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New *RAS*-Mutant Pancreatic Adenocarcinoma With Combined BRAF and MEK Inhibition for Metastatic Melanoma

Introduction

Combined BRAF and MEK inhibition improves the response rate (RR) and progression-free survival for patients with V600 *BRAF*-mutant metastatic melanoma over single-agent BRAF inhibition, with the advantage of causing fewer cutaneous squamous cell carcinomas (cuSCCs).[1] In addition to the superior efficacy, the decrease in oncogenic toxicities favor its use over single-agent BRAF inhibitors in both metastatic and adjuvant patients, particularly with recent case reports of propagation of pre-existing *RAS*-mutant leukemia,[2] gastric and colonic polyps,[3] and the possible increased risk of new *BRAF* wild-type primary cutaneous melanomas[4] with single-agent BRAF inhibitors. Herein, we report the first case of a new *KRAS*-mutant adenocarcinoma diagnosed in a patient while treated with the combination of dabrafenib and trametinib for V600 *BRAF*–mutant metastatic

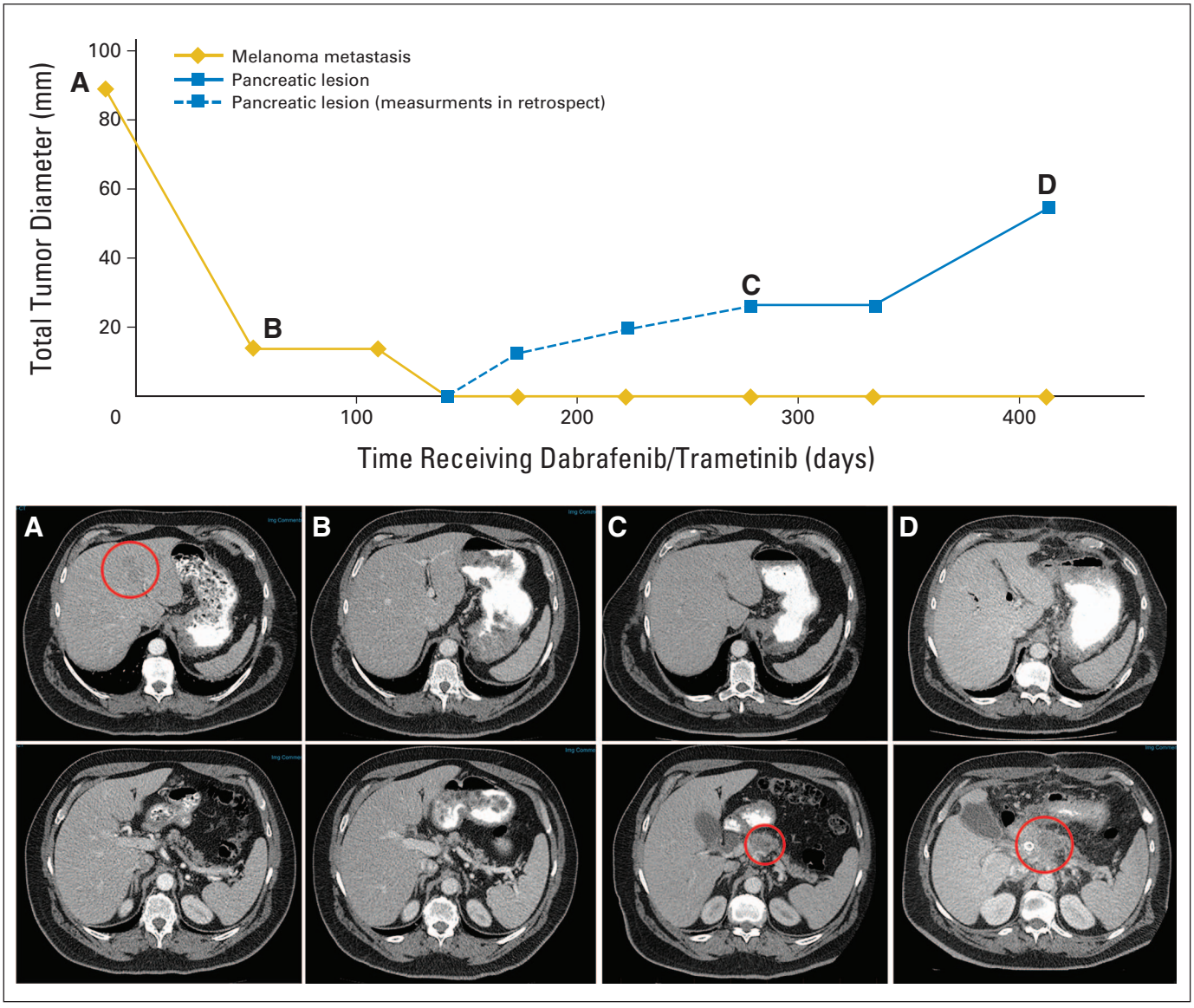

Fig 1.

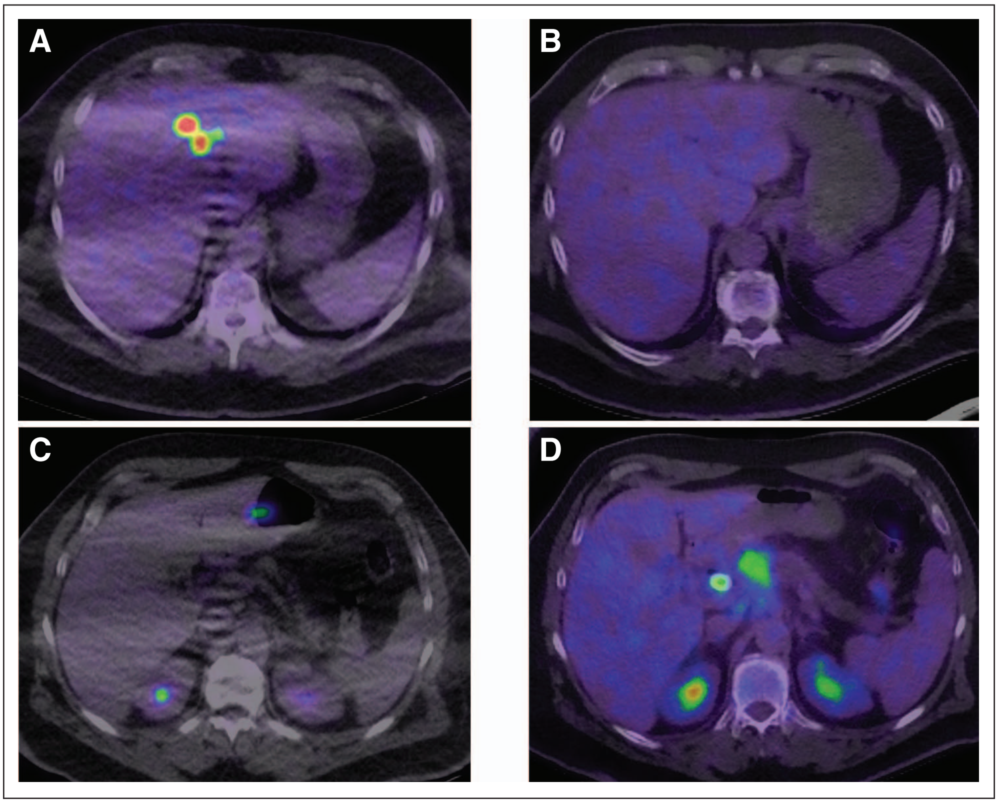

Fig 2.

melanoma that was not present before initiation of the combined BRAF and MEK inhibition.

Case Report

A well 63-year-old man with recently diagnosed metastatic melanoma of the liver and bone presented for an opinion regarding systemic therapy in October 2011. He had no risk factors for the development of pancreatic cancer; he had no family history of pancreatic cancer, was a life-long nonsmoker with no history of excessive alcohol consumption at baseline and had no history of pancreatitis. A left upper abdomen primary cutaneous melanoma was resected in 2007 (superficial spreading subtype, Breslow thickness 0.6 mm, Clark level III invasion with no ulceration, and no dermal mitoses identified). Four years later, in July 2011, he developed left axillary lymphadenopathy, confirmed to be melanoma on excision biopsy. Pathology of the cleared axilla showed a further involved lymph node, thus a total of two of 24 lymph nodes were involved, both with extranodal spread. The tumor cells stained positive for S100 and negative for melan-A and HMB45. Tumor mutation testing confirmed the presence of a *BRAF* Exon 15 V600E mutation (c.1799_1800delinsAA: p. V600E). Positron emission tomography/computed tomography with ^{18}F-labeled fluorodeoxyglucose (FDG PET/CT) showed multiple liver metastases, skeletal involvement at the fifth left rib and L2 and 3 vertebral bodies, and no focal abnormality in the pancreas.

The patient was enrolled in cohort D of the phase I trial BRF113220 of dabrafenib in combination with trametinib[1] in November 2011 as first-line systemic therapy. The patient was treated with single-agent dabrafenib at a dose of 75 mg twice daily for one cycle (28 days), and thereafter received dabrafenib 75 mg twice daily combined with trametinib 2 mg daily. The patient tolerated treatment with minimal toxicities. At first restaging at day 54, the patient had a RECIST 1.1[5] partial response (Fig 1B). Subsequently at day 141 the patient had a RECIST complete response by CT scan with resolution of all liver metastasis and sclerosis of the lytic lesion at L2 (Fig 1). At day 278, a 26 mm hypoattenuating lesion was identified in the head of the pancreas with associated biliary duct dilation on CT (Fig 1C). In retrospect this pancreatic lesion was present on CT at day 173, but not earlier (Fig 1, dashed red line). At day 307, he developed obstructive jaundice, which was relieved by endoscopic retrograde cholangiopancreatography and stenting (Fig 1D). At day 355, a further FDG PET/CT was performed revealing FDG-avid lesions within the anterior portion of the pancreatic body, the uncinate process, and celiac and precaval lymph nodes (Fig 2D, note physiological renal uptake). These were not present on the pretreatment PET/CT (Fig 2C) and full-dose CT scan (Fig 1A). The previously FDG-avid melanoma liver and skeletal metastasis at baseline (Fig 2A) were no longer visible (Fig 2B). Cytologic analysis of material obtained from an endoscopic ultrasound-guided biopsy confirmed adenocarcinoma and the combined dabrafenib and trametinib were ceased on day 365, October 2012. Cancer antigen (CA) 19.9 was elevated to 1,893 U/mL (normal range, 0-34 U/mL). In retrospect, the liver metastases diagnosed at the time of the left axillary melanoma recurrence were not considered to be pancreatic adenocarcinoma metastases because of their complete response to BRAF/MEK inhibition, and the lack of a pancreatic lesion on initial imaging.

In November 2012, a laparotomy was performed for a Whipple's procedure, but was abandoned as malignancy was encircling the celiac

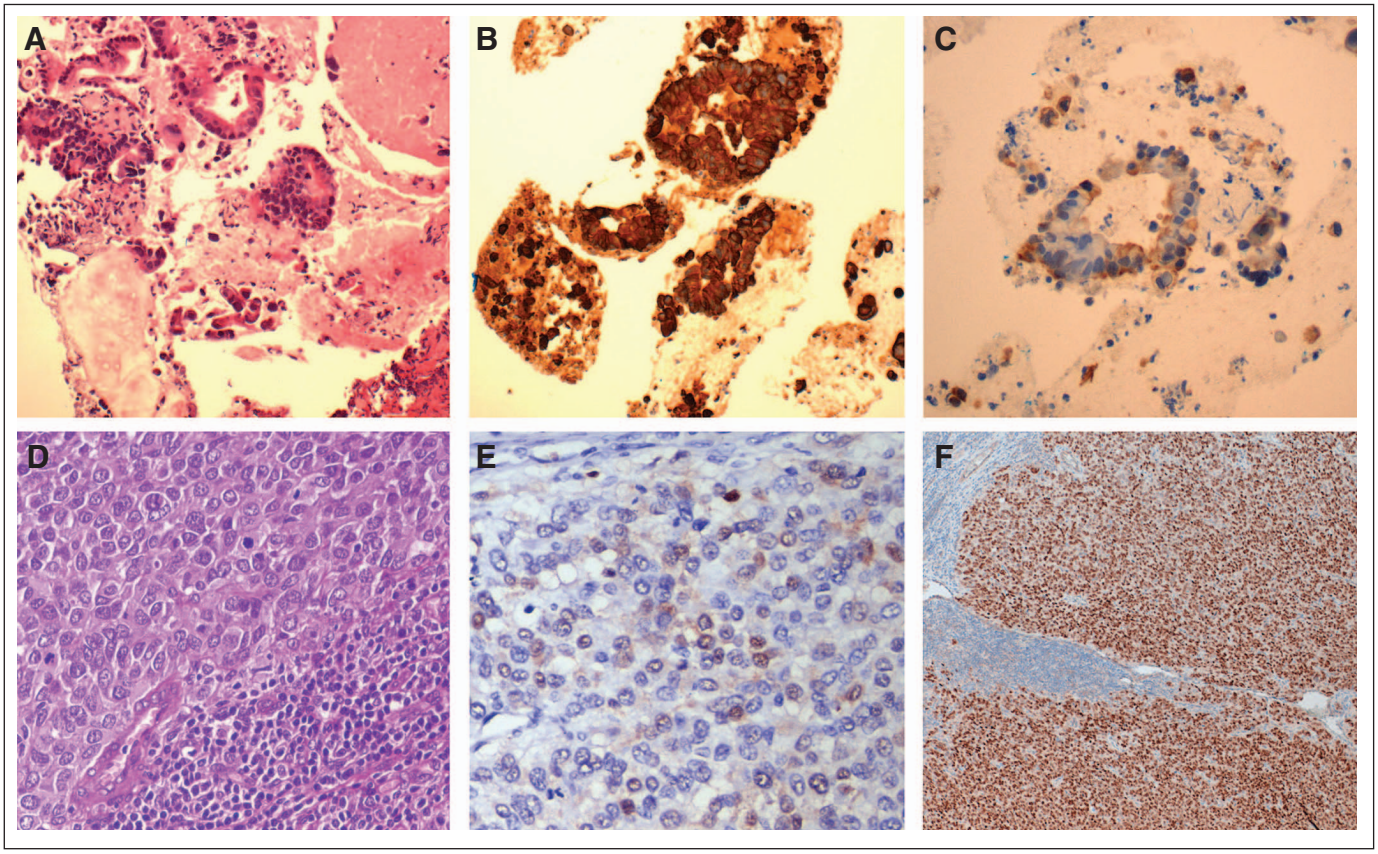

Fig 3.

trunk. A further biopsy via endoscopic ultrasound was performed for histological and molecular analysis. The tumor was confirmed to be an adenocarcinoma with well-formed glandular arrangements (Fig 3A). The tumor cells stained for cytokeratin (Fig 3B), and were negative for S100 (Fig 3C). In contrast the patients axillary melanoma had a differing histological appearance (Fig 3D), stained negative for cytokeratin and displayed positive for S100 (Fig 3E) and SOX-10 (Fig 3F). Subsequently deep amplicon-based sequencing was performed on the biopsy sample using the TruSEQ Amplicon Cancer panel targeting 225 loci across 48 known cancer genes to a depth of greater than 4,000 fold (Illumina, San Diego, CA).[6] The assay identified eight missense variants (Table 1) including *KRAS* (G12D) and *ERRB4* (R103C), while no variants were detected in either BRAF or CDKN2A. The *KRAS* mutation was confirmed using polymerase chain reaction and Sequenom Massarray mass spectrometer (Sequenom, San Diego, CA). The patient was screened for germline mutations in *CDKN2A*, as he had developed both melanoma and pancreatic cancer, and none were identified.

The patient was subsequently treated with gemcitabine and nanoparticle albumin-bound paclitaxel (Abraxane) with a rapid initial improvement in CA19.9 to 262 U/mL after two cycles of therapy. However, after the third cycle there was a rise of CA19.9 to 774 U/mL.

Table 1. Missense Variants Identified

Gene	NCBI Reference Sequence	Codon Change	Protein Substitution	Nonref Allele Frequency (%)	Reported on COSMIC*	dbSNP ID†	Minor Allele Frequency†
ERBB4	NM_005235	CGC to TGC	R103C	12.00	Yes	—	—
KIT	NM_001093772	ATG to CTG	M537L	57.00	Yes	rs3822214	0.064
RET	NM_020630	TAT to TTT	Y791F	42.00	Yes	rs77724903	N/A
KRAS	NM_033360	GGT to GAT	G12D	50.00	Yes	—	—
TP53	NM_001126116	CGC to TGC	R151C	25.00	No	—	—
TP53	NM_001126116	GCC to CCC	A27P	48.00	Yes	—	—
TP53	NM_001126113	CCG to CGC	P72R	99.00	No	rs1042522	0.398
STK11	NM_000455	TTC to TTG	F354L	59.00	Yes	rs59912467	0.013

Abbreviations: COSMIC, Catalogue of Somatic Mutations in Cancer; NCBI, National Center for Biotechnology Information; Nonref, nonreference.
*http://cancer.sanger.ac.uk/cancergenome/projects/cosmic/.
†http://www.ncbi.nlm.nih.gov/projects/SNP/.

A CT scan demonstrated a significant increase in the size of the pancreatic primary and the development of two new small liver metastases. Second-line chemotherapy was commenced with oxaliplatin, fluorouracil, and folinic acid.

Discussion

Targeting the mitogen-activated protein kinase pathway (MAPK) pathway has revolutionized the treatment of *BRAF*-mutant metastatic melanoma. Both single-agent BRAF and MEK inhibitors improve survival compared with chemotherapy,[7,8] however 4% to 31% of patients treated with type I BRAF inhibitors develop well-differentiated cuSCCs and keratoacanthomas.[9] These lesions develop early after the commencement of vemurafenib or dabrafenib and are due to the paradoxical activation of MAPK signaling in the setting of upstream activation of the pathway (eg, *HRAS* mutations).[10-13] Although these cuSCCs are easily managed and none have metastasized, their development highlights the possible risk of other malignancies with greater metastatic potential. There also appears to be an increased frequency in the development of new *BRAF* wild-type primary cutaneous melanomas in patients treated with BRAF inhibitors.[4,14] A case has been reported of accelerated progression of *RAS*-mutant leukemia after commencement of vemurafenib.[2] The rapid progression of a pre-existing pancreatic adenocarcinoma was reported recently in a patient treated with vemurafenib who had a family history of both melanoma and pancreatic adenocarcinoma, and was known to have a germline CDKN2A mutation.[15] The development of these malignancies soon after the initiation of BRAF inhibitor therapy suggests BRAF inhibitors potentiate the proliferation of a pre-existing malignancy rather than causing new malignancies. *RAS* wild-type gastric and colonic polyps have been reported to occur after prolonged therapy with vemurafenib, however no mutations in the MAPK pathway were identified, and most harbored mutations the *APC* gene or β-catenin. The mechanism of tumor development in these cases remains to be elucidated.[3]

Paradoxical MAPK pathway activation and the associated cuSCCs do not occur with single-agent trametinib treatment which inhibits wild-type MEK, the kinase downstream from the RAF kinases.[16] Combined BRAF (dabrafenib) and MEK (trametinib) inhibition improves the response rate and progression-free survival of patients with V600 *BRAF* mutant metastatic melanoma compared with BRAF inhibition alone with the added benefit of a reduced incidence of cuSCCs.[1] Despite this, propagation of a *KRAS*-mutant colon cancer by combined dabrafenib and trametinib was reported in a patient with metastatic melanoma and a history of resected Dukes stage B colonic adenocarcinoma.[17] The patient's serum carcinoembryonic antigen and symptoms due to the colonic metastases improved with treatment with single-agent trametinib, suggesting that BRAF inhibition was driving proliferation.[17] This case of colorectal cancer differs from our case of de novo pancreatic cancer, as it was a recurrence of a pre-existing malignancy. In retrospect the colon cancer was present as a malignant pleural effusion before initiating therapy with dabrafenib/trametinib.

Given the strong evidence for paradoxical activation of the MAPK pathway by BRAF inhibitors, the case described herein illustrates there is a risk of development or progression of malignancies of significant metastatic potential with BRAF inhibitor treatment, and the risk may not be completely negated by the addition of a MEK inhibitor. The pancreatic adenocarcinoma in this case was not clinically apparent by CT or PET scan before commencing treatment for metastatic melanoma and progressed to become locally advanced. Pre-existing microscopic disease may have been present that was propagated by BRAF inhibitor therapy but may have eventually developed irrespective of inhibitor therapy. Historically, this risk is of minimal clinical consequence given the poor prognosis of patients with metastatic melanoma.[18] With more effective systemic therapies, the risk has greater significance, particularly in patients such as this who have a complete response to therapy. Of more concern is the potential for malignancies to develop in patients treated with adjuvant MAPK inhibitors.

Lastly, this case highlights the need to biopsy any new radiological lesion that develops during BRAF inhibitor therapy, whether single agent or combined with other drugs, given the risk of a new or propagating malignancy. This is particularly important in patients whose pre-existing melanoma remains stable or is responding well.

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