

### LYMPHOID NEOPLASIA

# del(17p) without *TP53* mutation confers a poor prognosis in intensively treated newly diagnosed patients with multiple myeloma

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#### KEY POINTS

- del(17p) is an important prognostic factor in myeloma, even in the absence of *TP53* mutation.
- Isolated del(17p) should remain a high-risk feature in myeloma.

**Despite tremendous improvements in the outcome of patients with multiple myeloma in the past decade, high-risk patients have not benefited from the approval of novel drugs. The most important prognostic factor is the loss of parts of the short arm of chromosome 17, known as deletion 17p (del(17p)). A recent publication (on a small number of patients) suggested that these patients are at very high-risk only if del(17p) is associated with *TP53* mutations, the so-called "double-hit" population. To validate this finding, we designed a much larger study on 121 patients presenting del(17p) in > 55% of their plasma cells, and homogeneously treated by an intensive approach. For these 121 patients, we performed deep next generation sequencing targeted on *TP53*. The outcome was then compared with a large control population (2505 patients lacking del(17p)). Our results confirmed that the "double hit" situation is the worst (median survival = 36 months), but that del(17p) alone also confers a poor outcome**

**compared with the control cohort (median survival = 52.8 months vs 152.2 months, respectively). In conclusion, our study clearly confirms the extremely poor outcome of patients displaying "double hit," but also that del(17p) alone is still a very high-risk feature, confirming its value as a prognostic indicator for poor outcome. (Blood. 2021;137(9):1192-1195)**

## Introduction

Despite huge therapeutic progress in multiple myeloma (MM), prognosis is still poor for high-risk patients. It is of great importance to identify these patients at diagnosis, because after decades of treatment adapted only to a patient's age and renal function, the time has come to design risk-adapted treatment. Among all prognostic factors described for MM,<sup>1</sup> genetic abnormalities displayed by tumor plasma cells play a major role.<sup>2,3</sup> Deletion of the short arm of chromosome 17 (del(17p)) is a well-established high-risk feature in MM and is included in current disease staging criteria.<sup>4</sup> However, several questions are still debated. The first concerns the prognostic threshold for clone size, with some investigators defining del(17p) if detected in only

1% of plasma cells.<sup>5</sup> A recent meta-analysis of European data including more than 1000 patients with del(17p) clones of variable size confirmed previous data from the Intergroupe Francophone du Myélome (IFM),<sup>6</sup> with a significant clinical impact of the subclonality value of 55% to 60% by FISH.<sup>7</sup> With this threshold, del(17p) is observed in 8% of newly diagnosed MM (NDMM)<sup>6,8</sup> and clearly impacts both progression free survival (PFS, median just over a year) and overall survival (OS, median 2-3 years).<sup>6,7</sup> The second question addresses its molecular target; most studies have focused their analysis on the tumor suppressor gene *TP53*, localized in the minimal deleted region; however, the fact that the remaining allele is mutated in only a third of cases is surprising.<sup>9</sup> A third question, related to the previous one,

**Table 1. Characteristics of patients with deletion 17p**

	del(17p)/TP53wt	del(17p)/TP53mut	no del(17p)
Patients, n	76	45	2505
Age at diagnosis, median (range), y	61 (39-71)	59 (40-71)	58 (24-72)
<b>Sex, n (%)</b>			
Male	42 (55)	25 (56)	1451 (58)
Female	34 (45)	20 (44)	1054 (42)
<b>ISS, n (%)</b>			
1	18 (29)	7 (21)	692 (32)
2	28 (44)	11 (32)	950 (45)
3	17 (27)	16 (47)	484 (23)
Patients, n	63	34	2126
Missing, n	13	11	379
del(17p), median (range), %	76 (57-98)	82 (58-96)	—
<b>Treatment of multiple myeloma, n (%)</b>			
Doublet induction	26 (34)	17 (38)	846 (34)
Triplet induction	50 (66)	28 (62)	1659 (66)
<b>Lenalidomide maintenance, n (%)</b>			
No	66 (87)	36 (80)	2184 (87)
Yes	10 (13)	9 (20)	321 (13)
<b>Survival information</b>			
Deaths, n (%)	39 (51)	33 (73)	601 (24)
Median follow-up (range), mo	40.5 (1.8-148.4)	36.0 (3.7-125.4)	57.8 (1.6-185.2)

ISS, International Staging System.

concerns the mono- or biallelic nature of the prognostic abnormality. Indeed, recent data suggest that only patients with a “double hit” biallelic inactivation of *TP53* (del/mut most of the time) are at high-risk.<sup>10</sup> In the study presented here, we demonstrate that an isolated del(17p) present in the majority of plasma cells, even if less unfavorable than a double hit, is still a prognostic indicator for poor outcome in MM.

## Study design

The Toulouse Ethics Committee approved the study. Informed consent was obtained for all included patients. Clinical data were obtained from 121 NDMM patients displaying del(17p), treated by an intensive strategy, followed up for > 36 months, or having died of MM within 36 months post treatment, and for whom diagnosis was established between 2007 and 2017. We also established a control cohort of 2505 NDMM patients, defined by the absence of del(17p) (but including 307 patients (12.3%) displaying a translocation (4;14)), treated by intensive strategy, followed up for >36 months or having died of MM, and for whom diagnosis was established between 2007 and 2017. This control cohort was matched on age. All patients received either a doublet-based induction including bortezomib or a triplet-based induction including bortezomib and lenalidomide followed by ASCT and consolidation by the same regimen as induction, and some of them also received maintenance with lenalidomide (Table 1). Progression was determined based on criteria defined by the International Myeloma Working Group.<sup>11</sup> Rates of PFS and OS were estimated by the Kaplan-Meier method. Tests were two-

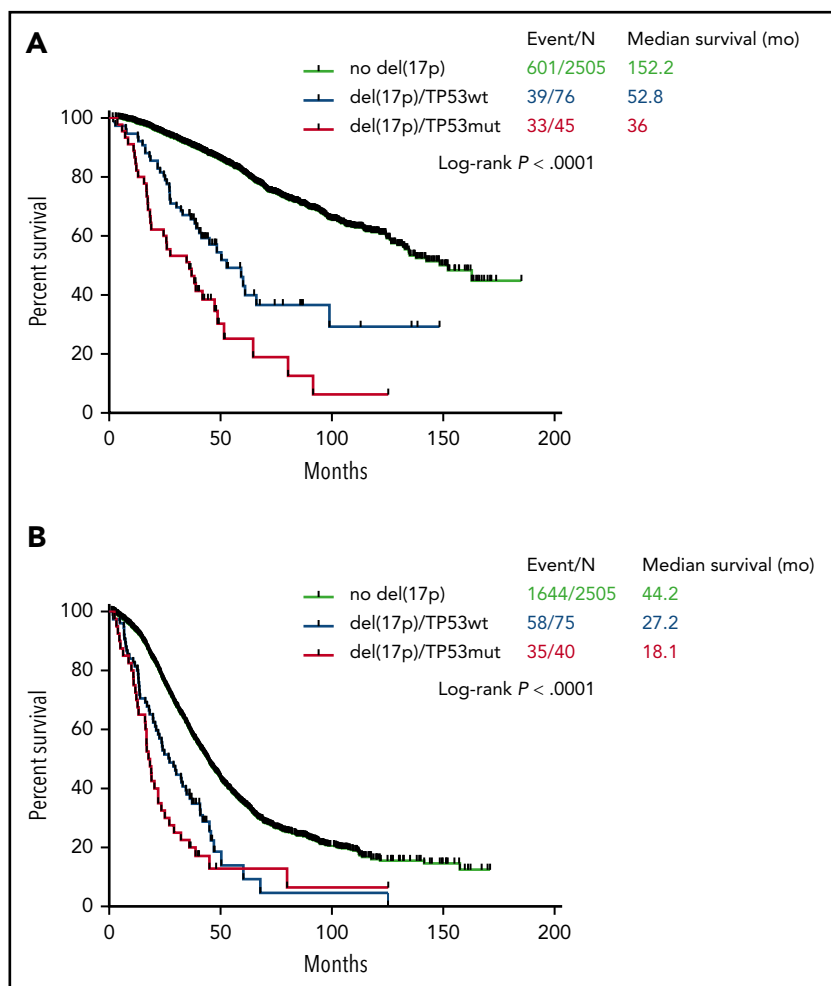
sided and  $P < .05$  were considered significant. Statistical analyses were conducted using Prism.

Bone marrow samples were obtained at diagnosis and shipped overnight to a central laboratory. Upon receipt, plasma cells were isolated using CD138+ MAC-Sorting (Miltenyi Biotec, Paris, France). Post-sorting purity was checked as previously described and only samples with  $\geq 70\%$  plasma cells after sorting were kept for the analysis. The mean purity was 96% and 93% of the samples displayed a purity  $\geq 90\%$ . Plasma cells were analyzed by FISH for t(4;14)(p16;q32) and del(17p) determination was by specific probe from Abbott Molecular (Paris, France) and Cytocell (Paris, France). Only del(17p) present in  $\geq 55\%$  plasma cells were taken into account.

For TP53 exonic mutations resequencing, a PCR amplicon based library was generated from multiplex PCR with 9 to 150ng genomic DNA input (11 specific couples of primers in supplemental Table, available on the *Blood* Web site). The library was sequenced with a MiSeq sequencer (Illumina, San Diego, USA) and Miseq Reagent kit V2 (paired-end sequencing  $2 \times 150$  cycles). Sequencing depths from 100X to 47800X were obtained depending on samples or exons. Alignment was performed using BWA aligner and variant calling was performed using FreeBayes and Mutect2 variant callers. They were all verified on IGV.

## Results and discussion

Characteristics of the patients are presented in Table 1.



**Figure 1. Kaplan-Meier survival of myeloma patients according to del(17p).** (A) Overall survival. (B) Progression-free survival. The blue curve corresponds to patients with del(17p) alone, the red curve to patients with biallelic inactivation of *TP53*, and the black curve to patients without del(17p) (control cohort).

Among 121 patients displaying del(17p), 45 (37.2%) also displayed a *TP53* mutation ("double hit" MM). Of note, previous data show that virtually all patients with *TP53* bi-allelic inactivation display the deletion in more than 55% of plasma cells.<sup>7</sup> The OS of patients with *TP53* biallelic inactivation was significantly shorter than patients with del(17p) alone (36.0 and 52.8 months, respectively,  $P = .004$ ) (Figure 1A). Conversely, there was no statistically significant difference in PFS between the 2 groups (18.1 and 27.2 months, respectively,  $P = .1$ ) (Figure 1B). Our data counteract an older study showing that 20 patients with *TP53* biallelic inactivation had a similar prognosis to 34 patients with del(17p) alone.<sup>12</sup> Conversely, in a dataset where both *TP53* and del(17p) status were available, patients with *TP53* biallelic inactivation ( $n = 7$ ) had shorter PFS and OS than patients with monoallelic inactivation ( $n = 26$ , del(17p) or *TP53* mutation).<sup>13</sup>

In this study, we chose a variant allelic fraction (VAF) cutoff of 0.4. Seven patients with del(17p) displayed a *TP53* mutation with a VAF ranging from 0.1 to 0.4; when transferred in the *TP53* biallelic inactivation group, the difference in PFS and OS between the 2 groups was similar (data not shown).

In order to determine if del(17p) alone can be considered a poor prognostic factor in MM, we compared the 76 patients with del(17p) alone to the 2505 patients without del(17p). We observed that PFS and OS were significantly shorter for patients

with isolated del(17p) (PFS 27.2 versus 44.2 months,  $P < .0001$ ; OS 52.8 versus 152.2 months,  $P < .0001$ ) (Figure 1).

One unresolved question is the prognostic role of isolated *TP53* mutations. Our control cohort was based on the absence of del(17p) and not on *TP53* status, which means that patients with *TP53* mutation only were included. It remains to be determined in large cohorts if monoallelic mutation of *TP53* is a poor prognostic feature in MM.<sup>9</sup> If the answer is yes, this mutation should be systematically sought in clinical practice. The NGS approach for routine risk assessment has the advantage of being informative on copy number abnormalities, translocations of interest such as t(4;14) and *TP53* mutations in one single technique.<sup>14</sup> Of note, 73% of the patients with del(17p) alone and 52% of those with *TP53* biallelic inactivation were not International Staging System (ISS)-3 (Table 1), which means that they were not classified in the Revised-ISS-3 subgroup.<sup>4</sup> Given the current number of available drugs, all patients with del(17p) in most of their plasma cells should benefit from the most powerful combinations as in other cancers. If evidence suggest that recent developments in immunotherapy such as CAR-T cells or T cell engagers are of particular interest to these patients, these approaches should be used in earlier lines. Of note, both del(17p) and *TP53* mutations can be acquired during evolution, meaning that both analyses should be performed again at the time of any relapse.

In summary, we demonstrate that an isolated del(17p) in more than 55% of plasma cells, even if less unfavorable than a biallelic inactivation of *TP53*, is still a poor prognosis factor in MM, and should remain the major factor to define high risk patients.

## Acknowledgments

This work was supported by National Institutes of Health, National Cancer Institute grants PO1-155258 and P50-100707 (H.A.-L.) and the Cancer Pharmacology of Toulouse and Region (CAPTOR) program. The Centre de Recherches en Cancérologie de Toulouse Team 13 is supported by the Fondation ARC (Association pour la Recherche sur la Cancer, grant PGA1\*20160203788).

## Authorship

Contribution: J.C., A.P., L.B., and H.A.-L. designed the research and analyzed data, J.C. and H.A.-L. wrote the manuscript, which was reviewed and edited by the other coauthors; J.C. performed the statistical analysis; and all other authors provided study samples and clinical data.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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## Footnotes

Submitted 22 July 2020; accepted 7 September 2020; prepublished online on *Blood* First Edition 20 October 2020. DOI 10.1182/blood.2020008346.

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No additional data will be made available by the authors.

The online version of this article contains a data supplement.

There is a *Blood* Commentary on this article in this issue.

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