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Therapeutic Implications of Detecting MAPK-Activating Alterations in Cutaneous and Unknown Primary Melanomas

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ABSTRACT

Purpose: Cutaneous and unknown primary melanomas frequently harbor alterations that activate the MAPK pathway. Whether MAPK driver detection beyond BRAF V600 is clinically relevant in the checkpoint inhibitor era is unknown.

Experimental Design: Patients with melanoma were prospectively offered tumor sequencing of 341–468 genes. Oncogenic alterations in 28 RTK-RAS-MAPK pathway genes were used to construct MAPK driver groups. Time to treatment failure (TTF) was determined for patients who received first-line programmed cell death protein 1 (PD-1) monotherapy, nivolumab plus ipilimumab, or subsequent genomically matched targeted therapies. A Cox proportional hazards model was constructed for TTF using driver group and clinical variables.

Results: A total of 670 of 696 sequenced melanomas (96%) harbored an oncogenic RTK-RAS-MAPK pathway alteration; 33% had ≥1 driver. Nine driver groups varied by clinical presentation and mutational burden. TTF of PD-1 monotherapy ($N = 181$) varied by driver, with worse outcomes for NRAS Q61 and BRAF V600 versus NF1 or other alterations (median 4.2, 7.5, 22, and not reached; $P < 0.0001$). Driver group remained significant, independent of tumor mutational burden and clinical features. TTF did not vary by driver for nivolumab plus ipilimumab ($N = 141$). Among 172 patients with BRAF V600 wild-type melanoma who progressed on checkpoint blockade, 27 were treated with genomically matched therapy, and eight (30%) derived clinical benefit lasting ≥6 months.

Conclusions: Targeted capture multigene sequencing can detect oncogenic RTK-RAS-MAPK pathway alterations in almost all cutaneous and unknown primary melanomas. TTF of PD-1 monotherapy varies by mechanism of ERK activation. Oncogenic kinase fusions can be successfully targeted in immune checkpoint inhibitor–refractory melanoma.

Introduction

Retrospective studies of cutaneous melanomas and melanomas of unknown primary have revealed frequent alterations, such as *BRAF* V600 and *NRAS* Q61 mutations, that induce MAPK pathway signaling (1). Several selective RAF and MEK inhibitors are now FDA approved for use in patients with BRAF V600 melanoma. A minority of chronically sun-exposed cutaneous melanomas also harbor *KIT* alterations that can be targeted with kinase inhibitors, like imatinib (2). While these results have prompted routine clinical testing for *BRAF* and *KIT* mutations, the clinical utility of broader sequencing panels for additional MAPK driver alterations in patients with melanoma remains unknown (3).

The Cancer Genome Atlas (TCGA) performed a multi-omics analysis of 318 cutaneous melanomas and proposed a classification schema on the basis of the presence of oncogenic mutations in *BRAF*, RAS (*N/H/KRAS*), or *NF1*, with the remainder classified as "triple wild-type" (1). Even after accounting for oncogenic alterations in *KIT, GNAQ*, and *GNA11*, 12% of cutaneous melanomas were "triple wild-type," and it is unclear whether these tumors lack MAPK drivers or whether such alterations went undetected because of stromal contamination or variable sequencing depth (1). Although *NF1* was defined as a genomically distinct subset, roughly one third of *NF1* mutants had MAPK coalterations, most often BRAF non-V600. More recent functional analyses have subdivided *BRAF* alterations into three classes based on their dimer and RAS dependence: class 1, which includes all V600 variants, are dimer and RAS independent; class 2, which are dimer dependent and RAS independent; and class 3, which are dimer and RAS dependent and require upstream activation of RAS via coalteration to induce MAPK activation (4, 5). These molecular insights suggest TCGA driver subgroup classification may need refinement.

Immune checkpoint inhibitors targeting programmed cell death protein 1 (PD-1; e.g., nivolumab and pembrolizumab) and cytotoxic

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Translational Relevance

The Cancer Genome Atlas defined four groups of MAPK alterations in cutaneous melanomas, BRAF, RAS, NF1, and "triple wild-type," that are widely used clinically. This system needs refinement to better reflect the improved understanding of mechanisms of ERK activation and to provide prognostic information for the checkpoint inhibitor era. We utilized a large, consecutive cohort of patients with cutaneous and unknown primary melanomas to construct nine mutually exclusive MAPK driver groups with distinct clinical features and tumor mutational burden (TMB). TMB was associated with overall survival with programmed cell death protein 1 (PD-1) treatment alone or with cytotoxic T-lymphocyte antigen-4 inhibition. Time to failure of PD-1 blockade was shorter for NRAS Q61 and BRAF V600 mutants versus NF1 or other alterations. Driver group remained significantly associated with time to treatment failure, independent of TMB and other clinical characteristics. For patients who progress on PD-1–based therapy, targeted inhibitors of rare kinase fusions can achieve durable complete responses. These refined MAPK driver groups offer prognostic information for clinicians and can improve the validity of preclinical genomic models of melanoma.

T-lymphocyte antigen-4 (CTLA-4; e.g., ipilimumab) are standard treatments for advanced melanoma (6–8). In the prospective randomized trial comparing nivolumab plus ipilimumab with PD-1 monotherapy, combination therapy was associated with improved objective response rate and progression-free survival at the expense of increased toxicity (8). The impact on overall survival (OS) remains unclear; thus, better predictive biomarkers are needed to select patients most likely to require combination therapy. High tumor mutational burden (TMB) has been linked to improved outcomes from checkpoint inhibitor therapy in melanoma, but its association with other clinical features, such as driver mutation status, age, sex, and primary tumor site, is not well understood (9–11).

To explore these questions, we retrospectively analyzed a prospective cohort of patients with melanoma who underwent clinical tumor sequencing using the MSK-IMPACT assay, a capture-based next-generation sequencing (NGS) platform (12). We integrated prospectively collected tumor genomic data with clinical and treatment response data to identify novel molecular drivers that could be targeted therapeutically and serve as prognostic biomarkers of benefit to PD-1–based therapy.

Materials and Methods

Institutional review board approval was obtained to collect clinical and treatment data for all patients with cutaneous and unknown primary melanoma sequenced using one of three versions (341, 410, or 468 genes) of MSK-IMPACT between January 2014 and April 2019 at two centers (Memorial Sloan Kettering Cancer Center, New York, NY and Lehigh Valley Medical Center, Bethlehem, PA; refs. 12, 13). All patients provided written informed consent, and the study was conducted in accordance with ethical principles described in the Declaration of Helsinki. Samples were excluded if estimated tumor purity was <20%, sequencing depth was <50 ×, if it was a duplicate from the same patient, or if the patient had received prior targeted therapy. For patients with >1 sample analyzed, we included the sample that, in order of priority, had higher purity, predated systemic treatment, and was metastatic rather than primary. For all analyses regarding driver alterations among 28 genes in the RTK-RAS-MAPK pathway (*BRAF, NF1, NRAS, KRAS, HRAS, KIT, PDGFRA, ERBB2, ERBB3, ERBB4, FGFR2, FGFR3, ALK, ROS1, NTRK1, NTRK3, CBL, SOS1, PTPN11, RASA1, SPRED1, ARAF, RAF1, RAC1, MAP2K1, MAP2K2, GNAQ*, and *GNA11*), only variants predicted to be oncogenic by the OncoKB knowledgebase (14) were included. There was no minimum variant allele fraction required for inclusion. FACETs (15), an allele-specific copy-number algorithm, was used to infer clonality of nonsynonymous variant driver mutations for a subset of samples with sufficiently high-quality data.

BRAF mutation classes were assigned as described previously (4, 5). TMB was estimated by calculating the number of nonsynonymous variants and dividing by the total sequenced exon length (13). Log_{10} transformation was used for all TMB analyses.

Clinical features collected included sex, age, primary tumor site, Eastern Cooperative Oncology Group (ECOG) performance status, and lactic dehydrogenase (LDH). Details on treatment initiation and survival were collected for patients who received first-line therapy with PD-1 inhibitor +/− ipilimumab for advanced or unresectable disease without prior adjuvant RAF or checkpoint inhibitors. Time to treatment failure (TTF) was defined as the interval between initiating therapy and the earliest of clinical progression, new locally directed or systemic treatment, or death, as described previously (16). OS was defined from initiation of therapy. Patients alive and free of treatment failure at last follow-up were censored.

Association between categorical variables was tested using Fisher exact test. For continuous variables, a nonparametric Wilcoxon rank-sum test or Kruskal–Wallis test was used for 2 and >2 groups, respectively. The multivariate Cox proportional hazards model was built by using backward selection of variables significant ($P < 0.05$) on

Table 1. Demographics.

Total patients	***n* = 696**
Melanoma type	
Cutaneous	556 (80%)
Unknown primary	140 (20%)
Age, median (range)	61 (8–95)
Sex	
Male	461 (66%)
Female	235 (34%)
Sample type	
Recurrent/metastatic	592 (85%)
Primary	104 (15%)
Sequenced sites	
Primary	104 (15%)
Regional LN/in-transit	271 (39%)
Distant LN/soft tissue	96 (14%)
Lung	88 (13%)
Brain	52 (7.5%)
Liver	36 (5.2%)
Bone	16 (2.3%)
Other visceral metastasis	33 (4.7%)
Cutaneous primary site	
Face	162 (29%)
Trunk	181 (32%)
Upper extremity	102 (18%)
Lower extremity	110 (20%)
Not available	1 (<1%)

Abbreviation: LN, lymph node.

univariate analysis. Differences in TTF and OS were evaluated using log-rank tests and Kaplan–Meier methods. All analyses were performed in R version 3.4.4.

All genomic and clinical data are accessible through the cBioPortal for Cancer Genomics (ref. 17; http://cbioportal.org/study?id=mel_ mskimpact_2020) and the MAF file is available as Supplementary Table S1.

Results

Between January 2014 and March 2019, 792 cutaneous and unknown primary melanomas were analyzed. Of these, 756 (95.4%) tumors were successfully sequenced, and 696 tumors from unique patients met inclusion criteria (Supplementary Fig. S1). Median sequencing depth was 709×.

Patient demographics are summarized in **Table 1** and Supplementary Table S2. A total of 556 patients (80%) had cutaneous melanomas, whereas 140 (20%) patients had melanomas of unknown primary. The majority of patients were men (66%). Median age at initial melanoma diagnosis was 61 years (range, 8–95). A total of 104 (15%) were primary melanomas; 46% were from distant metastatic sites. Cutaneous melanoma primary sites were relatively evenly divided between trunk, face, and the extremities.

Identification of RTK-RAS-MAPK pathway driver mutations

A total of 216 of 696 samples (31%) harbored BRAF V600E/K/R mutations. Non-V600 BRAF alterations were present in 97 tumors. A total of 55 (18%) of all *BRAF* alterations were class 2 mutants and 30 (10%) were class 3 mutants. Activating alterations in *NRAS, HRAS*, and *KRAS* were identified in 29%, 2%, and 1.3% of patients, respectively. Mutations in *MAP2K1* or *MAP2K2* were identified in 7% of patients. Predicted loss-of-function *NF1* alterations were identified in 23% of patients. Activating mutations in *KIT, GNAQ*, and *GNA11* were identified in 4%, 1%, and 0.4% of samples, respectively. In sum, 670 (96%) harbored a known or likely oncogenic alteration in ≥1 of 28 genes predicted to increase MAPK pathway activation (**Fig. 1A**).

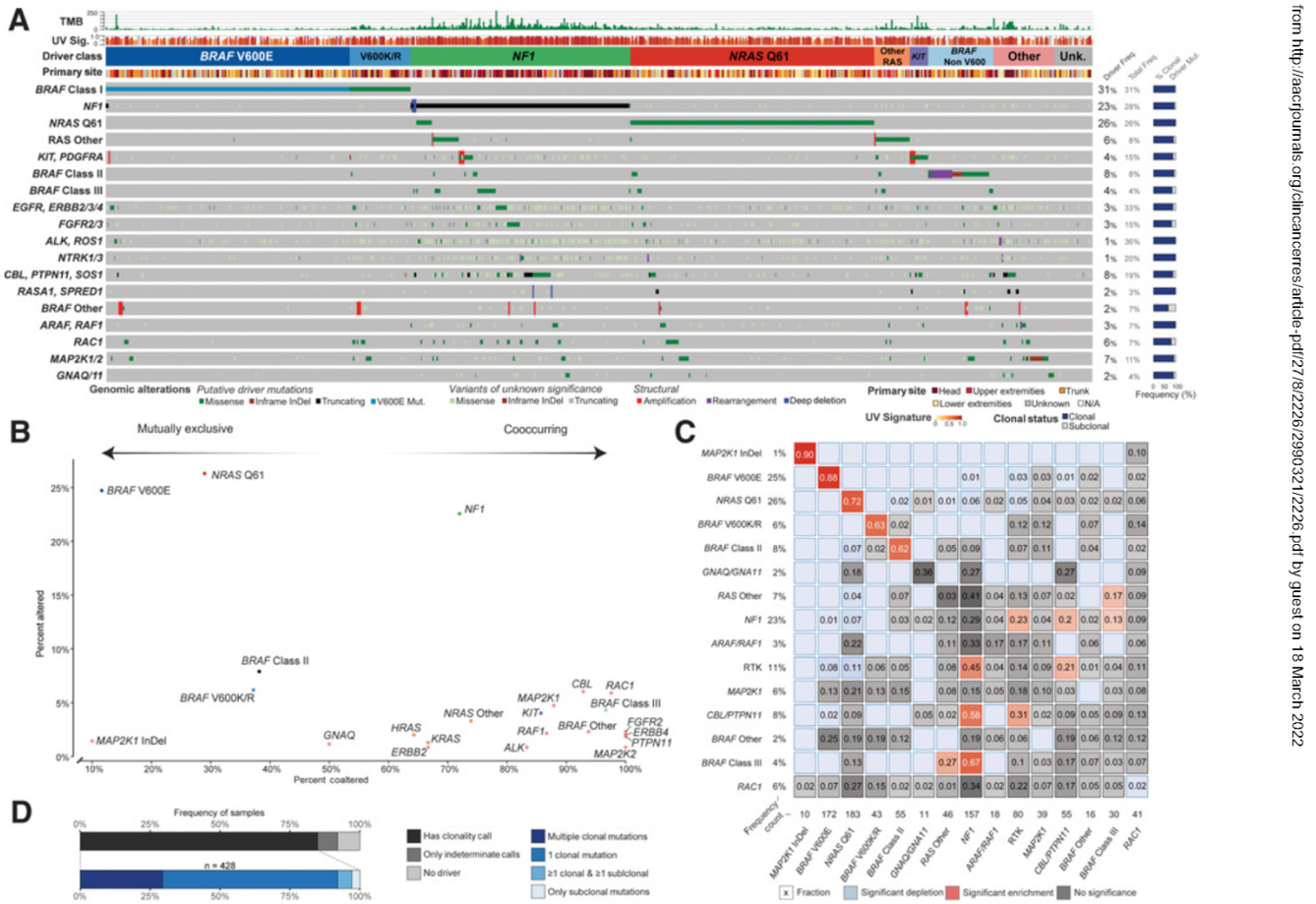

Figure 1.

A, Oncoprint of 696 cutaneous and unknown primary melanomas naïve to targeted therapy depicting nine mutually exclusive classes of RTK-RAS-MAPK pathway drivers and their relationship to primary site, percentage of mutations attributable to a UV signature, and TMB. **B,** Plotting the frequency of oncogenic and presumed oncogenic RTK-RAS-MAPK alterations and how often they are coaltered with other RTK-RAS-MAPK driver alterations identifies a rough dichotomy between "sole drivers," such as BRAF V600, NRAS Q61, BRAF class 2 alterations, and MAP2K1 indels, and frequently coaltered "backseat drivers," such as NRAS non-Q61 alterations, CBL, RAC1, and other RTKs. **C,** Plotting the frequency of specific pairs of validated alterations identifies an enrichment for NF1 coalterations with BRAF class 3 alterations, CBL, PTPN11/RASA1, and RTKs. **D,** Clonality was calculated among 428 cases with driver mutations only (no fusions or copy-number changes) and adequate sequencing quality. Of those, 92% had only clonal mutations, 5% had clonal and subclonal, and 3% had subclonal driver alterations only.

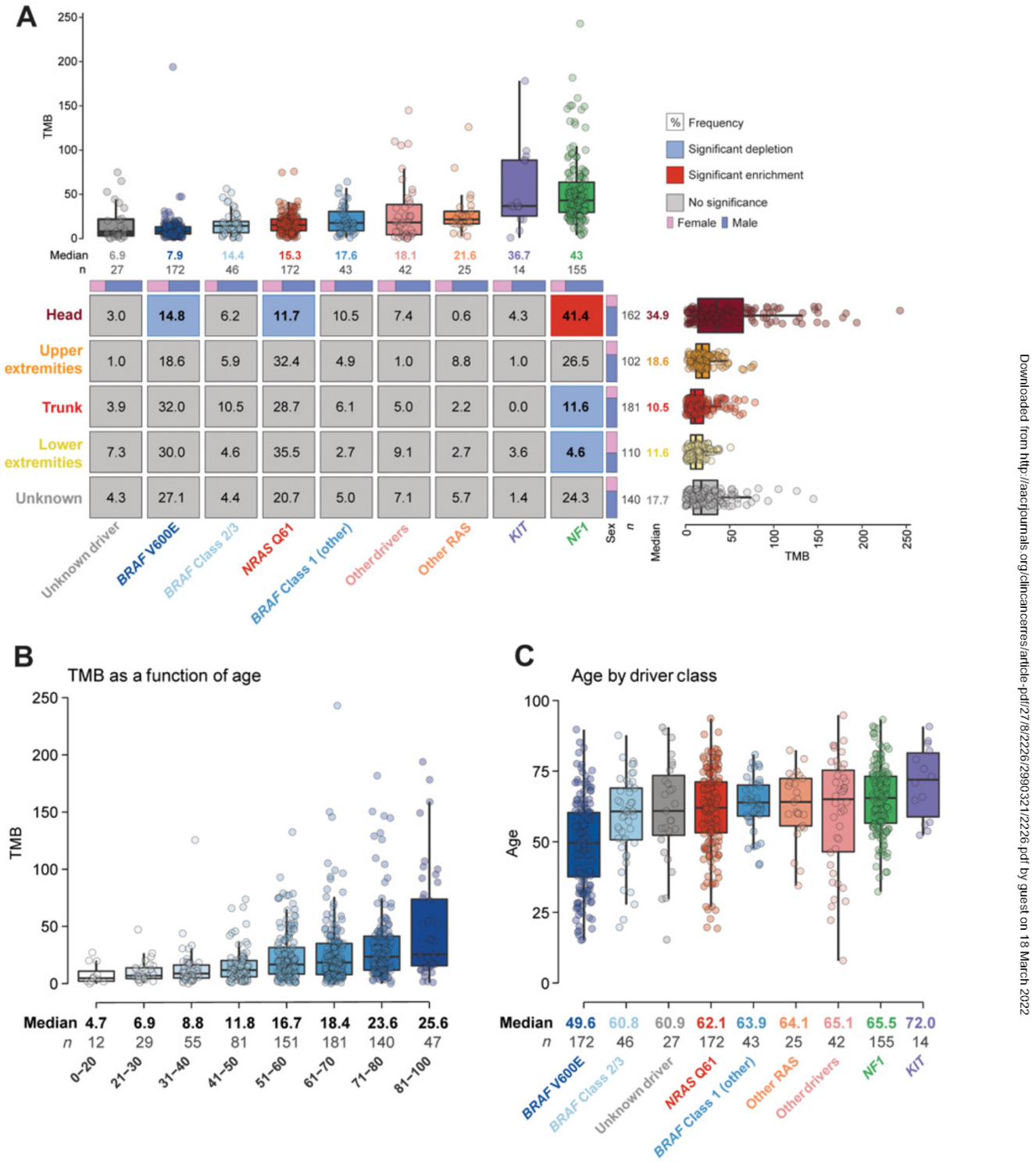

Figure 2.

A, The relationship between mutually exclusive driver class, primary site of melanoma, and TMB. NF1 is the driver with highest TMB and is enriched in head/neck primary sites. BRAF V600E and NRAS Q61 tumors are depleted in head/neck primary sites. **B,** TMB gradually rises with increasing patient age at time of primary melanoma diagnosis. **C,** Patients with BRAF V600E melanomas have the youngest median age at diagnosis, whereas those with KIT-mutant melanomas have the oldest median age.

The rate of MAPK drivers appeared similar between cutaneous melanomas and melanomas of unknown primary (Supplementary Fig. S2A).

Concurrent alteration of more than one of the 28 MAPK pathway genes was observed in 233 patients (33%), with coalteration rates varying by gene and in some cases by codon (**Fig. 1B**). Tumors with BRAF V600E mutations were less likely to harbor MAPK coalterations than tumors with other BRAF V600 alleles (K/R; 11.6% vs. 37%, respectively; $P = 0.0003$). Tumors with class 3 *BRAF* mutations were more likely to have a concurrent alteration in the MAPK pathway than tumors harboring class 2 alterations (97% vs. 38%, respectively; $P = 3.4e\text{-}08$). Similarly, NRAS Q61–altered samples harbored MAPK coalterations less frequently than tumors with other RAS alterations (29% vs. 70%, $P = 7.9e\text{-}07$; **Fig. 1B**). When specific drivers were compared, *NF1* was most often coaltered with RTK genes, *BRAF* class 3 alterations, or *CBL*. In contrast, class 1 or 2 *BRAF* alterations, NRAS Q61 mutations, and *MAP2K1* indels were likely to be the sole drivers of RTK-RAS-MAPK activation (**Fig. 1C**). *MAP2K1* missense mutations, however, commonly cooccurred with other MAPK drivers.

We investigated clonality in a subset of 428 samples with sufficient quality for FACETS analysis and at least one mutation in the 28 genes. Of these, 394 (92%) had only clonal alterations, 22 (5%) had both clonal and subclonal, and only 12 (3%) had exclusively subclonal MAPK alterations (**Fig. 1D**).

TMB varies as a function of MAPK driver

The median TMB (range) of all samples was 16.3 mutations (mut)/Mb (0–243 mut/Mb). To explore the association between TMB and the biologic basis of MAPK pathway activation, tumors were placed into nine mutually exclusive driver groups with BRAF

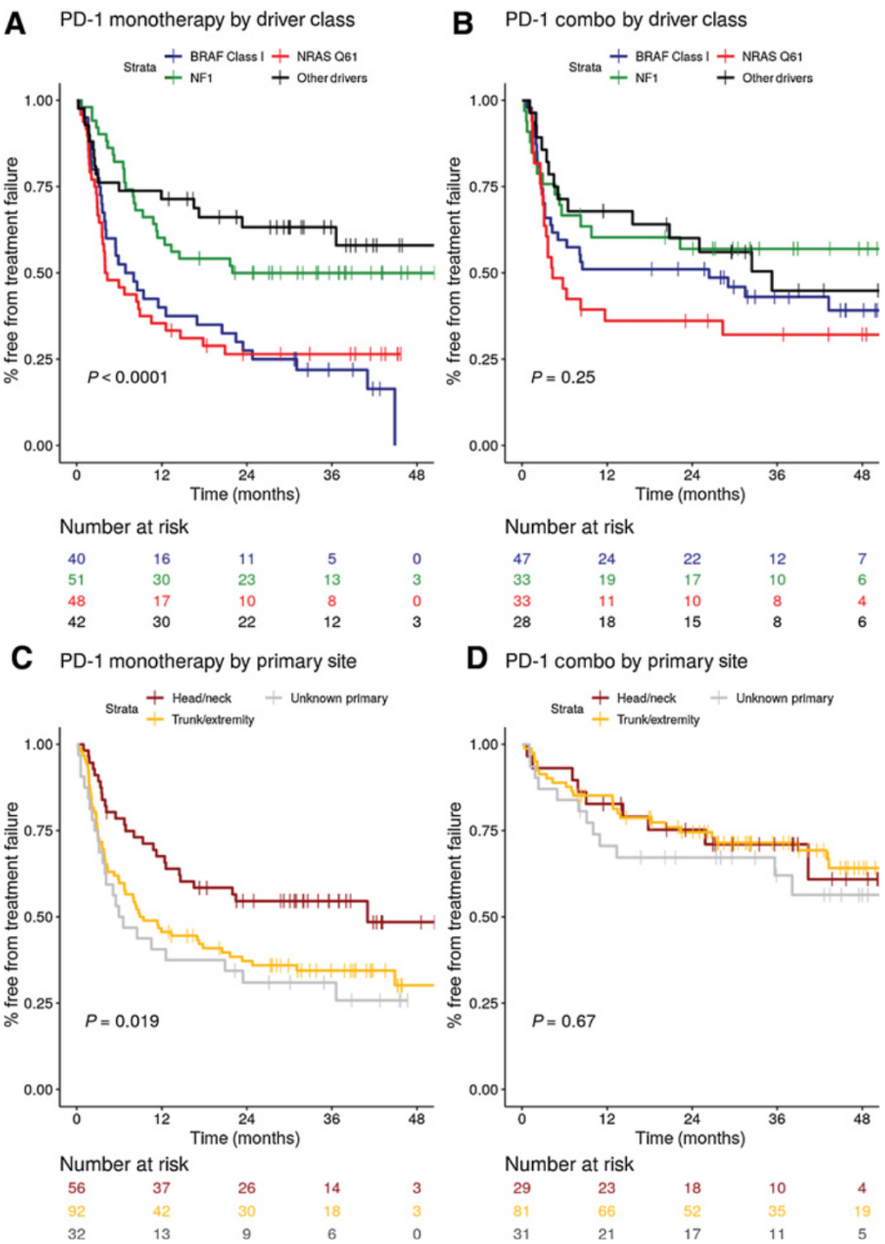

Figure 3.

A, TTF varied significantly by driver class for 181 patients treated with PD-1 monotherapy. Patients with tumors harboring BRAF V600 and NRAS Q61 alterations have inferior TTF than those with NF1 and other driver alterations. **B,** TTF did not vary by driver class for 141 patients treated with nivolumab plus ipilimumab. **C,** TTF of PD-1 monotherapy varied significantly by primary site of melanoma, with tumors arising from the head/neck faring better than those arising from other sites of the body or with unknown primary melanomas. **D,** TTF of nivolumab plus ipilimumab did not vary significantly by primary site of melanoma.

V600, NF1, and NRAS Q61 given the greatest weight within the classifier: BRAF V600E, BRAF V600K/R, NF1 alterations, NRAS Q61, other RAS family alterations, KIT, BRAF non-V600, other known driver, or "unknown driver." The median TMB varied significantly ($P = 9.5e-56$) among driver groups (**Fig. 2A**). TMB was lowest, 7–8 mut/Mb, in BRAF V600E and unknown driver tumors; intermediate (14–18 mut/Mb) in tumors harboring BRAF V600K or non-V600 alterations, NRAS Q61, or other known drivers; high-to-intermediate, 22 mut/Mb, in other RAS-mutant tumors, excluding NRAS Q61; and highest in *KIT*-mutant ($n = 14$; 37 mut/Mb) and *NF1*-mutant ($n = 155$; 43 mut/Mb) tumors.

Median TMB was significantly higher for primary melanomas located on the head (34.9 mut/Mb) versus the upper extremity (18.6 mut/Mb), lower extremity (11.6 mut/Mb), or trunk (10.5 mut/Mb; $P = 3.7e-21$; **Fig. 2A**). The association between sex and driver alteration varied by primary site; for example, head/neck melanomas were enriched for males (72% vs. 66% overall) and *NF1* alterations at the expense of NRAS Q61 and BRAF V600E alterations. Conversely, lower extremity melanomas were more likely to arise in females (52% vs. 34% overall) and lacked *NF1* alterations (**Fig. 2A**). Overall, melanomas in males had a higher median TMB than females (17.6 vs. 14.9; $P = 0.024$; Supplementary Fig. S2B). The fraction of alterations associated with a UV signature also varied significantly by driver group and primary site, with the lowest rates in BRAF V600E and lower extremities (70% and 74%, respectively) and the highest rates in NF1 and the head (88% and 85%, respectively; $P < 1.0e-9$ for both; Supplementary Fig. S2C and SCD).

The median TMB at age of diagnosis increased steadily by decade, from 4.7 mut/Mb in those younger than 20 years up to 25.6 mut/Mb for those older than 80 years (**Fig. 2B**). Median age at initial diagnosis varied by driver group (**Fig. 2C**), with BRAF V600E enriched in younger patients (50 years) and KIT alterations more often present in older patients (72 years).

Checkpoint inhibitor therapy outcomes vary by mechanism of ERK activation

A total of 322 tumors were collected prior to initial treatment with PD-1 monotherapy (pembrolizumab or nivolumab, $n = 181$) or combined nivolumab plus ipilimumab ($n = 141$; Supplementary Table S3). The median follow-up among those free of treatment failure was 36 months for PD-1 monotherapy and 39 months for nivolumab plus ipilimumab.

TTF varied significantly by TMB as a $\log_{10}$ transformed continuous variable for both PD-1 monotherapy [HR, 0.43 for every 10-fold mut/Mb increase, 95% confidence interval (CI), 0.29–0.62; $P < 0.0001$] and combined nivolumab plus ipilimumab treatment (HR, 0.51 for every 10-fold mut/Mb increase; 95% CI, 0.31–0.84; $P = 0.008$). TMB was also significantly associated with OS for both PD-1 monotherapy–treated (HR, 0.6 for every 10-fold mut/Mb increase; 95% CI, 0.37–0.97; $P = 0.039$) and combined nivolumab plus ipilimumab–treated patients (HR, 0.47 for every 10-fold mut/Mb increase; 95% CI, 0.25–0.89; $P = 0.021$).

To investigate the relationship between TTF and driver group, a simplified four-group system was investigated: BRAF V600 (median TMB, 9.3 mut/Mb), NRAS Q61 (15.3 mut/Mb), NF1 (43 mut/Mb), and other (17.6 mut/Mb). TTF for PD-1 monotherapy varied significantly by driver group ($P < 0.0001$; **Fig. 3A**). The median TTF was shorter for BRAF V600- and NRAS Q61–mutant tumors (7.5 and 4.2 months) and longer for NF1 (22 months) and other (not reached). In contrast, no significant difference in TTF by driver group was detected in those receiving nivolumab plus ipilimumab (**Fig. 3B**). For OS, these differences were not significant (Supplementary Fig. S3).

Table 2. Multivariate analysis of TTF with PD-1 monotherapy.

Variable		HR (95% CI)	P	
Log_{10} (TMB)		0.41 (0.25–0.67)	<0.001	
Driver class	NRAS Q61	1.38 (0.83–2.29)	0.20	<0.001
	NF1	1.04 (0.54–1.99)	>0.9	
	Other/unknown	0.35 (0.18–0.67)	0.002	
	BRAF V600	Reference		
ECOG performance status	2–3	4.98 (2.29–10.8)	<0.001	0.001
	1	1.33 (0.86–2.04)	0.20	
	0	Reference		
Stage	M1d	1.90 (1.09–3.33)	0.025	<0.001
	M1c	0.97 (0.57–1.64)	0.90	
	M1b	0.46 (0.27–0.81)	0.007	
	M0–M1a	Reference		

TTF with PD-1 monotherapy also varied when stratified by primary site (**Fig. 3C**); it was highest for patients with melanomas arising on the face, intermediate for other known cutaneous primary sites, and lowest for melanomas of unknown primary ($P = 0.019$). In contrast, primary site was not associated with TTF of nivolumab plus ipilimumab (**Fig. 3D**).

To investigate whether differences in TTF of PD-1 monotherapy among MAPK driver groups persisted after controlling for TMB and clinical features, a multivariate Cox proportional hazards model was built incorporating MAPK driver groups, $\log_{10}$ (TMB), primary site, ECOG performance status, LDH, American Joint Committee on Cancer (AJCC) 8th edition stage, and select hematologic parameters. TTF was significantly associated with MAPK driver groups after adjusting for TMB, ECOG performance status, and AJCC stage (**Table 2**; Supplementary Table S4).

Identification and treatment of patients with rare targetable drivers

A total of 172 patients with BRAF V600 wild-type tumors required systemic therapy beyond PD-1 and CTLA-4 blockade. Of these, 27 (16%) were treated with a therapy matched to the patient's MSK-IMPACT result. Genomically matched therapies included inhibitors of MEK or ERK targeted against RAS alterations ($n = 13$) or *BRAF* class 2 alterations ($n = 6$) and TRK inhibitors for tumors harboring *NTRK* fusions ($n = 3$; Supplementary Table S5). The median TTF of matched therapies was 3.2 months (range, 0.4–43.7 months). Eight patients remained free from treatment failure for greater than 6 months (**Fig. 4A** and **B**).

Four patients achieved complete responses to a genomically matched therapy given after progression on nivolumab plus ipilimumab: trametinib for BRAF K601E (class 2) and MEK1 (*MAP2K1*) E203K mutations, crizotinib for a ROS1 fusion, larotrectinib for an NTRK1 fusion, and PLX8394 for a BRAF fusion (class 2; **Fig. 4C–F**).

Overall, excluding the use of FDA-approved therapies for BRAF V600–mutant tumors, 99 samples were sequenced for each patient who derived 6 or more months of benefit from genomically matched therapy. Among patients who required additional therapy beyond PD-1 and CTLA-4 with known BRAF V600 wild-type tumors, the number needed to be sequenced was 21.

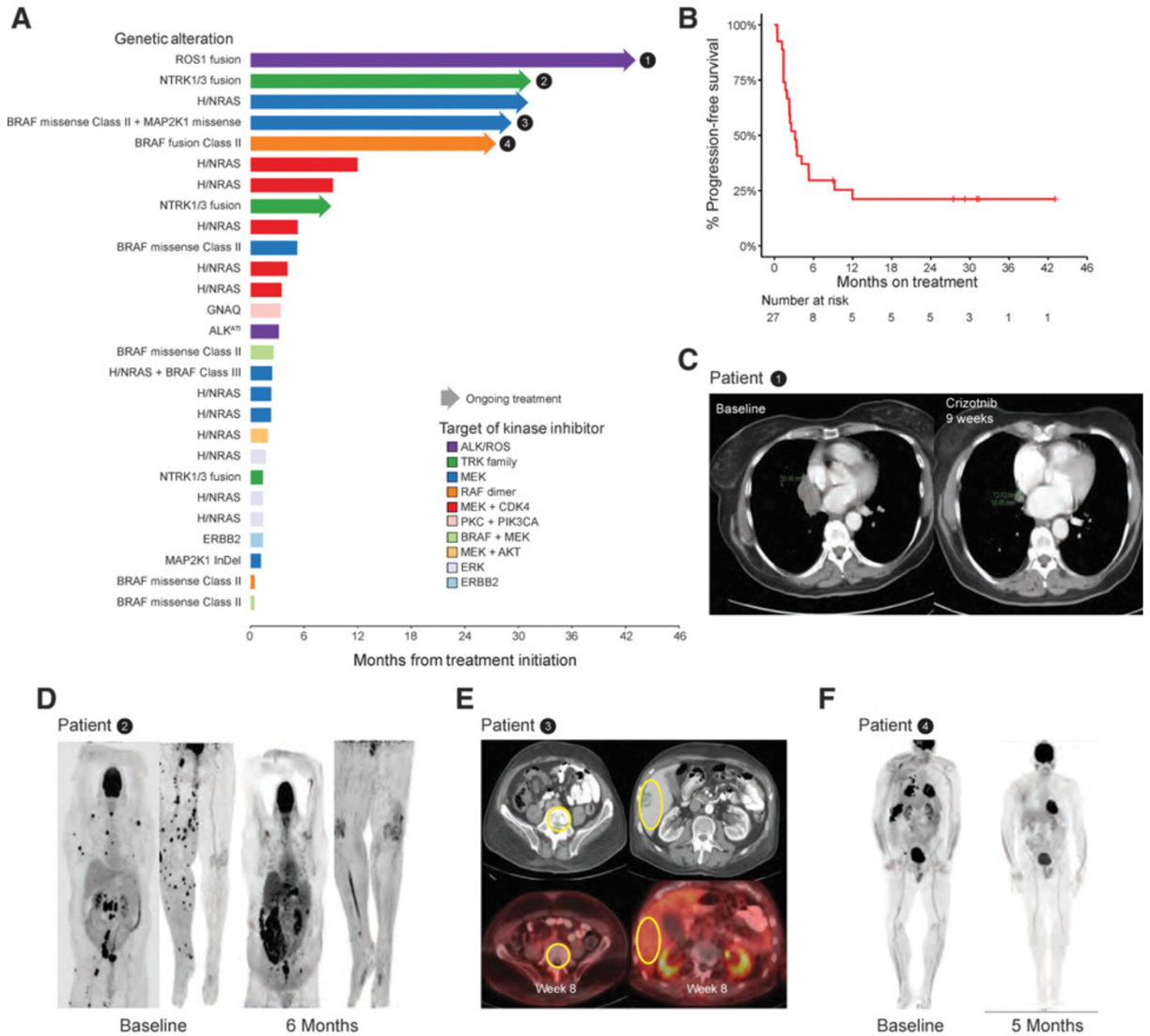

Figure 4.

A, Swimmer plot of 27 patients treated with genomically matched therapy, following progression on PD-1 +/− CTLA-4 therapy. Eight patients had a TTF >6 months and 4 patients achieved durable complete responses. **B,** Kaplan–Meier curve depicting a median TTF of 3.2 months. **C,** Radiographic partial response and pathologic complete response to crizotinib in a cutaneous melanoma harboring a ROS1 fusion. **D,** Rapid metabolic complete response to larotrectinib in a cutaneous melanoma with in-transit metastases harboring an *NTRK1* fusion. **E,** Rapid metabolic complete response in bone and liver to trametinib in a cutaneous melanoma harboring BRAF K601E and MEK1 (*MAP2K1*) E203K missense mutations. **F,** Metabolic complete response to PLX8394 in a patient with M1b cutaneous melanoma harboring a BRAF-AGK fusion.

Discussion

This cohort of patients with cutaneous and unknown primary melanoma represents the largest clinically annotated group to date with multigene sequencing results. Integration of clinical and molecular data revealed novel associations between genomic features, such as MAPK driver alteration status and TMB, and clinical features, such as age, sex, and primary melanoma location. Using the MSK-IMPACT platform, we detected a driver alteration in one of 28 genes predicted to activate the MAPK pathway in 96% of melanomas. A significant minority harbored more than one alteration in an MAPK pathway gene, and the vast majority of detected drivers were clonal. The rate of coalteration varied significantly among driver genes and occasionally within the same gene. Among BRAF-mutant tumors, MAPK driver coalteration was present in only 12% of V600E-mutant samples, but in 37% of V600K/R and in 97% of class 3 *BRAF* mutants, such as D594N. These findings validate prior findings in smaller cohorts (4, 5, 18). These coalterations might represent intrinsic resistance mechanisms for KIT, BRAF, or MEK inhibitors.

We hypothesize that the mechanism and magnitude of ERK activation by a driver alteration influence TMB by dictating how many additional alterations the melanocyte requires to become an invasive melanoma. This presumably influences the degree to which it is "immune edited" and can be successfully treated with PD-1

blockade. For example, BRAF V600E alterations signal as monomers, strongly activate ERK, and are insensitive to ERK-mediated feedback inhibition of RAS (19); hence, they frequently initiate benign nevi (20), require few additional alterations to become melanomas in younger patients with lower TMB, and have a shorter time to failure of PD-1 blockade. In contrast, NF1 alterations are relatively weaker activators of ERK (21) and require additional RTK or BRAF alterations to activate or disinhibit RAS. Thus, they are not found in benign nevi (20), arise in older patients with chronically sun-damaged skin with more UV-induced alterations, and appear more likely to be successfully treated with PD-1 monotherapy. One hypothesis for why adding ipilimumab to nivolumab significantly improved PFS for patients with BRAF-mutant, but not BRAF wild-type, tumors in a recent randomized trial is the lower median TMB in this subgroup (8). Among patients with cutaneous and unknown primary melanomas receiving first-line PD-1 monotherapy or combination therapy, increasing TMB is associated with a longer TTF and OS. This contrasts with the findings of a smaller cohort containing multiple melanoma subtypes and heterogeneous exposure to first-line CTLA-4 blockade that suggested this association with TMB and PD-1 blockade efficacy was confounded by histologic subtype (22).

We classified cutaneous and unknown primary melanomas using a detailed nine-driver group hierarchy that reflects the mechanism of ERK activation, the rate of pathway coalterations, and median TMB: BRAF V600E; BRAF V600K/R; NF1; NRAS Q61; other RAS; KIT; BRAF non-V600; another known driver (e.g., MAP2K1); and unknown driver. As more samples are analyzed, the two latter heterogeneous groups should be better defined. Some of these groups were too small to investigate outcomes to PD-1 blockade using this nine-group system. Using a simpler four-driver group system reliant on three genes, we show tumors that harbor BRAF V600 or NRAS Q61 alterations are associated with a shorter TTF with anti-PD-1 monotherapy than those with NF1 or other driver alterations. Driver class remained significantly associated even after accounting for TMB, ECOG performance status, and AJCC stage. One potential explanation is the mechanism of ERK activation may have downstream effects on T-cell inflammation, which has been independently associated with outcomes to PD-1 blockade (23). For example, NRAS Q61 strongly activates downstream PI3K signaling, which has been associated with reduced rates of tumor-infiltrating lymphocytes compared with BRAF V600–mutant melanomas (24, 25). These relationships between driver alteration, primary site, and TTF did not hold for patients receiving nivolumab plus ipilimumab. This may be due to the higher risk disease treated with PD-1 combination versus PD-1 monotherapy in this nonrandomized cohort, but also likely reflects distinct immunologic mechanisms underlying response to these agents (26, 27). These findings require validation in additional datasets, and a more detailed analysis of how the melanoma tumor microenvironment varies by MAPK driver is required to understand how it influences outcomes to checkpoint inhibition independently of TMB.

In the few extraordinary responses to targeted inhibition that we described, the tumors either had oncogenic fusions (NTRK, ROS1, and BRAF) or a coalteration in BRAF K601E and MEK1 E203K. MEK1 E203K is sensitive to feedback inhibition of RAF, in contrast to indels in the MAP2K1 inhibitory domain from amino acids 98–113 (28). Thus, this tumor requires both BRAF K601E, a class 2 RAS-independent kinase intact mutation, and MEK1 E203K, a RAF-dependent amplifier, to maximally amplify ERK output. This may explain why the tumor was uniquely sensitive to the allosteric MEK inhibitor, trametinib. Unfortunately, these durable responses were uncommon. In routine clinical practice at a tertiary care center, patients with BRAF V600 wild-type melanoma resistant to PD-1 and/or CTLA-4 inhibition have a roughly one in 21 chance of harboring a driver alteration on MSK-IMPACT testing that can be treated successfully for over 6 months. The largest unmet need remains successful targeting of the RAS pathway in RAS- and *NF1*-mutant tumors.

This analysis has some limitations. BRAF V600E–mutant tumors were likely underrepresented because patients were unlikely to be offered MSK-IMPACT if prior testing had detected this alteration. This series also had a relatively high rate of unknown primary melanomas, which may reflect referral bias to a tertiary cancer center. Nevertheless, this report shows cutaneous and unknown primary melanomas have a similar genomic profile. TTF for checkpoint inhibition in BRAF V600–mutant melanomas may be influenced by the unique availability of effective BRAF-MEK inhibitor therapy in the second-line setting. PD-L1 status was unknown for most samples, so its association with other clinical features could not be assessed. Mutation calls may vary by bioinformatic pipeline, so additional analyses using other NGS platforms should be performed to assess the reproducibility of these clinical–genomic correlations.

In summary, multigene tumor molecular profiling can identify MAPK driver alterations in almost all cutaneous and unknown primary melanomas. Strong ERK activators insensitive to RAS-mediated feedback inhibition, such as BRAF V600E, *BRAF* fusions, NRAS Q61, and *MAP2K1* indels, were typically the sole oncogenic alterations in the MAPK pathway, whereas melanomas with alterations reliant on RAS-mediated output, such as class 3 *BRAF* alterations and *MAP2K1* missense mutations, often contained alterations in other MAPK pathway drivers, such as *NF1* truncations. While melanomas with oncogenic fusions are rare, patients with these fusions exhibited deep, durable responses to the appropriate kinase inhibitor. This suggests broader panel NGS should be considered in all patients with BRAF V600 wild-type melanomas who progress through PD-1–based therapy. Finally, a hierarchical mutually exclusive driver classification system defined distinct groups of cutaneous and unknown primary melanomas that derived varying benefit from PD-1 monotherapy. Current trials enrolling PD-1–resistant melanomas will likely be enriched with RAS-activating mutations.

Authors' Disclosures

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Authors' Contributions

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