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Phenotypic and Genomic Determinants of Immunotherapy Response Associated with Squamousness

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Abstract

Advanced and metastatic squamous cell carcinomas (SCC) are common and difficult-to-treat malignancies. We assessed 75 immunotherapy-treated patients with SCC from a clinically annotated database of 2,651 patients, as well as 9,407 patients from a deidentified database for molecular features that might influence checkpoint blockade response. SCCs had higher tumor mutational burdens (TMB) than non-SCCs ($P < 0.0001$). Cutaneous SCCs had the highest TMB ($P < 0.0001$), with 41.3% demonstrating a very high TMB (≥50 mutations/Mb). In immunotherapy-treated patients with SCC, higher TMB (≥12 mutations/Mb) correlated with a trend to higher clinical benefit rate [stable disease ≥ 6 months or partial/complete remission; 60% vs. 29%; (high vs. low TMB); $P = 0.06$] and significantly longer median time-to-treatment failure (TTF; 9.9 vs. 4.4 months; $P = 0.0058$). Cutaneous SCCs had the highest clinical benefit [11/15 patients (73%) vs. 20/60 (33%) non-cutaneous ($P = 0.008$)], TTF ($P = 0.0015$), and overall survival ($P = 0.06$) with immunotherapy treatment. In conclusion, among a diverse set of SCCs, higher TMB and cutaneous disease associated with better immunotherapy outcome.

Introduction

Squamous cell carcinomas (SCC) occur in tissues that are lined with squamous epithelium. Common sites for SCC include the lung, head and neck, esophagus, and skin (1). Many types of SCCs are lethal, and several often present with locally advanced or metastatic disease. In contrast, the majority of cutaneous SCCs are cleared with local therapies (e.g., excision). However, cutaneous SCC can progress over time, leading to tissue destruction and morbidity. Rarely, cutaneous SCCs can metastasize to regional lymph nodes and distant sites (2, 3), and treatment options for locally advanced or metastatic cutaneous SCCs are suboptimal and consist of radiotherapy and chemotherapy, although various other treatments have been tried (4).

Studies of the genomic landscape of SCCs (or "squamousness") arising in diverse sites have suggested targeting the PI3K-AKT-mTOR and/or cyclin pathway components (5–8). Studies have suggested that SCCs from different organs can share patterns of molecular alterations (6, 7). Human papillomavirus (HPV) is a major cause of several SCCs including oropharyngeal, cervical, and cutaneous tumors (9). For the most part, HPV-negative SCCs harbor *TP53* and cyclin mutations, whereas HPV-positive patients harbor more PI3K pathway alterations (10, 11). Distinct mutation profiles in HPV-positive and HPV-negative SCCs of the head and neck identify subgroups with poor outcomes after adjuvant chemoradiation. Mutations in *TP53*, *NOTCH1*, *KDR*, and the PI3K pathway have been recognized as possible targets for subgroup-specific treatment regimens (12).

High tumor mutational burden (TMB) has been acknowledged as a response biomarker for PD-1/programmed death ligand 1 (PD-L1) blockade in multiple tumor types (13). Higher TMB correlates with better treatment outcomes, including higher response rates, longer progression-free survival, and longer overall survival (OS), in diverse cancers treated with immunotherapies compared with tumors with a low TMB (13). Patients with cancers harboring mismatch repair gene alterations, which are almost always associated with high TMB, also benefit from checkpoint inhibitors (14). Cutaneous SCCs have many molecular features that predict response to immunotherapy, including a high TMB, possibly due to ultraviolet (UV) light–driven mutations and an increased disease risk among patients with immunosuppression (15, 16). PD-1/PD-L1–blocking antibodies have been shown to be efficacious in the treatment of advanced SCCs. Nivolumab and pembrolizumab have both been approved for the treatment of advanced head and neck and lung SCC (17–19), and cemiplimab has been approved for the treatment of advanced

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cutaneous SCC (20). In this study, we explored the response to immunotherapy and the genomic features, including TMB, of a variety of SCCs. We observed high response rates in those tumors with a high TMB and in advanced cutaneous SCCs.

Materials and Methods

Patient selection

A total of 2,651 patients were reviewed from a clinically annotated University of California San Diego (UCSD) database. Data for those with SCC and treated with immunotherapy were extracted for analysis. All patients had undergone hybrid-capture–based next-generation sequencing (NGS; FoundationOne) and were treated at UCSD Moores Cancer Center (La Jolla, CA). Immunotherapy agents included anti-PD-1 and anti-PD-L1 and various combination regimens (Table 1). The TMB of 9,407 patients with SCC were reviewed from a large database [Foundation Medicine (FM)]. This study was performed in accordance with UCSD Institutional Review Board guidelines for data analysis (NCT02478931) and for any investigational treatments, for which patients provided written consent. The FM data were approved by the Western Institutional Review Board (Protocol No. 20152817). This study was conducted in accordance with the Declaration of Helsinki.

NGS and assessment of TMB

The FoundationOne assay was used [hybrid-capture–based NGS; 236 (if sequenced prior to August 2014) or 315 genes depending on the time period; http://www.foundationone.com//]. Formalin-fixed, paraffin-embedded tumor samples were submitted for NGS to FM by referring physicians as per their need to have NGS results on their patients. The methods and associated software information have been described previously (21). Average sequencing depth of coverage was greater than 250×, with >100× at >99% of exons.

TMB was measured in mutations per megabase (Mb). To assess TMB, somatic mutations detected by NGS (interrogating 1.2 Mb of the genome) were calculated, and the values were extrapolated to the whole exome utilizing a validated algorithm (13, 15). Bona fide oncogenic driver alterations and germline polymorphisms were excluded. TMB levels were divided into three groups (15): low (1–5 mutations/Mb), intermediate (6–19 mutations/Mb),

Table 1. Patient demographics by SCC type ($N = 75$ treated with immunotherapy; UCSD cohort)

Variable	All patients ($N = 75$)	Cutaneous SCC ($n = 15$)	Other SCC ($n = 60$)	P[a]
Median age (range) in years[b]	67 (33–90)	67 (45–79)	69 (33–90)	0.4663
Sex				
Male	54 (72%)	14 (93%)	40 (67%)	0.0534
Female	21 (28%)	1 (7%)	20 (33%)	
Ethnicity				
White	61 (81%)	14 (93%)	47 (78%)	0.2763
Hispanic	7 (9%)	1 (4%)	6 (10%)	1.0000
Black	3 (4%)	0 (0%)	3 (5%)	1.0000
Asian	3 (4%)	0 (0%)	3 (5%)	1.0000
Other	1 (2%)	0 (0%)	1 (2%)	1.0000
Disease status[b]				
Locally advanced	23 (31%)	5 (33%)	18 (30%)	0.7654
Metastatic	52 (69%)	10 (67%)	42 (70%)	
Genomics				
Median time (range) in months from biopsy used for genomic analysis to treatment with checkpoint blockade	7.9 (−16.1–43.2)	8.5 (−3.3–31.9)	6.5 (−16.1–43.2)	0.3535
Median number (range) of genomic alterations[c]	7	10 (5–22)	5 (0–12)	<0.0001
Median TMB (range) mutations/Mb[c]	10 (1–347)	63 (12–347)	6 (1–25)	<0.0001
TMB Low	11 (27%)	0 (0%)	11 (38%)	0.0175
TMB Intermediate	18 (44%)	2 (17%)	16 (55%)	0.0378
TMB High	12 (29%)	10 (83%)	2 (7%)	0.0001
MSI Status[d]				
MS-Stable	33 (97%)	7 (88%)	26 (100%)	0.2353
MS-High	1 (3%)	1 (12%)	0 (%)	
Treatment				
Median number (range) of prior systemic therapies	1 (0–5)	1 (0–3)	1 (0–5)	0.1993
PD-1/PD-L1 Blockade monotherapy	68 (91%)	15 (100%)	53 (88%)	0.3327
PD-1/PD-L1 Blockade + chemotherapy	1 (2%)	0 (0%)	1 (2%)	1.0000
PD-1/PD-L1 Blockade + targeted therapy	1 (2%)	0 (0%)	1 (2%)	1.0000
PD-1/PD-L1 Blockade + investigational agent	5 (6%)	0 (0%)	5 (8%)	1.0000
Median TTF (range) in months[a,b]	4.8 (0–32.1+)	Not reached (0–27.3+; median follow-up of 10.1 months)	4.2 (0.1–32.1+)	0.0015; HR, 0.3; 95% CI, 0.2–0.5
Median OS in months[a,b]	17.4 (0.1–32.1+)	Not reached (0.3–27.3+; median follow-up of 11.2 months)	12.5 (0.1–32.1+)	0.0593; HR, 0.5; 95% CI, 0.2–1.0
Overall benefit rate (SD for ≥ 6 months plus PR/CR)	31/75 (41%)	11/15 (73%)	20/60 (33%)	0.008; OR, 5.5; 95% CI, 1.6–16.9

Abbreviation: 95% CI, 95% confidence interval.

[a]Calculated using Student t test, Fisher exact test, and log-rank (Mantel–Cox) where applicable.

[b]At the time of initiation of checkpoint blockade.

[c]Not available ($N = 3$ patient with cutaneous SCC and $N = 31$ for other SCC).

[d]Not available ($N = 7$ patients with cutaneous SCC and $N = 34$ for other SCC).

and high (≥20 mutations/Mb), which divided approximately 50% of patients to low TMB, 40% intermediate TMB, and 10% high TMB. The number of patients with very high TMB (≥50 mutations/Mb) was also assessed. A total of 100 nonsynonymous mutations per exome were used previously as a threshold (15). The threshold of 20 coding mutations per Mb was roughly equivalent to 400 nonsynonymous mutations per exome (20 coding mutations/Mb × 30 Mb/exome × 2/3 nonsynonymous/coding).

The microsatellite instability (MSI) status was calculated using 114 loci determined to be useful in detecting evidence of polymerase slippage and, therefore, MSI (22). The information from these loci was then used in principal component analysis to produce an MSI score.

Statistical analysis and outcome evaluation

Student *t* test, Fisher exact test, and log-rank (Mantel–Cox) were used to assess categorical variables. $P \leq 0.05$ was considered significant. Stable disease (SD), partial and complete remission (CR and PR), and progressive disease (PD) were assessed on the basis of physician notation. Physicians generally used RECIST imaging criteria (23). Time-to-treatment failure (TTF) and OS were calculated by Kaplan–Meier survival analysis. Patients who died early were considered evaluable (as PD). For patients who received multiple immunotherapy regimens, the treatment with first immunotherapeutic was used in this analysis. TTF was defined as a composite endpoint measuring the time from immunotherapy origination to treatment discontinuation for any reason, including disease progression, treatment toxicity, or death. OS was defined as the time from initiation of the immunotherapy until patient death. Patients were censored at date of last follow-up for TTF and OS, if they had not progressed or died, respectively. Multivariate analyses were used to calculate independent variables associated with outcome. TMB was available on only 41 patients, and these were used in the calculation. Statistical analyses were carried out by S. Kato using GraphPad Prism version 7.0 and IBM SPSS Statistics version 24.

Results

Patient characteristics

Of the 2,651 patients with cancer of any histology and who had available data reviewed from a clinically annotated UCSD database, a total of 75 patients treated with immunotherapy for SCC were identified (Supplementary Fig. S1). Twenty-three patients had locally advanced disease, whereas 52 patients had metastatic SCC. Patients were treated with various immunotherapies, with the majority receiving anti-PD-1/PD-L1 monotherapy ($N = 68$; Table 1). Median age was 67 years (range, 33–90 years). Of the 75 patients, 15 had cutaneous SCC, and 60 had other types of SCC: head and neck cancer ($n = 35$), non–small cell lung cancer ($n = 7$), esophageal ($n = 3$), cervical ($n = 2$), anal ($n = 1$), rectal ($n = 1$), and urethral cancers ($n = 1$).

TMB and other molecular alterations

The median TMB for SCC versus non-SCCs of the entire UCSD cohort ($N = 2{,}651$) was 6 versus 2, respectively ($P < 0.0001$; Table 2; Supplementary Fig. S5). Overall, 33.9% of patients with SCC, compared with 9.8% of non-SCCs, had TMB ≥ 12 mutations/Mb ($P < 0.0001$), and 21.7% of patients with SCC, compared with 5.7% of patient with non-SCCs, had

Table 2. TMB in 9,407 patients with SCC

	Median TMB (IQR)	*P	Low TMB (<6 mutations/Mb)	Intermediate TMB (6–19 mutations/Mb)	High TMB (≥20–<50) mutations/Mb)	Very high TMB (≥50 mutations/Mb)	TMB < 12 mutations/Mb	TMB ≥ 12 mutations/Mb	P	OR (95% CI)
Tumors UCSD cohort ($N = 2{,}651$)			Percent of patients					Percent of patients		
Squamous tumors ($n = 180$)[a]	6 (13)	<0.0001	37.2	41.1	21.7	10.0	66.1	33.9	<0.0001	4.7 (3.3–6.7)
Nonsquamous tumors ($n = 2{,}471$)	2 (5)		74.8	19.5	5.7	2.5	90.2	9.8	<0.0001	0.2 (0.1–0.3)
Squamous tumors (FM cohort; $N = 9{,}407$)[a]										
Cutaneous ($n = 426$)	40 (68)	<0.0001	22.3	16.0	61.7	41.3	33.1	66.9	<0.0001	5.8 (4.7–7.2)
Noncutaneous ($n = 8{,}981$)	6 (9)		42.2	47.6	10.2	3.0	74.2	25.8	<0.0001	0.2 (0.1–0.2)
Squamous tumor subsets										
Cutaneous ($n = 426$)	40 (68)	<0.0001	22.3	16.0	61.7	41.3	33.1	66.9	<0.0001	5.8 (4.7–7.3)
Lung ($n = 4{,}096$)	8 (8)		28.1	61.9	10.0	1.6	66.3	33.7	<0.0001	1.8 (1.7–2.0)
Head and neck ($n = 1{,}938$)	4 (6)		57.8	31.3	10.9	5.4	82.0	18.0	<0.0001	0.5 (0.4–0.6)
Esophageal ($n = 423$)	5 (4)		57.2	40.7	2.1	0.5	89.8	10.2	<0.0001	0.3 (0.2–0.4)
Anal ($n = 390$)	5 (5)		58.2	38.0	3.8	0.8	89.0	11.0	<0.0001	0.3 (0.2–0.4)
Cervical ($n = 541$)	5 (5)		54.9	38.6	6.5	0.4	83.9	16.1	<0.0001	0.5 (0.4–0.6)
Urothelial ($n = 74$)	6 (6)		43.2	44.6	12.2	0.0	81.1	18.9	0.09	0.6 (0.3–1.1)

Abbreviation: 95% CI, 95% confidence interval.

*P of median TMB was determined using Mann–Whitney U test (for nonnormally distributed data) for two group comparisons and Kruskal–Wallis for multiple group comparisons. *P*, OR (≥12–<12), and 95% CI for TMB ≥ 12 comparisons were determined using Fisher Exact.

[a]The 180 UCSD patients with SCC and curated clinical data are a subset of the 9,407 patients with SCC from the FM deidentified database.

a TMB $\geq$ 20 mutations/Mb ($P < 0.0001$). Ten-percent of patients with SCC had a TMB $\geq$ 50 mutations/Mb compared with 2.5% of patients with non-SCC ($P < 0.0001$).

A total 9,407 patients with SCC had TMB testing performed (FM cohort; Table 2). Malignancies in this deidentified dataset (which included the 180 patients with SCC in the UCSD clinically annotated dataset, of which 75 received immunotherapy) included cutaneous ($n = 426$), lung ($n = 4{,}096$), head and neck ($n = 1{,}938$), esophageal ($n = 434$), anal ($n = 390$), cervical ($n = 541$), and urothelial ($n = 74$). The median TMB of cutaneous SCCs was 40 mutations/Mb compared with 8 (lung), 4 (head and neck), 5 (urothelial), and 5 mutations/Mb (esophageal, anal, and cervical; $P < 0.0001$). Overall, 66.9% of cutaneous SCCs had a TMB $\geq$ 12 compared with 33.7% of lung cancers ($P < 0.0001$), and 61.7% of cutaneous SCCs had a high TMB compared with 10% of lung cancers. A total of 41.3% of cutaneous SCCs had a very high TMB compared with 1.6% of lung cancers. Less than 1% of esophageal, anal, cervical, and urothelial tumors had a very high TMB.

Of the 41 patients who were treated with immunotherapy and whose tumors were analyzed for TMB (UCSD cohort), 11 (27%) were TMB low, 18 (44%) TMB intermediate, and 12 (29%) TMB high. Of those with high TMB, 4 (10%) had very high TMB (all cutaneous). For the 41 patients with TMB data available, dichotomizing TMB at <12 versus $\geq$12, yielded 21 patients in the lower group and 20 patients in the higher group. Of the 34 patients tested, only 1 patient had MSI high.

Supplementary Fig. S2 compares the molecular alterations in cutaneous versus noncutaneous SCC in the 41 immunotherapy-treated patients with available data in the UCSD cohort (with all alterations identified listed in Supplementary Tables S1 and S2). The most common alterations in cutaneous SCC involved the *TP53*, *NOTCH1*, *CDKN2A*, *LRP1B*, and *FAT1* genes, whereas the most common alterations in noncutaneous SCC were in the *TP53*, *CDKN2A/B*, *FAT1*, *TERT*, and *PIK3CA* genes (Supplementary Fig. S2).

Outcomes by TMB and histology

No difference in clinical benefit (SD $\geq$ 6 months or PR/CR), TTF, and OS between patients with SCC and non-SCC was seen. Less than half (41%, 31/75) of patients with SCC had clinical benefit. The median TTF for all patients was 4.8 months, and median OS was 17.4 months from time of first immunotherapy (Table 1). In comparison, the percent of immunotherapy-treated patients with non-SCC/non-melanoma ($N = 133$) who attained clinical benefit was 36% (48/133; $P = 0.4613$), and the median TTF and OS for this group were 3.7 months ($P = 0.2068$) and 12.2 months ($P = 0.4927$), respectively. All comparisons were to patients with SCC treated with immunotherapy.

In univariate analysis of patients with SCC treated with immunotherapy, TMB [dichotomized at $\geq$12 mutations/Mb ($N = 20$ patients) vs. <12 ($N = 21$ patients)] correlated with numerically higher rates of clinical benefit, although not statistically significant (SD $\geq$ 6 months or PR/CR; 60% vs. 29%; $\geq$12 vs. <12; $P = 0.06$) and OS (17.4 vs. 12.2 months; $P = 0.3$). Patients with a TMB $\geq$ 12 mutations/Mb did have a significantly longer median TTF (9.9 vs. 4.4 months; $P = 0.0058$; Table 3; Fig. 1; Supplementary Fig. S3).

In patients with SCC, when TMB was examined with a three-way stratification, TMB low (<6 mutations/Mb), intermediate (6–19 mutations/Mb), and high ($\geq$20 mutations/Mb), a similar pattern emerged. Immunotherapy-treated patients with high TMB tumors had a longer median TTF than those with intermediate or low TMB tumors (9.9, 5.3, and 4.4 months, respectively; $P = 0.0339$). Other associations were not statistically significant (Fig. 1; Supplementary Fig. S3; Supplementary Table S3). Cutaneous SCCs had better outcomes after immunotherapy than noncutaneous SCCs.

Comparing cutaneous to noncutaneous SCCs treated with immunotherapy (Table 3), we observed higher rates of clinical benefit (SD $\geq$ 6 months or PR/CR) for cutaneous disease, 73% (11/15) versus 33% (20/60; $P = 0.008$). The median TTF was longer (not reached vs. 4.2 months; $P = 0.0015$) and a trend to longer median OS was observed (not reached vs. 12.5 months; $P = 0.0593$; Table 2; Fig. 1) for patients with cutaneous SCC. In univariate analysis of SCCs, both high TMB and cutaneous SCC correlated with better outcomes after immunotherapy. However, in multivariate analysis, none of the comparisons reached significance, perhaps because of the limited number of patients ($N = 41$) with available TMB values (Table 3; Supplementary Table S2).

A case report of a patient with cutaneous SCC

A 64-year-old man developed progressive irritation in the socket of his right prosthetic eye. A CT scan demonstrated a hypervascular 3-cm right orbital mass that displaced the prosthesis 1.9 cm posteriorly. A biopsy of the mass was consistent with invasive cutaneous SCC. Further staging revealed disease involving his right parotid gland, and he underwent resection of the orbital tumor and a neck dissection. He was treated with adjuvant radiotherapy and cetuximab. However, he developed PD involving his right hilum. He was started on treatment with pembrolizumab and achieved a complete response (Supplementary Fig. S4) 7 months after starting therapy. Pembrolizumab was discontinued after 14 months, and he remains in an ongoing complete response. He experienced no treatment-related toxicities.

Discussion

This study evaluated the genomic landscape and mutational burden of diverse SCCs. Response to checkpoint blockade and correlation with histology and TMB were also assessed. Cutaneous SCC appeared to be sensitive to checkpoint blockade, indicated by frequent and durable responses. This finding is similar to the outcome reported in a phase I study of the PD-L1 inhibitor cemiplimab in advanced cutaneous SCC (20). Response rates of this magnitude to single-agent PD-1/PD-L1 inhibition have only otherwise been seen in classical Hodgkin lymphoma (24).

Response rates to checkpoint blockade in cutaneous SCC are likely driven by the high mutational burden of this disease. This study, as well as others, reported cutaneous SCC to have the highest TMB of all SCC malignancies (15). In our study, 41.3% of cutaneous SCCs had a very high TMB compared with 5.4% or less in other major subtypes of SCC. Both melanoma and cutaneous basal cell carcinoma have a high mutational burden and frequent responses to checkpoint blockade (25, 26). Indeed, TMB has been shown to be predictive of response to immunotherapy across diverse cancers (13). However, even among a group of SCCs, which in our study had high rates of clinical benefit after immunotherapy (rate of SD $\geq$ 6 months or PR/CR = 41%), higher TMB was shown to be associated with a longer TTF. Future prospective trials in patients with SCC may warrant stratification by TMB.

Table 3. Univariate and multivariate analysis of factors affecting outcome for patients with SCCs treated with PD-1/PD-L1 blockade (TMB < 12 and ≥12; see Supplementary Table S3 for analysis with TMB stratified by high, intermediate, and low; $N = 75$ patients)

Variable	Group (*N*)	SD ≥ 6 Months plus PR/CR[a] *N* = 31 (%)	OR[b] (95% CI)	*P* Univariate	*P* Multivariate[c]	Median TTF (months)	HR[b] (95% CI)	*P* Univariate	*P* Multivariate[c]	Median OS	HR[d] (95% CI)	*P* Univariate
Age (years)	<60 ($n = 18$)	6 (33%)	0.6 (0.2–1.8)	0.5844		3.9	1.8 (0.9–3.4)	0.0848		11.4	1.6 (0.8–3.4)	0.1724
	≥60 ($n = 57$)	25 (44%)	1.6 (0.5–5.0)			5.0	0.6 (0.3–1.2)			19.7	0.6 (0.3–1.3)	
Sex	Male ($n = 54$)	21 (39%)	0.7 (0.3–1.8)	0.6032		4.8	1.0 (0.5–1.7)	0.9261		17.4	1.0 (0.5–2.0)	0.9157
	Female ($n = 21$)	10 (48%)	1.4 (0.5–3.7)			4.8	1.0 (0.6–1.8)			17.4	1.0 (0.5–2.2)	
Ethnicity	White ($n = 61$)	25 (41%)	0.9 (0.3–3.2)	>0.9999		5.0	0.8 (0.4–1.5)	0.4328		17.4	1.0 (0.4–2.5)	0.9361
	Other ($n = 14$)	6 (43%)	1.1 (0.3–3.2)			2.9	1.3 (0.6–2.6)			11.4	1.0 (0.4–2.3)	
Disease status	Locally advanced ($n = 23$)	10 (43%)	1.1 (0.4–3.2)	0.8052		5.5	0.9 (0.5–1.6)	0.8419		17.4	0.7 (0.3–1.4)	0.3422
	Metastatic ($n = 52$)	21 (40%)	0.9 (0.3–2.2)			4.7	1.1 (0.6–1.8)			13.1	1.4 (0.7–2.9)	
TMB	TMB < 12 Mutations/Mb ($n = 21$)[c]	6 (29%)	0.3 (0.1–0.9)	0.0616	0.285	4.4	2.7 (1.3–5.7)	0.0058	0.209	12.2	1.6 (0.6–3.7)	0.3243
	TMB ≥ 12 Mutations/Mb ($n = 20$)[c]	12 (60%)	3.8 (1.1–12.3)			9.9	0.3 (0.2–0.7)			17.4	0.6 (0.2–1.5)	
Treatment	≤1 Prior therapy ($n = 46$)	20 (44%)	1.3 (0.5–3.4)	0.8101		4.8	0.9 (0.5–1.6)	0.7385		19.7	0.8 (0.4–1.5)	0.4657
	Two or more prior therapies ($n = 29$)	11 (38%)	0.8 (0.3–2.0)			5.2	1.1 (0.6–1.9)			12.2	1.2 (0.7–2.5)	
	PD-1/PD-L1 Blockade monotherapy ($n = 68$)	29 (43%)	1.9 (0.3–9.8)	0.6926		5.1	1.1 (0.4–2.6)	0.8822		17.4	0.7 (0.2–2.3)	0.5067
	PD-1/PD-L1 Blockade + other ($n = 7$)	2 (29%)	0.5 (0.1–2.9)			4.3	0.9 (0.4–2.3)			8.9	1.4 (0.4–4.7)	
Histology	Cutaneous SCC ($n = 15$)	11 (73%)	5.5 (1.5–16.9)	0.008	0.459	Not reached	0.3 (0.2–0.5)	0.0015	0.397	Not reached	0.5 (0.2–1.0)	0.0593
	Noncutaneous ($n = 60$)	20 (33%)	0.2 (0.1–0.6)			4.2	3.5 (2.0–6.2)			12.5	2.1 (1.0–4.5)	

NOTE: Other therapy: chemotherapy ($n = 1$), targeted therapy ($n = 1$), and investigational agent ($n = 5$).

[a]Response included patients with SD ≥ 6 months, partial responders, and complete responders.

[b]Calculated using Fischer exact test.

[c]Forty-one patients were included in the multivariate analysis. $P \leq 0.1$ in univariate were included in the multivariate analysis. Outcome numbers may be different than in Table 1 because only 41 patients with available TMB were included in the multivariate analysis in this table, whereas in Table 1, all 75 patients were analyzed.

[d]Calculated using log-rank (Mantel–Cox).

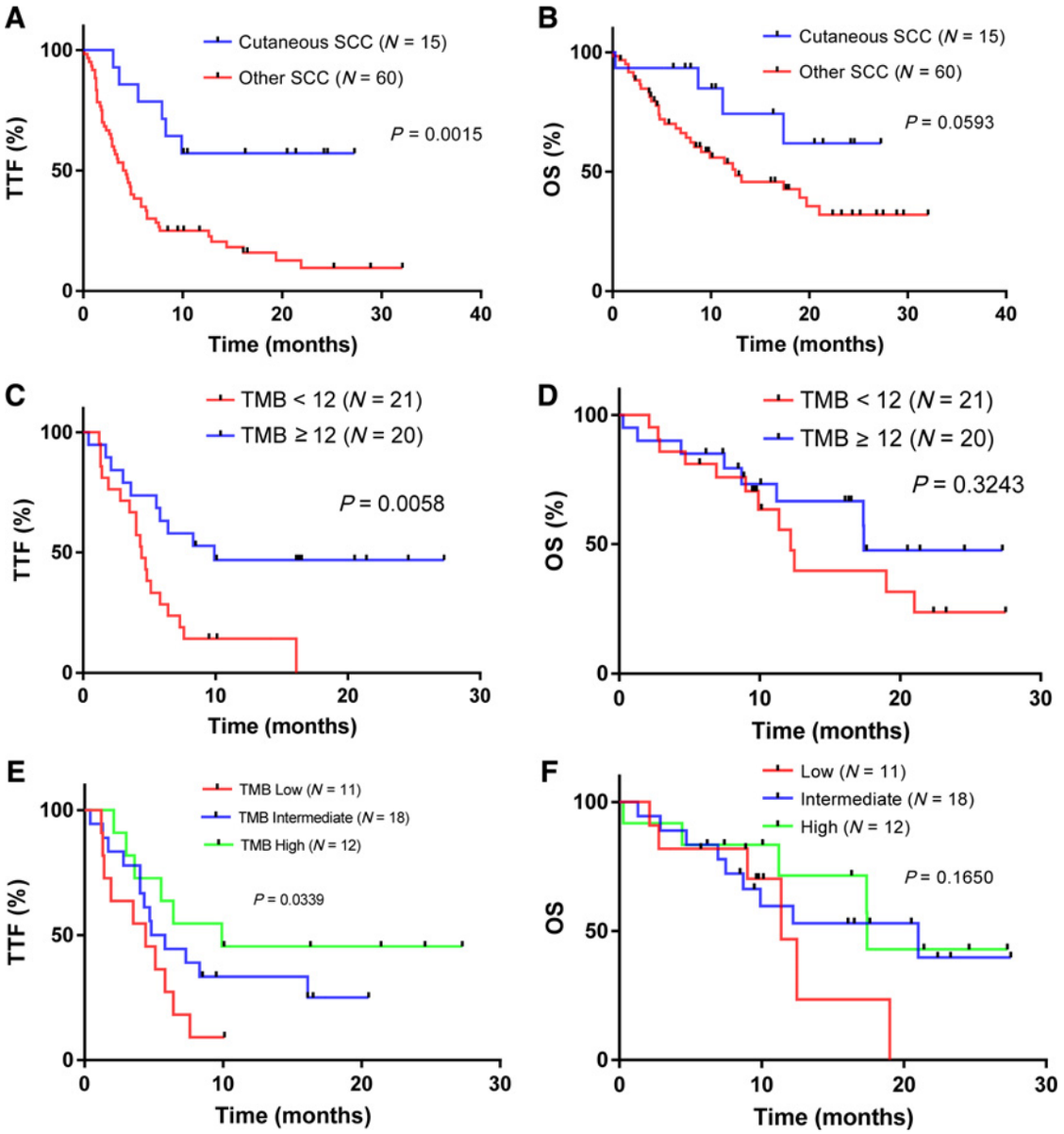

Figure 1.

TTF and OS for patients with advanced SCC treated with PD-1/PD-L1 blockade. **A,** Kaplan–Meier analysis of TTF for cutaneous SCC versus other SCCs. **B,** Kaplan–Meier analysis for OS for cutaneous SCC versus other SCCs. **C,** Kaplan–Meier analysis for TTF for TMB <12 versus ≥12 mutations/Mb. **D,** Kaplan–Meier analysis for OS for TMB < 12 versus ≥12. **E,** Kaplan–Meier analysis for TTF for all SCCs categorized by TMB low versus intermediate versus high. **F,** Kaplan–Meier analysis for OS for all SCCs categorized by TMB low versus intermediate versus high. Number of patients/group indicated.

Our study had several limitations. First, although univariate analysis demonstrated that both higher TMB and cutaneous SCCs were associated with better outcomes after immunotherapy, multivariate analysis was not able to determine whether either of these variables independently predicted outcome. This may have been due to the fact that the number of patients was relatively small and no patients with cutaneous SCC and a low TMB treated with checkpoint blockade were included. Therefore, it was not possible to determine whether TMB could segregate responders from nonresponders with cutaneous SCC. PD-L1 expression by IHC and MSI are both predictors of response to immunotherapy (14, 27). Unfortunately, we did not have this data available for the majority of our samples. Finally, patients in this study were assessed retrospectively and were treated with a variety of immunotherapeutics, although the majority (91%) received anti-PD-1/PD-L1 monotherapy.

In conclusion, SCCs appeared to have high clinical benefit rates after checkpoint blockade, which, in our series, were 73% for cutaneous SCC and 33% for noncutaneous SCC. The high clinical benefit rates, especially in cutaneous SCCs, may be, at least in part,

related to their relatively higher TMB than other SCCs, most likely due to the effects of UV light on cutaneous SCCs (28). As mentioned, TMB has been previously correlated with immunotherapy response (13). In our cohort of patients, 60% with SCCs having a TMB $\geq$ 12 mutations/Mb showed clinical benefit (vs. 29% of patients with TMB < 12 mutations/Mb).

Patients with high TMB and those with cutaneous SCC also showed significantly longer TTF, and cutaneous disease also was associated with a trend toward longer OS. Three patients with noncutaneous SCCs and low TMB also responded, perhaps due to other factors such as *CD274* (PD-L1) amplification, which has been reported to correlate with response to anti-PD1/PD-L1 immunotherapies (29, 30). Taken together, our results demonstrated that SCC, especially those of cutaneous origin and those with higher mutational burdens, are susceptible to checkpoint blockade.

Disclosure of Potential Conflicts of Interest

I.M. Saunders is a consultant/advisory board member for Takeda, Partner Therapeutics, and Genentech. G.M. Frampton has ownership interest (including stock, patents, etc.) in Roche Group. V.A. Miller is chief medical officer at Foundation Medicine/Roche Group, board member at Revolution Medicines, and has ownership interest (including stock, patents, etc.) in Foundation Medicine/Roche Group and Revolution Medicines. R. Kurzrock reports receiving commercial research grants from Incyte, Genentech, Merck Serono, Pfizer, Sequenom, Foundation Medicine, Guardant Health, Grifols, Konica Minolta, and OmniSeq (all institutional), has received speakers bureau honoraria from Roche, has ownership interest (including stock, patents, etc.) in IDbyDNA, CureMatch, Inc., and Soluventis, is a consultant/advisory board member for Gaido, LOXO, X-Biotech, Actuate Therapeutics, Roche, NeoMed, and Soluventis. No potential conflicts of interest were disclosed by the other authors.

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