Predictive Impact of Tumor Mutational Burden on Real-World Outcomes of First-Line Immune Checkpoint Inhibition in Metastatic Melanoma

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ABSTRACT

PURPOSE The choice of threshold and reliability of high tumor mutational burden (TMB) to predict outcomes and guide treatment choice for patients with metastatic melanoma receiving first-line immune checkpoint inhibitor (ICI) therapy in the real world is not well known.

METHODS Using a deidentified nationwide (US-based) melanoma clinicogenomic database, we identified a real-world cohort of patients with metastatic melanoma (N = 497) who received first-line monotherapy anti–PD-1 (n = 240) or dual anti–PD-1 and anti–CTLA-4 ICI (n = 257) and had a tissue-based comprehensive genomic profiling test TMB score.

RESULTS TMB-high (TMB-H; ≥10 mutations per megabase [muts/Mb], n = 352, 71%) was independently predictive of superior real-world progression-free survival and overall survival versus TMB-low (<10 mut/Mb, n = 145, 29%) in both mono ICI (hazard ratio [HR], 0.45 [95% CI, 0.32 to 0.63]; *P* < .001; HR, 0.61 [95% CI, 0.41 to 0.90]; *P* = .01, respectively) and dual ICI (HR, 0.67 [95% CI, 0.49 to 0.90]; *P* = .009; HR, 0.61 [95% CI, 0.42 to 0.88]; *P* = .007, respectively) patients. Dual ICI offered no significant advantage in *BRAF*wt patients and unexpectedly demonstrated greatest benefit in the TMB 10-19 mut/Mb group, identifying a TMB-very high (≥20 mut/Mb, n = 247, 50%) *BRAF*mut patient subgroup for whom mono ICI may be preferable.

CONCLUSION TMB-H predicts superior outcomes on ICI while coassessment of *BRAF* status and TMB may inform first-line regimen choice.

INTRODUCTION

Immune checkpoint inhibitors (ICIs) have transformed the treatment landscape of metastatic melanoma, but short-lived response and immune-related toxicity remain clinical limitations for which predictive biomarkers are needed. Tumor mutational burden (TMB), defined as the number of somatic mutations per megabase (muts/Mb) of an interrogated genomic sequence, is both validated and approved as a predictive molecular biomarker for ICI in the treatment of metastatic solid cancers. It is hypothesized that TMB correlates with the chance of responding to ICI, on the basis of the notion that numbers of immunogenic neoantigens and TMB are proportional[1] and latent immune responses to such neoantigens can be augmented or reinvigorated by ICI therapy.[2,3] The predictive utility of TMB for ICI has been demonstrated across multiple cancer types separately,[4-8] and in pan-cancer analyses.[9-11] In the prospective multicohort KEYNOTE-158 study, DNA mismatch repair enyzme (MMR) proficient patients with a high TMB (≥10 mut/Mb, TMB-high [TMB-H]) demonstrated superior response rate and progression-free survival (PFS) versus patients with low TMB (<10 mut/Mb, TMB-low [TMB-L]), measured using the FoundationOne CDx assay, leading to tumor-agnostic approval of this TMB-directed strategy by the US Food and Drug Administration (FDA).[12] Even in MMR deficient tumors for which ICI is highly effective,[9] TMB provided additional predictive information.[13] A threshold of 10 mut/Mb is supported by evidence of a plateau in objective response rates beyond this in patients with previously untreated non–small cell lung cancer receiving ICI in the CheckMate-568 study.[14] Conversely, application of an overall median-based TMB cutoff resulted in substantially variable ICI predictive performance for individual cancer types and lacks applicability to individual patients in the real world.[15]

CONTEXT

Key Objective

Does tumor mutation burden (TMB) predict immune checkpoint inhibitor (ICI) therapy outcome in real-world patients with advanced melanoma?

Knowledge Generated

High TMB (>10 mutations per megabase [muts/Mb]) independently predicts superior real-world progression-free and overall survival after first-line ICI. Unexpectedly, the relationship with TMB is not continuous for dual ICI, identifying a group of patients with very high TMB (≥20 muts/Mb) who may be best treated with monotherapy ICI.

Relevance

Comprehensive genomic profiling including TMB should be performed prior to first-line systemic therapy for advanced melanoma, as TMB results may help refine management choices.

In metastatic melanoma, increasing evidence supports TMB as a predictive biomarker of ICI efficacy yet equipoise remains about its value in routine clinical practice. An early retrospective analysis demonstrated greater clinical benefit for patients with a higher mutational load when treated with anti−CTLA-4 ICI.[16] In the CheckMate-067 phase III clinical trial of ipilimumab, nivolumab, or both in patients with unresectable or metastatic melanoma, high TMB (dichotomized at the median) was associated with improved survival and this effect was particularly apparent in *BRAF* wild-type patients.[17] In this study, we evaluated the impact of TMB on real-world outcomes of patients with advanced melanoma treated with mono- or dual-therapy ICI in the first-line setting using a large clinical database linked to matched tumor-based genomic profiling.

METHODS

Patient Cohort

This study used deidentified data collected as part of the nationwide (US-based, approximately 280 US cancer clinics incorporating approximately 800 sites of care) Flatiron Health-Foundation Medicine Inc (FMI) melanoma clinicogenomic database (CGDB) after institutional review board (IRB) approval with waiver of informed consent on the basis of the observational, noninterventional nature of the study (WCG IRB, Protocol No. 420180044). Participant inclusion in the CGDB required at least two visits to a Flatiron Health clinic site and issuance of a Foundation Medicine comprehensive genomic profiling (CGP) testing report, the latter almost always occurring after a participant has met the clinic attendance requirement and thus being the more critical time point for determination of delayed study entry (see Statistical Analyses). Relevant clinicopathologic variables were extracted from electronic health records (EHRs) for multivariable analyses, as described further in the Data Supplement (Methods). The data cutoff date was March 31, 2022.

Eligibility for inclusion or reasons for exclusion from the final study cohort are outlined in the flowchart (Fig 1). Lines of therapy were derived from EHR treatment data using an oncologist-defined, rule-based approach.[18] Patients must have had structured EHR activity within 90 days of their metastatic diagnosis date to ensure completeness of treatment data capture and therefore accuracy in the enumeration of lines of therapy. Patients must have received one of pembrolizumab, nivolumab, or ipilimumab plus nivolumab as the first line of therapy for metastatic melanoma, and needed to have a TMB score from a tissue-based FMI CGP test (FoundationOne or FoundationOne CDx).

CGP

Hybrid capture-based next-generation sequencing (NGS) was performed as a part of routine clinical care (Foundation Medicine, Cambridge, MA) using the 404-gene FoundationOne and the 324-gene FoundationOne CDx assays, which assess base substitutions, short insertions/deletions, rearrangements/fusions, and copy-number variations as well as genome-wide biomarkers such as TMB and microsatellite instability (MSI).[19,20] For this study, TMB <10 muts/Mb was considered TMB-L, TMB ≥10 muts/Mb was considered TMB-H, and TMB ≥20 muts/Mb was considered very high TMB (TMB-VH).

Statistical Analyses

This study included both prespecified and exploratory analyses. R version 4.2.1 software program was used for all statistical analyses.[21] A prospectively declared statistical analysis plan was developed and executed for all prespecified analyses consistent with International Society for Pharmacoeconomics and Outcomes Research guidelines,[22] covering inclusion and exclusion criteria, potential sources of bias, primary outcome measures, handling of missing data, and all methods described below, unless otherwise noted. Prespecified analyses considered TMB dichotomized at a
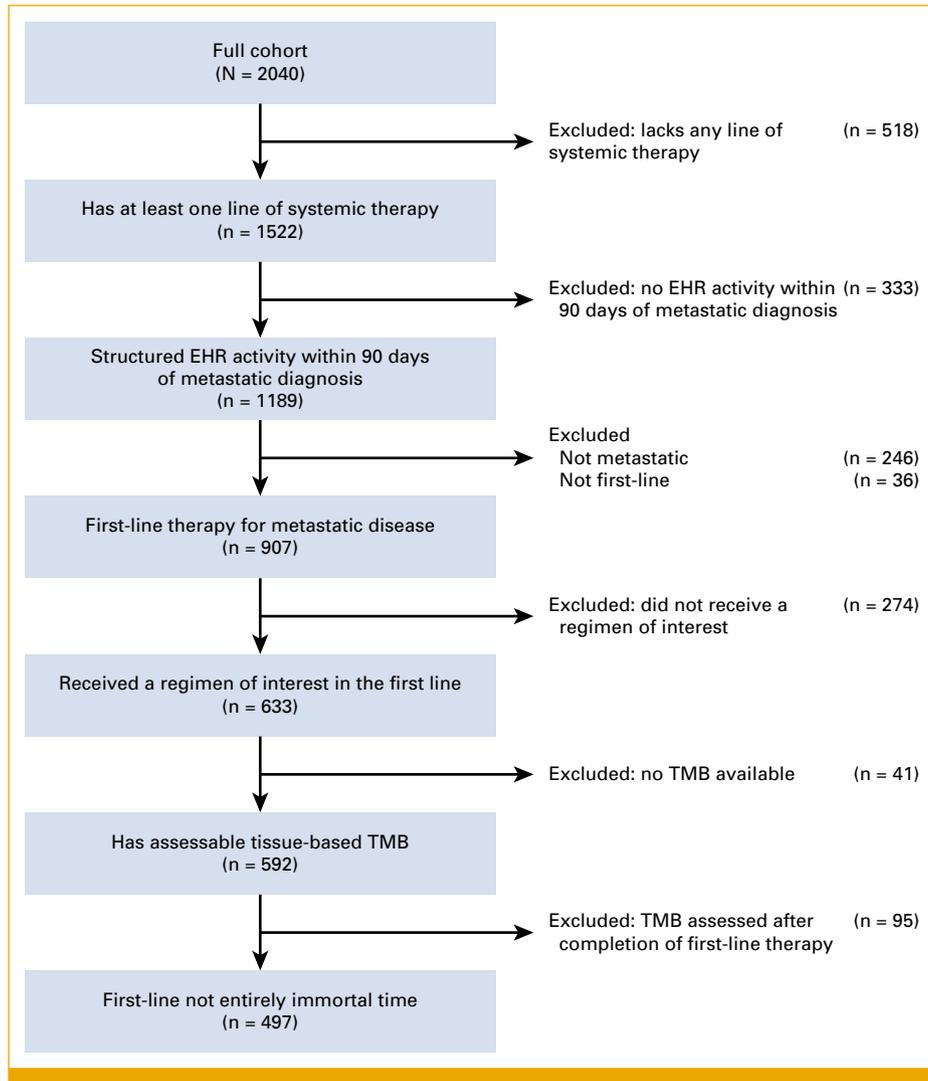
FIG 1. Flowchart of the melanoma study cohort. A total of 497 patients with melanoma were included in the study from 2,040 patients with melanoma from the deidentified Flatiron Health-Foundation Medicine melanoma CGDB after applying inclusion and exclusion criteria. Regimens of interest were nivolumab monotherapy, pembrolizumab monotherapy, and ipilimumab plus nivolumab combination therapy. CGDB, clinicogenomic database; EHR, electronic health record; TMB, tumor mutation burden.

10 mut/Mb threshold, in line with current, FDA-approved CDx indications for pembrolizumab.[12]

Handling of missing values and imputation were performed as described in the Data Supplement (Methods). All individual patient data were used in univariate and multivariable analyses after any missing value adjustments were applied. Overall survival (OS) was calculated from the start of treatment to death from any cause, and patients alive at last observation were censored. Real-world PFS (rwPFS) was calculated from the start of treatment to progression or death from any cause. Progression in this context refers to the real-world progression variable abstracted from EHRs as previously described.[23] Inclusion in this study cohort required issuance of a successful CGP report, which may have

occurred after the start of first line ICI therapy; thus, this data set was left-truncated for the purposes of OS analyses. To account for this, risk-set adjustment was performed, including only patients at each time point who met all inclusion criteria at that time point in Kaplan-Meier and Cox Model analyses (further details are provided in the Data Supplement, Methods). In the primary analysis, the composite end point of rwPFS was incompatible with risk-set adjustment as a patient can experience a nondeath progression event during their period of delayed entry and thus cannot be excluded from the risk set.

For this analysis, nivolumab monotherapy and pembrolizumab monotherapy were pooled together as monotherapy ICI. Patients receiving nivolumab and ipilimumab

were counted as dual ICI therapy. As the data cutoff occurred <2 weeks after its FDA approval, no patients in this study received coformulated nivolumab and relatlimab-rmbw.

The prognostic value of TMB was assessed in both univariable and multivariable contexts using the Kaplan-Meier method and corresponding Cox proportional hazards models, as described in detail in the Data Supplement (Methods).

Sensitivity analyses and exploratory analyses were not prospectively declared, and included assessment of TMB at 10 and 20 mut/Mb cutoffs and the effect of stratification by *BRAF* V600E/K mutations, as described further in the Data Supplement (Methods).

RESULTS

Patient Cohort Characteristics

Of 2,040 patients with melanoma captured by the CGDB, a total of 497 patients with metastatic melanoma received one of the first-line ICI regimens of interest (257 dual ICI, 240 monotherapy ICI) and had a tissue TMB score (Fig 1; Data Supplement, Table S1). Patients receiving monotherapy were older (median age at treatment initiation 71.0 and 63.0 years in monotherapy and dual ICI, respectively; $P < .001$), and had less brain metastases (prevalence 26.2% mono v 35.0% dual ICI; $P = .007$), steroid exposure immediately before initiating therapy (12.5% mono v 19.8% dual ICI; $P = .036$), or *BRAF*mut disease (*BRAF*wt in 62.1% mono v 51.4% dual ICI; $P = .005$), but baseline ECOG performance status did not differ between treatment groups. Patients of this real-world cohort were frailer than those studied in clinical trials such as CheckMate-067, evidenced by a lower proportion of patients with ECOG PS ≤1 and inclusion of patients (11%) with ECOG PS ≥2 (Data Supplement, Fig S1).

Dual ICI use significantly increased over time (25.0%-28.6% of all treatment regimens before 2018 v >65% after 2021) and trended higher in community compared with academic practice settings ($P = .095$). CGP reports were issued a median of 9 days before initiating first-line ICI but only 26.8% of patients received their reports more than 14 days before first dose (Data Supplement, Table S1), with most patients receiving these data after their first dose of ICI (Data Supplement, Fig S2A). However, nearly all (94.0%) CGP tests were performed on specimens collected before first dose of ICI, with a median of 40 days from specimen collection to first dose (Data Supplement, Fig S2B).

Clinicopathologic Associations With TMB

Of the 497 patients in the final cohort, 145 (29%) had low TMB (TMB-L, <10 mut/Mb) and 352 (71%) had TMB-H by the conventional threshold (≥10 mut/Mb), including 247 (50%) with TMB-VH by the exploratory threshold (≥20 mut/

Mb; Table 1). Patients with TMB-VH were more often male, *BRAF*wt, and given anti−PD-1 monotherapy. TMB-VH was enriched in tumor samples taken from brain or pulmonary metastatic sites. Patients with TMB-H were more likely to be *BRAF* V600K than other TMB subgroups and were least likely to have pulmonary metastases. TMB-L was associated with younger age and metastases limited to nonvisceral sites. Similar trends were observed when comparing characteristics strictly between the two subgroups defined by the conventional cutoff of TMB < or ≥10 mut/Mb (Data Supplement, Table S2). No significant association was observed between *CDKN2A/B* variant status or lactate dehydrogenase (LDH) level and TMB. Consistent with its role as the dominant carcinogen exposure relevant to cutaneous melanoma, the presence of ultraviolet (UV) mutation signatures was associated with high TMB, seen in only 17.9% of TMB-L patients, but 88.6% of TMB-H and 92.7% of TMB-VH patients ($P < .001$). The prevalence of *BRAF* non-V600E/K mutations increased with TMB, in keeping with these being largely nondriver *BRAF* mutations arising through the same nonspecific mutational processes leading to high TMB.

Prognostic Value of TMB and ICI Outcomes in First-Line Metastatic Melanoma

TMB-H was prognostic for improved rwPFS and OS in both monotherapy (hazard ratio [HR], 0.45 [95% CI, 0.32 to 0.63]; $P < .001$; HR, 0.61 [95% CI, 0.41 to 0.90]; $P = .01$, respectively) and dual ICI (HR, 0.67 [95% CI, 0.49 to 0.90]; $P = .009$; HR, 0.61 [95% CI, 0.42 to 0.88]; $P = .007$, respectively) patients (Fig 2). TMB-H remained prognostic for better rwPFS and OS outcomes even after adjusting for established prognostic features (Data Supplement, Fig S3). When repeated in exploratory subcohorts defined by the presence or absence of *BRAF* V600E/K mutations, a similar magnitude effect of TMB on rwPFS was seen regardless of *BRAF* status (TMB-H v -L, HR, 0.60 [95% CI, 0.41 to 0.89] in *BRAF*mut; HR, 0.54 [95% CI, 0.40 to 0.71], in *BRAF*wt; Data Supplement, Fig S4). OS results were likely confounded by the availability of effective second-line targeted therapy for *BRAF*mut patients, leading to better OS outcomes in *BRAF*mut patients than in their *BRAF*wt counterparts (Data Supplement, Fig S4). However, inspection of survival curves across the four strata defined by both TMB and *BRAF* status revealed this confounding effect to be relevant only in the TMB-L population. This may reflect a greater dependence on salvage therapy in the TMB-L subgroup who derive less benefit from first-line immunotherapy than TMB-H patients (Data Supplement, Fig S5).

To determine whether a more stringent cutoff for TMB delivered more predictive power, we examined ICI outcomes using stepwise TMB thresholds of 10 and 20 mut/Mb, designating TMB <10 low, TMB 10-19 high, and TMB ≥20 very high. When compared with TMB <10 in patients receiving monotherapy ICI, rwPFS and OS were highest in TMB ≥20 (HR, 0.40 [95% CI, 0.28 to 0.58]; and HR, 0.58 [95% CI, 0.39 to 0.87], respectively) and intermediate for TMB10-19 (Figs 3A and 3B). In patients receiving dual ICI, both TMB 10-19

TABLE 1. Clinicopathologic Characteristics of Patients With Melanoma Stratified by TMB Cutoffs of 10 or 20 Muts/Mb

Feature	All Patients (N = 497)	TMB-L <10 mut/Mb (n = 145)	TMB-H 10-19 mut/Mb (n = 105)	TMB-VH ≥20 mut/Mb (n = 247)	P[a] (overall)
Systemic regimen, No. (%)					<.001
Ipilimumab + nivolumab	257 (51.7)	89 (61.4)	63 (60.0)	105 (42.5)	
Nivolumab	120 (24.1)	25 (17.2)	17 (16.2)	78 (31.6)	
Pembrolizumab	120 (24.1)	31 (21.4)	25 (23.8)	64 (25.9)	
Mono or dual ICI therapy, No. (%)					<.001
Dual	257 (51.7)	89 (61.4)	63 (60.0)	105 (42.5)	
Mono	240 (48.3)	56 (38.6)	42 (40.0)	142 (57.5)	
Age at start of first-line systemic therapy, years, median (IQR)	67.0 (59.0-75.0)	63.0 (52.0-71.0)	67.0 (59.0-73.0)	70.0 (62.0-78.0)	<.001
Race, No. (%)					.045
White	384 (77.3)	100 (69.0)	83 (79.0)	201 (81.4)	
Other race	69 (13.9)	27 (18.6)	16 (15.2)	26 (10.5)	
Unknown/not documented	44 (8.9)	18 (12.4)	6 (5.7)	20 (8.1)	
ECOG performance score, No. (%)					.194
0	230 (46.3)	73 (50.3)	44 (41.9)	113 (45.7)	
1	157 (31.6)	47 (32.4)	32 (30.5)	78 (31.6)	
2	40 (8.1)	10 (6.9)	15 (14.3)	15 (6.1)	
≥3	18 (3.6)	5 (3.4)	3 (2.9)	10 (4.1)	
Unknown	52 (10.5)	10 (6.9)	11 (10.5)	31 (12.6)	
Sex, No. (%)					.007
Female	154 (31.0)	57 (39.3)	36 (34.3)	61 (24.7)	
Male	343 (69.0)	88 (60.7)	69 (65.7)	186 (75.3)	
Practice type, No. (%)					.217
Academic	59 (11.9)	12 (8.3)	12 (11.4)	35 (14.2)	
Community	438 (88.1)	133 (91.7)	93 (88.6)	212 (85.8)	
BRAF mutation status, No. (%)					<.001
No *BRAF* mutation detected	281 (56.5)	72 (49.7)	51 (48.6)	158 (64.0)	
V600E	109 (21.9)	59 (40.7)	28 (26.7)	22 (8.9)	
V600K	40 (8.1)	3 (2.1)	17 (16.2)	20 (8.1)	
Other mutation	56 (11.3)	4 (2.8)	7 (6.7)	45 (18.2)	
Unknown	11 (2.2)	7 (4.8)	2 (1.9)	2 (0.8)	
CDKN2A/B mutation status, No. (%)					.190
Negative	239 (48.1)	63 (43.4)	53 (50.5)	123 (49.8)	
Positive	229 (46.1)	68 (46.9)	48 (45.7)	113 (45.7)	
Unknown	29 (5.8)	14 (9.7)	4 (3.8)	11 (4.5)	
Lactate dehydrogenase, No. (%)					.500
≤ULN	181 (36.4)	47 (32.4)	35 (33.3)	99 (40.1)	

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TABLE 1. Clinicopathologic Characteristics of Patients With Melanoma Stratified by TMB Cutoffs of 10 or 20 Muts/Mb (continued)

Feature	All Patients (N = 497)	TMB-L <10 mut/Mb (n = 145)	TMB-H 10-19 mut/Mb (n = 105)	TMB-VH ≥20 mut/Mb (n = 247)	P^a (overall)
>ULN	96 (19.3)	30 (20.7)	19 (18.1)	47 (19.0)	
Unknown	220 (44.3)	68 (46.9)	51 (48.6)	101 (40.9)	
Sites of metastasis,[b] No. (%)					<.001
Nonvisceral	74 (14.9)	38 (26.2)	11 (10.5)	25 (10.1)	
Visceral pulmonary	128 (25.8)	35 (24.1)	20 (19.0)	73 (29.6)	
Visceral nonpulmonary	124 (24.9)	39 (26.9)	30 (28.6)	55 (22.3)	
Visceral NOS	6 (1.2)	0 (0.0)	3 (2.9)	3 (1.2)	
Brain	153 (30.8)	30 (20.7)	38 (36.2)	85 (34.4)	
Unknown	12 (2.4)	3 (2.1)	3 (2.9)	6 (2.4)	
Liver metastases before first-line ICI, n (%)					.910
Present	120 (24.1)	34 (23.4)	27 (25.7)	59 (23.9)	
Absent	377 (75.9)	111 (76.6)	78 (74.3)	188 (76.1)	
BMI, median (IQR)	27.7 (24.4-31.6)	27.6 (24.5-31.7)	28.9 (25.1-32.9)	27.4 (24.1-31.1)	.153
Year of start of first-line ICI,[c] No. (%)					.288
2015 or earlier	14 (2.8)	7 (50.0)	0 (0.00)	7 (50.0)	
2016	52 (10.5)	15 (28.8)	13 (25.0)	24 (46.2)	
2017	48 (9.7)	11 (22.9)	8 (16.7)	29 (60.4)	
2018	81 (16.3)	25 (30.9)	15 (18.5)	41 (50.6)	
2019	99 (19.9)	36 (36.4)	16 (16.2)	47 (47.5)	
2020	99 (19.9)	24 (24.2)	26 (26.3)	49 (49.5)	
2021	96 (19.3)	24 (25.0)	24 (25.0)	48 (50.0)	
2022	8 (1.6)	3 (37.5)	3 (37.5)	2 (25.0)	
Steroids immediately before therapy, No. (%)					.825
No steroids	416 (83.7)	123 (84.8)	86 (81.9)	207 (83.8)	
Received steroids	81 (16.3)	22 (15.2)	19 (18.1)	40 (16.2)	
Dominant tumor UV signature, No. (%)					<.001
Not observed	22 (4.4)	9 (6.2)	9 (8.6)	4 (1.6)	
Observed	348 (70.0)	26 (17.9)	93 (88.6)	229 (92.7)	
Unknown	127 (25.6)	110 (75.9)	3 (2.9)	14 (5.7)	
Site of TMB tumor specimen, No. (%)					<.001
Skin	95 (19.1)	30 (20.7)	15 (14.3)	50 (20.2)	
Nonvisceral	170 (34.2)	63 (43.4)	43 (41.0)	64 (25.9)	
Visceral pulmonary	97 (19.5)	13 (9.0)	18 (17.1)	66 (26.7)	
Visceral nonpulmonary	63 (12.7)	24 (16.6)	14 (13.3)	25 (10.1)	
Brain/CNS	54 (10.9)	10 (6.9)	9 (8.6)	35 (14.2)	
Other/unspecified	18 (3.6)	5 (3.5)	6 (5.7)	7 (2.8)	

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TABLE 1. Clinicopathologic Characteristics of Patients With Melanoma Stratified by TMB Cutoffs of 10 or 20 Muts/Mb (continued)

Feature	All Patients (N = 497)	TMB-L <10 mut/Mb (n = 145)	TMB-H 10-19 mut/Mb (n = 105)	TMB-VH ≥20 mut/Mb (n = 247)	P[a] (overall)
Days from tumor specimen to start of first-line ICI, median (IQR)	40.0 (22.0-78.0)	37.0 (15.0-70.0)	37.0 (22.0-72.0)	43.0 (23.0-91.0)	.105
Tumor specimen before or after initiation of ICI, No. (%)					.034
Before initiating ICI	467 (94.0)	130 (89.7)	101 (96.2)	236 (95.5)	
After initiating ICI	30 (6.0)	15 (10.3)	4 (3.8)	11 (4.5)	
Days from CGP report to start of first-line ICI, median (IQR)	−9.0 (−27.0 to 16.0)	−12.0 (−40.0 to 15.0)	−7.0 (−17.0 to 15.0)	−8.0 (−26.5 to 16.5)	.243
CGP report availability relative to start of first-line ICI, No. (%)					.969
Report issued at least 14 days before first dose of ICI	133 (26.8)	38 (26.2)	29 (27.6)	66 (26.7)	
Report issued after 14 days before first dose of ICI	364 (73.2)	107 (73.8)	76 (72.4)	181 (73.3)	

Abbreviations: CGP, comprehensive genomic profiling; ECOG, Eastern Cooperative Oncology Group; ICI, immune checkpoint inhibitor; mut/Mb, mutation per megabase; NOS, not otherwise specified; TMB, tumor mutation burden; TMB-H, TMB-high; TMB-L, TMB-low; TMB-VH, very high TMB; ULN, upper limit of normal; UV, ultraviolet radiation.

[a]P values indicate Kruskal-Wallis tests for continuous variables and chi-square/Fisher's exact tests for categorical variables.

[b]Site indicated describes the highest M subcategory (M1a/b/c/d)−associated metastatic site present per patient; patients are counted once only.

[c]Percentages shown in italics for this section are calculated row-wise (ie, percentage of all patients for that calendar year).
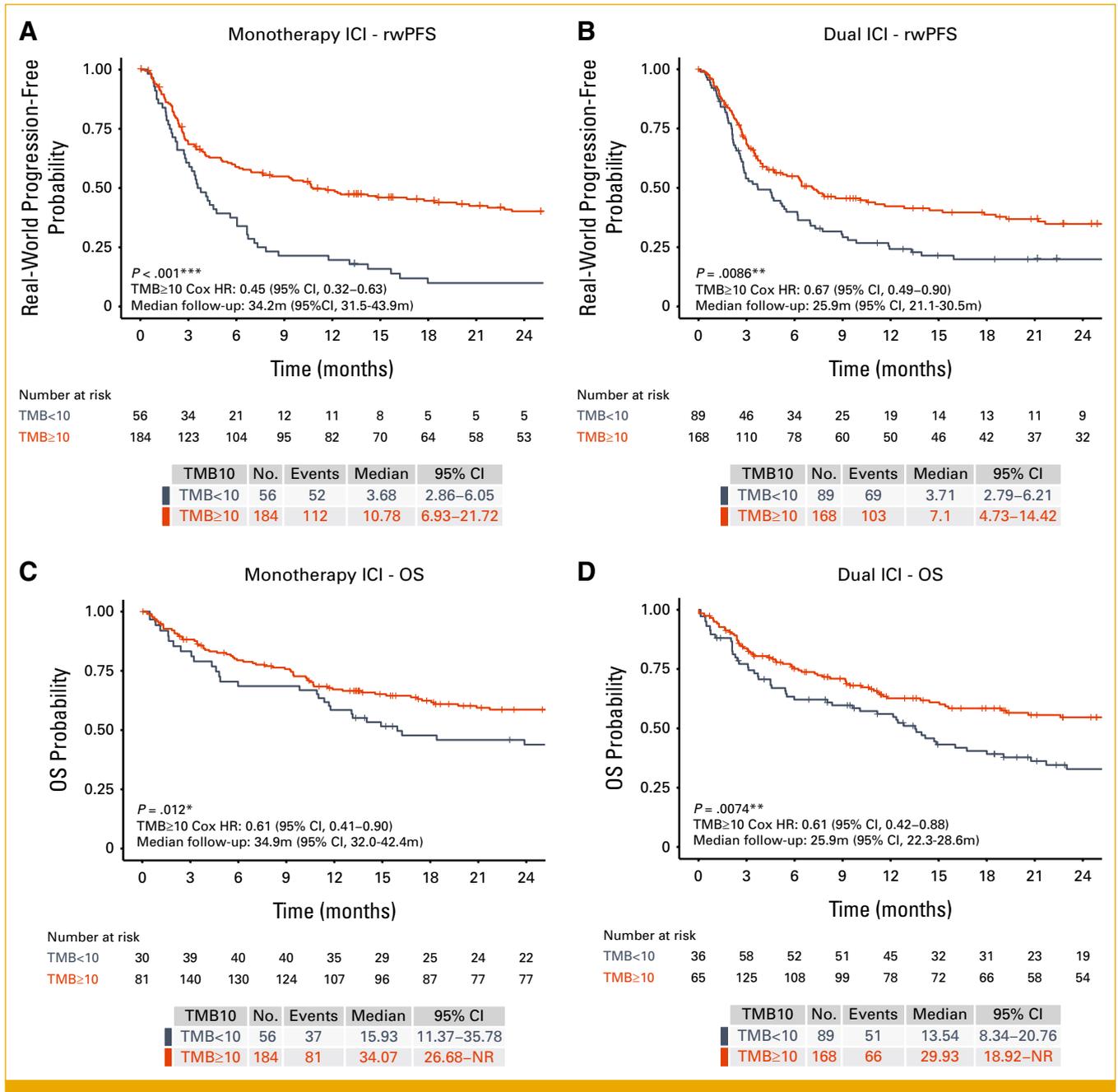
FIG 2. rwPFS and OS stratified by TMB using a 10 mutations per megabase cutoff (TMB10) and ICI regimen. Kaplan-Meier plots are shown stratified by TMB10 for (A) rwPFS in monotherapy ICI-treated patients, (B) rwPFS in dual ICI-treated patients, (C) OS in monotherapy ICI-treated patients, and (D) OS in dual ICI-treated patients. Significance levels: *P < .05, **P < .01, ***P < .001. HR, hazard ratio; ICI, immune checkpoint inhibitor; OS, overall survival; rwPFS, real-world progression-free survival; TMB, tumor mutation burden.

and TMB ≥20 patients demonstrated better rwPFS than TMB <10 patients but the effect was unexpectedly greatest, and statistically significant, for TMB 10-19 (HR, 0.55 [95% CI, 0.37 to 0.83]; and HR, 0.74 [95% CI, 0.53 to 1.03], for TMB 10-19 and TMB ≥20, respectively; Fig 3C). This inverted relationship between TMB and dual ICI outcome was also observed for OS (TMB 10-19: HR, 0.45 [95% CI, 0.26 to 0.76]; and TMB ≥20: HR, 0.72 [95% CI, 0.48 to 1.06], relative to TMB <10; Fig 3D). Significant clinicopathologic differences

in the dual-ICI treated cohort may have contributed to this unexpected result, with more *BRAF*mut (V600E/K 44.4% in TMB10-19 v 23.8% in TMB ≥20; P < .001) but less brain metastases (34.9% TMB 10-19 v 44.8% TMB ≥20; P = .003) and male patients (male 65.1% TMB10-19 v 79.0% TMB ≥20; P = .007) in the TMB10-19 group (Data Supplement, Table S3). In addition, with multivariable analysis, similar effect sizes were seen comparing either TMB ≥20 or TMB 10-19 versus TMB <10, suggesting that the higher TMB groups

A Monotherapy ICI - rwPFS

P < .001***
TMB 10-19 Cox HR: 0.64 (95% CI, 0.41–1.01)
TMB ≥20 Cox HR: 0.40 (95% CI, 0.28–0.58)
Median follow-up: 34.2m (95% CI, 31.5-43.9m)

Number at risk

TMB <10	56	34	21	12	11	8	5	5	5
TMB 10-19	42	21	18	18	16	14	10	9	8
TMB ≥20	142	102	86	77	66	56	54	49	45

TMB	No.	Events	Median	95% CI
TMB <10	56	52	3.68	2.86–6.05
TMB 10-19	42	30	5.45	2.43–17.74
TMB ≥20	142	82	12.52	8.21–33.31

B Monotherapy ICI - OS

P = .031*
TMB 10-19 Cox HR: 0.72 (95% CI, 0.41–1.25)
TMB ≥20 Cox HR: 0.58 (95% CI, 0.39–0.87)
Median follow-up: 34.9m (95% CI, 32.0-42.4m)

Number at risk

TMB <10	30	39	40	40	35	29	25	24	22
TMB 10-19	30	27	26	25	23	20	18	15	15
TMB ≥20	61	113	104	99	84	76	69	63	62

TMB	No.	Events	Median	95% CI
TMB <10	56	37	15.93	11.37–35.78
TMB 10-19	42	19	29.47	9.43–NR
TMB ≥20	142	62	38.01	26.68–NR

C Dual ICI - rwPFS

P = .014*
TMB 10-19 Cox HR: 0.55 (95% CI, 0.37–0.83)
TMB ≥20 Cox HR: 0.74 (95% CI, 0.53–1.03)
Median follow-up: 25.9m (95% CI, 21.1-30.5m)

Number at risk

TMB <10	89	46	34	25	19	14	13	11	9
TMB 10-19	63	42	32	28	21	19	17	14	12
TMB ≥20	105	68	46	32	29	27	25	23	20

TMB	No.	Events	Median	95% CI
TMB <10	89	69	3.71	2.79–6.21
TMB 10-19	63	34	11.04	4.01–NR
TMB ≥20	105	69	6.31	4.01–14.42

D Dual ICI - OS

P = .0078**
TMB 10-19 Cox HR: 0.45 (95% CI, 0.26–0.76)
TMB ≥20 Cox HR: 0.72 (95% CI, 0.48–1.06)
Median follow-up: 25.9m (95% CI, 22.3-28.6m)

Number at risk

TMB <10	36	58	52	51	45	32	31	23	19
TMB 10-19	54	51	40	37	31	28	25	22	20
TMB ≥20	37	74	68	62	47	44	41	36	34

TMB	No.	Events	Median	95% CI
TMB <10	89	51	13.54	8.34–20.76
TMB 10-19	63	19	NR	27.1–NR
TMB ≥20	105	47	19.22	11.17–NR

FIG 3. rwPFS and OS for first-line monotherapy or dual ICI stratified by TMB at 10 or 20 muts/Mb cutoffs. Kaplan-Meier plots stratified by TMB into <10, 10-19, and ≥20 muts/Mb are shown for (A) rwPFS in monotherapy ICI, (B) OS in monotherapy ICI, (C) rwPFS in dual ICI, and (D) OS in dual ICI patients. Both TMB-H and TMB-VH exhibited higher rwPFS and OS compared with TMB-L patients when treated with first-line ICI regimens. Survival outcomes were numerically greatest for TMB-VH patients after monotherapy ICI, but greatest in TMB-H patients after dual ICI. Significance levels: *P < .05, **P < .01, ***P < .001. HR, hazard ratio; ICI, immune checkpoint inhibitor; muts/Mb, mutations per megabase; OS, overall survival; rwPFS, real-world progression-free survival; TMB, tumor mutation burden; TMB-H, TMB-high; TMB-L, TMB-low; TMB-VH, very high TMB.

respond similarly to dual-ICI once adjusted for clinical covariates (Data Supplement, Fig S6).

Characteristics of Patients With Durable Benefit

A total of 24 patients fit the definition of exceptionally durable benefit, defined as >48 months of rwPFS; 92% (22/24) had TMB-H. These patients had several characteristics suggestive of better prognosis, including fewer brain and nonpulmonary visceral metastases, more pulmonary metastases, and less elevated LDH (Data Supplement, Table S4). These patients more commonly received pembrolizumab monotherapy.

Regimen, But Not TMB, Predicts Immune-Related Adverse Events to ICI

Taking on-therapy steroid use as a proxy for immune-related adverse events, we found no difference between TMB-H and TMB-L patients (10 mut/Mb threshold; $P = .28$). As expected, dual-ICI patients were more likely to use steroids on therapy than mono-ICI patients ($P < .001$; Data Supplement, Figs S7A and S7B), findings mirrored in the time-to-steroid analysis (Data Supplement, Figs S7C-S7F).

Predictive Value of Combined TMB and BRAF Assessment

We next performed exploratory multivariable modeling to compare effectiveness of treatment regimen, adjusted by established prognostic factors, within subcohorts defined by TMB at 10 or 20 mut/Mb thresholds. In the TMB ≥20 subgroup, the HR for dual ICI versus mono ICI was 1.54 (95% CI, 1.08 to 2.19) for rwPFS and 1.52 (95% CI, 0.99 to 2.33) for OS versus TMB <20, with wider separation of HR estimates than seen when dichotomized at the 10 mut/Mb threshold, suggesting that patients with TMB ≥20 may benefit from choosing monotherapy over dual ICI (Data Supplement, Fig S8). Subdividing these cohorts further by BRAF V600 mutation status, we observed that the significant rwPFS signal in the TMB-VH cohort was driven by BRAFmut patients (HR for dual ICI, 4.92 [95% CI, 1.4 to 17.2]), supported by a similar trend in OS (Fig 4; Data Supplement, Fig S9). Notably, while the majority of BRAFwt patients were TMB-VH (203 TMB-VH v 140 not TMB-VH), the reverse was true for BRAFmut patients (44 TMB-VH v 110 not TMB-VH; Data Supplement, Fig S10).

Sensitivity Analyses Limited to Patients Who Received Their CGP Results Before ICI Initiation

Sensitivity analyses repeating the main primary analyses were performed on a subcohort where all patients received their CGP results before ICI initiation (197 patients included). These findings showed directional alignment with our primary analysis results, although sample sizes and therefore statistical power were greatly reduced (Data Supplement, Figs S11-S14 and Tables S5 and S6), indicating that the

statistical measures undertaken to mitigate the effects of left truncation in the data set were sufficient.

DISCUSSION

Simple biomarkers are urgently needed to guide ICI risk-benefit decision making with patients, particularly for dual ICI. In this large, multicenter, real-world study of ICI in metastatic melanoma, we confirm that high TMB is a predictor of improved survival outcomes after ICI, even after adjusting for established prognostic factors, and was a feature of patients who derived exceptionally durable benefit (>48 months of rwPFS). The continuous association between TMB and rwPFS in patients receiving monotherapy ICI was expected and consistent with other studies[9,17,24-26] but not matched in patients receiving dual ICI. Patients with extreme levels of TMB (≥20 mut/Mb) benefited less from dual ICI than those with intermediate TMB (10 ≤ TMB < 20), suggesting not only that dual ICI may be unnecessary for patients with TMB-VH, but could be harmful. Furthermore, exploratory analyses identified a small subgroup of BRAFmut TMB-VH patients for whom anti−PD-1 monotherapy appears optimal but these results require further validation against the current standard-of-care recommendation for dual ICI in fit BRAFmut patients.
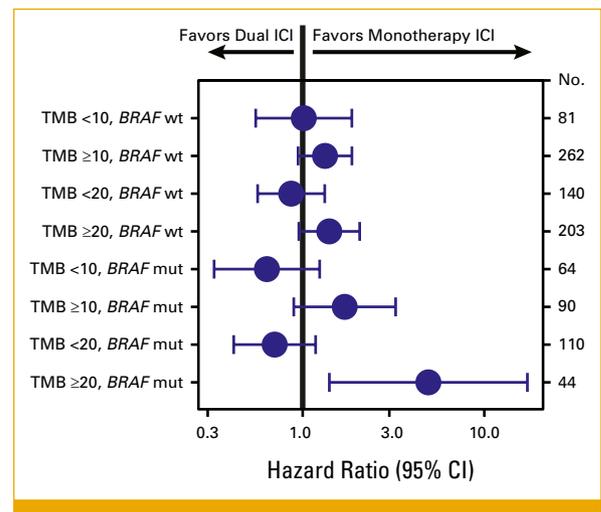
FIG 4. Dual versus monotherapy ICI outcomes stratified by TMB threshold and BRAF mutation status. Forest plot showing hazard ratios for dual versus monotherapy ICI in multivariable Cox proportional hazards modeling of rwPFS in subcohorts defined by TMB at various cutpoints and BRAF V600E/K mutation status as shown. Multivariable models using data for all 497 patients adjusted for age at start of therapy, race, ECOG performance status, sex, academic versus community practice type, CDKN2A/B status, LDH, sites of metastasis, and body mass index. Patients with melanoma with both a BRAF V600E/K mutation and TMB ≥20 mut/Mb may derive greater rwPFS from monotherapy than dual ICI. ECOG, Eastern Cooperative Oncology Group; ICI, immune checkpoint inhibitor; LDH, lactate dehydrogenase; mut/Mb, mutation per megabase; rwPFS, real-world progression-free survival; TMB, tumor mutation burden.

Our identification of inferior outcomes to dual ICI in TMB-VH patients was highly unexpected. TMB-VH patients more frequently had brain metastases at treatment initiation and were more frequently male, both likely to confer an overall worse prognosis. Conversely, TMB-VH patients less frequently harbored *BRAF* V600E/K mutations, consistent with findings from many cancer types, which suggest that founder driver mutations are associated with lower TMB.[27] Clinicopathologic differences in our cohort may therefore partly explain these results as evidenced by the diminished differences seen after multivariable analysis.

Our data confirm and extend upon post hoc analyses of the CheckMate-067 study and a recently reported registry study including 825 patients with *BRAF*wt melanoma treated with first-line ICI, by showing that dual ICI confers little additional benefit to patients with metastatic melanoma without *BRAF* V600E/K mutations at any TMB stratum, including in patients with brain metastases at baseline.[28,29] However, our real-world data incorporating TMB suggest that even in *BRAF* V600E/K-mutated cases, the incremental benefit of dual therapy may no longer hold true at very high levels of mutational burden. This may indicate distinct biology underlying the infrequent co-occurrence of *BRAF*mut and TMB-VH (9% of the total study cohort), suggesting that in these patients, *BRAF* V600 mutations are not classic driver events and could be expected to respond to ICI more like *BRAF*wt/TMB-VH than *BRAF*mut/TMB-L cases. Because of the small subset size, these findings should be interpreted cautiously, but further prospective evaluation of simultaneous TMB and *BRAF*-guided ICI regimen selection is warranted.

In our observational cohort, most patients commenced ICI before TMB results became available, indicating that treatment choice was not informed by TMB. The clinicians' reasons for requesting CGP evaluation are not known and may have been intended to inform subsequent lines of therapy. Our primary analyses therefore evaluate the relationship between TMB and ICI outcome, not whether clinician decision making on the basis of knowledge of the TMB improves survival outcomes. Nevertheless, our data suggest that coassessment of *BRAF* status and TMB could be informative to guide both prognostication and regimen choice in the first-line setting, thus CGP testing results should optimally be available before commencement of therapy.

It is also worth noting that our study cohort was treated before the March 2022 approval of coformulated nivolumab and relatlimab-rmbw—a LAG-3–targeting ICI—for patients with previously untreated advanced melanoma. Clinical trial data suggest that this new ICI combination has superior efficacy to nivolumab monotherapy and a safety profile favorable to ipilimumab plus nivolumab, and it has already been adopted into clinical practice guidelines as a copreferred frontline option with ipilimumab plus nivolumab.[30,31] Future work may consider replicating our study to include nivolumab plus relatlimab as an alternative dual ICI arm.

Two major technical considerations are relevant to studies of TMB. First, tumor-based TMB can be assessed by a wide variety of NGS assays of whole genome, whole exome, or a large targeted gene panel,[32] the last being cheaper, quicker, and more widely available.[2] These assays remain methodologically and analytically unstandardized,[33] making comparisons of TMB performance in different clinical settings and studies challenging,[2] despite efforts to deal with assay-based diversity in TMB estimation.[33] This study used the only current FDA-approved TMB companion diagnostic for an ICI. Second, although the use of a TMB biomarker threshold simplifies clinical decision making, the optimal threshold for patients with melanoma remains undefined. The ≥10 mut/Mb threshold validated using the FoundationOne CDx assay in the KEYNOTE-158 trial[3] has become widely used in melanoma, but evidence supports a more proportional effect of TMB in metastatic melanoma[1] and other cancer types[34] treated with PD-1 inhibitors. Our data emphasize that care is required when applying strict TMB thresholds to guide treatment selection, given the impact of additional clinical covariates and potential interaction with *BRAF* status at high levels of TMB.

Our findings confirm the real-world utility of TMB measurement to predict ICI outcome for patients with metastatic melanoma and it may have underutilized potential to impact regimen choice when considered together with *BRAF* status and established prognostic clinicopathologic factors. Further research is needed to integrate TMB metrics into advanced multifactor biomarker predictive tools as this may offer patients a safe pathway to ICI regimen deintensification and resulting improved tolerability.

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DATA SHARING STATEMENT

The data that support the findings of this study originated by Flatiron Health, Inc and Foundation Medicine, Inc. These deidentified data may

be made available upon request and are subject to a license agreement with Flatiron Health and Foundation Medicine; interested researchers should contact <cgdb-fmi@flatiron.com> and <dataaccess@flatiron.com> to determine licensing terms.

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