53* wild-type patients. Further, 36 out of 164 (24%) patients with wild-type *TP53* did not achieve CR, and for patients with *TP53* mutations it was 13/17 (78%) patients who did not achieve CR ($p < 0.001$, χ^2 test). In logistic regression, *TP53* mutation were significantly associated with lack of response after adjusting for age and risk group [OR 0.213 (95%CI 0.057-0.972), $p = 0.020$]. In Cox regression analysis, *TP53* mutation was significantly associated with decreased OS after adjusting for age, risk group, treatment and *MDM2*^{SNP309} genotype, HR 2.041 (95%CI 1.117-3.730), $p = 0.020$, **Table IV**.

***MDM2*^{SNP309} genotype analysis**

The *MDM2*^{SNP309} genotype distribution among the 189 AML patients was 76 T/T (40.2%), 93 T/G (49.2%) and 20 G/G (10.6%) compared to T/T 83 (38.9%), T/G 102 (47.7%) and G/G 29 (13.5%) in 214 healthy controls. Thus, there was no apparent or statistical significant difference between AML patients and healthy controls, nor a relationship between genotypes and age of diagnosis or other characteristics listed in **Table I**. There was an indication of an increased frequency of the homozygous G/G genotype in aberrant karyotype patients compared to CN-AML patients, 14.7% and 7.4%, respectively, which may indicate an impaired p53 function (OR 2.7 (95%CI 0.982-7.699), $p = 0.054$, for G/G compared to T/T). Kaplan-Meier curve and

log rank test disclosed a significant difference in OS between patients homozygous for the T allele and patients with at least one G allele (T/G+G/G), in the patient group with intermediate- or high risk cytogenetic aberrations (**Figure 2**, median OS 50 months and 9 months for T/T and T/G+G/G, respectively, $p=0.020$). In the Cox regression analysis, *MDM2*^{SNP309} genotype were not an independent factor for survival, but further analysis revealed an interaction between *MDM2*^{SNP309} genotype and cytogenetic risk group, indicating an additive effect with negative impact on OS in patients with combined *MDM2* T/G or G/G genotype and high risk cytogenetic aberrations; HR 4.377 (1.013-18.907), $p=0.048$; HR 1 for *MDM2* T/T genotype combined with intermediate risk (adjusted for age and treatment; low risk cytogenetics excluded due to small numbers). Patients with *TP53* mutation were excluded in the Kaplan Meier analysis and patients treated with allo-SCT were censored at the date for transplantation. A Kaplan Meier analysis showing the survival curves and data for *MDM2*^{SNP309} (all patients), and stratification in cytogenetics group/ *MDM2*^{SNP309} genotype combinations are provided in **Supplemental material A**.

Discussion

TP53 mutation analysis was performed in the DNA-binding domain (exon 5-8) of *TP53*, as approximately 95% of the *TP53* mutations were located in this region (2). We found a strong correlation between *TP53* mutations and aberrant karyotype. In patients with *TP53* mutations, 95% had aberrant cytogenetics and showed a dramatically impaired outcome and also resistance to chemotherapy. Our study supports other recent reports about *TP53* mutations as an independent prognostic factor and associated with an aberrant karyotype (1, 2, 11). Thus, *TP53* mutations have to be classified in the high risk group regardless of cytogenetic details and other clinical factors, such as age, *FLT3*-ITD or *NPM1* mutation.

In this study, we also analyzed the prognostic impact of a regulatory polymorphism, *MDM2* ^{SNP309}, in the 189 de novo AML patients, as an indication of an impaired p53 function. Overexpression of MDM2, the negative regulator of p53, is an alternative way to attenuate a cellular p53 response in the absence of *TP53* mutation/deletion. We found a negative influence on OS in *TP53* wild type AML patients with an intermediate/high risk karyotype and at least one G-allele of *MDM2* ^{SNP309}. This effect was neither observed in OS analysis of the entire group, nor in the CN-AML patients, suggesting that the SNP G-allele carriers may suppress function of wild-type p53 in cytogenetically altered tumors. Ellis et al. performed a study in therapy-related AML (t-AML) where the patients had previously been treated with chemotherapy and/or radiotherapy for other types of tumors, and no significant association between *MDM2* ^{SNP309} and t-AML was found (12). A Chinese group reported a 3.52 increased AML risk associated with the SNP G/G genotype compared to the SNP T/T genotype, but reported no association with the G/G genotype and earlier age of onset (13). The authors reported no data about treatment and survival. In contrast to the study by Xiong et al., we found no generally increased AML risk associated with *MDM2* ^{SNP309} genotype. However, there were a higher proportion of G/G cases in our cytogenetically altered AML cases, indicating that this *MDM2* variant may influence the normal repair systems via increased attenuation of normal p53 responses, thereby driving the disease towards a more high risk cytogenetic profile.

Allo-SCT in first complete remission improves the prognosis in AML patients with intermediate or high risk (9, 14). However, some patients, including those with monosomal karyotype, and, as seen in our study, patients with *TP53* mutation, are unlikely to reach CR with standard treatment. These patients may be considered for clinical trials with emerging novel therapies targeting the p53 signaling pathway. Drug candidates with the potential of restoring p53 activity in hematological malignances include small molecules such as the nutlins, RITA (“Reactivation of p53 and Induction of Tumour cell Apoptosis”) and PRIMA-1 (“p53

Reactivation and Induction of Massive Apoptosis”) (15-18). The nutlins act by inhibiting the p53 inhibitor MDM2, and are mainly an option in *TP53* wild-type patients with increased *MDM2* expression and activity. Based on our results, genotyping patients for the SNP309, which increase *MDM2* expression and p53 inhibitory activity, may be a feasible option for selecting the patients most likely to benefit from future treatment regimens with nutlins. Similarly, RITA induces p53 accumulation in AML cells, presumably by inhibiting p53-MDM2 interaction, with an effect predominantly in *TP53* wild-type cells (19). In contrast, PRIMA-1 has been shown to induce cytotoxic effects in AML cells by restoring the transcriptional activity of mutated p53 (16, 17, 19). Safety and pharmacokinetic profile appear favorable, and cross resistance with conventional chemotherapy drugs or P-glycoprotein mediated resistance appear unlikely (16, 20). Standard cytogenetic analysis is able to detect patients with loss of chromosome 17 material, but does not detect *TP53* mutations. Also, a small number of cytogenetically normal AML patients do harbor *TP53* mutations and have a poor prognosis similar to patients with high risk cytogenetics. Thus, the analysis of *TP53* mutations would provide important prognostic information in AML.

Conclusions

We have found *TP53* to be mutated in a high frequency of AML cases with aberrant, high risk cytogenetics, with a low rate of CR and markedly reduced OS. Also, aberrant karyotype patients with wild-type *TP53* but the variant allele of the *MDM2*^{SNP309} appears to have a reduced OS. Clinical trials as well as preclinical *in vivo* studies with nutlins, RITA and PRIMA-1 in hematological malignancies are in progress, and combination therapies with drugs targeting p53 or MDM2-p53 interactions and conventional drugs are likely to improve outcomes (20, 21). The exact dosing and most effective drug combinations remain to be identified, but our

study implies that analysis of polymorphisms affecting endogenous p53-inhibitors, such as the *MDM2*^{SNP309}, as well as the analysis of *TP53* mutational status should serve as important tools for prognostication and risk stratification, as well as selection of patients most likely to benefit from novel experimental therapies with drugs targeting the p53 signaling pathway.

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Author contributions

IJF: Research, *MDM2* genotyping, clinical data compilation and statistical analysis, manuscript writing; K.W: Research, *TP53* analysis, data compilation, manuscript writing; R.C: Clinical data compilation and consultation; J.L: Patient material and clinical data collection; H.N: Patient material and clinical data collection; M.H: *FLT3/NPM1* analysis, data collection; H.G: *MDM2* genotyping, data and statistical analysis; K.L: Research, patient material and clinical data collection; P.S: Conception and study design, research. All authors critically reviewed the manuscript.

Conflict of interest disclosure

The authors declare no competing financial interests.

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1 **Table I.** AML patient characteristics with *TP53* mutation and *MDM2*^{SNP309} distributions. P-values in italics indicating significant differences between groups (using
2 nonparametric independent samples median test, Chi² or Fisher's exact test).

CHARACTERISTIC		All (N=189)	<i>TP53</i>		<i>p</i>	<i>MDM2</i> 309T>G rs2279744			<i>p</i>
			Wild-type	Mutation		T/T	T/G	G/G	
Age median (range), years		64 (19-88)	62 (19-88)	71 (52-83)	<i>0.015</i>	61 (20-88)	65 (20-83)	68 (25-85)	0.34
Gender									
	Male	95	85	11	0.63	33	50	13	0.23
	Female	94	86	8		43	43	8	
Karyotype									
	Normal	108	107	1		50	50	8	
	Aberrant	75	59	16	<i><0.001</i>	25	39	11	0.12
	Undetermined	6	4	2		1	4	1	
Risk group									
	Low	32	32	0		14	16	2	
	Intermediate	87	84	3		41	38	8	0.51
	High	55	41	14	<i><0.001</i>	18	31	6	
	Undetermined	15	13	2		3	8	4	
<i>FLT3</i> status									
	<i>FLT3</i> wild-type	116	108	8		53	53	10	
	<i>FLT3</i> -ITD	37	36	1	0.63	12	19	6	0.26
	Undetermined*	36	26	10		11	21	4	
<i>NPM1</i> status									
	<i>NPM1</i> wild-type	99	91	8		41	47	11	
	<i>NPM1</i> mutation	52	52	0	<i>0.062</i>	23	24	5	0.94
	Undetermined*	38	27	11		12	22	4	
Treatment response									
	Complete response	132	128	4		52	63	17	
	Non-CR	49	36	13	<i><0.001</i>	19	27	3	0.41 [#]
	Undetermined/not reported	8	6	2 [§]		5	3	0	
<i>MDM2</i> 309T>G rs2279744									
	T/T	76	72	4					
	T/G	93	80	13	0.16				
	G/G	20	18	2					
<i>TP53</i>									
	Wild-type	170				72	80	18	0.16
	Mutation	19				4	13	2	

3 * *FLT3*-ITD and *NPM1* mutations were not routinely analyzed in all aberrant karyotype patients. [#]p=1.00 for T/T vs. T/G+G/G. [§] 1 patient (normal karyotype) dead two months after diagnosis; 1
4 patient dead one month after diagnosis.

5 **Table II.** Induction treatment regimes.

Regime	N (%)
Daunorubicine and Cytarabine (n=116) or Daunorubicine, cytarabine and mitoxantrone (n=2)	118 (62.4)
Idarubicine and Cytarabine (n=26) or Idarubicine, Cytarabine and Etoposide (n=3)	29 (15.3)
Idarubicine, Cytarabine and Cladribine	20 (10.6)
Mitoxantrone, cytarabine and Etoposide (n=7) or Mitoxantrone and Cytarabine (n=2)	9 (4.8)
Daunorubicine, Cytarabine and 6-Thioguanine	8 (4.2)
Other/unknown ¹	5 (2.6)

6 ¹Including 2 in clinical trial of combination therapy with Tipifarnib, and 1 with Fludarabine, Cytarabine
7 and G-CSF. 2 patients with unknown treatment but known curative intent.

8 **Table III.** *TP53* mutations in 19/189 AML patients.

Patient	Exon	Nucleotide change	Amino acid change	Mutation	Karyotype	Risk group ⁹
1	5	CAC>CGC	His168Arg	Missense	Aberrant (t(9;22), Ph+)	High
2	5	GTG>TTG	Val173Leu	Missense	Aberrant (complex, including monosomy 7)	High
3	6	GTG>ATG	Val216Met	Missense	Aberrant (complex, no further details)	High
4	6	GTG>TTG	Val216Leu	Missense	Aberrant (complex, no further details)	High
5	5	CGC>CAC	Arg175His	Missense	Normal (<i>FLT3</i> wt/ <i>NPM1</i> wt)	Intermediate
6	6	AGT>ATT	Ser215Ile	Missense	Undetermined	Undetermined
7	6	ATC>AAC	Ile195Asn	Missense	Undetermined	Undetermined
8	6	CAT>CGT	His214Arg	Missense	Aberrant (complex, including del(5))	High
9	8	GAG>AAG	Glu285Lys	Missense	Aberrant (complex, including del(5q))	High
10	8	CGT>CAT	Arg273His	Missense	Aberrant (complex, including monosomy 7, del(17p))	High
11	5	GAG>TAG	Glu171Stop	Nonsense	Aberrant (complex, including monosomies)	High
	7	CGG>CAG	Arg248Gln	Missense		
12	5	CGC>CAC	Arg175His	Missense	Aberrant (complex, including monosomies)	High
13	8	CGT>CAT	Arg273His	Missense	Aberrant (complex, including monosomy 5 and del(7q))	High
14	7	TAC>TGC	Tyr236Cys	Missense	Aberrant (complex, including del(5q) and del(17))	High
15	6	TAT>TGT	Tyr220Cys	Missense	Aberrant (-13, t(4;13)(q23;q31), t(13;17)(q21;q25))	High
16	7	CGG>TGG	Arg248Trp	Missense	Aberrant (complex, including monosomy 7, del17)	High
17	5	GAG>GGG	Glu171Gly	Missense	Aberrant (complex, including del(5q),del(7q), and del(17p))	High
	5	CCC>CTC	Pro177Leu	Missense		
18	6	ATC>ACC	Ile195Thr	Missense	Aberrant (complex, including monosomy 7)	High
19	6	TAT>CAT	Tyr220His	Missense	Aberrant (complex, including monosomy 7)	High

Table IV. Cox regression of overall survival, forced entry method.

<i>Covariates</i>	<i>N</i>	<i>HR</i>	<i>95%CI</i>	<i>p</i>
Age		1.022	1.002-1.042	0.032
Risk group				
Low risk	32	1		
Intermediate risk	87	2.652	1.349-5.212	0.005
High risk	55	3.698	1.806-7.570	<0.001
Treatment				
Chemotherapy	118	1		
Chemotherapy+allo-SCT	56	0.276	0.143-0.532	<0.001
<i>TP53</i>				
Wild type	157	1		
Mutated	17	2.041	1.117-3.730	0.020
<i>MDM2</i> SNP309				
T/T	72	1		
T/G+G/G	102	1.145	0.779-1.684	0.491

95%CI= 95% Confidence interval; HR=Hazard ratio.

Figure legends

Figure 1. Marked inferior survival in *TP53* mutated AML patients. Median OS 2 months and 15.5 months for *TP53* mutated and *TP53* wild-type patients, respectively ($p<0.001$).

Figure 2. Inferior survival in AML patients carrying the alternative *MDM2* SNP309 G allele compared to T/T patients; limited to cases with intermediate/high risk cytogenetic aberrations. Median OS 9 months and 50 months for T/G + G/G and T/T, respectively ($p=0.020$). *TP53* mutated cases excluded.

Figure 1.

Figure 2.

Supplemental material.

Combined effect of cytogenetic group and $MDM2^{SNP309}$ genotype, Kaplan-Meier analysis of overall survival (OS). $TP53$ mutated patients excluded (n=19). Risk group missing in 15 cases.

Overall survival times for cytogenetics/ $MDM2^{SNP309}$ genotype combinations (median).

Intermediate risk cytogenetics+MDM2 T/T (n=5): 49,7 months

Intermediate risk cytogenetics+MDM2 T/G or G/T (n=10): 18,2 months

High risk cytogenetics+MDM2 T/T (n=13): 13,3 months

High risk cytogenetics+MDM2 T/G or G/G (n=17): 9,1 months

CN-AML+MDM2 T/T (n=48): 15,5 months

CN-AML+MDM2 T/G or G/G (n=59): 17,4 months

All pairwise comparisons, log rank test.

Intermediate risk cyt.+MDM2 T/T vs.

Intermediate risk cyt.+MDM2 T/G or G/G: $p=0.079$

High risk cyt.+MDM2 T/T: $p=0.372$

High risk cyt.+MDM2 T/G or G/G: $p=0.023^*$

CN-AML+MDM2 T/T: $p=0.295$

CN-AML+MDM2 T/G or G/G: $p=0.274$

Intermediate risk cyt.+MDM2 T/G or G/G vs.

High risk cyt.+MDM2 T/T: $p=0.802$

High risk cyt.+MDM2 T/G or G/G: $p=0.152$

CN-AML+MDM2 T/T: $p=0.826$

CN-AML+MDM2 T/G or G/G: $p=0.590$

High risk cyt.+MDM2 T/T vs.

High risk cyt.+MDM2 T/G or G/G: $p=0.031^*$

CN-AML+MDM2 T/T: $p=0.836$

CN-AML+MDM2 T/G or G/G: $p=0.841$

High risk cyt.+MDM2 T/G or G/G vs.

CN-AML+MDM2 T/T: $p=0.023^*$

CN-AML+MDM2 T/G or G/G: $p=0.003^*$

CN-AML+MDM2 T/T vs.

CN-AML+MDM2 T/G or G/G: $p=0.87$

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3 **Overall survival in relation to MDM2SNP309 genotype, all patients.**

4 **MDM2 T/T:** 15.5 months (mean 39 months)

5 **MDM2 T/G or G/G:** 15.6 months (mean 21 months).

6 Patients with *TP53* mutation excluded (n=19).

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