Nivolumab in Patients with Advanced Platinum-resistant Urothelial Carcinoma: Efficacy, Safety, and Biomarker Analyses with Extended Follow-up from CheckMate 275

Matthew D. Galsky[1], Abdel Saci[2], Peter M. Szabo[2], G. Celine Han[2], Gary Grossfeld[2], Sandra Collette[2], Arlene Siefker-Radtke[3], Andrea Necchi[4], and Padmanee Sharma[3]

ABSTRACT

Purpose: We report efficacy and safety with extended follow-up, and exploratory biomarker analyses from the phase II CheckMate 275 trial to identify biomarkers of response to nivolumab in platinum-resistant metastatic or unresectable urothelial carcinoma (mUC).

Patients and Methods: Patients received nivolumab 3 mg/kg once every 2 weeks until disease progression, unacceptable toxicity, or other protocol-defined reasons. The primary endpoint was objective response rate (ORR) per blinded independent review committee (BIRC; using RECIST v1.1) in all treated patients and by tumor PD-L1 expression. Key secondary endpoints were progression-free survival (PFS) per BIRC using RECIST v1.1 and overall survival (OS) in all patients and by PD-L1 expression. Exploratory endpoints included safety and biomarker analyses of tumor mutational burden (TMB), PD-L1, and previously identified mutational signatures.

Results: Of 270 treated patients, 139 had evaluable TMB. With 33.7 months' minimum follow-up, ORR per BIRC, median PFS, and median OS [95% confidence interval (CI)] in all treated patients were 20.7% (16.1–26.1), 1.9 months (1.9–2.3), and 8.6 months (6.1–11.3), respectively. No new safety signals were identified. Higher TMB was associated ($P < 0.05$) with improved ORR [OR (95% CI): 2.13 (1.26–3.60)], PFS [HR: 0.75 (0.61–0.92)], and OS [HR: 0.73 (0.58–0.91)]. TMB combined with PD-L1 better predicted ORR, PFS, and OS than PD-L1 alone. Higher mutational signature 2 score was associated with better OS but did not improve the predictive value of TMB.

Conclusions: These results support the durable antitumor activity of nivolumab and suggest that TMB may enrich for better response in mUC. Future studies of TMB/PD-L1 as biomarkers for response to nivolumab in randomized trials are warranted.

See related commentary by Swami et al., p. 5059

Introduction

Immune checkpoint inhibitors have become the standard-of-care for patients with platinum-resistant metastatic or surgically unresectable urothelial carcinoma (mUC; ref. 1). The single-arm, phase II CheckMate 275 trial previously demonstrated the meaningful clinical benefit and manageable safety profile of nivolumab, a programmed death receptor 1 (PD-1) inhibitor, in patients with previously treated mUC. At a minimum follow-up of 6 months, the objective response rate (ORR) per blinded independent review committee (BIRC) using RECIST v1.1 was 19.6% [95% confidence interval (CI), 15.0–24.9], median duration of response (DOR) was not reached, median progression-free survival (PFS) per BIRC using RECIST v1.1 was 2.0 months (95% CI, 1.9–2.6), and median overall survival (OS) was 8.7 months (95% CI, 6.1–not estimable; ref. 2). Treatment-related adverse events (AEs) occurred in 64% of patients. The most common treatment-related AE of any grade was fatigue (17%). Grade 3–4 treatment-related AEs occurred in 18% of patients; most commonly grade 3 fatigue (2%) and diarrhea (2%). On the basis of these results, nivolumab is now approved in the United States and Europe for the treatment of patients with previously treated locally advanced or mUC (3, 4).

The identification of predictive biomarkers that could enrich for response to nivolumab specifically in this setting (as opposed to prognostic biomarkers that simply provide information about outcome regardless of treatment) will help identify which patients may optimally benefit from this treatment. High tumor mutational burden (TMB) has been shown to correlate with response to immune checkpoint inhibitors across multiple tumor types, including untreated non–small cell lung cancer (NSCLC), previously treated urothelial carcinoma, and small cell lung cancer (5–9). In advanced melanoma, high TMB has been associated with response in patients receiving ipilimumab, nivolumab followed by ipilimumab, and nivolumab in ipilimumab-naïve patients (9–12). In the CheckMate 066 (nivolumab vs. dacarbazine) and CheckMate 067 (nivolumab plus ipilimumab vs. nivolumab or ipilimumab monotherapy) trials in advanced melanoma, higher TMB was associated with improved OS and PFS (13). In addition, recent research has suggested that a predictive model utilizing a range of biomarkers in combination could inform clinical decision making with respect to the use of immune checkpoint inhibitors in mUC (14). Analysis of long-term clinical data is also needed to determine the durability of clinical benefit observed with immunotherapy-based regimens in platinum-resistant mUC, and there remains a need to identify optimal predictive biomarkers of response in this setting.

[1]Icahn School of Medicine at Mount Sinai/Tisch Cancer Institute, New York, New York. [2]Bristol Myers Squibb, Princeton, New Jersey. [3]MD Anderson Cancer Center, University of Texas, Houston, Texas. [4]Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy.

Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

Abdel Saci was an employee of BMS at the time the analysis was conducted.

Corresponding Author: Matthew D. Galsky, Icahn School of Medicine at Mount Sinai, 1470 Madison Ave., New York, NY 10029-6542. Phone: 212-659-5452; Fax: 212-659-5533; E-mail: matthew.galsky@mssm.edu

Clin Cancer Res 2020;26:5120–8

doi: 10.1158/1078-0432.CCR-19-4162

Translational Relevance

Our results are consistent with the initial report of this study, showing durable antitumor activity of nivolumab for patients with platinum-resistant metastatic or unresectable urothelial carcinoma and no new safety signals. In exploratory biomarker analyses, high tumor mutational burden (TMB ≥ 170 mutations per tumor) was associated with higher objective response rate, longer progression-free survival (PFS), and longer overall survival (OS) in patients treated with nivolumab, and patients with high TMB showed improved outcomes across baseline programmed death receptor 1 (PD-L1) expression levels. These results suggest that patients with higher TMB may have improved response versus patients with low TMB. The combination of TMB with PD-L1 was a better predictor of PFS and OS than TMB alone, highlighting the potential for a composite biomarker approach to help identify patients who could most benefit from nivolumab. Finally, higher mutational signature 2 score was associated with better OS but did not improve the predictive value of TMB.

Here, we report updated efficacy and safety, along with exploratory biomarker analyses of TMB, PD-L1, and mutational signatures, from CheckMate 275 with a minimum follow-up of 33.7 months.

Patients and Methods

Study design and treatment

CheckMate 275 is a multicenter, single-arm, phase II trial of nivolumab monotherapy for patients with platinum-resistant mUC. Full details of the study design were described previously (2). Patients received nivolumab 3 mg/kg every 2 weeks until documented disease progression, unacceptable toxicity, or other protocol-defined reasons (2). Treatment beyond disease progression was permitted if nivolumab was tolerated and clinical benefit was noted by investigator assessment. Dose modifications were not allowed, but dose delays due to AEs were permitted.

Patients

Patients ≥18 years of age with histologic evidence of metastatic or surgically unresectable, locally advanced urothelial carcinoma; measurable disease by CT or MRI per RECIST v1.1; and progression or recurrence after at least one platinum-based regimen for metastatic disease were included. Additional eligibility criteria were reported previously (2). Patients with active brain metastases, previous malignancy within the past 3 years, autoimmune disease, immunosuppressive treatment with corticosteroids or other drugs within 14 days of study drug administration, or previous treatment with agents targeting T-cell costimulation or immune checkpoint pathways were excluded from the study.

Endpoints and assessments

The primary endpoint was ORR (including DOR) per BIRC using RECIST v1.1 in all treated patients and in patients with tumor PD-L1 expression of ≥1% and ≥5%. Time to response and DOR were estimated in patients with confirmed complete or partial responses. Responses were confirmed by a second scan at least 4 weeks after criteria for objective response were met. Key secondary endpoints were PFS per BIRC using RECIST v1.1 and OS in all patients and in patients with ≥1% and ≥5% tumor PD-L1 expression. Exploratory endpoints included safety and biomarker analyses of TMB, PD-L1, and previously identified mutational signatures. A landmark analysis of OS by best response within the first 12 months was performed, which included 100 treated patients still alive at 12 months with best overall response (BOR) of complete response, partial response, stable disease, or progressive disease.

Disease assessments with CT or MRI were performed at baseline, every 8 weeks (±1 week) for 48 weeks, then every 12 weeks (±1 week) by BIRC using RECIST v1.1 until both disease progression and treatment discontinuation occurred. Safety was assessed on day 1 of each treatment cycle, at follow-up visits approximately 35 days after the last dose, and approximately 80 days after that. Patients were subsequently followed up every 3 months for survival assessment. AEs were graded according to NCI Common Terminology Criteria for Adverse Events (v4.0). Tumor PD-L1 membrane expression (≥1% or ≥5% tumor cell membrane staining) was assessed at a central laboratory using the Dako PD-L1 IHC 28-8 pharmDx Kit (Dako, an Agilent Technologies company).

Study oversight

This study was approved by the institutional review board or independent ethics committee at each center and conducted in accordance with Good Clinical Practice guidelines defined by the International Conference on Harmonisation. All patients provided written informed consent to participate based on the principles of the Declaration of Helsinki.

Statistical analyses and biomarker analyses

Continuous variables were summarized with descriptive statistics. ORRs and the corresponding 95% CIs were based on the Clopper and Pearson method (15). OS, PFS, and DOR were estimated using Kaplan–Meier methodology (16).

The TMB analysis included 139 TMB-evaluable patients. Tumor DNA was extracted from pretreatment archival tumor tissue (and matched with whole blood samples) and profiled by whole-exome sequencing. The majority of tissue samples were from primary tumors with less than 10% of samples from metastatic sites. In this *post hoc* exploratory analysis, TMB was defined as the total number of missense somatic mutations per tumor and evaluated as a continuous variable, by tertiles (low <85, medium 85–169, high ≥170), and using a median cutoff (low <113, high ≥113). Cox proportional hazards regression models were used to assess the dependence of PFS or OS on TMB alone and with PD-L1. The models included linear effects of each biomarker and the multiplicative interaction between them; the magnitudes of associations were summarized by HRs. Linear logistic regression models were used to assess the dependence of objective response on the biomarker scores; the magnitudes of associations were summarized by ORs. HRs and ORs were scaled to reflect the difference between the 75th and 25th biomarker percentiles. Two-sided 95% CIs for ORs were based on Wald test statistics, and two-sided 95% CIs for ORRs were estimated by the Clopper–Pearson exact method (15).

Likelihood-ratio tests were used to assess overall biomarker and interaction effects. Kaplan–Meier plots based on categorization of the biomarker scores were used to illustrate associations with PFS or OS. All TMB-related data analyses were performed with R 3.4.1 for Linux.

Mutational signatures are combinations of mutation types that arise from specific mutagenesis processes (17). A previous study identified 30 validated mutational signatures across human cancers (18). To examine mutational signatures in CheckMate 275, whole-exome

sequencing data and clinical annotations were collected and analyzed ($n = 139$). Reads were aligned to human genome reference hg19, and somatic mutations were detected using TNsnv. Percentages of mutations across 30 mutational signatures were generated using deconstructSigs, which infers signature activity with given known signatures (19). Association of the most prevalent mutational signatures with TMB, previously known clinical biomarkers, and clinical efficacy (OS, PFS, and BOR) was examined.

Data-sharing Statement

Bristol Myers Squibb policy on data sharing may be found at https://www.bms.com/researchers-and-partners/independent-research/data-sharing-request-process.html.

Table 1. Best overall response per BIRC in all treated patients and TMB-evaluable patients.

Response	All treated patients $N = 270$	TMB-evaluable patients $n = 139$
Objective response rate, % (95% CI)	20.7 (16.1–26.1)	21.6 (15.6–29.1)
Best overall response, *n* (%)		
Complete response	18 (6.7)	12 (8.6)
Partial response	38 (14.1)	18 (12.9)
Stable disease	56 (20.7)	27 (19.4)
Progressive disease	111 (41.1)	61 (43.9)
Not evaluable	47 (17.4)	21 (15.1)

Results

Patients

A total of 270 patients were treated with nivolumab. At a minimum follow-up of 33.7 months, 11 (4.1%) patients continued treatment with nivolumab. The primary reasons for treatment discontinuation were disease progression (60.7%) and AEs unrelated to nivolumab treatment (14.1%; Supplementary Fig. S1). Demographic and baseline characteristics were reported previously (2).

Efficacy

In all treated patients, the ORR (95% CI) per BIRC with extended follow-up was 20.7% (16.1–26.1), with complete responses in 6.7% of patients (**Table 1**). ORR (95% CI) was 16.4% (10.8–23.5) and 25.8% (18.4–34.4) in patients with PD-L1 expression <1% ($n = 146$) and ≥1% ($n = 124$), respectively, and 16.0% (11.1–22.1) and 31.3% (21.6–42.4) in patients with PD-L1 expression <5% ($n = 187$) and ≥5% ($n = 83$),

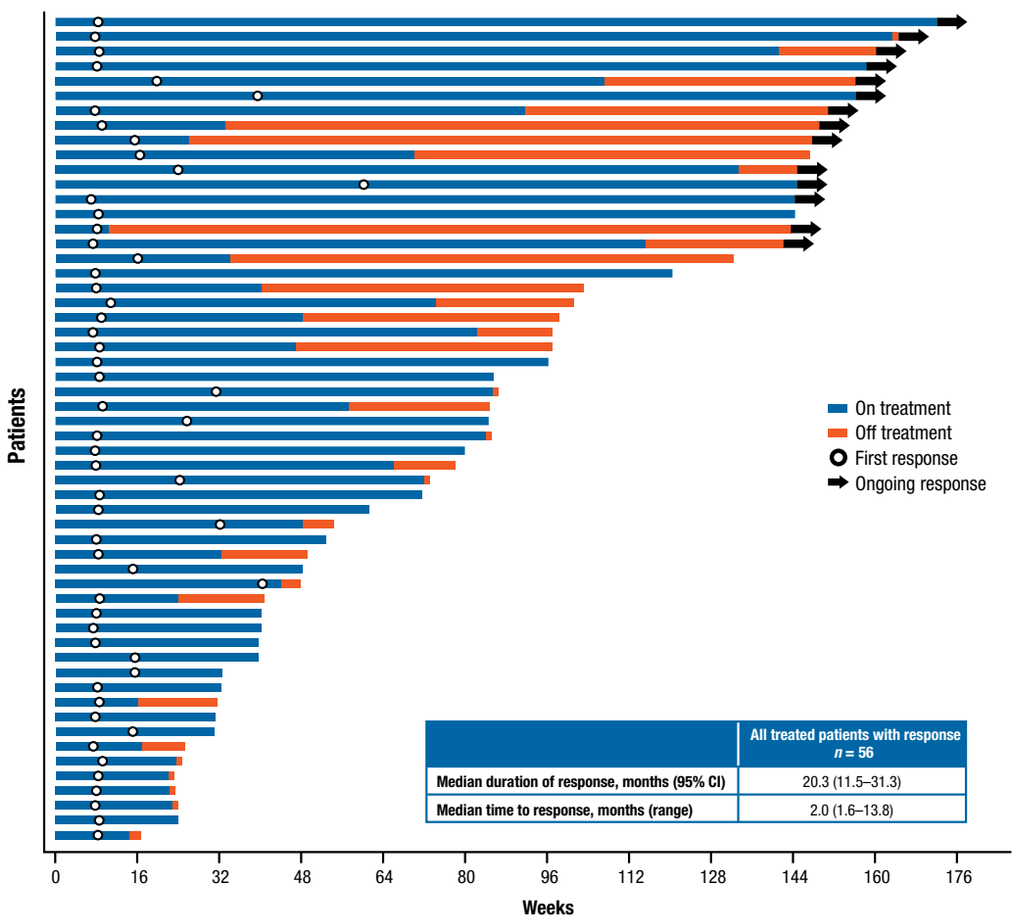

Figure 1.
Time to and duration of response per BIRC in all treated patients with response.

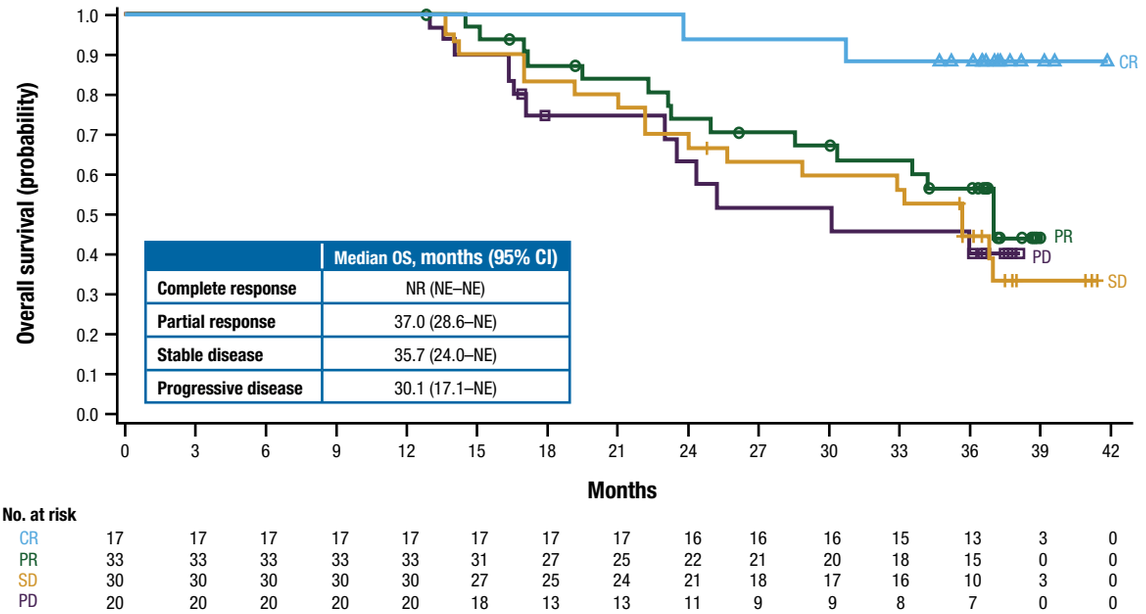

Figure 2.

Landmark analysis of overall survival by best response within the first 12 months. This analysis included 100 treated patients still alive at 12 months with BOR of CR, PR, SD, or PD within the first 12 months. CR, complete response; NE, not estimable; NR, not reached; PD, progressive disease; PR, partial response; SD, stable disease.

respectively (Supplementary Table S1). Median DOR was 20.3 months (95% CI, 11.5–31.3) in all treated patients with response ($n = 56$; **Fig. 1**). Of note, 41 of the 56 responders (73.2%) had responses lasting at least 6 months; in 33 responders (58.9%), the response lasted at least 12 months. Fourteen of the 56 responders (25.0%) had ongoing responses at the time of database lock. Among these 14 patients with ongoing responses, 9 had previously discontinued treatment.

A 12-month landmark survival analysis was performed to better define the impact of the depth of response on survival. In treated patients still alive at 12 months, median OS was >30 months regardless of BOR within the first 12 months (**Fig. 2**). However, patients achieving a complete response within the first 12 months experienced the best long-term outcomes, with 90% of this group alive at >30 months' follow-up.

Median PFS (95% CI) was 1.9 months (1.9–2.3) in all treated patients, and 1.9 months (1.7–2.0) and 3.5 months (1.9–3.7) in patients with PD-L1 expression <1% and ≥1%, respectively (Supplementary Fig. S2A). Median PFS data at the 5% PD-L1 expression cutoff were similar to the 1% data (Supplementary Fig. S2B).

Median OS (95% CI) was 8.6 months (6.1–11.3) in all treated patients (**Fig. 3A**); the 3-year OS rate (95% CI) was 22.3% (17.3–27.6). Patients with PD-L1 expression <1% had a median OS (95% CI) of 6.0 months (4.4–8.1) and patients with PD-L1 expression ≥1% had a median OS (95% CI) of 11.9 months (9.1–19.1; **Fig. 3A**). Median OS data by PD-L1 expression cutoff of 5% were similar to data at the 1% cutoff (Supplementary Fig. S2C).

Nivolumab efficacy and association with biomarkers

TMB and PD-L1

A total of 139 patients were evaluable for TMB analysis. Most baseline clinical variables were similar between the TMB-evaluable and the overall CheckMate 275 population; however, a slightly greater proportion of patients in the TMB-evaluable population had liver metastases present at baseline compared with the overall population (**Table 2**). The median and range of TMB in the evaluable subset were similar to that observed in The Cancer Genome Atlas population of patients with urothelial bladder cancer (data not shown).

ORR and complete response rate in the TMB-evaluable patients were similar to results obtained in all treated patients (**Table 1**). TMB showed a positive association with ORR [OR (95% CI): 2.13 (1.26–3.60), $P < 0.05$] with nivolumab regardless of baseline tumor PD-L1 expression (**Table 3**; **Fig. 4**). In an ROC analysis between objective response and TMB, the AUC was 65.1% (95% CI, 53.9–76.3). In patients with PD-L1 expression <1%, the AUC was 70.7% (95% CI, 56.5–85.0). In patients with PD-L1 expression ≥1%, the AUC was 59.2% (95% CI, 42.0–76.5). In the medium TMB group, ORR was slightly higher in patients with PD-L1 expression <1% than in patients with PD-L1 expression ≥1% (**Table 3**), which was possibly due to the small numbers of patients in these subgroups. ORR was 13.0% in patients with low TMB, 19.6% in patients with medium TMB, and 31.9% in patients with high TMB (**Table 3**). Furthermore, the population of patients with no PD-L1 expression and low TMB did not appear to benefit from treatment, as only one responder was observed in this category (Supplementary Fig. S3). Median DOR (range) among responders by TMB tertiles was 22.1 months (3.7–31.4; $n = 6$), 7.4 months (1.8–34.6; $n = 9$), and 25.9 months (4.1–37.6; $n = 15$) in patients with low, medium, and high TMB, respectively.

Median PFS data were similar between all treated patients and the TMB-evaluable patients (Supplementary Fig. S2A). TMB had a positive association with PFS [HR (95% CI): 0.75 (0.61–0.92), $P < 0.05$; **Table 3**]. Median PFS was longer in patients with higher TMB values than in patients with lower TMB regardless of PD-L1 expression level (Supplementary Fig. S4A and S4B). The combination of TMB and PD-L1 status was a better predictor of PFS than PD-L1 alone

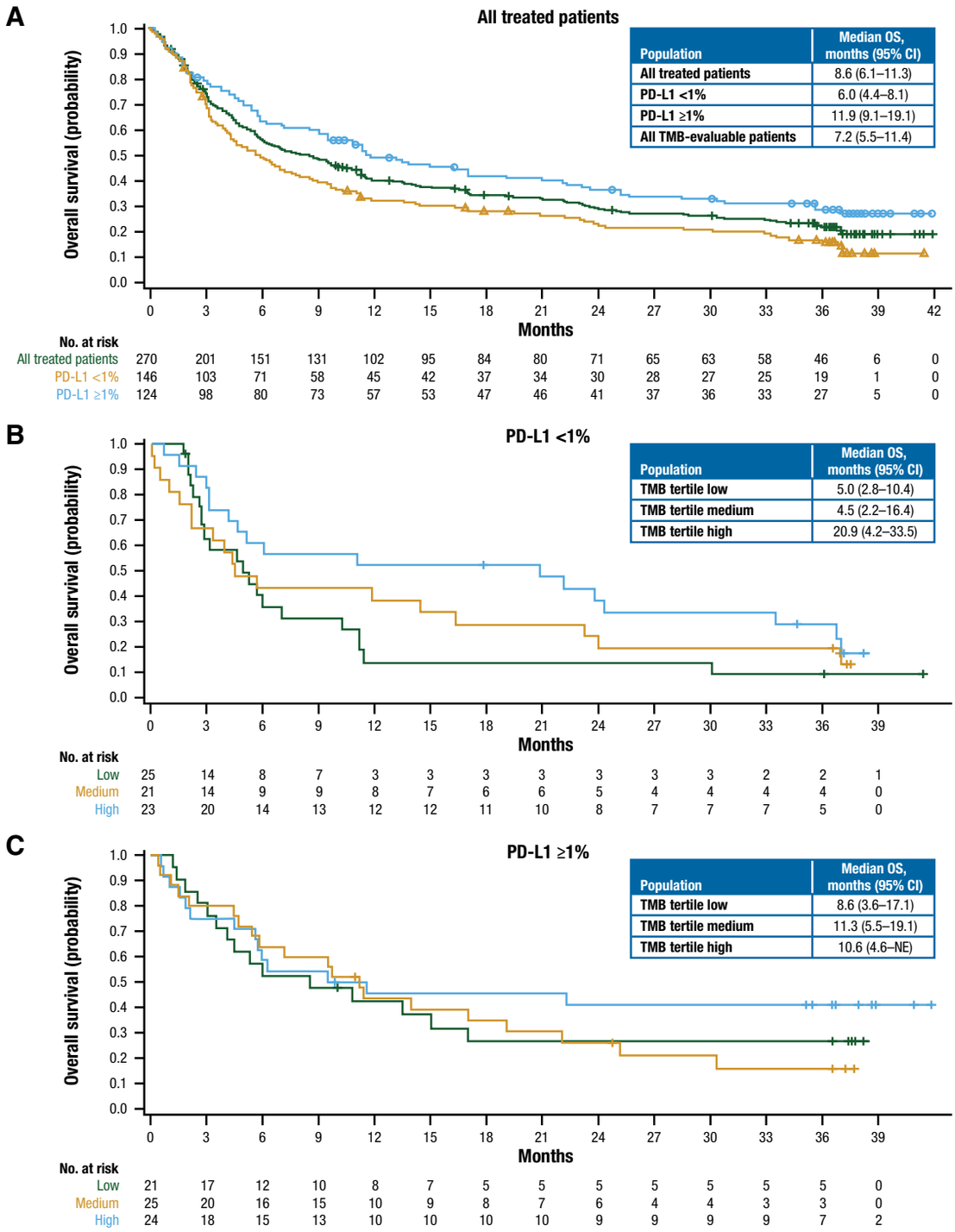

Figure 3.

Overall survival in all treated patients and by PD-L1 expression (**A**), and by TMB tertiles in patients with PD-L1 expression <1% (**B**) and ≥1% (**C**).

($P = 0.0056$) and we did not observe a significant interaction between TMB and PD-L1 ($P = 0.78$; Supplementary Fig. S4C and S4D).

Median OS data in the TMB-evaluable population were similar to the all-treated patient population (**Fig. 3A**). TMB was positively associated with OS [HR (95% CI): 0.73 (0.58–0.91), $P <$ 0.05; **Table 3**], and patients with higher TMB values had longer OS than patients with lower TMB regardless of PD-L1 status, although the tail of the curve indicated a longer survival benefit in patients with both high TMB and PD-L1 expression ≥1% (**Fig. 3B** and **C**). In addition, the combination of TMB and PD-L1 status was a better predictor of OS

Table 2. Comparison of clinical variables between all treated patients and TMB-evaluable patients.

Characteristic	All treated patients $N = 270$	TMB-evaluable patients $n = 139$
Mean age (range), years	65.0 (38–90)	64.5 (38–85)
Age group, %		
<65 years	45.2	45.3
≥65 and <75 years	40.7	42.4
≥75 years	14.1	12.2
Sex, %		
Male	78.1	78.4
Female	21.9	21.6
Baseline hemoglobin (g/dL), %		
<10	17.8	18.0
≥10	82.2	82.0
Liver metastases, %		
Yes	28.9	33.1
No	70.4	66.9
Not reported	0.7	–
Visceral metastases, %		
Yes	82.6	80.6
No	16.7	19.4
Not reported	0.7	–
Lymph node–only metastases, %		
Yes	16.7	19.4
No	82.6	80.6
Not reported	0.7	–
PD-L1 expression, %		
<1%	54.1	49.6
≥1%	45.9	50.4
TMB classification by tertiles, %		
High	–	33.8
Medium	–	33.1
Low	–	33.1

than PD-L1 alone ($P = 0.013$) and we did not observe a significant interaction between TMB and PD-L1 expression ($P = 0.98$; Supplementary Fig. S4C and S4D). TMB levels and PD-L1 expression were not correlated as objective response, OS, and PFS outcomes by TMB levels did not differ across PD-L1 levels (Supplementary Figs. S3 and S4D).

Mutational signatures

Potential correlations between mutational signatures and TMB were examined. The most common mutational signatures in this study included signature 1 (age-related), signatures 2 and 13 [endogenous mutagenesis-related apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like (APOBEC)], and signature 7 (ultraviolet induced; ref. 17). Of the top mutational signatures present in CheckMate 275, signature 1 showed negative correlation ($r = -0.46$) while signatures 2 and 13 showed moderate positive correlation with TMB ($r = 0.55$ and 0.54, respectively; Supplementary Fig. S5). Signature 7 showed low correlation with TMB ($r = 0.20$). Signature 2 had the highest association with TMB, relative to other top mutational signatures (Supplementary Fig. S5). Higher signature 2 scores were poor predictors for PFS (Supplementary Fig. S6A) and BOR (Supplementary Fig. S6B and S6C) but were positively associated with better OS (Supplementary Fig. S6D). Although signature 2 added predictive value to clinical biomarkers (including PD-L1 expression level, baseline hemoglobin level, and presence of liver metastases; $P = 0.0038$), it did not improve upon TMB as a predictive marker.

Safety

Any-grade treatment-related AEs occurred in 69.3% of patients treated with nivolumab (Supplementary Table S2). The most common treatment-related AEs were fatigue (19.3%), diarrhea (13.0%), and pruritus (11.9%). Grade 3 or 4 treatment-related AEs occurred in 24.8% of patients. The most common grade 3 or 4 treatment-related

Table 3. Overall response rate, overall survival, and progression-free survival in all patients and TMB/PD-L1 subgroups.

Population	ORR, %	Median PFS (95% CI), months	Median OS (95% CI), months	Tumor PD-L1 expression	ORR, %	Median PFS (95% CI), months	Median OS (95% CI), months
All treated patients $N = 270$	20.7	1.9 (1.9–2.3)	8.6 (6.1–11.3)	<1% $n = 146$	16.4	1.9 (1.7–2.0)	6.0 (4.4–8.1)
				≥1% $n = 124$	25.8	3.5 (1.9–3.7)	11.9 (9.1–19.1)
TMB-evaluable patients $n = 139$	21.6	1.9 (1.8–2.8)	7.2 (5.5–11.4)	<1% $n = 69$	18.8	1.8 (1.7–2.2)	5.7 (4.2–11.3)
				≥1% $n = 70$	24.3	2.3 (1.9–3.7)	10.3 (5.8–17.1)
TMB low $n = 46$	13.0	1.9 (1.8–3.1)	5.7 (3.6–10.9)	<1% $n = 25$	4.0	1.8 (1.6–2.0)	5.0 (2.8–10.4)
				≥1% $n = 21$	23.8	3.1 (1.9–5.7)	8.6 (3.6–17.1)
TMB medium $n = 46$	19.6	1.8 (1.7–2.3)	9.7 (4.5–16.4)	<1% $n = 21$	23.8	1.8 (1.4–3.7)	4.5 (2.2–16.4)
				≥1% $n = 25$	16.0	1.9 (1.6–3.4)	11.3 (5.5–19.1)
TMB high $n = 47$	31.9	3.5 (1.8–14.0)	11.6 (5.7–33.5)	<1% $n = 23$	30.4	2.5 (1.7–19.5)	20.9 (4.2–33.5)
				≥1% $n = 24$	33.3	3.5 (1.7–22.3)	10.6 (4.6–NE)

Abbreviation: NE, not estimable.

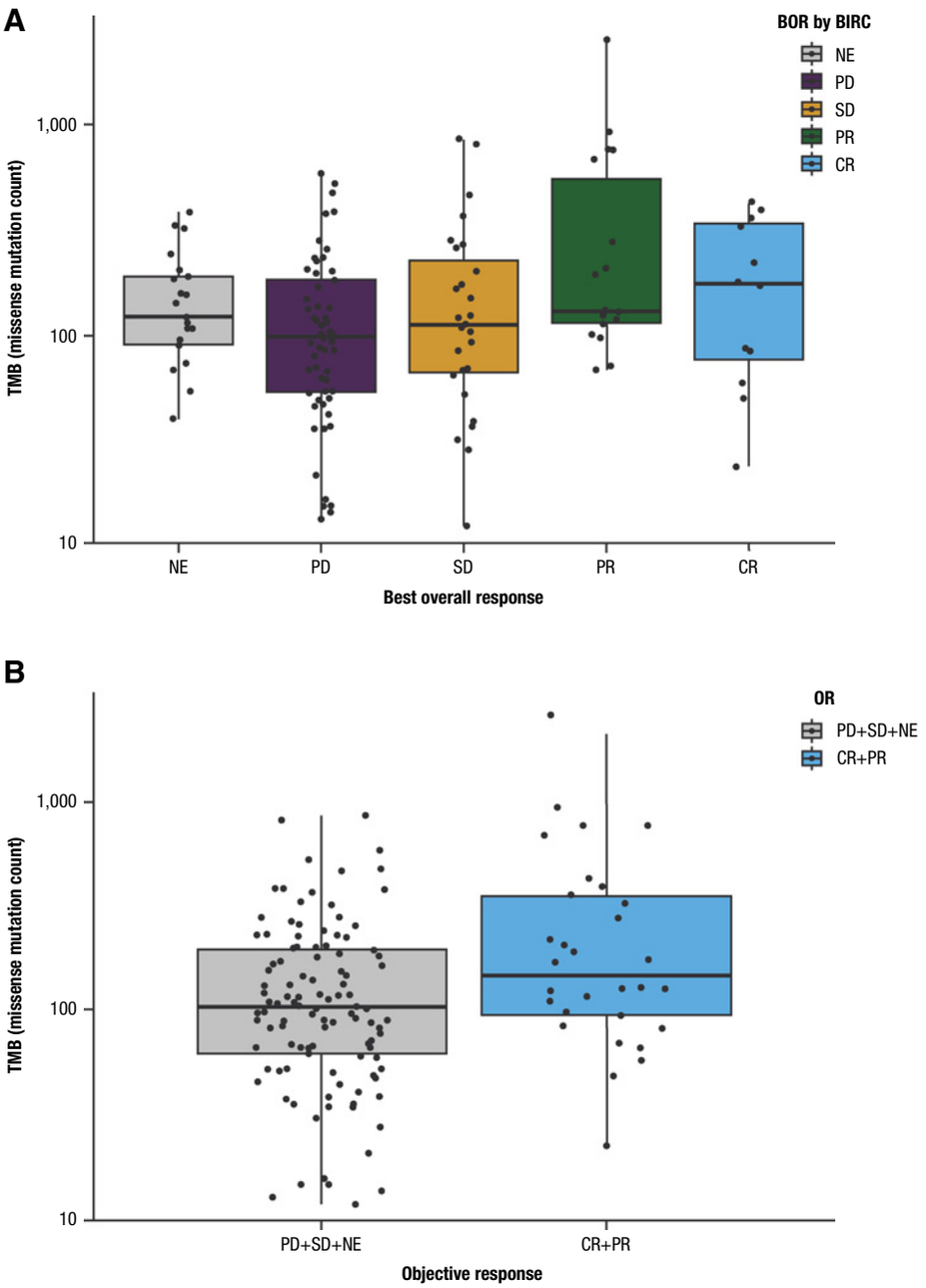

Figure 4.
Association of TMB with best overall response (**A**) and objective response (**B**). OR, objective response.

AEs were increased lipase (4.8%), increased amylase (3.0%), and diarrhea (3.0%; Supplementary Table S2). Three grade 5 treatment-related AEs were observed (one case each of pneumonitis, respiratory failure, and circulatory collapse), all of which were reported previously (2). Any-grade treatment-related AEs leading to discontinuation were observed in 10.0% (grade ≥3, 6.7%) of patients. The most common treatment-related select AEs (defined as AEs that may be immune-mediated, differ from those caused by nonimmunotherapies, may require immunosuppression for management, and whose early recognition may mitigate severe toxicity) of any grade were skin (23.7%), thyroid disorder (14.4%), and gastrointestinal (13.3%; Supplementary Table S2). The observed grade 5 case of pneumonitis was immune-related.

Discussion

Our results show that with approximately 3 years of follow-up from CheckMate 275, nivolumab continues to provide durable antitumor activity in patients with platinum-resistant mUC. Furthermore, the one additional complete response observed since the previous report of the study (20) shows the added benefit of nivolumab over time, and the potential relationship between depth of response and long-term survival is highlighted in a landmark analysis. Consistent with the initial report of this study, while ORR was numerically higher and median PFS and OS were numerically longer in patients with baseline tumor PD-L1 expression ≥1%, efficacy was observed regardless of PD-L1 expression (2). Furthermore, no new safety signals were observed with longer follow-up (2).

In the retrospective exploratory biomarker analyses, we found that high TMB categorization by tertiles as assessed by whole-exome sequencing was associated with higher ORR, longer PFS, and longer OS in patients treated with nivolumab, and patients with high TMB showed improved outcomes across baseline PD-L1 expression levels. In addition, TMB and PD-L1 expression were not correlated. These results suggest that patients with higher TMB by tertiles may have improved response to therapy relative to patients with low TMB. This is consistent with previous studies demonstrating that high TMB is a potential scientifically relevant biomarker for response to immune checkpoint inhibitors (5, 6, 13, 21). Furthermore, the analyses of TMB together with PD-L1 expression indicate that the combination of TMB and PD-L1 was a better predictor of PFS and OS than TMB or PD-L1 alone. These findings highlight the potential for a composite biomarker approach to help identify patients who could most benefit from nivolumab treatment.

To understand the underlying mutagenesis process of the population of patients in CheckMate 275, mutational signatures were examined. Of note, we found that mutational signature 2, an APOBEC-related mutational signature, had a positive association with TMB. APOBEC signatures have been found previously in bladder cancer as well as other cancer types, including breast, cervical, and lung cancer (22, 23). Consistent with our findings, mutational signature 2 was previously shown to be associated with both TMB and improved response to immune checkpoint inhibitors in patients with non–small cell lung cancer (24). We found that this signature improved the predictive value of some clinical biomarkers, such as baseline PD-L1 expression, hemoglobin level, and presence of liver metastases, but did not improve the predictive value of TMB. These results further underscore the importance of considering mutational signatures in the context of other biomarkers.

These analyses were limited by their exploratory nature and were not prespecified. It should be noted that the definition of TMB used in our study differs slightly from that used in studies in which TMB is determined by the FoundationOne CDx assay (measured in mutations per Mb; ref. 25) It should also be noted that CheckMate 275 is a single-arm trial, complicating the determination of any biomarker as being predictive versus prognostic. The evidence to date on the predictive value of TMB is inconsistent (6, 26–31). In addition, there is a lack of standardization among TMB assays, making it difficult to implement into clinical practice. For example, in the CheckMate 227 trial, PFS was improved in patients with high TMB who received nivolumab plus ipilimumab for NSCLC compared with those who received chemotherapy (25). In the KEYNOTE-010 and KEYNOTE-042 trials, high TMB was associated with ORR, PFS, and OS in patients receiving pembrolizumab for PD-L1–positive NSCLC (32). Powles and colleagues previously reported that immune checkpoint blockade was associated with improved survival versus chemotherapy in patients with platinum-resistant mUC with tumors harboring high (defined as a TMB level above the median), but not low, TMB (33). Future prospective analyses will be needed to validate the clinical utility of TMB as a predictive biomarker for immune checkpoint inhibitors, to demonstrate utility for TMB and PD-L1 together as biomarkers of response to checkpoint inhibitors in general and nivolumab specifically, and to develop predictive models for OS and PFS based on these biomarker combinations. In addition, further studies to potentially define a TMB cutoff for clinical use as a biomarker are needed. Future work could also examine biomarkers by tumor histology, as patients with rare histologies of bladder cancer have shown to have different biomarker levels compared with pure urothelial carcinoma (34).

In conclusion, these data with almost 3 years of minimum follow-up support the durable activity of nivolumab monotherapy for the treatment of mUC and suggest that TMB alone and/or combined with PD-L1 may enrich for response to nivolumab in this setting. Further study of TMB as a biomarker for response to nivolumab in patients with mUC is warranted.

Disclosure of Potential Conflicts of Interest

M.D. Galsky reports personal fees from BioMotive (advisor), Janssen (advisory board), GlaxoSmithKline (advisory board), Astellas (advisory board), Novartis (advisory board), Pfizer (advisory board), Seattle Genetics (advisory board), Incyte (advisory board), Aileron (advisory board), Dracen (advisory board), Inovio (advisory board), NuMab (advisory board), Dragonfly (advisory board), and Lilly (consultant) as well as grants and personal fees from Dendreon (advisory board), Merck (advisory board), Genentech (advisory board), Bristol-Myers Squibb Company (BMS; advisory board), and AstraZeneca (advisory board) outside the submitted work. P.M. Szabo reports other from BMS (employee and shareholder) during the conduct of the study. G.C. Han reports other from BMS (employee). G. Grossfeld reports other from BMS (employee and stock ownership) outside the submitted work. S. Collette reports personal fees from BMS (employee and stock ownership) during the conduct of the study. A. Siefker-Radtke reports personal fees from Merck, Bavarian Nordic, Seattle Genetics, Genentech, Mirati, AstraZeneca, Nektar Therapeutics, Pfizer, and Janssen outside the submitted work. A. Necchi reports grants and personal fees from BMS during the conduct of this study; grants from Roche and AstraZeneca; grants and personal fees from Merck, and personal fees from Bayer and Seattle Genetics outside the submitted work. P. Sharma reports personal fees from Oncolytics (consultancy), Jounce (consultancy), BioAtla (consultancy), Forty-Seven (consultancy), Polaris (consultancy), Marker (consultancy), Codiak (consultancy), ImaginAb (consultancy), Hummingbird (consultancy), Dragonfly (consultancy), Lytix (consultancy), Lava Therapeutics (consultancy), Achelois (consultancy), and Infinity (consultancy); other from Constellation (stock), Oncolytics (stock), Apricity Health (stock), Hummingbird (stock), Dragonfly (stock), Lytix (stock), Lava Therapeutics (stock), Achelois (stock), Jounce (stock), Neon (stock), BioAtla (stock), Forty-Seven (stock), Polaris (stock), Marker (stock), Codiak (stock), and ImaginAb (stock) outside the submitted work; in addition, P. Sharma is listed as a co-inventor of a patent related to the targeting of the ICOS pathway that is licensed to and owned by Jounce. No potential conflicts of interest were disclosed by the other author.

Authors' Contributions

M.D. Galsky: Resources, formal analysis, investigation, writing-original draft, writing-review and editing. **A. Saci:** Data curation, formal analysis, visualization, writing-original draft, writing-review and editing. **P.M. Szabo:** Data curation, formal analysis, visualization, writing-original draft, writing-review and editing. **G.C. Han:** Data curation, formal analysis, visualization, writing-original draft, writing-review and editing. **G. Grossfeld:** Formal analysis, writing-original draft, writing-review and editing. **S. Collette:** Data curation, formal analysis, writing-original draft, writing-review and editing. **A. Siefker-Radtke:** Resources, formal analysis, investigation, writing-original draft, writing-review and editing. **A. Necchi:** Resources, formal analysis, investigation, writing-original draft, writing-review and editing. **P. Sharma:** Resources, formal analysis, investigation, writing-original draft, writing-review and editing.

Acknowledgments

The authors would like the acknowledge the patients and families who made this study possible, the clinical study teams who participated in the study, the protocol manager(s) for this study, Dako, an Agilent Technologies, Inc. company, for collaborative development of the PD-L1 IHC 28-8 pharmDx assay (Santa Clara, CA), Bristol-Myers Squibb Company (Princeton, NJ), and ONO Pharmaceutical Company Ltd. (Osaka, Japan). The study was supported by Bristol-Myers Squibb Company. All authors contributed to and approved the manuscript; writing and editorial assistance was provided by Nicolette Belletier, PhD, of Parexel, funded by Bristol-Myers Squibb Company.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received January 23, 2020; revised May 12, 2020; accepted June 9, 2020; published first June 12, 2020.

References

1. National Comprehensive Cancer Network Clinical Practice Guidelines in Oncology (NCCN Guidelines). Bladder cancer, version 3.2019. Accessed April 23, 2019.
2. Sharma P, Retz M, Siefker-Radtke A, Baron A, Necchi A, Bedke J, et al. Nivolumab in metastatic urothelial carcinoma after platinum therapy (CheckMate 275): a multicentre, single-arm, phase 2 trial. Lancet Oncol 2017;18:312–22.
3. OPDIVO® (nivolumab) [package insert]. Princeton, NJ; Bristol-Myers Squibb Company; 2019.
4. OPDIVO. Available from: https://www.ema.europa.eu/en/medicines/human/EPAR/opdivo. Accessed June 27, 2019.
5. Rizvi NA, Hellmann MD, Snyder A, Kvistborg P, Makarov V, Havel JJ, et al. Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. Science 2015;348:124–8.
6. Samstein RM, Lee CH, Shoushtari AN, Hellmann MD, Shen R, Janjigian YY, et al. Tumor mutational load predicts survival after immunotherapy across multiple cancer types. Nat Genet 2019;51:202–6.
7. Carbone DP, Reck M, Paz-Ares L, Creelan B, Horn L, Steins M, et al. First-line nivolumab in stage IV or recurrent non-small-cell lung cancer. N Engl J Med 2017;376:2415–26.
8. Galsky MD, Saci A, Szabo PM, Azrilevich A, Horak C, Lambert A, et al. Impact of tumor mutation burden on nivolumab efficacy in second-line urothelial carcinoma patients: exploratory analysis of the phase II CheckMate 275 study. Ann Oncol 2017;28:v295–329.
9. Hellmann MD, Callahan MK, Awad MM, Calvo E, Ascierto PA, Atmaca A, et al. Tumor mutational burden and efficacy of nivolumab monotherapy and in combination with ipilimumab in small-cell lung cancer. Cancer Cell 2019;35:329.
10. Van Allen EM, Miao D, Schilling B, Shukla SA, Blank C, Zimmer L, et al. Genomic correlates of response to CTLA-4 blockade in metastatic melanoma. Science 2015;350:207–11.
11. Yusko E, Vignali M, Wilson RK, Mardis ER, Hodi FS, Horak C, et al. Association of tumor microenvironment T-cell repertoire and mutational load with clinical outcome after sequential checkpoint blockade in melanoma. Cancer Immunol Res 2019;7:458–65.
12. Riaz N, Havel JJ, Makarov V, Desrichard A, Urba WJ, Sims JS, et al. Tumor and microenvironment evolution during immunotherapy with nivolumab. Cell 2017;171:934–49.
13. Hodi FS, Wolchok JD, Schadendorf D, Larkin J, Qian M, Saci A, et al. Abstract CT037: Genomic analyses and immunotherapy in advanced melanoma. Cancer Res 2019;79:CT037.
14. van Dijk N, Funt SA, Blank CU, Powles T, Rosenberg JE, van der Heijden MS. The cancer immunogram as a framework for personalized immunotherapy in urothelial cancer. Eur Urol 2019;75:435–44.
15. Clopper PJ, Pearson ES. The use of confidence or fiducial limits illustrated in the case of the binomial. Biometrika 1934;26:404–13.
16. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. J Am Stat Assoc 1958;53:457–81.
17. Alexandrov LB, Nik-Zainal S, Wedge DC, Aparicio SA, Behjati S, Biankin AV, et al. Signatures of mutational processes in human cancer. Nature 2013;500:415–21.
18. Forbes SA, Beare D, Gunasekaran P, Leung K, Bindal N, Boutselakis H, et al. COSMIC: exploring the world's knowledge of somatic mutations in human cancer. Nucleic Acids Res 2015;43:D805–11.
19. Forbes SA, Beare D, Boutselakis H, Bamford S, Bindal N, Tate J, et al. COSMIC: somatic cancer genetics at high-resolution. Nucleic Acids Res 2017;45:D777–83.
20. Sharma P, Baron A, Necchi A, Plimack ER, Pal SK, Bedke J, et al. Abstract CT178: Nivolumab monotherapy in patients with advanced platinum-resistant urothelial carcinoma: Efficacy and safety update and association between biomarkers and overall survival in CheckMate 275. Cancer Res 2018;78:CT178.
21. Snyder A, Makarov V, Merghoub T, Yuan J, Zaretsky JM, Desrichard A, et al. Genetic basis for clinical response to CTLA-4 blockade in melanoma. N Engl J Med 2014;371:2189–99.
22. Roberts SA, Lawrence MS, Klimczak LJ, Grimm SA, Fargo D, Stojanov P, et al. An APOBEC cytidine deaminase mutagenesis pattern is widespread in human cancers. Nat Genet 2013;45:970–6.
23. Middlebrooks CD, Banday AR, Matsuda K, Udquim KI, Onabajo OO, Paquin A, et al. Association of germline variants in the APOBEC3 region with cancer risk and enrichment with APOBEC-signature mutations in tumors. Nat Genet 2016;48:1330–8.
24. Chen H, Chong W, Teng C, Yao Y, Wang X, Li X. The immune response-related mutational signatures and driver genes in non-small-cell lung cancer. Cancer Sci 2019;110:2348–56.
25. Hellmann MD, Ciuleanu TE, Pluzanski A, Lee JS, Otterson GA, Audigier-Valette C, et al. Nivolumab plus ipilimumab in lung cancer with a high tumor mutational burden. N Engl J Med 2018;378:2093–104.
26. Rizvi NA, Cho BC, Reinmuth N, Lee KH, Luft A, Ahn MJ, et al. Durvalumab with or without tremelimumab vs standard chemotherapy in first-line treatment of metastatic non-small cell lung cancer: the MYSTIC phase 3 randomized clinical trial. JAMA Oncol 2020;6:661–74.
27. Update on the phase III NEPTUNE trial of imfinzi plus tremelimumab in stage IV non-small cell lung cancer. Available from: https://www.astrazeneca.com/media-centre/press-releases/2019/update-on-the-phase-iii-neptune-trial-of-imfinzi-plus-tremelimumab-in-stage-iv-non-small-cell-lung-cancer-21082019.html.
28. Buttner R, Longshore JW, Lopez-Rios F, Merkelbach-Bruse S, Normanno N, Rouleau E, et al. Implementing TMB measurement in clinical practice: considerations on assay requirements. ESMO Open 2019;4:e000442.
29. Stenzinger A, Allen JD, Maas J, Stewart MD, Merino DM, Wempe MM, et al. Tumor mutational burden standardization initiatives: recommendations for consistent tumor mutational burden assessment in clinical samples to guide immunotherapy treatment decisions. Genes Chromosomes Cancer 2019;58:578–88.
30. Stenzinger A, Endris V, Budczies J, Merkelbach-Bruse S, Kazdal D, Dietmaier W, et al. Harmonization and standardization of panel-based tumor mutational burden (TMB) measurement: real-world results and recommendations of the QuIP study. J Thorac Oncol 2020;S1556-0864:30135-0.
31. Merino DM, McShane LM, Fabrizio D, Funari V, Chen SJ, White JR, et al. Establishing guidelines to harmonize tumor mutational burden (TMB): *in silico* assessment of variation in TMB quantification across diagnostic platforms: phase I of the Friends of Cancer Research TMB Harmonization Project. J Immunother Cancer 2020;8:e000147.
32. Herbst RS, Lopes G, Kowalski DM, Nishio M, Wu Y-L, de Castro Junior G, et al. Association between tissue TMB (tTMB) and clinical outcomes with pembrolizumab monotherapy (pembro) in PD-L1-positive advanced NSCLC in the KEYNOTE-010 and -042 trials. Ann Oncol 2019;30(suppl 5):v851–934.
33. Powles T, Duran I, van der Heijden MS, Loriot Y, Vogelzang NJ, De Giorgi U, et al. Atezolizumab versus chemotherapy in patients with platinum-treated locally advanced or metastatic urothelial carcinoma (IMvigor211): a multicentre, open-label, phase 3 randomised controlled trial. Lancet 2018;391:748–57.
34. Necchi A, Madison R, Raggi D, Jacob JM, Bratslavsky G, Shapiro O, et al. Comprehensive assessment of immuno-oncology biomarkers in adenocarcinoma, urothelial carcinoma, and squamous-cell carcinoma of the bladder. Eur Urol 2020;77:548–56.