Published in final edited form as:

J Cutan Pathol. 2019 March ; 46(3): 190–194. doi:10.1111/cup.13401.

NRAS Q61R and BRAF G466A Mutations in Atypical Melanocytic Lesions Newly Arising in Advanced Melanoma Patients Treated with Vemurafenib

Vishwas Parekh¹, Joseph Sobanko², Christopher J. Miller², Giorgos Karakousis³, Wei Xu^{4,5}, Richard Letrero⁶, Rosalie Elenitsas², Xiaowei Xu⁷, David E. Elder⁷, Ravi Amaravadi^{4,5}, Lynn M. Schuchter^{4,5}, Katherine L. Nathanson^{4,6}, Melissa A. Wilson^{#8}, and Emily Y. Chu^{#2}

¹Department of Pathology, City of Hope Medical Center, Duarte, CA.

²Department of Dermatology, Perelman School of Medicine at the University of Pennsylvania, 3400 Civic Center Boulevard, 1st Floor South Pavilion, Philadelphia, PA 19104

³Department of Surgery, Perelman School of Medicine at the University of Pennsylvania, 3400 Spruce Street, 4 Silverstein, Philadelphia, PA 19104

⁴Abramson Cancer Center, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA

⁵Hematology/Oncology, Department of Medicine, Perelman School of Medicine at the University of Pennsylvania, 3400 Civic Center Boulevard, 12 Penn Tower, Philadelphia, PA

⁶Translational Medicine and Human Genetics, Department of Medicine, Perelman School of Medicine at the University of Pennsylvania, 351 BRB 2/3, 421 Curie Blvd, Philadelphia, PA 19104

⁷Department of Pathology, Perelman School of Medicine at the University of Pennsylvania, 3400 Spruce Street, 6 Founders, Philadelphia, PA 19104

⁸Sidney Kimmel Cancer Center, Thomas Jefferson University, 1025 Walnut Street, Philadelphia, PA 19107

These authors contributed equally to this work.

Abstract

Background: BRAF inhibition has improved overall survival in patients with *BRAF* mutant melanoma but this is associated with a range of known and predictable cutaneous side effects, including squamous cell carcinomas associated with *RAS* mutations.

Methods: We identified three severely dysplastic nevi, one atypical intraepidermal melanocytic proliferation, and four melanoma *in situ* lesions, newly arising in four patients undergoing treatment with vemurafenib. To characterize mutations in these atypical melanocytic lesions, we used a custom iPLEX panel detecting 74 mutations in 13 genes known to play a role in melanoma pathogenesis.

Results: We identified an *NRAS* mutation at codon 61 (Q61R) and a rare *BRAF* exon 11 mutation (G466A) in atypical melanocytic lesions that arose in patients treated with vemurafenib.

Conclusions: There appears to be development or accelerated growth of atypical melanocytic lesions in the setting of BRAF inhibition. Our results underscore the need for careful surveillance for melanocytic lesions in patients on BRAF inhibitor therapy and shed light on potential mechanisms for melanoma pathogenesis in the context of BRAF pathway blockade. Further studies are warranted to demonstrate a causal relationship.

Keywords

Melanoma; *BRAF*; *NRAS*; Mutation; Vemurafenib

INTRODUCTION

Advances in melanoma therapy have led to improved overall survival in patients with advanced stage melanoma, which include immunotherapy and targeted therapy in *BRAF* mutant melanoma. BRAF inhibition with targeted therapy has demonstrated improved overall survival in patients with *BRAF* mutant melanoma (1–3).

The use of the selective BRAF inhibitors vemurafenib and dabrafenib are associated with a wide range of known and predictable cutaneous side effects. These include photosensitivity, maculopapular eruption, pruritus, folliculitis, burning feet, hair thinning, curly hair, nail changes, and squamoproliferative lesions ranging from keratosis pilaris, acanthopapilloma, plantar hyperkeratosis, verruca, actinic keratosis, keratoacanthoma and squamous cell carcinoma (4–6). Preclinical evidence indicates that BRAF inhibitors may give rise to non-melanoma tumors by promoting paradoxical MAPK activation in the tissues that lack mutant *BRAF* (7). The BRAF inhibitor toxicity profile also includes the de novo appearance of dysplastic nevi and primary melanomas, as well as changes in the existing melanocytic nevi (8–10). While the spectrum of morphologic and histopathologic features of BRAF inhibitor-associated melanocytic lesions has been relatively well-described, the genomic mutational landscape and molecular mechanisms driving the development of these lesions remains less clear. Herein, we report atypical melanocytic lesions arising in patients treated with the BRAF inhibitor vemurafenib, and discovery of associated mutations in *NRAS* and *BRAF* genes.

MATERIAL AND METHODS

The Institutional Review Board at the Perelman School of Medicine at the University of Pennsylvania approved the study (IRB# 703001). We performed a retrospective review of the medical records of all patients who received BRAF inhibitors between 2011 and 2013. Patient demographics, original indication for BRAF inhibitor therapy, dates of initiation of therapy and de novo clinical appearance of atypical melanocytic lesions, the location of the lesions, and biopsy diagnosis were recorded. We focused on patients who developed new lesions or changing lesions after the initiation of treatment with vemurafenib. In all, we identified three severely dysplastic nevi, one atypical intraepidermal melanocytic proliferation, and four melanoma *in situ* lesions in four patients undergoing treatment with

vemurafenib. Pre-treatment tumor samples and on-treatment melanocytic lesions were obtained from patients who provided written consent to use their tumor samples.

Genomic DNA isolation was performed as in Wilson et al (11). In summary, DNA was isolated from formalin-fixed paraffin embedded tumors by scraping from slides and extracted using sodium thiocyanate, digested with proteinase K, and precipitated with ammonium acetate. After resuspension in TE, DNA concentrations were determined using picoGreen (Quant-iT™ PicoGreen® dsDNA Reagent, Invitrogen). For the lesion containing MIS and SCC, tissue was macrodissected to isolate primarily MIS tissue for analysis. Three of the eight on-treatment melanocytic lesions did not have enough DNA for genotyping. A custom Sequenom panel designed to detect 74 mutations in 13 genes known to play a role in the pathogenesis of melanoma was applied to both melanoma and newly arising atypical melanocytic lesions for each patient. These genes included *AKT1*, *AKT3*, *BRAF*, *CDK4*, *CTNNB1*, *FBX04*, *GNA11*, *GNAQ*, *KIT*, *MEK1*, *MEK2*, *MET*, and *NRAS*.

RESULTS

We identified patients who developed new or changing skin lesions after the initiation of treatment with vemurafenib for previously diagnosed metastatic melanoma. The new or changing skin lesions were in some cases noted by the patients, and in others by their dermatologists during clinical exams. We observed the development of three severely dysplastic nevi, one atypical intraepidermal melanocytic proliferation, and four melanoma *in situ* lesions in four patients undergoing treatment with vemurafenib (Table 1, and Figures 1 and 2). Mutational analysis in both pre-treatment tumor samples and on-treatment melanocytic lesions was feasible in five samples from three of the four patients (Table 1). One patient with *BRAF*V600E metastatic melanoma developed three new melanoma *in situ* lesions while on vemurafenib, diagnosed at 10, 23, and 24 weeks after starting vemurafenib. Of these, two of the on-treatment melanoma *in situ* lesions yielded sufficient DNA for Sequenom analysis. Both were found to be *BRAF*wild-type, with a *NRAS*Q61R mutation detected in one of the two lesions. Another patient's pre-treatment metastatic melanoma demonstrated synchronous *BRAF*kinase domain mutations V600K and G466A, and *CTNNB1*D32E mutation, whereas a severely dysplastic nevus which developed after 25 weeks on vemurafenib in this patient showed a *BRAF*G466A mutation (Table 2).

DISCUSSION

The de novo appearance of atypical nevi and primary melanomas, as well as changes in the existing melanocytic nevi, is a recently observed adverse effect associated with the use of BRAF inhibitors (8–10). Our study confirms this observation. Several studies have noted that these lesions invariably lack *BRAF*V600E mutation (12–13). Although limited in number of samples, our study confirms this finding, which is to be expected mechanistically given the negative selective pressure that a BRAF inhibitor would be expected to have on growth of melanocytic lesions. *BRAF*G466A is a rare somatic mutation identified in a few large-scale genome sequencing projects, particularly in a cultured melanoma cell line and colonic adenocarcinoma (14–16). Dalle et al. have identified *NRAS*Q61R mutations in one of twenty-one second primary melanomas newly arising in patients with vemurafenib-

treated metastatic melanomas (12). We support this finding by observing the occurrence of this same mutation in one of the lesions in our study. *NRAS* Q61 mutations are considered mutually exclusive of *BRAF* mutations and are the second most common melanoma driver event (13–25% of primary melanomas) (17–19).

With respect to the mechanism of this phenomenon, increased levels of cyclin D1 and pAKT expression in *BRAF* inhibitor associated melanocytic lesions compared to control nevi have been described (9, 20). Several groups have reported an increased pERK expression by immunohistochemical analysis of these lesions (12–13, 21), suggesting paradoxical activation of MAPK pathway or induction of alternate signaling pathways in the presence of *BRAF* pathway blockade. Goppner et al. have additionally demonstrated increased expression of small GTPase Rac1 in its active form as Rac1-GTP in *BRAF* inhibitor associated melanocytic lesions by immunohistochemistry (13). Rac1 has been implicated in melanoma pathogenesis and is shown to be constitutionally activated in dysplastic nevi and melanomas (22–23). Interestingly, activated mutant *NRAS* Q61K has been shown to drive aberrant melanocyte signaling, survival, and invasiveness via a Rac1-dependent mechanism (24). Although limited in scale, these data suggest that in the presence of *BRAF* blockade, alternative pathways are activated, which include otherwise known players in melanoma pathogenesis such as *NRAS*, *RAC1*, *ERK*, Cyclin D1 and *PI3K-AKT* pathway.

In summary, we identified an *NRAS* Q61R mutation and a rare *BRAF* G466A kinase domain mutation in atypical melanocytic lesions that arose in patients treated with vemurafenib. Further studies are warranted to demonstrate a causal relationship between *BRAF* inhibition and the development or accelerated growth of atypical melanocytic lesions.

ACKNOWLEDGEMENTS

This research was funded in part by an NCI Cancer Center Research Training Program Grant T32 CA009615 (PI: Dr. John Maris) (MAW).

REFERENCES

1. Chapman PB, Hauschild A, Robert C, et al. Improved survival with vemurafenib in melanoma with braf v600e mutation. The New England journal of medicine. 2011;364:2507–2516 [PubMed: 21639808]
2. Sosman JA, Kim KB, Schuchter L, et al. Survival in braf v600-mutant advanced melanoma treated with vemurafenib. The New England journal of medicine. 2012;366:707–714 [PubMed: 22356324]
3. Hauschild A, Grob JJ, Demidov LV, et al. Dabrafenib in braf-mutated metastatic melanoma: A multicentre, open-label, phase 3 randomised controlled trial. Lancet. 2012;380:358–365 [PubMed: 22735384]
4. Chu EY, Wanat KA, Miller CJ, et al. Diverse cutaneous side effects associated with braf inhibitor therapy: A clinicopathologic study. Journal of the American Academy of Dermatology. 2012;67:1265–1272 [PubMed: 22609219]
5. Rinderknecht JD, Goldinger SM, Rozati S, et al. Rasopathic skin eruptions during vemurafenib therapy. PloS one. 2013;8:e58721 [PubMed: 23516541]
6. Curry JL, Tetzlaff MT, Nicholson K, et al. Histological features associated with vemurafenib-induced skin toxicities: Examination of 141 cutaneous lesions biopsied during therapy. The American Journal of dermatopathology. 2014;36:557–561 [PubMed: 24950418]
7. Hatzivassiliou G, Song K, Yen I, et al. Raf inhibitors prime wild-type raf to activate the mapk pathway and enhance growth. Nature. 2010;464:431–435 [PubMed: 20130576]

8. Dalle S, Poulalhon N, Thomas L. Vemurafenib in melanoma with braf v600e mutation. The New England journal of medicine. 2011;365:1448–1449; author reply 1450
9. Zimmer L, Hillen U, Livingstone E, et al. Atypical melanocytic proliferations and new primary melanomas in patients with advanced melanoma undergoing selective braf inhibition. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2012;30:2375–2383 [PubMed: 22614973]
10. Debarbieux S, Dalle S, Depaepe L, Poulalhon N, Balme B, Thomas L. Second primary melanomas treated with braf blockers: Study by reflectance confocal microscopy. The British journal of dermatology. 2013;168:1230–1235 [PubMed: 23302038]
11. Wilson MA, Zhao F, Letrero R, et al. Correlation of somatic mutations and clinical outcome in melanoma patients treated with carboplatin, paclitaxel, and sorafenib. Clinical cancer research : an official journal of the American Association for Cancer Research. 2014;20:3328–3337
12. Dalle S, Poulalhon N, Debarbieux S, et al. Tracking of second primary melanomas in vemurafenib-treated patients. JAMA dermatology. 2013;149:488–490 [PubMed: 23715012]
13. Goppner D, Muller J, Kruger S, et al. High incidence of naevi-associated braf wild-type melanoma and dysplastic naevi under treatment with the class i braf inhibitor vemurafenib. Acta dermato-venereologica. 2014;94:517–520 [PubMed: 24531394]
14. Davies H, Bignell GR, Cox C, et al. Mutations of the braf gene in human cancer. Nature. 2002;417:949–954 [PubMed: 12068308]
15. Giannakis M, Hodi E, Jasmine Mu X, et al. Rnf43 is frequently mutated in colorectal and endometrial cancers. Nature genetics. 2014;46:1264–1266 [PubMed: 25344691]
16. Sanz-Pamplona R, Lopez-Doriga A, Pare-Brunet L, et al. Exome sequencing reveals amer1 as a frequently mutated gene in colorectal cancer. Clinical cancer research : an official journal of the American Association for Cancer Research. 2015;21:4709–4718 [PubMed: 26071483]
17. Curtin JA, Fridlyand J, Kageshita T, et al. Distinct sets of genetic alterations in melanoma. The New England journal of medicine. 2005;353:2135–2147 [PubMed: 16291983]
18. Forbes SA, Bindal N, Bamford S, et al. Cosmic: Mining complete cancer genomes in the catalogue of somatic mutations in cancer. Nucleic acids research. 2011;39:D945–950 [PubMed: 20952405]
19. Xia J, Jia P, Hutchinson KE, et al. A meta-analysis of somatic mutations from next generation sequencing of 241 melanomas: A road map for the study of genes with potential clinical relevance. Molecular cancer therapeutics. 2014;13:1918–1928 [PubMed: 24755198]
20. Mudaliar K, Tetzlaff MT, Duvic M, et al. Braf inhibitor therapy-associated melanocytic lesions lack the braf v600e mutation and show increased levels of cyclin d1 expression. Human pathology. 2016;50:79–89 [PubMed: 26997441]
21. Mochel MC, Hammond MR, Frederick DT, et al. Melanocytic nevi excised during b-raf proto-oncogene (braf) inhibitor therapy: A study of 19 lesions from 10 patients. Journal of the American Academy of Dermatology. 2015;73:491–499 e492 [PubMed: 26190239]
22. Machesky LM, Sansom OJ. Rac1 in the driver's seat for melanoma. Pigment cell & melanoma research. 2012;25:762–764 [PubMed: 22882909]
23. Hodi E, Watson IR, Kryukov GV, et al. A landscape of driver mutations in melanoma. Cell. 2012;150:251–263 [PubMed: 22817889]
24. Li A, Ma Y, Jin M, et al. Activated mutant nras(q61k) drives aberrant melanocyte signaling, survival, and invasiveness via a rac1-dependent mechanism. The Journal of investigative dermatology. 2012;132:2610–2621 [PubMed: 22718121]

Figure 1:

Newly developed melanoma in situ arising within a pre-existing nevus in Patient 2, after 23 weeks of treatment for metastatic melanoma on vemurafenib. Confluent growth of single and nested melanocytes is observed along the dermal-epidermal junction. Extensive pagetoid spread is seen within this lesion. A. Hematoxylin and eosin stain, original magnification x55. B. MART-1 immunohistochemical stain, original magnification x55. C. Hematoxylin and eosin stain, original magnification x100. D. MART-1 immunohistochemical stain, original magnification x100.

Figure 2:

Newly developed compound dysplastic nevus with severe atypia in Patient 4, after 8 weeks of treatment for metastatic melanoma on vemurafenib. There is crowded growth of nested and single melanocytes along the dermal-epidermal junction with several mid-level pagetoid melanocytes observed. A. Hematoxylin and eosin stain, original magnification x100. B. MART-1 immunohistochemical stain, original magnification x100. C. Hematoxylin and eosin stain, original magnification x200. D. MART-1 immunohistochemical stain, original magnification x200.

Table 1:

Clinicopathologic characteristics of pre-treatment and on-treatment tumor samples.

Patient	Lesion Description	Sex	Age, yrs	Location
#1	Metastatic melanoma	F	81	Top of head
	Dysplastic nevus, 25 weeks on vemurafenib		83	Left dorsal foot
	Atypical intraepidermal melanocytic proliferation, 52 weeks on vemurafenib (No mutational analysis)		84	Right cheek
#2	Primary melanoma, 0.57mm	M	65	Left upper back
	Primary melanoma, 0.26mm		67	Left shoulder
	Primary melanoma, 1.06mm		69	Right upper back
	Primary melanoma, 0.5mm		71	Forehead
	Primary melanoma, 0.5mm		72	Left thigh
	Primary melanoma, 0.47mm		74	Right back
	Metastatic melanoma		75	Lung
	MIS, 10 weeks on vemurafenib, (MIS + SCC)		76	Left forearm
	MIS, 23 weeks on vemurafenib		76	Left back
	MIS, 24 weeks on vemurafenib (No mutational analysis)		76	Lower back
	Compound dysplastic nevus with severe atypia, 37 weeks on vemurafenib		77	Right chest
#3	Metastatic melanoma	M	52	Right lateral ankle
	MIS, 8 weeks on vemurafenib (No mutational analysis)		52	Chest
#4	Metastatic melanoma	F	71	Liver
	Compound dysplastic nevus with severe atypia, 8 weeks on vemurafenib		71	Left shoulder
			Median	Range
Age at diagnosis, years (newly developed melanocytic lesions)			73	52–84
Weeks on vemurafenib (BRAF inhibitor)			23.5	8–52

■, pre-treatment; ■, on-treatment; MIS, Melanoma in situ; SCC, Squamous cell carcinoma

Table 2:

Somatic mutations in pre-treatment and on-treatment tumor samples.

Patient	Lesion Description	Mutations					
		BRAF G466	BRAF V600	CDK4 R24	CTNNB1 D32	KIT N822	NRASQ61
#1	Metastatic melanoma	p.G466A	p.V600K	WT	p.D32E	WT	WT
	Dysplastic nevus, 25 weeks on vemurafenib	p.G466A	WT	WT	WT	WT	WT
#2	Primary melanoma, 0.57mm	WT	p.V600R	WT	WT	WT	WT
	Primary melanoma, 0.26mm	WT	WT	WT	WT	WT	WT
	Primary melanoma, 1.06mm	WT	p.V600E	WT	WT	WT	WT
	Primary melanoma, 0.5mm	WT	WT	WT	WT	p.N822I	WT
	Primary melanoma, 0.5mm	WT	p.V600E	WT	WT	WT	WT
	Primary melanoma, 0.47mm	WT	WT	WT	WT	WT	WT
	Metastatic melanoma	WT	p.V600E	p.R24C	WT	WT	WT
	MIS, 10 weeks on vemurafenib, (MIS + SCC)	WT	WT	WT	WT	WT	WT
	MIS, 23 weeks on vemurafenib	WT	WT	WT	WT	WT	p.Q61R
	Compound dysplastic nevus with severe atypia, on vemurafenib	WT	WT	WT	WT	WT	WT
#3	Metastatic melanoma	WT	p.V600E	WT	WT	WT	WT
#4	Metastatic melanoma	p.V600K	WT	WT	WT	WT	WT
	Compound dysplastic nevus with severe atypia, on vemurafenib	WT	WT	WT	WT	WT	WT

■,pre-treatment; ■,on-treatment; MIS, Melanoma in situ; SCC,Squamous cell carcinoma