Case Report/Case Series

Identification of a Novel Complex *BRAF* Mutation Associated With Major Clinical Response to Vemurafenib in a Patient With Metastatic Melanoma

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IMPORTANCE There is an increasing interest in *BRAF* V600 mutations in melanomas and their associated sensitivity to vemurafenib, a BRAF inhibitor. However, physicians cannot find information in the literature about vemurafenib response for rare and/or atypical *BRAF* mutations.

OBSERVATIONS We describe the identification of a novel complex *BRAF* mutation associated with major clinical response to vemurafenib in a patient with metastatic melanoma. Using a pyrosequencing method, we determined that the tumor positive for mutated *BRAF*, uncovering a novel c.1799_1803delinsAT; p.V600-K601>D variant. We uncovered this atypical *BRAF* mutation with 2 different sequencing methods, both in the primary lesion and in 1 metastasis. The patient was immediately treated with vemurafenib as monotherapy and achieved a prolonged (5.5-month) positive response.

CONCLUSIONS AND RELEVANCE We analyzed the consequences of the *BRAF* V600-K601>D mutation in terms of amino acids. We referred to the published data and databases to screen chemical properties of well-known *BRAF* V600 mutations and other complex *BRAF* mutations to find common features of activated *BRAF* mutations. Importantly, we highlighted that both the site of the mutation and the involved amino acids are important to predict vemurafenib response. Our conclusion is that complex *BRAF* mutation surrounding codon 600 could also be sensitive to BRAF inhibitors.

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The median survival time for patients with unresectable melanoma remains dramatically short, only 6 to 9 months from the time of diagnosis to death, with only 10% to 15% of patients living 3 years. Approximately 40% to 60% of cutaneous melanomas carry mutations in the *BRAF* gene, which is the most frequently mutated protein kinase in human cancers.[1] These activating mutations induce a constitutive *BRAF*-mediated signaling, leading to subsequent activation of the downstream mitogen-activated protein kinase (MAPK) pathway. These findings have led to the clinical development of specific and potent *BRAF*-mutated inhibitors.

Approximately 90% of *BRAF* mutations in melanoma cells occur at exon 15, codon 600, and result in the substitution of glutamic acid for valine (*BRAF* V600E), although other activating mutations are described at the same codon (eg, *BRAF* V600K, V600R, and V600D). The COSMIC database (Catalogue of Somatic Mutations in Cancer)[2] also describes other complex *BRAF* mutations situated between codons 587 and 602.

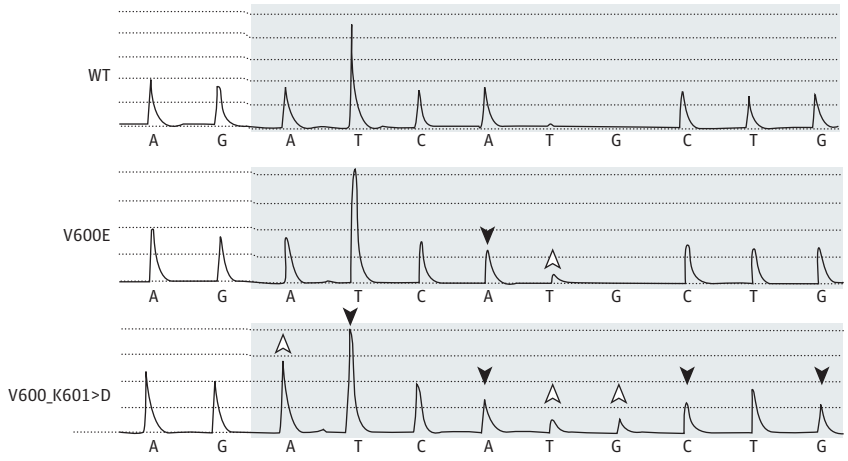
Vemurafenib (PLX4032) acts as an adenosine triphosphate-competitive inhibitor and has a marked antitumor effect against *BRAF* V600 mutated cancer cells but not against cells with wild-type *BRAF*.[3] Nevertheless, response to vemurafenib is frequently unknown for rare or complex *BRAF* mutations.

We report the case of a patient with a metastatic melanoma harboring a novel complex *BRAF* mutation who experienced a positive response 5.5 months' duration under vemurafenib therapy.

Report of a Case

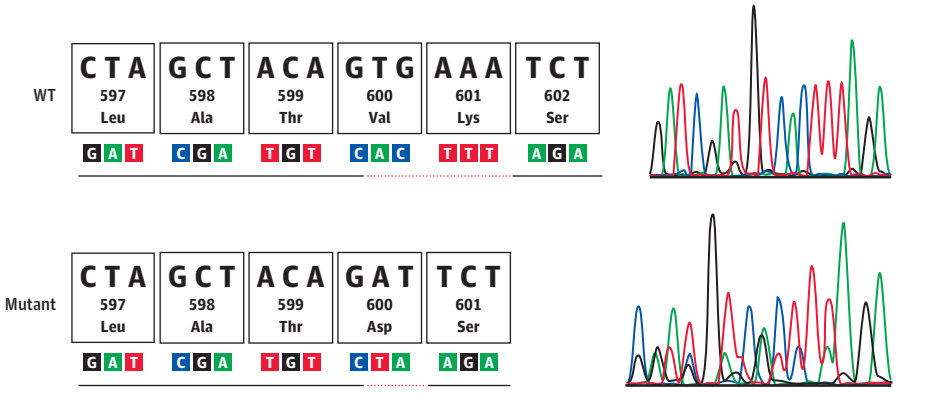
A man in his late 50s presented to our dermatology clinic for the resection of a cutaneous pigmented lesion on the left lumbar region. A local surgical resection was performed, and the subsequent histopathologic examination of the excised melanocytic lesion displayed features of ulcerated malignant melanoma, Clark level III, with Breslow thickness of 3.9 mm and a

Figure 1. *BRAF* Pyrograms After Reverse-Strand Pyrosequencing



The pyrograms correspond to the reference *BRAF* wild type (WT), *BRAF* V600E mutation, and the novel *BRAF* V600-K601>D mutation. Black arrowheads and open arrowheads indicate lower base incorporation and new/higher base incorporation, respectively, compared with the expected ratio.

Figure 2. *BRAF* Electropherograms After Sanger Sequencing



Both the wild type (WT) and the novel V600-K601>D mutant DNA were sequenced on the reverse strand. The corresponding amino acids, number of codons, and forward sequences are indicated in the boxes. Ala indicates, alanine; Asp, aspartate; Leu, leucine; Lys, lysine; Ser, serine; Thr, threonine.

mitotic rate of 1/mm^2. After sentinel lymph node dissection, the patient was staged as AJCC IIIc[4] and discharged home for quarterly clinical follow-up and bi-annual body computed tomography (CT) imaging evaluation, according to French standards of care.

Months later, the patient presented for his follow-up consultation and complained of axillary and inguinal lymphadenopathies, confirmed by physical examination, which revealed 6 subcutaneous nodules. A CT scan of the chest, abdomen, and pelvis was performed and confirmed the presence of multiple ipsilateral subcutaneous nodules (2 in the pectoral region, 5 in the axillary region, and 2 in the inguinal region). The CT scan of the brain showed no abnormalities. One pectoral nodule was excised for pathologic analysis, which confirmed melanoma metastasis.

Tumor DNA was screened for the oncogenic mutations in melanoma (ie, *BRAF*, *NRAS*, and *KIT*). Using a pyrosequencing method developed and clinically used for *KRAS* and *EGFR* testing,[5,6] we determined that the specimen was wild type for *NRAS* and wild type for *KIT*. However, the pyrogram was positive for mutated *BRAF*, uncovering a novel c.1799_1803delinsAT;p.V600-K601>D variant (**Figure 1**) which denotes the replacement of 5 consecutive nucleotides 1799 to1803 (TGAAA), by 2 other nucleotides (AT).

This mutation leads to the replacement of both the valine (V600) and the lysine (K601) by an aspartate (D600) in the mutated BRAF protein. Moreover, a retrospective pyrosequencing of the primary lesion revealed the same mutation, which was confirmed by classical Sanger sequencing of *BRAF* exon 15 (**Figure 2**). The experimental details for *BRAF* sequencing are listed in the **Table**.

To our knowledge, this complex *BRAF* V600-K601>D mutation has never been previously reported and was not described in the COSMIC database of the Wellcome Trust Sanger Institute.[7] Accordingly, we could not find any published information regarding whether such a *BRAF*-mutated melanoma should be treated with vemurafenib.

After multidisciplinary consultation, the patient began treatment with vemurafenib, 960 mg orally, twice per day. One month after the initiation of vemurafenib treatment,

the patient maintained a good performance status but complained of nausea, photosensitivity, and insomnia. Physical examination revealed multiple cutaneous lesions, and pathologic analysis identified 5 keratoacanthomas and 1 squamous cell carcinoma (SCC), which are frequently reported as adverse effects of anti-*BRAF* therapies.[8] Palpation showed a noticeable decrease in the size of the known nodules, already suggesting tumor response. To minimize nausea, we decreased the vemurafenib dose to 720 mg orally, twice per day.

After 3 monthly cycles of vemurafenib treatment, the patient's Eastern Cooperative Oncology Group (ECOG) performance status was 0, and CT scan documented the decrease in the overall sizes of both axillary (**Figure 3**A) and pectoral subcutaneous nodules (Figure 3B) from before vemurafenib treatment to after the third treatment cycle was completed.

Table. Primer Sequences and Nucleotides Dispensation Order Used for the *BRAF* Sequencing

Sequencing Type	Sequence	Process
Pyrosequencing	5′-CATAATGCTTGCTCTGATAGG-3′ 5′-TCTAGTAACTCAGCAGCATCTCAG-3′	First PCR
	5′-Biotin-AAAAATAGGTGATTTTGGTCTAGC-3′ 5′-GGCCAAAAATTTAATCAGTGGA-3′	Second PCR
	5′-GGACCCACTCCATCG-3′	Sequencing
	C,A,G,A,T,C,A,T,G,C,T,G	Nucleotide dispensation order
Sanger Sequencing	5′-GGCCAAAAATTTAATCAGTGGA-3′	Sequencing

Abbreviation: PCR, polymerase chain reaction.

Three months later, the patient presented with complaints of headaches, dizziness, and asthenia. However, neurologic examination findings were normal. The body CT imaging confirmed response to vemurafenib, but the CT scan of the brain demonstrated a solitary bleeding nodule in the right parietal region suggestive of a new metastasis. Unfortunately, the patient's neurologic function rapidly worsened leading to coma and subsequent death.

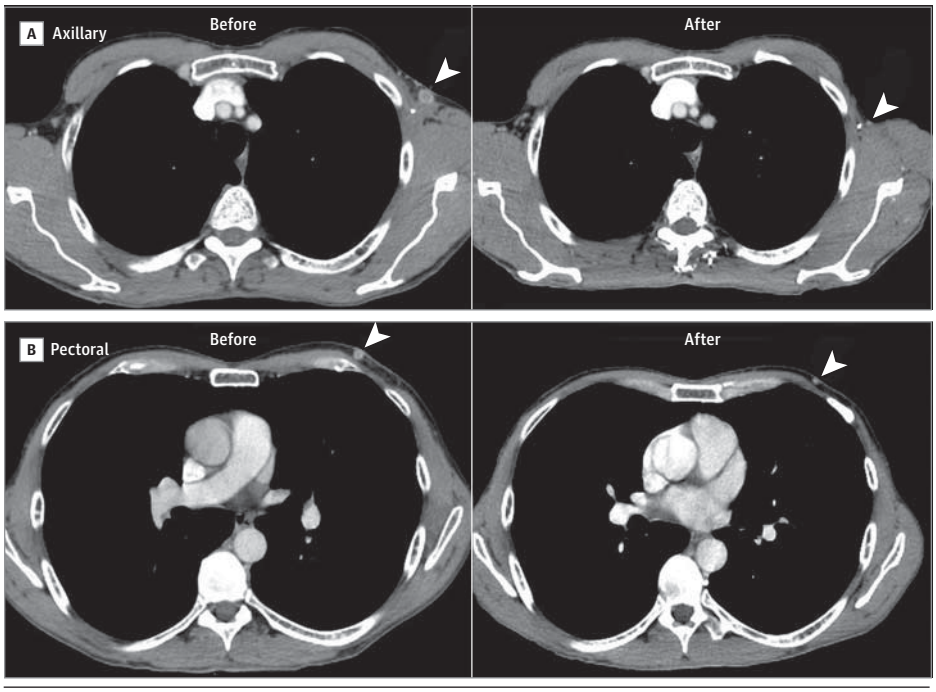
Discussion

In melanoma research, the finding that *BRAF* V600E mutation was strongly associated with antitumor effect of vemurafenib[1] prompted European and US agencies to approve vemurafenib after accelerated review. Therefore, *BRAF* testing is now mandatory, and vemurafenib is indicated as monotherapy for the treatment of adult patients with *BRAF* V600 mutation-positive unresectable or metastatic melanoma.

The database of patients with *BRAF* mutations surrounding codon V600 contains hundreds of point mutation cases,[2] eg, V600E, V600K, and V600R substitutions, associated with good response to vemurafenib.[3,9] However, almost no available data about vemurafenib response for tumors with complex *BRAF* mutations can be found in the literature, probably because of their low incidence rates.

BRAF V600E mutations respond to vemurafenib and are likely to result in 500-fold increased *BRAF* activity.[10] A similar but complex *BRAF* mutation pV600-K601>E identified in melanoma[11] and papillary thyroid carcinoma[12] was also found to induce an increased kinase activity.[13] Effectively, the negatively charged glutamate (E) residue mimics the structure of

Figure 3. Computed Tomographic (CT) Scans of the Patient Showing the Positive Response After 3 Months of Vemurafenib Treatment



Axillary (A) and pectoral (B) lesions (arrowheads) seen on CT scans provide evidence for tumor shrinkage after 3 months of vemurafenib treatment.

the phosphorylated loop of activated wild-type *BRAF*.[14] Similarly in V600D mutations, the replacement of valine by another negatively charged residue such as aspartate (D) is also associated with a 700-fold increase in *BRAF* activity,[10] and bioinformatic modeling of another *BRAF* complex in-frame mutation involving aspartate D600 confirmed that aspartate (D) replacement also leads to high kinase activity.[15]

Thus, it is easily conceivable that the novel in-frame complex *BRAF* V600-K601>D mutation observed in our patient's tumor has the same effect, since it leads to the replacement of the valine and lysine at positions 600 and 601 by a single aspartate (D600) in the mutated BRAF protein. Altogether, these data suggest that this new mutation is likely to induce a constitutively activated BRAF protein and should respond to *BRAF* inhibitors in the same way as the classic V600E mutation.

This hypothesis is in accordance with the good response of our patient to vemurafenib. This treatment rapidly and successfully induced an objective clinical response. The patient had a progression-free survival (PFS) of 5.5 months after beginning vemurafenib monotherapy, which precisely corresponds to the median PFS of 5.3 months shown in the phase III registration study of vemurafenib[1] for patients with metastatic melanoma.

In summary, given the fact that our patient achieved a prolonged response, vemurafenib treatment could also be evaluated for tumors with complex *BRAF* mutations surrounding exon 15, codon 600. Analysis of the location as well as the physical and chemical properties of the amino acids generated by the *BRAF* mutation might improve the predictability of vemurafenib sensitivity.

ARTICLE INFORMATION

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Study concept and design: Busser, Charles.
Acquisition of data: Busser, Gras-Combe, Bricault, Templier, Claeys, de Fraipont, Charles.
Analysis and interpretation of data: Busser, Leccia, Richard, de Fraipont, Charles.
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