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Phase I study of bortezomib with weekly paclitaxel in patients with advanced solid tumours

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ABSTRACT

Background: The combination of a proteasome inhibitor with a taxane has potential clinical synergism that prompted a clinical test.
Patients and methods: The maximum tolerated dose (MTD) and recommended dose (RD) of intravenous (i.v.) Bortezomib (B) (days 1, 4, 8, 11) and i.v. Paclitaxel (PTX) (days 1, 8) every 3 weeks was evaluated in patients with advanced solid tumours. The RD was tested in patients with breast, ovarian and prostate cancer. At the RD, microarray analysis of transcriptional profiles was carried out before and after the first dosing in peripheral blood mononuclear cells (PBMC).
Results: Thirty-one patients were enrolled and 22 were treated at the RD that corresponded to B 1.3 mg/m^2 and PTX 100 mg/m^2. The main toxicity was cumulative peripheral neuropathy (76% of patients; grade 3–4 in 9%) that required treatment discontinuation in six patients, followed by diarrhoea (55%) and fatigue (41%). Nine partial responses (30%) were observed (three breast cancer, four ovary, two prostate patients). Significant ($p < 0.05$) and consistent changes (>70% of patients) in transcriptome were observed.
Conclusions: The incidence of peripheral neuropathy and the anti-tumour activity comparable to that of single-agent PTX do not support further development of this regimen.

1. Introduction

The ubiquitin-proteasome pathway plays an essential role in the degradation of most short- and long-lived intracellular proteins in eukaryotic cells, including p53, cyclins, the cyclin-dependent kinase inhibitors p21 and p27, the oestrogen receptor, and the inhibitor (IkB) of nuclear factor kappa B (NF-kB). Central to this degradative pathway is the 26 S proteasome, an adenosine triphosphate-dependent multicatalytic protease present in both the cytoplasm and nucleus.

Bortezomib (B) is a small molecule potent proteasome inhibitor which acts by binding tightly to the chymotrypsin-like site of the 20 S core of the enzyme. B has the potential to affect multiple signalling pathways including cell growth, survival, apoptosis, gene expression related to cellular adhesion, migration and angiogenesis. It has been shown in experimental prostate tumours that the constitutive activation of NF-kB and the possibly associated PI3K/Akt activation could lead to a protection from apoptosis. Blockade of NF-kB in prostate tumours could inhibit angiogenesis and increase sensitivity to

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apoptosis. B single agent has been extensively studied in patients with haematological and solid malignancies; the most important clinical toxicities were peripheral neuropathy and diarrhoea[1–3]; fatigue was common but rarely severe, and myelosuppression was very limited with only thrombocytopenia.

Paclitaxel (PTX) promotes microtubule stabilisation and cell death, induces phosphorylation of BCL-2 and suppresses its antiapoptic function.[4] Combinations of B and taxanes are therefore of great interest because of the potential synergism in biological effects, confirmed by the report of increased activity of B and docetaxel in a human pancreatic xenograft.[5]

We report the results of a multicentric two-part study of B given twice a week in combination with weekly PTX to patients with advanced solid tumours (EudraCT Number 2004-003834-32). The first part of the study was a dose-escalation, the second an expansion phase, in which patients with breast, ovarian, and prostate cancer were treated at the dose defined as recommended (RD) for phase II. Primary objectives of the trial were the definition of the maximum tolerated dose (MTD) and recommended dose (RD). Secondary objectives were the definition of the safety profile and preliminary evaluation of the anti-tumour activity. Gene expression profiles were examined in peripheral blood mononuclear cells (PBMCs) before and after treatment in an attempt to identify drug induced changes and monitor the biological effects of the combination.

The study was approved by the Ethics Committees of the participating institutions and all patients gave their informed consent before starting the study.

2. Materials and methods

2.1. Eligibility criteria

Eligibility criteria were a histologically or cytologically confirmed diagnosis of solid tumours, progressive disease, ECOG performance status $\leqslant 1$, age $\geqslant 18$ and $\leqslant 70$ years, adequate haematological, liver and renal function (bilirubin within UNL; alkaline phosphatase $\leqslant 1.5 \times$ UNL; ALT, AST $\leqslant$ UNL or $\leqslant 2.5 \times$ UNL in case of liver metastases; albumin $\geqslant 2.5$ mg/dl; creatinine $\leqslant$ UNL), life expectancy of $\geqslant 3$ months. A maximum of two prior chemotherapy regimens for advanced disease was allowed in the dose escalation phase, one in the expansion phase. Disease measurability according to Response Evaluation Criteria in Solid Tumours (RECIST [6]) was required only for patients in the expansion phase.

Distinctive exclusion criteria of the study were resistance to taxane (defined as progression of disease while on treatment or within 6 months from the end of treatment), pre-existing peripheral neuropathy > grade 1, prior radiation to > 35% of bone marrow, presence of cirrhosis or active or chronic hepatitis, known history of allergy to cremophor, mannitol or boron.

The study was performed in two centres and coordinated by SENDO (Southern Europe New Drugs Organization); the protocol was approved by the local Ethical Committees and all patients signed an informed consent.

2.2. Study design and dose escalation schedule

PTX was administered intravenously (i.v.) by 1 h infusion after i.v. standard pre-medication on days 1 & 8; B was administered by i.v. push with a running i.v. of normal saline at 100 ml/h on days 1, 4, 8 & 11; the treatment was repeated every 3 weeks.

Table 1 reports the dose levels investigated.

At each dose level, the first two patients were enrolled simultaneously while the third could be entered once the first two had been observed for at least 3 weeks. Three additional patients were enrolled at each dose level if a DLT was observed in the first cycle. No intra-patient dose escalation was allowed. The MTD was defined as the dose at which at least two out of two to six patients experienced a first cycle DLT. The RD was defined as the dose closest to the MTD at which a DLT was observed in 0/3 or 1/6 patients. This dose was tested in the expansion phase, in which only patients with breast, ovarian or prostate cancer were treated.

2.3. Toxicity assessment

DLT was defined as an ANC $< 500 \times 10^9$/L lasting > 7 days, occurrence of febrile neutropenia, grade 4 thrombocytopenia or grade 3 thrombocytopenia requiring platelet transfusions, grade $\geqslant 2$ neuropathy, grade $\geqslant 3$ diarrhoea, or any other grade 3–4 non haematological toxicity except nausea and vomiting (unless nausea and vomiting occurred despite appropriate prophylaxis with $5HT_3$ antagonists), a > 2 week delay before starting a new cycle for grade $\geqslant 2$ persisting non haematological toxicities, failure to deliver $\geqslant 2$ doses of B in the first cycle due to toxicity.

Dose reductions were planned in case of severe haematological or non haematological toxicities. In particular, the

Table 1 – Number of patients and cycles per dose level and patient characteristics

Level	Bortezomib (B) dose mg/m^2 (day 1, 4, 8, 11)	Paclitaxel (PTX) dose mg/m^2 (day 1, 8)	No. of pts/cycles
1	0.70	80	3/11
2	1.0	80	3/9
3	1.3	80	3/14
4 (RD)	1.3	100	22/112
Patient characteristics		Patients at RD $N = 22$	All patients $N = 31$
Median age, range (years)		60 (27–70)	60 (27–77)
Sex (F; M)		15; 7	21; 10
ECOG performance status			
0		21 (95%)	29 (94%)
1		1 (4%)	2 (6%)
Tumour type			
Breast		10	13
Prostate		6	6
Ovary		5	8
Other		1	4
Prior chemotherapy		19 (86%)	28 (90%)
Taxanes		7 (32%)	8 (26%)
Platinum		–	3 (10%)
Taxanes & Platinum		5 (23%)	8(26%)
Other		7 (32%)	9 (29%)

RD=Recommended dose.
Pts = Patients.

combination doses had to be reduced to the previous dose level for > grade 1 motor or sensory neuropathy, permanently interrupted for grade 4 neuropathy or in the presence of both sensory and motor neuropathy of grade 3.

Treatment could be continued until there was a clinical benefit in absence of unacceptable toxicity.

2.4. Statistical methods

All patients who received at least one dose of the combination were included in summary statistics and in the safety analysis, whereas all eligible patients for whom the tumour response could be evaluated at least once while on treatment, and whom had received at least two cycles of treatment, were analysed for efficacy.

2.5. Pharmacogenomic assessment

Peripheral blood (2×2.5 ml) was collected into PAXgene tubes (Qiagen AG, Basel, Switzerland) immediately before B and PTX on day 1 (pre-therapy sample) and before B on day 4 (post-therapy sample) of cycle 1. RNA was extracted according to the Qiagen RNA isolation protocol for GeneChip array and subjected to the Ambion Globin reduction protocol to remove globin RNA. RNA was labelled and hybridised to GeneChip U133 2.0 plus oligonucleotides arrays using the standard Affymetrix protocol. Data normalisation and analysis were performed using the standard Gene Chip Operating System (GCOS) Change and Signal Log Ratio (SLR) algorithms as described.[7] Genes differentially expressed were defined on the basis of fold change ($\geqslant 2$) and Increase or Decrease Change call.[7] Gene ontology annotation was performed using DAVID.[7]

3. Results

From September 2004 to March 2006, a total of 31 patients with solid tumours were enrolled; of the 15 patients entered in the expansion part, six had breast, six had prostate and three had advanced ovarian cancer. Overall, 22 patients enrolled between the dose finding and the expansion phase were treated at the dose level PTX 100 mg/m^2 and B 1.3 mg/m^2 (Table 1).

The characteristics of the patients treated are shown in Table 1. All patients had received at least one systemic antitumour therapy, including chemotherapy, in all cases except for the three patients with prostate cancer who received prior hormonotherapy only; 16 patients had previous chemotherapy containing taxanes with/without platinum, while three had only received platinum compounds. All 31 patients were evaluable for safety, and 30 for efficacy (one patient enrolled in the expansion part was withdrawn at cycle 1 due to toxicity without tumour evaluation). The median number of cycles received was four (range: 1.0–14.0) and about 40% of patients received six cycles or more.

At the four dose levels tested in the dose finding part, no DLTs were observed and the dose of B 1.3 mg/m^2 and PTX 100 mg/m^2 was defined as the RD and selected for the expansion.

Doses of B higher than 1.3 mg/m^2 were not tested because this was the RD of the drug given as a single agent.

3.1. Safety

Table 2 reports the toxicity observed in 22 patients treated at the RD. Overall, 77% of patients had neutropenia, and of grade 3–4 in 14%; thrombocytopenia was less frequent (45%) and never of grade 3–4.

The most common drug related toxicities were peripheral neuropathy (86%), nausea (68%), diarrhoea (55%), fatigue (41%) and vomiting (45%) (the protocol did dot require anti-emetic prophylaxis in all patients).

Only one drug related serious adverse event was reported consisting of hypoesthesia grade 2 with a patient requesting withdrawal from the study after one dose.

In seven of the nine patients (41%) who required a decrease of the dose at the RD, the reason was peripheral neuropathy G2 in all cases apart from one case of G1.

Overall, seven out of 31 patients (22%) were withdrawn due to toxicity, all treated at the RD. Reasons for treatment discontinuation included peripheral neuropathy in 6/22 cases (19%) (neuropathic pain G3 and sensory neuropathy G2–3 in three cases each). Five of these patients went off study after five to seven cycles and neurotoxicity lasted for at least 2 months after the last B dose (in one case paraesthesia and neuropathic pain were still present after 5 months).

3.2. Efficacy

Among 30 evaluable patients, nine (30%) achieved a partial response (PR), 13 (43%) showed disease stabilisation (SD), and eight (27%) had tumor progression (PD) (Table 3). Eight responders were treated at the RD (two out of five patients with prostate cancer, five out of five with ovarian cancer, and three out of 10 with breast cancer).

Table 4 reports the characteristics of patients receiving $\geqslant$ six cycles and/or withdrawn for toxicity, the response achieved and the toxicity which prompted the discontinuation of treatment. Among nine responders, four had to be withdrawn because of neurological toxicity, i.e. severe neuropathic pain in three cases and paraesthesia G2 in one.

Table 2 – Most frequent toxicities (> 15%) in 22 patients treated at the RD (B 1.3 / PTX 100)

Treatment-related toxicity	N = 22 n (%)	G3–G4 Adverse events n (%)
Neutropenia	17 (77)	3 (14)
Thrombocytopenia	10 (45)	–
Sensory neuropathy	19 (86)	2 (9)
Neuropathic pain	7 (32)	3 (14)
Peripheral neuropathy (any type)	19 (86)	5 (23)
Nausea	15 (68)	–
Diarrhoea	12 (55)	3 (14)
Alopecia	15 (68)	
Fatigue	9 (41)	
Vomiting	10 (45)	
Myalgia	9 (41)	
Pyrexia	6 (27)	
Infection	5 (23)	
Headache	4 (18)	

RD = Recommended dose.

Table 3 – Therapeutic effects

Best overall response	No of eval. patients N = 30	Dose finding part (solid tumours)			
		Level 1 N = 3	Level 2 N = 3	Level 3 N = 3	Level 4 RD (B 1.3 / P 100) N = 7
(a) Tumour response in the dose finding partc					
Partial response, *n* (%)	9 (30.0)			1 (33.3)	4 (57.1)
Stable disease, *n* (%)	13 (43.3)	2 (67.7)	2 (67.7)	1 (33.3)	1 (14.2)
Progressive disease, *n* (%)	8 (26.7)	1 (33.3)	1 (33.3)	1 (33.3)	2 (28.6)
Median TTP, months 95%CI	4.1 2.7–6.3				
(b) Tumour response at the recommended dose (dose finding & activity part)					
Best overall response	No of eval. patients N = 21[a]	All patients treated at level 4 RD			
		Breast N = 10	Ovary N = 5 ‘	Prostate N = 5	Melanoma N = 1
Partial response, *n* (%)	8 (38.1)	3 (30%)	3 (60%)	2 (40.0)	
Stable disease, *n* (%)	8 (38.1)	4 (40%)	1 (20%)	3 (60.0)	
Progressive disease, *n* (%)	5 (23.8)	3 (30%)	1 (20%)		1

RD = Recommended dose;
TTP = Time to progression;
N = Number of patients evaluable for efficacy.
a Seven patients enrolled in the dose finding part and 15 in the activity part.

Table 4 – Characteristics of patients receiving ⩾ six cycles and/or withdrawn for toxicity

Pt. No.	Tumour type	Prior PTX (+/– CBDCA)	No. B+PTX cycles	Response to B+PTX	Duration of response (months)	Withdrawn due to drug-related toxicity?	Type of toxicity	B/PTX initial dose
10	Breast	Yes adjuvant	14	PR	19	No	–	RD
13	Breast	Yes adjuvant	7	PR	5.6	Yes	Paraesthesia G2	RD
14	Breast	Yes advanced disease	5	SD	–	Yes (subjective intolerance)	Neuropathic pain G2	RD
24	Breast	No	5	PR	5	Yes	Neuropathic pain G3	RD
20	Breast	No	2	SD	–	Yes	Rash G2	RD
30	Prostate	No	8	PR	6.7	No	–	RD
25	Prostate	No	6	PR	2.9	Yes	Neuropathic pain G3	RD
27	Prostate	No	6	SD	–	Yes	Dysaesthesia G3 + Dry eye G3	RD
26	Prostate	No	1	NE	–	Yes	Hypoesthesia G2	RD
15	Ovary	Yes advanced disease (+CBDCA)	12	PR	13.9	No	–	RD
11	Ovary	Yes advanced (+CBDCA)	6	PR	4	Yes	Neuropathic pain G3	RD
18	Ovary	Yes neo-adjuvant (+CBDCA)	6	PR	3	No	–	RD
7	Ovary	No	4	PR	1.4	No	–	1.3 / 80

Cumulative dose calculated from treatment start to treatment discontinuation.
RD = Recommended dose; B = Bortezomib;
CBDCA = Carboplatin; PTX = Paclitaxel;
PR = Partial response; SD = Stable disease;
NE = Not evaluable.

The median duration of response was 4.5 months (1.4–19 months).

3.3. *Pharmacogenomic analysis*

The analysis was performed on seven patients. The median number of differentially expressed transcripts in individual patients was 427 (range: 159 to 2253) for down-regulated genes and 195 (range: 116 to 1202) for up-regulated genes. To identify recurrent gene expression changes, the percentage of cases showing altered expression of individual genes was calculated. Overall, 100 transcripts were down-regulated in ⩾ 70% and 71 transcripts were up-regulated in ⩾ 57% of patients (Table S1 and S2). Eight transcripts, corresponding to six distinct genes, were down-regulated in all seven patients with a median signal log ratio from –1.4 to –2.6 (Fig. 1A and Table S1). An additional 35 transcripts were down-regulated in six out of seven patients (⩾ 80% of cases)

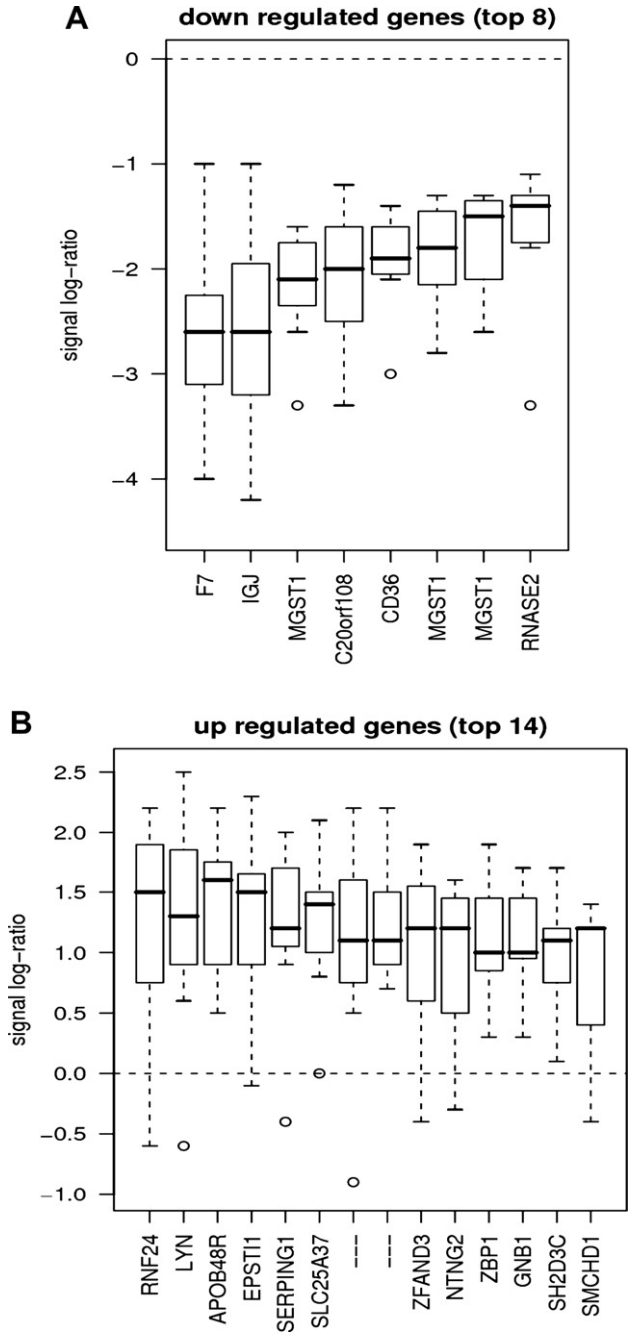

Fig. 1 – Signal log ratios of differentially expressed genes. (A) Top eight down-regulated transcripts. (B) Top 14 up-regulated transcripts. *Box-and-whiskers* plots: median signal log ratios (SLR) of individual genes: horizontal bars: *boxes:* data within 1st and 3rd quartile; *whiskers:* extreme data in the ⩽×1.5 interquartile range from the box. *Circles:* outliers.

with a median signal log ratio from −1.4 to −3.1 (Fig. S1 and Table S1). Among the up-regulated genes, 14 transcripts changed in ⩾ 70% of cases (five out of seven patients) with a median signal log ratios from 1.4 to 1.7 (Fig. 1B and Table S2). Functional annotation analysis of differentially expressed genes did not reveal clustering in any particular biological or functional class.

4. Discussion

The main rationale for combining B with PTX was their proapoptotic effect, possibly through blockade of PI3K/Akt activation by B and phosphorylation of Bcl-2 by PTX. The toxicity and the efficacy of escalating doses of B, given twice weekly for 2 consecutive weeks, with weekly PTX repeated every 3 weeks, were evaluated in 31 patients with advanced solid tumours.

The highest doses given in the dose finding part, of 1.3 mg/m^2 of B and 100 mg/m^2 of PTX - which were also the RDs - were evaluated in a total of 22 patients and 112 cycles and some conclusions on tolerability could be drawn.

Significant neutropenia was limited (14%) while grade 3–4 thrombocytopenia was never observed. Peripheral neurotoxicity, mainly of sensory type, and cumulative, was the most common non-haematological toxicity observed, of grade 3 in 23% of patients, and required treatment discontinuation at the RD in 27%. This incidence was higher than that reported with B and docetaxel in 42 patients with advanced prostate cancer in the study of Dreicer[8] and in seven patients with advanced solid tumours in the study of Lara.[9]

The incidence of the other non-haematological toxicities most likely related to B was lower in our study (diarrhoea 55%, fatigue 41%) than in that of Dreicer (diarrhoea 64%, fatigue 74%), possibly because these side effects were also related to docetaxel. One possible reason for the higher incidence of peripheral neuropathy in our study, however, could be that treatment was more prolonged because of the longer duration of response in patients with ovarian and breast cancer. In fact, among six patients who discontinued treatment due to neurotoxicity, four were responders.

Another reason could be a pre-treatment with neurotoxic agents. Five of the seven patients who had to decrease the dose of B because of neurotoxicity were pretreated with PTX or platinum, and in these cases a preexisting sub-clinical neurotoxicity cannot be excluded. However, the higher incidence of neurotoxicity observed in the present study as compared to the study of Dreicer suggest that this side effect could be due mostly to the combination and that the RD of 1.3 mg/m^2 of B, even though not associated with DLTs, cannot be safely given with neurotoxic agents like PTX for prolonged periods of time.

A potential overlapping of the neurotoxicity of B and PTX could be envisaged; in fact, in most trials with single agent B, neuropathy is not reported at doses < 1.2 mg/m^2 and DLT neuropathy starts at doses > 1.5 mg/m^2.

The overall response rate of 38% reported at the RD and in patients with ovary, breast and prostate cancer potentially sensitive to taxanes, appears to be in line with what is generally reported with single agent taxanes.

Analysis of treatment-induced changes in the peripheral blood transcriptome could provide sensitive means to monitor the biological effects of anticancer drugs in clinical trials. In this study many transcripts were significantly (fold change ⩾ 2) up- or down-regulated in ⩾ 70% of patients. A restricted group of genes was affected in all seven (eight transcripts) or in six out of seven (35 transcripts) analysed patients, suggesting a highly consistent response to the B and PTX combination. Although there is no formal proof that the observed changes in transcript levels are due to B and/or PTX, their

frequency and consistency suggest that they are likely to be treatment related. Their relevance to efficacy or toxicity of B and/or PTX will need to be investigated.

In conclusion, the lack of a clear cut clinical advantage in terms of efficacy of B with weekly PTX over single agent taxanes and the cumulative neurological toxicity of this regimen do not recommend its further clinical development.

5. Conflict of interest statement

None declared.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ejca.2008.05.022.

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