REVIEW

The ubiquitin–proteasome system as a molecular target in solid tumors: an update on bortezomib

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Abstract: The ubiquitin–proteasome system has become a promising molecular target in cancer therapy due to its critical role in cellular protein degradation, interaction with cell cycle and apoptosis regulation, and unique mechanism of action. Bortezomib (PS-341) is a potent and specific reversible proteasome inhibitor, which has shown strong in vitro antitumor activity as single agent and in combination with other cytotoxic drugs in a broad spectrum of hematological and solid malignancies. In preclinical studies, bortezomib induced apoptosis of malignant cells through the inhibition of NF-κB and stabilization of pro-apoptotic proteins. Bortezomib also promotes chemo- and radiosensitization of malignant cells in vitro and inhibits tumor growth in murine xenograft models. The proteasome has been established as a relevant target in hematologic malignancies and bortezomib has been approved for the treatment of multiple myeloma. This review summarizes recent data from clinical trials in solid tumors.

Keywords: proteasome, bortezomib, NF-κB, clinical studies, solid tumors

Introduction

Proteasome inhibitors represent a class of drugs that have anticancer activity through a variety of cellular mechanisms including induction of apoptosis, interference with cell cycle progression, inhibition of angiogenesis, and suppression of nuclear transcription factor kappa B (NF-κB).[1] The proteasome is a multicatalytic enzyme complex that degrades several intracellular proteins by a targeted and controlled mechanism.[2–4] The activity of proteasome in degradation of tumor-suppressing and proapoptotic protein targets known to be dysregulated in many human malignancies provides the rationale for its selection as a target for cancer therapy. The first-in-class proteasome inhibitor, bortezomib (PS-341; Velcade®; Millennium Pharmaceuticals), a boronic acid dipeptide derivative, received approval in the United States (2003) and Europe (2004) for relapsed and refractory multiple myeloma.[5–7] In the APEX phase III study, comparing bortezomib and dexamethasone in patients with multiple myeloma, the median time to progression was significantly increased from 106 days with dexamethasone to 189 days with bortezomib and the 1-year overall survival was also higher in the bortezomib arm (80% vs 66%).[7] Recently, the FDA approved bortezomib in relapsed mantle cell lymphoma.[8]

Bortezomib has also shown activity in preclinical studies of a variety of solid tumors, and this has paved the way for several phase I/II clinical studies of bortezomib either as single agent or in combination with cytotoxic and biologic agents.

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This review aims to highlight current knowledge of the anticancer effects of bortezomib with emphasis on recent clinical studies.

Mechanism of action of proteasome

The ubiquitin-mediated proteasome pathway regulates a group of intracellular proteins that govern cell cycle, tumor growth, and survival. Proteasome participates in the turnover and degradation of several pathways, or short-lived cellular regulatory proteins, including p53, cyclins and the cyclin-dependent kinase (CDK) inhibitors p21 and p27, the estrogen receptor, and the inhibitor (IκB) of NF-κ.[9–13] 26S proteasome consists of a multisubunit, cylindrical complex including a 20S core catalytic component and 19S regulatory particles that contain polyubiquitin-binding sites and isopeptidase activity for the cleavage and release of ubiquitin from the protein substrate.[14] The proteasome requires adenosine triphosphate (ATP) hydrolysis and regulates multicatalytic protease that selectively degrades polyubiquinated proteins. These proteins get marked for degradation by a multistep process. Prior to degradation, polyubiquitinated moieties are covalently attached to the target proteins in a multistep process involving three distinct enzymes: the ubiquitin-activating enzyme E1 binds ubiquitin and transfers it to the ubiquitin-conjugating enzyme E2. In the following step the ubiquitin ligase E3 catalyzes the transfer of the polyubiquitinated tails from E2 to lysine residues of the target protein, specifically marking them for proteolysis protein into small fragments in an ATP-dependent manner.[4,15] Activated p53 arrests cells in the G1-phase and promotes apoptosis to allow elimination of damaged cells through induction of the proapoptotic protein Bax, which, in turn, is also a proteasomal substrate. The final outcome of this process is proteasome inhibitor-induced stabilization of p53, p21^{Cip1}, p27^{Kip1}, and Bax, dysregulation of cell-cycle progression and, finally, apoptosis.[16]

The proteasome modulates also the activity of the transcription factor nuclear NF-κB. The NF-κB pathway is activated by a variety of cellular stress signals, and chemo- and radiotherapy, which lead to phosphorylation of a serine residue on IκB, which targets it for ubiquitination and proteasomal degradation. This process allows activated the NF-κB subunit to translocate into the nucleus, where it induces expression of a variety of genes encoding cell adhesion molecules and antiapoptotic factors (Figure 1).[17–19] More details have been provided in our previous review.[20]

Preclinical studies of proteasome inhibitors

Proteasome inhibitors have been shown to be cytotoxic against a variety of cancer cell lines *in vitro* and in *in vivo* models. The activity of bortezomib in solid tumors *in vivo* has been evaluated in a variety of xenograft models.[21,22]

Bortezomib also increases the sensitivity of tumor cells to chemotherapy and radiation and reverses chemoresistance. Bortezomib was two times more potent in inhibiting the growth of chemoresistant multiple myeloma cells compared with chemosensitive cells, in direct correlation with NF-κB activity.[23] In colon carcinoma cells, bortezomib inhibited the radiation-induced increase in NF-κB and enhanced radiosensitivity.[24]

Many chemotherapeutics induce NF-κB and thereby activate an antiapoptotic program that, if inhibited, can enhance the antitumor activity of the chemotherapeutic.[25] Inhibition of the proteasome was shown initially to increase the efficacy of CPT-11 (irinotecan) through blockade of NF-κB in a model of colon cancer.[26] In another study, gemcitabine caused a 59% reduction of pancreatic cancer volume compared with control, while the combination of gemcitabine and bortezomib increased growth inhibition to 75%.[27]

Taken together, results from preclinical studies show that bortezomib can induce apoptosis in a number of otherwise resistant tumor cells and can sensitize cancer cells to other cytotoxic agents and radiation therapy.

Clinical studies

The clinical feasibility of using bortezomib for treating solid malignancies has been explored in a number of phase I and II studies, the main of which are summarized below.

Phase I studies

A number of phase I trials have been carried out with different schedules of bortezomib.

A phase I clinical study evaluated the dose-limiting toxicity (DLT) and maximum-tolerated dose (MTD) of bortezomib as single agent administered as an intravenous bolus once-weekly for 4 out of 5 weeks in 53 patients, 48 of whom had advanced androgen-independent prostate cancer. The DLT was seen in 2 of 5 patients treated with a dose of 2.0 mg/m^2, and it included grade 3 diarrhea in both patients and grade 3 syncope and hypotension in one patient; so, the recommended phase II dose of bortezomib was 1.6 mg/m^2. Two patients with prostate cancer had prostate-specific antigen response, whereas 2 patients had partial response in lymph nodes. The biologic activity, such as inhibition of NF-κB related markers, was seen
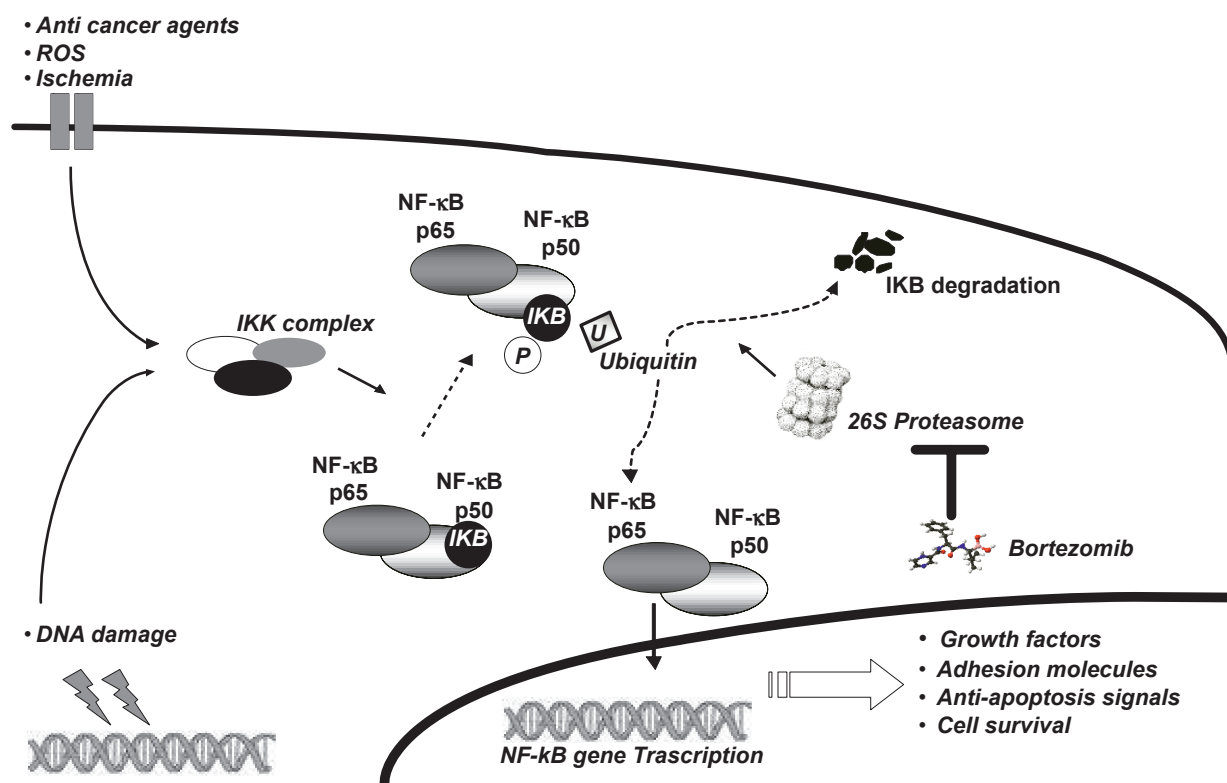
Figure 1 Several intra- and extracellular factors induce the intracellular increase of the IKK complex which phosphorylates the IKB protein. Phosphorylation of IKB causes its ubiquitination and degradation by 26S proteasome. NF-κB complex is then able to interact with its DNA ligand site, stimulating transcription of several genes which prompt apoptosis inhibition, growth factor increases, and cell survival. Bortezomib acts by inhibiting 26S proteasome.
Abbreviation: ROS, reactive oxygen species.

at tolerated doses of bortezomib. The maximum level of 20S inhibition was 70% to 75%, which suggests that the inhibition of proteasome is saturable.[28] Another phase I study tested two different schedules (schedule 1: twice weekly for 4 out 6 weeks; schedule 2: twice weekly for 2 out of 3 weeks) of bortezomib in 44 patients with advanced cancers. The most common toxicity was thrombocytopenia, which was dose limiting at 1.7 mg/m^2 (schedule 1) and 1.6 mg/m^2 (schedule 2), whereas the MTD was 1.5 mg/m^2

for both schedules. A patient with multiple myeloma had a partial response.[29] Another schedule of bortezomib (starting dose: 1.0 mg/m^2 on days 1, 4, 8, 11, every 3 weeks) was tested in a phase I/II study in 18 patients with unresectable hepatocellular carcinoma. Grade 2/3 toxicities included thrombocytopenia, fatigue, and neuropathy. MTD was considered to be 1.3 mg/m^2. In 7/15 evaluable patients, stable disease was observed.[30] The phase I single-agent studies with bortezomib are summarized in Table 1.

Table 1 Phase I single agent studies of bortezomib

Patient population	Prostate cancer	Advanced others solid tumors	Hepatocarcinoma
Number of patients	53	46	14
Schedule	Starting dose of 0.13 mg/m^2 iv once weekly for 4 weeks q5 weeks	Arm 1: starting dose of 0.13 mg/m^2 iv twice weekly for 4 weeks q6 weeks Arm 2: starting dose of 0.13 mg/m^2 iv twice weekly for 2 weeks q3 weeks	Starting dose of 1.0 mg/m^2 iv on days 1, 4, 8, 11 q3 weeks
Best response obtained	2 PR 2 SD	1 PR	7 SD
Toxicity observed	Diarrhea and hypotension	Neurotoxicity and fatigue	Thrombocytopenia, neurotoxicity and fatigue
MTD	1.6 mg/m^2	Arm 1: 1.7 mg/m^2 Arm 2: 1,6 mg/m^2	1.3 mg/m^2

Abbreviations: PR, partial response; SD, stable disease; MTD, maximum-tolerated dose.

Following preclinical studies which highlighted the synergy between bortezomib and taxanes,[31,32] a phase I trial of twice-weekly bortezomib and weekly docetaxel was carried out; the recommended doses were 0.8 mg/m^2 and 25 mg/m^2, respectively, every 21 days. The DLT were thrombocytopenia and febrile neutropenia. Clinical activity was low in this pretreated patient population, since only 4 patients had stable disease as best observed response.[33] In another phase I study, the combination of paclitaxel and bortezomib revealed no response rates.[34] Another phase I study was carried out to evaluate the combination between 5-fluorouracil (5-FU) 500 mg/m^2 and leucovorin (LV) 20 mg/m^2 with starting dose of bortezomib 0.5 mg/ m^2 twice weekly for 4 weeks, with 2 weeks rest. One partial response, 8 stable disease, and 10 progressive disease were achieved in 19 patients.[35] Another phase I clinical trial evaluated the safety and biologic effects of bortezomib and irinotecan coadministered in 51 patients. The MTD for the combination regimen was bortezomib 1.3 mg/m^2 twice a week and irinotecan 125 mg/m^2 on days 1, 8, followed by a 1-week rest.[36] In another study, escalating doses of bortezomib were administered along with standard FOLFOX-4 doses, in order to evaluate the DLT, toxicity profile, and activity of the combination. Among 13 evaluable patients, 5 had a partial response, 5 had stable disease, and 3 progressed. Two patients are long-term survivors after a combined chemosurgical approach.[37]

Findings from preclinical studies[38] prompted a phase I trial to determine the MTD of escalating doses of gemcitabine (1000 mg/m^2 given once a week for 2 weeks) with bortezomib (1.0 mg/m^2 given twice a week) every 21 days, in 31 patients with advanced solid tumors. This combination was well tolerated and a partial response was observed in a patient with advanced nonsmall-cell lung cancer (NSCLC), who had been pretreated with gemcitabine.[39] In another phase I study, a combination of bortezomib, gemcitabine, and carboplatin induced 4 partial responses and 5 stable diseases in 16 patients with advanced NSCLC.[40]

In addition to these studies, the administration of gemcitabine via fixed-dose rate (FDR) infusion at 10 mg/m^2/min has been shown to increase accumulation of the active phosphorylated metabolite of the drug. Recently, a phase I trial was set out to determine the safety, toxicity, and MTD of FDR gemcitabine with bortezomib in advanced solid tumors refractory to standard therapy. One partial response lasting 12 months was observed in breast cancer, and 6 stable disease were observed in 29 patients. The combination of FDR gemcitabine with bortezomib may warrant further studies in advanced breast, lung, ovarianm, and pancreatic cancer patients.[41]

A marked effect of escalating dose of bortezomib administered twice weekly for 2 weeks every 21 days in combination with a fixed dose of carboplatin (AUC = 5) was assessed in 15 patients with advanced ovarian cancer who had received upfront chemotherapy and up to 2 prior chemotherapy regimens for recurrent disease. The overall response rate to this combination was 47%, with 2 complete responses and 5 partial responses, including 1 complete response in a patient with platinum-resistant disease.[42] A Gynecologic Oncology Group phase II trial of single-agent bortezomib in recurrent ovarian cancer is currently ongoing. Kubicek and co-workers have recently presented the preliminary results of a phase I trial of bortezomib, cisplatin, and radiotherapy for advanced head and neck cancer. The combination proved feasible; main toxicity was trombocytopenia; no clinical relevant peripheral neuropathy occurred; and most of the other observed toxicities were clearly attributable to cisplatin and radiation therapy.[43] The final paper is awaited with full details on safety and activity.

Phase I combination studies with bortezomib are summarized in Tables 2 and 3.

Phase II studies

A large number of phase II studies of single-agent bortezomib have been carried out or are currently underway. Phase II trials of bortezomib as single agents in patients with melanoma,[44] recurrent or metastatic sarcomas,[45] neuroendocrine tumors,[46] colorectal cancer,[47] advanced renal cancer,[48,49] and advanced breast cancer[50,51] revealed no significant response rates.

Bortezomib was safe, the most significant clinical adverse event being a peripheral sensory neuropathy. Phase II studies of single agent bortezomib are summarized in Table 4.

A randomized phase II study was conducted in 87 patients with metastatic pancreatic cancer, who were randomized to receive bortezomib alone (1.5 mg/m^2 twice weekly for 2 weeks every 3 weeks) or a combination of bortezomib (1.0 mg/m^2 twice weekly for 2 weeks every 3 weeks) plus gemcitabine (1000 mg/m^2 on days 1, 8 every 3 weeks). The response rate was 0% in the arm with bortezomib alone, with median survival of 2.5 months and median time to progression of 1.2 months. Four patients achieved a partial response in the combination arm, but the benefit obtained with bortezomib alone or in combination with gemcitabine was low.[52]

Table 2 Phase I combination studies of bortezomib in advanced solid tumors

Number of patients	21	14	25	51	31	29
Schedule	Starting dose of 0.5 mg/m² iv twice weekly + 5-FU 500 mg/m² + leucovorin 20 mg/m² on day 1 q2 weeks	Starting dose of 0.8 mg/m² iv on days 2, 5, 9, 12 + docetaxel 25 mg/m² on days 1, 8 q3 weeks	Starting dose of 0.6 mg/m² iv on days 2, 5, 9, 12 + paclitaxel 80 mg/m² on days 1, 8 q3 weeks	Starting dose of 1.3 mg/m² iv twice a week + irinotecan 125 mg/m² on days 1, 8 q3 weeks	Starting dose of 1.0 mg/m² iv twice a week + gemcitabine 1000 mg/m² on days 1, 8 q3 weeks	Starting dose of 1.0 mg/m² iv on days 1, 4, 8, 11 + FDR gemcitabine 750, 1000, 1250 mg/m² on days 1, 8 q3 weeks
Best response obtained	1 PR 8 SD	4 SD	1 PR	10 SD	1 PR 7 SD	1 PR 6 SD
Toxicity observed	Abdominal pain and diarrhea	Hematologic	Neurotoxicity and fatigue	Diarrhea, nausea and vomiting	Abdominal pain and hematologic	Neutropenia and thrombocytopenia

Abbreviations: FDR, fixed dose rate; PR, partial response; SD, stable disease; 5FU, 5-fluorouracil.

Fanucchi et al investigated the safety and efficacy of bortezomib monotherapy (arm A) compared with the combination of bortezomib and docetaxel (arm B) as second-line therapy in 155 patients with locally advanced and metastatic NSCLC. Overall response rate was 8% in arm A and 9% in arm B. Time to response was 36 to 83 days in arm A, 5 of 6 patients responding within 40 days, and 38 to 99 days in arm B, 2 of 7 patients responding within 41 days.[53] Bortezomib plus docetaxel seemed to demonstrate modest benefit compared with bortezomib monotherapy. Phase II combination studies of bortezomib are summarized in Table 5.

Conclusion and future perspectives

The 26S proteasome acts as a housekeeper to eliminate damaged or misfolded proteins. In addition, many regulatory proteins governing the cell cycle, transcription factor activation, apoptosis, and cell trafficking are the substrates for proteasome-mediated degradation. Five years after entering clinical trials, bortezomib has demonstrated efficacy for the treatment of patients with recurrent and refractory multiple myeloma. 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 therapy is undergoing active investigation. In contrast to the results of myeloma, the treatment of solid tumors with either single-agent bortezomib or bortezomib in combination with conventional chemotherapy agents has not yet yielded significant improvements in treatment response. Probably, a more extensive investigation of the survival signals induced by proteasome inhibition may offer further insight into the poor responses of solid malignancies.

The dysregulation of a variety of pathways, such as NF-κB, epidermal growth factor receptor (EGFR),

Table 3 Phase I disease specific combination studies of bortezomib

Patient population	Advanced NSCLC	Advanced ovarian tumors	Advanced head and neck cancer	Advanced colorectal cancer
Number of patients	16	15	17	13
Schedule	Starting dose of 1.0 mg/m² iv on days 1, 4, 8, 11 + gemcitabine 800 mg/m² on days 1, 8 + CBDCA AUC 5 on day 1 q3 weeks	Starting dose of 0.75 mg/m² iv on days 1, 4, 8, 11 + CBDCA AUC 5 on day 1 q3 weeks	Starting dose of 0.7 mg/m² iv on days 1, 4, 8, 11 + CDDP 30 mg/m² on day 1 q3 weeks	Starting dose of 1.03 mg/m² iv on days 1, 8, 15 + FOLFOX-4 q2 weeks
Best response obtained	4 PR 5 SD	2 CR 5 PR	NR	5 PR 5 SD
Toxicity observed	Myelosuppression	Diarrhea	Thrombocytopenia	Myelosuppression and diarrhea

Abbreviations: NSLC, nonsmall-cell lung cancer; PR, partial response; SD, stable disease; NR, not reported; CBDCA, carboplatin; CDDP, cisplatin.

Table 4 Phase II single agent studies of bortezomib

Patient population	Metastatic melanoma	Recurrent/metastatic soft tissue sarcoma	Metastatic neuroendocrine tumors	Metastatic colorectal cancer	Advanced renal tumors	Metastatic breast cancer
Number of patients	27	25	16	19	60	24
Best response obtained	6 SD	1 PR	11 SD	3 SD	5 PR 14 SD	1 SD
Toxicity observed	Neurotoxicity, fatigue and thrombocytopenia	Neurotoxicity, myalgia and fatigue	Neurotoxicity, diarrea and vomiting	Neurotoxicity and myalgia	Neurotoxicity	Thrombocytopenia and fatigue

Abbreviations: PR, partial response; SD, stable disease.

and Ras/PI3K/Akt, is very common in solid tumors. It is known that bortezomib also interferes with the p44/42 mitogen-activated protein kinase (MAPK), a downstream effector of EGFR pathway that communicates proliferative signals, and induces accumulation of cyclin-dependent kinase inhibitors p21[Cip1] and p27[Kip1].[54] Signaling by the EGFR family occurs through several downstream pathways to promote cell proliferation and inhibit apoptosis,[55,56] suggesting that EGFR itself might be subjected to ubiquitination and subsequent proteolityc breakdown, and that this process might be affected by proteasome inhibition. On the basis of this assumption, a preclinical study was carried out to evaluate the effect of proteasome inhibitor on EGFR survival signaling in pancreatic cancer cells.[57] Intriguingly, the addition of EGFR inhibition only enhanced proteasome inhibitor effects in vivo but not in vitro, suggesting that some other paracrine response may be involved in the effects of EGFR inhibition in vivo. An and Rettig have described the importance of the sequence of drug administration when bortezomib is used in combination with an EGFR tyrosine kinase inhibitor in renal carcinoma cells.[58] They concluded that pre-treatment of renal carcinoma cells with the EGFR

tyrosine kinase inhibitor prior to bortezomib was cytotoxic, whereas an antagonist interaction resulted with the reverse schedule. The authors also postulated that the diminished AKT and NF-κB, inhibition observed when renal carcinoma cells were pretreated with bortezomib, might result from decreased degradation of signaling proteins that function to converge on the EGFR/PI3K/AKT/NF-κB pathway, although direct evidence to support or refute this hypothesis does not yet exist.

In summary, these findings show that proteasome inhibitor treatment activates several mitogenic signaling pathways that blunt the full potential of the apoptotic response to proteasome inhibitor treatment. As an alternative or addition to EGFR inhibition, selective inhibition of these downstream mitogenic signaling pathways may increase the apoptotic response to proteasome inhibition and further overcome the drug resistance mechanisms in solid tumors. These preclinical studies might identify new drug combinations to enter clinical trials.

Disclosures

The authors disclose no conflicts of interest.

Table 5 Phase II combination studies of bortezomib

Patient population	Metastatic colorectal cancer	Metastatic pancreatic carcinoma	Pretreated NSCLC
Number of patients	68	87	155
Schedule	Arm 1: 1.5 mg/m² on days 1, 4, 8, 11 q21 days Arm 2: 1.3 mg/m² on days 1, 4, 8, 11 + CPT 11 125 mg/m² on day 1 q21 days	Arm 1: 1.5 mg/m² on days 1, 4, 8, 11 q21 days Arm 2: 1.3 mg/m² on days 1, 4, 8, 11 + gemcitabine 1000 mg/m² on day 1 q21 days	Arm 1: 1.5 mg/m² on days 1, 4, 8, 11 q21 days Arm 2: 1.3 mg/m² on days 1,4,8,11 + docetaxel 75 mg/m² on day 1 q21
Response rates %	NA	10% RR	42% RR
Toxicity observed	Hematologic, neurotoxicity and fatigue	Abdominal pain, fatigue and thrombocytopenia	Neutropenia, neurotoxicity and fatigue

Abbreviations: RR, response rate; CPT, irinotecan.

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