Ipilimumab and Radiation in Patients with High-risk Resected or Regionally Advanced Melanoma

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ABSTRACT

Purpose: In this prospective trial, we sought to assess the feasibility of concurrent administration of ipilimumab and radiation as adjuvant, neoadjuvant, or definitive therapy in patients with regionally advanced melanoma.

Patients and Methods: Twenty-four patients in two cohorts were enrolled and received ipilimumab at 3 mg/kg every 3 weeks for four doses in conjunction with radiation; median dose was 4,000 cGy (interquartile range, 3,550–4,800 cGy). Patients in cohort 1 were treated adjuvantly; patients in cohort 2 were treated either neoadjuvantly or as definitive therapy.

Results: Adverse event profiles were consistent with those previously reported with checkpoint inhibition and radiation. For the neoadjuvant/definitive cohort, the objective response rate was 64% (80% confidence interval, 40%–83%), with 4 of 10 evaluable patients achieving a radiographic complete response. An additional 3 patients in this cohort had a partial response and went on to surgical resection. With 2 years of follow-up, the 6-, 12-, and 24-month relapse-free survival for the adjuvant cohort was 85%, 69%, and 62%, respectively. At 2 years, all patients in the neoadjuvant/definitive cohort and 10/13 patients in the adjuvant cohort were still alive. Correlative studies suggested that response in some patients were associated with specific $CD4^+$ T-cell subsets.

Conclusions: Overall, concurrent administration of ipilimumab and radiation was feasible, and resulted in a high response rate, converting some patients with unresectable disease into surgical candidates. Additional studies to investigate the combination of radiation and checkpoint inhibitor therapy are warranted.

Introduction

Survival after resection of melanoma varies widely by stage, with the most important prognostic factors being the depth of the primary tumor and status of the lymph nodes (1). While early-stage melanoma is often associated with a good prognosis, the prognosis for patients with regionally advanced disease can be poor as the risk of melanoma recurrence approaches 90% in patients who have multiple involved nodes removed (1). In addition, some regionally advanced melanomas may be unresectable, even in the absence of distant metastatic disease. In each of these situations, clinical practice can vary widely with limited consensus regarding a standard approach to treatment.

Traditionally, definitive surgery has been thought to represent the best chance for a cure in patients with resectable disease, with adjuvant modalities playing a more limited role. For decades, the only adjuvant systemic therapy available for clinical use was interferon-alfa-2B (IFN). Some studies demonstrated an improvement in both disease-free and overall survival (OS), but these improvements were modest, and the considerable toxicity associated with IFN limited its widespread use (2). Similarly, radiotherapy has had a more limited and less well-defined role in the management of resected cutaneous melanoma, with only one randomized, prospective trial conducted to date (3). Consideration of adjuvant radiotherapy for regionally advanced cutaneous melanoma has been largely based on specific clinical features, such as location, size, and number of involved lymph nodes, and the presence of extracapsular extension or perineural invasion. While data suggest radiotherapy has the potential to improve regional control, there is no evidence that adjuvant radiotherapy impacts distant recurrence or OS, and risks of long-term toxicity pose concerns (3).

The anti-CTLA antibody, ipilimumab, was the first agent to demonstrate an improvement in OS in a randomized phase III trial in patients with metastatic melanoma (4). Subsequent studies demonstrated increased efficacy and improved tolerability of checkpoint inhibitors that target the programmed cell death protein 1 (PD-1) pathway, and these agents, either alone or in combination with ipilimumab, are now considered standard front-line therapy for patients with advanced or unresectable melanoma (5, 6). More recently, these advances have also translated into shifts in the management of stage III disease. Ipilimumab administered in the adjuvant setting resulted in an improvement in relapse-free survival (RFS) and OS compared with placebo in patients with stage III melanoma with at least 1 mm of nodal disease (7). Adjuvant anti-PD-1–based therapy has demonstrated an improvement in RFS and a more favorable toxicity

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Translational Relevance

Relapse after surgical resection is common in patients with regionally advanced melanoma. For patients with locally advanced or unresectable disease, systemic treatment options can be limited. Preclinical data have suggested the potential for synergy between radiotherapy and immune checkpoint inhibition, though the optimal use in the clinical setting remains undefined. In this study, we investigated the concurrent administration of ipilimumab and radiotherapy in 2 distinct patient cohorts: as adjuvant therapy in patients at high risk of relapse after surgery and as neoadjuvant or definitive treatment in patients with locally advanced disease. We found the combination to be feasible, with an acceptable safety profile. Promising clinical activity was seen, particularly in patients who were treated as neoadjuvant or definitive therapy. Correlative analyses suggested that responses in some patients may be associated with the presence of specific $CD4^+$ T-cell subsets. This study suggests that combined modality approaches using immunotherapy and radiotherapy could be of benefit in selected subsets of patients with regionally advanced melanoma.

profile compared with ipilimumab and is now considered a standard-of-care option for patients with high-risk resected melanoma (8). Despite the improvements in outcome, however, nearly 40% of patients will recur by 2 years after receiving adjuvant nivolumab (9). In recent years, there has been significant interest in investigating the combination of immunotherapy and radiotherapy in a number of tumor types, particularly in melanoma. Preclinical models support the role of radiotherapy as a potential immune modulator: studies demonstrate increased T-cell infiltration, enhanced antigen presentation, and increased cytokine production (10). Synergy with immune checkpoint inhibitors has also been seen, with one preclinical model demonstrating that radiotherapy combined with CTLA-4 blockade resulted in improved survival compared with either modality alone (11). Radiotherapy dose and fractionation are likely important, though conflicting preclinical data exist. Some studies suggest more favorable immunologic effects with ablative radiotherapy; however, fractionated dosing resulted in both improved local and distant tumor control when combined with CTLA-4 inhibition (12, 13). Clinically, data have suggested that the combination of stereotactic body radiotherapy (SBRT) is safe when administered either concurrently or sequentially with ipilimumab in metastatic solid tumors, though not all sites of disease were treated with SBRT (14). A combined modality approach offers potential for improved locoregional as well as distant control in melanoma subtypes considered to be at high risk of both local and distant recurrence.

Therefore, we designed this prospective trial to evaluate the safety of concurrent ipilimumab and radiotherapy in two distinct cohorts of patients with melanoma with a historically poor prognosis: those at high risk of local and distant recurrence after surgery and those with locally advanced disease, including those considered inoperable. Secondary objectives of this study were to evaluate overall response rate (ORR), 6- and 12-month RFS/progression-free survival (PFS), 6- and 12-month OS in the individual cohorts, as well as assessment of immune profiling signatures in peripheral blood.

Patients and Methods

Patients

Patients were enrolled from December 2013 to August 2016. Eligible patients were ≥18 years of age with a confirmed pathologic diagnosis of melanoma, and an Eastern Cooperative Oncology Group performance status of 0 or 1. Patients were required to undergo baseline imaging of the neck, chest, abdomen, and pelvis. Patients with resected stage IIIC and those with unresectable disease were required to have a brain MRI.

Adjuvant patients were included in cohort 1 if pathologic analysis following surgery revealed any of the following high-risk features: melanoma of mucosal origin, desmoplastic melanoma, or primary melanoma of the head and neck with lymph node involvement. Patients with non–head and neck melanomas with macroscopic nodal involvement were also included provided at least one of the following high-risk criteria were met: regional nodal involvement of at least two axillary nodes, three groin nodes, extracapsular extension, or lymph nodes ≥3 cm in size. In addition to the clinical parameters specified in cohort 1, patients with locally advanced disease could be enrolled into cohort 2 if there was recurrent macroscopic nodal disease irrespective of number and size of nodes or if disease was deemed inoperable due to radiographic evidence of tumor invasion into surrounding local structures. Patients in cohort 2 were required to have measurable disease according to RECIST version 1.1 (15). Key exclusion criteria included patients with uveal melanoma, the presence of brain metastases, a history of autoimmune disease, prior treatment with ipilimumab/CTLA-4 inhibitor, and a history of radiotherapy for melanoma. Written informed consent was obtained for all subjects. The study was performed in accordance with provisions of the Declaration of Helsinki and Good Clinical Practice guidelines. The protocol was reviewed and approved by the Duke Institutional Review Board.

Study design and treatments

For all patients, local-regional treatment sites were required to be encompassed within a reasonable radiotherapy treatment volume as determined by the radiation oncology Principal Investigator of the study.

All patients were treated with radiotherapy with three-dimensional conformal and intensity-modulated radiotherapy techniques utilized. Dose and total dose were governed by the anatomic region being treated and whether the region contained gross or subclinical microscopic disease. For the head and neck, a daily fraction size of 300 cGy was utilized (4,200 cGy for node negative and negative margins at primary site, 4,500–4,800 cGy for node positive or positive margin at the primary site, and 5,100–5,400 cGy for gross disease). For the axilla or mediastinum, a daily dose of 250 cGy was administered (3,500 cGy if node positive with no gross residual disease and 4,000 cGy for gross disease). For the abdomen/pelvis, a daily fraction size of 225 cGy was delivered (3,600 cGy for subclinical microscopic disease and 4,050 cGy for gross disease). Patients received concurrent ipilimumab at a dose of 3 mg/kg every 3 weeks for a total of four doses; ipilimumab was required to start within 3 days of the start of radiotherapy, but not prior to radiotherapy initiation.

Patients could be considered for resection after the completion of ipilimumab and radiation if curative surgery was considered feasible by the treating surgeon. During trial accrual, anti-PD-1 therapy became standard front-line therapy for patients with unresectable melanoma; thus, an amendment allowed the last remaining slot on this study (cohort 2) to be used for an adjuvant patient. Subsequent disease assessments with clinical exam and CT imaging were performed for evidence of recurrence or progression at week 16 and then every 12 weeks for up to 2 years. For cohort 2, response evaluations were

assessed as ORR by RECIST and immune-related response criteria (irRC; ref. 16).

Cellular analysis and flow cytometry of immune subsets

Blood was collected at four time points: baseline (week 1) prior to administration of ipilimumab, prior to third cycle of ipilimumab (week 7), after completion of ipilimumab (week 12), and at disease progression or week 24. Blood was obtained by venipuncture and collected in acid-citrate-dextrose tubes (BD Vacutainer). Peripheral blood mononuclear cells (PBMC) were isolated by Ficoll density gradient centrifugation (GE Healthcare), resuspended in a 90% FBS (Gemini) and 10% DMSO (Sigma-Aldrich) solution, and cryopreserved in vapor phase liquid nitrogen until batch testing.

Cellular analysis of immune cell subsets in isolated PBMCs was performed by polychromatic flow cytometry. For cell staining, thawed cells were first incubated with a Zombie viability dye (BioLegend) to detect dying cells, followed by a surface stain procedure with an antibody cocktail consisting of anti-CD3 (SK7; BD), CD4 (SK3; BioLegend), CD8 (SK1; BD), PD-1 (EH12.2H7; BioLegend), TIM-3 (7D3; BD), HLA-DR (G46-6; BD), CD56 (HCD56; BioLegend), CD19 (HIB19; BioLegend), CD16 (3G8; BD), CD11b (M1/70; BD), CD14 (M5E2; BD), and CD33 (P67.6; BioLegend). The unbound antibodies were washed out by centrifugation and stained cells were fixed with 1% paraformaldehyde prior to acquisition on an LSR Fortessa flow cytometer (BD Biosciences), and data were analyzed using Flowjo software (BD Biosciences).

Statistical analysis

The primary objective of this study was to evaluate the safety of ipilimumab administered concurrently with radiation. To that endpoint, adverse events (AE) were categorized as treatment-related or not and summarized using descriptive statistics. AEs could be attributed to radiation, ipilimumab, or both; a relationship to any component of study therapy was defined as a treatment-related event. Grade 3+ toxicities were further categorized as early (occurring within 90 days of radiation start) or late (occurring 91 days or more from start of radiation). The percent of patients who experienced a grade 3+ treatment-related toxicity was calculated with an exact binomial 80% confidence interval (CI).

Other study objectives were to evaluate ORR, 6- and 12-month PFS and OS, and the disease control rate (DCR). Response was determined using the irRC and the ORR calculated as the percent of patients who met the criteria for complete or partial response. ORR with an exact binomial 80% CI was calculated among all treated patients in cohort 2; unevaluable patients were treated as nonresponders. DCR was calculated similarly to ORR, but as the percent of patients who met response or stable disease (SD) criteria. PFS and OS were calculated using the Kaplan–Meier method. PFS was defined as the time from enrollment to progression or death. OS was defined as the time from enrollment to death. Where possible, median PFS and OS were estimated along with their 80% confidence limits. All calculations were performed in SAS (9.4) and R (3.6.1).

Results

Twenty-four patients with regionally advanced melanoma were enrolled onto this propsecitive trial: 13 in the adjuvant cohort (cohort 1) and 11 in the neoadjuvant/definitive cohort (cohort 2; Supplementary Fig. S1).The median age for patients in cohort 1 was 63.6 years (range, 20.1–83.4) and was 56.2 years (range, 47.4–70.0) for cohort 2. Five patients in cohort 1 (39%) and 7 (64%) in cohort 2 had stage IIIC disease. The majority of patients had cutaneous melanoma; 2 patients in cohort 1 and 1 patient in cohort 2 had mucosal melanoma. Four patients (2 in each cohort) had melanoma of unknown primary, with macroscopic lymph node involvement. One patient in each cohort had received prior systemic therapy for melanoma, and 1 patient in cohort 2 received prior radiotherapy for a separate diagnosis. More details on patient characteristics are presented in **Table 1**.

Table 1. Patient characteristics at study entry.

Variable	Cohort 1	Cohort 2	All
Median age, yr (range)	63.6 (20.1–83.4)	56.2 (47.4–70)	60.1
	$n = 13$	$n = 11$	$n = 24$
Sex, *n* (%)			
Female	9 (69)	5 (45)	14 (58)
Male	4 (31)	6 (55)	10 (42)
Race, *n* (%)			
Black or African American	1 (8)	0 (0)	1 (4)
White	12 (92)	11 (100)	23 (96)
Disease stage, *n* (%)			
Stage III NOS	3 (23)	1 (9)	4 (17)
IIIB	4 (31)	3 (27)	7 (29)
IIIC	5 (39)	7 (64)	12 (50)
IVB[a]	1 (8)	0 (0)	1 (4)
Melanoma type			
Unknown primary	2 (15)	2 (18)	4 (17)
Cutaneous	9 (69)	8 (73)	17 (71)
Mucosal	2 (15)	1 (9)	3 (13)
Prior systemic therapy, *n* (%)			
No	12 (92)	10 (91)	22 (92)
Yes	1 (8)	1 (9)	2 (8)
Prior radiation, *n* (%)			
No	13 (100)	10 (91)	23 (96)
Yes	0 (0)	1 (9)	1 (4)
Treatment site[b], *n* (%)			
Head and neck	5 (38)	3 (27)	8 (33)
Axilla/mediastinum	6 (46)	8 (72)	14 (58)
Abdomen pelvis	2 (15)	1 (9)	3 (13)

Abbreviations: NOS, not otherwise specified; yr, years.
[a]This patient had a head and neck mucosal melanoma.
[b]Total sites and percentages may add up to more than total *N* or 100% due to more than one site being treated.

Safety

Overall, the combination of ipilimumab and radiotherapy had an AE profile that was comparable with that seen with either modality alone. While a high percentage of patients in each cohort experienced an AE felt to be at least possibly related to treatment, the majority of these were grade 1/2. Only 15% (80% CI, 4%–36%) of patients in cohort 1 and 18% (80% CI, 5%–42%) of patients in cohort 2 experienced a grade 3 treatment-related AE; there were no grade 4 or 5 events. The most common AE reported on study was fatigue, reported in 62% ($n = 8$) and 64% ($n = 7$) of patients in cohorts 1 and 2, respectively. Pruritis was reported in 54% of patients in cohort 1 and 64% of patients in cohort 2. Rash was reported in 54% of patients in cohort 1, with 1 grade 3 event, and occurred in 45% of patients in cohort 2, with 2 grade 3 events. Radiation dermatitis was reported in 46% and 73% of patients in cohorts 1 and 2, respectively, and all were grades 1–2. Diarrhea was the most common gastrointestinal AE, occurring in 23% of patients in cohort 1 and 27% of patients in cohort 2, and all cases were grade 1. Two patients in cohort 2 developed

Table 2. Treatment-related AEs.[a]

	Grades 1/2 *n* (%)	Grade 3 *n* (%)	RT *n* (%)[b]	IPI *n* (%)[b]	Grades 1/2 *n* (%)	Grade 3 *n* (%)	RT *n* (%)[b]	IPI *n* (%)[b]
General								
Fatigue	8 (62)	0 (0)	5 (63)	6 (75)	7 (64)	0 (0)	4 (50)	5 (63)
Fever	0 (0)	0 (0)	0 (0)	0 (0)	2 (18)	0 (0)	1 (50)	1 (50)
Dermatologic								
Pruritis	7 (54)	0 (0)	1 (14)	6 (86)	7 (64)	0 (0)	1 (14)	6 (86)
Rash	6 (46)	1 (8)	0 (0)	7 (100)	2 (18)	2 (18)	0 (0)	4 (100)
Vitiligo	1 (8)	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)
Radiation dermatitis	6 (46)	0 (0)	6 (100)	0 (0)	8 (73)	0 (0)	8 (100)	0 (0)
Endocrine disorders								
Hypophysitis	1 (1)	0 (0)	0 (0)	1 (100)	2 (18)	0 (0)	0 (0)	2 (100)
Hypothyroidism	1 (8)	0 (0)	0 (0)	1 (100)	1 (9)	0 (0)	0 (0)	1 (100)
Diabetes mellitus	1 (8)	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)
Gastrointestinal								
Diarrhea	3 (23)	0 (0)	0 (0)	3 (100)	3 (27)	0 (0)	1 (33)	2 (67)
Oral mucositis	4 (31)	0 (0)	4 (100)	0 (0)	2 (18)	0 (0)	2 (100)	0 (0)
Nausea	2 (15)	0 (0)	1 (50)	1 (50)	0 (0)	0 (0)	0 (0)	0 (0)
Dysgeusia	3 (23)	0 (0)	3 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Respiratory								
Pneumonitis	0 (0)	0 (0)	0 (0)	0 (0)	1 (9)	0 (0)	0 (0)	1 (100)
Rheumatologic								
Periorbital myositis	0 (0)	1 (8)	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)

Abbreviations: IPI, ipilimumab; RT, radiotherapy.

[a]Includes AEs at least possibly related to either ipilimumab or radiotherapy occurring in more than 10% of patients. Also includes grade 3 events and immune-related adverse events, even if incidence less than 10%.

[b]For AE attribution, the percentage reported refers to the percent of patients with the noted AE that was attributed to either ipilimumab or radiotherapy; percentages may add up to more than 100% as AEs could be attributed to both ipilimumab and radiotherapy.

hypophysitis after completing ipilimumab and radiotherapy; 1 patient in cohort 1 developed hypophysitis and type 1 diabetes, also after completion of therapy. One patient in cohort 2 developed pneumonitis after one dose of ipilimumab which resolved with steroids; mean radiotherapy lung dose was 400 cGy. One patient in cohort 1 developed grade 3 periorbital myositis after two doses of ipilimumab; this eventually resolved over several months after the administration of two doses of infliximab. Additional details regarding AEs are presented

Table 3. Patient characteristics post progression.

Subject	Time to recurrence (months)	Type of recurrence	Recurrence substage	Subsequent treatment	Best response	Outcome at end of study
Cohort 1						
012	3.5	Distant	M1d	Pembrolizumab	PD	
				Ipilimumab/nivolumab	PR	
				Dabrafenib/trametinib	PR	On therapy; continued PR
010	3.4	Distant	M1c	Dabrafenib/trametinib	PR	
				Pembrolizumab	PD	Died because of PD
022	25.5	Distant	M1c	Surgical resection of isolated liver metastasis	NA	NED
015	7.9	Regional	N1c	Pembrolizumab	PD	
				TVEC	PD	
				Ipilimumab/nivolumab	PR	Died because of PD 8.4 months after initiation of therapy
023	15.4	Distant	M1a	Pembrolizumab	CR	Off therapy with NED
006 (mucosal)	9.1	Distant	M1c	Nivolumab	PD	Died because of PD 2.4 months after initiation of nivolumab
Cohort 2						
017 (mucosal)	3.5	Distant	M1b	Nivolumab	PD	
				Ipilimumab/nivolumab	PR	On therapy
014	25.1	Regional	N1b	Resection	NA	NED
030	3.5	Distant	M1c	Ipilimumab/nivolumab	PR	Off therapy; continued PR
024	5.3	Distant	M1a	Pembrolizumab	CR	Off therapy; continued CR

Abbreviations: CR, complete response; NA, not applicable; NED, no evidence of disease; PD, progressive disease; PR, partial response; TVEC, talimogene laherparepvec.

in **Table 2**. All grade 3 AEs occurred within 90 days of starting radiation treatment.

Efficacy

Median follow-up was 25.8 months (80% CI, 25.3–26.2) for cohort 1 and 25.3 months (80% CI, 25.1–25.8) for cohort 2. There were 10 relapses or progressions during this study. No patient in either cohort recurred within the radiation field.

Of the 6 patients who recurred in cohort 1, 5 had distant metastases, including 1 of the 2 patients with mucosal melanoma; 1 patient recurred with regional in-transit disease. The majority of the patients recurred within the first year after therapy, with an additional 2 patients recurring at 16 and 26 months, respectively. Additional details of subsequent treatment are reviewed in **Table 3**. For cohort 1, median RFS was 26.7 months (80% CI, 8.9–26.7; **Fig. 1A**) and the 6-, 12-, and 24-month RFS rates were 85% (66%–93%), 69% (50%–82%), and 62% (42%–76%), respectively. Three of the 13 patients in cohort 1 died by the end of the study (**Fig. 2A**).

For cohort 2, the global ORR at 16 weeks was 64% (80% CI, 40%–83%), with 4/10 evaluable patients achieving a radiographic complete response (**Table 4**). Three patients had a partial response (PR) and 1 had SD, for a DCR of 73% (80% CI, 49%–90%). One patient was considered unevaluable for response, as the baseline lymph node measurements did not meet measurable size criteria per RECIST but remained free of disease progression at the end of study follow-up. Two patients had progressive disease (PD) as best response in both cases

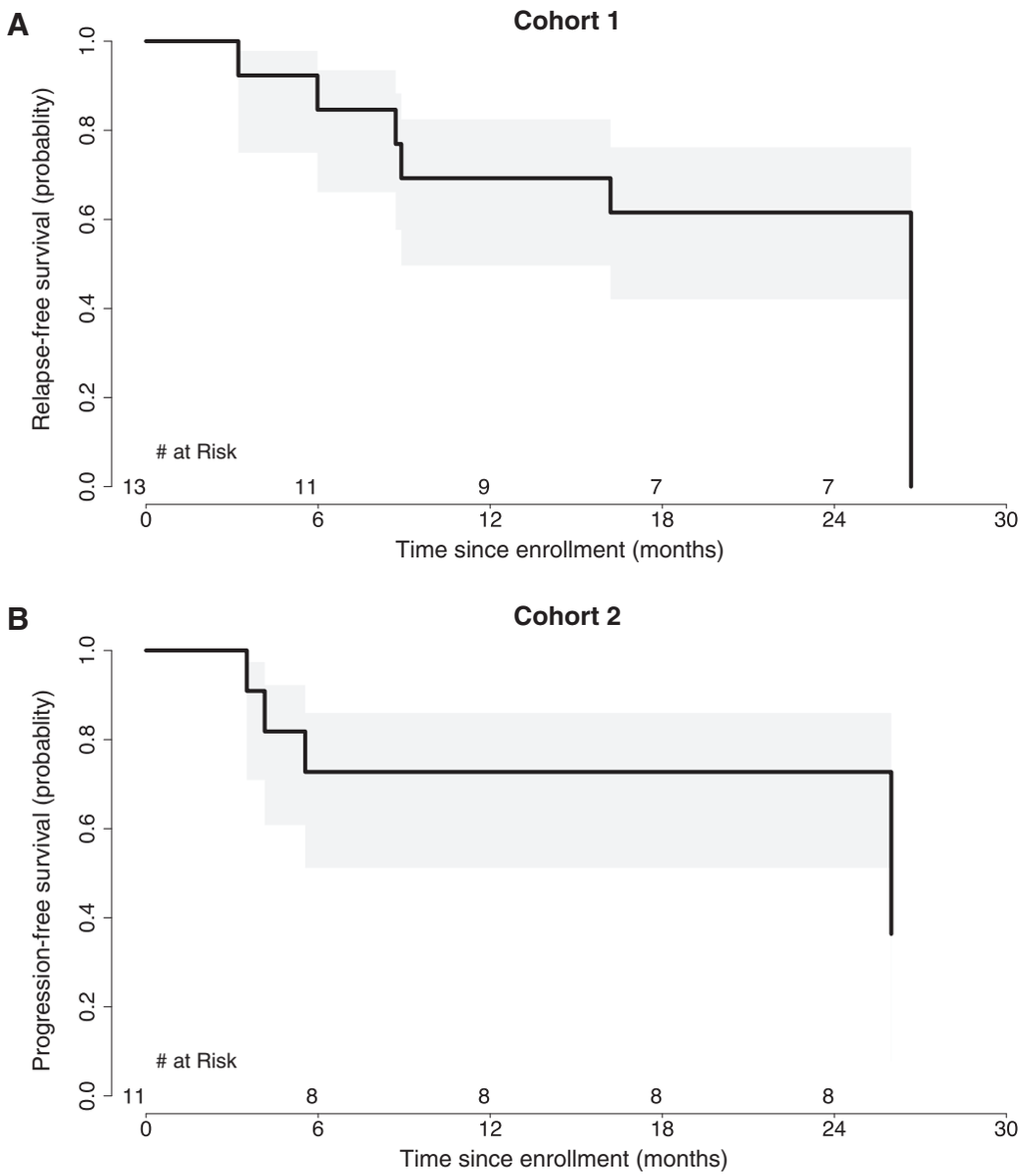

Figure 1.
RFS in cohort 1 (**A**). PFS in cohort 2 (**B**).

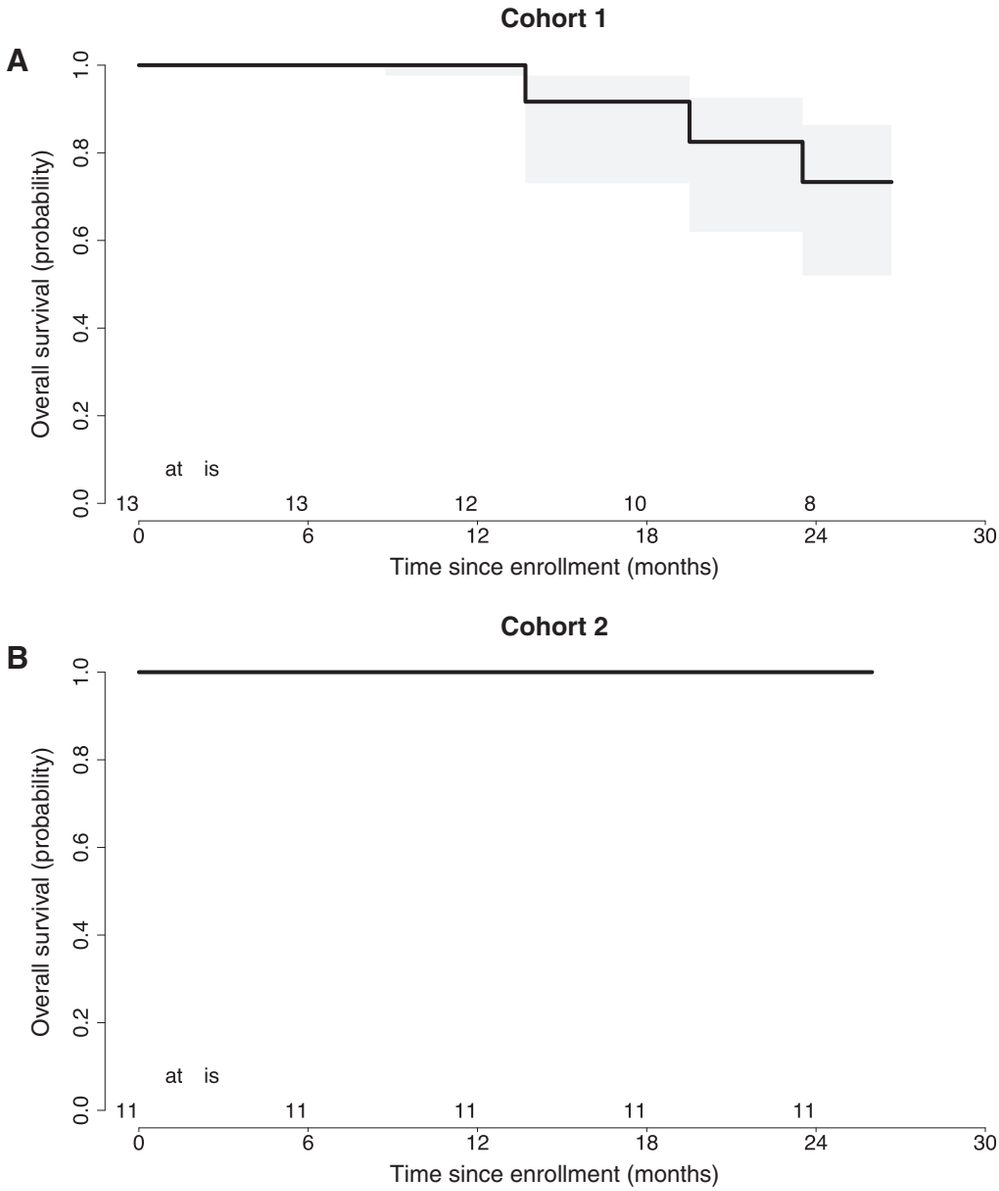

Figure 2.
OS for cohort 1 (**A**). OS for cohort 2 (**B**).

due to distant recurrence, including 1 patient with unresectable mucosal melanoma.

Three patients, all with a PR by RECIST criteria after the completion of ipilimumab and radiation in cohort 2, underwent resection at the completion of study therapy. Two of the patients received all four cycles of ipilimumab, and underwent surgical resection at weeks 17 and 19, respectively. The third patient developed grade 2 pneumonitis after the first dose of ipilimumab and was treated with a steroid taper, but completed the planned radiotherapy course. Response assessment was performed at week 12, and resection performed at week 13. Pathologic assessment revealed a pathologic CR (pCR) in two specimens; a third specimen demonstrated extensive tumoral melanosis in 22/38 lymph nodes, with the largest node containing necrotic tumor. This patient received all four doses of ipilimumab on study prior to surgery, and then received ipilimumab 10 mg/kg adjuvantly at the discretion of the treating physician off protocol. At the completion of the study follow-up period, all 3 of the surgically resected patients remained free of recurrence.

For cohort 2, in addition to the 2 patients with initial PD, 2 more progressed during the course of the study: 1 patient with initial SD progressing distantly at month 6, and another with unresectable scalp in transit disease with an initial CR progressing at 24 months within the cervical nodes. This patient was a candidate for subsequent resection. All 3 patients who progressed with distant disease were treated with anti-PD1–based therapy at the time of recurrence, with continued disease control at the time of study completion (**Table 3**). The 6- and

Table 4. Response to treatment in cohort 2.

Variable, *n* (%)	Cohort 2 *n* = 11
Best overall response	
Complete response	4 (36)
Partial response	3 (27)
Stable disease	1 (9)
Progressive disease	2 (18)
Unevaluable[a]	1 (9)
Objective response rate	7 (64)
Disease control rate (CR +PR + SD)	8 (73)

[a]Baseline disease not measurable per RECIST criteria.

12-month PFS for cohort 2 was 73% (80% CI, 56%–90%; **Fig. 1B**), and was unchanged at 24 months. At the completion of study follow-up, all patients in cohort 2 were still alive (**Fig. 2B**) with a median follow-up of 25.3 months (80% CI, 25.1–25.8).

Cellular analysis of immune subsets

With progressors and nonprogressors in each treatment cohort, cellular immune profiling of PBMCs was conducted at the baseline timepoint (within 1 week prior to starting radiotherapy and ipilimumab) to search for an immune signature that was predictive of treatment outcomes. The flow cytometry panel consisted of markers that distinguished B cells, natural killer (NK) cells, monocytes, myeloid-derived suppressor cells (MDSC), and subsets contained within $CD4^+$ and $CD8^+$ T cells. At baseline, the frequency of B cells, NK cells, monocytes, MDSCs, dendritic cells, and $CD8^+$ T-cell subsets were comparable between progressors and nonprogressors in cohorts 1 and 2 (Supplementary Fig. S2). Examination of the $CD4^+$ T-cell subsets revealed a significant enhancement in the frequency of PD-1^+ $CD4^+$ T cells in the progressor group in cohort 2 (**Fig. 3A** and **B**; $P = 0.0242$). Further analysis showed a disparity in the frequency of PD-1^+ HLA-DR^- $CD4^+$ T cells in cohort 2 progressors (**Fig. 3C**; $P = 0.0424$).

Discussion

To our knowledge, the prospective study reported here is the first and only study evaluating the concurrent administration of a checkpoint inhibitor and radiotherapy in either the adjuvant or neoadjuvant/definitive setting for locally advanced melanoma. In this study, we demonstrated that the concurrent administration of ipilimumab and radiotherapy is safe in patients with high-risk resected and regionally advanced melanoma, and consistent with the safety profiles of radiotherapy and ipilimumab alone, with relatively low rates of severe AEs. In the adjuvant cohort, the 6- and 12-month RFS was 85% and 69%, respectively, which is similar to other adjuvant studies using ipilimumab (7). In addition, we identified the combination of ipilimumab and radiotherapy appeared to be active in patients with unresectable/locally advanced disease, with an ORR of 64% and a DCR rate of 73%, higher than historic response rates for ipilimumab alone, with all patients alive at 2 years (4). Finally, we identified differences in PBMCs

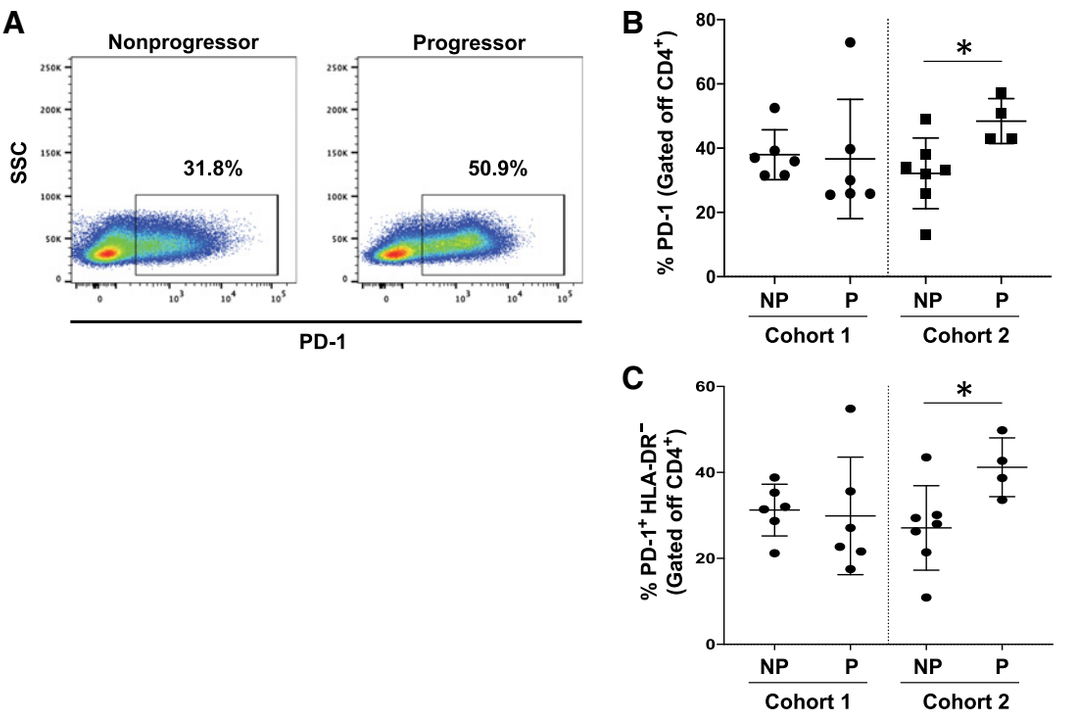

Figure 3.

Progressors in cohort 2 demonstrate enhanced baseline frequencies of PD-1^+ $CD4^+$ T cells. Cellular analysis by flow cytometry analysis was performed on PBMCs collected at baseline (within 1 week prior to starting radiotherapy and ipilimumab). **A,** Representative surface stain analysis of PD-1 expression in a nonprogressor and progressor patient from cohort 2. Gated $CD4^+$ T cells are shown and percentage of PD-1^+ cells are reported. **B,** Composite data of nonprogressors and progressors in cohorts 1 and 2 showing baseline frequencies of PD-1^+ $CD4^+$ T cells. **C,** Composite data of nonprogressors and progressors in cohorts 1 and 2 showing baseline frequencies of PD-1^+ HLA-DR^- $CD4^+$ T cells. Statistical significance is represented as *, $P < 0.05$.

with responders having a higher frequency of PD-1$^+$ HLA-DR$^-$ CD4$^+$ T cells.

One of the most important findings from our study was the promising signal that in unresectable patients, the combination of ipilimumab and radiotherapy was associated with favorable disease control and survival. Not only were high local response rates seen with radiotherapy, but also no patient in this study recurred within the radiation field. The activity of the combination is highlighted with 4 patients having a radiographic CR following protocol therapy. Furthermore, an additional 3 initially unresectable patients went on to have resection of their regional nodal disease, with 2 of 3 patients demonstrating a pCR, and all remaining disease free at the completion of the study. Interestingly, this initial benefit appeared to translate into favorable long-term clinical outcomes, with all patients in this high-risk cohort alive with controlled disease at the time of study completion.

Since the initiation of this study, the use of systemic therapies in melanoma continues to evolve rapidly. While ipilimumab was considered standard first-line therapy at the initiation of this trial, this was subsequently replaced by anti-PD-1 therapies due to improved efficacy and tolerability (5). Similarly, the landscape for adjuvant options for regionally advanced melanoma has shifted in recent years with the development of more effective and better tolerated therapies (8, 17). Adjuvant anti-PD-1–based therapy with nivolumab was subsequently shown to result in an improved RFS compared with ipilimumab in the adjuvant setting, and also has an improved tolerability profile, establishing these agents as the preferred immunotherapy in the adjuvant setting (8). However, even with these advancements, many patients still recur despite therapy. Current adjuvant studies are examining combined checkpoint inhibition with ipilimumab and nivolumab, which has the potential to result in increased immune-mediated AEs (NCT03068455). In this patient population with potentially curable, albeit high-risk disease, side effects of systemic therapy factor significantly into clinical decision-making. The RFS and OS data of this small cohort, 39 percent of which had stage IIIC melanoma and the remainder having macroscopic nodal disease, are promising in light of the historically poor prognosis of patients with these high-risk melanoma subtypes. Further defining optimal synergism between local and systemic modalities may allow for future deescalation of systemic therapy in selected patients.

Recently, the sequencing of systemic therapies as part of the management of regionally advanced melanoma has begun to shift, with systemic therapies taking a more prominent role in the initial management of stage III disease. The concept of a neoadjuvant approach, long considered standard of care in many other malignancies, is emerging as a promising strategy in the management of high risk, but potentially resectable, patients with regionally advanced melanoma. However, randomized prospective data regarding sequencing and optimal length of therapy are lacking. As in the metastatic and adjuvant settings, additional efforts have focused primarily on the combination of systemic agents. In a randomized trial of neoadjuvant nivolumab versus ipilimumab and nivolumab, the combination yielded high response rates but also resulted in high rates of grade 3/4 AEs, which occurred in over 70% of patients (18). Similar outcomes were also seen in a separate study of neoadjuvant ipilimumab and nivolumab (19). While our study focused on a patient population with unresectable disease, the integration of local therapy with modified doses of newer systemic agents could be applicable in the neoadjuvant setting where the risks of systemic therapies should be carefully considered. Current data suggest that favorable pathologic responses to neoadjuvant therapy is prognostic of subsequent outcomes (20, 21). However, there is currently limited data reporting on the use of checkpoint inhibitors as a strategy to "downstage" or convert patients with unresectable disease into surgical candidates. Although this strategy was not a primary objective of our study, 3 of 11 patients (27%) were ultimately able to undergo resection. Each of these patients had either a pCR or marked evidence of antitumor activity and remained disease free at 2 years.

We also sought to identify potential blood-based biomarkers of response. Our previous study of ipilimumab in NSCLC revealed a global T-cell activation in all patients receiving ipilimumab (22). Both CD4$^+$ and CD8$^+$ T cells had significant increase in the expression of ICOS, HLA-DR, CTLA-4, and PD-1. In this study, we observed similar increases in ICOS and PD-1 expression in all our patients, demonstrating a systemic effect of ipilimumab on circulating T cells. In another melanoma study, MHC-II expression, measured by HLA-DR, was associated with a therapeutic response to anti-PD-1 therapy, PFS, OS, and T-cell infiltration (23). In our dataset, disparities in total HLA-DR expression were not observed; however, progressors in cohort 2 exhibited a higher frequency of CD4$^+$ T cells expressing PD-1$^+$ that lack HLA-DR expression. HLA-DR is an activation marker that is synonymous with effector T cells and the absence of this marker on PD-1$^+$ cells may suggest an increase in PD-1–expressing exhausted CD4$^+$ T cells (24). A larger cohort is required to validate our findings and to demonstrate the ability to identify predictive biomarkers in the peripheral blood.

Limitations of this study include the small sample size, as this was a pilot study to assess the feasibility and safety of concurrent administration of ipilimumab and radiotherapy. At the time the majority of the patients were enrolled, ipilimumab was considered standard first-line therapy for patients with unresectable melanoma, and later as a standard option for adjuvant therapy. While ipilimumab has largely been replaced by PD-1–based regimens in these settings, the concept of integrating radiotherapy with systemic agents still has important implications in today's treatment landscape. Post PD-1 progression, retrospective series suggest modest activity with ipilimumab monotherapy, and a recent prospective study demonstrated a response rate of 30% with the combination of pembrolizumab and low-dose ipilimumab (25, 26). Primary and acquired resistance to anti-PD-1 therapy continue to remain major challenges, and limited data exist to guide subsequent treatment strategies. Given the favorable outcomes with long-term follow-up in our study, particularly in patients with locally advanced disease, we feel this further adds to the support of investigations using a combined immune checkpoint and radiotherapy approach in selected patients.

In summary, the concurrent administration of ipilimumab and radiotherapy is feasible and safe. This approach leads to long-term disease control in a subset of patients with regionally advanced melanoma. Future studies of combined modality therapy, particularly in the neoadjuvant setting, with additional systemic agents should be explored for selected patients with high-risk melanoma.

Authors' Disclosures

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Authors' Contributions

A.K.S. Salama: Conceptualization, resources, formal analysis, funding acquisition, investigation, writing-original draft. **M. Palta:** Conceptualization, supervision, investigation, writing-original draft, writing-review and editing. **C.N. Rushing:** Data curation, formal analysis, writing-original draft, writing-review and editing. **M.A. Selim:** Data curation, investigation, writing-review and editing, pathologist who reviewed clinical specimens and performed staining of slides. **K.N. Linney:** Data curation, project administration, writing-review and editing. **B.G. Czito:** Data curation, investigation, writing-review and editing. **D.S. Yoo:** Data curation, investigation, writing-review and editing. **B.A. Hanks:** Data curation, investigation, writing-review and editing. **G.M. Beasley:** Data curation, investigation, writing-review and editing. **P.J. Mosca:** Data curation, investigation, writing-review and editing. **C. Dumbauld:** Data curation, formal analysis, investigation. **K.N. Steadman:** Data curation, formal analysis, investigation. **J.S. Yi:** Data curation, formal analysis, investigation, writing-original draft. **K.N. Weinhold:** Conceptualization, resources, supervision, writing-review and editing. **D.S. Tyler:** Conceptualization, resources, funding acquisition, investigation, writing-review and editing. **W.T. Lee:** Conceptualization, resources, formal analysis, supervision, writing-review and editing. **D.M. Brizel:** Conceptualization, supervision, funding acquisition, investigation, writing-original draft, writing-review and editing.

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