Investigation of bendamustine HCL in a Phase 2 study in women with resistant ovarian cancer

Amanda F. Baker · Denise J. Roe · Cynthia Laughren ·
Janice L. Cohen · Heather M. Wright ·
Mary C. Clouser · Haiyan Cui · David S. Alberts ·
Setsuko K. Chambers

Received: 13 March 2012 / Accepted: 25 April 2012 / Published online: 13 May 2012
© Springer Science+Business Media, LLC 2012

Summary We investigated the safety and efficacy of 90 mg/m² bendamustine HCL, administered intravenously on days 1 and 2 every 28 days in 10 women with platinum and taxane resistant epithelial ovarian cancer. There were no objective tumor responses observed; 2 patients had stable disease. Plasma samples collected at pre-treatment and end of cycle one were analyzed for changes in circulating total cytokeratin 18 and caspase cleaved cytokeratin 18 as exploratory early biomarkers of bendamustine-induced tumor cell death. All patients had measureable levels of both total and cleaved caspase 3 cytokeratin 18, but no relationship with response was possible due to the lack of clinical benefit in treated patients. Due to the high incidence of adverse events and absence of objective responses, only ten patients were treated as predefined by the Simon Two-Stage Design in the protocol. Overall, the regimen was not well tolerated and was associated with fatigue and a greater number of gastrointestinal side effects as compared to previously reported experiences in different patient populations. However, our study subjects did experience less bone marrow suppression. The lack of tolerability could reflect the degree of tumor burden within the peritoneal cavity as well as the high number of prior regimens (median of 5) received by the patients participating in this study.

Keywords Bendamustine · Ovarian cancer · Cytokeratin 18 · Alkylating agent

Introduction

Ovarian cancer is the leading cause of gynecologic cancer-related deaths in the United States, with approximately 22,280 women expected to be diagnosed with ovarian cancer in 2012, and with 15,500 estimated deaths in the same year [1]. Platinum-based chemotherapy in combination with taxanes is the primary treatment for epithelial ovarian cancer [2]. Although a majority of women with advanced ovarian cancer will demonstrate an objective or subjective response to these drug combinations, the responses are generally of limited duration. Second-line chemotherapy for ovarian cancer has in general been a disappointment in the setting of platinum and taxane-resistant disease with the best single agents yielding approximately 20 % response rates [3]. Targeted therapeutic approaches using antiangiogenesis and poly ADP ribose polymerase (PARP) inhibitors offer new alternatives [4, 5]. However, there is still a pressing need to investigate new chemotherapeutic agents for the treatment of ovarian tumors, specifically for women with platinum-resistant disease.

Continued clinical testing of novel anti-neoplastic agents that are non-cross resistant with platinum is critical for the development of effective salvage and primary treatment regimens for ovarian cancer. Bendamustine HCL is a multifunctional alkylating agent developed in the early 1960s which shows only partial cross-resistance to other alkylating agents [6–8]. Due to its unique structure, it can also act as a purine analog. The mechanism of action of bendamustine HCL is still incompletely understood, but treatment is broadly associated with marked DNA damage. In myeloma cell lines, bendamustine HCL induces apoptosis via cleavage of caspase 3, and results in G2 cell cycle arrest [9]. In chronic lymphocytic and mantle cell lymphoma cell lines, bendamustine HCL has been shown to activate both the

A. F. Baker · D. J. Roe · C. Laughren · J. L. Cohen ·
H. M. Wright · M. C. Clouser · H. Cui · D. S. Alberts ·
S. K. Chambers (✉)
Arizona Cancer Center,
University of Arizona College of Medicine,
Tucson, Arizona
e-mail: schambers@azcc.arizona.edu

mitochondrial cell death pathway and caspase-dependent apoptosis. The generation of reactive oxygen species (ROS) were implicated in these studies as an important mediator influencing the type of death signaling activated, and bendamustine HCL was active in p53 deficient and mutated cell lines [10]. In addition, bendamustine HCL has been shown to inhibit mitotic checkpoints and to induce mitotic catastrophe, a necrotic form of cell death [6]. (For a recent comprehensive review of bendamustine HCL the reader is referred [11]). The ability for bendamustine HCL to work independent of p53 status and induce cell death via a non-apoptotic mechanism may contribute to its activity in the setting of cisplatin resistance.

Currently, bendamustine HCL is approved by the US Food and Drug Administration (FDA) for the treatment of chronic lymphocytic leukemia and rituximab-refractory indolent non-Hodgkin lymphoma. Clinical activity has also been reported in multiple other tumor types, but to our knowledge there are no former reports of bendamustine HCL activity in epithelial ovarian cancer. Bendamustine HCL has been reported to have cytotoxic activity against several ovarian cell lines in vitro, and to be cross-resistant to other alkylating agents including cisplatin [12]. Bendamustine has been clinically investigated in cisplatin-refractory germ cell cancer and was shown to have no clinical activity in this setting [13].

The current study was designed to investigate the safety and efficacy of bendamustine HCL in women with platinum and taxane resistant ovarian cancer. Unfortunately, in this heavily pre-treated ovarian cancer patient population, bendamustine treatment was associated with dose-limiting side effects and no clinically observed benefit.

Patients and methods

Patient selection

Patients with epithelial carcinoma of the ovary, fallopian tube or peritoneum were eligible for participation in this study if they had relapsed within 6 months of completing chemotherapy, or had a best response of increasing disease during any number of prior chemotherapy regimens with a platinum (either cisplatin or carboplatin) and a taxane (paclitaxel or docetaxel). These agents may have been administered concurrently or sequentially. Any number of additional regimens for recurrent disease was allowed, as long as the performance status was ≤ 1 as characterized by the Eastern Cooperative Oncology group (ECOG). Patients were required to have measurable or evaluable disease (i.e. elevated serum CA-125 marker). Prior radiation which encompassed no more than 25 % of the bone marrow was allowed. Debulking surgery for relapsed disease was allowed as long

as the patient had measurable or evaluable disease remaining after the surgery. The patient must have recovered from all side effects of surgery. Additional inclusion criteria were adequate liver function (serum bilirubin $\leq 2.0 \times$ the IULN, SGOT or SGPT $\leq 2.5 \times$ the IULN), adequate renal function (serum creatinine $\leq 1.5 \times$ the IULN), hemoglobin of ≥ 9 gm/dL, ANC ≥ 1000 , and platelets $\geq 100,000$.

Exclusion criteria included ovarian tumors of low malignant potential and mixed mesodermal tumors; unstable pre-existing major medical conditions; life-threatening complications of their malignancies; known severe and/or uncontrolled concurrent medical disease; evidence of uncontrollable nausea, presence of central nervous system or brain metastases; or known hypersensitivity to any component of bendamustine HCL. Pregnant or lactating women were excluded. Patients must not have received chemotherapy, biologic therapy or any other investigational drug within 28 days prior to registration, or have had a major surgery within 14 days.

All patients gave informed consent before study enrollment. The study was approved by the Institutional Review Board at the Arizona Cancer Center, University of Arizona, and was conducted in accordance with institutional and federal guidelines.

Study design and treatment

The study was a non-randomized, open-label, single-center phase 2 trial. The study was approved by the Arizona Institutional Review Board for Human Subject Protection. Safety monitoring, oversight, and reporting was performed by the University of Arizona Cancer Center Data and Safety Monitoring Board. The primary objective of the study was to evaluate the response rates (confirmed, complete and partial), and response durations. Secondary end points were progression-free survival (PFS), toxicity evaluation, and correlative studies utilizing blood samples.

Initially, patients were treated with bendamustine HCL 90 mg/m^2 intravenously (IV) on days 1 (± 1 day) and 2 (± 1 day) every 28 days. If no grade ≥ 3 hematologic adverse event was experienced, the dose was escalated to 120 mg/m^2 on days 1 (± 1 day) and 2 (± 1 day) every 28 days at cycle 2. The first 3 patients who were dose escalated in this manner, found the 120 mg/m^2 dose to be intolerable. In fact, 2 of the 3 patients refused to receive day 2 of the 120 mg/m^2 dose. These patients mainly experienced grade 2 and 3 gastrointestinal toxicities despite supportive medications given to prevent and alleviate these toxicities. Because this was a heavily pre-treated ovarian cancer population, it was thought that these patients were highly susceptible to gastrointestinal side effects. Further, presentations consistent with ‘cytokine release’, consisting of fevers, rigors and chills, asthenia, muscle weakness/

cramping were observed. The only grade 3 hematologic toxicity among this first cohort was leukopenia in one patient.

Since the 90 mg/m² dose of bendamustine HCL was better tolerated in these patients, the study was amended to remove the 120 mg/m² dose and to allow for one level dose reduction to 60 mg/m² should grade ≥ 3 non-hematologic toxicities be experienced. No re-escalation was allowed once dose reduction was made.

Treatment was to be discontinued secondary to disease progression; clinically significant deterioration of the patient's condition; persistent (≤ 3 weeks) grade 3 adverse event(s); investigator determination that it was not safe or in the patient's best interest to continue participation; and all grade 4 events thought to be related to bendamustine HCL. No grade 4 events were observed.

Efficacy assessment

Baseline CA125 and computed tomography (CT) scans of the chest, abdomen, and pelvis were obtained, along with physical examination, and laboratory values. Patients were evaluated every cycle (defined as an interval of 28 days). Evaluation included adverse event assessment, physical examination, and laboratory values including CA125. CT scans were performed for disease assessment every 3 cycles, or earlier if needed. Blood was banked at baseline and prior to cycle 2 for correlative studies. CA125 response in evaluable patients ($N=9$) was analyzed using the modified Gynecologic Cancer Intergroup (GCIg) criteria (Rustin). Five patients were also evaluable for response by the Response Evaluation Criteria in Solid Tumors (RECIST 1.1) criteria. Toxicity was evaluated using the Common Terminology Criteria for Adverse Events 3.0. After the treatment was discontinued, all but one patient was followed for continuation of care.

Statistical analysis

PFS was defined as the time from the start of therapy to the time of first documentation of progression, or death due to any cause; PFS and overall survival (OS) were estimated using the Kaplan-Meier method. Statistical analysis was carried out using the SAS statistical package, version 9.2 (Cary, NC).

With a target response rate of 25 % (versus a null hypothesis rate of 5 %) and a Simon two-stage design (5 % alpha level with 80 % power), one response among 9 patients was required to continue to the second stage.

Plasma biomarker assessment (M30/M65)

Whole blood (10 ml in a sodium heparin coated collection tube) was collected at baseline and at the end of cycle 1. Samples were centrifuged at 150 x g for 10 min and

immediately frozen. Determination of caspase-3 generated cytokeratin 18 fragment and uncleaved cytokeratin in patient plasma (baseline, and 4 weeks post-treatment) was performed in duplicate using the M30-Apoptosense and M65 sandwich ELISA kits (all of the same lot number) obtained from PEVIVA AB (Bromma, Sweden). Assay was performed according to manufacturer's instructions.

Results

Patient characteristics

Ten of 13 patients who consented to the protocol were treated with bendamustine HCL. Three consented patients were not treated; two were found to be ineligible due to hospitalization for small bowel obstructions and the third withdrew consent. Baseline characteristics for the 10 patients are shown on Table 1. All were heavily pre-treated with a median of 5 prior regimens, which included one biologic regimen in 6 of the patients and 3 biologic regimens in one patient who had also received 5 prior chemotherapeutic regimens.

Treatment administration

After the experience with the first 3 patients, who were dose escalated after cycle 1 to 120 mg/m² bendamustine HCL, all subsequent patients received the 90 mg/m² dose only. There

Table 1 Patient and disease characteristics

Characteristic	Patients ($N=10$)
Median age, y (range)	58 (34–82)
ECOG performance status, n (%)	
0	9 (90.0)
1	1 (10.0)
Race/Ethnicity, n (%)	
White, Hispanic	5 (50.0)
White, Non-Hispanic	5 (50.0)
Primary site, n (%)	
Ovarian	9 (90.0)
Peritoneal	1 (10.0)
Cell type, n (%)	
Serous	6 (60.0)
Endometrioid	3 (30.0)
Clear cell	1 (10.0)
Tumor grade, n (%)	
2	2 (20.0)
3	8 (80.0)
Median number of prior regimens, (range)	5 (3–10)

Abbreviation: ECOG, Eastern Cooperative Oncology Group

were no further dose reductions necessary. Two patients who experienced grade 3 leukopenia were supported by granulocyte colony stimulating factor. The 10 patients completed a median of 3 cycles of treatment (range 1–7). One patient unfortunately withdrew consent after 2 cycles secondary to family pressure, and therefore no post treatment CA125 or CT scans were obtained and response evaluation was not possible, although we were able to obtain follow-up survival data.

Toxicity

There was no grade 4 toxicity (Table 2). The most common grade 3 toxicity was fatigue, affecting 30 % of the patients. Grade 2 or 3 gastrointestinal toxicities were common, and included nausea, vomiting, mucositis and dysgeusia. Grade 3 leukopenia or neutropenia was present in 30 % of patients. Two patients refused to continue the regimen due to toxicity. One patient (120 mg/m² dose) experienced grade 3 fatigue along with symptoms consistent with cytokine release syndrome and subsequently enrolled on a phase 1 trial prior to progression. The other patient (90 mg/m² dose) also experienced grade 3 fatigue as well as grade 3 hypokalemia, grade 2 body pain, and grade 2 nausea, diarrhea, and mucositis. She was subsequently treated with hexamethylmelamine prior to progression. In general, these patients did not feel well on this regimen, as compared to their other regimens, and had to be convinced to continue.

Efficacy

Nine patients were evaluable for response. There were no responses observed. Four patients progressed by CA125 criteria alone, and 2 patients progressed by RECIST criteria alone. One patient presented with partial small bowel obstruction confirmed by CT scan and was found to have significant progression of disease at the time of surgery. Of the 2 patients who refused to continue treatment because of toxicity, one had stable disease by both CA125 and RECIST criteria for 2.5 months, and the other had stable disease by CA125 criteria for 1 month and was unevaluable by RECIST 1.1.

With a median follow-up of 12.9 months, the median PFS was 4.6 months and median OS was 13.7 months (Fig. 1). The PFS at 4-months was 60 % and at 6-months, 20 %.

Plasma biomarker data (M30/M65)

Total cytokeratin 18 levels reflect non-apoptotic cell death, and caspase-3 cleaved cytokeratin 18 reflect apoptotic cell death. Both total (M65) and caspase-3 cleaved cytokeratin 18 (M30) were measured in plasma samples collected pre-treatment and at the end of cycle 1. No significant increase

Table 2 Treatment-related toxicity for 90–120 mg/m² dose levels by grade (N=10)

Adverse event	Patients With Event, n (%)			
	Grade 1	Grade 2	Grade 3	Grade 4
<i>Constitutional</i>				
Fatigue	0 (0)	1 (10.0)	3 (30.0)	0 (0)
Fever	1 (10.0)	1 (10.0)	1 (10.0)	0 (0)
Rigors/Chill	0 (0)	2 (20.0)	0 (0)	0 (0)
Weight loss	1 (10.0)	0 (0)	0 (0)	0 (0)
Hypotension	1 (10.0)	0 (0)	0 (0)	0 (0)
<i>Dermatologic</i>				
Pruritus	1 (10.0)	0 (0)	0 (0)	0 (0)
Other	1 (10.0)	0 (0)	0 (0)	0 (0)
<i>Gastrointestinal</i>				
Anorexia	1 (10.0)	1 (10.0)	0 (0)	0 (0)
Constipation	2 (20.0)	0 (0)	0 (0)	0 (0)
Dehydration	0 (0)	1 (10.0)	0 (0)	0 (0)
Diarrhea	0 (0)	1 (10.0)	0 (0)	0 (0)
Distention	0 (0)	1 (10.0)	0 (0)	0 (0)
Dysgeusia	1 (10.0)	2 (20.0)	0 (0)	0 (0)
Nausea	1 (10.0)	3 (30.0)	2 (20.0)	0 (0)
Oral mucositis	0 (0)	2 (20.0)	0 (0)	0 (0)
Vomiting	1 (10.0)	3 (30.0)	1 (10.0)	0 (0)
Other—GI	2 (20.0)	0 (0)	0 (0)	0 (0)
<i>Hematologic</i>				
Leukocytes	0 (0)	4 (40.0)	2 (20.0)	0 (0)
Neutrophils	2 (20.0)	2 (20.0)	1 (10.0)	0 (0)
Hemoglobin	1 (10.0)	4 (40.0)	0 (0)	0 (0)
Platelets	5 (50.0)	0 (0)	0 (0)	0 (0)
<i>Infection</i>				
Cellulitis	0 (0)	1 (10.0)	0 (0)	0 (0)
<i>Metabolic</i>				
Creatinine	0 (0)	1 (10.0)	0 (0)	0 (0)
Hyperbilirubinemia	1 (10.0)	0 (0)	0 (0)	0 (0)
Hypokalemia	0 (0)	0 (0)	1 (10.0)	0 (0)
Hyponatremia	1 (10.0)	0 (0)	0 (0)	0 (0)
<i>Musculoskeletal</i>				
Muscle weakness/cramping	0 (0)	2 (20.0)	0 (0)	0 (0)
<i>Neurologic</i>				
Dizziness	1 (10.0)	1 (10.0)	0 (0)	0 (0)
Sensory neuropathy	1 (10.0)	1 (10.0)	0 (0)	0 (0)
<i>Pain</i>				
Abdominal, chest, back pain	0 (0)	3 (30.0)	0 (0)	0 (0)
Headache	1 (10.0)	0 (0)	0 (0)	0 (0)
<i>Pulmonary</i>				
Cough/Dyspnea	2 (20.0)	0 (0)	0 (0)	0 (0)

in either biomarker post-bendamustine treatment was observed in the seven paired samples analyzed (Fig. 2).

Fig. 1 Progression free (a; PFS, $N=10$, median PFS=4.6 months) and Overall (b; OS, $N=10$, median OS=13.7 months) survival for all evaluable patients. Censored observations are designated with a "+" at the time of censoring

Discussion

This phase 2 study explored the efficacy and safety of bendamustine HCL in patients with resistant epithelial

ovarian cancer. The mean progression free survival (4.6 months) and overall survival rates (13.7 months) were typical of a heavily pretreated ovarian cancer population [14]. No objective responses were observed in the first nine evaluable patients, therefore the study was discontinued.

The rate of GI toxicity and fatigue in this patient population was higher and the rate of bone marrow related side effects less than expected based on what has been previously reported for patients with hematological malignancies treated with bendamustine HCL [15]. Due to the high incidence of GI side effects at 120 mg/m², a dose of 90 mg/m² was established for this patient population. For the treatment of NHL, the recommended monotherapy dose of bendamustine HCL is 120 mg/m² on days 1 and 2 of a 28 day cycle. However, in combination with rituximab a dose of 90 mg/m² is used in patients with NHL [15]. Because we did not perform pharmacokinetic measurements, we were not able to investigate dose–response relationships. It is possible that the need to use a lower dose (90 mg/m² vs 120 mg/m²) of bendamustine HCL may have impacted the clinical benefit of this regimen. A pharmacokinetic profile study in indolent non-Hodgkin's lymphoma looking at exposure–response relationships for efficacy and safety was conducted with a bendamustine HCL dose of 120 mg/m² on days 1 and 2 of a 21 day cycle (a higher dose than our current dose of 90 mg/m²) [16]. Correlations between bendamustine HCL exposure and responder occurrence of neutropenia, thrombocytopenia, fatigue, nausea and vomiting were examined. The study found no correlation between exposure and safety or efficacy measures, likely because of the limited range of exposures after 120 mg/m² administration. The study, however, did find that the $C_{(max)}$ was a statistical significant predictor of the nausea, which is a known and expected toxicity of bendamustine HCL.

Fig. 2 Measurement of secreted caspase-3 cleaved (M30) and total cytokeratin 18 (M65) in plasma from patients at baseline (day 0) and at the end of cycle 1 of bendamustine HCL therapy (C2D1)

Based on the mechanism of action of bendamustine HCL, which includes alkylation of DNA, it is possible that tumors with DNA homologous recombination deficiencies, including mutations in BRCA1 and BRCA2 may have greater benefit from this cytotoxic agent. Interestingly, a recent integrated genomic analyses study suggests that homologous recombination is defective in almost half of serous ovarian cancers [17]. Due to the inability to collect fresh tumor tissue at time of study, which would reflect the current molecular profile as compared to tumor tissue obtained at time of diagnosis, we did not profile patient tumors to assess their DNA repair capabilities. We did explore the expression of circulating cytokeratin 18 and caspase 3 cleaved cytokeratin 18 as biomarkers of epithelial (tumor) cell death. This assay has been formerly validated using plasma from ovarian cancer patients receiving carboplatin, and was found to be a reliable pharmacodynamic biomarker assay to monitor drug effects [18]. Both proteins were measurable in the seven paired samples analyzed, however no relationship with response to therapy was observed. In patients with breast cancer, caspase 3 cleaved cytokeratin 18 levels have been reported to show a greater increase in clinical responders to anthracycline based neoadjuvant chemotherapy as opposed to nonresponders when measured 24 h following treatment [19]. In our study we compared cytokeratin levels at baseline vs end of the first treatment cycle (28 days post-first treatment). The inclusion of an earlier time point would have allowed for the examination of any early effects of bendamustine HCL on tumor cell death. Our data does support the feasibility of future studies investigating the use of these biomarkers to monitor response to therapy in ovarian cancer patients. The levels of cytokeratin proteins have recently been shown to predict progression free survival in gastric cancer [20]. Similar studies in ovarian cancer should be considered.

This is the first study to report on the safety, tolerability, and efficacy of bendamustine HCL in patients with ovarian cancer. The lack of tolerability and efficacy observed in heavily pretreated advanced ovarian cancer patients is disappointing. However, it is conceivable that bendamustine HCL may be better tolerated in ovarian cancer patients having received fewer prior treatments.

Ethical standards

The studies reported on in this manuscript were conducted according to the current laws governing human research within the State of Arizona and the United States of America.

Acknowledgements Partial funding for this study was provided by an investigator initiated grant from Teva, Frazer, Pennsylvania. Additional support was provided by P50 CA95060 from the National

Cancer Institute including contributions from the Clinical Research and Biometry shared services of the Arizona Cancer Center, Tucson, AZ. The contents of this manuscript are solely the responsibility of the authors and do not necessarily represent the official views of Teva or the National Cancer Institute. We would like to thank Erin A. Maine, B.S. technical assistance.

Conflicts of Interest None of the authors have any conflicts to disclose.

References

1. Siegel R, Naishadham D, Jemal A (2012) Cancer statistics, 2012. *CA Cancer J Clin* 62:10–29
2. Han ES, Lin P, Wakabayashi M (2009) Current status on biologic therapies in the treatment of epithelial ovarian cancer. *Curr Treat Options Oncol* 10:54–66
3. Sehoul J, Stengel D, Oskay-Oezcelik G, Zeimet AG, Sommer H, Klare P, Stauch M, Paulenz A, Camara O, Keil E, Lichtenegger W (2008) Nonplatinum topotecan combinations versus topotecan alone for recurrent ovarian cancer: results of a phase III study of the North-Eastern German Society of Gynecological Oncology Ovarian Cancer Study Group. *J Clin Oncol* 26:3176–3182
4. Burger RA, Brady MF, Bookman MA, Fleming GF, Monk BJ, Huang H, Mannel RS, Homesley HD, Fowler J, Greer BE, Boente M, Birrer MJ, Liang SX (2011) Incorporation of bevacizumab in the primary treatment of ovarian cancer. *N Engl J Med* 365:2473–2483
5. Mantia-Smaldone GM, Edwards RP, Vlad AM (2011) Targeted treatment of recurrent platinum-resistant ovarian cancer: current and emerging therapies. *Cancer Manag Res* 3:25–38
6. Leoni LM, Bailey B, Reifert J, Bendall HH, Zeller RW, Corbeil J, Elliott G, Niemeyer CC (2008) Bendamustine (Treanda) displays a distinct pattern of cytotoxicity and unique mechanistic features compared with other alkylating agents. *Clin Cancer Res* 14:309–317
7. Gandhi V (2002) Metabolism and mechanisms of action of bendamustine: rationales for combination therapies. *Semin Oncol* 29:4–11
8. Teichert J, Baumann F, Chao Q, Franklin C, Bailey B, Hennig L, Caca K, Schoppmeyer K, Patzak U, Preiss R (2007) Characterization of two phase I metabolites of bendamustine in human liver microsomes and in cancer patients treated with bendamustine hydrochloride. *Cancer Chemother Pharmacol* 59:759–770
9. Gaul L, Mandl-Weber S, Baumann P, Emmerich B, Schmidmaier R (2008) Bendamustine induces G2 cell cycle arrest and apoptosis in myeloma cells: the role of ATM-Chk2-Cdc25A and ATM-p53-p21-pathways. *J Cancer Res Clin Oncol* 134:245–253
10. Roue G, Lopez-Guerra M, Milpied P, Perez-Galan P, Villamor N, Montserrat E, Campo E, Colomer D (2008) Bendamustine is effective in p53-deficient B-cell neoplasms and requires oxidative stress and caspase-independent signaling. *Clin Cancer Res* 14:6907–6915
11. Leoni LM (2011) Bendamustine: rescue of an effective antineoplastic agent from the mid-twentieth century. *Semin Hematol* 48(1):S4–S11
12. Balfour JA, Goa KL (2001) Bendamustine. *Drugs* 61:631–638
13. Kollmannsberger C, Gerl A, Schleucher N, Beyer J, Kuczyk M, Rick O, Casper J, Sosada M, Rie C, Kanz L, Bokemeyer C (2000) Phase II study of bendamustine in patients with relapsed or cisplatin-refractory germ cell cancer. *Anticancer Drugs* 11:535–539
14. Chambers SK, Clouser MC, Baker AF, Roe DJ, Cui H, Brewer MA, Hatch KD, Gordon MS, Janicek MF, Isaacs JD, Gordon AN, Nagle RB, Wright HM, Cohen JL, Alberts DS (2010) Overexpression of tumor vascular endothelial growth factor A may portend an increased likelihood of progression in a phase II trial of

- bevacizumab and erlotinib in resistant ovarian cancer. *Clin Cancer Res* 16:5320–5328
15. Tageja N (2011) Bendamustine: safety and efficacy in the management of indolent non-hodgkins lymphoma. *Clin Med Insights Oncol* 5:145–156
 16. Owen JS, Melhem M, Passarell JA, D'Andrea D, Darwish M, Kahl B (2010) Bendamustine pharmacokinetic profile and exposure-response relationships in patients with indolent non-Hodgkin's lymphoma. *Cancer Chemother Pharmacol* 66:1039–1049
 17. Integrated genomic analyses of ovarian carcinoma. *Nature* 474:609–615, 2011
 18. Cummings J, Ranson M, Lacasse E, Ganganagari JR, St-Jean M, Jayson G, Durkin J, Dive C (2006) Method validation and preliminary qualification of pharmacodynamic biomarkers employed to evaluate the clinical efficacy of an antisense compound (AEG35156) targeted to the X-linked inhibitor of apoptosis protein XIAP. *Br J Cancer* 95:42–48
 19. Demiray M, Ulukaya EE, Arslan M, Gokgoz S, Saraydaroglu O, Ercan I, Evrensel T, Manavoglu O (2006) Response to neoadjuvant chemotherapy in breast cancer could be predictable by measuring a novel serum apoptosis product, caspase-cleaved cytokeratin 18: a prospective pilot study. *Cancer Invest* 24:669–676
 20. Bilici A, Ustaalioglu BB, Ercan S, Orcun A, Seker M, Salepci T, Gumus M (2011) Is there any impact of plasma M30 and M65 levels on progression-free survival of patients with advanced gastric cancer? *Cancer Chemother Pharmacol* 68:309–316