

A phase II study of the combination of endocrine treatment and bortezomib in patients with endocrine-resistant metastatic breast cancer

X.B. TRINH^{1,2}, L. SAS¹, S.J. VAN LAERE¹, A. PROVÉ¹, I. DELEU¹, M. RASSCHAERT¹,
H. VAN DE VELDE³, P. VINKEN³, P.B. VERMEULEN¹, P.A. VAN DAM¹,
A. WOJTASIK¹, P. DE MESMAEKER¹, W.A. TJALMA² and L.Y. DIRIX¹

¹Oncology Centre/Translational Cancer Research Unit Antwerp, St. Augustinus Hospital, GZA Hospitals, Oosterveldlaan 24, 2610 Antwerp; ²Department of Gynaecological Oncology, Antwerp University Hospital, Wilrijkstraat 10, 2650 Edegem;

³Johnson & Johnson Pharmaceutical Research and Development, Turnhoutseweg 30, 2340 Beerse, Belgium

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Abstract. The majority of patients with hormone receptor-positive metastatic breast cancer die from disease progression despite different types of anti-hormonal treatments. Preclinical studies have indicated that resistance to anti-hormonal therapies may be the result of an activated NF- κ B signalling pathway in breast cancer. Bortezomib is a proteasome inhibitor that blocks the NF- κ B pathway. Recent pharmacodynamic and pharmacokinetic xenograft studies have shown that drug exposure may be a crucial factor for the efficacy of bortezomib in solid tumours. The aim was to investigate whether the addition of bortezomib to anti-hormonal therapy would result in regained antitumour activity in patients with progressive and measurable disease being treated with an endocrine agent. Clinical benefit was defined as patients obtaining stable disease, partial response or complete response after 2 cycles, lasting for at least another five weeks. Bortezomib was administered on Days 1, 8, 15 and 22 of a 5-week regimen (1.6 mg/m²). Eight patients received an aromatase inhibitor and bortezomib, while one received tamoxifen and bortezomib. There were 3 grade 3 gastrointestinal toxicities. Median time to treatment failure was 69 days (range, 35-140). Two out of the 9 patients had stable disease for more than 10 weeks. Despite an effective target inhibition, suggested in peripheral blood mononuclear cells and available tumour samples, no objective antitumour responses were observed. Addition of a proteasome inhibitor to anti-hormonal therapy resulted in a clinical benefit rate of 22% in a limited number of patients with endocrine resistant

and progressive metastatic breast cancer. The demonstrated proteasome inhibition in tumour tissue provides evidence that the lack of clinical responses is not attributed to deficient drug exposure.

Introduction

Endocrine manipulation is the most important systemic treatment option, regardless of disease stage, for patients with hormone receptor-positive breast cancer (1). Anti-hormonal medication for advanced disease in general is given continuously until the patient experiences disease progression. A substantial number of patients will experience progression although their cancer cells continue to express the estrogen receptor (ER) and/or the progesterone receptor (PR). Other patients, while initially obtaining a clinical benefit from a particular type of endocrine treatment, will unfortunately experience further cancer growth or tumour progression. Mechanisms of primary or acquired endocrine resistance are manifold: overexpression and activation of other growth factor pathways (enhanced EGFR/HER2 and subsequent downstream MAPK and PI3K/AKT activation), and other mechanisms (2-4). Estrogen receptor mutations are only rarely observed, suggesting that even in patients with progressive disease the estrogen-ER interaction remains potentially intact (5).

Different strategies are currently being investigated in order to re-establish the clinical endocrine responsiveness of breast cancer (6). Bi-directional inhibitory interactions of ER and nuclear factor κ B (NF- κ B) have been documented by some authors in preclinical breast cancer models, resulting in hormone-resistance and enhanced tumour growth (7-17). These interactions suggest that the lack of efficacy of an agent inhibiting the ER pathway in a subset of patients may be the result of an activated NF- κ B signalling pathway. The proteasome inhibitor bortezomib has been shown to interfere with the NF- κ B signalling pathway in haematological malignancies, such as multiple myeloma and diffuse large B-cell lymphoma (18-21). Theoretically, and as described in breast cancer cell line

Correspondence to: Dr L.Y. Dirix, Oncology Centre, St. Augustinus Hospital, Oosterveldlaan 24, 2610 Wilrijk-Antwerp, Belgium
E-mail: luc.dirix@gza.be

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experiments (22), blocking the NF- κ B pathway in this setting, e.g. by bortezomib, might restore SERM (selective estrogen receptor modulator) or AI (aromatase inhibitor) sensitivity in breast cancer.

In this study the question asked was whether inhibition of the proteasome by bortezomib might lead to regained disease control in patients with either primary endocrine resistance or acquired endocrine resistance being treated with a SERM or an AI. Bortezomib is a small molecule proteasome inhibitor developed as a novel agent to treat human malignancies (23–28). Bortezomib is approved by regulatory authorities for the treatment of patients with multiple myeloma and with relapsed mantle cell lymphoma in a treatment schedule of 1.3 mg/m² on Days 1, 4, 8 and 11 every 3 weeks. Several studies suggest that the alternative schedule of 1.6 mg/m² on Days 1, 8, 15 and 22 every 5 weeks has similar antitumour activity and is better tolerated (29,30). By inhibiting a single molecular target, the proteasome, bortezomib affects multiple signalling pathways, including inhibition of the NF- κ B pathway. The antineoplastic effect of bortezomib likely involves several distinct mechanisms, including inhibition of cell growth and survival pathways, induction of apoptosis, and inhibition of the expression of genes that control cellular adhesion, migration, and angiogenesis (19,31).

Materials and methods

This study was a single arm phase II study. The aim was to investigate whether the addition of bortezomib to either a SERM or an AI would result in documented activity in patients with progressive and measurable disease on the identical endocrine agent. This endpoint was evaluated according to the RECIST 1.0 criteria every 5 weeks. Clinical benefit was prospectively defined by the patients obtaining at least either stable disease, or a partial or a complete response according to the RECIST 1.0 criteria after two cycles, lasting for at least five weeks. The study enrolled postmenopausal patients with ER and/or PR-positive metastatic breast cancer who suffered from radiological and/or clinical (with digital photography for skin metastasis) documented progression of disease according to the RECIST 1.0 criteria. The hypothesis was that clinical benefit should occur in at least 2 patients in the first 14 evaluable patients which equals to a clinical benefit of >14.2%.

The secondary objectives were to define the activity of the proteasome and the effect on the NF- κ B pathway in peripheral blood and if possible in tumour tissue. Bortezomib was administered on Days 1, 8, 15 and 22 of a 5-week regimen at a dose of 1.6 mg/m² on each treatment day. Patients attended the following study visits: i) screening visit(s) at maximum 21 days prior to the first dose of study drug; ii) on Days 1, 8, 15 and 22 of each treatment cycle; iii) an end-of-treatment visit within 10 days after the administration of the last study drug dose. Patients who participated in the study had measurable disease by RECIST 1.0. Patients needed to be on tamoxifen or an AI for at least 3 months in order to clearly document endocrine resistance and exclude late responses. Bortezomib was kindly provided by the manufacturer. The study was approved by the Institutional Review Board and was registered by Eudract number 2006-004144-23. All patients gave written informed consent to participate in the study.

Translational studies. Whole blood, plasma, serum and if possible tumour biopsies were collected for translational purposes. These were collected at baseline (before administration of bortezomib) and on Day 22 within 4 h after the administration of bortezomib. Plasma and serum samples were stored at -80°C until batched analysis. Tissue samples were immediately put on RNAlater (Ambion, Lennik, Belgium) and also stored at -80°C according to the manufacturer's manual.

Enzyme-linked immunosorbent assay. Quantikine ELISA kits for human IL6, IL8 and VEGF-A were used according to the manual of the kit; all measurements were performed in duplicate (R&D Systems, Abingdon, UK) with the Tecan Sunrise absorbance reader and Tecan Columbus Plate washer (Tecan Ltd., Mechelen, Belgium). Standards provided with the kit were used for dilution series for a standard curve (R^2 for all dilution series were >0.99). Concentrations were calculated using the standard curve. Wilcoxon signed-rank tests were performed to compare baseline samples vs. samples of patients during treatment (Day 22).

Proteasome inhibition analysis. The 20S proteasome activity was determined by measuring the rate of proteolytic hydrolysis of a fluorescent-tagged peptide substrate Suc-Leu-Leu-Val-Tyr-AMC*, by the sample and normalizing the activity to the amount of protein present in the tumour tissue lysate. To measure the release of free AMC with time, a spectrofluorometer (SpectraMax M5, VA, USA) was used with the following settings: read interval 5 min, read length 2 h at 37°C. Protein concentrations were determined using a Pierce BCA protein assay kit (R^2 >0.99) (Pierce, Etten-Leur, The Netherlands). The specific activity of each sample was calculated in pmol AMC/min/mg protein applying the slope of an AMC standard curve as conversion factor. Similarly the activity was determined in peripheral whole blood samples. The measurements of the whole blood samples were performed on a Safire2 instrument (Tecan) with read interval 90 sec and read length 25 min at 37°C.

Results

Patient characteristics. Clinical data of the 9 participating patients enrolled in this trial are summarized in Table I. These patients were using an endocrine treatment (8/9 AI and 1/9 tamoxifen) and all were diagnosed with documented progressive disease (10 months, median time to progression) on the prior endocrine treatment when entered in this study. Five of these patients had already received one line of chemotherapy. They all had a good performance status [median Karnofsky index (KI), 90%; range, 70–100%], with the majority (7/9) suffering from visceral disease. All 9 patients seemed to have benefited to some extent of the current endocrine treatment. None of these patients had been treated with fulvestrant. All patients had baseline complete blood counts and bilirubin levels within normal ranges.

Treatment administered. The individual treatment durations are visualized in Fig. 1. The median duration of treatment was 63 days (range, 35–140), and the median number of cycles was

Table I. Baseline clinical data of patients who participated.

Baseline clinical data	n
Total no. of patients	9
Stratum A	1
Stratum B	8
Median age at initial diagnosis (range), years	58 (45-73)
Previous chemotherapy	
No prior chemotherapy	4
One line of chemotherapy	5
Line of endocrine therapy at trial recruitment	
First	2
Second	4
Third	3
Median time to progression after last line of endocrine therapy (range), months	10 (4-32)
Patients with non-visceral metastases	2
Patients with visceral metastases	7
Performance status	
KI 70%	1
KI 80%	1
KI 90%	3
KI 100%	4

Stratum A patients were patients who had progressive disease during tamoxifen treatment. Stratum B patients are patients who had progressive disease during aromatase inhibitor treatment. KI, Karnofsky index.

2 (range, 1-4). A total of 60 treatment doses of bortezomib were administered. Four doses were withheld due to toxicity.

Response. After 10 weeks of addition of bortezomib to their endocrine therapy, the tumour status according to the RECIST 1.0 criteria was determined. At that time, 6/9 patients had been withdrawn from the study either due to progressive disease (4/6) or due to adverse events (2/6). There were no objective antitumour responses observed. Two patients (22.2%) had stable disease that lasted for more than 10 weeks. These two patients were considered as patients in whom the experimental addition of bortezomib to their endocrine treatment resulted in clinical benefit. Remarkably, the 2 patients who met the endpoint of the study both had progressive disease of their visceral metastasis and the AI treatments at baseline were in second and third lines of endocrine therapy. The median time to treatment failure was 63 days (range 35-140), either due to toxicity (3/9) or (eventual) progression (6/9) (Fig. 2).

Toxicity. All adverse events, which were observed during the study period, are reported in Table II. There were 3 grade 3 toxicities (diarrhoea) during the study period, which led to treatment discontinuation and study withdrawal in these 3 patients. Other toxicities were predominantly gastrointestinal (anorexia, nausea and abdominal pain). Additionally, there

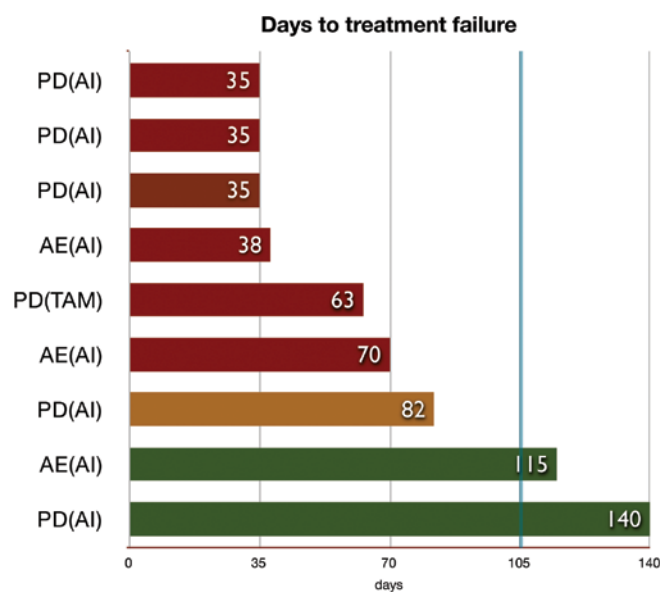


Figure 1. Days to treatment failure are reported for each patient. The reason for treatment failure at the time of study withdrawal is noted on the left. Between brackets is the endocrine treatment which was administered in combination with bortezomib. The cut-off at 105 days (in blue) is the threshold for clinical benefit. Patients needed to have partial response/complete response/stable disease at week 10 (70 days) continuing for at least another 5 weeks (total 105 days). Patients who are displayed in red failed to reach this threshold due to progressive disease or adverse events. Patients displayed in orange had stable disease at week 10, but progressed during the following 5 weeks. Patients displayed in green met the predefined clinical benefit with lasting stable disease above the threshold. (AI, aromatase inhibitor; TAM, tamoxifen; PD, progressive disease; AE, adverse events).

were only few grade 1-2 haematological and neurological adverse events.

Serum/plasma levels of cytokines. We analyzed serum and plasma levels of cytokines as potential surrogate endpoints of bortezomib activity in paired samples in all patients. The results are summarized in Fig. 2. Enzyme-linked immunosorbent assays revealed that the addition of bortezomib to endocrine treatment resulted in a moderate VEGF-A increase (trend, $p=0.066$ and $p=0.068$) of serum and plasma levels. Serum IL6 levels decreased significantly ($p=0.04$) on Day 22, while no significant change was observed for plasma IL6, serum IL8 and plasma IL8.

Proteasome activity in PBMC and tumour samples. 20S proteasome inhibition studies revealed a significant ($p=0.028$) downregulation when measured in peripheral blood mononuclear cells (7/9 paired samples available for analysis). The baseline values of all patients showed little variation (mean = 0.079; range, 0.067-0.089 in pmol AMC/sec/mg protein). After exclusion of one obvious outlier value, there was a mean inhibition of $66\pm7.1\%$ (SD) of proteasome activity in PBMCs (Fig. 2).

Likewise, in two-paired tumour samples, there was a decrease of proteasome activity. Four tumour samples (a supraclavicular lymph node, one skin and two liver metastases) were available at baseline for analysis, of which 2 were paired with on-treatment biopsies (lymph node and liver metastasis).

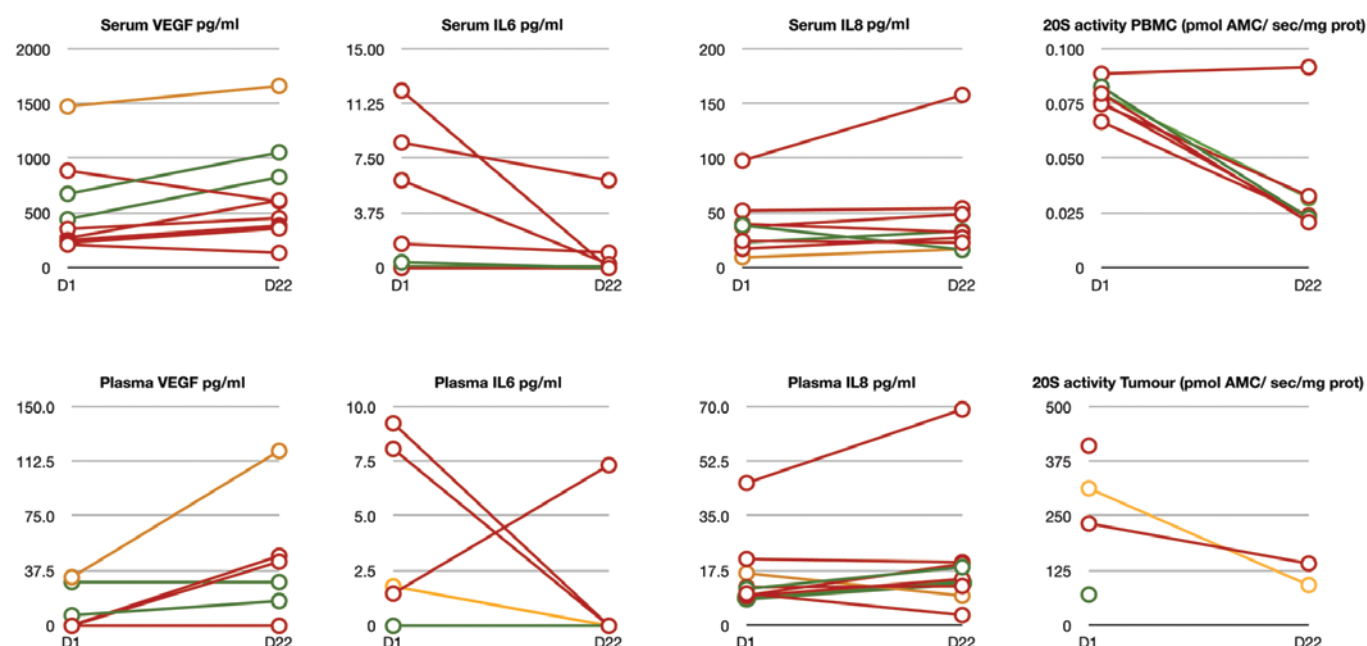


Figure 2. Serum and plasma levels are shown for VEGF, IL6 and IL8 at baseline and on Day 22 (post treatment). Post-treatment samples were taken within 4 h after bortezomib administration. There was a trend of increase of serum and plasma VEGF. There was a significant decrease in serum IL6. For plasma IL6 and serum/plasma IL8, no changes could be demonstrated. The 20S activity assay showed a significant downregulation on Day 22 in PBMCs. Two-paired tumour samples were available and concordantly showed a decrease of 20S activity. The same colour code is used as in Fig. 1.

Table II. Adverse events during the whole study period conforming to the NCI CTCAE3.0.

	National Cancer Institute Common Toxicity Criteria AE v3.0		
	Grade 1	Grade 2	Grade 3
Abdominal pain	2	2	0
Anorexia	1	1	0
Arthralgia	0	3	0
Conjunctivitis	1	2	0
Diarrhoea	1	5	3
Fatigue	3	4	0
Flu-like symptoms	2	0	0
Headache	1	0	0
Hematological toxicities (anemia/thrombocytopenia)	2	0	0
Nausea	1	6	0
Peripheral neuropathy	1	0	0
Skin toxicity	0	1	0
Vomiting	1	6	0

Three grade 3 toxicities were observed.

Baseline proteasome activity values were higher in tumour and showed a wider variation among samples with higher values than in PBMCs. The median activity was 4.57 pmol/sec/mg with a range of 1.18-6.87. One patient showed a 39.2% inhibition

while another patient showed a 70.4% proteasome inhibition (Fig. 2). These 2 patients (with respectively 63 and 82 days to treatment failure in Fig. 1) failed to reach the clinical benefit threshold.

Discussion

No clinical responses were observed after the addition of bortezomib to endocrine treatment. Nonetheless, the observed clinical benefit rate of stable disease in 2 out of 9 patients was higher than the predefined threshold of 2/14. Although this was a small study, the chance that in these patients the addition of bortezomib was not responsible for the obtained stabilisation of the disease is unlikely. These two patients were receiving second and third line anti-hormonal therapy for progression of visceral metastasis. Therefore, this observation is consistent with the hypothesis that the addition of a proteasome inhibitor to endocrine therapy can stabilise their disease progression. It remains a possibility that the observed disease stabilisation could be the result of single agent bortezomib activity. The results of the proteasome inhibition in peripheral blood mononuclear and in two tumours proved the target inhibition with this regimen in a surrogate tissue and tissue of interest.

Two previous phase II studies with single agent bortezomib in metastatic breast cancer have only reported disease stabilisation. Yang *et al* (32) reported on a phase II study with bortezomib using a biweekly regimen of 1.5 mg/m² followed by one week rest every 21 days in 12 patients, all suffering from measurable metastatic breast cancer. One (8.3%) of these 12 patients experienced stable disease for up to 5 cycles of 21 days. Engel *et al* (33) reported on another 12 patients, using the same regimen but at the standard dose of 1.3 mg/m² on Days 1, 4, 8 and 11 every 2 days, in a more heavily pretreated setting. Three

patients (25%) received three 21-day cycles before disease progression was documented. Our study results are in line with these data, and therefore single agent activity cannot be ruled out. The inclusion criterion of having radiological documented disease progression whilst on the same hormonal agent renders credibility to our observation of disease stabilisation in 2 patients with visceral disease. A hypothetical explanation for the observation that there were no partial or complete responses in this study, besides the small sample size, may be attributed to the fact that endocrine therapy is mainly an anti-proliferative treatment rather than a pro-apoptotic causing therapy. It has been shown in human breast cancer samples that the administration of neoadjuvant hormonal therapy reduces the proliferation rate and has an anti-apoptotic effect (34). This may partly explain why stable disease was more likely to occur than a reduction in tumour volume. On the other hand, in multiple myeloma, single agent bortezomib is able to induce complete and partial responses (26,27).

In the present phase II study, we chose the weekly regime of bortezomib (25,35). This regimen was considered more feasible as it allowed once weekly outpatient clinic visits. In the phase I study by Papandreou (25) *et al* dose-limiting toxicity occurred at 2.0 mg/m². At the dose 1.6 mg/m², these investigators also observed diarrhoea of any grade in 12 out of 13 patients. Neuropathy was observed with this weekly regimen, albeit mainly grade 1 and 2 (38%). Use of this alternative weekly schedule in the present study resulted in almost no neurological toxicity but more frequent diarrhoea events than the standard twice-weekly bortezomib regimen. The largest experience with 1.6 mg/m² weekly regimen is the phase III study of rituximab with or without bortezomib in relapsed follicular lymphoma. In the rituximab plus bortezomib arm (N=334), diarrhoea of any grade occurred in 52% with grade 3 diarrhoea in 7% (36). This would have been relevant if they had liver impairment, which was not the case (37) or CYP2C19 cytochrome leading to decreased clearance and therefore higher bortezomib exposure (38-42).

After a first interim analysis the decision was made to terminate the study. Two reasons contributed to this decision. First, the endpoint threshold of the study was obtained. At least 2 patients of the first 14 enrolled patients experienced clinical benefit. Second, the toxicity, although manageable, was found to be considerably high since it was the reason for study withdrawal in 3/9 patients. Particularly the occurrence of grade 3 diarrhoea was difficult to manage in this elderly population. Since bortezomib's working mechanism through proteasome inhibition has more effects than merely NF- κ B inhibition, forthcoming studies need to clarify whether a more specific target inhibition of NF- κ B may be more beneficial. Several other new compounds and even different natural products are known to inhibit the NF- κ B pathway (43). Furthermore, other mechanisms of endocrine resistance have been described.

The translational studies provide data that there was an effective proteasome inhibition in mononuclear cells in all except for one patient. In our study, only two coupled pre- and on-therapy tumour samples were available and also showed similar inhibition. The activation of the proteasome measured in PBMCs showed relatively low variation pre- and post-treatment, while in the tumour samples the variation of activity seemed to be larger with much higher values. Previous studies

have also shown large variation and increased proteasome activity in breast cancer samples compared to patient-matched adjacent normal tissue (44). Another consideration is that samples were derived from different sites (liver, skin and lymph node metastasis), which could also explain the higher variation.

Notably, there was a considerable proteasome inhibition in available bortezomib-treated tumour samples. Prior xenograft models have identified prominent differences in vessel perfusion, permeability, and architecture that ultimately resulted in variations in bortezomib tumour exposure. Comparing and contrasting the differences between a bortezomib-responsive and a bortezomib-resistant model with these techniques allowed the authors to establish a relationship among tumour perfusion, drug exposure, pharmacodynamic response and efficacy, and provided a hypothesis for why some solid tumour models did not respond to bortezomib treatment (45). We provide data that there was a significant proteasome inhibition and that the lack of an objective antitumour response in these two patients could not be attributed to insufficient target inhibition in the tumour. Few other studies have provided clinical data of similar proteasome inhibition after bortezomib treatment in other solid tumours (25,46,47).

In future studies, biopsies and thus assessment of intratumoural interaction changes between the ER and the NF- κ B pathway will be made mandatory. We also analysed several downstream targets of the NF- κ B pathway. Indirectly, these data provide evidence that bortezomib interfered with NF- κ B pathway in the patients who participated. After the addition of bortezomib, there is a trend for upregulation of VEGF-A, measured by ELISA in serum and plasma. These findings are concordant with earlier pancreatic adenocarcinoma xenograft findings where an upregulation of VEGF-A was also observed (48). Serum IL6 was significantly downregulated in patients while this was not observed in plasma possibly due to one outlier observation of a patient who had an increase in plasma IL6. No significant changes in IL8 plasma and serum levels could be detected. These IL6/IL8 findings confirm for previous findings (32). Yang *et al* found a significant upregulation of plasma IL6 after bortezomib treatment while for plasma IL8 there was a no difference (32).

In conclusion, although the sample size of the study was small, the addition of bortezomib to anti-hormonal therapy with progressive disease resulted in disease stabilisation in 2 out of 9 patients, which equals a clinical benefit rate of 22%. Despite an effective target inhibition, which was suggested by increased toxicity, and by effective proteasome inhibition in peripheral blood mononuclear cells and available tumour tissue samples, no objective antitumour responses were observed. The demonstrated proteasome inhibition in tumour tissue provides evidence that the lack of clinical responses is not attributed to deficient drug exposure.

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References

- Veronesi U, Boyle P, Goldhirsch A, Orecchia R and Viale G: Breast cancer. *Lancet* 365: 1727-1741, 2005.
- Arpino G, Green SJ, Allred DC, Lew D, Martino S, Osborne CK and Elledge RM: HER-2 amplification, HER-1 expression, and tamoxifen response in estrogen receptor-positive metastatic breast cancer: a southwest oncology group study. *Clin Cancer Res* 10: 5670-5676, 2004.
- Atalay G, Cardoso F, Awada A and Piccart MJ: Novel therapeutic strategies targeting the epidermal growth factor receptor (EGFR) family and its downstream effectors in breast cancer. *Ann Oncol* 14: 1346-1363, 2003.
- Osborne CK, Shou J, Massarweh S and Schiff R: Crosstalk between estrogen receptor and growth factor receptor pathways as a cause for endocrine therapy resistance in breast cancer. *Clin Cancer Res* 11: S865-S870, 2005.
- Herynk MH and Fuqua SAW: Estrogen receptor mutations in human disease. *Endocr Rev* 25: 869-898, 2004.
- Johnston SR: New strategies in estrogen receptor-positive breast cancer. *Clin Cancer Res* 16: 1979-1987, 2010.
- Biswas DK, Cruz AP, Gansberger E and Pardee AB: Epidermal growth factor-induced nuclear factor kappa B activation: a major pathway of cell-cycle progression in estrogen-receptor negative breast cancer cells. *Proc Natl Acad Sci USA* 97: 8542-8547, 2000.
- Biswas DK, Dai SC, Cruz A, Weiser B, Graner E and Pardee AB: The nuclear factor kappa B (NF-kappa B): a potential therapeutic target for estrogen receptor negative breast cancers. *Proc Natl Acad Sci USA* 98: 10386-10391, 2001.
- Biswas DK, Shi Q, Baily S, Strickland I, Ghosh S, Pardee AB and Iglehart JD: NF-kappa B activation in human breast cancer specimens and its role in cell proliferation and apoptosis. *Proc Natl Acad Sci USA* 101: 10137-10142, 2004.
- Chadwick CC, Chippari S, Matelan E, Borges-Marcucci L, Eckert AM, Keith JC, Albert LM, Leathurby Y, Harris HA, Bhat RA, Ashwell M, Trybulski E, Winneker RC, Adelman SJ, Steffan RJ and Harnish DC: Identification of pathway-selective estrogen receptor ligands that inhibit NF-kappaB transcriptional activity. *Proc Natl Acad Sci USA* 102: 2543-2548, 2005.
- Ghisletti S, Meda C, Maggi A and Vegeto E: 17beta-estradiol inhibits inflammatory gene expression by controlling NF-kappaB intracellular localization. *Mol Cell Biol* 25: 2957-2968, 2005.
- Liu H, Liu K and Bodenner DL: Estrogen receptor inhibits interleukin-6 gene expression by disruption of nuclear factor kappaB transactivation. *Cytokine* 31: 251-257, 2005.
- Nakshatri H, Bhat-Nakshatri P, Martin DA, Goulet RJ and Sledge GW: Constitutive activation of NF-kappaB during progression of breast cancer to hormone-independent growth. *Mol Cell Biol* 17: 3629-3639, 1997.
- Pratt MAC, Bishop TE, White D, Yasvinski G, Ménard M, Niu MY and Clarke R: Estrogen withdrawal-induced NF-kappaB activity and bcl-3 expression in breast cancer cells: roles in growth and hormone independence. *Mol Cell Biol* 23: 6887-6900, 2003.
- Van Laere SJ, Van der Auwera I, Van den Eynden GG, Elst HJ, Weyler J, Harris AL, van Dam P, Van Marck EA, Vermeulen PB and Dirix LY: Nuclear factor-kappaB signature of inflammatory breast cancer by cDNA microarray validated by quantitative real-time reverse transcription-PCR, immunohistochemistry, and nuclear factor-kappaB DNA-binding. *Clin Cancer Res* 12: 3249-3256, 2006.
- Van Laere SJ, Van der Auwera I, Van den Eynden GG, van Dam P, Van Marck EA, Vermeulen PB and Dirix LY: NF-kappaB activation in inflammatory breast cancer is associated with oestrogen receptor downregulation, secondary to EGFR and/or ErbB2 overexpression and MAPK hyperactivation. *Br J Cancer* 97: 659-669, 2007.
- Zhou Y, Eppenberger-Castori S, Marx C, Yau C, Scott GK, Eppenberger U and Benz CC: Activation of nuclear factor-kappaB (NFkappaB) identifies a high-risk subset of hormone-dependent breast cancers. *Int J Biochem Cell Biol* 37: 1130-1144, 2005.
- Dunleavy K, Pittaluga S, Czuczman MS, Dave SS, Wright G, Grant N, Shovlin M, Jaffe ES, Janik JE, Staudt LM and Wilson WH: Differential efficacy of bortezomib plus chemotherapy within molecular subtypes of diffuse large B-cell lymphoma. *Blood* 113: 6069-6076, 2009.
- Hideshima T, Chauhan D, Richardson P, Mitsiades C, Mitsiades N, Hayashi T, Munshi N, Dang L, Castro A, Palombella V, Adams J and Anderson KC: NF-kappa B as a therapeutic target in multiple myeloma. *J Biol Chem* 277: 16639-16647, 2002.
- Lam LT, Wright G, Davis RE, Lenz G, Farinha P, Dang L, Chan JW, Rosenwald A, Gascoyne RD and Staudt LM: Cooperative signaling through the signal transducer and activator of transcription 3 and nuclear factor-kB pathways in subtypes of diffuse large B-cell lymphoma. *Blood* 111: 3701-3713, 2008.
- Palombella VJ, Rando OJ, Goldberg AL and Maniatis T: The ubiquitin-proteasome pathway is required for processing the NF-kappa B1 precursor protein and the activation of NF-kappa B. *Cell* 78: 773-785, 1994.
- deGraffenried LA, Chandrasekar B, Friedrichs WE, Donzis E, Silva J, Hidalgo M, Freeman JW and Weiss GR: NF-kappa B inhibition markedly enhances sensitivity of resistant breast cancer tumor cells to tamoxifen. *Ann Oncol* 15: 885-890, 2004.
- Adams J: The development of proteasome inhibitors as anti-cancer drugs. *Cancer Cell* 5: 417-421, 2004.
- Orlowski RZ, Stinchcombe TE, Mitchell BS, Shea TC, Baldwin AS, Stahl S, Adams J, Esseltine DL, Elliott PJ, Pien CS, Guercioli R, Anderson JK, Depcik-Smith ND, Bhagat R, Lehman MJ, Novick SC, O'Connor OA and Soignet SL: Phase I trial of the proteasome inhibitor PS-341 in patients with refractory hematologic malignancies. *J Clin Oncol* 20: 4420-4427, 2002.
- Papandreou CN, Daliani DD, Nix D, Yang H, Madden T, Wang X, Pien CS, Millikan RE, Tu SM, Pagliaro L, Kim J, Adams J, Elliott P, Esseltine D, Petrusich A, Dieringer P, Perez C and Logothetis CJ: Phase I trial of the proteasome inhibitor bortezomib in patients with advanced solid tumors with observations in androgen-independent prostate cancer. *J Clin Oncol* 22: 2108-2121, 2004.
- Richardson PG, Barlogie B, Berenson J, Singhal S, Jagannath S, Irwin D, Rajkumar SV, Skralovic G, Alsina M, Alexanian R, Siegel D, Orlowski RZ, Kuter D, Limentani SA, Lee S, Hideshima T, Esseltine DL, Kauffman M, Adams J, Schenkein DP and Anderson KC: A phase 2 study of bortezomib in relapsed, refractory myeloma. *N Engl J Med* 348: 2609-2617, 2003.
- Richardson PG, Sonneveld P, Schuster MW, Irwin D, Stadtmauer EA, Facon T, Harousseau JL, Ben-Yehuda D, Lonial S, Goldschmidt H, Reece D, San-Miguel JF, Bladé J, Boccadoro M, Cavenagh J, Dalton WS, Boral AL, Esseltine DL, Porter JB, Schenkein D, Anderson KC and Assessment of Proteasome Inhibition for Extending Remissions (APEX) Investigators: Bortezomib or high-dose dexamethasone for relapsed multiple myeloma. *N Engl J Med* 352: 2487-2498, 2005.
- San-Miguel JF, Schlag R, Khuageva NK, Dimopoulos MA, Shpilberg O, Kropff M, Spicka I, Petrucci MT, Palumbo A, Samoilova OS, Dmoszynska A, Abdulkadyrov KM, Schots R, Jiang B, Mateos MV, Anderson KC, Esseltine DL, Liu K, Cakana A, van de Velde H, Richardson PG and VISTA Trial Investigators: Bortezomib plus melphalan and prednisone for initial treatment of multiple myeloma. *N Engl J Med* 359: 906-917, 2008.
- de Vos S, Goy A, Dakhil SR, Saleh MN, McLaughlin P, Belt R, Flowers CR, Knapp M, Hart L, Patel-Donnelly D, Glenn M, Gregory SA, Holladay C, Zhang T and Boral AL: Multicenter randomized phase II study of weekly or twice-weekly bortezomib plus rituximab in patients with relapsed or refractory follicular or marginal-zone B-cell lymphoma. *J Clin Oncol* 27: 5023-5030, 2009.
- Reece DE, Sanchirawala V, Hegenbart U, Merlini G, Palladini G, Femand JP, Vescio RA, Liu X, Elsayed YA, Cakana A, Comenzo RL and VELCADE CAN2007 Study Group: Weekly and twice-weekly bortezomib in patients with systemic AL amyloidosis: results of a phase 1 dose-escalation study. *Blood* 114: 1489-1497, 2009.
- Sunwoo JB, Chen Z, Dong G, Yeh N, Crowl Bancroft C, Sausville E, Adams J, Elliott P and Van Waes C: Novel proteasome inhibitor PS-341 inhibits activation of nuclear factor-kappa B, cell survival, tumor growth, and angiogenesis in squamous cell carcinoma. *Clin Cancer Res* 7: 1419-1428, 2001.
- Yang CH, Gonzalez-Angulo AM, Reuben JM, Booser DJ, Pusztai L, Krishnamurthy S, Esseltine D, Stec J, Broglio KR, Islam R, Hortobagyi GN and Cristofanilli M: Bortezomib (VELCADE) in metastatic breast cancer: pharmacodynamics, biological effects, and prediction of clinical benefits. *Ann Oncol* 17: 813-817, 2006.

33. Engel RH, Brown JA, Von Roenn JH, O'Regan RM, Bergan R, Badve S, Rademaker A and Gradishar WJ: A phase II study of single agent bortezomib in patients with metastatic breast cancer: a single institution experience. *Cancer Invest* 25: 733-737, 2007.
34. Dowsett M, Smith IE, Ebbs SR, Dixon JM, Skene A, Griffith C, Boeddinghaus I, Salter J, Detre S, Hills M, Ashley S, Francis S, Walsh G and A'Hern R: Proliferation and apoptosis as markers of benefit in neoadjuvant endocrine therapy of breast cancer. *Clin Cancer Res* 12: S1024-S1030, 2006.
35. Papandreou CN and Logothetis CJ: Bortezomib as a potential treatment for prostate cancer. *Cancer Res* 64: 5036-5043, 2004.
36. Coiffier B, Osmanov EA, Hong X, Scheliga A, Mayer J, Offner F, Rule S, Teixeira A, Walewski J, de Vos S, Crump M, Shpilberg O, Esseltine DL, Zhu E, Enny C, Theocharous P, van de Velde H, Elsayed YA, Zinzani PL and the LYM-3001 study investigators: Bortezomib plus rituximab versus rituximab alone in patients with relapsed, rituximab-naïve or rituximab-sensitive, follicular lymphoma: a randomised phase 3 trial. *Lancet Oncol* 12: 773-784, 2011.
37. Venkatakrishnan K, Ramanathan R, Sarantopoulos J, Mulkerin D, Shibata S, Hamilton A, Dowlati A, Mani S, Rudek MA, Ivy P, Takimoto CH, Neuwirth R, Parasuraman S and Lorusso PM: Pharmacokinetics and safety of bortezomib in patients with advanced malignancies and varying degrees of liver dysfunction: results of the phase I National Cancer Institute Organ Dysfunction Working Group Study NCI 6432. *Blood (ASH Annual Meeting abstracts)* 116: 3975, 2010.
38. Brauch H, Murter T and Eichelbaum M: Pharmacogenomics of tamoxifen therapy. *Clin Chem* 55: 1770-1782, 2009.
39. Desta Z, Ward BA, Soukhova NV and Flockhart DA: Comprehensive evaluation of tamoxifen sequential biotransformation by the human cytochrome P450 system in vitro: prominent roles for CYP3A and CYP2D6. *J Pharmacol Exp Ther* 310: 1062-1075, 2004.
40. Kamdem LK, Flockhart DA and Desta Z: In vitro cytochrome P450-mediated metabolism of exemestane. *Drug Metab Dispos* 39: 98-105, 2011.
41. Murai K, Yamazaki H, Nakagawa K, Kawai R and Kamataki T: Deactivation of anti-cancer drug letrozole to a carbinol metabolite by polymorphic cytochrome P450 2A6 in human liver microsomes. *Xenobiotica* 39: 795-802, 2009.
42. Uttamsingh V, Lu C, Miwa G and Gan LS: Relative contributions of the five major human cytochromes P450, 1A2, 2C9, 2C19, 2D6, and 3A4, to the hepatic metabolism of the proteasome inhibitor bortezomib. *Drug Metab Dispos* 33: 1723-1728, 2005.
43. Nakanishi C and Toi M: Nuclear factor-kappaB inhibitors as sensitizers to anticancer drugs. *Nat Rev Cancer* 5: 297-309, 2005.
44. Chen L and Madura K: Increased proteasome activity, ubiquitin-conjugating enzymes, and eEF1A translation factor detected in breast cancer tissue. *Cancer Res* 65: 5599-5606, 2005.
45. Williamson MJ, Silva MD, Terkelsen J, Robertson R, Yu L, Xia C, Hatsis P, Bannerman B, Babcock T, Cao Y and Kupperman E: The relationship among tumor architecture, pharmacokinetics, pharmacodynamics, and efficacy of bortezomib in mouse xenograft models. *Mol Cancer Ther* 8: 3234-3243, 2009.
46. Hamilton AL, Eder JP, Pavlick AC, Clark JW, Liebes L, Garcia-Carbonero R, Chachoua A, Ryan DP, Soma V, Farrell K, Kinchla N, Boyden J, Yee H, Zeleniuch-Jacquotte A, Wright J, Elliott P, Adams J and Muggia FM: Proteasome inhibition with bortezomib (PS-341): a phase I study with pharmacodynamic end points using a day 1 and day 4 schedule in a 14-day cycle. *J Clin Oncol* 23: 6107-6116, 2005.
47. Van Waes C, Chang AA, Lebowitz PF, Druzgal CH, Chen Z, Elsayed YA, Sunwoo JB, Rudy SF, Morris JC, Mitchell JB, Camphausen K, Gius D, Adams J, Sausville EA and Conley BA: Inhibition of nuclear factor-kappaB and target genes during combined therapy with proteasome inhibitor bortezomib and reirradiation in patients with recurrent head-and-neck squamous cell carcinoma. *Int J Radiat Oncol Biol Phys* 63: 1400-1412, 2005.
48. Märten A, Zeiss N, Serba S, Mehrle S, von Lilienfeld-Toal M and Schmidt J: Bortezomib is ineffective in an orthotopic mouse model of pancreatic adenocarcinoma. *Mol Cancer Ther* 7: 3624-3631, 2008.