Melanocytes, like many other human cells, express the BRAF gene. However, mutation of BRAF in melanocytes occurs at high frequency in melanocytic proliferations such as nevi (70%-82%) and melanomas (50%-60%).1,2 Mutation of BRAF results in a defect of the mitogen-activated protein kinase (MAPK) pathway causing oncogenic proliferation and avoidance of apoptosis.3 Most frequently, BRAF mutations occur at the V600E position (74%-90%), and the next most common mutation occurs at V600K (16%-29%);4 together, these sites account for 95% of all BRAF mutations.

Initial breakthrough treatments were made with vemurafenib, a selective inhibitor of BRAF<sup>V600E</sup>-mutated kinase. The inhibition of BRAF<sup>V600E</sup> initially induces tumor growth arrest and partial or complete tumor regression in metastatic melanoma.5 Given the frequency of BRAF<sup>V600E</sup> mutations in benign nevi,2 it is also not surprising that changes have been observed in existing melanocytic nevi and that new nevi appear during BRAF<sup>V600E</sup> inhibitor therapy.6,7 Recently, a study of 42 patients treated with vemurafenib for a mean duration of 6.7 months described a high level of dermoscopic change in pre-existing lesions such as color changes, appearance and disappearance of globules, dermoscopic island pigmentation, and increases in size of nevi.8 New primary melanomas have also been reported during the early stages of vemurafenib treatment, arising from new erupting melanocytic proliferations or rapidly changing existing nevi.9 Zimmer et al,9 Dalle et al,7 and Perierre-Muzet et al8 report that these new primary melanomas arising during vemurafenib therapy are BRAF wild type.

Herein we describe an example of nevus volatility and propose the molecular involvement in a patient undergoing BRAF<sup>V600E</sup> inhibition therapy and who participated in a nevus surveillance study. All patients in the surveillance study provided written consent, and the study followed the Declaration of Helsinki protocols and was approved by the Princess Alexandra Hospital human research ethics committee.

Author Affiliations: Dermatology Research Centre, The University of Queensland, School of Medicine, Translational Research Institute, Brisbane, Queensland, Australia (McClenahan, Lin, Tan, Flewell-Smith, Schaid, Jagirdar, Prow, Sturm, Soyer); The University of Queensland, Institute for Molecular Biosciences, Brisbane, Queensland, Australia (Jagirdar, Sturm); Medical Oncology, Princess Alexandra Hospital, Woolloongabba, Queensland, Australia (Atkinson); The University of Queensland, Translational Research Institute, Brisbane, Queensland, Australia (Lambie); IQ Pathology, Brisbane, Queensland, Australia (Lambie).

Corresponding Author: H. Peter Soyer, MD, FACD, Level 5, Translational Research Institute (TRI), 37 Kent St, Woolloongabba QLD 4102, Australia (h.p.soyer@uq.edu.au).
While participating in a nevus surveillance study, 1 of the patients, a man in his 30s who had been diagnosed 5 years earlier as having a superficial melanoma (Clark level 3, Breslow index 0.64 mm), developed metastases in the pancreas, liver, and mesenteric lymph nodes. Two months later, he was enrolled in a clinical trial of dabrafenib with or without trametinib therapy. Dabrafenib is a BRAF inhibitor similar to vemurafenib, and it was being tested with trametinib, a MEK (MAPK kinase) inhibitor that targets the same MAPK pathway. The trial was blinded and still ongoing at the time of the present report, and so it is unknown whether this patient’s treatment regimen included trametinib.

The patient presented with Fitzpatrick skin type III, dark brown hair, and green eyes. He underwent imaging with a FotoFinder system (FotoFinder Systems GmbH) of all nevi larger than 2 mm on the back and larger than 5 mm on the rest of the body. No significant changes were observed dermoscopically throughout. Ten nevi larger than 5 mm were identified on the body, while 25 nevi larger than 2 mm were identified on the back, for a total of 31 nevi included in our analysis. There were 2 globular, 15 reticular, and 14 nonspecific/homogeneous nevi. Full-body and dermoscopic imaging was conducted 5 times over the next 27 months at roughly 7-month intervals, and no significant dermoscopic changes were identified by assessment of imaged nevi at the 7- or 14-month visits. However, at the 21-month visit, 6 months after he commenced participation in the BRAF inhibitor trial, assessment revealed significant dermoscopic changes 16 nevi (51% of total) (Figure 1). The nevi changes predominantly involved involution and a decrease in pigmentation and size. In addition, in concurrence with other reports, flattening of raised nevi was also observed. By dermoscopic pattern, 4 reticular, 10 homogeneous, and 2 globular nevi showed signs of involution. Therefore, 71% of the unspecific and 26% of the reticular nevi showed signs of involution, while both raised globular nevi decreased in pigmentation and flattened.

By the time of final imaging at 27 months’ surveillance (12 months into the BRAF inhibitor trial), the nevi had generally not further changed, but 5 nevi had continued to involute: 3 reticular and 2 homogeneous nevi. Again, no increase in pigmentation was observed in any lesions, and no new nevi were observed. The patient had an otherwise excellent systemic response to the targeted therapy and an excellent partial response to the point of almost a complete response, with the exception of a small unchanged node near the pancreas that was seen on computed tomographic imaging.

There are a number of external and endogenous factors influencing changes and appearance of nevi over time. These
Figure 2. Histopathologic Images and Molecular Sequencing Charts for \( \text{BRAF} \) \(^{\text{V600E}} \) Status of 1 Involuting Nevus and 1 Noninvoluting Nevus

A-D, Workup of an involuting nevus. E-H, Workup of a noninvoluting nevus. C and G, Dermoscopic images show microbiopsy sites 1 through 5 (scale bar = 1 mm); site 6 in each panel is a control biopsy site adjacent to the nevus. D and H, Molecular analysis charts for microbiopsy sites shown in panels C and G, respectively. A and E, Histopathologic images of the nevi, neither of which shows any histopathological criteria for melanoma (scale bars = 200 μm; boxes enclose areas shown at higher magnification in panels B and F).

The involuted nevus in panel A is a benign, predominantly junctional nevus with few discrete nests of nonpigmented nevus cells at the dermal-epidermal junction; subtle lymphatic infiltration around suprapapillary vascular plexus; and no obvious signs of fibrosis or regression; sequencing (D) reveals that the nevus is heterogeneous for \( \text{BRAF} \) \(^{\text{V600E}} \) mutation at sites 1 and 5.

The noninvoluted nevus in panel E is a benign lentiginous melanocytic nevus with elongated pigmented rete ridges and slightly increased numbers of melanocytes at the dermal-epidermal junction; small junctional nests of melanocytes are also present; and sequencing (H) reveals no presence of \( \text{BRAF} \) \(^{\text{V600E}} \) mutation. B and F, Greater magnifications of the boxed areas of panels A and E, respectively (scale bars = 200 μm).
Diagnosis of shave excisions were performed on 1 involuted nevus and 1 unchanged nevus (Figure 1). The histopathologic diagnosis for the involuted lesion was a predominantly junctional compound nevus without significant inflammation or fibrosis, and the unchanged lesion was characterized as a junctional nevus with a lentiginous melanocytic pattern. Microbiopsy specimens were taken from 6 locations on both the excised nevi (Figure 2). DNA samples extracted from microbiopsy specimens were subjected to polymerase chain reaction amplification using selected forward and reverse primers to flank the BRAF exon 15 and NRAS exon 2 mutation hotspots. Molecular sequencing of the samples for BRAF and NRAS mutations were performed after extraction of amplified products from the DNA gel. Sequencing revealed heterogeneous BRAFV600E mutation in the involuting nevus and BRAF wild type in the unchanged nevus, while both lesions were NRAS wild type.

Discussion

The involution of nevi in BRAFV600E inhibitor therapy has been reported, but herein we report findings that support the hypothesis that these nevi are BRAFV600E positive. This is related to decreased MAPK activity due to BRAF inhibition. In contrast to reports in vemurafenib-treated patients of increased size and pigmentation in some nevi and the appearance of new BRAF wild-type melanoma through paradoxical BRAF activation, we observed no increase in pigmentation of nevi or suspect changes in our patient. Because we could not know whether our dabrafenib-treated patient was also receiving trametinib, conclusions regarding the combination regimen cannot be drawn. However, our long-term monitoring prior to and during therapy combined with confirmation of involuting nevi possessing BRAFV600E mutation adds another component to the dermoscopic changes in long-term therapy with BRAFV600E inhibitors. Larger-scale and longer-term trials will give a broader and more accurate description of specific medication effects required for dermatologic follow-up.