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Safety and clinical activity of combined PD-1 (nivolumab) and CTLA-4 (ipilimumab) blockade in advanced melanoma patients

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Abstract

Background—In patients with melanoma, ipilimumab (anti-CTLA-4) prolongs overall survival and nivolumab (anti-PD-1) produced durable tumor regressions in a phase 1 trial. Based on their distinct immunologic mechanisms of action and supportive preclinical data, we conducted a phase 1 trial of nivolumab combined with ipilimumab in advanced melanoma patients.

Methods—Patients received nivolumab and ipilimumab every 3 weeks for 4 doses, followed by nivolumab alone every 3 weeks for 4 doses (concurrent regimen). Combined treatment was subsequently continued every 12 weeks for up to 8 doses. In a sequenced regimen, patients previously treated with ipilimumab received nivolumab every 2 weeks.

Results—Fifty-three patients received concurrent nivolumab/ipilimumab and 33 received sequenced treatment. The objective response rate, for all concurrent-regimen patients was 40% (modified WHO criteria). Evidence of clinical activity (conventional, unconfirmed, or immune-related response or stable disease ≥ 24 weeks) was observed in 65% of patients. At the maximum tolerated dose (1 mg/kg nivolumab + 3 mg/kg ipilimumab), 53% of patients achieved an objective response, all with ≥ 80% tumor reduction. Grade 3–4 related adverse events occurred in 53% of concurrent-regimen patients, but were qualitatively similar to historical monotherapy experience and were generally reversible. Among sequenced-regimen patients, 18% had grade 3–4 related adverse events and the objective response rate was 20%.

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Conclusions—Concurrent nivolumab/ipilimumab had a manageable safety profile and achieved clinical activity that is distinct from published monotherapy data, with rapid and deep tumor regressions in a substantial number of patients.

INTRODUCTION

Escape from immune surveillance is a recognized hallmark of cancer; therefore, development of potent therapies to enhance tumor immunity is an imperative.^{1, 2} Immune checkpoint blockade is an effective means to induce durable regressions in several types of cancer. Ipilimumab, a fully human IgG1 monoclonal antibody blocking CTLA-4, improved overall survival in patients with advanced melanoma.^{3, 4} Nivolumab, a fully human IgG4 antibody blocking PD-1, produced durable objective responses in patients with melanoma, renal-cell and non-small-cell lung cancer.⁵

CTLA-4 and PD-1 appear to play complementary roles in regulating adaptive immunity. While PD-1 appears to contribute to T-cell exhaustion in peripheral tissues, CTLA-4 inhibits at earlier points in T-cell activation. In preclinical models, coordinate blockade of PD-1 and CTLA-4 achieved more pronounced antitumor activity than blockade of either pathway alone.^{6, 7} Based on these observations, we conducted a phase 1 study to investigate the safety and efficacy of combined CTLA-4 (ipilimumab) and PD-1 (nivolumab) blockade in patients with advanced melanoma. Data for 86 patients treated on this ongoing study are reported.

METHODS

Study Design

In this phase I study, successive cohorts of patients were treated with escalating doses of intravenous nivolumab and ipilimumab administered concurrently (concurrent regimen, Figure S1, supplementary appendix). Two cohorts of patients treated previously with ipilimumab received nivolumab alone (sequenced regimen, Figure S1). After completion of therapy, patients without confirmed disease progression were followed for up to 2.5 years. Patients with complete response [CR], partial response [PR], or stable disease [SD] 24 weeks and subsequent disease progression could be retreated with the original regimen.

Safety evaluation was performed per protocol. The severity of adverse events (AEs) was graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 3.0.⁸ Disease assessment, using CT and/or MRI as appropriate, was performed per protocol.

Study Oversight

The protocol was approved by the respective institutional review boards and the study was conducted in accordance with the Declaration of Helsinki and International Conference on Harmonization Guidelines for Good Clinical Practice. An independent Early Development Advisory Committee was utilized for additional safety oversight. The committee was responsible for reviewing and adjudicating individual high-grade AEs as potentially dose-

limiting, and guiding the study team on decisions for dose escalation and cohort expansion. All participating patients gave written informed consent.

This study was designed by the senior academic authors and the sponsor, Bristol-Myers Squibb. Data were collected by the sponsor and analyzed and interpreted in collaboration with the academic authors. Manuscript drafts were prepared by the authors with editorial assistance from a professional medical writer paid by the sponsor. All authors vouch for the accuracy and completeness of the data. The protocol, including statistical analysis plan, is available with the full text of this article at NEJM.org.

Dose Escalation and Cohort Expansion

The study was initially planned to evaluate the concurrent regimen using a standard 3 + 3 design for the dose escalation phase, followed by cohort expansion to a total of up to 16 patients at the maximum tolerated dose or the maximum administered dose. The dose-limiting toxicity (DLT) evaluation period for dose escalation was 9 weeks. A modified definition for DLT determination was incorporated in the protocol (NEJM.org). No intra-patient dose escalation was allowed and patients who experienced DLT were discontinued from therapy. Patients who withdrew from study during the DLT evaluation period for reasons other than drug-related toxicity could be replaced. The protocol was amended to permit expansion of any concurrent regimen cohort during dose escalation to N = up to 12 patients with approval by the Early Development Advisory Committee. Two sequenced-regimen cohorts (6 to 16 patients each) were later added; patients were treated with nivolumab (1 mg/kg or 3 mg/kg) after having received prior ipilimumab.

Patients

Eligible patients were ≥ 18 years of age and had a diagnosis of measurable, unresectable stage III or IV melanoma; Eastern Cooperative Oncology Group performance status of 0-1;⁹ adequate organ function; and life expectancy of ≥ 4 months. Patients with active, untreated central nervous system metastasis; history of autoimmune disease; prior therapy with T-cell modulating antibodies (excluding ipilimumab for sequenced-regimen cohorts); HIV; or hepatitis B or C were excluded.

In the sequenced-regimen cohorts, patients were required to have received ≥ 3 prior doses of ipilimumab, with the last dose administered within 4–12 weeks of initiation of nivolumab. Patients with CR, progression with evidence of clinical deterioration, or a history of high-grade AEs related to ipilimumab were excluded (see protocol, NEJM.org).

PD-L1 Immunohistochemistry

Pre-treatment PD-L1 expression was measured by immunohistochemistry in formalin-fixed, paraffin-embedded tumor specimens using a rabbit monoclonal anti-human PD-L1 antibody (clone 28-8) and an automated assay developed by Dako (Carpinteria, CA). Antibody specificity was assessed by Western blotting against recombinant PD-L1 protein and lysates from PD-L1 expressing and non-expressing cell lines. An immunohistochemistry assay with and without antigen competition and an assessment of staining patterns in normal human tissues was performed. Analytical sensitivity, specificity, repeatability, reproducibility, and

robustness of the immunohistochemistry assay were tested and met all pre-specified acceptance criteria. Two pathologists, blinded to outcome, independently read and adjudicated scores for all clinical specimens. A sample was defined as PD-L1-positive if 5% of tumor cells exhibited membrane PD-L1 staining of any intensity in a section with 100 evaluable cells.^{5, 10}

Statistical Analysis

All treated patients (N=86) as of February 15, 2013 were used to describe baseline characteristics, safety, and absolute lymphocyte count (ALC). Analysis of PD-L1 staining included tumor specimens available as of February 28, 2013. The efficacy population consisted of 82 response-evaluable patients who received at least one dose of study therapy, had measurable disease at baseline, and one of the following: 1 on-treatment tumor evaluation, clinical progression, or death prior to the first on-treatment tumor evaluation. AEs were coded using Medical Dictionary for Regulatory Activities (MedDRA), version 15.1. Select AEs with potential immunologic etiologies, those that require more frequent monitoring and/or unique intervention, were identified using a pre-defined list of MedDRA terms. These are similar to events previously described as immune-related adverse events or adverse events of special interest.⁵ Best overall responses were derived programmatically from tumor-measurements provided by the study- site radiologist and investigators per modified WHO (mWHO) or immune-related response criteria.¹¹ Complete and partial responses were confirmed by at least one subsequent tumor assessment. Unconfirmed responses as of the date of this analysis were also included in an estimate of aggregate clinical activity (Table 3A).

RESULTS

Baseline Patient Characteristics

Eighty-six patients were treated between December 2009 and February 2013, 53 with the concurrent regimen and 33 with the sequenced regimen. Baseline patient characteristics are shown in Table 1. In the concurrent and sequenced regimens, 38% and 100% of patients, respectively, received prior systemic therapy. The majority of patients had M1c disease and >30% had elevated serum lactate dehydrogenase (LDH). Most patients enrolled to the sequenced-regimen cohorts demonstrated radiographic progression (73%) with prior ipilimumab treatment.

Safety

For the concurrent regimen (n=53), AEs of any grade, irrespective of attribution, were observed in 98% of patients (Table S2-A). Treatment-related AEs were observed in 93% of patients with the most common being rash (55%), pruritus (47%), fatigue (38%), and diarrhea (34%, Table S2-B). Grade 3–4 AEs, irrespective of attribution, were observed in 72% of patients, while grade 3–4 treatment-related events were noted in 53%, with the most common being elevations of lipase (13%), aspartate aminotransferase (AST; 13%) and alanine aminotransferase (ALT; 11%). Six of 28 (21%) patients had grade 3–4 dose-limiting treatment-related events. Treatment-related serious AEs were reported in 49% of patients (Table S2-C). Common grade 3–4 treatment-related select AEs included hepatic (15%),

gastrointestinal (9%), and renal (6%) events (Table 2). Isolated cases of pneumonitis and uveitis were seen (Table S2B) consistent with historical monotherapy experiences. Eleven (21%) patients discontinued due to treatment-related AEs (Table S3).

Cohort 3 (3 mg/kg nivolumab + 3 mg/kg ipilimumab) exceeded the MTD (3 of 6 patients experienced asymptomatic grade 3–4 elevated lipase that persisted for 3 weeks). Cohort 2 (1 mg/kg nivolumab + 3 mg/kg ipilimumab) was identified as the MTD (grade 3 uveitis, grade 3 elevated AST/ALT in 1 patient each).

For the sequenced regimen (n=33), AEs of any grade, irrespective of attribution, were observed in 29 (88%) patients (Table S2-A). Treatment-related AEs were observed in 24 (73%) patients, with the most common including pruritus (18%) and lipase elevation (12%, Table S2-B). Grade 3–4 AEs, irrespective of attribution, were observed in 11 (33%) patients, while grade 3–4 treatment-related AEs were observed in 6 (18%) patients, with lipase elevation as the most common event (6%). Treatment-related serious AEs were reported in 7 (21%) patients (Table S2-C). Grade 3–4 endocrine events were noted as treatment-related select AEs in 2 patients (Table 2). One patient had grade-2 pneumonitis. Three (9%) patients discontinued due to treatment-related AEs (Table S3).

For both the concurrent and sequenced regimens, treatment-related AEs were manageable and generally reversible with immunosuppressants and/or replacement therapy (for endocrinopathies) per previously established algorithms.¹² Amongst the 86 patients treated on the study, 28 of 73 patients (38%) with drug-related adverse events required management with systemic glucocorticoids. Three patients required additional immunosuppressive therapy with infliximab (2 patients) or mycophenolate mofetil (1 patient). No treatment-related deaths were reported.

Clinical Activity

Clinical activity was observed with both regimens (Tables 3A and B). In the concurrent-regimen cohorts, confirmed objective responses (OR) by mWHO criteria were observed in 21 of 52 (40%; 95% CI: 27-55) response-evaluable patients across all doses. After noting several patients who demonstrated major responses (approaching CR), we assessed the number of patients with 80% tumor reduction. This type of response was uncommon in published studies of checkpoint blockade.^{3, 5} Sixteen patients had 80% tumor reduction at 12 weeks, including 5 CRs (Table 3A, Figures 1A, 2, and S2A-C). In addition to the 21 patients with OR by mWHO criteria, 4 patients experienced an objective response by immune-related response criteria and 2 patients had unconfirmed responses. These patients were not included in the calculation of objective response rates. For the concurrent regimen, overall evidence of clinical activity (conventional, unconfirmed, or immune-related response or SD 24 weeks) was observed in 65% (95% CI: 51-78; Table 3A). The profound impact of the concurrent combination can best be appreciated in the waterfall plot (Figure 1B). Responses were ongoing among 19 of 21 responders, with durations ranging from 6.1+ to 72.1+ weeks at the time of data analysis (Table S1). For patients treated at the MTD (cohort 2, 1 mg/kg nivolumab + 3 mg/kg ipilimumab), OR occurred in 9 of 17 (53%; 95% CI: 28-77) patients, including 3 CRs. All 9 responders achieved 80% tumor reduction at their first scheduled on-treatment assessment (Table 3A and Figure 1A).

For patients in the sequenced-regimen cohorts, 6 of 30 patients achieved OR (20%; 95% CI: 8-39) including 1 CR. Four (13%) patients achieved 80% tumor reduction at 8 weeks (Table 3B and Figures 1C and S2D). Additional patients had immune-related (n=3) or unconfirmed (n=3) responses. When objective, immune-related or unconfirmed responses or SD 24 weeks are considered, evidence of clinical activity for the sequenced regimen was observed in 43% (95% CI: 26-63). The waterfall plot reveals that patients who did not respond to prior ipilimumab can respond to subsequent nivolumab (Figure 1D).

Assessment of Tumor PD-L1 Expression and Absolute Lymphocyte Count

Tumor PD-L1 expression and alterations in the peripheral blood ALC have been explored as biomarkers for nivolumab and ipilimumab monotherapy, respectively.^{5, 13-16} To investigate the relationship of these observations to our current results with the nivolumab/ipilimumab combination, we characterized tumor PD-L1 expression via immunohistochemical staining and analyzed pharmacodynamic changes in peripheral blood ALC. Using a 5% cut off to define PD-L1 positivity, tumor specimens from 21 of 56 (38%) patients were PD-L1-positive (Table S4; representative images, Figure S3). Objective responses were seen in patients with either PD-L1-positive (6/13) or PD-L1-negative (9/22) tumors amongst patients treated with the concurrent regimen (post-hoc P-value >0.99; Fisher's exact test). In sequenced-regimen cohorts, a numerically higher number of OR were seen in patients with PD-L1-positive tumor samples (4/8) compared with patients with PD-L1-negative tumors (1/13).

In contrast to observations with ipilimumab monotherapy, a consistent rise in ALC from baseline was not detected in patients treated with the concurrent combination or in patients treated with nivolumab following ipilimumab therapy (Table S5). In the concurrent-regimen cohorts, patients with a low ALC at weeks 5 to 7 (<1000 cells/ μ L¹⁴) had similar OR (43%) compared with patients with a normal ALC at weeks 5 to 7 (40%; Table S6). Likewise, in the sequenced-regimen cohorts, 17% of patients with low ALC had OR and 23% of patients with normal or high ALC had OR.

DISCUSSION

The immune system is coordinately regulated to ensure effective elimination of foreign pathogens, while minimizing damage to normal tissues. Until recently, cancer immunotherapy has focused substantial effort on approaches that enhance anti-tumor immune responses by adoptive-transfer of activated effector cells, immunization against relevant antigens, or providing non-specific immune-stimulatory agents such as cytokines. In the past decade, agents that block inhibitory T-cell checkpoints have shown substantial clinical antitumor activity, including antibodies blocking CTLA-4,^{3, 4, 17-19} PD-1,^{5, 20, 21} and PD-L1.²² Given that immunologic checkpoints are non-redundant and can inhibit T-cell activation, proliferation and effector function within lymph nodes and/or the tumor microenvironment, we hypothesized that combined blockade of CTLA-4 and PD-1 could produce greater anti-tumor activity than single agents.²³

Although not formally compared in this study, the concurrent nivolumab/ipilimumab regimen achieved objective response rates that exceed the prior reported experience with

either nivolumab or ipilimumab alone^{3, 5}. Most importantly, rapid and deep responses were achieved in a substantial portion of treated patients, with the majority of responding patients having achieved 80% tumor regression at the time of the initial tumor assessment, including some with extensive and bulky tumor burden. Particularly striking was the observation that 31% of response-evaluable patients treated across the concurrent-regimen cohorts demonstrated 80% tumor regression by week 12. At the MTD for the concurrent regimen, all 9 responding patients demonstrated 80% tumor regression with 3 CRs. In contrast, in clinical experience to date, <3% melanoma patients who received nivolumab or ipilimumab at 3 mg/kg achieved a CR.^{3, 5} The overall activity of this immunotherapy combination compares favorably with that of other agents approved or being developed for advanced melanoma including the targeted inhibitors,²⁴ although we recognize that these results must be interpreted with caution given that this is a phase 1 trial which is subject to biases, including patient selection and small patient numbers. The potential advantage of this immunotherapy combination is the durability of response which is characteristic of immunotherapies, as demonstrated in prior trials.^{25, 26}

These initial data suggest that rapid responses of a greater magnitude may be achieved in patients treated with the nivolumab/ipilimumab combination compared with the historical experience of either agent alone.^{3, 5} Responses were generally durable, and were observed even in patients whose treatment was terminated early, secondary to a DLT. Responding patients included those with elevated LDH, M1c disease and bulky multi-focal tumor burden. Similar to prior reports regarding ipilimumab^{3, 4} or nivolumab⁵ monotherapy, conventional objective response rates may not fully capture the spectrum of clinical activity and potential benefit in patients treated with the concurrent nivolumab/ipilimumab regimen in that a number of patients experienced either long-term SD or unconventional “immune-related” patterns of response. Indeed, even among the 7 patients in the concurrent regimen with SD 24 weeks or irSD 24 weeks as best response, 6 demonstrated meaningful tumor regression of at least 19%, and the seventh patient has declining tumor burden after prolonged stable disease. Prior experience with checkpoint blockade monotherapy supports the observation that some patients may survive for extended periods of time with SD as the best objective response, lending credence to the hypothesis that re-establishment of the equilibrium phase of immune surveillance is a desirable outcome.¹

The observation that patients can achieve OR when treated sequentially with nivolumab after prior ipilimumab indicates that lack of response to CTLA-4 blockade does not preclude clinical benefit from PD-1 blockade and further supports the non-redundant nature of these co-inhibitory pathways. Notably, prior data suggest a potential association between the occurrence of OR and tumor PD-L1 expression in patients receiving nivolumab⁵ and correlation between overall survival and increases in peripheral ALC in patients treated with ipilimumab.^{13–16} In this study of the nivolumab/ipilimumab combination, OR were observed in patients irrespective of ALC or baseline tumor PD-L1 expression. PD-L1 expression was measured using an immunohistochemistry antibody and assay that is different from those in published reports,^{5, 10, 27} and factors including assay conditions, biopsy samples, and tumor heterogeneity may have affected these results. However, the PD-L1 positivity rate for tumor specimens in this study (38%) was similar to those for published studies in metastatic melanoma (43% to 40%).^{10, 27} Thus, our results suggest that baseline tumor PD-L1

expression and ALC may be less relevant in the setting of active combination regimens capable of inducing rapid and pronounced tumor regression. Prospective exploration of these observations is planned for future randomized studies.

The spectrum of adverse events observed among patients treated with the concurrent regimen was qualitatively similar to historical experience with nivolumab or ipilimumab monotherapy, although the rate of AEs was increased in patients treated with the combination. We observed grade 3–4 treatment-related AEs in 53% of patients treated with the concurrent nivolumab/ipilimumab regimen, compared with historical rates of 20% in patients treated with ipilimumab monotherapy at a dose of 3 mg/kg and 15% in patients treated with nivolumab alone.^{3, 5} In the sequenced-regimen cohorts, 18% of patients experienced grade 3–4 treatment-related AEs. AEs experienced by patients treated with the concurrent and sequenced regimens were manageable and/or generally reversible using existing treatment algorithms.¹²

Collectively, these results suggest that nivolumab and ipilimumab can be administered concurrently with a manageable safety profile. Materially distinct and superior patterns of clinical response were observed in patients treated with the combination compared with the historical experience with either single agent, including increased rates and depth of OR even in patients with bulky advanced disease, as well as increased rates of overall disease control. Responses are durable, although longer follow-up is needed in some cohorts. The impact of this regimen on the overall survival of patients treated with this combination remains to be defined. The results of the current study support a randomized phase 3 trial to investigate the clinical efficacy of nivolumab versus ipilimumab versus concurrent nivolumab/ipilimumab in patients with advanced melanoma.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Clinical activity of concurrent and sequenced regimens of nivolumab and ipilimumab
Representative spider plots show changes from baseline in the tumor burden, measured as the sum of products of perpendicular diameters of all target lesions, in patients who received the concurrent regimen of 1 mg/kg nivolumab + 3 mg/kg ipilimumab, the maximum tolerated dose, (panel A) and in patients who received the sequenced regimen of 1 mg/kg nivolumab after prior ipilimumab therapy (panel C). Red triangles indicate the first occurrence of a new lesion. Representative waterfall plots show maximum percentage response in baseline target lesions in all patients who received the concurrent regimen (panel B) or the sequenced regimen (panel D). For sequenced regimen cohorts (panel D), “*” denotes patients who had radiographic progression with prior ipilimumab treatment.

Figure 2. Tumor regressions of patients who received concurrent nivolumab and ipilimumab
 In Panel A, regression of tumors in a 52-year old patient who received 1 mg/kg nivolumab + 3 mg/kg ipilimumab, the maximum tolerated dose. This patient presented with extensive neck, mediastinal, axillary, abdominal and pelvic lymphadenopathy, bilateral pulmonary nodules, small bowel metastasis, peritoneal implants and diffuse subcutaneous nodules. Baseline lactate dehydrogenase (LDH) was $2.25 \times$ upper limit of normal, hemoglobin was 9.7 g/dL, and symptoms included nausea and vomiting. Within 4 weeks of treatment the LDH normalized, symptoms improved (appetite increased, nausea decreased), and cutaneous lesions were regressing. At week-12 scans, there was marked reduction in all areas of disease. Arrows denote location of metastatic disease. In Panel B, regression of tumors in a 61-year-old patient who received 0.3 mg/kg nivolumab + 3 mg/kg ipilimumab. The patient presented with stage IV (M1c) melanoma of an unknown primary site, metastatic to the stomach and the mesentery. LDH was 225 and hemoglobin was 9.6 g/dL after a recent

transfusion. Twelve weeks after the initiation of study treatment, a CT scan showed an 86% reduction in the bulky disease burden.

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Table 1

Baseline Characteristics and Prior Therapy of All Treated Patients*

Variable	Concurrent Treatment (n=53)	Sequenced Treatment (n=33)
Age - yr		
Median	58	64
Range	22-79	23-89
Sex – no. (%)		
Male	32 (60)	18 (55)
Female	21 (40)	15 (45)
ECOG performance status – no. (%)		
0	44 (83)	22 (67)
1	8 (15)	11 (33)
Unknown	1 (2)	0
M-stage at study entry – no. (%)		
M1a	8 (15)	5 (15)
M1b	11 (21)	5 (15)
M1c	30 (57)	18 (55)
Unknown	4 (8)	5 (15)
Lactate dehydrogenase level – no. (%)		
Upper limit of the normal range	33 (62)	21 (64)
>Upper limit of the normal range	20 (38)	12 (36)
Nature of prior therapy – no. (%)		
Surgery	51 (96)	31 (94)
Radiotherapy	11 (21)	17 (52)
Systemic cancer therapy	20 (38)	33 (100)
Immunotherapy	9 (17)	33 (100)
Interleukin-2	8 (15)	1 (3)
B-RAF inhibitor	3 (6)	2 (6)
Number of prior systemic cancer therapies – no. (%)		
0	33 (62)	0
1	14 (26)	18 (55)
2	5 (9)	10 (30)
3	1 (2)	5 (15)
Lesions at baseline – no. (%)		
Bone	5 (9)	1 (3)
Central nervous system/brain	0	1 (3)
Liver	16 (30)	13 (39)
Lung	25 (47)	16 (48)
Lymph node	26 (49)	8 (24)

Variable	Concurrent Treatment (n=53)	Sequenced Treatment (n=33)
Soft tissue/other organs	34 (64)	19 (58)

* ECOG denotes Eastern Cooperative Oncology Group.

Treatment-Related Select Adverse Events, Worst Grade, That Occurred in at Least One of All Treated Patients

Table 2

Select Adverse Event *	0.3 mg/kg Nivolumab + 3 mg/kg Ipilimumab (n=14)		1 mg/kg Nivolumab + 3 mg/kg Ipilimumab (n=17)		3 mg/kg Nivolumab + 1mg/kg Ipilimumab (n=16)		3 mg/kg Nivolumab + 3 mg/kg Ipilimumab (n=6)		Concurrent Treatment, All (n=53)		1 mg/kg Nivolumab + Prior Ipilimumab (n=17)		3 mg/kg Nivolumab + Prior Ipilimumab (n=16)		Sequenced Treatment, All (n=33)	
	All Gr	Gr 3-4	All Gr	Gr 3-4	All Gr	Gr 3-4	All Gr	Gr 3-4	All Gr	Gr 3-4	All Gr	Gr 3-4	All Gr	Gr 3-4	All Gr	Gr 3-4
Category	number of patients (percent)															
Pulmonary †	1 (7)	0	2 (12)	1 (6)	0	0	0	0	3 (6)	1 (2)	0	0	1 (6)	0	1 (3)	0
Pneumonitis	1 (7)	0	2 (12)	1 (6)	0	0	0	0	3 (6)	1 (2)	0	0	1 (6)	0	1 (3)	0
Endocrinopathies †	1 (7)	0	3 (18)	0	1 (6)	0	2 (33)	1 (17)	7 (13)	1 (2)	3 (18)	2 (12)	0	0	3 (9)	2 (6)
Hypothyroidism	0	0	2 (12)	0	0	0	0	0	2 (4)	0	1 (6)	0	0	0	1 (3)	0
Hypophysitis	0	0	1 (6)	0	0	0	1 (17)	1 (17)	2 (4)	1 (2)	1 (6)	0	0	0	1 (3)	1 (3)
Thyroiditis	0	0	1 (6)	0	1 (6)	0	1 (17)	0	3 (6)	0	0	0	0	0	0	0
Adrenal insufficiency	0	0	2 (12)	0	0	0	0	0	2 (4)	0	1 (6)	1 (6)	0	0	1 (3)	1 (3)
Hyperthyroidism	0	0	1 (6)	0	0	0	1 (17) ‡	0	2 (4) ‡	0	0	0	0	0	0	0
Thyroid function test abnormal	1 (7)	0	0	0	0	0	0	0	1 (2)	0	0	0	0	0	0	0
Hepatic †	4 (29)	3 (21)	5 (29)	3 (18)	2 (13)	1 (6)	1 (17)	1 (17)	12 (23)	8 (15)	0	0	1 (6)	0	1 (3)	0
Aspartate aminotransferase increased	4 (29)	3 (21)	4 (24)	2 (12)	2 (13)	1 (6)	1 (17)	1 (17)	11 (21)	7 (13)	0	0	0	0	0	0
Alanine aminotransferase increased	3 (21)	2 (14)	5 (29)	3 (18)	2 (13)	0	1 (17)	1 (17)	11 (21)	6 (11)	0	0	1 (6)	0	1 (3)	0
Gastrointestinal †	5 (36)	1 (7)	6 (35)	2 (12)	6 (38)	2 (13)	3 (50)	0	20 (38)	5 (9)	2 (12)	0	1 (6)	0	3 (9)	0
Diarrhea	5 (36)	0	5 (29)	1 (6)	6 (38)	2 (13)	3 (50)	0	19 (36)	3 (6)	2 (12)	0	1 (6)	0	3 (9)	0

Select Adverse Event *	0.3 mg/kg Nivolumab + 3 mg/kg Ipilimumab (n=14)		1 mg/kg Nivolumab + 3 mg/kg Ipilimumab (n=17)		3 mg/kg Nivolumab + 1mg/kg Ipilimumab (n=16)		3 mg/kg Nivolumab + 3 mg/kg Ipilimumab (n=6)		Concurrent Treatment, All (n=53)		1 mg/kg Nivolumab + Prior Ipilimumab (n=17)		3 mg/kg Nivolumab + Prior Ipilimumab (n=16)		Sequenced Treatment, All (n=33)	
	All Gr	Gr 3-4	All Gr	Gr 3-4	All Gr	Gr 3-4	All Gr	Gr 3-4	All Gr	Gr 3-4	All Gr	Gr 3-4	All Gr	Gr 3-4	All Gr	Gr 3-4
Colitis	1 (7)	1 (7)	2 (12)	1 (6)	1 (6)	0	1 (17)	0	5 (9)	2 (4)	0	0	0	0	0	0
Renal [‡]	1 (7)	1 (7)	1 (6)	1 (6)	1 (6)	1 (6)	0	0	3 (6)	3 (6)	0	0	0	0	0	0
Blood creatinine increased	1 (7)	1 (7)	1 (6)	1 (6)	1 (6)	1 (6)	0	0	3 (6)	3 (6)	0	0	0	0	0	0
Renal failure, acute	0	0	1 (6)	1 (6)	1 (6)	1 (6)	0	0	2 (4)	2 (4)	0	0	0	0	0	0
Renal failure	0	0	1 (6)	1 (6)	0	0	0	0	1 (2)	1 (2)	0	0	0	0	0	0
Tubulointerstitial nephritis	1 (7)	0	0	0	0	0	0	0	1 (2)	0	0	0	0	0	0	0
Skin [‡]	10 (71)	1 (7)	14 (82)	0	10 (63)	1 (6)	3 (50)	0	37 (70)	2 (4)	4 (24)	0	4 (25)	0	8 (24)	0
Rash	8 (57)	1 (7)	11 (65)	0	7 (44)	1 (6)	3 (50)	0	29 (55)	2 (4)	3 (18)	0	0	0	3 (9)	0
Pruritus	6 (43)	0	11 (65)	0	7 (44)	0	1 (17)	0	25 (47)	0	2 (12)	0	4 (25)	0	6 (18)	0
Urticaria	0	0	0	0	1 (6)	0	0	0	1 (2)	0	0	0	0	0	0	0
Blister	0	0	1 (6)	0	0	0	0	0	1 (2)	0	0	0	0	0	0	0
Infusion reaction [‡]	0	0	1 (6)	0	0	0	0	0	1 (2)	0	0	0	0	0	0	0
Infusion-related reaction	0	0	1 (6)	0	0	0	0	0	1 (2)	0	0	0	0	0	0	0

* Adverse events that require more frequent monitoring and/or unique intervention, based on a prespecified list of MedDRA terms.¹¹

[‡]The numbers reported for the specific adverse events within an organ category may be greater than the number reported for the organ category because patients who had more than one adverse event were counted for each event but were counted only once for the organ category.

[‡]One patient with unknown grade.

Table 3A

Clinical Activity of Patients Who Received the Concurrent Regimen of Nivolumab and Ipilimumab*

Cohort	Dose	Response-Evaluable Patients [†] , n	CR n	PR n	uPR [‡] n	irPR [§] n	SD 24 wk n	irSD [§] 24 wk n	Objective Response Rate [¶] % [95% CI]	Aggregate Clinical Activity Rate [¶] % [95% CI]	80% Tumor Reduction at 12 wk n (%)
1	0.3 mg/kg nivolumab + 3 mg/kg ipilimumab	14	1	2	0	2	2 (14)	0	21 [5-51]	50 [23-77]	4 (29)
2	1 mg/kg nivolumab + 3 mg/kg ipilimumab	17	3	6	0	0	0	2 (12)	53 [28-77]	65 [38-86]	7 (41)**
2a	3 mg/kg nivolumab + 1 mg/kg ipilimumab	15	1	5	2	1	2 (13)	0	40 [16-68]	73 [45-92]	5 (33)
3	3 mg/kg nivolumab + 3 mg/kg ipilimumab	6	0	3	0	1	0	1 (17)	50 [12-88]	83 [36-100]	0
All	Concurrent treatment	52	5	16	2	4	4 (8)	3 (6)	40 [27-55]	65 [51-78]	16 (31)

* CR denotes complete response, PR partial response, uPR unconfirmed partial response, irPR immune-related partial response, SD stable disease, irSD immune-related stable disease.

[†] Response-evaluable patients were those who received at least one dose of study therapy, had measurable disease at baseline, and one of the following: 1) at least one on-treatment tumor evaluation, 2) clinical progression, or 3) death prior to the first on-treatment tumor evaluation.

[‡] Patients who had a PR after one tumor assessment but did not have sufficient follow-up time for confirmation of the initial PR.

[§] Patients who had target tumor-lesion reduction in the presence of new lesions, consistent with immune-related PR or SD.¹¹

[¶] $\frac{[(CR + PR) / \text{no. response-evaluable patients}] \times 100}{[(CR + PR + uCR + uPR + SD + 24 \text{ wk} + irSD + 24 \text{ wk}) / \text{no. response-evaluable patients}] \times 100}$. Confidence intervals were estimated by the Clopper-Pearson method.

** Two additional patients in cohort 2 achieved 80% tumor reduction at their first scheduled assessment, which was conducted after week 12.

Table 3B

Clinical Activity of Patients Who Received the Sequenced Regimen of Nivolumab and Ipilimumab*

Cohort	Dose	Response-Evaluable Patients [†] n	CR n	PR n	uPR [‡] n	irPR [§] n	SD 24 wk n	irSD [§] 24 wk n	Objective Response Rate [¶] % [95% CI]	Aggregate Clinical Activity Rate [¶] % [95% CI]	80% Tumor Reduction at 8 wk n (%)
6	1 mg/kg nivolumab + prior ipilimumab	16	1	5	2	2	1 (6)	0	38 [15-65]	69 [41-89]	4 (25)
7	3 mg/kg nivolumab + prior ipilimumab	14	0	0	1	1	0	0	0	14 [2-43]	0
All	Sequenced treatment	30	1	5	3	3	1 (3)	0	20 [8-39]	43 [26-63]	4 (13)

* CR denotes complete response, PR partial response, uPR unconfirmed partial response, irPR immune-related partial response, SD stable disease, irSD immune-related stable disease.

[†] Response-evaluable patients were those who received at least one dose of study therapy, had measurable disease at baseline, and one of the following: 1) at least one on-treatment tumor evaluation, 2) clinical progression, or 3) death prior to the first on-treatment tumor evaluation.

[‡] Patients who had a PR after one tumor assessment but did not have sufficient follow-up time for confirmation of the initial PR.

[§] Patients who had target tumor-lesion reduction in the presence of new lesions, consistent with immune-related PR or SD.¹¹

[¶] $\frac{[(CR + PR) / \text{no. response-evaluable patients}] \times 100}{[(CR + PR + uCR + uPR + irPR + SD - 24 \text{ wk} + irSD - 24 \text{ wk}) / \text{no. response-evaluable patients}] \times 100}$. Confidence intervals were estimated by the Clopper-Pearson method.