MB4-2 breakpoint in MMSET combined with del(17p) defines a subset of t(4;14) multiple myeloma with very poor prognosis

Multiple myeloma (MM) is a clonal plasma cell disorder, which remains incurable. The t(4;14) translocation is present in 15% of patients with symptomatic disease and, despite recent therapeutic improvements such as bortezomib treatment, still indicates a poor prognosis.[1,2] However, t(4;14) MM is a heterogeneous group, containing both "high risk" and "good risk" patients.[3] In addition, the translocation is also detected in some cases of indolent (stage I) MM and even in monoclonal gammopathy of undetermined significance (MGUS).[4,5] Prognostic tools capable of predicting the evolution of the different forms of t(4;14) monoclonal gammopathies are currently lacking.

The t(4;14) translocation deregulates two potential oncogenes, *FGFR3* and *MMSET/WHSC1*. Previous studies have shown that *FGFR3* expression, which is lost in a subset of t(4;14) MM, does not have a significant impact on patients' survival.[6-9] The *MMSET* gene, which is overexpressed in all t(4;14) MM, encodes for a histone methyltransferase which is involved in tumor progression and genomic instability.[8,10-12] Three major breakpoints within the 5'coding region of *MMSET* (MB4-1, MB4-2 and MB4-3) have been observed at 4p16 on chromosome der(4).[7,11] Each breakpoint overexpresses a specific *IGH/MMSET* fusion transcript. While MB4-1 produces a full length MMSET protein, MB4-2 and MB4-3 give different truncated proteins. The aim of this study was to clarify the prognostic significance of each MB4 breakpoint in a large cohort of patients.

We investigated the MB4 breakpoint distribution and prognostic value in a cohort of 294 patients with t(4;14) monoclonal gammopathies including 38 asymptomatic patients (MGUS or stage I MM according to the Durie and Salmon classification) and 256 symptomatic MM patients diagnosed at the Hematology Laboratory of Paris Saint Louis and Nantes. In all cases, quantitative reverse transcriptase polymerase chain reaction (RT-PCR) was performed using cDNA from purified CD138$^+$ bone marrow plasma cells at diagnosis to analyze expression of *FGFR3* and the *IGH-MMSET* fusion transcripts resulting from the three different breakpoints MB4-1, MB4-2 and MB4-3, as described previously.[13]

Among the 38 asymptomatic (MGUS/stage I MM) patients [(14 males and 24 females; median age 61 years (range, 35-78) with a median follow-up since diagnosis of 56 months)], RT-PCR analysis of the different *IGH/MMSET* fusion transcripts revealed a low percentage of the MB4-2 subtype (5%), compared to the MB4-1 and MB4-3 subtypes (74% and 21%, respectively). In contrast, among the patients with symptomatic MM, MB4-2 transcripts were expressed in 21% of cases, as compared to 62% for MB4-1, and 17% for MB4-3. Thus, the MB4-2 breakpoint is rarely observed in t(4;14) indolent monoclonal gammopathy, while it is significantly more frequent in t(4;14) symptomatic MM (P=0.0228) (Table 1). The characteristics of patients with symptomatic MM, including *FGFR3* expression, were similar in all MB4 subtypes at diagnosis (Table 1).

In each subgroup, about two-thirds of patients received

Table 1. Characteristics of MGUS/stage I MM (n=38) and patients with symptomatic MM (n=256), according to the MB4 breakpoint.

Breakpoint	MB4-1	MB4-2	MB4-3
MGUS/stage I MM n=38 (%)	28 (74)	2 (5)	8 (21)
Symptomatic MM n=256 (%)	159(62)	53 (21)	44 (17)
Median age (years)	58 (36-91)	56 (33-76)	59 (46-74)
IgA isotype n=101/235 (%)	57 (38)	25 (53)	19 (47)
Median % of bone marrow plasma cells (range)	22 (1-90)	29 (0-81)	36 (1-94)
Calcemia >2.7 mmol/L n=30/137 (%)	19 (22)	7 (27)	4 (17)
Creatinine>170 µmol/L n=27/138 (%)	14 (16)	5 (19)	8 (35)
Elevated lactate dehydrogenase n=29/102 (%)	16 (25)	4 (21)	3 (21)
Anemia (hemoglobin<10 g/dL) n=74/137 (%)	46 (52)	13 (52)	15 (65)
International Staging System (ISS) n=134 (%)			
ISS 1	20 (30)	6 (33)	4 (20)
ISS 2	27 (41)	4 (22)	8 (40)
ISS 3	19 (29)	8 (44)	8 (40)
FGFR3 expression n=256 (%)			
Yes	129 (81)	43 (81)	36 (82)
No	30 (19)	10 (19)	8 (18)
Treatment n=256 (%)			
Bortezomib-based regimen	109 (69)	41 (77)	31 (70)
Bortezomib+immunomodulatory drug	28 (18)	17 (32*)	4 (10)
High dose therapy + ASCT	113 (71)	40 (75)	34 (77)
Response n=142 (%)			
Overall response	68 (78)	24 (89)	20 (76)
>Very good partial response	44 (49)	19 (70*)	12 (44)
Partial response	24 (29)	5 (19)	8 (32)
Stable disease	8 (10)	0 (0)	2 (8)
Progressive disease	8 (10)	3 (11)	3 (12)

**Significant differences between the three groups: percentage of response superior to very good partial response (P=0.049) and percentage of immunomodulatory drug-based regimen (P=0.0228).*

a bortezomib-containing regimen in frontline therapy and two-thirds of patients had an autologous stem cell transplant. Only four patients received such a transplant after relapse. A combined immunomodulator-bortezomib regimen was used more frequently in MB4-2 patients (32%) than in MB4-1 (18%) (P=0.026) or MB4-3 (10%) patients (P=0.0014).

Overall response rates to the first-line therapy, according to International Myeloma Working Group statements[12] were 78%, 89% and 76% in the MB4-1, MB4-2 and MB4-3 sub-groups, respectively. Interestingly, 70% of MB4-2 patients achieved a very good partial response or better, as compared to 49% and 44% of patients with MB4-1 and MB4-3, respectively (P=0.049). Thus, MB4-2 breakpoint was associated with a better quality of response to the front-line treatment, possibly as a result of a more frequent use of a combination of immunomodulatory drugs plus bortezomib.

The prognostic impact of each breakpoint (MB4-1, MB4-2 or MB4-3) was analyzed by comparing patients' outcome (event-free survival, overall survival, survival after the first relapse) using Kaplan-Meier analysis. As shown in Figure 1B, with a median follow-up of 33 months, there was no evidence that event-free survival of the three subgroups was different (P=0.26). In contrast, the overall survival of patients with the MB4-2 breakpoint was shorter than that observed in patients with the other breakpoints (P=0.022) (Figure 1A). Accordingly, survival after first relapse was reduced in the MB4-2 subgroup (median overall survival: 14.6 months *versus* 23.7 months, P=0.036) (Figure 1C). In multivariate analysis testing MB4 breakpoints, hemoglobin level, calcemia, serum beta-2 microglobulin level and International Staging System score as survival parameters, MB4-2 was an independent prognostic factor for survival [hazard ratio (HR)=1.8; 95% confidence interval (CI): 1.05-3.08; P=0.03)] along with hypercalcemia (HR=3.4; 95%CI: 2.04-5.81; P<0.0001). Thus, symptomatic t(4;14) MM patients with the MB4-2 breakpoint are sensitive to first-line therapy, but develop chemo-resistant relapse and have a poorer outcome.

To further investigate the impact of WHSC1 breakpoints on the outcome of patients with t(4;14), deletion of chromosome 17 at p13 [del(17p)], which is associated with high-risk MM, was analyzed by fluorescence in situ hybridization at diagnosis in 162/256 cases for which tumor plasma cells were available. Overall, del(17p) was detected in 34 out of 162 cases (21%). Only 5/34 patients (2 MB4-1, 1 MB4-2 and 2 MB4-3) had a del(17p) in less than 60% of plasma cells (31-45%). The presence of del(17p) was associated with a shortened survival (median survival: 21 *versus* 40 months, P=0.00766) (*data not shown*). The del(17p) was statistically more frequently

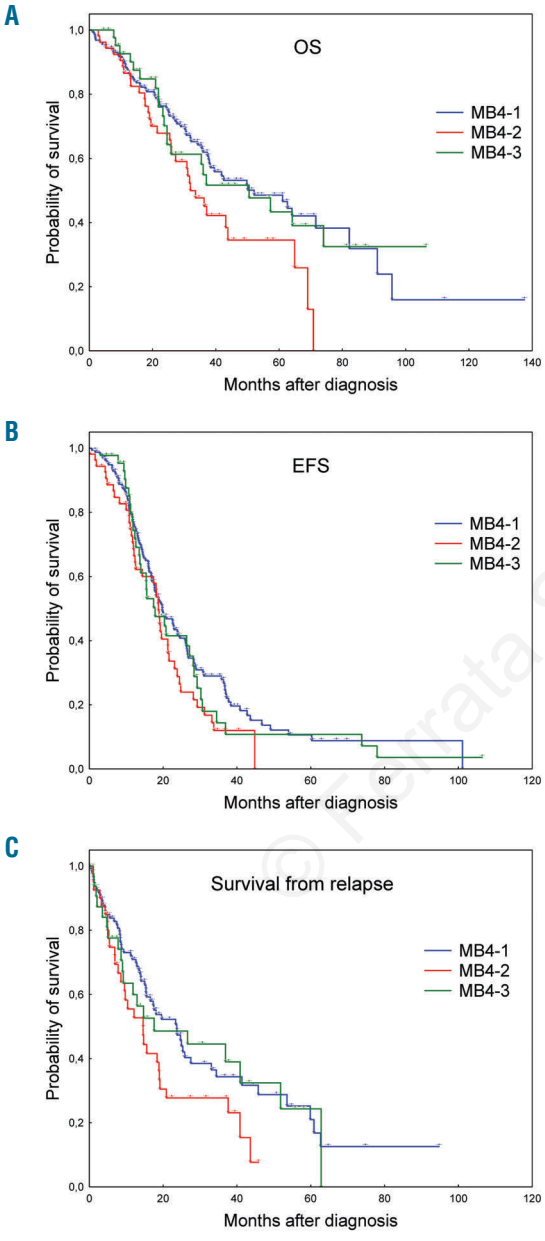

Figure 1. (A) Overall survival (B) event-free survival and (C) survival after the first relapse of the 256 patients with symptomatic MM according to the MB4 breakpoint. MB4-1 (n=159): blue curve, MB4-2 (n=54): red curve and MB4-3 (n=43): green curve. In (B) and (C), statistical significance between MB4-2 and the two other subgroups were P=0.022 and P=0.036, respectively (Kaplan-Meier analysis).

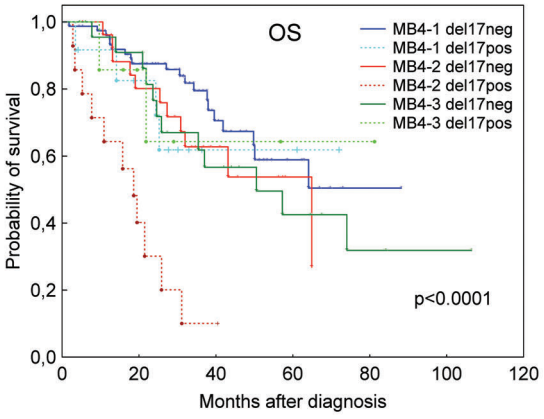

Figure 2. Overall survival of the 162 patients in whom fluorescence *in situ* hybridization 17p analysis was available, according to the MB4 breakpoint and del(17p). Statistical significance between MB4-2 del(17p) positive (pos.) and the other subgroups was P<0.0001. (Kaplan-Meier analysis) (MB4-1: del17 neg: n=77, del 17 pos n=13; MB4-2: del17 neg n=27, del17 pos n=14; MB4-3: del17 neg n=24, del17 pos n= 7).

found in tumor cells with MB4-2 (14/41, 34%) as compared to MB4-1 breakpoint (13/90, 14%) (P=0.0216) but was found at a similar rate in plasma cells with MB4-3 breakpoints (7/31, 28%) (P=0.292). The frontline therapy was similar in the del(17p)-positive and -negative MB4 groups. Interestingly, among patients with del(17p), those with the MB4-2 breakpoint had a shorter overall survival than those with the MB4-1 or MB4-3 breakpoint with a median overall survival of 18.6 months (P<0.0001) (Figure 2). In contrast, the MB4 breakpoint had no impact on overall survival in the absence of del(17p) (Figure 2). In multivariate analysis of patients for whom del(17p) status was determined (n=50), MB4-2 was an independent marker of survival (HR: 2.12; 95% CI: 1.20-3.73; P=0.009). These results indicate that patients with del(17p) and the MB4-2 breakpoint constitute a subset of very high risk patients with a very poor prognosis.

Our results contrast with those of a previous study by Keats *et al*., who found a similar overall survival in the different MB4 subgroups[11]. However, in that study, survival analysis was performed on a smaller number of patients (n=43). In addition, the survival analysis compared patients with MB4-1 (n=30) to the pooled MB4-2 and MB4-3 subgroup (n=13), according to their ability to encode a full length or a truncated MMSET protein. Pooling MB4-2 and MB4-3 cases may have masked the specific prognostic value of the MB4-2 breakpoint.

At present, the molecular basis for the particularly poor prognosis associated with the MB4-2 breakpoint is not clear. Recent studies have shown that over-expression of MMSET triggers a genome-wide increase of H3K36 dimethylation and drives oncogenic properties *in vitro*.[9-13] In addition, increased H3K36 dimethylation was reported in a series of children with pediatric acute lymphoblastic leukemia expressing mutated or MB4-2-like truncated MMSET.[14,15] Thus, it is possible that the higher genome-wide level of H3K36me2 resulting from over-expression of a truncated MB4-2 MMSET protein could render tumor plasma cells more sensitive to first-line therapy, but could eventually drive the emergence of chemoresistant clones that could shorten patients' survival.

MMSET has also been implicated in the cellular response to DNA damage through its H4K20 histone methyltransferase activity.[10] This function requires the phosphorylation of serine 102 by the ATM protein, which facilitates the binding of MMSET at DNA double-strand breaks and the recruitment of 53BP1.[10] The removal of Ser102 in truncated MMSET isoforms might therefore enhance genomic instability and promote the emergence of resistant clones in patients. However, Ser102 is deleted in the forms derived from both MB4-2 and MB4-3, and so differential effects on DNA damage are unlikely to account for the different clinical outcomes observed for these two breakpoints. One potentially important difference between MB4-2 and MB4-3 could relate to the position of these breakpoints relative to the 5' PWWP domain involved in protein-protein interactions. While the MB4-3 breakpoint leads to a truncated MMSET protein lacking the entire PWWP domain, the product expressed from MB4-2 product retains part of this domain.[11] Future studies will need to address whether these truncated MMSET proteins could interact with different partner proteins and exert different activities on histone H3 and H4, with specific impact on patients' outcome.

In our study, a high frequency of del(17p) was observed in plasma cells overexpressing the MB4-2-derived truncated form of MMSET. The combination of both genetic abnormalities severely impairs patients' outcome. In this retrospective study, del(17p) was specifically included as it was the only poor prognostic marker which had been determined in a large proportion of patients. It is now important to investigate the clinical interactions between the different MMSET breakpoints and other genetic markers associated with poor prognosis, including gain of 1q, del(12p) and del(17p), in prospective studies.

Together, our results indicate that the breakpoint within the MMSET locus may explain, in part, the prognostic heterogeneity of t(4;14) gammopathies. Thus, a systematic identification of the MB4 breakpoint, along with screening for a del(17p), may be useful in the management of patients with t(4;14) MM and may pave the way for specific therapies targeting MMSET activity.

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