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Clinicopathological features and clinical outcomes associated with *TP53* and *BRAF*$^{Non\text{-}V600}$ mutations in cutaneous melanoma patients

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Abstract

Background—*BRAF*^{V600}, *NRAS*, *TP53* and *BRAF*$^{Non\text{-}V600}$ are among the most common mutations detected in non-acral cutaneous melanoma (CM) patients. While several studies have identified clinical and pathologic features associated with *BRAF*^{V600} and *NRAS* mutations, limited data is available regarding the correlates and significance of *TP53* and *BRAF*$^{Non\text{-}V600}$ mutations.

Methods—We analyzed the patient demographics, primary tumor features, and clinical outcomes of a large cohort (n=926) of non-acral cutaneous melanoma patients that had undergone clinically-indicated molecular testing.

Results—The prevalence of *BRAF*^{V600}, *NRAS*, *TP53*, and *BRAF*$^{Non\text{-}V600}$ mutations were 43%, 21%, 19%, and 7%. The presence of a *TP53* mutation was associated with older age (p=0.019), head and neck primary tumor site (p=0.0001), and longer overall survival (OS) from the diagnosis of stage IV disease on univariate (p=0.039) and multivariate (p=0.015) analyses. *BRAF*$^{Non\text{-}V600}$ mutations were associated with older age (p=0.005), but no primary tumor features nor OS from stage IV. Neither *TP53* nor *BRAF*$^{Non\text{-}V600}$ mutations correlated significantly with OS with frontline ipilimumab treatment, and *TP53* status was not significantly associated with outcomes with frontline BRAF inhibitor therapy. Eleven patients with *BRAF*$^{Non\text{-}V600}$ mutations were treated with a BRAF inhibitor. Three patients were not evaluable for response due to treatment cessation for toxicities; the remaining patients had disease progression as the best response to therapy.

Conclusions—These results add to the understanding of the clinical features associated with *TP53* and *BRAF*$^{Non\text{-}V600}$ mutations in advanced CM patients, and they support the rationale to evaluate the prognostic significance of *TP53* in other cohorts of melanoma patients.

Keywords

Melanoma; mutations; *TP53*; *BRAF*^{V600}; *BRAF*$^{Non\text{-}V600}$

Introduction

One of the most impactful discoveries in melanoma was the identification of missense mutations in the *BRAF* gene. Approximately 95% of these mutations result in substitutions for valine at position 600 of the BRAF protein (BRAF^{V600}), most commonly with glutamic acid (BRAF^{V600E}).[1] These mutations markedly activate the RAS-RAF-MAPK signaling pathway. In addition to improving the understanding of the molecular pathogenesis of melanoma, this discovery led to the development of highly effective targeted therapies for patients with *BRAF*^{V600} mutations, including the mutant-selective BRAF inhibitors (BRAFi) vemurafenib and dabrafenib and the MEK inhibitors (MEKi) trametinib and cobimetinib. Together these developments strongly support the rationale to interrogate the significance of other somatic mutations in this disease to identify additional personalized therapeutic strategies.

Recently, we reported the results of a clinical next-generation sequencing (NGS) panel that encompassed commonly mutated regions in 46 genes in a large cohort of advanced melanoma patients.[2] The most common mutations detected in the cutaneous melanoma

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patients were *BRAF*^{V600} (41%), *NRAS* (22%), *TP53* (17%) and *BRAF*$^{Non\text{-}V600}$ (7%). Previous studies have that interrogated the clinical and pathological features that correlate with *BRAF*^{V600} and *NRAS* mutations have identified a number of significant associations.[3] However, very little is known about features of melanomas that are associated with *TP53* mutations, which correlate with poor clinical outcomes in head and neck cancer[4] and hematologic malignancies.[5] Preclinical studies support that *BRAF*$^{Non\text{-}V600}$ mutations are a potential therapeutic target,[6] and clinical trials are ongoing to determine the efficacy of trametinib (MEKi) in melanoma patients with these mutations (NCT02296112). However, little is known at this time about the clinical features or outcomes associated with *BRAF*$^{Non\text{-}V600}$ mutations in melanoma patients.

We have performed a retrospective analysis of a large single-institution cohort of advanced cutaneous melanoma patients with clinical NGS testing results, which encompassed regions of prevalent hotspot mutations in 50 genes. We have interrogated this cohort to identify clinical and pathological features that are associated with the presence of *TP53* and *BRAF*$^{Non\text{-}V600}$ mutations to improve our understanding of their significance in this disease.

Materials and Methods

Patient Selection and Clinical Data Collection

Under an Institutional Review Board-approved protocol, the results of clinically indicated molecular testing performed at The University of Texas MD Anderson Cancer Center (MDACC) from April of 2012 to November of 2014 for patients with non-acral cutaneous melanoma were reviewed. Patient demographics, primary tumor characteristics, treatments received, and overall survival were collected.

Mutation Testing

Molecular testing by a pan-cancer NGS panel of hotspot regions in 50 genes [Supplemental Table 1] was performed on DNA extracted from formalin-fixed, paraffin-embedded tissues from melanoma primary tumors or metastases using the AmpliSeq sequencing panel (CMS50; Life Technologies) as previously described.[2, 7]

Among detected mutations in the panel, *TP53* mutations were further classified based on physicochemical or functional consequences including truncating mutations, missense mutations, DNA-binding domain mutations and UV signature mutations. Truncating mutations are mutations leading to a stop codon, frameshift and splice defect.[8] In contrast, missense mutations are characterized by an amino acid change which results in a dominant-negative or a gain-of-function.[9] DNA-binding domain mutations are mutations in codons 102 to 292 which induce inactivation of TP53 by eliminating DNA-binding contacts or altering structural stability of the core domain.[9] Missense mutations are further stratified into high (≥75) and low risk (<75) by an evolutionary action score system (http://mammoth.bcm.tmc.edu/EAp53/) to predict highly deleterious TP53 functions which was validated in head and neck squamous cell carcinoma.[10]

In order to verify the coverage of CMS50 panel, potential hotspot mutations in cutaneous melanoma The Tumor Genome Atlas (TCGA) data[11] were identified using the HotSpotter

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method as previously described which allows rapid and easy visualization of mutation data sets and identification of potential gene mutation hotspot sites and/or regions.[12] The identified potential hotspot mutations by the HotSpotter analysis were compared to CMS50 panel.

Statistical Methods

The association between continuous parameters and mutational status was assessed by analysis of variance (ANOVA). Fisher's exact test was used to assess the association between categorical variables (primary tumor site, ulceration, elevated LDH) and mutational status. Wilcoxon rank sum tests were used to assess the association between continuous and ordinal variables (age, Breslow thickness, M stage) and mutational status. The method of Kaplan and Meier was used to estimate the distribution of overall survival, and log-rank testing was performed to determine the significance of observed differences. All statistical analyses were performed using R version 3.1.1. All statistical tests used a significance level of 5%. No adjustments for multiple testing were made.

Results

Patients Demographics and Mutation Frequency—A total of 926 patients with a non-acral cutaneous primary melanoma and clinical NGS testing results using the CMS50 panel were identified [Table 1]. The median age of the patients was 56 years (range 12-94) and 67.4% were male. Superficial spreading melanoma was the most common melanoma subtype, and the trunk was the most common primary tumor site. 531 patients (57.3%) were diagnosed with stage IV disease.

A $BRAF^{V600}$ mutation was detected in 398 patients (43.0%), *NRAS* mutation in 195 (21.1%), *TP53* mutation in 180 (19.4%), and $BRAF^{Non\text{-}V600}$ mutation in 60 (6.5%) [Table 2]. The prevalence of the specific $BRAF^{V600}$, *NRAS*, *TP53* and $BRAF^{Non\text{-}V600}$ substitutions detected in the cohort are presented in Supplemental Tables 2 – 5. *TP53* mutations were detected in 11.3% of melanomas with a $BRAF^{V600}$ mutation, 19.4% of tumors with an *NRAS* mutation, and 28.9% of tumors without a $BRAF^{V600}$ or *NRAS* mutation [Supplemental Table 6 and Supplemental Fig 1A]. A $BRAF^{Non\text{-}V600}$ mutation was detected in 0.5% of melanomas with a $BRAF^{V600}$ mutation, 4.1% of tumors with an *NRAS* mutation, and 14.9% of tumors without a $BRAF^{V600}$ or *NRAS* mutation [Supplemental Table 7 and Supplemental Fig 1B]. *TP53* mutations were present in 8.9% of tumors with a $BRAF^{Non\text{-}V600}$ mutation.

Clinical and pathologic characteristics associated with TP53 mutations— Analysis of the full patient cohort showed that the presence of a *TP53* mutation that was detectable by the clinical CMS50 panel was associated with increased age at diagnosis (median 58 versus 56 years, p=0.019) [Table 3]. *TP53* mutations were also significantly associated with primary tumor site (p=0.0001), as they were more common in tumors of the head and neck (27.4%) than the trunk (15.9%) or extremity (14.7%). *TP53* mutations were not significantly associated with primary tumor thickness or ulceration status. Among the 531 patients that had developed stage IV disease, there was no significant association

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between *TP53* mutation status and M stage or serum LDH at the diagnosis of stage IV disease.

Similar analyses were performed to assess the characteristics associated with *TP53* mutations separately in melanomas with a concurrent $BRAF^{V600}$ mutation, in melanomas with a concurrent *NRAS* mutation, and in melanomas without a $BRAF^{V600}$ or *NRAS* mutation [Supplemental Table 6]. There were no significant associations with *TP53* mutation status in tumors with $BRAF^{V600}$ mutations or with *NRAS* mutations. Significant associations with age at diagnosis (p=0.001) and primary tumor site (p=0.009) were observed in tumors without a $BRAF^{V600}$ or *NRAS* mutation.

TP53 mutations and overall survival from stage IV—In order to avoid bias due to inclusion of patients with unusually long survival intervals prior to molecular testing, the analysis of outcomes after stage IV diagnosis were limited to patients with clinical NGS testing performed within 12 months of stage IV diagnosis (n=417). The prevalence of *TP53* mutations detected by the CMS50 panel among these patients was 21.1%. Patients with a *TP53* mutation had longer overall survival (OS) from the diagnosis of stage IV on univariate analysis (median 18.8 versus 15.3 months, p=0.039) [Fig. 1A]. OS from stage IV was very similar for patients with a $BRAF^{V600}$ mutation with or without a *TP53* mutation (median 17.5 versus 16.9 months, p=0.89) [Supplemental Fig. 2A]. In contrast, non-significant trends for longer OS with *TP53* mutation were observed among patients with *NRAS* mutations (median 21.7 versus 13.0 months, p=0.07) and among patients without a $BRAF^{V600}$ or *NRAS* mutation (median 19.3 versus 15.8 months, p=0.08) [Supplemental Fig. 2B/C].

To further evaluate the prognostic significance of *TP53* mutations, outcomes with FDA-approved therapies among the stage IV cohort were reviewed. A total of 146 patients with clinical NGS testing performed within 12 months of stage IV diagnosis were treated with frontline ipilimumab, including 35 patients with a *TP*53 mutation. No significant correlation was observed between *TP53* mutation status and OS from the start of ipilimumab treatment in the full cohort (median 11.1 months for *TP53* mutant versus 13.9 months for *TP53* mutation not detected, p=0.46) [Fig. 1B]. There was also no significant association with OS from the start of ipilimumab by *TP53* mutation among the cohorts defined by the concurrent $BRAF^{V600}$ and *NRAS* mutation status [Supplemental Fig. 3A, B and C].

Among the patients with a $BRAF^{V600}$ mutation, 64 patients in the cohort were treated with an FDA-approved BRAF inhibitor as first-line therapy for stage IV disease [Supplemental Table 8]. The objective response rate (ORR) did not differ significantly between the patients with (n=9, ORR 55.6%) and without a *TP53* mutation (n=55, ORR 47.3%, p=0.7). *TP53* mutation status was also not significantly associated with progression-free survival (PFS) (p=0.42) or OS (p=0.49) with BRAF inhibitor treatment [Supplemental Fig. 4A and B].

The outcomes associated with *TP53* mutations were further characterized by analyzing the nature of the observed mutations in the stage IV patients, as different types of mutations in *TP53* may have different prognostic significance. Consistent with the strong association of non-acral cutaneous melanoma with UVR exposure, 58.2% of the detected *TP53* mutations in the cohort were substitutions that are associated with UVR-related DNA damage. This

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rate is relatively similar to the rate observed in the cutaneous melanoma TCGA cohort (45.3%).[11] Patients with UVR-related (n=55, median OS 17.5 months) and non-UVR related *TP53* mutations (n=31, median OS 24.0 months) had trends for improved OS from stage IV compared to patients without a detectable *TP53* mutation (median OS 15.3 months), but these differences did not reach statistical significance (p=0.09 and p=0.11, respectively) [Supplemental Fig. 5A]. Most *TP53* mutations were missense mutations (n=61, median OS 19.3 months), and patients with such mutations had improved OS compared to patients without *TP53* mutations detected by the CMS50 panel (p=0.002). In contract, the presence of a truncating mutation in *TP53* was less common (n=21) and was not associated with a significant difference in survival from stage IV (median OS 12.9 months, p=0.93) [Supplemental Fig. 5B]. The majority (n=74; 84.1%) of the mutations detected in *TP53* affected the DNA binding domain of the protein, and the presence of such mutations was associated with improved OS compared to patients without a mutation detected (median OS 18.6 months, p=0.05). Although mutations in other domains of *TP53* were not significantly associated with survival from stage IV (p=0.39), there was very limited power for this analysis due to the small number of such patients in this cohort (n=11) [Supplemental Fig. 5C]. Finally, categorization of the *TP53* mutations into two groups based on evolutionary score[10] showed that high risk evolutionary score (N=51) correlated significantly with OS from stage IV (median OS 21.7 months, p=0.04) but not low risk (N=10, median OS 19.3 months, p=0.16) [Supplemental Fig. 5D].

Clinical and pathologic characteristics associated with $BRAF^{Non\text{-}V600}$ mutations—Twenty seven different $BRAF^{Non\text{-}V600}$ mutations were detected in a total of 60 patients in the cohort [Supplemental Table 5]. $BRAF^{Non\text{-}V600}$ mutations were detected in 2 patients with $BRAF^{V600}$ mutations (0.5%), 8 patients with *NRAS* mutations (4.1%), and 50 patients with neither $BRAF^{V600}$ nor *NRAS* mutations (14.9%) [Supplemental Fig. 1B]. In the full cohort, the presence of a $BRAF^{Non\text{-}V600}$ mutation (6.5%) was associated with significantly older age at diagnosis (median 60 versus 56, p=0.005) [Table 3]. No other significant correlations with patient demographics or primary tumor characteristics were identified. Among the 531 patients diagnosed with stage IV disease, the presence of a $BRAF^{Non\text{-}V600}$ mutation (n=41, 7.7%) was not associated significantly with M1 stage or serum LDH levels.

Clinical and pathologic characteristics of $BRAF^{Non\text{-}V600}$ mutations were examined separately in melanomas with a concurrent $BRAF^{V600}$, a concurrent *NRAS* mutation, and in melanomas without a $BRAF^{V600}$ or *NRAS* mutation (Supplemental Table 7). Non-significant trends for deeper Breslow thickness with $BRAF^{Non\text{-}V600}$ mutation were observed in tumors without a $BRAF^{V600}$ or *NRAS* mutation (p=0.08), but no statistically significant associations were detected.

$BRAF^{Non\text{-}V600}$ mutations and overall survival from stage IV—A $BRAF^{Non\text{-}V600}$ mutation was detected in 25 (6.0%) of patients with mutation testing performed within 12 months of the diagnosis of stage IV disease. There was no significant difference in OS from stage IV between patients with (median 17.9 months) and those without (median 16.1 months, p=0.53) a $BRAF^{Non\text{-}V600}$ mutation [Figure 1C]. There was no patient with a

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BRAF^{V600} mutations who had mutation testing within 12 months of the diagnosis of stage IV diagnosis who had a concurrent *BRAF*$^{Non\text{-}V600}$ mutation, and only 1 among 86 patients with an *NRAS* mutation. Analysis of patients without a concurrent *BRAF*^{V600} or *NRAS* mutation failed to detected a significant association between OS from stage IV and *BRAF*$^{Non\text{-}V600}$ mutation status (p=0.95) [Supplemental Figure 6].

Twenty one (8.9%) of the patients treated with ipilimumab had a *BRAF*$^{Non\text{-}V600}$ mutation. There was no significant correlation between *BRAF*$^{Non\text{-}V600}$ mutation status and OS from the start of ipilimumab treatment was observed (median 12.5 versus 13.5 months, p=0.44) [Figure 1D].

Eleven patients with a *BRAF*$^{Non\text{-}V600}$ mutation were treated with an FDA-approved BRAF inhibitor (vemurafenib, n=9; dabrafenib, n=1; dabrafenib + trametinib, n=1). All of the patients had stage 4 disease, and none of the patients had a concurrent *BRAF*^{V600} or *NRAS* mutation [Table 4]. Three patients treated with vemurafenib were not evaluable for response due to early severe drug related toxicities; the remaining 8 patients had disease progression as their best response to therapy. The median PFS and OS were 1.9 months (95% CI: 1.48-2.32) and 7.1 months (95% CI: 4.54-9.66), respectively [Supplemental Figure 7A and B]. Since *BRAF K601* and *L597* mutations are located in the activating kinase domain of *BRAF*, and a BRAF inhibitor demonstrated some antitumor activity in a patient with a *BRAF L597* mutant melanoma,[13] clinical outcomes of patients with *BRAF K601* and *L597* mutations and other *BRAF*$^{Non\text{-}V600}$ mutations with BRAF inhibitor treatment. There was no difference in PFS and OS between patients with *BRAF K601* and *L597* mutations and the other *BRAF*$^{Non\text{-}V600}$ mutations [supplemental Figure 7C and D]. No responses were observed among 3 patients with *BRAF*$^{Non\text{-}V600}$ mutations (*BRAF L597R*, *G466E*, *G469E*) treated with trametinib (MEKi) monotherapy [Table 4].

Multivariate analysis

Multivariate Cox regression modeling was performed to identify significant predictors of OS from stage IV diagnosis, including gender, M1 stage, LDH, and *BRAF*^{V600}, *NRAS*, *TP53* and *BRAF*$^{Non\text{-}V600}$ mutation status [Table 5]. Age and the presence of stage IV M1c disease were associated with non-significant trend of shorter OS from stage IV (p=0.05 and p=0.08). The presence of a *TP53* mutation was associated with improved OS from stage IV (HR 0.62, 95% CI 0.42-0.91, p=0.015).

Discussion

A number of previous studies have described the clinical and pathologic features associated with *BRAF*^{V600} and *NRAS* mutations,[3, 14, 15] which are the most common hotspot oncogenic mutations detected in cutaneous melanomas. While many other mutations have been identified in melanoma, the ability to assess their clinical associations has been limited by their relative scarcity. In this study, we have utilized a retrospective cohort of 926 non-acral cutaneous melanoma patients that underwent clinically indicated NGS testing to expand the integrated analysis of molecular and clinical features in this disease. This analysis focused on *TP53* and *BRAF*$^{Non\text{-}V600}$ mutations, as they are particularly common in

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cutaneous melanomas without *BRAF*^{V600} or *NRAS* mutations, and as preclinical data supports potential functional roles for both events in this disease.

TP53 encodes a tumor suppressor gene that is altered in many cancer types. Similar with the rate observed in the melanoma TCGA (15%),[11] we identified *TP53* mutations in 19% of the full cohort of cutaneous melanoma patients who had undergone molecular testing with the clinical CMS50 NGS panel. Compared to patients who did not have an alteration detected by this panel, *TP53* mutations were associated with older age and increased head and neck primary tumor location. These clinical associations are consistent with the observed correlation between ultraviolet radiation (UVR)-induced DNA damage and *TP53* mutations in cutaneous melanoma.[16, 17] In this cohort, 58% of *TP53* mutations were consistent with UV-induced DNA damage, which is again similar to the rate reported in the melanoma TCGA (45.3%).[11] While preclinical studies have shown that *TP53* mutations accelerate *BRAF*^{V600}-driven melanomagenesis,[17, 18] we observed that *TP53* mutations were associated with a significantly improved survival from the diagnosis of stage IV disease on univariate and multivariate analyses. The observed association of improved outcomes in melanomas with *TP53* mutations contrasts with associations with poor clinical outcomes in a number of other cancers, including breast cancer,[19] head and neck cancer,[4] and hematologic malignancies.[5] However, these results are consistent with a previous study that identified improved clinical outcomes in melanomas with overexpression of the P53 protein,[20] which is usually associated with mutations in the *TP53* gene. Our analysis also showed that missense substitution mutations in *TP53*, mutations that affected the DNA binding domain of the P53 protein and high risk evolutionary score of *TP53* mutations were associated with significant improvements in OS from stage IV, but truncating mutations, mutations affecting other domains and low risk evolutionary score were not. While these findings may further help to interpret the results of *TP53* molecular testing results, the analysis of truncating mutations, mutations affecting other domains and low risk evolutionary score were limited by the small number of patients with such alterations in this cohort.

At this time it is unclear why *TP53* mutations are associated with improved survival in stage IV melanoma patients. The presence of a somatic mutation in *TP53* was also associated with a trend for improved OS from initial diagnosis in the full melanoma TCGA cohort, as patients with a *TP53* mutation (n=64) had a median OS of 170 months, while patients without a *TP53* mutation (n=350) had a median OS of 114 months (p=0.44). Analysis limited to the TCGA patients with stage III or IV metastases also showed a trend for improved OS with a *TP*53 mutation (median OS Not reached versus 72.8 months, p=0.25). While the observed differences are interesting, the TCGA cohort included stage II-IV melanomas, many tumors were collected after previous recurrences, the date of stage IV disease is unknown for the overwhelming majority of patients, and there is not sufficient data to evaluate associations with known stage-specific prognostic factors for the cohort. Thus, studies of additional cohorts with appropriate power and annotation will need to be evaluated in the future to further assess the prognostic significance of *TP53* mutations in melanoma. Notably, our analysis of survival was limited to patients with molecular testing performed within 12 months of stage IV diagnosis to avoid biasing results by including patients who survived for a prolonged period of time before undergoing molecular testing, another factor to consider in the evaluation of molecular prognostic factors. Recent studies

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support that increased missense mutation burden correlates with improved survival in metastatic melanoma patients.[21] As *TP53* mutations are associated with increased mutation burden, including in the melanoma TCGA cohort ($p<0.0001$ by Mann-Whitney test), it is possible that the presence of this mutation signifies the likelihood of increased mutation burden, and therefore enhanced anti-tumor immune surveillance. While this is a reasonable hypothesis, we did not observe a significant association between the presence of a *TP53* mutation and an improvement in overall survival with ipilimumab. As the relatively low response and long-term survival rates for ipilimumab are modest, this analysis was somewhat limited to detect a positive association. Additional studies may be performed in the future to examine outcomes in patients treated with more active immunotherapy regimens such as anti-PD-1 antibodies and adoptive T cell therapy.

BRAF$^{Non\text{-}V600}$ mutations were detected in 6% of the full cohort of cutaneous melanomas in this study, also consistent with our previous report[2] and the melanoma TCGA. *BRAF*$^{Non\text{-}V600}$ mutations were extremely rare in cutaneous melanomas with a concurrent *BRAF*^{V600} mutation (0.5%), but they were more frequent in melanomas with an *NRAS* mutation (4%) and in melanomas with neither of those oncogenic driver mutations (15%). In contrast to the *TP53* mutation analysis, there was no significant association observed between *BRAF*$^{Non\text{-}V600}$ mutations and overall survival from stage IV diagnosis or with ipilimumab treatment.

Consistent with previous clinical data,[22, 23] patients with *BRAF*$^{Non\text{-}V600}$ mutations in this cohort that were treated with FDA-approved BRAF inhibitors had no clinical responses. The failure of a selective BRAF inhibitor in *BRAF*$^{Non\text{-}V600}$ mutant melanoma can be explained by preclinical data which showed activation of endogenous CRAF in *BRAF*$^{non\text{-}V600}$ mutant melanoma.[6] In contrast to other *BRAF*$^{Non\text{-}V600}$ mutations, *BRAF K601* and *L597* mutations are located in the activating domain of BRAF, and a clinical response to a BRAF inhibitor has previously been reported in one metastatic melanoma patient with a *BRAF L597* mutation.[13] However, we did not observe any clinical responses in this study in patients with a *BRAF*$^{Non\text{-}V600}$ mutation. While there is evidence that MEK inhibitors may be an effective strategy for melanomas with *BRAF*$^{Non\text{-}V600}$ mutations, and objective response to a selective MEK inhibitor was reported in *BRAF L597S* mutant melanoma,[6, 24] no responses were seen among 3 patients with *BRAF L597R*, *G466E* or *G469E* mutations in this cohort that were treated with trametinib. A prospective, open-label phase II study (NCT02296112), is currently ongoing to further characterize the safety and activity of trametinib in patients with *BRAF*$^{Non\text{-}V600}$ mutations, and particularly to interrogate if clinical activity is enhanced with specific mutations. Notably the findings that *BRAF*$^{Non\text{-}V600}$ mutations do not correlate significantly with overall survival from stage IV or with ipilimumab treatment is beneficial for the interpretation of the clinical results seen in that study.

As our study was conducted at a single tertiary cancer center, it is possible that different results may be detected in other melanoma patient populations. Indeed, we do note that serum LDH, which is part of the AJCC staging system for stage IV melanoma, was not prognostic in the cohort of patients with molecular testing with 12 months of the diagnosis of stage IV. The reason for this finding in this cohort is unknown. However, our cohort is quite large, and thus likely represents significant clinical heterogeneity that is seen at

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melanoma centers. Notably, the clinical NGS panel did not perform NGS of the entire encoding region of *TP53* or *BRAF* [Supplemental Table 1]. Thus, the actual prevalence of *TP53* and $BRAF^{Non\text{-}V600}$ mutations could be higher than were detected using this clinical panel. However, the observed rates of *TP53* and $BRAF^{Non\text{-}V600}$ mutations are very similar to those reported in the initial report of the melanoma TCGA effort,[11] which included whole exome sequencing (WES). This is likely due to the fact that the regions that were sequenced in the clinical panel were selected based on existing data about the location of common mutations. Indeed, the residues in *TP53* that are assessed for mutations in the CMS50 panel largely overlap with the mutations that were detected in the melanoma TCGA [Supplemental Fig. 8]. However, we do note the limitation that the clinical CMS50 panel is not optimal to detect medium and long deletions, nor chromosomal copy number changes, affecting the *TP53* gene. Based on our observations, there is a strong rationale for a comprehensive evaluation of the *TP53* gene in other cohorts of clinically annotated stage IV melanoma patients.

In conclusion, this study reports the analysis of the largest single-institution cohort of clinically and molecularly characterized advanced cutaneous melanoma patients to date. The size of this cohort has allowed us to interrogate the clinical and pathological features that are significantly associated with *TP53* and $BRAF^{Non\text{-}V600}$ mutations. Our results suggest that in contrast to many other tumor types, *TP53* mutations may correlate with a favorable prognosis in advanced cutaneous melanoma patients, at least for those who undergo molecular testing within 12 months of stage IV diagnosis. In addition, our studies show that $BRAF^{Non\text{-}V600}$ mutations are not prognostic in stage IV melanoma patients, information which will augment the design and interpretation of current and future clinical trials in this patient population. Further studies including prospective and mechanistic analysis are needed to validate our findings.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Hodis E, Watson IR, Kryukov GV, et al. A landscape of driver mutations in melanoma. Cell. 2012; 150:251–263. [PubMed: 22817889]
2. Siroy AE, Boland GM, Milton DR, et al. Beyond BRAF(V600): clinical mutation panel testing by next-generation sequencing in advanced melanoma. J Invest Dermatol. 2015; 135:508–515. [PubMed: 25148578]
3. Ellerhorst JA, Greene VR, Ekmekcioglu S, et al. Clinical correlates of NRAS and BRAF mutations in primary human melanoma. Clin Cancer Res. 2011; 17:229–235. [PubMed: 20975100]
4. Poeta ML, Manola J, Goldwasser MA, et al. TP53 mutations and survival in squamous-cell carcinoma of the head and neck. N Engl J Med. 2007; 357:2552–2561. [PubMed: 18094376]

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5. Wattel E, Preudhomme C, Hecquet B, et al. p53 mutations are associated with resistance to chemotherapy and short survival in hematologic malignancies. Blood. 1994; 84:3148–3157. [PubMed: 7949187]
6. Wan PT, Garnett MJ, Roe SM, et al. Mechanism of activation of the RAF-ERK signaling pathway by oncogenic mutations of B-RAF. Cell. 2004; 116:855–867. [PubMed: 15035987]
7. Singh RR, Patel KP, Routbort MJ, et al. Clinical validation of a next-generation sequencing screen for mutational hotspots in 46 cancer-related genes. J Mol Diagn. 2013; 15:607–622. [PubMed: 23810757]
8. Petitjean A, Mathe E, Kato S, et al. Impact of mutant p53 functional properties on TP53 mutation patterns and tumor phenotype: lessons from recent developments in the IARC TP53 database. Hum Mutat. 2007; 28:622–629. [PubMed: 17311302]
9. Petitjean A, Achatz MI, Borresen-Dale AL, Hainaut P, Olivier M. TP53 mutations in human cancers: functional selection and impact on cancer prognosis and outcomes. Oncogene. 2007; 26:2157–2165. [PubMed: 17401424]
10. Neskey DM, Osman AA, Ow TJ, et al. Evolutionary Action Score of TP53 Identifies High-Risk Mutations Associated with Decreased Survival and Increased Distant Metastases in Head and Neck Cancer. Cancer Res. 2015; 75:1527–1536. [PubMed: 25634208]
11. Cancer Genome Atlas N. Genomic Classification of Cutaneous Melanoma. Cell. 2015; 161:1681–1696. [PubMed: 26091043]
12. Roszik J, Woodman SE. HotSpotter: efficient visualization of driver mutations. BMC Genomics. 2014; 15:1044. [PubMed: 25435088]
13. Bahadoran P, Allegra M, Le Duff F, et al. Major clinical response to a BRAF inhibitor in a patient with a BRAF L597R-mutated melanoma. J Clin Oncol. 2013; 31:e324–326. [PubMed: 23715574]
14. Jakob JA, Bassett RL Jr, Ng CS, et al. NRAS mutation status is an independent prognostic factor in metastatic melanoma. Cancer. 2012; 118:4014–4023. [PubMed: 22180178]
15. Long GV, Menzies AM, Nagrial AM, et al. Prognostic and clinicopathologic associations of oncogenic BRAF in metastatic melanoma. J Clin Oncol. 2011; 29:1239–1246. [PubMed: 21343559]
16. Zerp SF, van Elsas A, Peltenburg LT, Schrier PI. p53 mutations in human cutaneous melanoma correlate with sun exposure but are not always involved in melanomagenesis. Br J Cancer. 1999; 79:921–926. [PubMed: 10070891]
17. Viros A, Sanchez-Laorden B, Pedersen M, et al. Ultraviolet radiation accelerates BRAF-driven melanomagenesis by targeting TP53. Nature. 2014; 511:478–482. [PubMed: 24919155]
18. Terzian T, Torchia EC, Dai D, et al. p53 prevents progression of nevi to melanoma predominantly through cell cycle regulation. Pigment Cell Melanoma Res. 2010; 23:781–794. [PubMed: 20849464]
19. Olivier M, Langerod A, Carrieri P, et al. The clinical value of somatic TP53 gene mutations in 1,794 patients with breast cancer. Clin Cancer Res. 2006; 12:1157–1167. [PubMed: 16489069]
20. Essner R, Kuo CT, Wang H, et al. Prognostic implications of p53 overexpression in cutaneous melanoma from sun-exposed and nonexposed sites. Cancer. 1998; 82:309–316. [PubMed: 9445187]
21. Gupta S, Artomov M, Goggins W, Daly M, Tsao H. Gender Disparity and Mutation Burden in Metastatic Melanoma. J Natl Cancer Inst. 2015; 107
22. Hong DS, Vence L, Falchook G, et al. BRAF(V600) inhibitor GSK2118436 targeted inhibition of mutant BRAF in cancer patients does not impair overall immune competency. Clin Cancer Res. 2012; 18:2326–2335. [PubMed: 22355009]
23. Falchook GS, Long GV, Kurzrock R, et al. Dabrafenib in patients with melanoma, untreated brain metastases, and other solid tumours: a phase 1 dose-escalation trial. Lancet. 2012; 379:1893–1901. [PubMed: 22608338]
24. Dahlman KB, Xia J, Hutchinson K, et al. BRAF(L597) mutations in melanoma are associated with sensitivity to MEK inhibitors. Cancer Discov. 2012; 2:791–797. [PubMed: 22798288]

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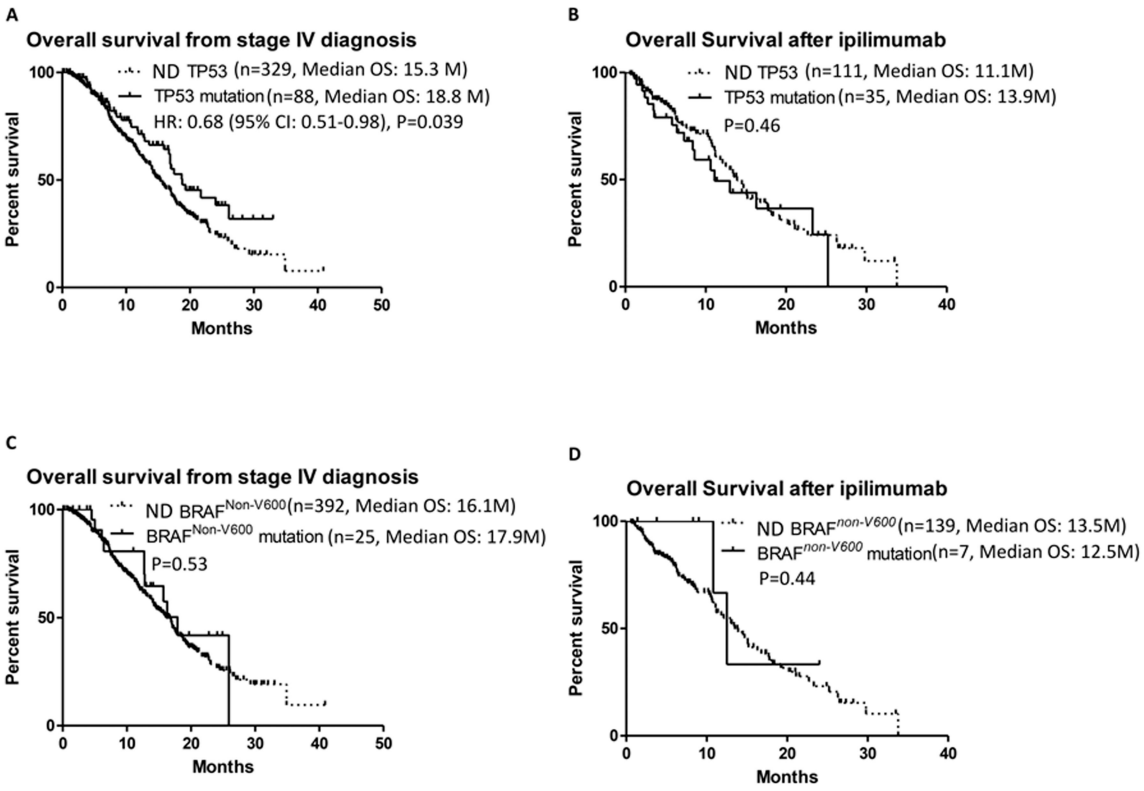

Figure 1.

Kaplan Meier analysis of survival after stage IV diagnosis (A) and after frontline ipilimumab treatment (B) based on *TP53* mutation status. Kaplan Meier survival curve after stage IV diagnosis (C) and after frontline ipilimumab treatment (D) based on *BRAF*$^{Non\text{-}V600}$ mutation status. *Dashed line*, not detected gene mutation (ND); *solid line*, gene mutation present.

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Table 1

Patients Demographics

Variable	All patients (N=926)
Median Age (range)	56 (12-94)
Gender	
Male	624 (67.4%)
Female	302 (32.6%)
Subtype	
superficial spreading	313 (33.8%)
nodular	184 (19.9%)
lentigo maligna	88 (9.5%)
desmoplastic	7 (0.8%)
unclassified	82 (8.9%)
not documented	252 (27.2%)
Primary site	
Trunk	339 (36.6%)
extremity	256 (27.6%)
head and neck	317 (34.2%)
other	14 (1.5%)

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Table 2

Prevalence of mutations in full cohort of non-acral cutaneous melanomas.

Mutation	No. of pt (%)	Mutation	No. of pt (%)
BRAF^{V600}	398 (43.0%)	*HNF1A*	9 (1.0%)
NRAS	195 (21.1%)	*RET*	9 (1.0%)
TP53	180 (19.4%)	*STK11*	8 (0.9%)
BRAF$^{Non\text{-}V600}$	60 (6.5%)	*CSF1R*	7 (0.8%)
CDKN2A	59 (6.4%)	*GNAS*	7 (0.8%)
PTEN	36 (3.9%)	*EZH2*	6 (0.6%)
KIT	35 (3.8%)	*MLH1*	5 (0.5%)
ERBB4	34 (3.7%)	*SMAD4*	5 (0.5%)
EGFR	31 (3.3%)	*SMARCB1*	5 (0.5%)
KDR	30 (3.2%)	*SMO*	5 (0.5%)
IDH1	27 (2.9%)	*ABL1*	4 (0.4%)
CTNNB	24 (2.6%)	*AKT1*	4 (0.4%)
KRAS	24 (2.6%)	*FGFR1*	4 (0.4%)
ATM	18 (1.9%)	*NOTCH1*	4 (0.4%)
APC	16 (1.7%)	*GNAQ*	3 (0.3%)
PKI3CA	16 (1.7%)	*SRC*	3 (0.3%)
RB1	16 (1.7%)	*JAK2*	2 (0.2%)
FLT3	15 (1.6%)	*JAK3*	2 (0.2%)
MET	15 (1.6%)	*VHL*	2 (0.2%)
PTPN11	15 (1.6%)	*ALK*	1 (0.1%)
FBXW7	13 (1.4%)	*ERBB2*	1 (0.1%)
HRAS	13 (1.4%)	*GNA11*	1 (0.1%)
FGFR2	11 (1.2%)	*MPL1*	1 (0.1%)
PDGFRA	11 (1.2%)	*NPM1*	1 (0.1%)
FGFR3	9 (1.0%)		

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Table 3

Demographics, primary tumor features, and clinical outcomes of melanoma patients with *TP53* or *BRAF*$^{Non\text{-}V600}$ mutations

Variable	All (N=926)					
	***TP53* mutant**	**ND *TP53***	***P value***	***BRAF*$^{Non\text{-}V600}$ mutant**	**ND *BRAF*$^{Non\text{-}V600}$**	***P value***
No. of Patients (%)	180 (19.4%)	746 (80.6%)		60 (6.5%)	866 (93.5%)	
Median Age (range)	58 (12-89)	56 (13-94)	*0.019*	60 (29-89)	56 (12-94)	0.005
Gender (%)			*0.052*			0.88
Male	132 (21.2%)	492 (78.8%)		41 (6.6%)	583 (93.4%)	
Female	48 (15.9%)	254 (84.1%)		19 (6.3%)	283 (93.7%)	
Primary site (%)			*0.0001*			0.38
Trunk	54 (15.9%)	285 (84.1%)		21 (6.2%)	318 (93.8%)	
Extremity	38 (14.8%)	218 (85.2%)		12 (4.7%)	244 (95.3%)	
Head and neck	87 (27.4%)	230 (72.6%)		26 (8.2%)	291 (91.8%)	
Other	1 (7.1%)	13 (92.9%)		1 (7.1%)	13 (92.9%)	
Breslow thickness (mean)	3.31mm	3.35mm	*0.9*	2.9mm	3.38mm	0.32
Ulceration (%)			*0.9*			0.63
Yes	47 (18.7%)	205 (81.3%)		18 (7.1%)	234 (92.9%)	
No	80 (19.2%)	337 (80.8%)		25 (6.0%)	392 (94.0%)	
Not available	53 (20.6%)	204 (79.4%)		17 (6.6%)	240 (93.4%)	
Patients with stage IV	N=531					
No. of Patients (%)	107 (20.1%)	424 (79.9%)		41 (7.7%)	490 (92.3%)	
M stage (%)			*0.5*			0.56
M1a	5 (15.2%)	28 (84.8%)		1 (3.0%)	32 (97.0%)	
M1b	22 (23.9%)	70 (76.1%)		8 (8.7%)	84 (91.3%)	
M1c	80 (19.7%)	326 (80.3%)		32 (7.9%)	374 (92.1%)	
LDH at stage IV			*0.6*			0.3
Elevated (%)	57 (19.3%)	239 (80.7%)		26 (8.8%)	270 (91.2%)	
WNL (%)	46 (20.4%)	180 (79.6%)		14 (5.9%)	212 (94.1%)	
Missing (%)	4 (44.4%)	5 (55.6%)		1 (11.1%)	8 (88.9%)	

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Variable	All (N=926)					
	***TP53* mutant**	**ND *TP53***	***P value***	***BRAF*$^{Non-V600}$ mutant**	**ND *BRAF*$^{Non-V600}$**	***P value***
Patients with stage IV (tested within 12 mo)				N=417		
Median OS from stage IV (Months, 95% CI)	18.8 (14.0-23.6)	15.3 (13.4-17.2)	*0.039*	17.9 (14.7-17.9)	16.1 (14.3-17.9)	0.53

CI: confidence interval, LDH: lactate dehydrogenase, No.: Number, OS: overall survival, WNL: within normal limit, ND: not detected mutation

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Table 4

Clinical outcomes of patients with *BRAF*$^{non\text{-}V600}$ mutations treated with a selective BRAF inhibitor and/or a MEK inhibitor

Patient #	Age	Gender	*BRAF*$^{Non\text{-}V600}$ mutation	Stage	Treatment	Response	PFS (Months)	OS (Months)
1	84	M	G469R	M1c	Vemurafenib	NE	NE	NE
2	35	M	K601E	M1c	Vemurafenib	NE	NE	NE
3	85	M	G469R	M1a	Vemurafenib	NE	NE	NE
4	56	F	L597R	M1c	Vemurafenib	PD	4.3	11.2
5	51	M	S467L	M1c	Vemurafenib	PD	1.9	5.6
6	69	F	G466V	M1c	Vemurafenib	PD	0.9	3.7
7	44	M	L597Q	M1c	Vemurafenib	PD	2.0	5.4
8	70	M	G469R	M1c	Vemurafenib	PD	3.5	9.1
9	74	M	G466E	M1c	Vemurafenib	PD	1.9	+ 30.6
10	52	M	K601E	M1c	Dabrafenib	PD	0.7	+ 64.5
11	36	M	K601E	M1c	Dabrafenib and trametinib	PD	2.0	+ 4.5
12	57	F	L597R	M1c	Trametinib	PD	1.7	4.8
13	76	M	G466E	M1c	Trametinib	PD	1.5	5.2
14	66	M	G469E	M1c	Trametinib	PD	1.3	7.1

PFS, progression free survival; OS, overall survival; PD, progressive disease; NE, not evaluable.

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Table 5
Multivariate analysis of survival from diagnosis of stage IV melanoma

	HR	95% CI	P value
Age	1.01	1.00-1.02	0.05
Gender			
Female			
Male	0.82	059-1.14	0.23
stage IV M1 classification			
M1a			
M1b	0.91	0.42-1.97	0.8
M1c	1.92	0.92-4.00	0.08
LDH			
WNL			
elevated	1.01	0.70-1.47	0.94
unknown	0.8	0.11-5.93	0.83
Mutation			
BRAF^{V600} mutation (vs *BRAF*^{V600} ND)	0.93	0.64-1.34	0.7
NRAS mutation (vs *NRAS* ND)	1.2	0.82-1.76	0.35
BRAF$^{Non\text{-}V600}$ mutation (vs *BRAF*$^{Non\text{-}V600}$)	0.74	0.39-1.76	0.37
TP53 mutation (vs *TP53* ND)	0.62	0.42-0.91	0.015

CI: confidence interval, HR: hazard ratio, LDH: lactate dehydrogenase, WNL: within normal limit, ND: not detected