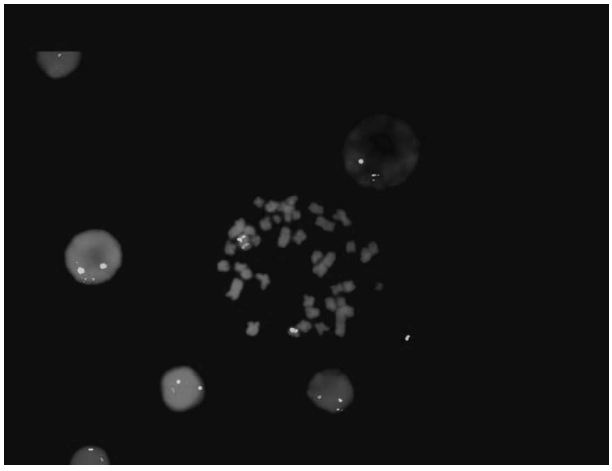

Figure 1. Methaphase and interphase FISH analysis of a representative MDS case with 5q- and deletion of *NPM1* gene. Spectrum red: *NPM1* specific BAC (RP11-117L6). Spectrum green: 5ptel48 probe.

In our series, none of the 7 cases with *NPM1* deletions showed at karyotypic examination a deletion that included the *NPM1* gene locus at 5q35. However, it should be noted that the inaccuracy of the deleted region assignment by chromosome banding has been reported for 5q deletions in both MDS and AML.[11]

Our findings suggest that NPM1 haploinsufficiency may have a role in myeloid malignancies associated with large 5q- deletions contributing to MDS development likely through genetic instability. This would be in line with the finding that *NPM1* is haploinsufficient in the control of centrosome duplication in the mouse model, as well as with evidence that *NPM1* hypomorphic mouse embryonic fibroblasts reveal high levels of tetraploidy and aneuploidy.[4] Alternatively, NPM1 deletions may represent secondary events linked to the progression of the disease in MDS.

Given the low number of cases and heterogeneity of treatments, we were unable to determine here the clinical significance of *NPM1* deletion in MDS with 5q-. Hence a larger series of cases including homogeneously treated patients is needed to investigate the prognostic impact of these abnormalities.

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Prognostic value of the International Scoring System and response in patients with advanced Waldenström macroglobulinemia

Waldenström macroglobulinemia (WM) is a lymphoproliferative disorder characterized by the production of serum monoclonal immunoglobulin (Ig) M and lymphoplasmacytic bone marrow infiltration.[1] Median survival ranged from 60 to 77 months[1,2] and the International Scoring System for WM (ISSWM) identified 3 risk subgroups with significantly different survival after starting first treatment.[2]

Despite this difference in outcome, almost all patients progressed, even low-risk patients (15% of cases), although their median survival was estimated to be 12 years and few events occurred during their first four years of follow up.[2] Therefore, it is unlikely that improving prognostic assessment at the initiation of first-line therapy will identify a large subset of patients requiring new treatment approaches. In order to identify later during the follow up the patients who may need reinforced therapy or an innovative approach, we assessed the role of ISSWM in patients who required second or a subsequent line of therapy.

Eighty-two WM patients have been included in one of 3 trials evaluating the following combination regimens: fludarabine and cyclophosphamide[3] (n=47), fludarabine, cyclophosphamide and rituximab[4] (n=29), and fludara-

bine and rituximab[5] (n=6). Fifty-one of these 82 patients were enrolled for the treatment of relapsing or primary refractory WM and ISSWM was available before the initiation of one of these regimens. The median survival of these 51 patients was not significantly different from that of the remaining patients registered for salvage therapy. Response (complete, partial or minor) was evaluated according to the updated recommendations of the third International Workshop on WM.[6] Subsequent survival was calculated from the date of inclusion in the study for salvage therapy to death, the stopping date or the last follow up.

Main patient characteristics before inclusion are reported in Table 1. Forty-two (82%) of the 51 patients had received one or more regimens with alkylating agents, 10 (19%) one or more regimens with purine analog, 12 (24%) one or more doxorubicin containing regimens, and 6 (11%) a rituximab-based therapy.

Treatment was initiated because of cytopenia, hyperviscosity, constitutional symptoms, IgM related symptoms and organomegaly in 68%, 27%, 23%, 16% and 15% of patients, respectively. ISSWM-risk, as assessed before inclusion for salvage therapy, was low, intermediate and high in 11 (22%), 17 (33%) and 23 (45%) patients, respectively. Patients at low risk before inclusion had a shorter time from first therapy to registration in the study than other patients (P=0.04) and they were younger, as expected from the low-risk criteria (P<0.01, Table 1). At the end of salvage therapy, the overall response rate was 80% (Table 1), in agreement with previously reported rates from 56% to 94%, with similar therapies.[3,7,8] Stable or progressive disease was more frequently recorded in high-risk patients (32% *vs.* 7%, P=0.06). Twenty-four events occurred during follow up. Relapse or progression was observed in 19 patients (6 low-risk, 7 intermediate-risk and 6 high-risk patients). Transformation in diffuse large B-cell lymphoma occurred in one low-risk patient. Five patients died without evidence of relapse or progression (3 patients from acute myelogenous leukemia (AML), one from infection and one from a solid tumor). Median subsequent event-free survival (EFS) was 43 months (95% confidence interval [CI_{95}]: 27-73). At the date of analysis, 13 patients have died (progression n=7 including one histological transformation, AML n=3, infection n=2 and cancer one). Subsequent survival was estimated to be 67% (CI_{95} [54-81]) at 48 months with a median follow up of 27 months. High-risk patients had a significantly shorter subsequent survival than low- or intermediate-risk patients (66% *vs.* 96% at two years, P=0.019), whereas no difference was found between low- and intermediate-risk patients (Figure 1A). High-risk patients also had a significantly shorter subsequent cause-specific survival than low- or intermediate-risk patients (P=0.05, Figure 1B).

Few studies focused on the prognostic factors for the outcome after progression or relapse in WM patients. By contrast with the present study, the Lille system split into 3 risk groups with very different outcome (P<0.0001) in WM patients who received fludarabine or the cyclophosphamide, doxorubicin and prednisone combination at the time of their first progression.[9] As indicated above, patients reported in the present study were more heavily treated before registration. Salvage combination regimen included fludarabine in all patients and rituximab in 51% of cases. Therefore, it would have been useful to check the effectiveness of the Lille system in this series, but albumin was not available for a large subset of patients. In the present study, the time elapsed from first therapy to inclusion was shorter in patients at low risk before salvage therapy. Reluctance of physicians to propose an intensive salvage regimen trial in cases of late progression without adverse characteristics may explain this difference. This discrepancy in characteristics of patients at low risk before registration may explain, at least in part, the lack of difference in subsequent outcome between low- and intermediate-risk patients. In addition, the long-term events reported after fludarabine therapy[10] may play a role; indeed, one (9%) of the 11 low-risk patients died from AML and another patient died from progression after transformation in diffuse large B-cell lymphoma.

Although time elapsed from first therapy to inclusion in the study was shorter in the absence of initial response (median 12 months *vs.* 61 months, P<0.001), this time duration had no influence on response rate after salvage therapy. In addition, response rates achieved after a salvage trial were similar in cases of previous response and in cases of primary refractory disease. Moreover, response to salvage therapy significantly prolonged subsequent survival (2-year survival at 91% *vs.* 55%, P=0.005). Thus, response to previous therapy and time between first-line therapy and inclusion in the study were not significant prognostic factors for subsequent EFS and survival. Similarly, patients with WM who achieved a minor response have been reported to do as well as those achieving an objective response after rituximab single agent therapy.[11] Further analyses should

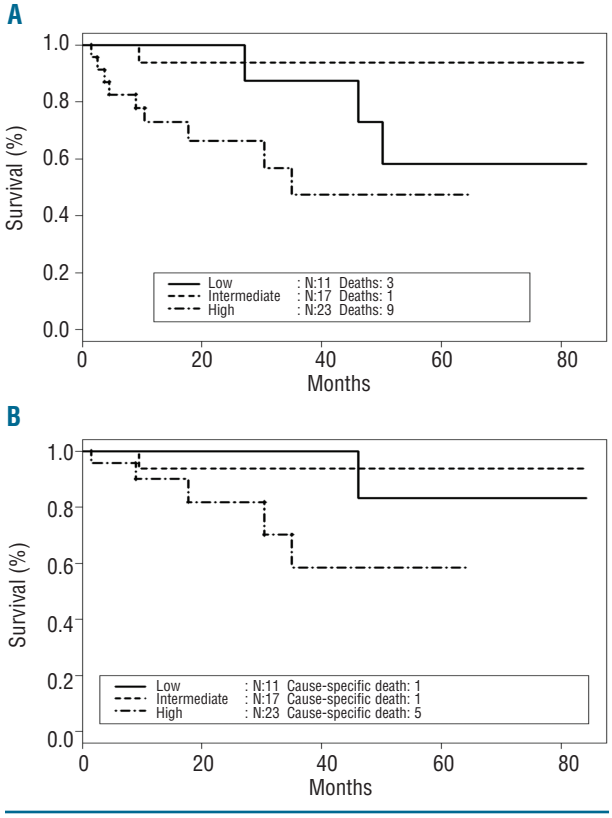

Figure 1. Subsequent survival and cause-specific survival in patients with advanced Waldenström macroglobulinemia according to the International Scoring System risk evaluated at the time of salvage therapy. (A) Survival: *P* value was 0.046 for the overall comparison of the 3 subgroups and 0.019 for the comparison of high-risk and remaining patients (intermediate or low-risk). (B) Cause-specific survival. *P* value was 0.05 for the comparison of high-risk and remaining patients (intermediate or low-risk).

Table 1. Characteristics at the time of initiation of salvage therapy and outcome of 51 patients with advanced Waldenström macroglobulinemia.

Characteristics	Total	Low Risk (n=11)	Intermediate Risk (n=17)	High Risk (n=23)	*P* value (low-risk *vs.* other)
Sex ratio: Male/Female	35/16	8/3	12/5	15/8	
Median age (years)		50.4 (35-64)	69.7 (47-76)	69.7 (40-82)	<0.01
Other factors of the International Scoring System					
Hemoglobin (g/dL)					
≤ 11.5	41	7 (64%)	11 (65%)	23 (100%)	
> 11.5	10	4 (36%)	6 (35%)	0	
Platelet count (10^9/L)†					
≤ 100	14	0	1 (6%)	13 (81%)	
> 100	29	11 (100%)	15 (94%)	3 (19%)	
Serum β2-microglobulin (mg/L)†					
> 3	28	2 (18%)	8 (50%)	18 (90%)	
≤ to 3	19	9 (82%)	8 (50%)	2 (10%)	
Serum monoclonal protein (g/dL)					
≤ 7.0	50	11 (100%)	17 (100%)	22 (95%)	
> 7.0	1	0	0	1 (5%)	
Median time from diagnosis to relapse/progression (months)		16.2 (4-85)	48 (4-217)	45 (10-188)	0.004
Median time from first therapy to relapse/progression (months)		12.3 (3-68)	22.4 (4-205)	27.9 (2-122)	0.04
Number of previous lines of therapy					
One	32	7 (64%)	10 (59%)	15 (65%)	
More than one	19	4 (36%)	7 (41%)	8 (35%)	
Status					
Primary refractory	20	4 (36%)	7 (41%)	9 (39%)	
Relapse	31	7 (64%)	10 (59%)	14 (61%)	
Treatment					
FC	25	8 (73%)	3 (18%)	14 (61%)	
RFC	20	3 (27%)	10 (59%)	7 (30%)	
RF	6	0	4 (23%)	2 (9%)	
Response					
Complete and Partial Response	34	7 (70%)	12 (70%)	15 (68%)	
Minor response	4	2 (24%)	2 (20%)	0	
Stable and Progression	9	1 (10%)	1 (6%)	7 (32%)	
NE	4	1	2	1	
Two-year subsequent EFS*	67 (56-78)	87 (67-100)	68 (48-96)	47 (29-78)	0.79
Two-year subsequent survival*	86 (74-92)	100 (67-100)	94 (83-100)	66 (48-91)	0.04
Outcome					
Number of events	24	7	7	10	
Number of relapses (incl DLBCL)	19	6	7	6	
DLBCL	1	1	0	0	
Number of deaths without relapse	5	1	0	4	
AML	3	1	0	2	
Number of deaths	13	3	1	9	
Causes of death					
WM progression	7	1	1	5	
Infection	2	1	0	1	
AML	3	1	0	2	
Cancer	1	0	0	1	

*Percentages in parentheses. CI: confidence interval. †Data were unavailable for some patients, without consequence on risk assessment according to International Scoring System. F: fludarabine, C: cyclophosphamide, R: rituximab. NE not evaluable, EFS: Event-free survival, DLBCL: diffuse large B-cell lymphoma, AML: acute myelogenous leukemia; * 95% confidence intervals are indicated in parentheses.*

assess the interactions between the prognostic factors observed before salvage therapy and response; they might be particularly useful for identifying high-risk patients who may require intensive therapy, especially allogeneic stem cell transplantation,[12] despite the achievement of good response after salvage therapy.

Although the small numbers of patients and events mean that conclusions cannot be reached, especially on intermediate- and low-risk patients, ISSWM was effective for identifying high-risk patients in advanced WM. Therefore, ISSWM should be reported for all treatment studies in WM patients. Achievement of response was also an important prognostic factor for subsequent survival in patients with advanced WM.

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