AMP case report: An unusual BRAF mutation in a patient with melanoma, February 2017

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CAP TODAY and the Association for Molecular Pathology have teamed up to bring molecular case reports to CAP TODAY readers. AMP members write the reports using clinical cases from their own practices that show molecular testing's important role in diagnosis, prognosis, and treatment. The following report comes from Brigham and Women's Hospital. If you would like to submit a case report, please send an email to the AMP at amp@amp.org. For more information about the AMP and all previously published case reports, visit amp@amp.org.

February 2017—An activating BRAF mutation is found in 40 to 60 percent of melanoma patients.[1] *BRAF* (B-Raf proto-oncogene) encodes a protein-kinase that activates the MAP kinase/ERK signaling pathway, a pathway that regulates cell differentiation, growth, and survival. Another protein, NRAS, normally activates *BRAF*. A mutated BRAF, however, can act independently of NRAS and skew cell activity toward growth and survival and away from differentiation.[2]

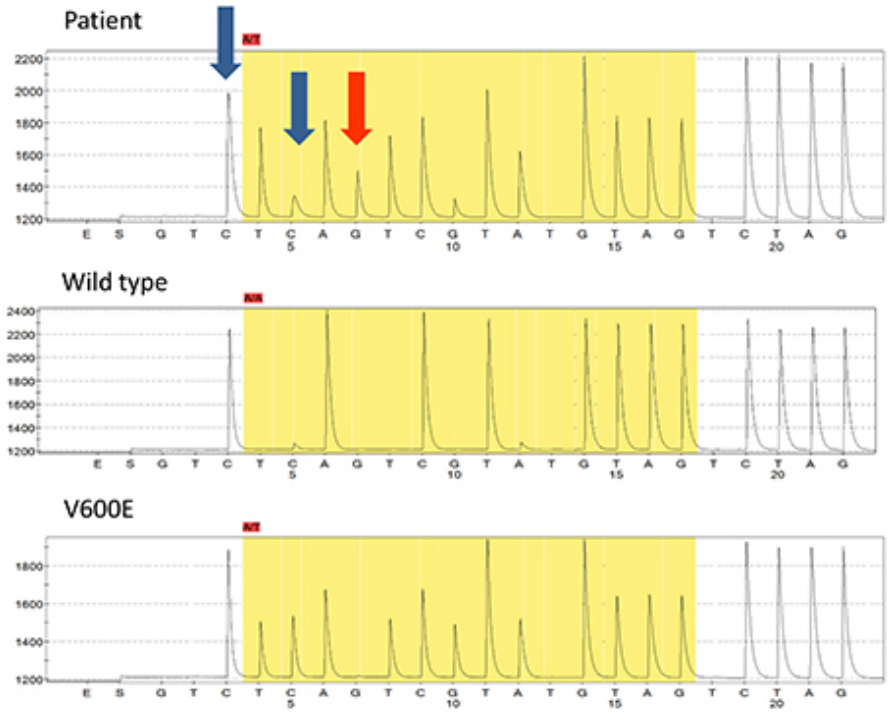


Fig. 1. The pyrosequencing peak pattern for the patient sample does not match the wild type or the V600E controls. The sizes of the first two C peaks are unusual (blue arrows), and the first G peak is not consistent with V600E (red arrow).

In melanoma patients, approximately 90 percent of the activating *BRAF* mutations are V600E (a change from valine to glutamic acid at amino acid 600 in exon 15).[1] A number of BRAF inhibitors have been developed that specifically target the V600E mutation. Generally, patients with metastatic melanoma are tested for *BRAF* mutations to determine if they might be candidates for BRAF inhibitor therapy.

Case presentation. A 57-year-old Caucasian woman with a history of metastatic melanoma (with metastases to the liver and brain) presented with five weeks of pleural congestion and one to two weeks of worsening shortness of breath. She reported decreased energy and appetite, but denied fever, chills, hemoptysis, night sweats, and weight loss.

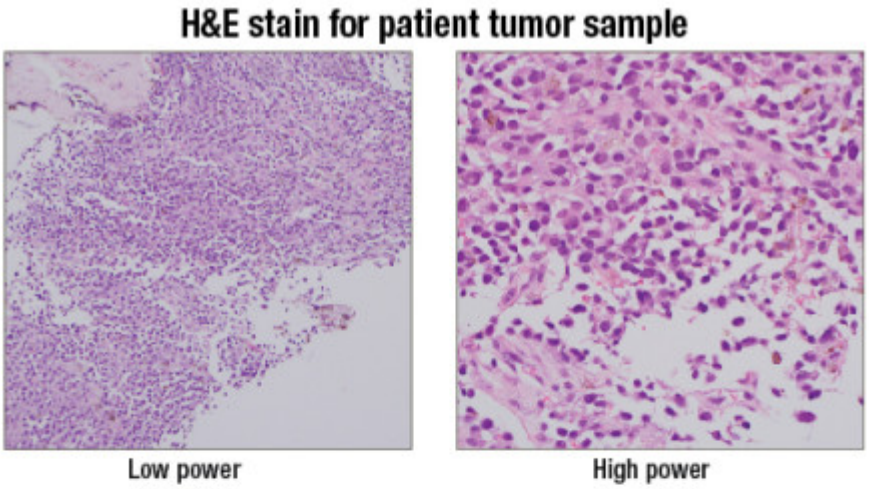
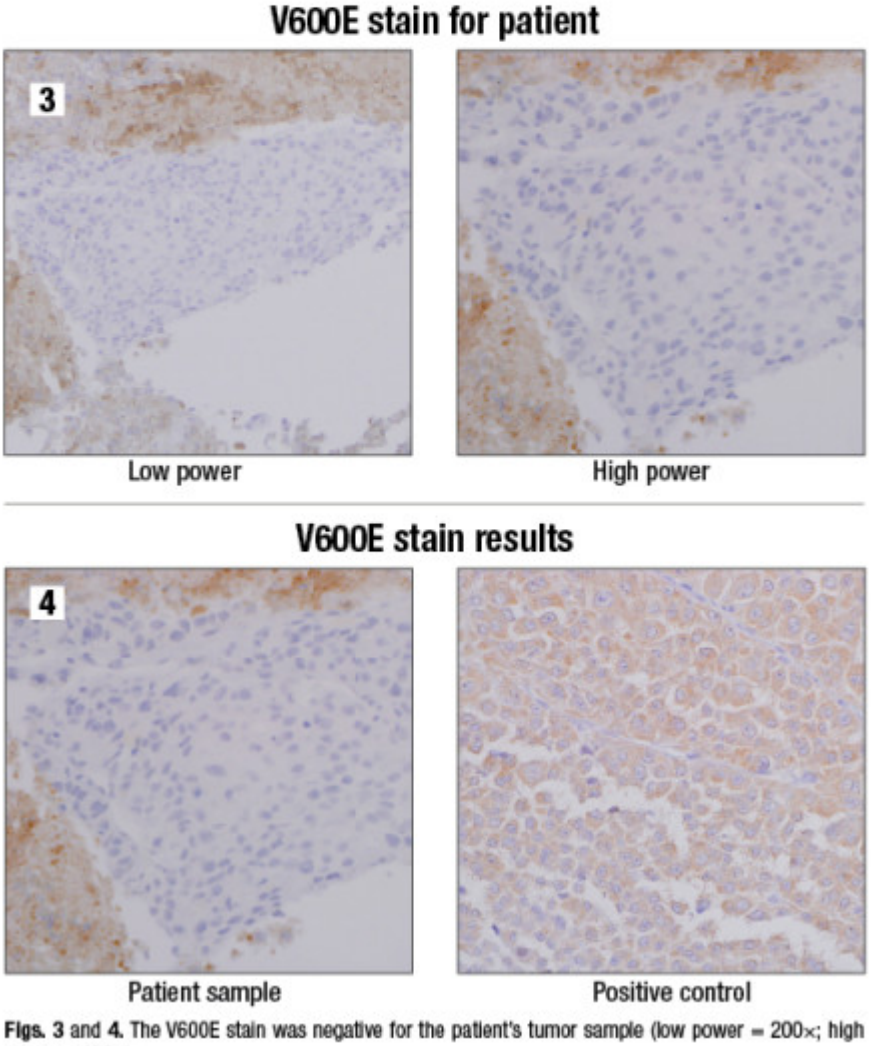


Fig. 2. H&E stain on the patient's tumor sample showed approximately 90 percent cellularity (low power = 100×; high power = 400×).



Figs. 3 and **4.** The V600E stain was negative for the patient's tumor sample (low power = 200×; high power = 400×).

A computed tomography scan of the chest showed a right-sided pleural effusion and a right-sided collapsed lung. A thoracentesis was performed, and tissue was sent to pathology for *BRAF* mutation testing.

A pyrosequencing *BRAF* test was performed on formalin-fixed, paraffin-embedded tumor tissue. A pattern consistent with V600E was present, but this could not explain all of the peaks (**Fig. 1**). It appeared that more than one alteration may have been present. A satisfactory interpretation consisting of one or two alterations was not identified, even with the aid of a computational peak prediction program. Therefore, the test was interpreted as inconclusive after it was repeated with the same result.

Given that the V600E alteration may have been present, a V600E immunohistochemical stain was performed. This new stain is a mutation specific monoclonal mouse antibody raised against a synthetic peptide representing the V600E sequence from amino acids 596–606.[3] In this case, the V600E stain was negative (**Figs. 2–4**). Next-generation sequencing was then performed. (The NGS method used employs hybrid capture with an Agilent SureSelect custom probe set and massively parallel sequencing on an Illumina HiSeq 2500.[4]) It showed that two alterations were present: V600R and S602T (**Fig. 5**). The allele frequency was 44 percent. For all of the reads, both alterations were either present or absent; no reads had only one of the alterations.

In light of the *BRAF* test results, the patient was enrolled in a translational study of ipilimumab and nivolumab. Progression of disease was noted, however, and so the patient was then switched to dabrafenib and trametinib. To date, she has responded well to this regimen.

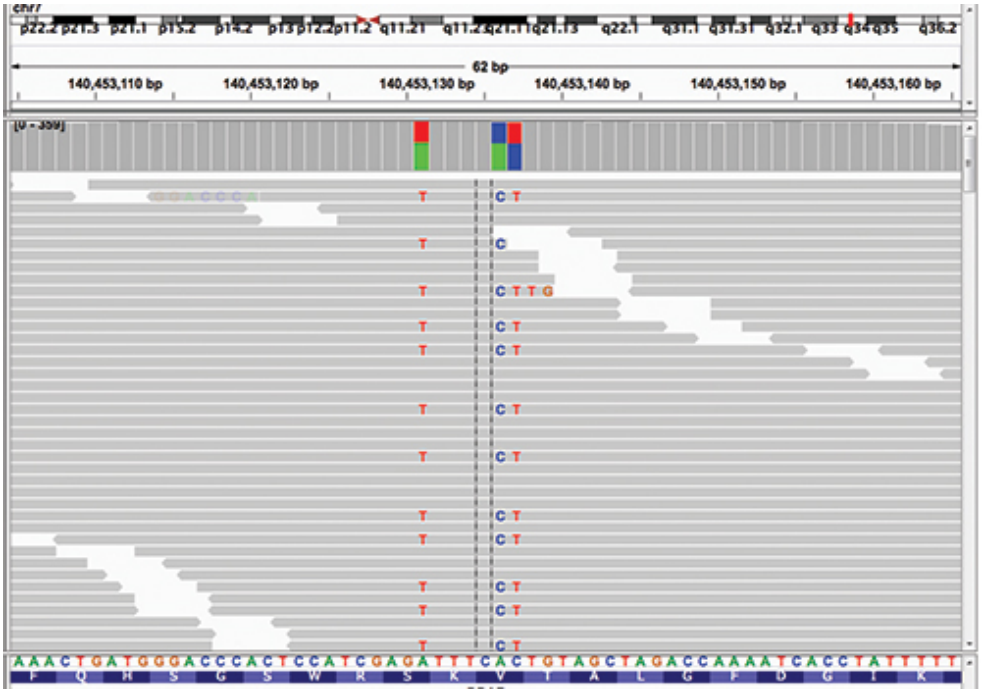


Fig. 5. The next-generation sequencing results for the patient showed two alterations: CAC to TCC at position 600 (for which the wild type is valine [V]) and AGA to TGA at position 602 (for which the wild type is serine [S]). Note: the amino acid numbering is from right to left in the figure.

Discussion. Pyrosequencing is the preferred method for BRAF testing in many institutions because of its rapid turnaround time (approximately one day) and because it is highly sensitive and specific (the sensitivity is greater than 99 percent and the specificity is greater than 90 percent for allele frequencies greater than five percent).

In this case, the pyrosequencing was inconclusive because it was not possible to interpret the peaks in a satisfactory way. It would have been a mistake to report a V600E alteration even though the peaks appeared to be consistent with a V600E along with a second alteration. Additional studies were warranted.

After the next-generation sequencing identified two alterations, one possible explanation for the peak pattern is that the S602T alteration occurred under the pyrosequencing primer, and thus may have caused a delayed start to

primer elongation because of a mismatch in the primer binding site. If this hypothesis is correct, the pattern of the peaks is plausible (**Fig. 6**).

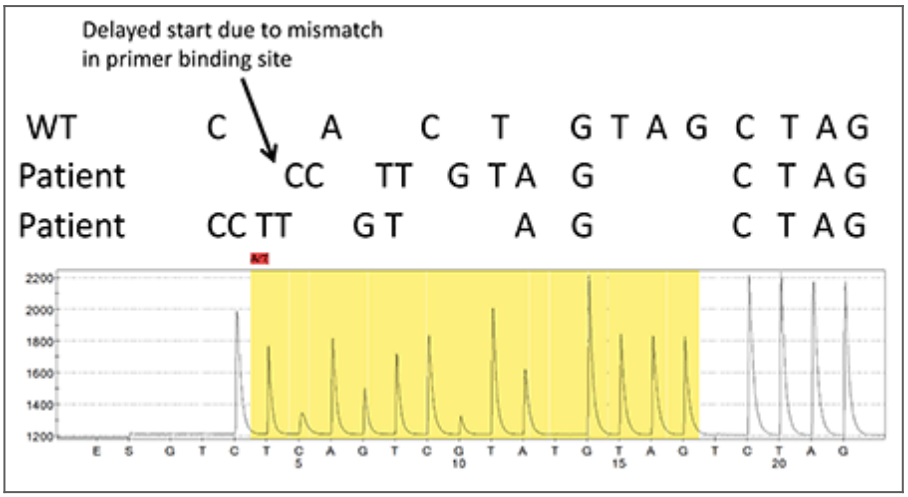


Fig. 6. The S602T alteration was under the pyrosequencing primer sequence. This may have caused the primer to bind inefficiently. A delayed start to elongation may account for the second C peak (position 5 in the strip). Thus, the first C may or may not have been incorporated. If it was not incorporated, then the second C might have been incorporated and this would explain the small second C peak and the G peak in position 7.

To our knowledge, there is only one case in the literature of a patient with V600R and S602T. It occurred in a patient with V600E-negative hairy cell leukemia.[5]

1. Flaherty KT, Puzanov I, Kim KB, et al. Inhibition of mutated, activated BRAF in metastatic melanoma. *N Engl J Med.* 2010;363(9):809–819.
2. Huang PH, Marais R. Cancer: melanoma troops massed. *Nature.* 2009;459(7245):336–337.
3. Capper D, Preusser M, Habel A, et al. Assessment of BRAF V600E mutation status by immunohistochemistry with a mutation-specific monoclonal antibody. *Acta Neuropathol.* 2011;122(1):11–19.
4. Howitt BE, Sholl LM, Dal Cin P, et al. Targeted genomic analysis of Müllerian adenosarcoma. *J Pathol.* 2015;235(1):37–49.
5. Tschernitz S, Flossbach L, Bonengel M, et al. Alternative BRAF mutations in BRAF V600E-negative hairy cell leukaemias. *Br J Haematol.* 2014;165(4):529–533.

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Test yourself

Here are three questions taken from the case report.
Answers are online now at www.amp.org/casereports and will be published next month in CAP TODAY.

1. What percentage of melanoma patients have an activating *BRAF* mutation?

a) 20–40 percent

b) 40–60 percent

c) 60–80 percent

d) 80–100 percent

2. At present, why isn't next-generation sequencing the first-line test for *BRAF*?

a) It has a low sensitivity.

b) It has a low specificity.

c) It has a longer turnaround time compared with other tests.

3. Why is it important to test the *BRAF* mutational status in melanoma patients?

a) Some BRAF inhibitors are specific to V600E.

b) *BRAF* mutations can confirm a melanoma diagnosis.

c) It provides useful information about tumor staging.

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Dr. McDonald completed the molecular genetic pathology fellowship at Harvard Medical School. Dr. Kuo is an associate professor of pathology, Brigham and Women's Hospital, Boston.