Tumor Mutational Burden as an Independent Predictor of Response to Immunotherapy in Diverse Cancers

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Abstract

Immunotherapy induces durable responses in a subset of patients with cancer. High tumor mutational burden (TMB) may be a response biomarker for PD-1/PD-L1 blockade in tumors such as melanoma and non–small cell lung cancer (NSCLC). Our aim was to examine the relationship between TMB and outcome in diverse cancers treated with various immunotherapies. We reviewed data on 1,638 patients who had undergone comprehensive genomic profiling and had TMB assessment. Immunotherapy-treated patients ($N = 151$) were analyzed for response rate (RR), progression-free survival (PFS), and overall survival (OS). Higher TMB was independently associated with better outcome parameters (multivariable analysis). The RR for patients with high (≥ 20 mutations/mb) versus low to intermediate TMB was 22/38 (58%) versus 23/113 (20%; $P = 0.0001$); median PFS, 12.8 months vs. 3.3 months ($P \leq 0.0001$); median OS, not reached versus 16.3 months ($P = 0.0036$). Results were similar when anti-PD-1/PD-L1 monotherapy was analyzed ($N = 102$ patients), with a linear correlation between higher TMB and favorable outcome parameters; the median TMB for responders versus nonresponders treated with anti-PD-1/PD-L1 monotherapy was 18.0 versus 5.0 mutations/mb ($P < 0.0001$). Interestingly, anti-CTLA4/anti-PD-1/PD-L1 combinations versus anti-PD-1/PD-L1 monotherapy was selected as a factor independent of TMB for predicting better RR (77% vs. 21%; $P = 0.004$) and PFS ($P = 0.024$). Higher TMB predicts favorable outcome to PD-1/PD-L1 blockade across diverse tumors. Benefit from dual checkpoint blockade did not show a similarly strong dependence on TMB. *Mol Cancer Ther; 16(11); 2598–608. ©2017 AACR.*

Introduction

Immunotherapeutics, including high-dose IL2 and antibodies that block programmed death receptor-1 (PD-1)/programmed death-ligand 1 (PD-L1) and cytotoxic T-lymphocyte–associated protein 4 (CTLA4) can induce durable responses across numerous types of solid tumors (1–7) and hematologic malignancies (8, 9). However, the majority of unselected patients will not respond to immunotherapy, even among those with responsive tumor types. For example, response rates to single-agent PD-1/PD-L1 inhibition in patients with melanoma, non–small cell lung cancer (NSCLC), and renal cell carcinoma (RCC) are 40% (1, 10), 25% (2, 3), and 19% (4), respectively.

There is an unmet need for biomarkers that will identify patients more likely to respond to PD-1/PD-L1 blockade as well as other immunotherapeutics (11). The use of tumor PD-L1 expression as a biomarker has been studied extensively. In general, across all tumor types, anti-PD-1/PD-L1 therapy results in response rates of 0% to 17% in patients with PD-L1-negative tumors, whereas in those with tumors that express PD-L1, response rates range from 36% to 100% (12). However, widespread use and standardization of PD-L1 as a biomarker has been limited by the different detection methods used in practice [immunohistochemistry (IHC), flow cytometry, versus mRNA expression] (9). In addition, there is no standard definition as to what level of PD-L1 expression defines positivity (13). Furthermore, many tumors not only express PD-L1 on malignant cells, but also on the nonmalignant cells within the tumor microenvironment (14). Finally, PD-L1 expression is only applicable to patients treated with PD-1/PD-L1 blockade and not other types of immunotherapy.

Cancers are caused by the accumulation of somatic mutations that can result in the expression of neoantigens (15). Neoantigens occasionally elicit successful T-cell–dependent immune responses against tumors by activating $CD8^+$ CTLs. Primed CTLs can recognize target antigen that is peptide bound to MHC class I (MHC I) and presented on tumor cells, and hence initiate tumor cell lysis (16).

The most robust responses to PD-1/PD-L1 blockade have been seen in melanoma and NSCLC, which are both tumors with a high tumor mutational burden (TMB; ref. 17). Higher nonsynonymous mutational burden in NSCLC, assessed by whole exome

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sequencing (WES), is associated with an improved overall response rate (RR), durable clinical benefit, and progression-free survival (PFS) in patients treated with anti-PD-1/PD-L1 therapy (18). Despite the proven utility of WES in measuring TMB and predicting response to PD-1/PD-L1 blockade, it has many limitations. WES is expensive, time consuming, and labor intensive, and, therefore, difficult to incorporate into clinical practice (19).

Hybrid capture-based next-generation sequencing (NGS) permits simultaneous identification of all classes of DNA alterations (base substitutions, indels, gene rearrangements, and copy number changes) and TMB from a single specimen (20–25). TMB, measured by hybrid-based NGS, has been shown to correlate with response to PD-1/PD-L1 blockade in patients with melanoma (19, 26), NSCLC (26, 27), and urothelial carcinoma (28, 29). Patients with colorectal cancer and mismatch repair gene anomalies (which are generally associated with high TMB) also commonly respond to PD-1/PD-L1 blockade (30). However, it is unknown whether TMB serves as a useful biomarker for predicting response to other forms of immunotherapy and to PD-1/PD-L1 blockade in other tumor histologies (31–33). We hypothesized that TMB, measured by hybrid capture-based NGS, would prove clinically useful in predicting response to immunotherapy across a wide array of tumor histologies.

Materials and Methods

Patient selection

We reviewed the charts of 1,638 cancer patients who had undergone hybrid capture-based NGS (Foundation Medicine) at UC San Diego Moores Cancer Center (October 2012 to August 2016). Only patients treated with immunotherapy were further analyzed. Immunotherapy agents included anti-PD-1/PD-L1, anti-CTLA4, combination anti-CTLA4/anti-PD-1/PD-L1, high-dose IL2, and other agents (see Table 1). This study was performed and consents were obtained in accordance with UCSD Institutional Review Board guidelines for data analysis (NCT02478931) and for any investigational treatments.

NGS and assessment of tumor mutational burden

Formalin-fixed paraffin-embedded tumor samples were submitted for NGS to Foundation Medicine [clinical laboratory improvement amendments (CLIA)-certified lab]. The FoundationOne assay was used (hybrid-capture-based NGS; 182, 236, or 315 genes, depending on the time period; http://www.foundationone.com/). The methods have been previously described (20). Average sequencing depth of coverage was greater than 250×, with >100× at >99% of exons.

For TMB, the number of somatic mutations detected on NGS (interrogating 1.2 mb of the genome) are quantified and that value extrapolated to the whole exome using a validated algorithm (19, 28). Alterations likely or known to be bona fide oncogenic drivers and germline polymorphisms are excluded. TMB was measured in mutations per megabase (mb). TMB levels were divided into three groups based off the Foundation Medicine official reports: low (1–5 mutations/mb), intermediate (6–19 mutations/mb), and high ($\geq$20 mutations/mb), which in a large cohort divided approximately 50% of patients to low TMB, 40% intermediate TMB, and 10% high TMB (34). One hundred nonsynonymous mutations per exome was used previously as a threshold in other articles. Our threshold of 20 coding mutations per megabase is roughly equivalent to 400 nonsynonymous mutations per exome (20 coding mutations/MB * 30 MB / exome * 2/3 nonsynonymous/coding).

For outcome analyses, comparisons were made between both low to intermediate versus high and low versus intermediate to high TMB. In addition, the linearity of TMB across all levels was assessed.

Statistical analysis and outcome evaluation

The Fisher exact test was used to assess categorical variables. P values $\leq$ 0.05 were considered significant. Responses were assessed based on physician notation; physicians used RECIST criteria. PFS and OS were calculated by the method of Kaplan and Meier [P values by log-rank (Mantel–Cox) test]. Linear regressions were performed using the least squares method. Patients who died early were considered evaluable (as progressive disease). For patients who received multiple immunotherapy regimens, the treatment with the longest PFS was chosen for analysis (however, a second analysis that included all treatments given to all patients was also performed). OS was defined as the time from initiation of the immunotherapy with longest PFS until patient death. Patients were considered inevaluable for inclusion in the survival analysis if they were lost to follow up before their first restaging. Patients were censored at date of last follow up for PFS and OS, if they had not progressed or died, respectively. Statistical analyses were carried out by S. Kato using GraphPad Prism version 7.0 and IBM SPSS Statistics version 24.

Results

Patient characteristics

Overall, 151 patients treated with various immunotherapies were evaluable for outcome (Supplementary Fig. S1). Median age was 59 years (range, 19–88 years). The most common tumor types were melanoma and NSCLC (N = 52 and 36 patients, respectively). Sixty-three patients had 19 other tumor types (Tables 1 and 2). All patients had locally advanced or metastatic disease. Thirty-seven patients received multiple lines of immunotherapy (range 2–5; Supplementary Table S1). The outcome data are compiled for the immunotherapy with best PFS (see Materials and Methods) unless otherwise stated. The most common treatment evaluated was anti-PD-1/PD-L1 monotherapy (N = 102; anti-PD1 = 99 and anti-PD-L1 = 3).

Of the 151 patients, 65 (43%) had low TMB (1–5 mutations/mb); 48 (32%), intermediate (6–19 mutations/mb); and 38 (25%), high TMB ($\geq$20 mutations/mb). The median time from biopsy for NGS/TMB to immunotherapy initiation was 8.0, 9.2, and 6.4 months for tumors with low, intermediate, and high TMB (P = 0.2208). The median TMB was 6 mutations/mb (range, 1–347). The median TMB for patients with melanoma (n = 52) was 10.5 (range, 1–133); for NSCLC (n = 36), 5 (range, 1–57); and for tumors other than melanoma or NSCLC (N = 63), median TMB was 6 (range, 1–347).

Among the 151 patients, the number who attained CR/PR was 45 (30 %); median PFS, 4.6 months; median OS, 25.4 months (Table 1).

Outcome by TMB

When TMB was dichotomized by high vs. low to intermediate, age $\geq$60 (P = 0.0014), male sex (P = 0.0349), and Caucasian ethnicity (P = 0.0104) were all associated with a high TMB,

Table 1. Patient demographics by TMB low or intermediate versus high ($N = 151$)[a]

Variable	Group	Number for all patients $N = 151$ (%)	TMB low to intermediate $n = 113$ (%)	TMB high $n = 38$ (%)	P[b]
Age	<60 years	78 (52%)	67 (59%)	11 (29%)	**0.0014**
	≥60 years	73 (48%)	46 (41%)	27 (71%)	
Sex	Men	93 (62%)	64 (56%)	29 (76%)	**0.0349**
	Women	58 (38%)	49 (44%)	9 (24%)	
Ethnicity	Caucasian	111 (74%)	77 (68%)	34 (89%)	**0.0104**
	Hispanic	18 (12%)	18 (16%)	0 (0%)	**0.0070**
	Asian	9 (6%)	7 (6%)	2 (5%)	1.0000
	African American	9 (6%)	8 (7%)	1 (3%)	0.4505
	Other	4 (3%)	3 (3%)	1 (3%)	1.0000
Tumor type	Melanoma	52 (34%)	34 (30%)	18 (47%)	0.0748
	NSCLC[c]	36 (24%)	33 (29%)	3 (8%)	**0.0077**
	Other tumors[d]	63 (42%)	46 (41%)	17 (45%)	0.7060
Type of immunotherapy	Anti-PD-1/PD-L1 alone	102 (68%)	80 (71%)	22 (58%)	0.1631
	Anti-CTLA4 alone	15 (10%)	10 (9%)	5 (13%)	0.5308
	High-dose IL2	9 (6%)	7 (6%)	2 (5%)	1.0000
	Anti-CTLA4/Anti-PD-1/PD-L1	17 (11%)	11 (10%)	6 (16%)	0.3732
	Other immunotherapy[e]	8 (5%)	5 (3%)	3 (8%)	0.4157
Response	CR/PR	45 (30%)	23 (20%)	22 (58%)	**0.0001** (OR 5.38; 95% CI, 2.44–11.58)[f]
	SD or PD	106 (70%)	90 (80%)	16 (42%)	**0.0001** (OR 0.19; 95% CI, 0.09–0.41)[f]
PFS	Median (months)	4.6	3.3 (2.98)	12.8 (0.34)	<**0.0001** (HR 0.34; 95% CI, 0.23–0.50)[f]
OS	Median (months)	25.4	16.3 (3.03)	Not reached (median f/u of 10.5 mos)	**0.0036** (HR 0.33, 95% CI, 0.19–0.58)[f]

NOTE: Significant P values were bolded.

Abbreviations: CI, confidence interval; CR, complete response; PD, progressive disease; PR, partial response; SD, stable disease.

[a]See Supplementary Table S2 for patient demographics by TMB low versus intermediate to high.

[b]Calculated using Fisher exact test and log-rank (Mantel–Cox) test where appropriate.

[c]Histologies included the following: adenocarcinoma ($N = 30$) and squamous cell carcinoma ($N = 6$).

[d]Tumors included the following: adrenal carcinoma ($n = 1$), appendix adenocarcinoma ($n = 1$), basal cell carcinoma ($n = 2$), bladder transitional cell carcinoma ($n = 4$), breast cancer ($n = 3$), cervical cancer ($n = 2$), colon adenocarcinoma ($n = 5$), cutaneous squamous cell carcinoma ($n = 8$), hepatocellular carcinoma ($n = 3$), head and neck ($n = 13$), Merkel cell carcinoma ($n = 2$), ovarian carcinoma ($n = 2$), pleural mesothelioma ($n = 1$), prostate cancer ($n = 1$), renal cell carcinoma ($n = 6$), sarcoma ($n = 3$), thyroid cancer ($n = 3$), unknown primary squamous cell carcinoma ($n = 2$), and urethral squamous cell carcinoma ($n = 1$).

[e]Other immunotherapy: OX40 ($n = 3$), anti-CD73 ($n = 1$), Talimogene laherparepvec ($n = 2$), OX40 + anti-PD-L1 ($n = 1$), and IDO + anti-PD-1 ($n = 1$).

[f]OR >1.0 implies higher chance of response; HR <1.0 implies less chance of progression or death; OR and HR refer to TMB high versus TMB low to intermediate.

whereas age <60 ($P = 0.0014$), female sex ($P = 0.0349$), Hispanic ethnicity ($P = 0.0070$), and NSCLC histology ($P = 0.0077$) were associated with a low to intermediate TMB (Table 1). CR/PR rates were 22/38 (58%) versus 23/113 (20%; $P = 0.0001$); median PFS, 12.8 months versus 3.3 months ($P \leq 0.0001$); median OS, 16.3 months versus not reached ($P = 0.0036$). Supplementary Table S2 shows similar results when TMB was dichotomized by low versus intermediate and high (except that age and sex are no longer significantly associated with TMB stratification).

The median TMB was 19 versus 5 mutations/mb for responders versus nonresponders for all 151 patients ($P \leq 0.0001$; Fig. 1); it was 32 versus 6 mutations/mb for the 63 patients that did not include melanoma and NSCLC ($P = 0.0001$), and it was 16 versus 5 mutations/mb for the 88 melanoma and NSCLC patients ($P < 0.0003$; Supplementary Table S3).

Multivariate analysis of factors predicting outcome after immunotherapy

All tumor types considered together. The key independent factors affecting outcome in multivariate analysis of all 151 patients included having a high versus low to intermediate TMB (CR/PR rate = 58% vs. 20%; $P < 0.001$), and receiving combination therapy with anti-CTLA4/anti-PD-1/PD-L1 versus anti-PD-1/PD-L1 alone (CR/PR rate = 77% vs. 21%; $P = 0.004$; Table 2). Independent factors correlating with longer PFS included hav-

ing melanoma ($P = 0.035$), combination therapy with anti-CTLA4/anti-PD-1/PD-L1 versus anti-PD-1/PD-L1 alone ($P = 0.024$), and TMB high versus low to intermediate ($P < 0.001$). It is interesting that the combined anti-CTLA4/anti-PD-1/PD-L1 remained an independent factor predicting outcome despite the fact that all but one patient receiving the combination had melanoma (note that 16 of 52 patients with melanoma received the combination immunotherapy regimen). Independent predictors of longer OS included having melanoma ($P = 0.006$) and TMB high versus low to intermediate ($P = 0.016$). Identical independent factors were selected for predicting outcome when TMB was dichotomized by low versus intermediate plus high (Supplementary Table S4).

Tumor types other than melanoma and NSCLC. For 63 patients with tumor types other than melanoma and NSCLC, only TMB (high vs. low to intermediate; Table 3) was selected for independently predicting RR (CR/PR rate = 47% vs. 9%; $P = 0.005$) and PFS [median PFS 10 months vs. 2.1 months; $P = 0.0007$; but not OS ($P = 0.1847$)]. Similar results were seen when TMB was dichotomized by intermediate to high versus low (Supplementary Table S5).

Melanoma and NSCLC analysis. Supplementary Tables S6 and 7 show that TMB, dichotomized either as high versus low to

Table 2. Univariate and multivariate analysis of factors affecting outcome for all patients treated with immunotherapy agents (TMB low or intermediate vs. high; $N = 151$)[a]

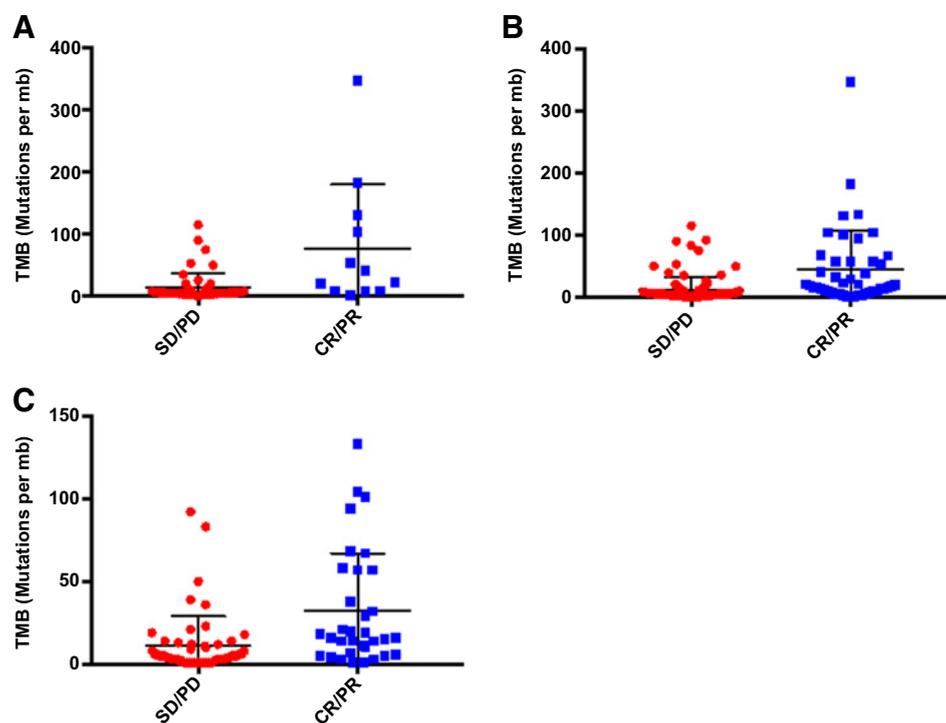| Variable	Group (N)	PR/CR N (%)	OR (95% CI)[b]	P univariate (PR/CR)[c]	P multivariate (PR/CR)	Median PFS (mos)[d]	HR (95% CI) (PFS)[b]	P univariate (PFS)[d]	P multivariate (PFS)	Median OS (mos)[d]	HR (95% CI) (OS)[a]	P univariate (OS)[e]	P multivariate (OS)
Age	≤60 years (n = 78)	20 (26%)	0.66 (0.32–1.33)	0.2873		4.0	1.12 (0.77–1.63)	0.5396		28.4	1.03 (0.61–1.72)	0.9165	
	>60 years (n = 73)	25 (34%)	1.51 (0.75–3.14)			5.7	0.89 (0.61–1.29)			25.4	0.97 (0.58–1.63)		
Gender	Men (n = 93)	33 (36%)	2.11 (0.97–4.57)	0.0675	0.235	5.8	0.70 (0.47–1.03)	0.0572	0.362	28.4	0.88 (0.51–1.50)	0.6204	
	Women (n = 58)	12 (21%)	0.47 (0.22–1.03)			3.6	1.44 (0.97–2.12)			16.3	1.14 (0.67–1.95)		
Ethnicity	Caucasian (n = 111)	38 (34%)	2.45 (0.99–6.29)	0.0685	0.604	5.9	0.58 (0.36–0.92)	0.0066	0.983	28.4	0.65 (0.35–1.20)	0.1192	
	Hispanic (n = 18)	3 (17%)	0.43 (0.13–1.57)	0.2745		2.6	1.38 (0.73–2.61)	0.2543		15.6	1.46 (0.64–3.30)	0.2927	
	Asian (n = 9)	1 (11%)	0.28 (0.02–1.94)	0.2808		2.0	2.61 (0.86–7.90)	0.0063	0.083	Not reached (median f/u 3.4 mos)	1.84 (0.49–7.01)	0.2265	
	African American (n = 9)	3 (33%)	1.19 (0.31–4.45)	1.0000		3.9	1.30 (0.55–3.07)	0.5002		Not reached (median f/u 6.5 mos)	1.26 (0.35–4.60)	0.6916	
	Other (n = 4)	0 (9%)	0 (0–2.40)	0.3181		4.1	1.54 (0.45–5.23)	0.3902		38.33	1.02 (0.25–4.27)	0.9730	
Tumor type	Melanoma (n = 52)	26 (50%)	3.68 (1.71–7.82)	0.0007	0.562	9.3	0.36 (0.25–0.51)	<0.0001	0.035	Not reached (median f/u of 15.6 mos)	0.26 (0.16–0.43)	<0.0001	0.006
	NSCLC (n = 36)	7 (19%)	0.49 (0.19–1.23)	0.1460		2.8	2.00 (1.21–3.27)	0.0007	0.992	8.0	1.90 (0.97–3.72)	0.0213	0.794
	Other tumors[5] (n = 63)	12 (19%)	0.39 (0.18–5.51)	0.0087	0.301	2.9	1.67 (1.12–2.50)	0.0056	0.992	11.2	2.13 (1.23–3.69)	0.0022	0.794
TMB	Low to intermediate (n = 113)	23 (20%)	0.19 (0.09–0.41)	0.0001	<0.001	3.3	2.98 (2.02–4.41)	<0.0001	<0.001	16.3	3.03 (1.72–5.33)	0.0036	0.016
	High (n = 38)	22 (58%)	5.38 (2.44–11.58)			12.8	0.34 (0.23–0.50)			Not reached (median f/u of 10.5 mos)	0.33 (0.19–0.58)		
Type of immunotherapy	Anti-PD-1/PD-L1 monotherapy (n = 102)	21 (21%)	0.27 (0.13–0.58)	0.0006	0.743	3.3	2.41 (1.65–3.50)	<0.0001	0.588	15.7	2.74 (1.63–4.59)	0.0005	0.820
	Anti-CTLA4 alone (n = 15)	2 (13%)	0.33 (0.07–1.40)	0.2329		4.7	1.08 (0.58–2.01)	0.7957		28.4	0.87 (0.39–1.95)	0.7529	
	High dose IL2 (n = 9)	5 (56%)	3.19 (0.88–10.72)	0.1270		37.0	0.40 (0.23–0.71)	0.0146	0.070	Not reached (median f/u of 34.6 mos)	0.37 (0.17–0.80)	0.0614	0.284
	Anti-CTLA4/anti-PD-1/PD-L1 13 (77%) (n = 17)	13 (77%)	10.36 (3.05–30.18)	<0.0001	0.004	Not reached (median f/u 9.2 mos)	0.27 (0.16–0.44)	0.0006	0.024	Not reached (median f/u of 16.3 mos)	0.20 (0.10–0.40)	0.0107	0.172
	Other immunotherapy[9] (N = 8)	4 (50%)	2.49 (0.69–8.82)	0.2392		8.9	0.77 (0.32–1.87)	0.6044		Not reached (median f/u of 5.5 mos)	0.81 (0.23–2.92)	0.7694	

NOTE: Significant P values were bolded.

Abbreviations: CI, confidence interval; CR, complete response; PD, progressive disease; PR, partial response; SD, stable disease.

Abbreviations: CI, confidence interval; CR, complete response; PR, partial response.

[a]All univariate P values of ≤0.1 were included in the multivariate analysis. For a similar analysis by TMB low vs. intermediate to high, see Supplementary Table S4.

[b]OR >1.0 implies higher chance of response; HR <1.0 implies less chance of progression or death.

[c]Calculated using Fisher exact test.

[d]All medians for PFS and OS calculated by Kaplan–Meier.

[e]Calculated using log-rank (Mantel-Cox) test.

[f]Other tumors: Adrenal carcinoma (n = 1), appendix adenocarcinoma (n = 1), basal cell carcinoma (n = 2), bladder transitional cell carcinoma (n = 2), breast cancer (n = 3), cervical cancer (n = 3), colon adenocarcinoma (n = 4), ovarian carcinoma (n = 2), pleural mesothelioma (n = 1), prostate cancer (n = 1), renal cell carcinoma (n = 6), sarcoma (n = 3), thyroid cancer (n = 3), unknown primary squamous cell carcinoma (n = 2), and urethral squamous cell carcinoma (n = 1).

[g]Other immunotherapy: OX40 (n = 3), anti-CD73 (n = 1), Talimogene laherparepvec (n = 2), OX40+anti-PD-1/PD-L1 (n = 1), and IDO+anti-PD-1/PD-L1 (n = 1).

Goodman et al.

Figure 1.
Forest plots comparing TMB for patients treated with immunotherapy agents: responders vs. nonresponders. The mean with SD is represented. **A,** Patients with all tumors excluding melanoma and NSCLC ($N = 63$; $P < 0.0001$). **B,** Patients with all tumors, including melanoma and NSCLC ($N = 151$; $P = 0.0001$). **C,** Patients with melanoma or NSCLC ($N = 88$; $P = 0.0003$). CR, complete response; PD, progressive disease; PR, partial response; SD, standard deviation.

intermediate or as intermediate to high versus low, was also an independent predictor of outcome (RR and PFS) when only the 88 patients with melanoma and NSCLC were included. Treatment with combined anti-CTLA4/anti-PD1/PD-L1 also predicted significantly better outcomes (RR and PFS; P values ranged from 0.042 to 0.003). For OS, the only factor that showed a trend to predict a better outcome was TMB high versus low to intermediate ($P = 0.055$).

Treatment with anti-PD1/PD-L1 monotherapy and outcome by TMB

All tumor types considered together. For the 102 patients treated with single-agent anti-PD-1/PD-L1 antibodies, high TMB correlated with better outcomes as compared with low to intermediate TMB (CR/PR rate = 46% vs. 14%; $P = 0.0025$; PFS = 10 months vs. 2.2 months; $P = 0.0005$; OS = 11.1 months vs. not reached, $P = 0.0557$; Supplementary Table S8 and Fig. 2B and E). Similar results were obtained when TMB was dichotomized at intermediate to high versus low (Supplementary Table S9: $P = 0.0002$, $P < 0.0001$, and $P = 0.0103$, respectively; Supplementary Fig. S2B and S2E).

For anti-PD-1/PD-L1 monotherapy, the response rate was 4% (2/46) for low TMB, 26% (9/34) for intermediate TMB, and 45% (10/22) for high TMB. For patients with very high TMB (which we designate as >50 mutations/mb), the response rate was 67% (8/12). Furthermore, as demonstrated in Supplementary Tables S10 and S11, and Fig. 3, as the cutoff used to dichotomize TMB between low and high increases, the outcome improves in a linear fashion, favoring the TMB high group. This can be seen both for the OR for response (Fig. 3A), the HR for PFS (Fig. 3B), and the HR for OS (Fig. 3C).

Tumor types other than melanoma and NSCLC. When melanoma and NSCLC were excluded (55 patients analyzed; Supplementary

Table S12; Fig. 2A and D for PFS and OS), the CR/PR rate for TMB high versus low to intermediate was 40% versus 8% ($P = 0.0086$); median PFS was 10 months versus 2.1 months ($P = 0.0033$), but median OS did not differ significantly. When comparing this same group of patients and separating them by TMB intermediate to high versus low, the RR and PFS was 26% versus 4% ($P = 0.0620$) and 6.2 versus 2.0 ($P < 0.0001$), respectively (Supplementary Fig. S2A and S2D; Supplementary Table S13).

Melanoma and NSCLC analysis. Finally, when only melanoma and NSCLC were included, CR/PR rates, PFS, and OS all showed either a strong trend or significantly better outcomes as TMB increased (Supplementary Tables S14 and S15; Supplementary Fig. S3). For instance, when TMB was dichotomized as intermediate to high versus low (Supplementary Table S15), CR/PR rate was 44% versus 5% ($P = 0.0023$), PFS (median 5.7 months vs. 1.9 months; $P = 0.0023$) and OS (median not reached vs. 8.0 months; $P = 0.0791$; Fig. 2C and F; Supplementary Fig. S2C and S2F).

When analyzing the 102 patients treated with anti-PD1/PD-L1 monotherapy, including individuals with melanoma and NSCLC, the median TMB for responders versus nonresponders was 18.0 and 5.0 mutations/mb ($P < 0.0001$; Supplementary Table S16). For the 55 patients with tumors other than melanoma and NSCLC, the median TMB for responders versus nonresponders was 53.0 mutations/mb versus 5.5 mutations/mb ($P < 0.0001$). For 47 patients with melanoma and NSCLC, the median TMB for responders versus nonresponders was 15.5 mutations/mb versus 5 mutations/mb ($P = 0.0005$).

Treatment with a combination of anti-CTLA4 and anti-PD-1 therapy. Seventeen patients received combination therapy. All but one of these patients had melanoma. Thirteen (77%) achieved CR/PR. The median TMB for responders versus nonresponders did not

Table 3. Univariate and multivariate analysis of factors affecting outcome for patients with all tumor types excluding melanoma and NSCLC treated with immunotherapy agents (TMB low or intermediate vs. high; N = 63)[a]

Variable	Group (N)	PR/CR N (%)	OR (95% CI)[b]	P univariate (PR/CR)[c]	P multivariate (PR/CR)	Median PFS (mos)[d]	HR (95% CI) (PFS)[b]	P univariate (PFS)[e]	P multivariate (PFS)	Median OS (mos)[d]	HR (95% CI) (OS)[b]	P-value univariate (OS)[e]	P-value multivariate (OS)
Age	≤60 years (n = 33)	4 (12%)	0.38 (0.12–1.35)	0.2017		3.4	1.44 (0.82–2.54)	0.2033		11.1	1.25 (0.60–2.65)	0.5449	
	>60 years (n = 30)	8 (27%)	2.64 (0.74–8.55)			2.7	0.69 (0.39–1.22)			11.2	0.80 (0.39–1.66)		
Gender	Men (n = 41)	11 (27%)	7.7 (1.11–86.57)	**0.0433**	0.219	2.9	0.70 (0.38–1.29)	0.2128		11.1	1.23 (0.56–2.69)	0.6085	
	Women (n = 22)	1 (5%)	0.13 (0.01–0.90)			3.5	1.43 (0.78–2.63)			Not reached (median f/u of 5.4 mos)	0.81 (0.37–1.77)		
Ethnicity	Caucasian (n = 40)	10 (25%)	3.50 (0.77–17.00)	0.1830	0.254	3.6	0.64 (0.35–1.18)	0.1179	0.499	11.2	0.63 (0.29–1.38)	0.2047	
	Hispanic (n = 7)	0 (0%)	0 (0–2.58)	0.3292		1.9	1.38 (0.52–3.65)	0.4515		3.1	2.82 (0.76–10.44)	**0.0168**	0.053
	Asian (n = 7)	1 (14%)	0.68 (0.05–5.40)	1.0000		2.0	1.62 (0.57–4.56)	0.2597		Not reached (median f/u of 3.7 mos)	1.15 (0.32–4.07)	0.8179	
	African American (n = 6)	1 (17%)	0.70 (0.06–5.51)	1.0000		5.0	1.047 (0.41–2.69)	0.9212		Not reached (median f/u of 6.0 mos)	0.80 (0.22–2.97)	0.7625	
	Other (n = 3)	0 (0%)	0 (0–4.99)	1.0000		3.6	1.66 (0.38–7.21)	0.3849		Not reached (median f/u of 8.6 mos)	0.72 (0.13–4.05)	0.7491	
TMB	Low to intermediate (n = 46)	4 (9%)	0.11 (0.03–0.44)	**0.0016**	0.006	2.1	3.31 (1.86–5.91)	**0.0007**	0.003	11.1	1.88 (0.84–4.22)	0.1847	0.362
	High (n = 17)	8 (47%)	9.33 (2.28–31.23)			10.0	0.30 (0.17–0.54)			11.2	0.53 (0.24–1.20)		
Type of immunotherapy	Anti-PD-1/PD-L1 monotherapy (n = 55)	9 (16%)	0.33 (0.06–1.43)	0.1700	0.120	2.9	0.97 (0.38–2.48)	0.9477		11.2	1.23 (0.46–3.28)	0.6633	
	Other immunotherapy[5] (n = 8)	3 (38%)	3.07 (0.70–15.99)			2.6	1.03 (0.40–2.63)			25.4	0.81 (0.31–2.17)		

NOTE: Significant P values were bolded.

Abbreviations: CI, confidence interval; CR, complete response; PR, partial response.

[a]All univariate P values of ≤0.2 were included in the multivariate analysis. For a similar analysis by TMB low versus intermediate to high, see Supplementary Table S5. For an analysis of melanoma and NSCLC on their own, see Supplementary Tables S20–S23. Tumors included the following: adrenal carcinoma (n = 1), appendix adenocarcinoma (n = 1), basal cell carcinoma (n = 2), bladder transitional cell carcinoma (n = 4), breast cancer (n = 3), cervical cancer (n = 2), colon adenocarcinoma (n = 5), cutaneous squamous cell carcinoma (n = 8), head and neck (n = 13), Merkel cell carcinoma (n = 3), hepatocellular carcinoma (n = 3), ovarian carcinoma (n = 2), pleural mesothelioma (n = 1), prostate cancer (n = 1), renal cell carcinoma (n = 6), sarcoma (n = 3), thyroid cancer (n = 3), unknown primary squamous cell carcinoma (n = 2), and urethral squamous cell carcinoma (n = 1).

[b]OR >1.0 implies higher chance of response; HR <1.0 implies less chance of progression or death.

[c]Calculated using Fisher exact test.

[d]All medians for PFS and OS calculated by Kaplan–Meier.

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

[f]Other immunotherapy: OX40 (n = 2), anti-CD73 (n = 2), anti-CTLA4 (n = 1), anti-CTLA4 (n = 2), OX40 + anti-PD-L1 (n = 1), anti-CTLA4/anti-PD-1/PD-L1 (n = 1), anti-CTLA4/anti-PD-L1 (n = 1), and IDO + anti-PD-1/PD-L1 (n = 1).

Goodman et al.
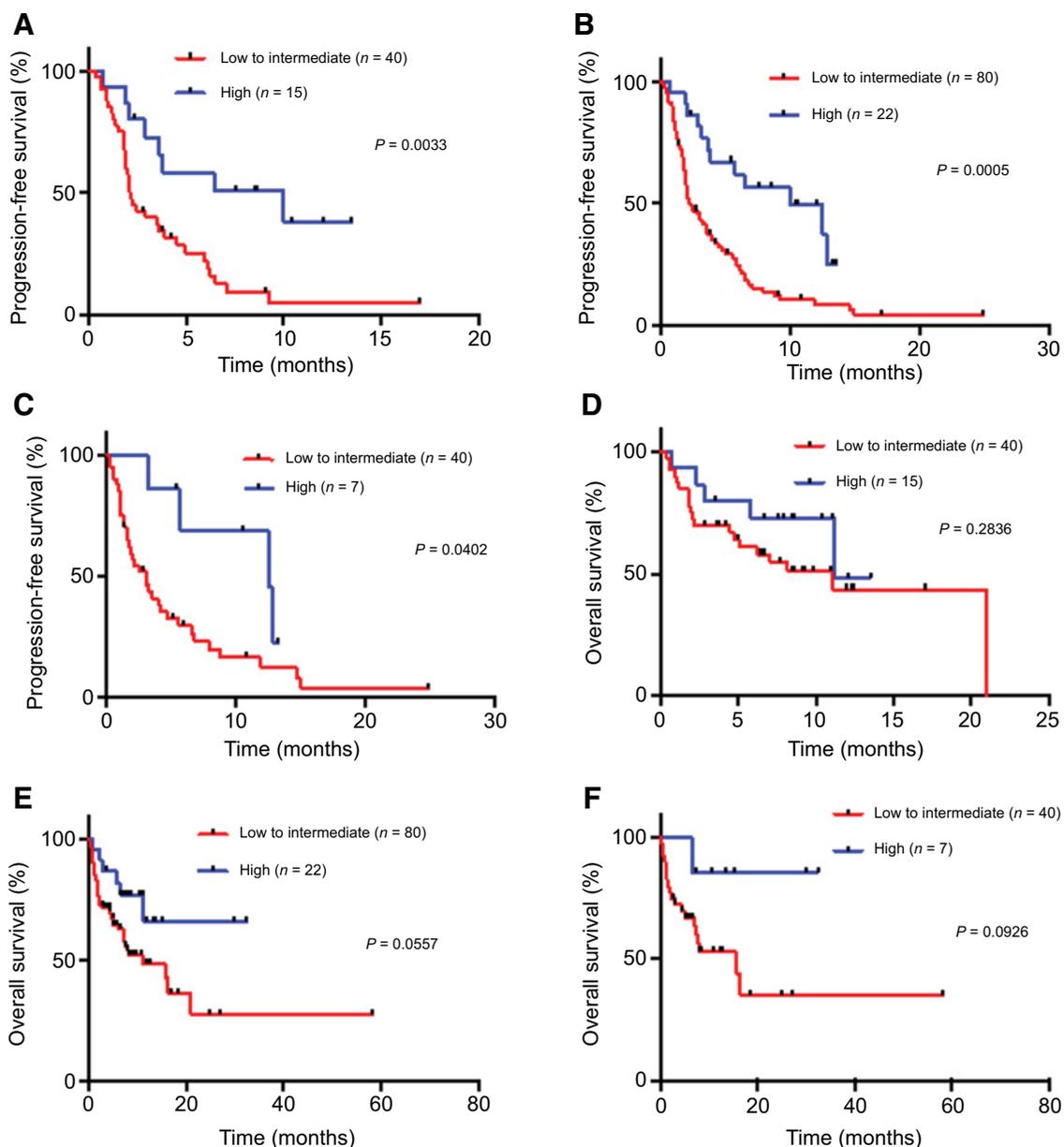
Figure 2.
Kaplan–Meier curves for PFS and OS (for patients treated with anti-PD-1/PD-L1 monotherapy). Tick marks represent patients at the time of censoring, and P values were calculated using log-rank (Mantel–Cox) test. For a similar analysis by TMB low versus intermediate to high, see Supplementary Fig. S2. **A,** PFS for patients with all tumor types excluding melanoma and NSCLC – TMB low to intermediate vs. high ($P = 0.0033$; HR 0.35; 95% CI, 0.19–0.64). For TMB low to intermediate, $N = 40$ with 35 events. For TMB high, $N = 15$ with 8 events. **B,** PFS for patients with all tumor types, including melanoma and NSCLC – TMB low to intermediate vs. high ($P = 0.0005$; HR 0.36; 95% CI, 0.23–0.58). For TMB low to intermediate, $N = 80$, with 66 events. For TMB high, $N = 22$ with 12 events. **C,** PFS for patients with melanoma or NSCLC – TMB low to intermediate vs. high ($P = 0.0402$; HR = 0.36; 95% CI, 0.17– 0.77). For TMB low to intermediate, $N = 40$ with 31 events. For TMB high, $N = 7$ with 4 events. **D,** OS for patients with all tumor types excluding melanoma and NSCLC – TMB low to intermediate vs. high for all tumor types excluding melanoma and NSCLC ($P = 0.2836$; HR 0.59; 95% CI, 0.25–1.40). For TMB low to intermediate, $N = 40$ with 20 events. For TMB high, $N = 15$ with 5 events. **E,** OS for patients with all tumor types, including melanoma and NSCLC – TMB low to intermediate vs. high ($P = 0.0557$; HR 0.44; 95% CI, 0.23–0.87). For TMB low to intermediate, $N = 80$ with 36 events. For TMB high, $N = 22$ with 6 events. **F,** OS for patients with melanoma or NSCLC – TMB low to intermediate vs. high ($P = 0.0926$; HR 0.21; 95% CI, 0.07–0.63). For TMB low to intermediate, $N = 40$ with 16 events. For TMB high, $N = 7$ with one events. CI, confidence interval.

differ ($P = 0.6535$). Among the 17 patients, 6 had a high TMB and, of these 5 (83%) responded; 11 had a low or intermediate TMB and of these, 8 (67%) responded ($P = 1.0000$).

Because of the relatively small number of patients in the above analysis which, as per Materials and Methods, included only patients whose best PFS was on combination treatment,
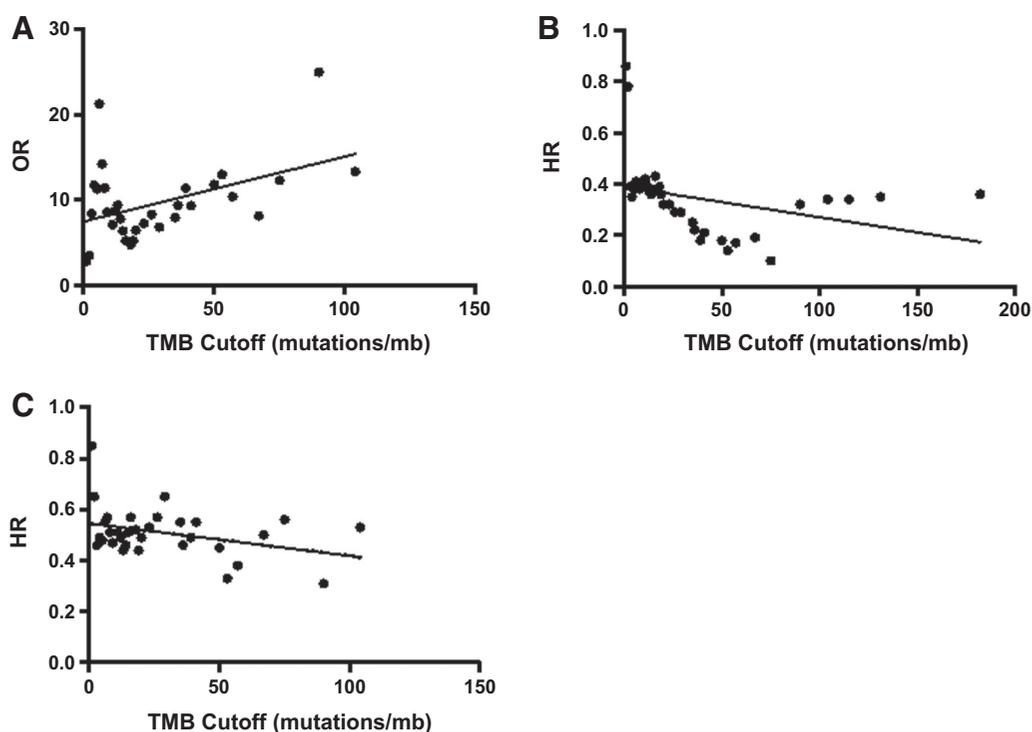
Figure 3.
Linear correlation[a] between TMB cutoff for OR[b] for CR/PR rates and HR[c] for PFS, and OS depending on TMB for patients treated with anti-PD-1/PD-L1 monotherapy ($N = 102$). **A,** OR for CR/PR rate depending on TMB cutoff ($R^2 = 0.1985$, $P = 0.0106$, $Y = 0.07617X + 7.494$). **B,** HR for PFS depending on TMB cutoff ($R^2 = 0.1246$, $P = 0.0487$, $Y = -0.001184X + 0.3886$). **C,** HR for OS depending on TMB cutoff ($R^2 = 0.1985$, $P = 0.0476$, $Y = -0.001275X + 0.5462$). [a], Linear regression performed using the least squares method. [b], OR >1.0 implies higher chance of response. The OR was calculated by comparing RR above and below the cut-off for each value. [c], HR < 1.0 implies less chance of progression or death. The HR was evaluated by comparing OS above and below the cut-off for each value. CR, complete response; PD, progressive disease; PR, partial response; SD, stable disease.

we repeated the analysis with all instances of combination treatment ($n = 27$; Supplementary Table S17). There were 16 responders (59%). Median TMB for responders was 9.5 mutations/mb (range, 1–133); for nonresponders, 6 (1–83; $P = 0.4061$). Median PFS also did not differ by TMB ($P = 0.3051$).

Treatment with other modalities: anti-CTLA4 and IL2. When considering therapy with best PFS in each patient, there were 15 patients treated with anti-CTLA4 monotherapy. Their CR/PR rate was 13% (2/15 patients). The TMB of responders was 20 and 68 mutations/mb; median TMB of nonresponders was 8 mutations/mb (range, 2–92). We also assessed the total treatments with anti-CTLA4 alone ($n = 29$; Supplementary Table S17). There were six responders (21%). Median TMB (mutations/mb) for responders versus nonresponders was 20.5 (range, 16–68) versus 8 (range, 1–92; $P = 0.24$). Median PFS for high versus low to intermediate TMB was 6.4 months versus 2.7 months (HR 0.38; 95% CI, 0.17–0.81; $P = 0.0144$).

When considering therapy with best PFS, there were 9 patients treated with high-dose IL2. Their CR/PR rate was 56% (5/9 patients). TMB of responders was 1, 3, 4, 38, and 58 mutations/mb (median = 4); for nonresponders, 1, 2, 4, and 9 mutations/mb (median = 3). We also assessed all treatments with high-dose IL2 ($N = 22$; Supplementary Table S17). There were nine responders (41%). Median TMB (mutations/mb) for responders versus nonresponders was 16 (range, 1–58) versus

5 (1–16) ($P = 0.056$). Median PFS for high versus low to intermediate TMB was 38.9 months versus 4.2 months ($P = 0.1$; HR 0.24; 95% CI, 0.08–0.77).

Discussion

To our knowledge, this is the first study evaluating the utility of TMB as a biomarker of response to immunotherapy in patients with diverse tumor histologies treated with various types of immunotherapy. Our results suggest that TMB, measured by hybrid capture-based NGS interrogating 1.2 mb of the genome, can predict better outcomes after anti-PD-1/PD-L1 immunotherapy in many tumor types, in addition to melanoma and NSCLC.

Although NGS technology is young, oncologists are beginning to effectively customize treatment for patients by matching targeted therapies with cognate alterations (35–37). NGS also has the ability to recognize alterations that can predict response to immunotherapy by identifying mutations in mismatch repair genes (21), microsatellite instability (MSI; refs. 24, 25, 30, 38, 39), and PD-L1 amplification (40).

Supplementary Table S18 summarizes many of the published abstracts and manuscripts that have evaluated somatic mutational burden in cancer. Most of these studies are descriptive and do not correlate outcome after immunotherapy to TMB. Two published manuscripts (19, 26) and one abstract (27) suggest that TMB

measured by NGS predicts response to anti-PD-1/PD-L1 monotherapy in melanoma and NSCLC. In addition, patients with urothelial carcinoma, who responded to treatment with atezolizumab (anti-PD-L1), had a significantly increased TMB compared to nonresponders (12.4 mutations/mb versus 6.4 mutations/mb, respectively). Finally, patients with colorectal cancer and mismatch repair defects (which are known to result in high TMB) also respond to PD-1/PD-L1 blockade (30).

Herein, we confirm the correlation between TMB and outcome for patients with NSCLC and melanoma, and suggest that this correlation holds true in other tumor histologies (Tables 1–3 and Fig. 1). Patients with a high TMB had significantly higher response rates, and longer PFS and OS than those with a lower TMB, and the correlation between TMB and outcome was linear for patients treated with PD-1/PD-L1 monotherapy blockade (Fig. 3). The association between higher TMB and better response rates and PFS remained significant when we excluded melanoma and NSCLC patients; however, OS did not (though the smaller number of patients may have precluded finding significance).

Patients with rare tumors generally have limited treatment options (41). Utilizing TMB as a biomarker may help select such patients for immunotherapy. For example, in our study, patients with cervical high-grade neuroendocrine carcinoma, metastatic basal cell carcinoma (42), and undifferentiated pleomorphic sarcoma, all of whom had failed multiple prior treatments and had intermediate to high TMB, responded to PD-1/PD-L1 blockade (Supplementary Table S19). Prospective basket trials evaluating patients with uncommon tumors harboring high TMB are needed.

Not surprisingly, TMB is not a perfect predictor of response to anti-PD-1/PD-L1 therapy. In our study, 2 of 46 patients (4.3%) with a low TMB responded to PD-1/PD-L1 blockade, whereas 12 of 22 patients (54.5%) with a high TMB did not achieve an objective response. Of the two patients with a low TMB who responded, one patient had squamous cell NSCLC [TMB = 5 mutations/mb (the cutoff for intermediate TMB is ≥ 6 mutations/mb)]. The other patient had Merkel cell carcinoma (TMB = 1 mutation/mb). Virus-associated Merkel cell carcinomas are known to carry a low mutational burden (43–45); however, these tumors are responsive to PD-1/PD-L1 blockade (46). Viral disease, which may upregulate specific genes such as APOBEC (responsible for mRNA editing; ref. 47), could create immunogenic neoantigens (48). Furthermore, other biological mechanisms (e.g. PD-L1 amplification), in addition to TMB, contribute to immunotherapy response.

In 17 of our patients, anti-PD-1/PD-L1 combined with anti-CTLA4 was the immunotherapy with the best PFS; all but one had melanoma. In these patients, combination therapy was a significant predictor of response and PFS, independent of TMB (multivariate analysis). We also evaluated all treatments with combination therapy ($n = 27$). Median TMB for responders did not differ from that in nonresponders ($P = 0.4061$), and outcome data remained unrelated to TMB. Our analysis suggests that combinations of anti-PD-1/PD-L1/CTLA4-blocking antibodies can induce responses regardless of the TMB level. This observation is supported by prior studies reporting that combined ipilimumab and nivolumab produced similar response rates in PD-L1–expressing and nonexpressing tumors (49), which is relevant because increased PD-L1 expression correlates with higher TMB (50). The number of patients treated with combination therapy

was, however, small in our study, and the implications of TMB level for combination therapy requires validation in larger cohorts.

We used the immunotherapy treatment with best PFS in each patient to assess outcome. However, because anti-CTLA4 or high-dose IL2 were therefore chosen for assessment in only a few patients, we also evaluated all treatments in all patients with these agents. Higher TMB showed a significant correlation or a strong trend to associate with better outcomes [anti-CTLA4 monotherapy ($n = 29$ treatments); high-dose IL2 ($n = 22$ treatments)]. These results are consistent with those previously reported for ipilumumab in melanoma (32, 33).

Our study has several limitations. First, it is retrospective. Furthermore, only 151 patients could be analyzed for immunotherapy response. Second, the number of patients for any given malignancy (other than melanoma and NSCLC) and immunotherapy agent (other than anti-PD-1/PD-L1) were low. For this reason, we also assessed the total number of treatments given, which confirmed our observations. Third, cancers are not static, and can acquire mutations as they evolve. NGS is often performed on old biopsy specimens, and samples tested may therefore not accurately reflect the current mutational burden of a tumor. In our study, the median time to treatment with immunotherapy from biopsy was similar among TMB groups [median 8.0, 9.2, and 6.4 months for TMB low, intermediate, and high, respectively ($P = 0.2208$)]. Even so, it would be ideal to have TMB assessment on tissue obtained immediately prior to therapy.

In conclusion, our study suggests that, across tumor diagnoses, cancers with a higher TMB, measured by comprehensive genomic profiling, have a higher likelihood of immunotherapy response, especially with PD-1/PD-L1 blockade. Similar findings were demonstrated with single-agent anti-CTL4 or high-dose IL2, albeit in small numbers of patients. Outcome after anti-PD-1/PD-L1/anti-CTLA4 combinations appeared to be independent of TMB. Our observations should be validated in prospective cohorts, and clinical trials should incorporate TMB as a biomarker for assigning patients to single-agent immunotherapies such as checkpoint inhibitors. Larger studies are also needed to confirm if dual checkpoint inhibition is less reliant on higher TMB for response.

Disclosure of Potential Conflicts of Interest

Authors' Contributions

Conception and design: A.M. Goodman, R. Kurzrock
Development of methodology: A.M. Goodman, G.M. Frampton,
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): A.M. Goodman, S. Kato, L. Bazhenova, S.P. Patel, G.M. Frampton,
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): A.M. Goodman, S. Kato, L. Bazhenova, G.M. Frampton, G.A. Daniels, R. Kurzrock
Writing, review, and/or revision of the manuscript: A.M. Goodman, S. Kato, L. Bazhenova, S.P. Patel, G.M. Frampton, V. Miller, P.J. Stephens, G.A. Daniels, R. Kurzrock

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