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Brief Communication

BRIEF COMMUNICATION

Co-occurring Genomic Alterations and Association With Progression-Free Survival in BRAF^{V600}-Mutated Nonmelanoma Tumors

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Abstract

BRAF^{V600} mutations occur in multiple nonmelanoma tumors, but no US Food and Drug Administration–approved BRAF-targeted therapies exist for these cancers. BRAF inhibitor vemurafenib was recently found to demonstrate activity across various BRAF-mutated nonmelanoma cancer types. However, most tumors ultimately become resistant to BRAF-targeted monotherapy. To identify whether co-occurring genomic alterations drive resistance to BRAF-targeted therapies, we analyzed next-generation sequencing data from 30 advanced BRAF-mutated nonmelanoma cancers treated with BRAF inhibitor monotherapy. Kaplan-Meier survival analysis and Cox proportional hazard regression analysis were performed and hazard ratios (HR) with 95% confidence intervals (CI) were calculated. All statistical tests were two-sided. We identified a strong association between co-occurring PI3K-mTOR pathway aberrations and primary resistance to BRAF-targeted therapy. PI3K-mTOR pathway aberrations were associated with a statistically significant reduction in progression-free survival (HR = 15.0, 95% CI = 3.6 to 63.0, $P < .001$) and overall survival (HR = 19.2, 95% CI = 3.7 to 100.0, $P < .001$). This suggests that co-occurring genomic alterations may predict response and resistance to BRAF inhibitor therapy and identify subgroups of BRAF-mutated nonmelanomas cancers.

BRIEF COMMUNICATION

The BRAF oncogene is mutated in 50% of cutaneous melanomas and up to 10% of nonmelanomas, leading to constitutive activation of mitogen-activated protein kinase (MAPK) signaling (1). While vemurafenib, cobimetinib, dabrafenib, and trametinib are US Food and Drug Administration (FDA)–approved BRAF^{V600}-targeted therapies for metastatic melanoma with response rates higher than 50% (2), no FDA-approved therapy exists for BRAF-mutated nonmelanoma cancers. In many of these tumors, the BRAF^{V600} mutation is associated with an aggressive phenotype and decreased progression-free survival (PFS) and overall survival (OS). A recent "basket" study of vemurafenib in BRAF^{V600}-mutated nonmelanoma cancers demonstrated clinical activity (3). However, many patients exhibit primary and secondary resistance. The role of concurrent genomic alterations leading to alternative survival pathway activation has yet to be clinically examined and mechanisms of resistance to BRAF-targeted therapy remain unknown in nonmelanomas harboring a BRAF mutation. To elucidate mechanisms of resistance to BRAF-targeted monotherapy, we analyzed the genomic landscape of these tumors and the association between co-occurring mutations and resistance to therapy, PFS, and OS.

We analyzed Clinical Laboratory Improvement Amendments (CLIA)-certified next-generation sequencing data from BRAF^{V600} nonmelanoma cancers treated with BRAF inhibitor monotherapy at UT MD Anderson Cancer Center between May 2012 and January 2016 (3). All patients provided written informed consent,

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Table 1. Table indicating tumor histology, all aberrations, progression-free survival (in days) and disease status at last follow-up of each BRAF-mutated nonmelanoma cancer evaluated

Histology	Co-occurring mutations	Progression-free survival, d	Disease progression
Non–small cell lung	TP53 (E286V)	61	Yes
Non–small cell lung	–	98	Yes
Non–small cell lung	TP53 (G154V)	57	Yes
Non–small cell lung	SMAD4 (C499R)	425	No
Non–small cell lung	–	121	Yes
Non–small cell lung	SETD2 (H2514fs)	554	No
Non–small cell lung	–	350	No
Non–small cell lung	TP53 (R248W)	216	No
Non–small cell lung	AKT (D46E)	279	No
Non–small cell lung	NNF1 (3314 + 1G>A (splice)), NTRK3 (L560H), STK11 (920 + 1G>T (splice)), ITGB3 (S85N), KEAP1 (G333S), LPHN3 (N1311K), LRP1B (D2307E), UBR5 (R1427S)	126	Yes
Non–small cell lung	IDH1 (R132C), ARID2 ((splice site 92 + 1gA))	131	Yes
Colorectal	TP53 (R175H)	56	Yes
Colorectal	–	112	Yes
Colorectal†	TP53 (R213*), MLL3 (G2568R), TLR4 (G480F), SMAD4 (P356R), PTPRT (V239F), WHSC1 (R976K)	252	Yes
Colorectal†	PTEN (R130*), TP53 (R248Q), SMAD4 (D124fs*), ATM (R3008C)	77	Yes
Colorectal†	PIK3CA (E545K), TP53 (C176Y), SMAD4 (C361C)	72	Yes
Cholangiocarcinoma	IDH1 (R132H)	100	Yes
Cholangiocarcinoma	–	236	Yes
Cholangiocarcinoma	LKB1 (F354L), MYC amplification, MYST3 amplification	305	No
Cholangiocarcinoma	PIK3R1 (splice site 1119-1 G>A)	17	Yes
Anaplastic thyroid	PIK3CA (I391M)	56	Yes
Anaplastic thyroid	TP53 (R280K)	184	Yes
Papillary thyroid	–	86	Yes
Erdheim Chester Disease	–	66	Yes
Erdheim Chester Disease	–	799	No
Erdheim Chester Disease	–	376	No
Salivary gland carcinoma	–	84	Yes
Glioblastoma	PTEN (P339fs*2)	44	Yes
Glioblastoma	CDK2NA (H66fs*54), NOTCH1 (E1567K), KRAS (G13D)	455	Yes
Unknown primary	TP53 (R110C), TP53 (P278S)	100	Yes

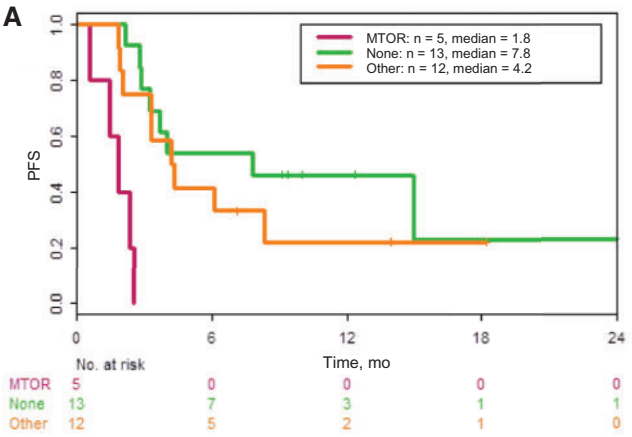
†Treated with cetuximab in addition to BRAF inhibitor. - = no co-occurring mutation present.

and institutional review board authorization was obtained. Kaplan-Meier survival analysis and Cox proportional hazard regression analysis were performed and hazard ratios with 95% confidence intervals calculated in order to determine whether survival on BRAF-inhibitor monotherapy was associated with co-occurring mutation status. All statistical tests were two-sided, and a *P* value of less than .05 was considered statistically significant. *P* values for the hazard ratios were computed via Wald tests from the Cox proportional hazard models. The proportional hazards assumption was verified using plots and tests based on the rescaled Schoenfeld residuals. TIBCO Spotfire S + 8.2 for Windows was used to make calculations.

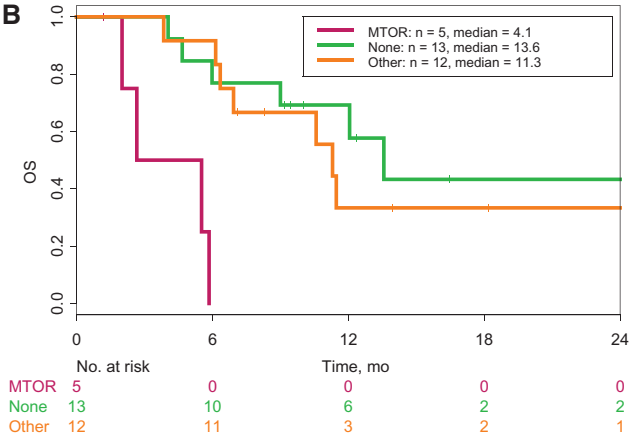
Of the 30 patients with $BRAF^{V600}$-mutated tumors treated with BRAF inhibitor monotherapy at our institution, 11 (36.7%) had non–small cell lung cancer, five (16.7%) had colorectal cancer, four (13.3%) had cholangiocarcinoma, three (10.0%) had thyroid cancer, three (10.0%) had Erdheim Chester disease, two (6.7%) had glioblastoma, one (3.3%) patient had salivary gland carcinoma, and one (3.3%) patient had unknown primary. Three of the five colorectal cancers, including two with PI3K-mTOR pathway co-occurring alterations, were treated with BRAF inhibitor in combination with cetuximab. Three subsets of co-occurring genomic alterations were identified: 14 (46.6%) had no co-occurring alterations, five (16.6%) had PI3K-mTOR pathway alterations, and 11 (36.7%) had "other" mutations, most commonly TP53 (n = 11), SMAD4 (n = 4), LKB1 (n = 2), and IDH1 (n = 2). The tumor type breakdown, co-occurring alterations, and progression-free survival are shown in Table 1.

To investigate whether co-occurring mutations are associated with survival, we analyzed PFS and OS in each patient. Eight patients (26.7%) had ongoing response without progression on BRAF inhibitor monotherapy (range PFS = 222–805 days). Six of eight durable responders have no co-occurring alterations, two of eight have "other" mutations. Both patients with non-mTOR pathway somatic gene mutations who have yet to progress have metastatic adenocarcinoma of the lung, one with a SMAD4 C499R mutation and the other with a TP53 R248W mutation. The six patients without co-occurring mutations had non–small cell lung cancer (n = 3), Erdheim-chester disease (n = 2), and cholangiocarcinoma (n = 1). All patients with PI3K-mTOR pathway mutations progressed within 77 days (metastatic colorectal cancer [n = 2], anaplastic thyroid cancer [n = 1], glioblastoma [n = 1], and cholangiocarcinoma [n = 1]).

Kaplan-Meier analyses demonstrate a statistically significant reduction in PFS (hazard ratio [HR] = 15.0, 95% confidence interval [CI] = 3.6 to 63.0, $P = .0002$) and OS (HR = 19.2, 95% CI = 3.7 to 100.0, $P < .001$) in patients whose tumors harbored co-occurring PI3K-mTOR pathways mutations compared with



Co-occurring mutations	HR (95% CI)	P
MTOR vs None	15.0 (3.6 to 63.0)	<.001
Other vs None	1.5 (0.6 to 3.9)	.42
MTOR vs Other	10.2 (2.5 to 42.0)	.001
PFS difference between 3 groups, $P = .003$		
After a median follow-up of 16 months, 5/30 patients have yet to progress.		



Co-occurring mutations	HR (95% CI)	P
MTOR vs None	19.2 (3.7 to 100.0)	<.001
Other vs None	1.6 (0.5 to 4.6)	.40
MTOR vs Other	12 (2.5 to 59.0)	.002
OS difference between all 3 groups, $P = .003$		
After a median follow-up of 16 months, 9/30 patients remain alive.		

Figure 1. Progression-free survival and overall survival by co-occurring mutations in patients with BRAF-mutated nonmelanoma cancers treated with BRAF-inhibitor monotherapy. Kaplan-Meier survival analysis and Cox proportional hazard regression analysis for progression-free survival **(A)** and overall survival **(B)** were performed in 30 patients with BRAF-mutated nonmelanoma cancers treated with BRAF inhibitor monotherapy. Patients were stratified by tumors harboring no co-occurring mutations (None) in **green**, mTOR pathway mutations (MTOR) in **purple**, or other mutations (Other) in **orange**. Statistical tests were two-sided. CI = confidence interval; HR = hazard ratio; OS = overall survival; PFS = progression-free survival.

those with "other" co-occurring mutations and no co-occurring mutations, as seen in Figure 1. Tumors with co-occurring PI3K-mTOR pathway aberrations had a median PFS of 1.8 months (95% CI = 1.4 to not reached [NR]) compared with tumors with other co-occurring mutations (4.2 months, 95% CI = 3.3 to NR) and no co-occurring mutations (7.8 months, 95% CI = 3.2 to NR). OS in patients with tumors harboring co-occurring mTOR pathway aberrations had a median OS of 4.1 months (95% CI = 2.0 to NR) compared with tumors with other co-occurring mutations (11.3 months, 95% CI = 6.9 to NR) and without co-occurring genomic alterations (13.6 months, 95% CI = 9.0 to NR).

While BRAF mutations, amplifications, and fusions have been identified in a number of nonmelanoma cancers, the mutational landscape of these tumors has yet to be defined. We identified three distinct subsets of co-occurring molecular alterations in BRAF-mutated nonmelanoma cancers and found a statistically significant association between co-occurring mutations and PFS and OS. Specifically, 20% of our patients' tumors across all histologies had mutations in PI3K-mTOR pathway genes, and this subset was associated with de novo resistance to BRAF inhibitor monotherapy and statistically significantly shorter PFS and OS (Figure 1).

In melanoma, resistance to BRAF-targeted therapy results from reactivation of the MAPK pathway, activation of parallel signaling pathways including the PI3K-mTOR pathway, or ineffective modulation of the immune system (4). Our study suggests that parallel activation of mTOR signaling may be one mechanism contributing to de novo resistance to BRAF-targeted therapy in nonmelanoma cancers as well. In preclinical colorectal cancer models, parallel activation of the PI3K-mTOR pathway has been implicated as a mechanism of resistance to BRAF inhibition (5). Clinically, resistance to BRAF inhibition may be mediated through the epidermal growth factor receptor pathway as well (6). In our cohort, despite the addition of cetuximab, survival was statistically significantly worse in tumors harboring PI3K-mTOR pathway mutations. Given the known crosstalk between the MAPK and mTOR signaling pathways (7), it is plausible that mTOR activation may also mediate acquired resistance to BRAF-targeted therapy in the patients who initially respond to BRAF inhibiton as well. This remains to be studied and also highlights the utility of re-biopsying a patient at the time of progression.

We acknowledge that the lack of serial biopsies was a limitation of our study, as was the sample size. While PI3K-mTOR pathway co-occurring alterations were identified across tumor

types in our study, a larger sample size would allow for a better understanding of the role of tumor histology in response and resistance to BRAF-targeted therapy. Additionally, hot spot next-generation sequencing was utilized in our study, and whole-genome sequencing may lead to a more comprehensive pathway analysis in this subset of tumors in the future.

Co-occurring genomic alterations may help predict response and resistance to targeted therapies in BRAF-mutated cancers in a histology-independent manner. This study highlights the importance of implementing next-generation sequencing testing and enrolling BRAF-mutated cancer patients in larger genotype-matched trials when feasible (8). A phase I trial combining BRAF inhibitor vemurafenib and mTOR inhibitor everolimus is underway (NCT01596140).

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