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ORIGINAL ARTICLE

# Early tumor-cell gene expression changes may predict the response to first-line bortezomib-based therapy in patients with newly diagnosed multiple myeloma

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## *Summary*

***Purpose:*** *Maximizing the response rate to first-line therapy in patients with multiple myeloma (MM) is important because it leads to improved outcome. Gene-expression studies have identified prognostic gene sets in patients receiving bortezomib-based therapy. Comparison of the lists of genes derived from two gene-expression-based models (GEP70, GEP80) showed that they overlap in three genes, namely PSMD4, BIRC5, and KIAA1754. An unanswered question is whether early gene-expression changes can be used as predictors of the response to first-line bortezomib. In this study we aimed to examine the predictive value of gene expression changes for the depth of response after bortezomib-based therapy in newly diagnosed MM.*

***Methods:*** *We prospectively assessed the relation between early PSMD4, BIRC5, and KIAA1754 gene expression changes (before therapy and one week later) and the response rate after bortezomib-based therapy in 25 patients with newly diagnosed MM. Gene expression was studied by RT-PCR on CD138-selected plasma cells, and changes were recorded as upregulation, downregulation, or unchanged.*

***Results:*** *Whereas baseline prognostic factors including genetic lesions and stage were not predictive of the response rate, we found that early BIRC5 and KIAA1754 gene-expression changes were significantly associated with the depth of response to bortezomib ($p=0.001$ and $p<0.001$, respectively). PSMD4 was not predictive of the depth of response. KIAA1754 upregulation was linked to complete remission (CR) or very good partial remission (VGPR). BIRC5 upregulation was linked to stable disease (SD) or progressive disease (PD). We also observed that BIRC5 upregulation was associated with worse progression-free survival (PFS).*

***Conclusions:*** *Our results suggest that BIRC5 and KIAA1754 gene-expression changes may predict the response to bortezomib-based therapy. These data may have relevance for the stratification and early adaptation of first-line treatment in patients with newly diagnosed MM.*

***Key words:*** *autophagy, bortezomib, gene expression, multiple myeloma, pharmacogenomics, survivin*

## Introduction

The achievement of complete remission or very good partial remission after initial treatment of patients with newly diagnosed MM is an important therapeutic goal and prognostic factor for overall survival [1]. Therefore, the aim of primary therapy is to maximize response rates in all patients to reach complete remission or very good partial remission. The use of bortezomib in first-line therapy accounts for much of the recent progress in MM. Bortezomib targets the proteasomal

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degradation of ubiquitinated proteins leading to cytotoxic injury in myeloma cells, and results in high remission rates in patients with newly diagnosed MM [2,3]. However, the efficacy of first-line bortezomib is variable and unpredictable and almost one third of the patients respond poorly. Much of the clinical research in MM focuses on the mechanisms of resistance to bortezomib.

New evidence from genomic studies indicates that *in vivo* tumor-cell gene expression changes following short-term exposure to anticancer agents may be related to survival [4-6]. Notably, such pharmacogenomic changes seem to be specific for the class of anticancer agent used. There is a basis for the belief that an evaluation of the pharmacogenomic changes may help us make informed choices about the management of patients with cancer and also lead to improvements in our understanding of the molecular mechanisms of drug resistance [4-6].

Global tumor-cell gene expression studies in MM have revealed a number of genes whose expression changed after brief exposure to bortezomib [6]. Based on these findings, changes in the expression of *PSMD4* (encodes a non-ATPase regulatory subunit of the 26S proteasome called Psmd4), *BIRC5* (encodes an anti-apoptosis protein of the IAP family called survivin), and *KIAA1754* (encodes a protein involved in inositol trisphosphate [IP3] signaling called inositol 1,4,5-trisphosphate receptor interacting protein [ITPRIP] or Danger) genes appear to be particularly consistent in predicting overall survival after bortezomib-based therapy. However, the value of these genes in predicting the depth of response to first-line bortezomib-based therapy remains unknown. We therefore asked whether early changes in *PSMD4*, *BIRC5*, and *KIAA1754* gene expression could be used as biomarkers for the response to first-line bortezomib-based therapy in patients with MM. The three genes were chosen for this study because they were the only genes overlapping in two validated gene-expression profiling (GEP) models, the 70-gene [GEP70] model and the 80-gene [GEP80] model, which are widely accepted as valid tools capable of predicting outcome in MM [6,7]. Notably, the three genes were not included among the genes whose expression changed after dexamethasone test dose in another study [5]. Although other GEP models exist such as the EMC-92 gene score based on the HOVON65/GMMG-HD4 trial [8] and the IFM GEP15 based on the IFM-99 trials [9], there is no existing information about the variation in gene expression after bortezomib-based therapy for these GEP scores.

## Methods

*Patients*

In this prospective study, we examined the relationship between early *PSMD4*, *BIRC5*, and *KIAA1754* gene expression changes and depth of response after bortezomib-based therapy in 25 patients with newly diagnosed MM. All patients met the IMWG criteria for symptomatic MM [10]. All patients started therapy between March 2009 and March 2010. Follow-up continued until January 2014 (mean follow-up, 35±14 months). To be eligible for inclusion, patients had to present with bone-marrow plasma cells (BMPC) >20%, ensuring sufficient isolation of plasma cells from aspirated samples. Patients with plasma-cell leukemia were excluded from this study.

Patients underwent risk status assessment based on the mSMART classification which includes detection of t(4;14), t(11;14), t(14;16), del(13q), and del(17p) with interphase fluorescent *in situ* hybridization (FISH) analysis and estimation of the plasma-cell labeling index (PCLI) [11]. In this study, PCLI (i.e., the percentage of plasma cells in the S-phase of the cell cycle) was calculated by means of flow cytometry using propidium iodide; a PCLI value of ≥2.5% was considered to indicate a high proliferation rate. Other prognostic parameters included the International Staging System (ISS) stage (stages I/II vs stage III) [12], the BMPC count (<50% vs ≥50%), the serum lactate dehydrogenase (LDH) concentration (normal vs elevated), and the M-protein quantifications in serum and urine for response assessment.

All 25 patients received biweekly intravenous bortezomib: 19 patients were treated with a combination of bortezomib and dexamethasone (VD) for 4 courses, followed by high-dose melphalan and autologous stem-cell transplantation (ASCT), and 6 patients who were not eligible for transplantation were treated with a combination of bortezomib, melphalan, and prednisolone (MPV) to a total of 9 cycles [13,14]. Response assessment was performed after 4 cycles of treatment based on IMWG criteria [10]. For the purposes of our study, a bone-marrow aspiration sample was collected before treatment (day 0) and another sample was collected one week later (day 7). We selected the above-mentioned timepoints in order to ensure patient adherence and to allow time for gene-expression changes to occur after two doses of bortezomib. Ethical approval for this study was obtained from the Institutional Review Board of Alexandra Hospital in Athens. Written informed consent was obtained from all patients in accordance to the principles of the Declaration of Helsinki.

*Isolation of plasma cells, RNA extraction, and PCR analysis*

CD138+ plasma cells were selected through the

use of immunomagnetic beads coated with an anti-CD138 monoclonal antibody (MACS CD138 microbeads, Miltenyi-Biotec GmbH, Bergisch Gladbach, Germany). Isolated plasma cells were of sufficient quantity (≥0.5×10$^6$ CD138+ cells per ml) and purity (≥95%), as confirmed by two-color (CD45/CD138) flow cytometry and cytospin preparations stained with May-Gruenwald-Giemsa. Total RNA was recovered from the plasma cell suspensions with the use of TRIzol reagent obtained from Invitrogen (California, USA). Gene expression was assessed with quantitative RT-PCR using a customary kit (Pre-validated RT qPCR Primer Assay) obtained from Qiagen Ltd., Crawley, UK. All PCR primers were developed by Qiagen to cover exons which are always expressed (catalog number PPH22763A-200 for *PSMD4*, catalog number PPH08788A-200 for *KIAA1754*, and catalog number PPH00271E-200 for BIRC5). The cDNA product was amplified with Taq DNA polymerase using standard protocols. The concentrations of *PSMD4*, *KIAA1754*, and *BIRC5* mRNA were normalized to those of β-actin mRNA in the corresponding sample.

*Gene expression changes between day 0 and day 7*

The expression (mRNA) of the three plasma-cell genes *PSMD4*, *KIAA1754*, and *BIRC5* was measured before the initiation of bortezomib-based therapy (day 0) and one week later (day 7). Each sample was measured in triplicate and gene expression was expressed as a mean value. We calculated the change in gene expression on day 7 in comparison to the patient's own sample on day 0. The change in gene expression was expressed as a ratio by dividing day 7 mRNA by the mRNA level on day 0, and results were interpreted as follows: a ratio of 0.8-1.2 was considered unchanged, >1.2 was defined as upregulation, and <0.8 was defined as downregulation.

*Statistics*

Data were analyzed with the Pearson's chi-square test and Fisher's exact test. All tests were two-sided. A p value of less than 0.05 was considered to indicate statistical significance. Survival curves were generated according to Kaplan-Meier method and compared with the use of log-rank test. PFS was calculated from the date of initiation of therapy until the date of relapse or death from any cause. The impact of gene expression changes in PFS was estimated with the use of Cox regression analysis.

## Results

*Clinical characteristics*

Our study included 25 patients with newly diagnosed symptomatic MM, with a male:female ratio of 1.3:1. The median age at time of presentation was 61 years (range 43-75). Five patients had initial diagnosis of MGUS before the development of symptomatic MM. Five patients had stage I, 10 had stage II, and 10 had stage III disease. The median hemoglobin level was 10.2 g/dl (range 6.0-14.0). Renal impairment (serum creatinine level >2.0 mg/dl) and hypercalcemia were seen in 24% of the patients at presentation. Serum LDH concentration was elevated in 32% of the patients. Demographic and baseline clinical characteristics of the patients are shown in Table 1.

After 4 cycles of bortezomib-based therapy, 32% of the patients achieved CR or a VGPR, 52% achieved PR, and 16% of the patients had SD or PD.

*Prediction of response to bortezomib-based therapy*

Our analysis showed that the baseline prognostic factors were not capable of predicting the response to bortezomib therapy, including BMPC count (p=0.173), ISS stage (p=0.122), PCLI

**Table 1.** Demographic and clinical characteristics of patients

| *Characteristics* | *Patients. N (%)* |
|---|---|
| Gender | |
| Male | 14/25 (56) |
| Female | 11/25 (44) |
| Age (years) | |
| <65 | 17/25 (68) |
| 65-70 | 3/25 (12) |
| >70 | 5/25 (20) |
| Multiple myeloma type | |
| IgG | 12/25 (48) |
| IgA | 7/25 (28) |
| Light-chain | 6/25 (24) |
| International Staging System | |
| Stage I | 5/25 (20) |
| Stage II | 10/25 (40) |
| Stage III | 10/25 (40) |
| Bone marrow plasma cells (%) | |
| <50 | 8/25 (32) |
| 50-80 | 12/25 (48) |
| >80 | 5/25 (20) |
| Genetic lesions* | |
| t(11;14) | 6/25 (24) |
| t(4;14) | 5/25 (20) |
| t(14;16) | 1/25 (4) |
| del(13q) | 7/25 (28) |
| del(17p) | 3/25 (12) |
| Genetic risk status† | |
| Standard risk | 17/25 (68) |
| High risk | 8/25 (32) |
| Plasma cell labeling index (PCLI) | |
| Low proliferation rate | 18/25 (72) |
| High proliferation rate | 7/25 (28) |

*Genetic lesions were detected with FISH analysis. Some patients had more than one genetic lesion. †Genetic risk status was assessed on the basis of the mSMART (Mayo Stratification of Myeloma and Risk-Adapted Therapy) classification system

(p=0.076), serum LDH concentration (p=0.08), and genetic risk status (p=0.158).

In contrast, we found that early changes in the level of *BIRC5* (p=0.001) and *KIAA1754* (p<0.001) expression were strongly associated with the depth of response to first-line bortezomib. *KIAA1754* upregulation correlated with achievement of CR or VGPR, whereas *BIRC5* upregulation was predictive of poor response (SD or PD). In cases with *BIRC5* upregulation, the mean (±SD) *BIRC5* mRNA ratio was 3.81±2.84 (median 3.96; range 1.28-9.91). In cases with *BIRC5* downregulation, the corresponding value was 0.41±0.20 (median 0.28; range 0.19-0.75). With regard to *KIAA1754* mRNA, the mean (±SD) mRNA ratio was 3.98±2.11 and the median ratio was 4.18 (range 1.27-7.15) in patients with upregulated gene expression. In cases characterized by *KIAA1754* downregulation, the mean (±SD) *KIAA1754* mRNA ratio was 0.49±0.21 and the median mRNA ratio was 0.47 (range 0.27-0.72).

Unlike *BIRC5* and *KIAA1754* expression, our analysis showed that early changes in *PSMD4* expression were not significantly associated with the depth of response to first-line bortezomib therapy (p=0.132). Figure 1 shows the relationship between early gene expression changes and response to treatment with bortezomib. In comparing the data between the two bortezomib groups (patients receiving VD vs patients receiving MPV), we found no significant between-group differences in *BIRC5*, *KIAA1754*, and *PSMD4* gene-expression changes.

Also, we noticed that the combination of *BIRC5* and *KIAA1754* gene expression was highly predictive of the efficacy of first-line treatment. Patients who achieved maximum response (CR or VGPR) after 4 cycles of bortezomib-based therapy had a *BIRC5*/*KIAA1754* combination of either downregulation/upregulation or unchanged/upregulation, respectively. Conversely, patients who had a poor response (SD or PD) to bortezomib-based initial therapy presented with a combination of *BIRC5* and *KIAA1754* gene expression corresponding to upregulation/downregulation, upregulation/unchanged, or unchanged/downregulation, respectively. The achievement of PR was usually associated with parallel changes (i.e., upregulation/upregulation, downregulation/downregulation, or unchanged/unchanged) of *BIRC5* and *KIAA1754* gene expression. Figure 2 illustrates the combination of *BIRC5* and *KIAA1754* gene expression in association with the response to first-line bortezomib-based therapy.

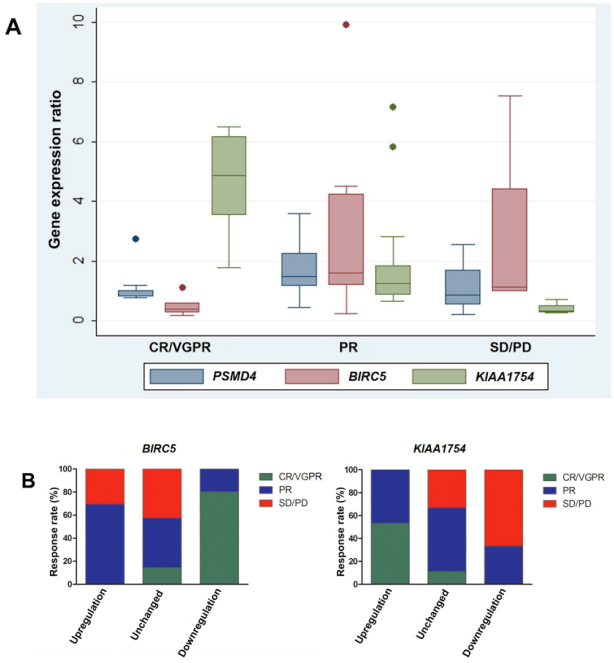


**Figure 1.** Early tumor-cell gene expression changes are related to the response rate to first-line bortezomib-based therapy in newly diagnosed multiple myeloma. **A**: Relationship between early *PSMD4*, *KIAA1754 BIRC5* gene-expression changes and response to initial bortezomib-based therapy. *KIAA1754* upregulation correlates with CR/VGPR (p<0.001), whereas *BIRC5* upregulation correlates with SD/PD (p=0.001). *PSMD4* expression was not a predictor of treatment response (p=0.132). **B**: *BIRC5* and *KIAA1754* gene expression in CR, PR, and SD/PD patients. For abbreviations see text.

Next, we investigated the relationship between *PSMD4*, *BIRC5*, and *KIAA1754* gene expression changes and baseline prognostic factors including ISS stage, genetic risk group, PCLI, and BMPC count. Within the limits of this study, we found no correlation for each of the three genes (*PSMD4*, *BIRC5*, and *KIAA1754*) with the disease stage, BMPC count, PCLI, genetic risk status, or specific cytogenetic abnormalities.

*Gene expression changes and PFS*

Although the prediction of response to first-line bortezomib was the aim of this study, we also examined whether early changes in the expression of *PSMD4*, *BIRC5*, and *KIAA1754* might be related to PFS. Our analysis showed that the upregulation of BIRC5 gene was associated with a shorter PFS, including patients who underwent ASCT, although the effect did not attain statistical significance (hazard ratio, 1.85; 95% confidence interval [CI], 0.94-3.68; p=0.06) (Figure 3).

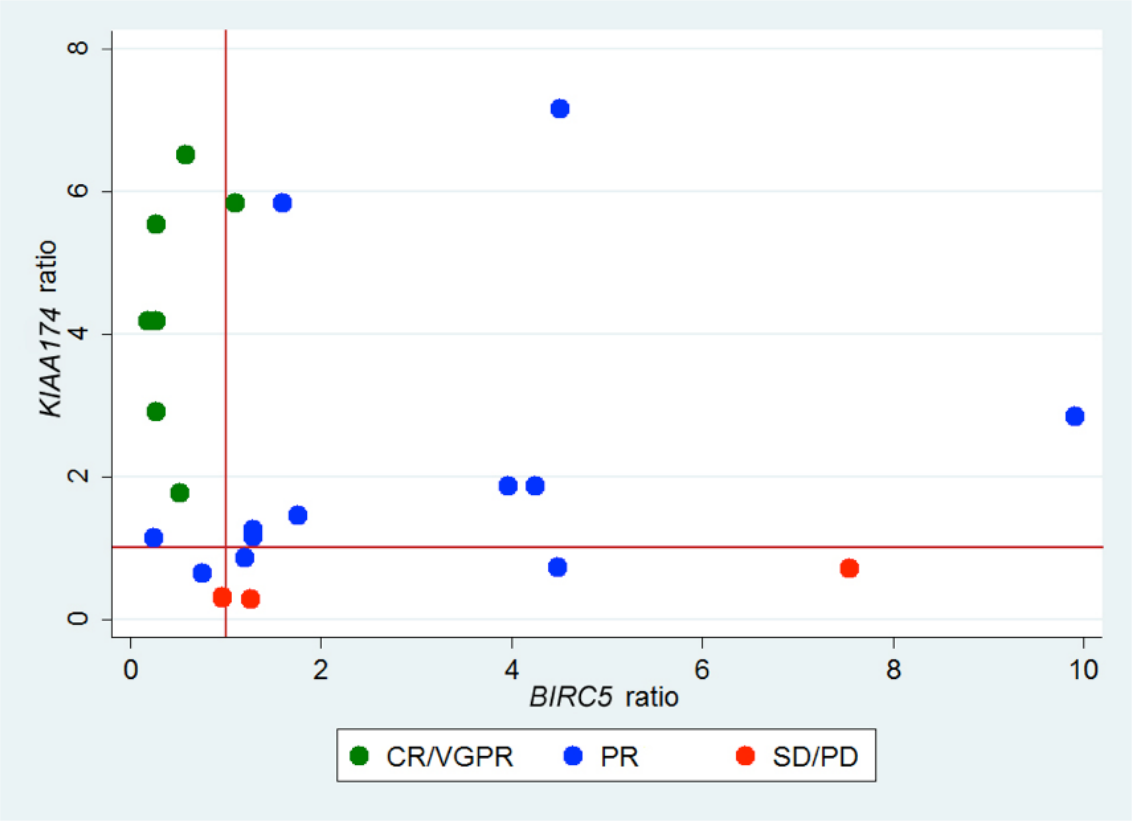


**Figure 2.** Combination of *KIAA1754* and *BIRC5* gene-expression changes. A combination of *KIAA1754* upregulation and *BIRC5* downregulation was associated with major response (CR/VGPR) after first-line bortezomib-based therapy. For abbreviations see text

This finding suggests that the overexpression of survivin in tumor tissue might be associated with more aggressive forms of myeloma characterized by low response rate to bortezomib, early relapse after ASCT, and adverse outcome. Further analysis showed that changes in the expression of *PSMD4* (hazard ratio, 1.108; 95% CI, 0.42-2.92; p=0.837) and *KIAA1754* (hazard ratio, 1.502; 95% CI, 0.553-4.08; p=0.125) genes were not associated with PFS. The fact that more than one third of the patients in this study did not undergo ASCT after initial therapy might account for the lack of correlation of *KIAA1754* expression with PFS, since consolidation of the initial response by means of ASCT significantly prolongs PFS, especially in patients with high-risk cytogenetics.

## Discussion

A predictive technique in the management of patients with newly diagnosed MM could improve the therapeutic index by allowing better individualization of treatment. In the present study, we were able to confirm that the baseline risk factors currently used such as ISS status and genetic lesions, cannot adequately predict the depth of response to primary bortezomib-based therapy. One possible explanation for this may be the fact that bortezomib can overcome the negative impact of several high-risk factors - including the presence of a t(4;14), t(14;16) translocation or a 17p deletion - leading to a higher response rate in the first-line treatment [14-16]. Instead, we found that early changes (between day 0 and day 7) in the mRNA level of *BIRC5* and *KIAA1754* genes may serve as useful predictors of the depth of response to first-line bortezomib-based therapy. Our findings extend the observations of previous studies [6,7], and indicate that early *BIRC5* and *KIAA1754* gene-expression changes may represent potential biomarkers for the susceptibility of the neoplastic plasma cells to bortezomib that is worthy of fur-

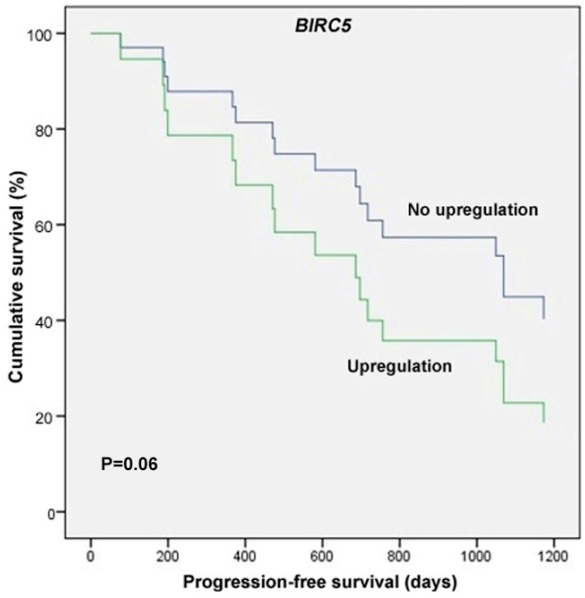


**Figure 3.** Progression-free survival and *BIRC5* expression. In the present study, we found an association of PFS with *BIRC5* gene-expression changes (hazard ratio, 1.85; 95% confidence interval, 0.94 to 3.68; p=0.06).

ther investigation.

The context in which these genes are upregulated and downregulated provides insights into the signaling pathways that are activated following proteasome inhibition by bortezomib. There is a growing suspicion that the nature of bortezomib resistance is more multifaceted and complex than previously thought [17,18]. At a mechanistic level, the results of this study indicate potential molecular mechanisms for primary or early resistance to bortezomib. Our observations about the clinical significance of early *BIRC5* and *KIAA1754* expression changes argue that survivin and the aggresome-autophagosome pathway may be involved in early bortezomib resistance, and invite speculation that these pathways might be clinically more relevant than the effect of increased synthesis of proteasome subunits such as Psmd4 in the development of early resistance.

Survivin encoded by the *BIRC5* gene, is a key bifunctional regulator of cell death and cell proliferation expressed in many cancers such as non-Hodgkin lymphoma, myeloma, colorectal cancer, and neuroblastoma [19,20]. Stimulation of survivin expression results in inhibition of apoptosis due to the inactivation of caspase 3 and 9, enhanced mitotic-spindle assembly, and p53 downregulation [20,21]. Gene products controlling apoptosis can modulate cancer's variable course. Therefore, the inhibition of caspases by survivin may suggest an important mechanism by which early bortezomib resistance develops. In agreement with our findings, survivin overexpression has been linked to bortezomib resistance among patients with mantle cell lymphoma [22].

Proteasome inhibition by bortezomib causes endoplasmic reticulum (ER) stress due to the accumulation of unfolded or misfolded proteins [6,18]. In response to ER stress, a protective pathway is upregulated in tumor cells in which unfolded proteins are aggregated into aggresomes. However, an excess of aggresomes may also trigger oxidative damage and apoptotic death so that aggresome removal by autophagy is essential for tumor-cell survival [23]. Molecular evidence suggests that the calcium-calmomodulin–dependent serine-threonine kinase DAPK-1 plays a major role in the initiation of autophagy [24,25]. The ITPRIP/Danger protein encoded by the *KIAA1754* gene directly binds and inhibits the catalytic activity of DAPK-1 and thus inhibits autophagy [26,27]. Thus, we may speculate that tumors that downregulate *KIAA1754* gene during bortezomib-induced metabolic stress may possess a greater ability for initiation of autophagy and bortezomib resistance. What has clinical implications is that these findings provide supportive evidence that survivin and the aggresome-autophagy pathway may contribute to early bortezomib resistance and could be useful therapeutic targets. YM155, an investigational small molecule that targets survivin, and several autophagy inhibitors are currently undergoing development for use in many cancers including MM [28-30].

Several lines of evidence suggest that survivin expression is a negative prognostic factor in MM associated with a more aggressive disease and a poor outcome [31-33]. These findings are consistent with our observation that *BIRC5* gene upregulation correlated with worse PFS in patients treated with first-line bortezomib-based therapy.

Although our study has recruited a relatively small number of patients, the findings about the changes in the expression of *BIRC5* and *KIAA1754* genes are indicative of potential clinical usefulness as markers of early response. However, our results should be viewed with caution. Additional studies with larger numbers of patients are required to determine the accuracy of a PCR-based strategy for the prediction of response to borte-

zomib-based initial therapy in patients with newly diagnosed MM. Whether intensification of treatment or switch to an alternative regimen, based for example on immunomodulatory drugs, should be pursued when <VGPR is expected should also be evaluated. Of additional interest is to evaluate second-generation proteasome inhibitors such as carfilzomib and ixazomib as to their effects in the expression of *BIRC5* and *KIAA1754* levels and whether these changes are a class effect or bortezomib-specific, given the differences in the pharmacodynamics of the various proteasome inhibitors [34].

In conclusion, our preliminary findings suggest that early changes in *BIRC5* and *KIAA1754* gene expression detected by RT-PCR could be useful predictive markers for the depth of response to bortezomib. RT-PCR is widely available and less expensive than gene-expression profiling (GEP) by DNA-array techniques. In a context in which the focus of clinical care is moving towards personalized medicine to improve drug safety and efficacy [35,36], the data from this study may have relevance for the early stratification and adaptation of primary treatment in newly diagnosed MM. Of perhaps greater interest is that this study supports the idea that pharmacogenomic changes of gene expression may have the ability to predict treatment response after chemotherapy and warrant further exploration for use in patients with MM.

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## References

1. Röllig C, Knop S, Bornhäuser M. Multiple myeloma. Lancet 2014 Dec 22. pii: S0140-6736(14)60493-1 [Epub ahead of print].
2. Terpos E, Roussou M, Dimopoulos MA. Bortezomib in multiple myeloma. Expert Opin Drug Metab Toxicol 2008;4:639-654.
3. Kastritis E, Charidimou A, Varkaris A, Dimopoulos MA. Targeted therapies in multiple myeloma. Target Oncol 2009;4:23-36.
4. Cheok MH, Yang W, Pui CH, et al. Treatment-specific changes in gene expression discriminate in vivo drug response in human leukemia cells. Nat Genet 2003;34:85-90.
5. Burington B, Barlogie B, Zhan F, Crowley J, Shaughnessy JD Jr. Tumor cell gene expression changes following short-term in vivo exposure to single agent chemotherapeutics are related to survival in multiple myeloma. Clin Cancer Res 2008;14:4821-4829.
6. Shaughnessy JD Jr, Qu P, Usmani S et al. Pharmacogenomics of bortezomib test-dosing identifies hyperexpression of proteasome genes, especially PSMD4, as novel high-risk feature in myeloma treated with Total Therapy 3. Blood 2011;118:3512-3524.
7. Shaughnessy JD Jr, Zhan F, Burington BE et al. A validated gene expression model of high-risk multiple myeloma is defined by deregulated expression of genes mapping to chromosome 1. Blood 2007;109:2276-2284.
8. Kuiper R, Broyl A, de Knegt Y et al. A gene expression signature for high-risk multiple myeloma. Leukemia 2012;26:2406-2413.
9. Decaux O, Lode L, Magrangeas F et al. Prediction of survival in multiple myeloma based on gene expression profiles reveals cell cycle and chromosomal instability signatures in high-risk patients and hyperdiploid signatures in low-risk patients: a study of the Intergroupe Francophone du Myelome. J Clin Oncol 2008;26:4798-4805.
10. Durie BG, Harousseau JL, Miguel JS et al. International uniform response criteria for multiple myeloma. Leukemia 2006;20:1467-1473.
11. Kumar SK, Mikhael JR, Buadi FK et al. Management of newly diagnosed symptomatic multiple myeloma: updated Mayo Stratification of Myeloma and Risk-Adapted Therapy (mSMART) Consensus Guidelines. Mayo Clin Proc 2009;84:1095-1110.

12. Greipp PR, San Miguel J, Durie BG et al. International staging system for multiple myeloma. J Clin Oncol 2005;23:3412-3420.
13. Harousseau JL, Attal M, Leleu X et al. Bortezomib plus dexamethasone as induction treatment prior to autologous stem cell transplantation in patients with newly diagnosed multiple myeloma: results of an IFM phase II study. Haematologica 2006;91:1498-1505.
14. San Miguel JF, Schlag R, Khuageva NK et al. Bortezomib plus melphalan and prednisone for initial treatment of multiple myeloma. N Engl J Med 2008;359:906-917.
15. Bergsagel PL, Mateos MV, Gutierrez NC, Rajkumar SV, San Miguel JF. Improving overall survival and overcoming adverse prognosis in the treatment of cytogenetically high-risk multiple myeloma. Blood 2013;121:884-892.
16. Nahi H, Sutlu T, Jansson M, Alici E, Gahrton G. Clinical impact of chromosomal aberrations in multiple myeloma. J Intern Med 2011;269:137-147.
17. Lü S, Wang J. The resistance mechanisms of proteasome inhibitor bortezomib. Biomark Res 2013;1:13.
18. Kumar S, Rajkumar SV. Many facets of bortezomib resistance/susceptibility. Blood 2008;112:2177-2178.
19. Li F, Ambrosini G, Chu EY et al. Control of apoptosis and mitotic spindle checkpoint by survivin. Nature 1998;396:580-584.
20. Fukuda S, Pelus LM. Survivin, a cancer target with an emerging role in normal adult tissues. Mol Cancer Ther 2006;5:1087-1098.
21. Altieri DC. Survivin, versatile modulation of cell division and apoptosis in cancer. Oncogene 2003;22:8581-8589.
22. Paydas S. Can survivin expression predict the response to bortezomib in cases with mantle cell lymphoma? Leuk Lymphoma 2006;47:2412-2413.
23. Mathew R, Karantza-Wadsworth V, White E. Role of autophagy in cancer. Nat Rev Cancer 2007;7:961-967.
24. He C, Klionsky DJ. Regulation mechanisms and signaling pathways of autophagy. Annu Rev Genet 2009;43:67-93.
25. Hison B, Kraus M, Burch L et al. DAPK-1 binding to a linear peptide motif in MAP1B stimulates autophagy and membrane blebbing. J Biol Chem 2008;283: 9999-10014.
26. van Rossum DB, Patterson RL, Cheung KH et al. DANGER, a novel regulatory protein of inositol 1,4,5-trisphosphate-receptor activity. J Biol Chem 2006;281:37111-37116.
27. Kang BN, Ahmad AS, Saleem S et al. Death-associated protein kinase-mediated cell death modulated by interaction with DANGER. J Neurosci 2010;30:93-98.
28. Wagner V, Hose D, Seckinger A et al. Preclinical efficacy of sepantronium bromide (YM155) in multiple myeloma is conferred by down regulation of Mcl-1. Oncotarget 2014;5:10237-10250.
29. Mobahat M, Narendran A, Riabowol K. Survivin as a preferential target for cancer therapy. Int J Mol Sci 2014;15:2494-2516.
30. Liu B, Bao JK, Yang JM, Cheng Y. Targeting autophagic pathways for cancer drug discovery. Chin J Cancer 2013;32:113-120.
31. Romagnoli M, Trichet V, David C et al. Significant impact of survivin on myeloma cell growth. Leukemia 2007;21:1070-1078.
32. Wagner V, Hose D, Seckinger A et al. Survivin in multiple myeloma: prognostic and therapeutic implications. Blood 2011;118 (Supplement): abstr no.137.
33. Tsubaki M, Satou T, Itoh T et al. Overexpression of MDR1 and survivin, and decreased Bim expression mediate multidrug-resistance in multiple myeloma cells. Leuk Res 2012;36:1315-1322.
34. Kubiczkova L, Pour L, Sedlarikova L, Hajek R, Sevcikova S. Proteasome inhibitors – molecular basis and current perspectives in multiple myeloma. J Cell Mol Med 2014;6:947-961.
35. Russell SJ, Rajkumar SV. Multiple myeloma and the road to personalised medicine. Lancet Oncol 2011;12:617-619.
36. Mirnezami R, Nicholson J, Darzi A. Preparing for precision medicine. N Engl J Med 2012;366:489-491.