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A Phase I trial of Bortezomib and Interferon Alfa-2B in Metastatic Melanoma

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Abstract

The possibility that cytokine administration could enhance the anti-tumor effects of proteasome inhibition was explored. It was found that co-administration of bortezomib and interferon- α (IFN- α) induced synergistic apoptosis in human melanoma cell lines and prolonged survival in a murine model of melanoma. A phase I study was conducted to determine the tolerability and the maximally tolerated dose (MTD) of bortezomib when administered in combination with interferon- α -2b to patients with metastatic melanoma. Patients were treated on a 5 week cycle. In week 1 of cycle 1 patients received 5 MU/m² IFN- α subcutaneously thrice weekly. During weeks 2–4 of cycle one bortezomib was administered intravenously weekly along with IFN- α thrice weekly. There was a treatment break during week 5. After cycle 1, bortezomib was administered in combination with IFN- α . Bortezomib was administered in escalating doses (1.0, 1.3, or 1.6 mg/m²) to cohorts of three patients. 16 patients were treated (8 female, 8 male; median age 59 years). Common grade 3 toxicities included fatigue (5), vomiting (3), and diarrhea (3). Grade 4 toxicities included fatigue (3) and lymphopenia (1). The MTD for bortezomib was 1.3 mg/m². One patient had a partial response and 7 had stable disease. Progression-free survival was 2.5 months and overall survival was 10.3 months. Bortezomib administration did not augment the ability of IFN- α to induce phosphorylation of STAT1 in circulating immune cells; however, it did lead to reduced plasma levels of pro-angiogenic cytokines. The combination of bortezomib and IFN- α can be safely administered to melanoma patients.

Keywords

Bortezomib; Melanoma; Interferon; Phase I

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INTRODUCTION

The incidence of melanoma is rising faster than any other cancer in the United States.¹ In 2012, it was estimated that there will be over 76,000 new cases of melanoma in the United States and nearly 10,000 deaths from the disease.² Traditional biological and chemotherapeutic regimens including dacarbazine, temozolomide, high-dose interleukin-2 (IL-2), and paclitaxel with or without cisplatin or carboplatin have demonstrated only modest response rates (<20%).^{3,4} Recently, novel therapies including ipilimumab (a monoclonal antibody directed against cytotoxic T lymphocyte antigen-4) and vemurafenib (a BRAF inhibitor) have received FDA-approval for the treatment of metastatic melanoma. However, both agents possess limitations. Phase III trials involving ipilimumab revealed a potential for serious autoimmune toxicity, with immune-related events occurring in 60% of patients. Moreover, the overall response rate remains less than 20%.⁵ Vemurafenib has high clinical response rates (40–50%), but its use is limited to patients with tumors expressing a V600 mutated BRAF gene. In addition, the median duration of response is only 5–6 months.⁶ These regimens highlight the need for new therapies with improved toxicity profiles. There is a need for therapies in BRAF negative populations or BRAF refractory tumors.

The ubiquitin-proteasome signaling pathway (UPS) is important for the ordered degradation of transcription factors, cyclins, and cyclin dependent kinase inhibitors required for cell cycle progression.⁷ Dysregulation in the UPS pathway is linked to the pathogenesis of various human diseases and therefore targeting components of the UPS represents a novel therapeutic treatment strategy in cancer. Proteasome inhibition results in the stabilization and accumulation of cell regulatory proteins, cell cycle disruption, activation of apoptotic pathways, and, ultimately, cell death.^{8,9} Bortezomib is a reversible inhibitor of the 26S proteasome. Cells treated with bortezomib accumulate in the G2-M cycle and some undergo apoptosis.^{10,11}

Bortezomib was shown to be safe in phase I studies for advanced solid malignancies with the maximum tolerated dose (MTD) in the original phase I trial being 1.56 mg/m² twice weekly on a 14 day cycle.^{12,13} Markovic *et al.* performed the first phase II study evaluating single-agent bortezomib for the treatment of metastatic malignant melanoma. Bortezomib (1.5 mg/m²) was administered by i.v. bolus twice weekly for 2 out of every 3 weeks. However, the study was closed at the time of the interim analysis due to insufficient clinical efficacy. Of the twenty-seven patients accrued to the study, 22% achieved stable disease (SD) at the 18 week time point. Bortezomib was generally well tolerated in this patient population. The median time to disease progression was 1.5 months with a median overall survival (OS) of 14.5 months. It was determined that single-agent bortezomib had minimal activity in malignant melanoma.¹⁴

To date, a great deal of effort has been expended in identifying the optimal manner in which to give targeted agents with cytotoxic chemotherapy. The possibility that immune-modulatory agents could enhance the effects of these drugs was explored. While the mechanism of apoptotic resistance in melanomas is not completely understood, a role for Bcl-2, Mcl-1 and Fas has been described.⁷ IFN- α has been shown to induce apoptosis in

some cell types and is able to sensitize others to apoptosis.¹⁵ Our group has shown that bortezomib and IFN- α act synergistically to induce apoptosis in melanoma cell lines by activation of caspase 8 through the association of Fas and the Fas-Associated protein with Death Domain (FADD). The combination of these agents was even effective at inducing apoptosis in cells that over-expressed the pro-survival proteins Bcl-2 and Mcl-1. Combination treatment also led to increased survival and inhibited tumor growth in a murine tumor model of human melanoma.⁷ Furthermore, it was shown that bortezomib enhanced the direct cytotoxic effect of IFN- α on melanoma cells through the induction of IFN- α response genes and increased phosphorylation of STAT1.¹⁶ IFN- α is used for the adjuvant therapy of melanoma patients who have undergone complete excision of their tumor but are at high-risk for recurrence. Side effects usually consist of flu like symptoms such as fever, fatigue, nausea, vomiting and myalgias. The dose chosen for this clinical trial was 5 million unit/m² instead of the 10 million unit/m² standard subcutaneous dose used in the adjuvant setting because of prior work by our group showing equal potency of the two doses of interferon.^{17,18}

A phase I trial of the combination of bortezomib and IFN- α was conducted to determine the safety, tolerability and dose-limiting toxicity (DLT) of these agents in patients with metastatic melanoma. The effect of bortezomib on the ability of IFN- α ability to phosphorylate STAT1 in patient PBMCs was evaluated as were levels of circulating inflammatory cytokines.

PATIENTS AND METHODS

Eligibility Criteria

A Millennium Inc. supported phase I trial of bortezomib and interferon-alpha-2b (IFN- α) was conducted at The Ohio State University Comprehensive Cancer Center under IRB approval. Millennium Inc. provided bortezomib and some support for conduct of the trial. Interferon (INTRON A) was obtained from a commercial supply. The correlative work was supported by an NCI R21 funding mechanism (to WEC) and a U01 mechanism. The protocol was registered with [ClinicalTrials.gov](https://clinicaltrials.gov) and was compliant with ICH-GCP. All patients were provided written informed consent. Eligible patients had histologically or cytologically confirmed malignant melanoma, evidence of measurable metastatic disease and met the following criteria: ECOG status ≤ 2 , normal organ function, and ability to provide informed consent. Patients were permitted an unrestricted number of prior chemotherapy regimens as long as they had recovered from the reversible side effects of the prior regimen. Prior adjuvant IFN- α was allowed if >6 months had passed since the last dose. Patients with brain metastases were eligible for the study, but must have received definitive therapy and be stable both clinically and by repeat head CT scan or MRI four weeks following definitive therapy. Patients without a history of brain metastases were required to undergo a CT scan or MRI of the brain prior to enrollment. Patients with significant brain metastases, a central nervous system disorder, or grade ≥ 2 peripheral neuropathy were excluded from participation in the study.

Study Design: Treatment Regimen and Toxicity Assessment

The primary objective of the study was to determine the safety tolerability and DLT of bortezomib when administered in combination with IFN- α -2b to patients with metastatic melanoma. The secondary objectives of this study were to document any objective anti-tumor responses that may occur in response to this treatment regimen, determine the time to tumor progression in patients receiving the regimen and measure plasma levels of bFGF and VEGF and other factors. Lastly, the protocol specified to monitor the effects of proteasome inhibition on the biological activity of IFN- α within immune cells by measuring Jak-STAT signal transduction in patient PBMCs.

Bortezomib was administered intravenously according to the schedule reported previously where the MTD of bortezomib was 1.6 mg/m²/dose on a weekly dosing regimen.¹⁹ Treatment was administered on a 5 week cycle using a standard 3*3 design (Supplementary Figure 1). During the first week of the first cycle, patients received IFN- α 5 MU/m² subcutaneously on days 1, 3, and 5 in order to identify interferon specific side effects. During the first cycle, bortezomib was administered at a dose of 1.0, 1.3, or 1.6 mg/m² intravenously on day 1 of weeks 2–4 in combination with IFN- α on days 1, 3 and 5. During week 5 of the first cycle the patients received a one week treatment break. During all subsequent cycles, bortezomib was administered at a dose of 1.0, 1.3, or 1.6 mg/m² intravenously on day 1 of weeks 1–4 in combination with IFN- α on days 1, 3 and 5 of weeks 1–4. Patients received a one week treatment break during week 5. This 5 week cycle was repeated for a total of 6 months. The maximum possible dose of bortezomib for this study was chosen as 1.6 mg/m² based on the MTD determined in phase I studies.^{12,13,19} While the MTD of bortezomib in combination with temozolamide was shown to be 1.3 mg/m², it was hypothesized that the MTD in combination with IFN might be higher due to the fact that the intermediate dose IFN is relatively well tolerated. Toxicity was assessed using the NCI Common Toxicity Criteria version 3.0. Patients with bortezomib-related grade 4 hematological toxicities or grade 3 non-hematologic toxicities (except neuropathies) had treatment held for 2 and 3 weeks, respectively. If the toxicity resolved to grade 1, bortezomib was resumed at a 25% reduced dose. Patients experiencing peripheral sensory neuropathy had their dose adjusted or held based on the NCI CTC Grade. Patients experiencing a grade 3 non-hematologic IFN- α related toxicity had treatment held for 2 weeks. Subsequently, the IFN- α was resumed at a reduced dose (3 MU/m² s.c). Patients who experienced non-hematological grade 4 toxicities or grade 3 toxicities that recurred following dose reduction were removed from therapy and were considered to have experienced a DLT.

Assessment of Disease Response

Patients were staged after cycle 1 (5 weeks) and every 2 cycles (10 weeks) thereafter by CT scan. Patients with PD were removed from trial therapy. Patients exhibiting a clinical response or SD by RECIST criteria were continued on the regimen until disease progression.

Flow Cytometric Analysis of Phosphorylated STAT1

PBMCs were isolated from patient blood via centrifugation with Ficoll-Paque Plus (Amersham Pharmacia Biotech). The phosphorylated (p) form of STAT1 (Tyr⁷⁰¹) in

cryopreserved PBMC was measured by intracellular flow cytometry as previously described.^{20,21} Anti-pSTAT1 (Tyr⁷⁰¹) conjugated antibody and isotype control antibody were obtained from BD Biosciences Pharmingen (San Jose, CA).

Plasma Cytokine Analysis

Plasma was procured via centrifugation of peripheral blood samples of study patients and stored at -80° until analysis. For cytokine profiling, plasma samples were thawed at room temperature and then assayed in duplicate wells using the premixed Bio-Plex® Pro Human Cytokine Array (Bio-Rad) according to manufacturer's instructions. Normal donor plasma samples were purchased from Innovative Research, Inc (Novi, MI).

Statistical methods

Analysis was performed on the 16 patients recruited for this phase I study. Estimates of progression free survival (PFS) and overall survival (OS) were generated by the Kaplan-Meier method and compared via the log-rank test.^{22,23} PFS time was defined as the length of time from the date of enrollment until the date of disease progression or the end of follow up. OS was defined as the time from enrollment to the time of death or the end of follow up. Patients were considered censored for PFS if they had not progressed and censored for OS if they remained alive at the completion of follow up. Final follow up was completed as of 10/26/2010. Samples were analyzed for cytokine levels in plasma obtained prior to the administration of bortezomib and/or interferon and again 1 hour following the administration of IFN- α 2b. Analysis of the change in cytokine levels before and after therapy was made through regression modeling, accounting for correlation within subjects over time by estimating robust (sandwich) standard errors.^{24,25} Cytokine levels and IFN- α response measurements (phosphorylation of STAT1 as measured by flow cytometry) were transformed to the natural log scale for analysis as the values are heavily skewed. Results remain on the log scale. All presented p-values are two-sided and are unadjusted for multiple comparisons. Analyses were carried out in Stata (Version 10.1, Strata-Corp, College Station, TX).

RESULTS

Sixteen patients were accrued to the study (8 women, 8 men). Their median age was 58.5 years (range 34–82). All patients had metastatic disease at entry (Table 1). The majority of patients had M1c disease (n=10, 62%). Metastatic sites of disease included the following: lung (10), subcutaneous nodules (5), lymph nodes (9), soft tissue (5), brain (3), skin (5), viscera (5), and bone (2). The average time from excision of the primary to the diagnosis of metastasis was 5.9 yrs. All but four patients (n = 12, 75%) had received at least one prior medical therapy for metastatic disease. Six patients (38%) received one prior therapy; two patients (13%) had 4 prior therapies.

Dose Escalation

Five patients were accrued to the level I dose (1.0 mg/m²). Dose level I (1.0 mg/m²) was expanded to 5 patients despite the lack of DLT in order to gain experience with the drug combination. Since the combination of a targeted agent and an immune activator was novel

at the time this protocol was developed, the protocol provided the principle investigator with the ability to expand the first cohort in order to gain additional clinical experience with this regimen prior to escalating the dose of bortezomib. Six patients were accrued to the level II dose. There was one grade 4 toxicity of fatigue at the level II dose that was associated with grade 3 hypotension and confusion. Therefore the second dose level cohort was expanded to six patients. Five total patients were accrued to the level III dose (1.6 mg/m²). Accrual to dose level III was halted when two patients experienced a DLT (fatigue, lymphopenia). The level II dose (1.3 mg/m²) was therefore determined to be the maximum-tolerated dose (MTD).

Toxicities

Toxicities are listed in Table 2. Overall the regimen was well-tolerated. Common grade 3 toxicities included fatigue (n=5), vomiting (n=3) and diarrhea (n=3). Observed grade 4 toxicities were fatigue (n=3) and lymphopenia (n=1). Bortezomib-related neuropathy was limited to grade 1 and 2 sensory neuropathy in three patients. There was one grade 4 toxicity of fatigue in the second cohort that was classified as being possibly related to study drug. Notably, this patient died of disease progression within two weeks of the development of this symptom. Two patients experienced grade 4 fatigue in the level III dose cohort. In one patient the toxicity was felt to be unrelated to the study drug. The second patient with fatigue at this dose level had a past medical history of COPD and a 30-pack-year smoking history and developed grade 3 dyspnea associated with grade 4 fatigue that did not respond to a three week rest period. This adverse event was felt to be drug-related and was classified as a DLT. This event triggered the expansion of dose level III. The fifth patient on dose level III experienced a DLT of grade 4 lymphopenia. This led to the conclusion that dose level II (1.3 mg/m²) was the maximally tolerated dose of bortezomib when given in combination with interferon alpha-2B. The majority of the grade 3 and 4 toxicities were encountered by patients at dose level III. Four patients in the level three cohort had their treatment held or had their dose reduced as a result of toxicities.

Response to Therapy

Outcome data are listed in Table 3. Seven patients exhibited SD after one cycle of therapy. One patient who exhibited SD after 1 cycle of therapy received no further treatments or imaging scans and so the timing of disease progression is unknown. One patient had a partial response (PR) to therapy after 1 cycle of therapy. Overall, the median PFS was 2.5 months (95% CI: 1.4 – 3.7). PFS did not vary substantially by dose level (overall log rank p-value=0.22). The median OS was 10.3 months (95% CI: 5.5–12.8) (Figures 1A and B).

Effect of Bortezomib on the IFN- α response of PBMC

The effect of bortezomib on the host IFN- α response during the first cycle of therapy (week 1–4) was measured in 8 patients. Interferon signaling results in phosphorylation of STAT1 and activation of an anti-tumor immune response by human immune cells. The phosphorylation of STAT1 in PBMCs was determined by flow cytometry before and after treatment with IFN- α on day one of each week of the cycle. A statistically significant increase in phosphorylated STAT1 (pSTAT1) was identified after treatment with IFN- α regardless of whether bortezomib was being administered concurrently. In week 1 levels of

pSTAT1 (as measured by MFI) increased significantly following IFN- α administration (95% CI: (1.82, 5.0); $p < .001$) (Figure 2). A similar induction of p-STAT1 was also observed in weeks 2–4 (Supplementary Table 1). IFN- α therapy at this dose level resulted in increased levels of pSTAT1. However, bortezomib did not appear to enhance or inhibit the ability of IFN- α to pSTAT1 in PBMCs.

Effect of Bortezomib and IFN- α on Serum Cytokines

A panel of cytokines that were known to be modulated by IFN and/or bortezomib (PDGF, IL-1 β , IL-4, IL-6, IL-8, IL-9, IL-17, FGF, GCSF, IFN- α , IP-10, MCP-1 and VEGF) was evaluated using patient plasma obtained pre-therapy and one hour post-therapy with bortezomib and interferon alfa-2b during cycle one (Supplementary Tables 2 and 3). During cycle one, the effects of the treatment on circulating levels of cytokines was examined and several significant trends were observed for the entire patient group. Levels of pro-angiogenic cytokines such as VEGF and IL-8 were significantly higher at baseline in melanoma patients than in normal controls (Table 4, Figure 3). For this group of patients as a whole, there was no statistically significant difference in cytokine levels when comparing baseline values to end of study values. However, when comparing cytokine values that span the start of bortezomib infusions (start of week 2 vs. start of week 3) we find statistically significant reductions in levels of IP-10 and IFN-gamma and an increase in levels of MCP-1 (Table 5). An analysis of the cytokine levels in the patient who experienced a PR was instructive and revealed marked declines in levels of VEGF, IL-8 and IL-6 during week two of the initial cycle. Baseline levels of VEGF were 121.0 pg/mL. During week 2 of cycle 1 VEGF levels were 53.6 ± 2.5 pg/ml and 1 hour post treatment levels of VEGF decreased to 30.8 ± 0.4 pg/ml. Similar results were seen for IL-8 and IL-6 in this patient (Data not shown). There were no statistically significant trends in cytokine levels for patients that experienced SD in response to the treatment; however, there was a trend toward decreased levels of FGF and IL-17. Notably, an analysis of the patients with PD revealed that levels of FGF and IP-10 decreased significantly at one point during cycle 1. This is an unexpected finding that may not have clinical significance in this patient population.

DISCUSSION

Based on the results of preclinical data demonstrating synergistic effects between interferon and bortezomib, a phase I clinical trial evaluating combination therapy with bortezomib and IFN- α for the treatment of metastatic melanoma was conducted. The combination of bortezomib and IFN- α was generally well-tolerated with toxicities similar to those seen with bortezomib and/or IFN- α treatment alone. The grade 3 and 4 events encountered in this study were temporally associated with the bortezomib infusions and thus were ascribed to bortezomib. Grade 4 toxicities included fatigue and lymphopenia, which were observed in 4 of 16 patients (25%). The most common grade 3 toxicities included fatigue ($n=5$), vomiting ($n=3$) and diarrhea ($n=3$). Nearly all grade 3 and 4 toxicities occurred in patients who received the highest bortezomib dose (1.6 mg/m^2). Of the 16 patients accrued to the study, one patient (6.3%) experienced a PR and seven patients (43.8%) exhibited SD. Median PFS and OS were 2.5 months and 10.3 months, respectively.

Recombinant IFN- α has been used in the treatment of metastatic malignant melanoma and mediates the regression of metastatic disease in about 10% of patients. This cytokine remains the only FDA-approved agent for the adjuvant therapy of patients who have undergone complete excision of their tumor but are at high-risk for recurrence.^{17,26–29} IFN- α has direct anti-tumor effects as well as immune-stimulatory effects. The pro-apoptotic effects of IFN- α are generally weak. Our group has shown that bortezomib can synergize with IFN- α to induce apoptosis in melanoma cells and exhibits anti-tumor activity in vivo.⁷ This data suggested that bortezomib and IFN- α acted through the extrinsic pathway of apoptosis via FADD-induced caspase-8 activation to initiate melanoma tumor cell death. Additional data suggested that the combination might have enhanced the IFN-responsiveness of melanoma cells and their ability to phosphorylate STAT1 in response to IFN- α treatment.¹⁶ Bortezomib administration at the doses employed in the current trial did not affect the ability of IFN- α to induce phosphorylation of STAT1 in PBMCs obtained from treated patients. A distinct enhancement of STAT1 activation might be obtainable with higher doses of bortezomib or the use of an alternate preparation with a better pharmacodynamic and pharmacokinetic profile.

VEGF is thought to be directly related to the pathogenesis of melanoma as melanoma is a highly vascular tumor and increased VEGF levels in tumor or peripheral blood predict poorer outcomes. In a recent trial, bevacizumab therapy led to increased PFS only in melanoma patients with elevated LDH. Patients with increased LDH likely have a hypoxic tumor environment and tumor growth may be driven by a VEGF-dependent process.³⁰ Bortezomib therapy could possibly reverse the pro-angiogenic properties of VEGF and slow cancer progression by inhibiting the development of new blood vessels.³¹ Cytokine analysis of patient plasma samples suggested that the combination of bortezomib and interferon alfa-2b is potentially anti-angiogenic. In the current study, levels of the pro-angiogenic cytokines IL-8 and VEGF were significantly increased at baseline in patients with melanoma, and there was a decrease in the levels of IL-8 during week 2 of therapy in the patient group as a whole. Of note, in the one patient with a partial response, the levels of VEGF, IL-6 and IL-8 all decreased with treatment compared to their baseline values. The combination of IFN-alfa-2b and bortezomib also produced a small but significant decrease in the levels of the proangiogenic factor bFGF. However, levels of VEGF were not significantly different at the completion of bortezomib and interferon therapy compared to baseline across the entire patient cohort. Lack of decrease in VEGF may be secondary to other pathways stimulating production of VEGF in the presence of bortezomib or it could be a function of the fact that most patients had advanced disease and received prior therapy.

Bortezomib in combination with other melanoma therapeutic agents has been investigated. Preclinical data on the combination of temozolomide and bortezomib in a murine model of melanoma demonstrated an improvement in response to the combination that appeared to be mediated through the inhibition of NF κ B. In a phase I trial of the combination, 1 of 19 patients exhibited a PR that persisted for 8 months. The MTD or recommended phase II dose was determined to be 1.3 mg/m² bortezomib and 75 mg/m² temozolomide. Dose-limiting toxicities included neurotoxicity, fatigue, diarrhea, and rash. PFS was 2.1 mos and OS was 6.3 months.³² Correlative studies revealed inhibition of proteasome activity in PBMCs 1 hour after dosing with bortezomib, however, this did not correlate with circulating

chemokine levels or NF κ B activity. In a phase II trial of bortezomib, paclitaxel and carboplatin, 11% of patients exhibited a partial response (PR). Progression free survival (PFS) was 3.2 months (months) and OS was 7 months.³³ Thus, the bortezomib/chemotherapy combination has not been further pursued in the setting of melanoma.

Proteasome inhibition may be a good strategy to augment the direct anti-tumor effects of interferons or other cytokines produced by the innate immune system. In co-culture experiments utilizing natural killer cells and primary hepatoma cell lines, bortezomib therapy decreased IFN- γ production but did not alter natural killer cell cytotoxicity.³⁴ The rationale for the present clinical trial of interferon alfa-2b and bortezomib was that interferon synergized with bortezomib to increase apoptosis in melanoma cells. In the past few years it has been shown in vitro that bortezomib sensitizes cancer cells to NK-mediated cytotoxicity in multiple myeloma, lymphoma, renal cell carcinoma, and prostate cancer.^{34–39} Furthermore, bortezomib treatment can improve the cytotoxicity of adoptively infused NK cells.^{40–42} The fact that changes in cytokine levels were observed in the present study suggests that proteasome inhibition in combination with interferon treatment can modulate the host immune response.

Combined therapy with bortezomib and IFN- α represents a novel immune based treatment strategy for malignant melanoma and other solid tumors. The combination of bortezomib and IFN- α is generally well tolerated and can be safely administered to melanoma patients including those patients with treated CNS metastases. Unfortunately, tumor regression was only seen in a single patient. The fact that changes in cytokine levels were observed in the present study suggests that proteasome inhibition in combination with IFN treatment can possibly promote an immune response with anti-tumor effects in addition to its direct pro-apoptotic effects. Future trials may combine oral proteasome inhibitors with long acting IFN preparations to provide more sustained levels of the two treatments. One might consider the use of orally available proteasome inhibitor that provides superior systemic levels. This study also raises the possibility that other immune based therapies may benefit by being combined with bortezomib.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Kaplan-Meier Survival curves. (A) Progression free survival with 95% confidence intervals (---). (B) Overall survival with 95% confidence intervals (---).

Figure 2.

A) A representative scatter plot of PBMCs obtained from peripheral blood. B) A histogram measuring pSTAT1 from a representative patient before and after treatment with interferon.

Figure 3.

Levels of IL-8, IL-6, and VEGF in all patients. Patient plasma was collected prior to treatment and 1 hour after week 2 treatment with IFN- α -2B and bortezomib.

Table 1

Demographics

Number of patients	16
Sex (n)	
Male	8
Female	8
Age (years)	
Mean	58
Median	58.5
Range	38–82
Adjuvant Therapy (n)	
High-Dose IFN	3
Radiation	2
M Stage Tumor (n)	
M1a	2
M1b	4
M1c	10
Number of prior therapies for Metastatic disease (n)	
1	6
2	3
3	1
4	2
Median Time to 1st Distant Metastasis	5.9 years
ECOG Performance Status (n)	
0	4
1	10
2	1
Unknown	1

Table 2

Toxicities

Toxicity	Grade 1/2 (n)	Grade 3/4 (n)
Neurologic		
Neuropathy – sensory	4	1
Neuropathy – motor		1
Syncope		1
Dizziness	2	
Depression	2	1
Confusion		1
All other toxicities Grade 3	Grade 3 (n)	Grade 4 (n)
Cardiovascular/Pulmonary		
Hypotension	2	
Dyspnea	1	
Gastrointestinal		
Anorexia	1	
Diarrhea	3	
Dehydration	1	
Nausea/Vomiting	4	
Constitutional		
Fatigue	5	3
Fever/chills	1	
Laboratory Abnormalities		
Anemia	1	
Hyponatremia	1	
Hypokalemia	1	
Platelets	1	
Proteinuria		
Lymphopenia	1	1
AST/ALT		
Other		
Insomnia	1	
Pain	1	
Weakness	1	

Table 3

Outcome Data

Best Response to Therapy (n)	
PR	1
SD	7
PD	8
PFS (mo)	
Median (95% CI)	2.5 (1.38, 3.68)
Overall Survival (mos)	
Median (95% CI)	10.3 (5.49, 12.78)

Table 4

Levels of pro-angiogenic cytokines are increased in the plasma of melanoma patients. All analyses were conducted on the natural log scale. The median and the minimum and maximum are given above on the natural log scale. A total of 16 melanoma and 4 control patients were used for this analysis.

	Melanoma Patients	Normal Controls	Wilcoxon Rank-sum test p-value
VEGF	4.8 (.3.8–5.7)pg/mL	2.9 (2.3–3.5) pg/mL	.003
IL-6	2.1 (0.7–4.7) pg/mL	1.3 (0.6–2.2) pg/mL	.19
IL-8	3.5 (1.9–6.4) pg/mL	1.8 (1.1–3.0) pg/mL	.03

Table 5

Statistical trends in cytokines over the course of treatment with IFN- α and bortezomib. The above analyses present the changes in cytokines identified through regression modeling, accounting for correlation within individual patients over time. Note that each estimate is from a separate model for each cytokine and patient. 95% confidence intervals (CI) are given in parenthesis. All analyses were conducted on the natural log scale.

Cytokine	Time Period	Trend (CI)
<i>All Patients</i>		
bFGF	Week 3 (Pre vs 1 hour Post-infusion)	-0.36 (-0.72, -0.01) p = 0.05
IL8	Week 2 (Pre vs 1 hour Post-infusion)	-0.41 (-0.75, 0.06) p = 0.03
MCP-1	Week 3 (Pre vs 1 hour Post-infusion)	-0.27 (-0.51, -0.03) p = 0.03
MCP-1	Week 3 Pre vs Week 2 Pre	0.16 (0.01, 0.32) p = 0.04
IFN- γ	Week 3 (1 hour Post-infusion) vs Week 2 Pre	-0.21 (-0.41, -0.01) p = 0.05
IP10	Week 2 (1 hour Post-infusion) vs Week 3 (1 hour Post-infusion)	-0.22 (-0.37, -0.07) p = 0.01