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An EORTC phase I study of Bortezomib in combination with oxaliplatin, leucovorin and 5-fluorouracil in patients with advanced colorectal cancer

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ABSTRACT

The combination of oxaliplatin, leucovorin and 5-fluorouracil (FOLFOX-4) is still a reference regimen in advanced colorectal cancer; however, the addition of new biologic compounds represents a significant way forward. Bortezomib is an inhibitor of proteasome, a multicatalytic enzyme complex that degrades several intracellular proteins. In this study, escalating doses of Bortezomib were administered along with the standard FOLFOX-4 doses, in order to evaluate the dose-limiting toxicity (DLT), toxicity profile and activity of the combination.

Patients with advanced colorectal cancer, unpretreated for metastatic disease, were enroled in the study. Bortezomib starting dose was 1.3 mg/m^2, which was to be escalated in the subsequent steps according to the toxicities observed after first cycle.

Exploratory pharmacogenetics research was conducted by analysing the association between clinical outcomes and polymorphisms in candidate genes for response to each of the used drugs. Correlation between tumour marker changes and response was also investigated.

One mg/m^2 (DL-1) was defined as being the maximum tolerated dose since only 1 DLT was observed in 6 patients. The main toxicities were haematologic, neuropathy, diarrhoea and fatigue. Amongst 13 evaluable patients, five had a partial response, five had a stable disease and three patients progressed. Two patients are long-term survivors after a combined chemosurgical approach.

Further trials of the current combination may be justified.

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1. Introduction

The treatment of advanced colorectal cancer (CRC) has changed significantly in the past decade with improvements in the response rates and the survival achieved, in part, by the introduction of new chemotherapeutic agents. Combinations of oxaliplatin, leucovorin (LV) and 5-fluorouracil (5-FU) represent a reference regimen for the treatment of advanced colorectal

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cancer in first line and salvage setting.[1] Further advances have been seen with the introduction of agents designed to target specific proteins within pathways vital to maintaining the malignant phenotype. Bevacizumab, a humanised monoclonal antibody against vascular endothelial growth factor (VEGF), shows significant benefits in first-line therapy of advanced colorectal cancer when combined with established irinotecan-based and oxaliplatin-based regimens.[2,3] Monoclonal antibodies targeting the epidermal growth factor receptor (EGFR), such as cetuximab and panitumumab, when combined with irinotecan or oxaliplatin regimens also show improved outcome.[4] Despite these advances, there remains a significant need for further improvements in the treatment of advanced CRC.

Bortezomib (Velcade) is a potent and reversible inhibitor of the proteasome, a multicatalytic enzyme complex that degrades intracellular proteins by a targeted and controlled mechanism.[5,6] It is licensed in the United States and Europe for the treatment of multiple myeloma patients who have received at least one prior therapy.[7–9] Numerous studies have shown that Bortezomib can enhance the effect of standard chemotherapies in solid tumour xenograft models.[10] This has paved the way to Bortezomib evaluation in a number of phases I and II trials in solid tumours, both alone and in combination. In these trials, Bortezomib toxicity, mainly consisting of sensory neuropathy, thrombocytopaenia and fatigue has been moderate.[10] Iqbal and colleagues have recently carried out a phase I combination study of 5-FU 500 mg/m^2 and leucovorin 20 mg/m^2 with Bortezomib starting dose 0.5 mg/m^2 twice weekly for four weeks, followed by a two-week rest. Nineteen patients in total were evaluated for response; amongst them, seven patients with colorectal cancer had stable disease.[11] To the best of our knowledge, no preclinical or clinical data of the combination of Bortezomib and oxaliplatin have ever been presented.

In this present study, Bortezomib was given at escalating doses in combination with standard oxaliplatin, 5-fluorouracil, leucovorin (FOLFOX-4) regimen (1) in first-line treatment of advanced colorectal cancer with the aim of evaluating the feasibility and the tolerability of the combination.

2. Patients and methods

2.1. Patient selection

Patients aged ⩾18 years with histologically proven advanced/metastatic colorectal cancer, amenable to the first-line treatment with oxaliplatin and 5-FU/LV, were eligible for the study. Patients were ineligible if having received prior chemotherapy for advanced disease; non-oxaliplatin-based adjuvant chemotherapy was allowed if completed more than 6 months before registration. Patients were required to have an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1, and an adequate bone marrow, hepatic and renal function defined as absolute neutrophil count (ANC) ⩾2000×10^9/l, platelets ⩾$100{,}000 \times 10^9$/l, bilirubin ⩽1.5 mg/dl, alkaline phosphatase and serum transaminases ⩽2.5 times the upper limit of reference range (ULRR) in the absence of liver metastases or <5 times the ULRR in the presence of liver metastases, serum creatinine ⩽150 μmol/l (1.7 mg/dl). Additional exclusion criteria included the presence of pre-existing neuropathy ⩾grade 1; evidence of brain metastases; prior radiotherapy or major surgery (within prior 4 and 2 weeks, respectively); history or the presence of malignancies at other sites (with the exception of cone biopsied carcinoma of the cervix and adequately treated basal or squamous cell skin carcinoma); unstable systemic diseases; active uncontrolled infections and electrocardiogram (ECG) changes with the history of ischemic heart disease in the past 6 months. The study was conducted in compliance with the International Conference on Harmonization for Good Clinical Practice. The protocol was approved by the European Organisation for Research and Treatment of Cancer (EORTC) Protocol Review Committee and by the Ethics Committee of each participating centre. All patients provided written informed consent.

2.2. Treatment plan

The study was conducted in three centres of the New Drug Development Group (NDDG) of the EORTC. Patients with advanced colorectal cancer were registered at the EORTC Headquarters prior to the start of treatment and after the verification of the eligibility criteria. The doses of oxaliplatin, 5-FU and LV were maintained unchanged, whilst Bortezomib dose was escalated according to the schedule shown in Table 1. The appropriate amount of Bortezomib was administered as an intravenous (IV) push over 3–5 s followed by a standard saline flush or through a running IV on days 1, 8 and 15; oxaliplatin was administered intravenously at the fixed dose of 85 mg/m^2 in 250 ml glucose 5%, 2-h infusion, on days 1 and 15; LV was administered at the fixed dose of 200 mg/m^2 (DL form) or 100 mg/m^2 (L form) in 250 ml glucose 5% solution, 2-h infusion, on days 1, 2, 15 and 16 and was immediately followed by 5-FU administered at the fixed dose of 400 mg/m^2 as intravenous bolus, on days 1, 2, 15 and 16; and at the fixed dose of 600 mg/m^2 as 22-h continuous infusion on days 1, 2, 15 and 16; the duration of one cycle was 4 weeks. Treatment was administered until disease progression, patient refusal, unacceptable toxicity or patient's best interest according to treating physician.

Cohorts of three patients were treated; dose escalation proceeded if no patients had dose-limiting toxicity (DLT) at cycle 1. DLT was defined (using CTC version 3) as grades 3–4 non-haematologic toxicity, with the exclusion of alopecia, nausea, vomiting and fever controlled after 48 h with appropriate measures, or haematologic toxicities, including ANC <0.5×10^9/l lasting for ⩾5 d; febrile neutropaenia defined as

Table 1 – Bortezomib dose escalation scheme

Dose levels	Bortezomib (mg/m^2)
−1[a]	1.0
1 (starting dose)	1.3
2	1.6
3	1.8
>3	0.2 + previous Dose[b]

a A dose level-1 was planned in case of toxicity at DL1.
b Increments of 0.2 mg/m^2 were planned after this until reaching the MTD.

ANC $<1.0 \times 10^9$/l and fever at least 38.5 °C or thrombocytopaenia $\leqslant$25,000/μl. Any toxicity which prevented the combination from being given according to the planned schedule at cycle 1 also qualified for DLT. If one patient in the initial cohort had DLT, up to a maximum of 6 patients were included at that particular dose level. If two patients or more, out of a maximum of six patients, experienced a DLT, dose escalation was stopped, and six additional patients were included at the dose below which represented maximum tolerated dose (MTD) provided no more than one DLT was observed in 6 patients during cycle 1.

As of cycle 2, in case of any non-haematological or haematological grade 2 toxicity, the treatment was interrupted for a maximum of 2 weeks until resolution to grade 1 or better, and restarted at the same dose. In case of grade >2 toxicity, treatment was delayed for a maximum of 2 weeks and restarted at the next lower dose level of Bortezomib. Patients treated at the first dose level and experiencing a grade >2 toxicity, which resolved within 2 weeks, were treated at dose level 1 (1.0 mg/m^2).

Exploratory pharmacogenetics research was conducted to evaluate the association of germline polymorphisms with clinical outcome and toxicity. Peripheral blood samples from all enroled patients were used for genotyping polymorphisms in DNA repair, i.e. excision repair cross-complementation group-1 (ERCC 1) and X-ray cross-complementing-1 (XRCC 1), which have a role in the development of pharmacoresistance to platinum derivatives and drug metabolism pathway (uridine diphosphate-glucuronosyltransferases-A1 (UGT1 A1)).[12]

2.3. *Patient evaluation*

At enrolment, patients were evaluated by a complete history and physical examination, performance status recording, heart rate and blood pressure, ECG, body temperature and the evaluation of all clinical symptoms. Complete blood cell (CBC) count and serum chemistries had to be performed less than 14 d before the start of treatment. Full tumour evaluation was required within 21 d prior treatment start, and consisted of chest X-rays, total body computed tomography (CT) scan and/or MRI and tumour markers (CEA, CA 19.9) assessment.

Patients were monitored throughout the treatment by clinical examination, toxicity assessment, biochemistry, ECG at the end of each cycle of therapy; complete blood counts were performed weekly. The disease was evaluated every 8 weeks: all sites that were found to be involved at the initial assessment were re-evaluated by the same method. In patients with measurable disease at entry, response was assessed according to the Response Evaluation Criteria in Solid Tumours (RECIST), and tumour marker changes were also correlated with tumour response. National Cancer Institute's (NCI) Common Toxicity Criteria (CTC), version 3.0, were used to grade toxicity. For the patients who discontinued treatment prematurely due to toxicity, follow-up was continued on a weekly basis after the completion of treatment until any associated toxicity had resolved. However, the patients who discontinued treatment for any other reason than toxicity and before documented disease progression were to be followed-up every 8 weeks until the first sign of progression or start of a new anticancer treatment.

2.4. *Statistical methods*

The standard '3+3' design was used for the dose escalation.

Kaplan–Meier analysis was used for the evaluation of progression-free survival and overall survival.[13]

The Wilcoxon test was used to assess a correlation between tumour marker changes and response.[14]

Fisher exact test was used to evaluate the correlation between gene polymorphism and treatment outcome.[15]

3. Results

3.1. *Patient characteristics*

Between January 2005 and January 2007, 16 patients were enroled in the study. Ten patients were male, six patients female. Median age was 62 years (ranges 38–78). Performance status was 0 in the majority of patients. Eleven patients had previously undergone surgery, whereas five patients had received prior adjuvant chemotherapy. The characteristics of the eligible patients are detailed in Table 2.

3.2. *Dose escalation results*

Forty-eight cycles of treatment were given (mean 3 cycles, ranges 1–6). At Bortezomib dose level 1 (1.3 mg/m^2), none of the first 3 patients had a DLT. Therefore, next patients were treated at dose level 2. Since DLT was observed in 2 of 4 patients at dose level 2, dose escalation was halted, and next 3 additional patients were treated again at dose level 1. Two DLT were observed amongst them and dose level 1 was therefore investigated. As one DLT was observed amongst 6 patients, 1 mg/m^2 was defined as the recommended Bortezomib dose.

In detail, both patients who had a DLT at dose level 2 (1.6 mg/m^2) experienced a toxicity, which prevented treatment from being given as scheduled. In particular, the first patient had grade 3 febrile neutropaenia, which caused days 15 and 16 of treatment to be delayed for one week and Bortezomib dose to be reduced to 1.3 mg/m^2; the second patient had day 8 Bortezomib dose skipped due to persistent grade 2 peripheral neuropathy and myalgia.

The 2 DLT observed at 1.3 mg/m^2 consisted of grade 3 neutropaenia, which prevented treatment from being given as scheduled in cycle 1. In particular, one patient had days 15 and 16 doses not given, whilst in the other patient day 15 dose was reduced. The only DLT observed at dose level 1 consisted in grade 3 neutropaenia, which prevented treatment from being given as scheduled in cycle 1.

3.3. *Toxicity*

The main reason for stopping treatment was toxicity in 8 patients. In particular, 5 patients had grade 3 or persistent grade 2 neutropaenia; one patient had grade 3 neuropathy, whereas another patient had grade 3 fatigue, weakness and diarrhoea. Grade 3 myalgia and paresthaesia was observed in the eighth

Table 2 – Characteristics of eligible patients (total no. 16)

Median age (years) – range	62 (38–78)
Sex	
M	10
F	6
Performance status	
0	10
1	6
Liver metastasis	
Yes	7
No	9
Prior surgery	
Yes	12
No	4
Weight loss over the last 3 months	
<5%	14
5% to <10%	1
10% to <20%	1
Histopathological grading	
G1 well differentiated	2
G2 moderately differentiated	12
G3 poorly differentiated	1
GX unknown grade of differentiation	1
Prior radiotherapy	
Yes	1
No	15
Site of primary	
Ascendens	6
Transversum	1
Descendens	1
Sigmoid	4
Rectum	1
Combination	3
Prior adjuvant chemotherapy	
Yes	5
No	11

patient. Four patients left the study for medical decision (2 for patients best interest; one for venous access problems; one for hepatic embolisation, right lobectomy and hemicolectomy), whereas 3 patients had disease progression.

The most frequently reported toxicities in cycle 1 were nausea, sensory neuropathy and fatigue (63%), diarrhoea and muscle pain (27%), anorexia and mucositis (13%) and grades 2–3 neutropaenia (29%). Grade 4 haematological toxicity was not observed. One patient had grade 3 febrile neutropaenia. Table 3 details all grades haematological toxicities observed at the three dose level after first cycle, whereas the worst haematological and non-haematological toxicities by dose level observed across all treatment cycles are detailed in Tables 4 and 5, respectively.

The overall drug-related toxicity observed in this study was consistent with that expected and included neutropaenia, fatigue, diarrhoea, nausea/vomiting, sensory neuropathy, mucositis and anorexia.

3.4. *Response*

Thirteen patients were evaluable for response. Three patients were not evaluable for response because they received less than 2 cycles of therapy. Five patients had a partial response with a median duration of 7.7 months (range 64–593 d). One patient with partial response who underwent intestinal and liver surgery after chemotherapy followed by 8 cycles of adjuvant capecitabine is currently disease free after a two-year follow-up. A second patient underwent a hepatic segmentectomy followed by adjuvant treatment with FOLFOX and is also currently in complete response after a four-month follow-up. Stable disease and disease progression were observed in 5 and 3 patients, respectively. The best overall response by dose level is summarised in Table 6.

Neither response to treatment nor toxicity correlated with ERCC1, XRCC1 and UGT1A1 polymorphisms in the 12 patients whose data were collected ($p = 1.0000$).

Responses were correlated with a decrease in CA19.9 marker level as compared to baseline values (The Wilcoxon test $p = 0.004$) (Fig. 1). However, the analysis was performed only in 12 evaluable patients, one of whom had no baseline value. Missing data precluded a similar analysis for CEA levels.

4. Discussion

Degradation via ubiquitin-mediated proteasome pathway helps to regulate the intracellular levels of short-lived proteins including those that govern the cell cycle, tumour growth and survival.[10] For example, proteins regulating the cell cycle such as p53, cyclins and the cyclin-dependent kinase (CDK) inhibitors p21 and p27 are principally degraded by the S26 proteasome, as is the proapoptic protein Bax. Experimental data suggest that proteasome inhibition results in the stabilisation of these proteins with the subsequent dysregulation of cell-cycle progression and increased apoptosis.[10] Furthermore, the proteasome is also of crucial importance in

Table 3 – Haematological toxicity observed at three dose levels after 1st cycle

	DL1 ($n = 6$)			DL2 ($n = 4$)			DL-1 ($n = 6$)			
Toxicity grade	1	2	3	1	2	3	1	2	3	4
Anaemia	4	1	–	4	1	–	4	–	–	–
Neutropaenia	–	1	2[b]	–	1	1[a,b]	2	1	–	1[b]
Leucopaenia	–	–	2[b]	–	–	–	2	–	1	–
Thrombocytopaenia	1	–	–	–	–	–	2	–	–	–

a Patient (1) with grade 3 febrile neutropaenia.
b DLT.

Table 4 – Worst haematological toxicity by dose level across all treatment cycles

	DL1 (n = 6, 13 cycles)			DL2 (n = 4, 14 cycles)			DL-1 (n = 6, 20 cycles)			
Toxicity grade	1	2	3	1	2	3	1	2	3	4
Neutropaenia	1	–	3	–	1	3	–	1	2	3
Leucopaenia	1	1	2	2	–	1	–	5	1	–
Thrombocytopaenia	5	1	–	3	1	–	4	2	–	–
Febrile neutropaenia	–	–	–	–	–	1	–	1	–	–

Table 5 – Worst non-haematological toxicity by dose level across all treatment cycles

	DL1 (n = 6, 13 cycles)		DL2 (n = 4, 14 cycles)			DL-1 (n = 6, 20 cycles)		
Toxicity grade	1	2	1	2	3	1	2	3
Fatigue	–	2	–	3	1	3	2	–
Fever	1	–	2	–	–	–	1	–
Weight loss	–	–	–	1	–	–	1	–
Anorexia	–	1	1	1	–	3	–	–
Dehydratation	–	–	–	–	1	1	–	–
Diarrhoea	1	1	2	–	1	5	1	–
Mucositis	1	–	–	1	–	2	1	–
Nausea	3	–	2	–	–	1	3	–
Vomiting	2	–	1	–	–	1	1	–
Gamma–GT	–	–	2	–	–	1	–	–
Dizziness	–	–	1	–	–	1	–	–
Motor neuropathy	–	–	–	1	–	–	–	1
Sensory neuropathy	3	1	–	2	1	3	2	–
Pain	1	1	2	1	–	–	–	1

Table 6 – Best overall response by dose level

Response	DL-1 (n = 6)	DL1 (n = 6)	DL2 (n = 4)
Partial response	2	1	2
Stable disease	3	–	2
Progression disease	–	3	–
Not applicable	1	2	–

modulating the activity of nuclear transcription factor kappa (NF-kappaB), which plays a main role in oncogenesis by stimulating cell proliferation, blocking apoptosis and inducing angiogenesis. In quiescent cells, the regulatory protein inhibitor IkB binds NFkB in the cytoplasm and prevents its translocation into the nucleus. The NFkB pathway is activated by many chemotherapeutics which there by switch on an anti-apoptotic programme that, if inhibited, can enhance the anti-tumour activity of the cytotoxic drug.

The clinical results in multiple myeloma provide proof of concept for proteasome inhibition as an anticancer therapy, and the role of Bortezomib in other types of cancer and in different settings is under active investigation.

In particular, a number of phases I and II studies of Bortezomib, both as single-agent and in combination, have been undertaken in solid tumours.[11,16–35] Single-agent Bortezomib has not shown substantial activity, but toxicity has been acceptable, the most significant clinical adverse events being a peripheral sensory neuropathy and gastrointestinal disturbances, mainly diarrhoea. The above toxicity profile mandates caution in combining Bortezomib with agents that may have overlapping neurologic or gastrointestinal toxicities.

Preclinical data providing a possible mechanism of action of Bortezomib in colorectal cancer have been recently presented. Uddin and colleagues[36] have demonstrated that Bortezomib induces p27kip1 expression through the degradation of SKP2, a F-box protein which targets cell cycle regulators. In this study, the role of SKP2 and its ubiquitin-proteasome pathway was investigated in a panel of colorectal carcinoma cell lines, clinical samples and NUDE mouse model. Immunohistochemical analysis on a large tissue microarray showed an inverse association of SKP2 expression with p27kip1 protein levels. The subset of tumours with high SKP2 and low p27kip1 expression showed a decreased overall survival (p = 0.0057). The treatment of colorectal cancer cell lines with Bortezomib caused the downregulation of SKP2, the accumulation of p27kip1 and apoptosis. Treatment of colorectal cancer cell lines xenografts with Bortezomib resulted in growth inhibition of tumours in NUDE mice via the downregulation of SKP2 and the accumulation of p27kip1. Altogether, these results suggest that ubiquitin-proteasome pattern may be a potential target for therapeutic intervention in colorectal cancer.

Only one single-agent study of Bortezomib in advanced colorectal cancer has been reported, and no objective responses have been observed.[28] In addition, the literature on the use of Bortezomib-based combinations in advanced colorectal cancer is very scarce. Apart from the previously mentioned Iqbal study, which was targeted to patients with advanced solid tumours,[11] only another phase II combination

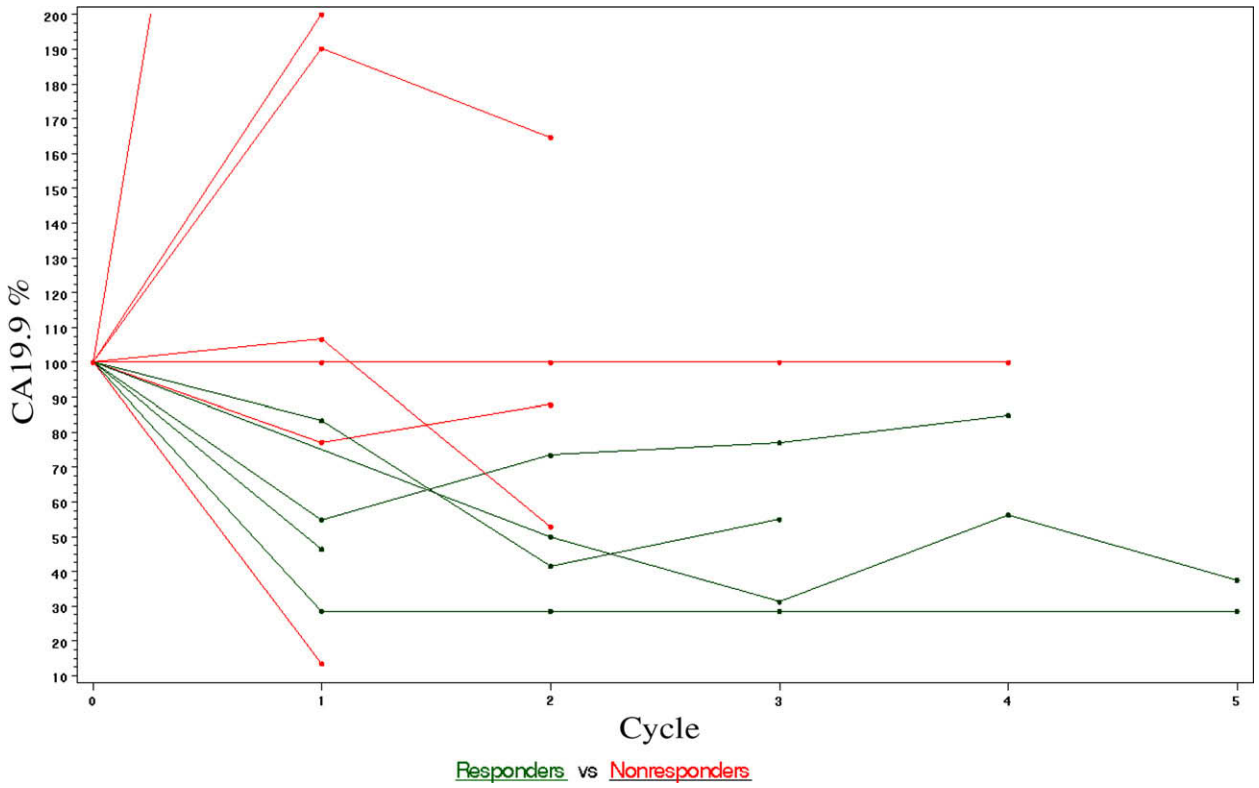

Fig. 1 – Per patient CA19.9 as a percentage of baseline across 5 treatment cycles in responders *versus* non-responders.

study (irinotecan + Bortezomib) has been reported in advanced colorectal cancer, with clear evidence of lack of activity.[33]

Our study, aimed at testing the feasibility and activity of the combination of Bortezomib with oxaliplatin, 5-FU and leucovorin, determined a tolerable Bortezomib dose of 1.0 mg/m^2, much lower than Bortezomib recommended dose as single-agent (1.5 mg/m^2 on days 1, 4, 8 and 11 every 3 weeks). The predominant dose-limiting toxicity was the prolongation of neutropaenia preventing the administration of chemotherapy in cycle 1 within the allowed schedule, and no unpredictable toxicities were observed. Thus, future studies of this combination exploring different doses/schedules of Bortezomib may be warranted. The toxicity profile in our study was predictable and included, in particular, neutropaenia, thrombocytopaenia, sensory neuropathy and gastrointestinal disturbances. The previously mentioned Iqbal study[11] used lower doses, but no objective signs of clinical activity were observed in colorectal cancer patients. The Bortezomib schedule we used in this study should be probably reconsidered, since in most cases the skipment of one or more drug doses, with subsequent failure to administer treatment appropriately, represented DLT. The low number of evaluable patients makes any conclusion on activity impossible; however, despite delays and dose reductions due to toxicity, five of 13 patients had a confirmed partial response. In addition, 2 patients had a long lasting complete response after combined chemosurgical approaches. CEA and Ca 19-9 pretreatment levels have been previously found to be an independent prognostic factor in patients with colorectal carcinoma. However, only the combined elevation of the two markers seemed to be significant.[37,38] In our study, CA19.9 levels were assayed, and a correlation between marker levels and response was observed.

4.1. *Is there a way forward for Bortezomib in colorectal cancer?*

As many novel targeted that therapeutic agents are currently available licensed [2,4] or are being developed in the setting of advanced colorectal cancer, the results of this and the previous Bortezomib clinical studies in this setting are not encouraging for further development. However, using Bortezomib in combination with other biological agents through an understanding of its effects on different signalling pathways may prove more fruitful.

For example, Bortezomib interferes with the p44/42 mitogen-activated protein kinase (MAPK), a downstream effector of EGFR pathway that communicates proliferative signals.[39] Bortezomib has been combined with EGFR tyrosine kinase inhibitors (gefitinib or erlotinib) resulting in the significant enhancement of apoptosis.[40] Furthermore, a significant synergistic antiproliferative effect has been observed with the combined treatment of Bortezomib and either gefitinib or cetuximab in a number of cancer cell lines.[41] These findings support the translation of the combination of the proteasome inhibitor with an EGFR inhibitor into a clinical setting.

Conflict of interest statement

None declared.

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