

Mutations in *TP53* are exclusively associated with del(17p) in multiple myeloma

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ABSTRACT

Deletion of the 17p13 chromosomal region [del(17p)] is associated with a poor outcome in multiple myeloma. Most of the studies have targeted the *TP53* gene for deletion analyses, although no study showed that this gene is the deletion target. In order to address this issue, we sequenced the *TP53* gene in 92 patients with multiple myeloma at diagnosis, 54 with a del(17p) and 38 lacking del(17p). At least one mutation was found in 20 patients, all of them presenting a del(17p). The analysis of the mutation location showed that virtually all of them occurred in highly conserved domains involved in the DNA-protein interactions. In conclusion, we showed that 37% of the myeloma patients with del(17p) present a *TP53* mutation versus 0% in patients lacking the del(17p). The prognostic significance of these mutations remains to be evaluated.

Key words: mutations, *TP53*, del(17p), multiple myeloma.

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Introduction

The prevalence of *TP53* mutations differs considerably between tumor types and stages of cancer, and approximately 50% of all tumors present mutations. In multiple myeloma (MM), mutations of the *TP53* gene is rarely detected at diagnosis, although it becomes more frequent in advanced disease¹ and human myeloma cell lines.² In other hematologic malignancies, like diffuse large B-cell lymphomas (DLBCL),^{3,4} follicular lymphoma⁵ or chronic lymphocytic leukemia (CLL)⁶ mutations in *TP53* correlate with unfavorable prognosis and chemotherapy resistance, especially when located in DNA binding domain. Furthermore, a strong correlation between 17p deletions and *TP53* mutations has been shown in CLL.

In multiple myeloma, we previously showed that deletion of the *TP53* gene (located at 17p13) was present in 7% of the patients enrolled in the IFM99 trials and tested by FISH. After a median follow up of 56 months, univariate statistical analyses showed that del(17p) negatively impacted both the event

free survival and the overall survival.⁷ However, it is unknown whether p53 signaling is still functional in those myeloma cells or if p53 is totally inactivated through mutations on the other allele. We therefore set out to clarify the prevalence of *TP53* mutations in del17p MM patients and compared it to prevalence in a series of non-del(17p) MM patients.

Design and Methods

Patients

Primary myeloma cells were obtained from bone marrow aspirates after Ficoll density gradient centrifugation followed by separation of myeloma cells with CD138 microbeads (StemCell Technologies, Vancouver, Canada). Cytospins of purified samples stained according to the MGG method routinely confirmed plasma cell morphology for more than 90% of cells. All primary cells were obtained from routine diagnostic samples after informed consent was provided by the patients. Fluorescence *in situ* hybridization (FISH) analysis using a *TP53*-specific probe (Abbott, Rungis, France) was routinely performed

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for patients enrolled in the IFM99 trials. RNA from 92 untreated patients was obtained from purified plasma cells. This study was conducted in accordance with the Declaration of Helsinki.

TP53 mutation analysis

We screened cDNA samples (exons 2-11) for *TP53* mutations by direct sequencing as described previously.⁸ Two overlapping fragments spanning the *TP53* coding region were amplified by PCR, purified, and were bidirectionally sequenced, using the same primers and the Big Dye Terminator kit on the Applied Biosystems 3130xl Genetic Analyser (Applied Biosystems, Foster City, CA, USA). Data were analyzed by visual inspection of electropherograms on Seqscanner software and compared to reference sequence NM_000546.2 (NCBI Nucleotide) using Seqscape software (Applied Biosystems, Foster City, CA, USA). *TP53* mutations found in patients were compared to the UMD p53 Web site (<http://p53.free.fr/>)⁹ and analyzed using MUT-MAT 2.0, a verification spreadsheet to certify p53 mutations.¹⁰

Results and Discussion

Deletion of the short arm of chromosome 17 was detected in 11% of newly diagnosed patients.⁷ However, we did show that a short survival was predicted only in patients with a deletion present in at least 60% of the plasma cells (7% of the patients at the time of diagnosis). We sequenced cDNA coding for the *TP53* gene in 54 of those patients. Twenty-one hemizygous *TP53* mutations were detected in 20 of these 54 cases (37%) of MM with del(17p) (Table 1) including 19 single nucleotide missense

mutations, one single nucleotide nonsense mutation and one single nucleotide nonsense insertion (Table 2), unlike Chng *et al.* who found a majority of deletions and insertions.¹¹ We compared these cases with 38 patients lacking del(17p). No *TP53* mutation was found in those cases of newly diagnosed MM without del(17p) ($P < 0.0001$).

The distribution of the 21 mutations was one insertion and one point mutation in exon 4, 6 point mutations in exon 5, 2 in exon 6, 5 in exon 7, and 6 in exon 8 (Figure 1). No mutations were identified in exons 2, 3, 9, 10, or 11. All missense mutations were previously reported in the UMD *TP53* mutation database, most of them were frequent and 5 were infrequent. When the *TP53* mutation distribution pattern was analyzed, these included 2 mutations in LSH1 (loop-sheet-helix1), 2 mutations in Loop-L1, 4 mutations in Loop-L2, 5 mutations in Loop-L3, 5 mutations in the LSH 2 regions (Table 2); more than half of the *TP53* mutations found in our series were located to DNA-binding domain, as described in diffuse large B-cell lymphomas (DLBCL).^{3,4}

Indeed, as described by Levine, loops 1 and 3 and the

Table 1. Results of *TP53* mutation analysis.

	del 17p	no del 17p
Mut p53	20 (37%)	0 (0%)
No Mut p53	34 (63%)	38 (100%)
Total (n=92)	54	38

TP53 mutations were observed in 37% of the patients with del(17p), and 0% of patients lacking del(17p).

Table 2. Description of the 21 *TP53* mutations in 20 patients according to Ref Seq for p53: GenBank: NM_000546.2.

Sample N.	Mutation		Exon	Codon number	TP53 domain	Frequency in the database	Residual activity
5809	c.158G>A	p.W53X	4	53	NA	16	ND
3390	c.376T>A	p.Y126N	5	126	LSH1	9	11.53
2964	c.357_358insC	p.K120QfsX29	4	129	LSH1	NA	0
3294	c.395A>T	p.K132M	5	132	Loop1 (DNA-bd)	10	14.93
3690	c.404G>T	p.C135F	5	135	Loop1 (DNA-bd)	67	10.37
3107	c.528C>G	p.C176W	5	176	Loop2	25	15.16
5225	c.530C>A	p.P177H	5	177	Loop2	4	48.75
8186	c.535C>T	p.H179Y	5	179	Loop2	142	13.27
5954	c.569C>T	p.P190L	6	190	Loop2	54	10.12
3400	c.632C>T	p.T211I	6	211	NA	25	13.49
3843	c.711G>C	p.M237I	7	237	Loop3 (DNA-bd)	204	0.43
3390	c.722C>G	p.S241C	7	241	Loop3 (DNA-bd)	39	0.00
7843	c.733G>A	p.G245S	7	245	Loop3 (DNA-bd)	500	0.00
6680	c.743G>A	p.R248Q	7	248	Loop3 (DNA-bd)	1016	0.00
5975	c.749C>T	p.P250L	7	250	Loop3 (DNA-bd)	58	-0.50
4048	c.785G>T	p.G262V	8	262	NA	17	11.71
5887	c.811G>A	p.E271K	8	271	LSH2 (DNA-bd)	45	8.55
3987	c.836G>A	p.G279E	8	279	LSH2 (DNA-bd)	50	0.27
3275	c.839G>C	p.R280T	8	280	LSH2 (DNA-bd)	105	0.29
3336	c.839G>C	p.R280T	8	280	LSH2 (DNA-bd)	105	0.29
6073	c.845G>C	p.R282P	8	282	LSH2 (DNA-bd)	28	0.00

(DNA-bd: DNA-binding domain, LSH: Loop-sheet-helix). Location of the *TP53* mutations and expected results on the *TP53* activity.

Loop-Sheet-Helix (LSH) region from codons 272 to 287 make direct contacts with the DNA adjacent to a gene that is regulated by the p53 transcription factor, while loop 2 is required for folding and stabilization of this DNA-binding domain and is not in direct contact with DNA.¹²

As recently reported by Young *et al.* in DLBCL,⁴ position of the mutation seems to determine the outcome. Such correlation has not yet been described in MM. In 2 cases, the mutations were localized to codons involved in the zinc-binding site (Cys176 and Cys 179). Seventeen mutations were localized to highly conserved areas: 4 mutations in area II (codons 117-142), 3 mutations in area III (codons 171-181), 5 mutations in area IV (codons 234-258), and 5 mutations in area V (codons 270-286). According to the UMD *TP53* mutation database, virtually all p53 mutants had low remaining activity (Table 2). Survival analyses did not reveal any difference between patients presenting a *TP53* mutation and those with a germline *TP53*, in patients with del(17p) (Figure 2). However, the numbers are too small to draw any definitive conclusion.

We did not find *TP53* mutations in the vast majority

(63%) of del(17p) hemizygous patients, suggesting that normal p53 protein is still present and still functional in these non-mutated patients and potentially overcome the poor effect of del(17p). However, epigenetic modifications may occur that alter the p53 pathway. Absence of response to treatment in patients with del(17p), especially in chronic lymphocytic leukemia (CLL) treated with fludarabine, is strongly in favor of the implication of the *TP53* pathway. Also in CLL, *TP53* mutations have as poor a prognosis as del(17p), suggesting a similar mechanism, probably because of the high incidence of *TP53* mutations in patients with del(17p).⁶

We may have underestimated the frequency of *TP53* mutations: as we sequenced cDNA, we could not estimate frequency of mutations in introns. However, very few mutations have been described in introns so far³ and we doubt many intron mutations have been missed. However, direct sequencing (similarly to PCR-SSCP, DHPLC, or HRM assay frequently used to detect *TP53* gene mutations) has low sensitivity and may miss some minor mutant subclones which could subsequently be

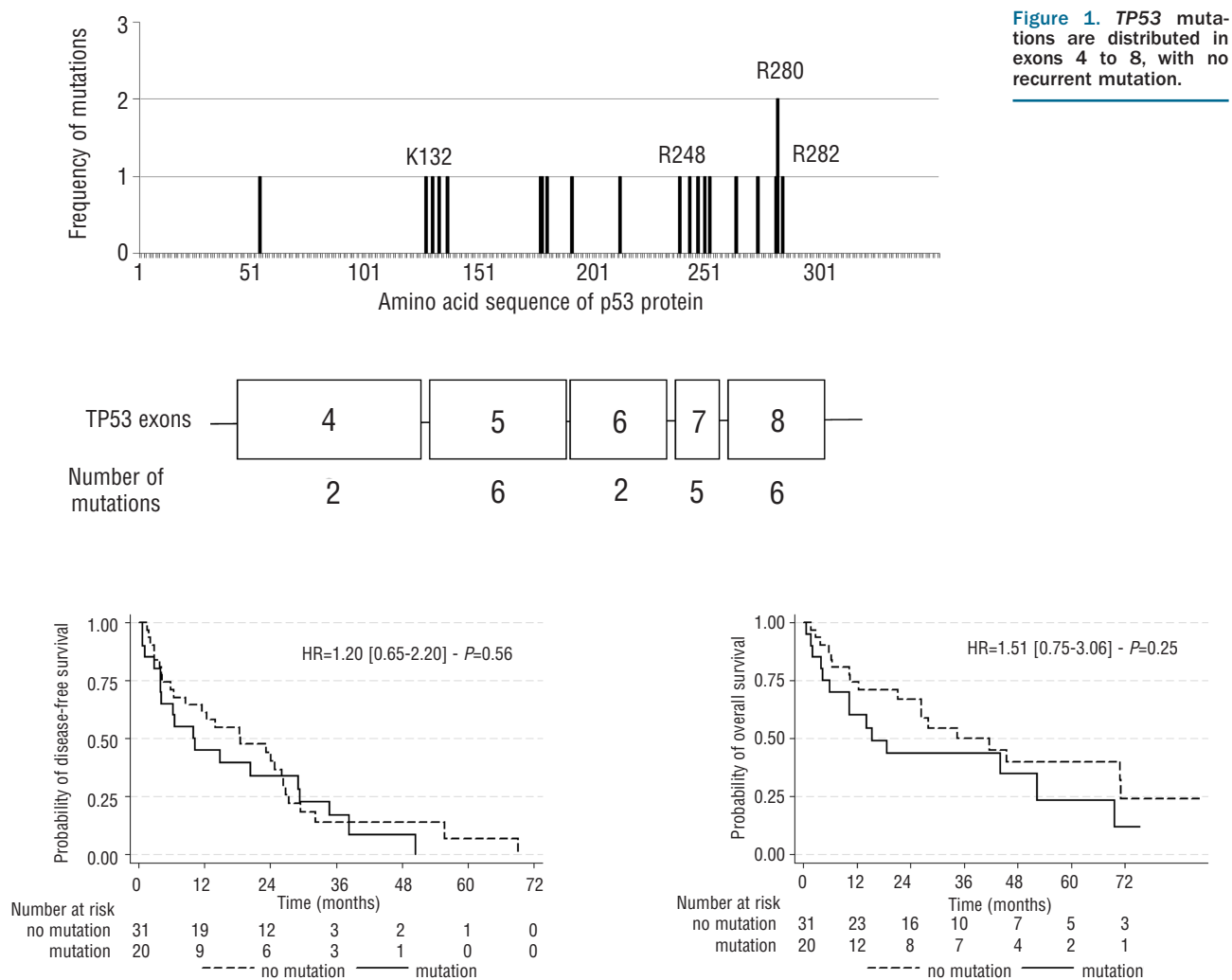


Figure 2. Patients with *TP53* mutations do not present different outcome.

responsible for relapse and explain poor prognosis of del(17p). Indeed, some studies have demonstrated a mutation in up to 40% of patients with end-stage disease and plasma cell leukemia.^{1,13,14}

As a result, more sensitive technologies such as Next-Generation Sequencing technology could help in finding some minor mutant subclones, and functional impairment of p53 in del(17p) patients has to be explored through other effectors of the p53 DNA repair pathway (like MDM2¹⁵ or ATM¹⁶). Recently, Xiong *et al.* reported on genes regulated by p53, and confirmed that low expression of p53 was associated with a poor outcome.¹⁷ In our small series (92 patients, 54 [del(17p)]), it was not possible

to explore the prognostic impact of TP53 mutations. Analysis of mutations in purified plasma cells in other larger series is required to address this issue.

Authorship and Disclosures

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