Sequencing Ipilimumab Immunotherapy Before or After Chemotherapy (Nab-Paclitaxel and Bevacizumab) for the Treatment of BRAFwt (BRAF Wild-Type) Metastatic Malignant Melanoma

Results of a Study of Academic and Community Cancer Research United (ACCRU) RU261206I

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Objectives: With the introduction of novel immune therapeutics for the treatment of disseminated malignancies, we sought to evaluate whether deliberate sequencing of immunotherapy before/after conventional cytotoxic chemotherapy would have an impact on clinical outcomes in patients with previously treated metastatic melanoma. We sought to evaluate whether or not ipilimumab immunotherapy administered before or after cytotoxic chemotherapy (nab-paclitaxel+bevacizumab, AB) would impact clinical outcomes.

Methods: We conducted a randomized phase 2 clinical trial of patients with BRAF wild-type metastatic melanoma (up to 2 prior therapies) who received either: (A) AB followed by ipilimumab therapy at progression; or (B) ipilimumab followed by AB treatment at progression. The primary goal of the study was a comparison of AB versus ipilimumab progression-free survival, with secondary clinical and laboratory endpoints.

Results: This study did not reach full accrual due to concurrent Food and Drug Administration approval of anti-programmed cell death 1 agents. Nevertheless, the available data suggests a cumulative therapeutic advantage to the sequential use of ipilimumab followed by AB. Correlative laboratory data revealed a favorable effect on systemic immune homeostasis in patients receiving AB therapy, of potential interest in further investigations, especially in the context of chemotherapy/immunotherapy combinations.

Conclusion: Albeit limited in scope, our data suggest that cytotoxic therapy with nab-paclitaxel and bevacizumab appear to favorably alter systemic parameters of immune function of potential benefit in combination T-cell directed immune checkpoint inhibitor therapy.

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Recent developments in clinical cancer immunotherapy have decisively changed the landscape of modern cancer therapeutics. The century-old promise that therapeutic modulation of the patient's own immune system would help treat cancer, is finally coming to fruition. Clinical results from large randomized phase III trials using immune checkpoint inhibitors (ICIs) have laid the foundation of cancer immunotherapy as an equal arm of cancer therapy alongside surgery, radiation, and chemotherapy.

Even with the remarkable results in the treatment of patients with metastatic malignant melanoma, ICIs yield long-term benefits in ~20% of patients. Albeit a dramatic improvement over prior therapies, these outcomes suggest that the majority of these patients require further therapy. For those in whom BRAF-targeted therapy is not an option (BRAF wild-type melanoma), salvage treatments are limited to experimental options or palliative cytotoxic chemotherapy. Thus, for many patients, postimmunotherapy salvage treatments are critically needed.

Considering that the antitumor activity of modern ICIs is dependent on preexisting tumor-specific T cells, we sought to test the hypothesis that cytotoxic/antiangiogenic therapy–induced modulation of systemic immunity (and the tumor microenvironment) before versus after ICI treatments could differentially impact antitumor efficacy of ICI therapy. Taxanes have demonstrated favorable immune-modulating properties in preclinical and clinical settings,[1] as have vascular endothelial growth factor (VEGF) antagonists.[2] Thus, we designed a clinical study comparing the clinical efficacy of the first Food and Drug Administration (FDA) approved ICI, ipilimumab (IPI),[3] administered either before or after therapy with nab-paclitaxel/bevacizumab (AB) in patients with metastatic malignant melanoma (BRAFwt). We hypothesized that sequential administration of different, but, potentially complementary systemic treatments could positively impact overall clinical outcomes.

Herein, we present the clinical results and immunologic correlative studies of a randomized phase 2 clinical trial in patients with BRAFwt metastatic melanoma treated with either: (1) AB followed by IPI at time of progression; or (2) IPI

followed by AB at time of progression. At the time of study development, IPI was the only FDA approved ICI for the treatment of metastatic melanoma. Of note, due to the rapid development of anti-programmed cell death 1 (PD1) agents and the demonstration of their improved clinical efficacy over single-agent IPI during the time-frame of our study, we were unable to complete study accrual. Thus, our findings can only be viewed as hypothesis-generating.

MATERIALS AND METHODS

Eligibility and Enrollment

This study enrolled individuals 18 years of age or above with histologically or cytologic confirmation of surgically unresectable stage IV malignant melanoma who received at most 2 prior courses of systemic treatment for metastatic disease. Additional eligibility criteria included measurable disease by the RECIST criteria, Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 1, and adequate hematologic, renal and hepatic function. Exclusion criteria included: radiographic documentation of tumor invading major blood vessels; brain metastases found by magnetic resonance imaging or computed tomography; grades 2 to 4 peripheral sensory neuropathy; anticancer therapy (including immunotherapy) or investigational agents within 30 days of registration; prior treatment with agents disrupting VEGF activity or targeting VEGF receptor; IPI or taxane-based chemotherapy, other medical conditions including but not limited to: active infection requiring parenteral antibiotics; poorly controlled high blood pressure, history of hypertensive crisis, or hypertensive encephalopathy; liver disease; history of central nervous system disease, clinically significant stroke, or transient ischemic attack within 6 months of registration; New York Heart Association (NYHA) class II to IV congestive heart failure; myocardial infarction or unstable angina within 6 months of registration, ongoing antiplatelet treatment other than low dose aspirin; ongoing need for oral or parenteral anticoagulation; clinically significant peripheral vascular disease; deep vein thrombosis or pulmonary embolus within a year of registration; history of abdominal fistula, gastrointestinal perforation, or intra-abdominal abscess with 6 months of registration; active bleeding or pathologic condition that carries a high risk of bleeding; hemoptysis within 30 days of registration; know autoimmune disease; systemic corticosteroid use within 14 days of registration, another invasive malignancy within 5 years of enrollment, pregnancy, or breast feeding.

This study was approved by the Mayo Foundation Institutional Review Board and assurances were filed with the Department of Health and Human Services. Written informed consent was required for enrollment. The study was registered at Clinicaltrials.gov as NCT01879306.

Study Treatment

Patients were randomized to receive either a regimen of bevacizumab and nab-paclitaxel or IPI alone. Specifically, patients randomized to Arm A received 10 mg/kg of bevacizumab infused over 60 minutes followed by 150 mg/m^2 of nab-paclitaxel infused over 30 minutes on days 1 and 15 and only 150 mg/m^2 nab-paclitaxel infused over 30 minutes on day 8 of a 28-day cycle until disease progression. Treatment was omitted on day 15 if the patient developed grades 3 to 4 neutropenia or grades 2 to 4 thrombocytopenia. Treatment was discontinued if the patient developed any grade of colonic perforation, myocardial infarction, angina, cerebrovascular ischemia, transient ischemic attack, arterial thromboembolic event, wound dehiscence, adult respiratory distress syndrome, or bronchospasm; grades 2 to 4 bronchopulmonary hemorrhage; grades 3 to 4 bowel obstruction; grade 4 left ventricular systolic dysfunction or thromboembolic event or proteinuria; or any clinically significant grade 4 adverse event attributable to bevacizumab.

Patients randomized to Arm B received 3 mg/kg of IPI infused over 90 minutes on day 1 of a 21-day cycle for a maximum of 4 cycles. Treatment was discontinued if the patient developed grades 3 to 4 diarrhea, hypothyroidism, nervous system disorder, maculopapular rash, increase in aspartate aminotransferase, increase in alanine aminotransferase, increase in blood bilirubin, or pneumonitis or any grade of colonic perforation.

At the time of progression, patients were allowed to cross-over to the other treatment arm if they meet the eligibility criteria for study entry. Patients who discontinued protocol treatment for reasons other than progression were followed for disease progression and survival for a maximum of 5 years post-registration.

Tests and Procedures While on Protocol Treatment

Within 14 days of study registration, patients underwent a physical examination, assessment of performance status, blood chemistries, and metabolic panel, urinalysis for proteinuria, thyroid-stimulating hormone (TSH), toxicity assessments (using Common Terminology Criteria for Adverse Events [CTCAE], v. 4.0), and research blood draws for immunologic profiling. These evaluations were repeated before each cycle of treatment for both regimens and for those randomized to IPI every 8 weeks after completion of 4 cycles of treatment. Disease status was radiographically evaluated at registration, at the completion of cycle 3 (~week 12), and every other cycle until disease progression.

Statistical Considerations

Progression-free survival (PFS) time was defined as the time from randomization to the documentation of disease progression (using RECIST criteria) during the initial course of treatment and as the time from reregistration at cross-over to the documentation of disease progression. The distribution of PFS times was estimated using the Kaplan-Meier method where patients who discontinued protocol treatment for reasons other than disease progression were censored at the time of their last disease evaluation or when an non-protocol anticancer therapy was administered. The study was designed to assess whether there is a reduction in the hazard of progression of at least 40% with the combination of bevacizumab and nab-paclitaxel relative to IPI alone. With a sample size of 53 patients per arm enrolled over a 12-month period and followed for a minimum of 12 months after the completion of enrollment, a 1-sided $\alpha = 0.10$ log-rank test would have a 90% chance of detecting a 40% decrease in the hazard of progression in bevacizumab+nab-paclitaxel arm relative to the IPI alone arm, when the median PFS in the IPI is 3.0 months. Data lock occurred May 30, 2018.

RESULTS

Patient Characteristics

This study was closed to enroll on November 19, 2015, after having enrolled 24 patients (12 patients/arm) due to the FDA approval of new agents for this patient population. One patient randomized to IPI alone was found to be ineligible as TSH testing was not performed before registration. Table 1 summarizes the patient, disease, and symptom characteristics of all 24 of the patients enrolled.

Treatment Course

Arm A: Nab-Paclitaxel+Bevacizumab Course

The median number of treatment cycles among the 12 patients randomized to Arm A was 3 (range: 2 to 17; total: 62).

TABLE 1. Patient Characteristics at Registration

	n (%)	
	Arm A (N = 12)	Arm B (N = 12)
Age, median (range)	60 (37-77)	61 (38-81)
Male	8 (67.3)	7 (58.3)
Primary site		
Ocular	5 (41.7)	2 (16.7)
Skin	5 (41.7)	6 (50.0)
Rectum	1 (8.3)	0
Mucosal	0	2 (16.7)
Unknown	1 (8.3)	2 (16.7)
Sites of metastases		
Lung	7 (58.3)	8 (66.7)
Liver	4 (33.3)	4 (33.3)
Bone	3 (25.0)	1 (8.3)
Nodes	3 (25.0)	4 (33.3)
Subcutaneous	1 (8.3)	4 (33.3)
Prior RT	4 (33.3)	3 (25.0)
Prior systemic therapy	3 (25.0)	3 (25.0)
ALC		
Below LLN	3 (25.0)	1 (8.3)
Within NR	8 (66.7)	11 (91.7)
Unknown	1 (8.3)	0
LDH		
Below LLN	1 (8.3)	0
Within NR	10 (83.3)	7 (58.3)
Above ULN	1 (8.3)	5 (41.7)
TSH		
Within NR	12 (100)	9 (75.0)
Above ULN	0	3 (25.0)
AMC		
Within NR	11 (91.7)	9 (75.0)
Above ULN	0	3 (25.0)
Unknown	1 (8.3)	0
Grade 1 fatigue	2 (16.7)	3 (25.0)
Grade 1 neurosensory difficulties	2 (16.7)	0
Grade 1 anemia	1 (8.3)	3 (25.0)
Grade 1 arthralgia	1 (8.3)	1 (8.3)
Grade 1 nausea	0	1 (8.3)
Grade 1 abdominal pain	0	2 (16.7)
Grade 1 ALK	0	2 (16.7)
Grade 1 AST	0	1 (8.3)

ALC indicates absolute lymphocyte count; ALK, serum alkaline phosphatase; AMC, absolute monocyte count; AST, serum aspartate aminotransferase; LDH, lactate dehydrogenase; LLN, institutional lower limit of normal; NR, normal range; RT, radiation therapy; TSH, thyroid-stimulating hormone; ULN, institutional upper limit of normal.

The most common severe toxicities reported during the course of treatment included neutropenia (grade 3—5 patients; grade 4—2 patients) and leukopenia (grade 4—1 patient; grade 3—2 patients). All grades 2 to 4 toxicities reported are found in Table 2. Seven patients reduced their nab-paclitaxel dose at least once over the course of their treatment due to severe neutropenia (grade 3—3 patients; grade 4—2 patients), grade 3 hypertension (1 patient), or grade 3 fatigue (1 patient). All patients have discontinued nab-paclitaxel+bevacizumab treatment. The reasons treatment was discontinued included disease progression (8 patients), desire for alternative treatment (1 patient); and intolerability due to persistent neutropenia with memory impairment and peripheral sensory neuropathy (1 patient), rectal hemorrhaging (1 patient), or photo recall phenomenon (1 patient). There were 2 complete and 1 partial radiographic responses among these 12 patients. The median PFS time was 139 days (Fig. 1).

Arm A: Cross-Over to IPI

Of the 8 patients who discontinued nab-paclitaxel +bevacizumab due to progression, 4 did not cross-over to IPI due to refusal (1 patient) or their clinical condition was such that they no longer met eligibility criteria to cross-over (3 patients).

Only 1 of the 4 patients who crossed over to IPI completed all 4 cycles of treatment. The other 3 patients discontinued IPI due to disease progression (2 patients) or intolerability (1 patient). Specifically, 1 patient who developed a lesion in the pancreas while on nab-paclitaxel+ bevacizumab discontinued IPI after 1 cycle of treatment due progression to the brain; 1 patient who developed lesions in the lung and soft tissue while on nab-paclitaxel+ bevacizumab discontinued IPI after 2 cycles of treatment due grade 3 diarrhea with grade 2 fatigue and dehydration, and 1 patient who progressed while on nab-paclitaxel+bevacizumab due to increase in size of liver lesions discontinued IPI after 2 cycles of treatment due to continued increase in size of the lesions (Fig. 2).

Arm A: Survival

Two patients were alive at 40.5 and 42.2 months after registration. The remaining 10 patients died due to disease. The median survival time from registration was 1.5 years (Fig. 3).

Arm B: IPI Course

Of the 12 patients randomized to IPI alone, 6 (50.0%) patients completed all 4 cycles of IPI treatment. The reasons that 4 cycles of IPI were not completed included disease progression (3 patients), patient request to discontinue due to fatigue (1 patient), worsening macropapular rash (1 patient), and increasing ALK and AST (1 patient). The most common severe toxicities reported during the course of IPI treatment included acute kidney injury (grade 3—2 patients; grade 4—1 patient) and neutropenia (grade 3—3 patients). All grades 2 to 4 toxicities reported are found in Table 2. There was 1 partial radiographic response among these 12 patients. The median time to progression was 94 days (Fig. 1).

Arm B: Cross-Over to Nab-Paclitaxel+Bevacizumab Course

Six of the 12 patients who progressed during IPI or during the observation period following discontinuation of IPI did not cross-over to nab-paclitaxel+bevacizumab as they entered hospice care (1 patient), received radiation therapy (1 patient), refused (1 patient), or their clinical condition was such that they no longer met eligibility criteria to cross-over (3 patients).

A median of 7 cycles of nab-paclitaxel+bevacizumab (range: 1 to 22 cycles) were received among the remaining 6 patients. All of these patients discontinued nab-paclitaxel +bevacizumab therapy. The reasons included: grade 2 retinopathy (1 patient); grade 2 fatigue with peripheral sensory neuropathy (1 patient); grade 2 fatigue with grade 1 arthralgia, myalgia, dyspnea, limb edema, epistaxis, and dysgeusia (1 patient); grade 2 wound infection (1 patient), and disease progression (3 patients). There was 1 partial response and 2 patients remained stable for at least 6 months.

Arm B: Survival

Four patients were alive at 27.1, 38.9, 39.8, and 47.8 months after registration. The remaining 8 patients died due to disease. The median survival time from registration was 2.25 years (Fig. 3).

TABLE 2. Grades 2 to 5 Toxicities Reported (Before Cross-Over, if Applicable)

	Arm A (%)			Arm B (%)		
	Grade 2	Grade 3	Grade 4	Grade 2	Grade 3	Grade 4
Allergic reaction	8.3	—	—	—	—	—
Abdominal pain	—	—	—	25.0	—	—
Acute kidney injury	—	—	—	—	16.7	8.3
Alkaline phosphatase increase	—	—	—	8.3	8.3	—
Anemia	8.3	—	—	8.3	—	—
Anorexia	16.7	—	—	—	—	—
Anxiety	8.3	—	—	—	—	—
Arthralgia	25.0	—	—	—	—	—
Aspartate aminotransferase increase	—	—	—	8.3	8.3	—
Back pain	—	—	—		8.3	—
Blood bilirubin increase	—	—	—	—	8.3	—
Cataract	—	8.3	—	—	—	—
Catheter-related infection	—	—	—	8.3	—	—
Cough	—	—	—	16.7	—	—
Creatinine increase	8.3	—	—	—	—	—
Dehydration	8.3	—	—	—	—	—
Diarrhea	8.3	8.3	—	—	—	—
Dysgeusia	8.3	—	—	—	—	—
Dyspnea	—	8.3	—	—	—	—
Fatigue	41.7	8.3	—	16.7	—	—
Hemolytic uremic syndrome	—	8.3	—	—	—	—
Hypertension	25.0	16.7	—	8.3	8.3	—
Infection	—	—	—	8.3	—	—
Lymphocyte count decrease	—	—	—	—	—	8.3
Myalgia	8.3	—	—	8.3	—	—
Neutrophil count decrease	16.7	41.7	16.6	—	—	—
Nausea	—	—	—	8.3	—	—
Oral mucositis	8.3	—	—	—	—	—
Pain in extremity	—	—	—	8.3	—	—
Papulopustular rash	8.3	—	—	—	—	—
Peripheral sensory neuropathy	—	8.3	—	—	—	—
Proteinuria	8.3	—	—	—	—	—
Pruritus	—	—	—	8.3		
Rash maculopapular	—	—	—	8.3	8.3	—
Rectal hemorrhage	—	8.3	—	—	—	—
Sinusitis	8.3	—	—	—	—	—
Thromboembolic event	—	—	—	—	8.3	—
White blood cell count decrease	41.7	16.7	8.3	—	—	—

Correlative Studies

Immune Cell Phenotypes

Differences in the immune profile after 1 cycle of treatment between those receiving AB (Arm A) and those receiving IPI (Arm B) were examined. Each immune subset was examined in terms of the ratio of its postcycle 1 value to its pretreatment value. For $CD25^+FoxP3^+$; $CD14^+CD11c^-$; $CD14^+CD206^+$ and $DR^{lo}CD33^+CD11b^+$ this ratio tended to be lower among those receiving AB than those receiving IPI (Table 3). Of note, no significant change was observed when comparing the pre–post-treatment frequencies of major T-cell subsets ($CD3^+/CD4^+$ helper T cells, $CD3^+/CD8^+$ cytotoxic T cells or $CD3^+/CD69^+$ activated T cells) in either treatment arm.

Among the 10 patients who crossed over to the other treatment arm after disease progression, 5 patients (2 Arm A; 3 Arm B) had paired blood samples available for determination of immune cell subsets before the start and after 1 cycle of cross-over treatment. With the minimal available data the only observation we are able to make is that the regulatory T cells ($CD25^+/FoxP3^+$) appeared to be more profoundly depleted if AB was administered upfront, rather than after IPI (data not shown). The effect on myeloid cell subset was inconsistent among this small set of patients.

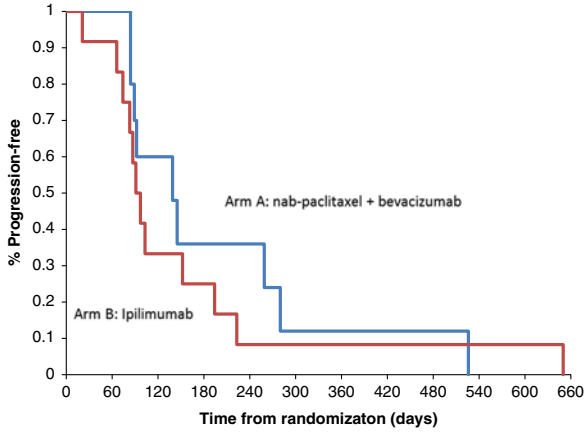

FIGURE 1. Progression-free survival.

DISCUSSION

The introduction of ICIs in cancer therapy has dramatically changed clinical outcomes in an increasing number of patients suffering from a wide range of malignant disorders.

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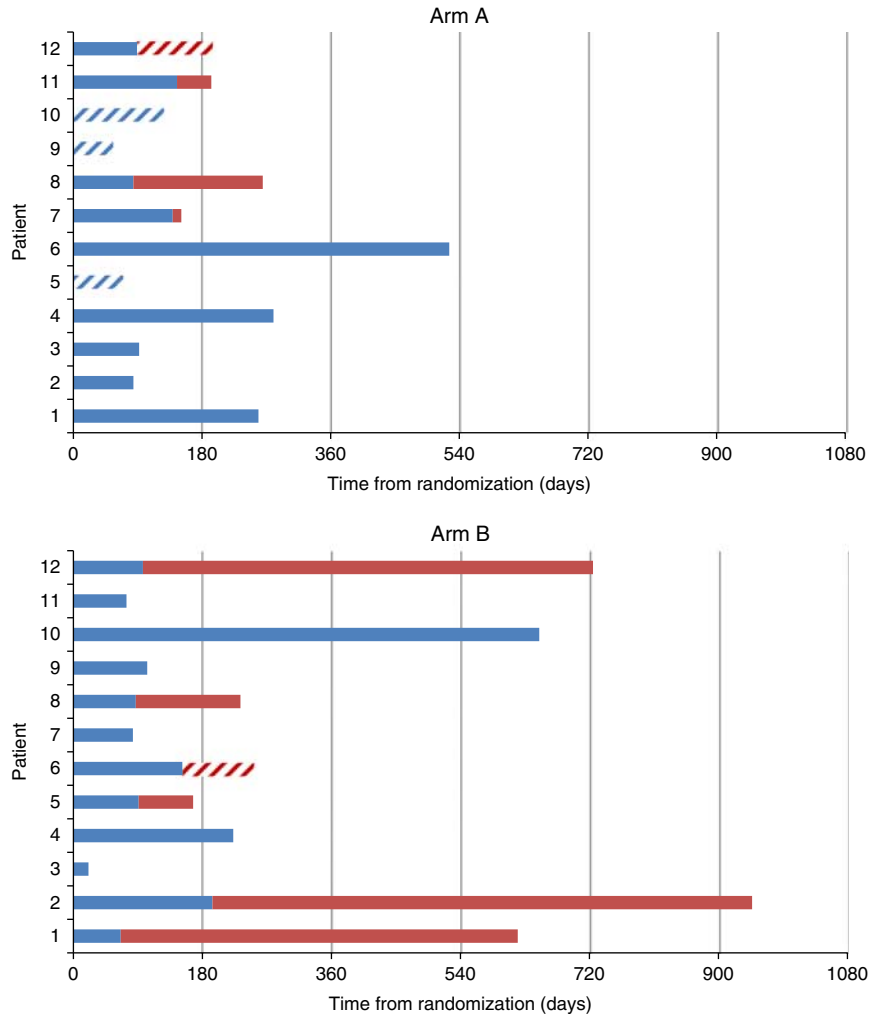

FIGURE 2. Progression-free survival by treatment phase. Blue: Progression-free survival during the initial treatment phase. Red: Progression-free survival following cross-over to second treatment phase. Dashed lines: Progression-free. Solid: Progressed.

Albeit very effective, for many of these patients (especially those with metastatic melanoma treated with IPI), overall survival benefits remain limited.[3] Soon after the FDA approval of IPI for the treatment of metastatic melanoma (2011), it was clear that even though a portion of patients had experienced long-term treatment success, the majority still required additional therapy for tumor progression. At that time, nonexperimental salvage therapy options for patients with metastatic melanoma failing IPI treatments primarily included conventional cytotoxic therapy (dacarbazine or taxane-based combinations). Anti-PD1 agents were not yet FDA approved, and for patients with BRAF V600e mutated melanoma (~35% of patients), targeted agents (vemurafenib) had just become available.[4]

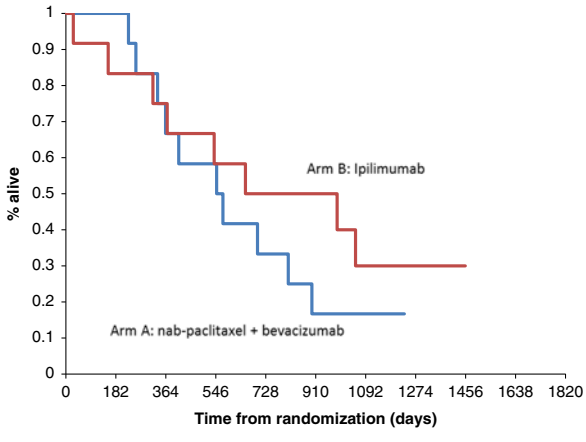

FIGURE 3. Overall survival.

Considering that the antitumor efficacy of ICIs (IPI as well as later developed PD1/programmed death-ligand 1 blocking agents) is primarily dependent on their ability to recover endogenous antitumor T-cell immunity, we postulated that the best IPI-associated cytotoxic therapy regimen would be one that would exert toxic injury to cancer cells, while preserving/favorably modulating the T-cell immune compartment that may have been beneficially altered (albeit insufficiently to control cancer) by prior IPI therapy. Taxanes are among the most studied conventional cytotoxic agents that exhibit a range of favorable immune-modulating effects in cancer (clinical and preclinical settings) primarily depleting tumor-associated myeloid-derived suppressor cells (MDSC), with minimal negative effects on effector cytotoxic T lymphocytes.[5] Taxanes,

TABLE 3. Peripheral Blood Immune Cell Profiles

	Median (IQR)		
Postcycle 1 Value/Pretreatment Value	**Arm A (n = 12)**	**Arm B (n = 10)**	**Wilcoxon Rank-Sum Test *P***
$CD3^+CD4^+$	1.02 (0.94-1.16)	1.03 (0.84-1.15)	0.843
$CD3^+CD8^+$	0.95 (0.72-1.12)	1.07 (0.97-1.27)	0.531
$CD3^+CD69^+$	1.16 (0.88-1.30)	1.03 (0.97-1.26)	0.717
$CD4^+CD294^+$	1.16 (0.80-1.58)	1.15 (0.94-1.74)	0.717
$CD4^+CD25^+FoxP3^+$	0.74 (0.43-1.25)	1.09 (1.05-1.73)	0.075
$CD14^+CD11c^-$	0.74 (0.48-1.30)	1.40 (1.15-2.0)	0.056
$CD14^+CD206^+$	0.97 (0.72-1.28)	1.42 (1.14-2.16)	0.065
$DR^{lo}CD33^+CD11b^+$	0.97 (0.68-1.24)	1.69 (0.76-2.19)	0.086

IQR indicates interquartile range.

especially nab-paclitaxel, have also demonstrated single-agent activity against metastatic melanoma,[6,7] and when combined with bevacizumab (anti-VEGF neutralizing antibody) the therapeutic benefit appeared to be improved (N0775).[8–10] In addition, VEGF antagonists themselves have demonstrated immune-modulating properties favorable to promoting antitumor immunity.[11] Thus, we reasoned, a salvage treatment regimen of nab-paclitaxel plus bevacizumab when administered to patients that were failing front line IPI therapy could potentially preserve any positive T-cell modulating effects of IPI (insufficient to control the malignancy on their own) while introducing cytotoxic injury to the tumor and tumor-associated immune suppressor cells (MDSC). Conversely, it was also possible that upfront therapy with a cytotoxic regimen may "debulk" the tumor mass, and dampen the negative immune regulatory effects of MDSC, potentially allowing greater clinical efficacy of IPI therapy. Thus, we designed a clinical trial where patients with metastatic melanoma (treatment-naive, or having failed 1 prior therapy regimen) were randomized to 1 of 2 treatment arms of sequential systemic therapies. In Arm A, patients underwent upfront chemotherapy with AB followed by IPI at time of progression; in Arm B IPI was administered first, followed by AB at time of progression. Concurrent administration of all 3 agents was considered but not pursued due to the risk of additive gastrointestinal toxicities of concurrent IPI and bevacizumab therapy. At the time, this study was the first sequential systemic therapy clinical trial in metastatic melanoma involving an immunotherapeutic agent. Although this study was developed in a timely fashion, within months of activation, a new generation of immune therapeutic agents (anti-PD1) demonstrating clinical efficacy superior to that of IPI became clinically available. As we were no longer able to justify single-agent IPI therapy in the face of superior anti-PD1 agents (pembrolizumab), our trial could not complete accrual. However, our limited data did suggest that sequential administration of IPI followed by AB ultimately translated into longer survival, and that the AB regimen did appear to favorably modulate certain aspects of systemic immune homeostasis, diminishing the frequencies of circulating immune suppressor cells ($CD4^+/CD25^+/FoxP3^+$ regulatory T cells), with little impact on total circulating $CD8^+$ counts (including tumor-specific cytotoxic T lymphocytes) (Table 3), suggesting an overall immunologically beneficial outcome with the use of immune-modulating chemotherapy. Specific to Arm A, we could not correlate the observed depletion of circulating regulatory T cells (postcycle 1) to any meaningful clinical benefit. The very small sample size of this subset of patients is a limitation to drawing more definitive conclusions. Another potential explanation of a relative lack of clinical benefit noted in Arm A would be the higher proportion of ocular melanoma patients in Arm A as compared with Arm B (41.7% vs. 16.7%). Ocular melanoma typically demonstrates a lack of response to cytotoxic chemotherapy or immune checkpoint inhibition. The observed decline in circulating regulatory T cells noted with the AB regimen is still an immunologically meaningful finding. Arm B did demonstrate an improved therapeutic effect in terms of longer survival, despite the relatively higher median posttreatment/pretreatment circulating regulatory T-cell ratio in this arm (Table 3). It can be hypothesized that the introduction of immune-modulating chemotherapy could have potentially "rescued" an antitumor immunologic response (previously generated by IPI), resulting in an improved clinical outcome as noted in Arm B. In contrast, upfront immune-modulating chemotherapy (AB, Arm A) could deplete immune-suppressive T cells, but would lack the antitumor effector T-cell response in the absence of IPI.

The concept of combining immune-modulating agents with conventional cytotoxic chemotherapeutics is not new. The most prominent example of this strategy in advanced melanoma was the combination of cisplatin, vinblastine, and dacarbazine (CVD) with interleukin-2 and interferon α.[12] Patients were treated with CVD chemotherapy (cisplatin 20 $mg/m^2/d\times4$, vinblastine 1.6 $mg/m^2/d\times5$, and dacarbazine 800 $mg/m^2\times1$), and biotherapy (interleukin-2: 1×10^6 $IU/m^2/d\times4$, and interferon: 5×10^6 $U/m^2/d\times5$). The CVD and biotherapy components were administered either as alternating regimens every 3 weeks, or immediately sequential (CVD/Bio or Bio/CVD). Although toxic, these regimens produced significant objective responses and ultimately led to the selection of CVD/Bio sequential administration as the most favorable (objective response rate 69%); subsequently confirmed in a larger follow-up phase 2 trial of 53 patients (objective response rate 64%).[13] This regimen was definitively tested in a randomized phase III clinical trial of 395 patients with metastatic melanoma, comparing CVD (n = 195) versus CVD/Bio (n = 200) [14] that failed to demonstrate a survival advantage (8.7 vs. 9.0 mo), despite a slight advantage in objective response rates (13.8% vs. 19.5%) and PFS (2.9 vs. 4.8 mo) favoring the CVD/Bio arm. From today's perspective, the unmet expectations of the CVD/Bio regimen could be attributed to the limited therapeutic efficacy of the biological agents of the time, as well as the profound treatment-related leukopenia as a surrogate of immune competence (≥ grade 3 in 78% of CVD/Bio patients). Recently, successful combination of an effective immunotherapeutic agent (anti-PD1, pembrolizumab) with conventional platinum-based chemotherapy for the treatment of patients with metastatic non–small cell lung cancer has yielded promising results.[15] Patients were treated with either pembrolizumab (200 mg/d) or placebo, combined with pemetrexed ($500\ mg/m^2/d$) and investigator

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choice of cisplatin (75 mg/m^2/d) or carboplatin (5 area under the curve/d), every 3 weeks. Platinum agents were administered for only 4 cycles, whereas pemetrexed and pembrolizumab were continued every 3 weeks (maintenance therapy). The results of the study demonstrated a 12-month survival rate at 69.2% for the pembrolizumab+chemotherapy arm, versus 49.4% for the chemotherapy arm alone, with median PFSs 8.8 versus 4.9 months, respectively. Thus, the combination of effective chemotherapy and immunotherapy resulted in superior clinical outcomes and a new standard of care. A similar effort in metastatic melanoma using a combination of paclitaxel, carboplatin, and pembrolizumab has also been reported.[16,17]

In summary, it is increasingly clear that combination treatments of modern ICI therapy with certain cytotoxic agents can yield superior clinical outcomes. At the time of our study design, we selected the first clinically available ICI (IPI) demonstrating clinical efficacy in metastatic melanoma and combined it with a cytotoxic/antiangiogenic treatment regimen that had demonstrated modest clinical benefit with potentially favorable immune-modulating properties. Albeit incomplete, our data illustrated favorable immune-modulatory properties of AB therapy (depletion of circulating regulatory T cells without any impact on total peripheral CD8 T cells) and a suggestion of therapeutic advantage of IPI followed by AB sequential therapy. Thus, further understanding of the immune-modulatory properties of conventional cytotoxic agents may uncover innovative application of existing drugs with established toxicity profiles that could further improve clinical benefits of modern ICI therapy.

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