High *XBP1* expression is a marker of better outcome in multiple myeloma patients treated with bortezomib

Multiple myeloma (MM) is a hematologic tumor characterized by accumulation of monoclonal plasma cells (PCs) in the bone marrow (BM) producing antigen-specific immunoglobulins. The transcription factor X box binding protein 1 (XBP1), the interferon regulatory factor 4 (IRF4) and the transcriptional repressor B lymphocite-induced maturation protein 1 (BLIMP1) are essential to drive physiological plasmacytic differentiation.[1,2] XBP1 is particularly required for the last stages of B-cell differentiation into PC, and, consistently, XBP1-deficient mice display normal B-lymphocite development up to germinal center, but are unable to produce PCs.[2] High mRNA levels of *IRF4*, *BLIMP1* and *XBP1* have been detected in malignant PC and are negative prognostic factors in patients treated with standard chemotherapy or thalidomide.[3,4] Lenalidomide seems to overcome the negative prognostic impact of *IRF4* overexpression, due to its rapid downregulation following treatment. Bortezomib induces better responses in patients with high levels of *XBP1*.[5,6]

We assessed the prognostic role of gene-driven plasmacytic differentiation in a large cohort of patients treated with bortezomib. RNA expression of three genes involved in PCs differentiation was investigated in purified PCs ($CD138^+$ BM fraction) of well-characterized patients with newly diagnosed MM. One hundred and fifty-one patients enrolled in two multicenter clinical trials (the PAD-MEL100-LP-L and the VMP-VMPT) were assessed.[7,8] PCs were purified using anti-CD138-coated magnetic MicroBeads and AutoMACS Pro Separator (Miltenyi Biotech GmbH, Germany) following manufacturer specifications. Gene expression was investigated on isolated PCs with more than 90% of purity assessed by flow cytometry. RNA was extracted using the DNA/RNA Purification Kit (Norgen, Thorold, Canada). Complementary DNA was produced using High capacity cDNA RT Kit (Applied Biosytem, Foster Ciy, CA, USA). Quantitative PCR to measure RNA expression of *XBP1*, *IRF4* and *BLIMP1* was performed with the Abi Prism 7900HT (Applied Biosystems, Carlsbad, CA, USA) using a relative quantification based on ΔΔCt approach and *GUSB* (β-glucuronidase) as housekeeping gene. All RNA determinations were performed using the following assays: Hs00231936_m1 (*XBP1*), Hs01056533_m1 (*IRF4*), Hs00153357_m1 (*BLIMP1*). Patients were divided accord-

Table 1. Univariate and multivariate analysis.

	Univariate analysis - PFS			Univariate analysis - OS		
	HR	(95% CI)	P	HR	(95% CI)	P
Age, years						
>70	1.11	0.73 - 1.67	0.637	0.96	0.50 - 1.85	0.896
FISH risk						
high	1.01	0.66 - 1.54	0.955	1.08	0.55 - 2.09	0.828
ISS						
I *vs.* II *vs.* III			0.063			0.195
II *vs.* I	1.22	0.72 - 2.08	0.463	1.39	0.56 - 3.46	0.475
III *vs.* I	1.99	1.10 - 3.62	**0.024**	2.37	0.90 - 6.27	0.082
Response to therapy*						
≤VGPR *vs.* CR	62.55	8.08 - 484.10	**<0.001**	32.89	1.64 - 657.91	**0.022**
XBP1 expression						
high	0.50	0.33 - 0.76	**0.001**	0.54	0.28 - 1.06	0.071
IRF4 expression						
high	0.58	0.38 - 0.87	**0.009**	0.79	0.42 - 1.52	0.485
BLIMP1 expression						
high	0.85	0.57 - 1.28	0.445	0.81	0.43 - 1.55	0.530

	Multivariate analysis - PFS			Multivariate analysis - OS		
	HR	(95% CI)	P	HR	(95% CI)	P
ISS						
I *vs.* II *vs.* III			0.285			0.569
II *vs.* I	1.29	0.75 - 2.21	0.355	1.33	0.53 - 3.33	0.538
III *vs.* I	1.68	0.88 - 2.20	0.114	1.76	0.62 - 4.96	0.289
Response to therapy						
≤VGPR *vs.* CR	**58.42**	**7.00 - 487.58**	**<0.001**	**22.41**	**1.14 - 439.44**	**0.041**
XBP1 expression						
high	**0.53**	**0.33 - 0.86**	**0.010**	**0.47**	**0.21 - 1.02**	**0.055**
IRF4 expression						
high	**0.61**	**0.38 - 0.99**	**0.043**			

High risk FISH is defined as having at least one of the following abnormalities: del(17p) or t(4;14) or t(14;16). HR: hazard ratio; CI: confidence interval; ISS: International Staging System; VGPR: very good partial response; CR: complete response. Response to therapy has been treated as independent variable.

ing to gene expression using the median value as cut off. Response to therapy and clinical outcome were assessed following IMWG criteria.[9] The progression-free survival (PFS) and overall survival (OS) were estimated by the Cox proportional hazard model, comparing the risk factors by the Wald test; best response was treated as a time-dependent variable. Patients' characteristics were compared by the Fisher's exact test for the categorical variables and the Mann-Whitney test for the continuous ones. All reported *P*-values were two-sided, at the conventional 5% significance level. Data were analyzed as of January 2013 by SPSS 21.0.0 and R 3.0.1 package survivalROC.

No differences in base-line β2-microglobulin and albumin levels have been observed between patients according to gene expression. A higher proportion of patients with high *XBP1* had ISS I (n=24) compared to patients with low *XBP1* (n=12, *P*=0.03). No differences in FISH karyotype were observed between patients with high and low *XBP1* expression.

Though a recent study found an association between *XBP1* RNA expression levels and response to treatment in patients receiving bortezomib-based therapy,[6] in our study, no correlation between *XBP1* expression and response to bortezomib-containing regimens was observed. Patients achieving a complete response had median *XBP1* RNA expression (8.14; QR 4.68–13.76) similar to that of patients obtaining very good partial response (8.73; QR 3.56–12.72), partial response (8.26; IQR 3.82–9.93), or stable disease (7.68; IQR 4.11–11.12). The discrepancy between our study and the previous study[6] may be due to differences in the: i) inclusion criteria (previously untreated *versus* relapsed patients, respectively); and ii) interval between BM investigation and start of bortezomib treatment (short *vs.* heterogeneous, respectively).

In our study, the 3-year PFS was 59% for patients with high *XBP1* RNA expression and 28% for patients with low *XBP1* (*P*=0.001), translating into a higher 3-year OS probability (86% *vs.* 74%; *P*=0.067) for patients with high *XBP1* levels. High *IRF4* RNA expression identified patients with better PFS (51% *vs.* 36% respectively; *P*=0.008) but with only slightly and not significantly improved OS (85% *vs.* 75% respectively; *P*=0.484) (Figure 1). No differences in PFS (*P*=0.444) and OS (*P*=0.529) differences have been observed according to *BLIMP1* RNA expression. Similar results were obtained when only patients receiving the same treatment were analyzed even if no statistical significance was reached due to the low number of events in each subgroup.

In univariate analysis, response to therapy, *XBP1* expression and *IRF4* expression were the main predictors of PFS. Response to therapy also significantly correlated with OS, while *XBP1* expression almost reached statistical significance (Table 1). In Cox multivariate analysis, response to therapy, *XBP1* and *IRF4* expression were shown to be independent predictors of PFS.

Although no correlation between *XBP1* RNA expression

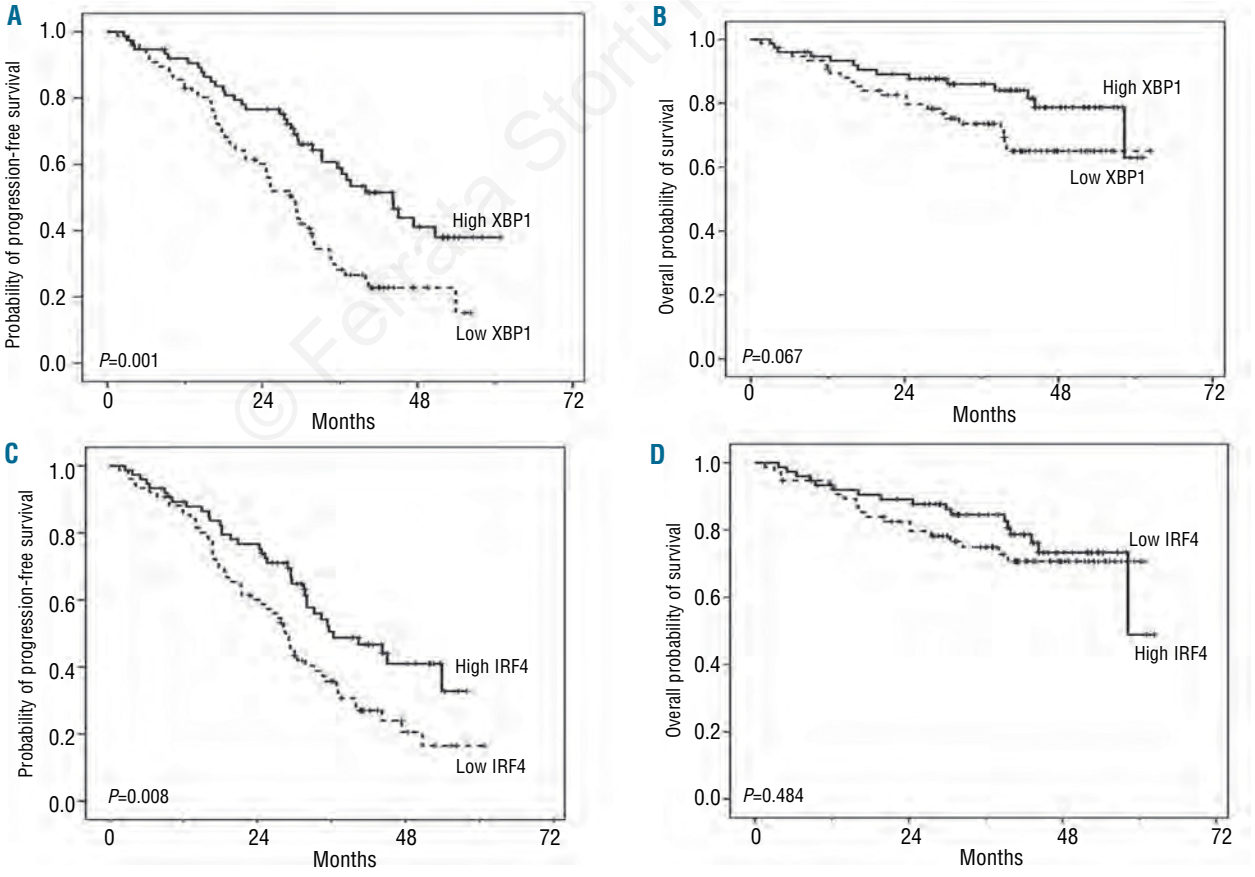

Figure 1. Clinical outcome according to *XBP1* and *IRF4* RNA value. PFS (A) and OS (B) in 151 MM patients according to XBP1 RNA expression. Median *XBP1* RNA value has been used as cut off. PFS (C) and OS (D) in 151 MM patients according to IRF4 RNA expression. Median *IRF4* RNA value has been used as cut off.

and response to therapy was found, our results evidenced that patients with high *XBP1* expression who received bortezomib-based therapy have a better outcome. Bortezomib inhibits the proteasome activity and induces apoptosis determining reduction of protein degradation and accumulation of misfolded proteins. In MM, the amount of immunoglobulin production (controlled also by XBP1) correlates with bortezomib sensitivity and *XBP1* RNA decreases after bortezomib administration.[10] Bortezomib is more effective in patients with high *XBP1* expression, probably due to its key role in the unfolded protein response and in immunoglobulin production, suggesting that bortezomib could reduce protein degradation leading to immunoglobulin accumulation and finally to cell damage.

High expression of *IRF4* was associated with poor prognosis in MM patients treated with standard chemotherapy, but lenalidomide can overcome its negative prognostic impact.[3-5] *IRF4* is one of the target genes of lenalidomdie and is necessary for the drug activity. Our study highlighted the prognostic role of *IRF4* in MM patients receiving bortezomib, suggesting that all novel drugs can overcome the negative impact of high IRF4 expression.

High *XBP1* showed to be a marker of improved outcome in MM patients treated with bortezomib. The combination of *XBP1* expression and response to therapy further predicts better clinical outcome. Additional analyses are required to confirm these data in an independent cohort, to clarify the action of novel drugs on genes involved in plasmacytic differentiation and to evaluate the opportunity to include drugs targeting this pathway in the myeloma therapy.

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