Efficacy of Vemurafenib in Hairy-Cell Leukemia

TO THE EDITOR: The *BRAF V600E* mutation is present in nearly all cases of hairy-cell leukemia.[1] This finding has led to the introduction of BRAF inhibitors for the treatment of chemotherapy-resistant hairy-cell leukemia, and patients have had a good response to the oral inhibitor vemurafenib.[2-4] Constitutive phosphorylation of both extracellular signal-regulated kinase (ERK) and mitogen-activated protein–ERK kinase (MEK) has been considered to be a direct consequence of BRAF activation, with BRAF inhibition resulting in cell death through suppression of this pathway in hairy-cell leukemia. However, data to support this theory are limited, since most patients present with pancytopenia.

We evaluated a patient with purine analogue–refractory hairy-cell leukemia who had biallelic *BRAF V600E* mutations and a high leukemic burden during treatment with vemurafenib. Because of the high numbers of circulating hairy-cell leukemia cells, it was possible to study the effects of vemurafenib directly in vivo. Vemurafenib induced complete clinical remission with reduction of the viability of CD103+ hairy-cell leukemia cells during therapy (Fig. 1A). A pull-down and kinase assay showed inhibition of BRAF in leukemic cells in vivo (Fig. 1B). However, BRAF inhibition was not associated with any major changes in phosphorylation of either MEK or ERK in vivo, as shown by means of both immunoblot and flow cytometry (Fig. 1C), despite prolonged exposure to vemurafenib.

Our experiments showed an unanticipated uncoupling between the decrease in BRAF activity (together with increased cell death) and MEK–ERK inhibition in vivo; this was not dependent on the duration of exposure to vemurafenib. We cannot rule out the possibility that BRAF inhibition in vivo eventually resulted in suppression of ERK activation in some anatomical compartment other than the blood before leukemic cell death, but this possibility appears to be unlikely. First, our in vivo data clearly showed inhibition of BRAF without any change in phosphorylated ERK levels in leukemic cells, as shown by means of both immunoblot and flow cytometry, while cells were dying (as shown by the increasing level of propidium iodide staining). Second, the lack of effect of BRAF inhibitors on phosphorylated MEK and ERK was previously reproduced during prolonged incubation in vitro of hairy-cell leukemia cells obtained from the same patient, whereas the MEK 1 and MEK 2 inhibitor PD325901 successfully blocked ERK phosphorylation and induced significant cell death.[5]

An alternative signaling pathway, as yet uncharacterized, may therefore be affected by vemurafenib, either directly or through BRAF inhibition, and it may have a strong impact in hairy-cell leukemia cell survival in vivo. These data have implications for the design of possible combination therapies.

Figure 1 (facing page). Extracellular Signal-Regulated Kinase (ERK) and Mitogen-Activated Protein–ERK (MEK)–Independent Mechanism of Action of Vemurafenib in Refractory Hairy-Cell Leukemia.

This 72-year-old man had had hairy-cell leukemia since he was 50 years of age. After an initial splenectomy at that time, he remained in good health for 14 years. A third relapse 21 years after the initial diagnosis was treated with rituximab and 2-chlorodeoxyadenosine, which induced a complete response. However, a florid relapse occurred in less than a year. A DNA sequence showed a biallelic *BRAF V600E* mutation, and treatment with vemurafenib at a dose of 240 mg twice daily was initiated in April 2013 (22 years after the initial diagnosis). The only clinically significant toxic effect was alopecia. Panel A shows an initial increase followed by a rapid decrease in the patient's peripheral white-cell count and viability of CD103+ cells after administration of vemurafenib. Hematologic recovery began after 2 months of treatment (the complete blood count was monitored daily in samples obtained from the patient before the first daily dose of vemurafenib). Panel B shows a decrease in BRAF kinase activity after treatment with vemurafenib in a kinase cascade assay. BRAF activity was reduced by 75% 1 day after treatment and reduced by 90% by day 8. The T bars indicate standard deviations. In Panel C, a Western blot study (top) showed no changes in the levels of either phosphorylated MEK or ERK after treatment with vemurafenib. Flow cytometry (bottom) showed that ERK1 and ERK2 phosphorylation was also not reduced in CD103+ cells.

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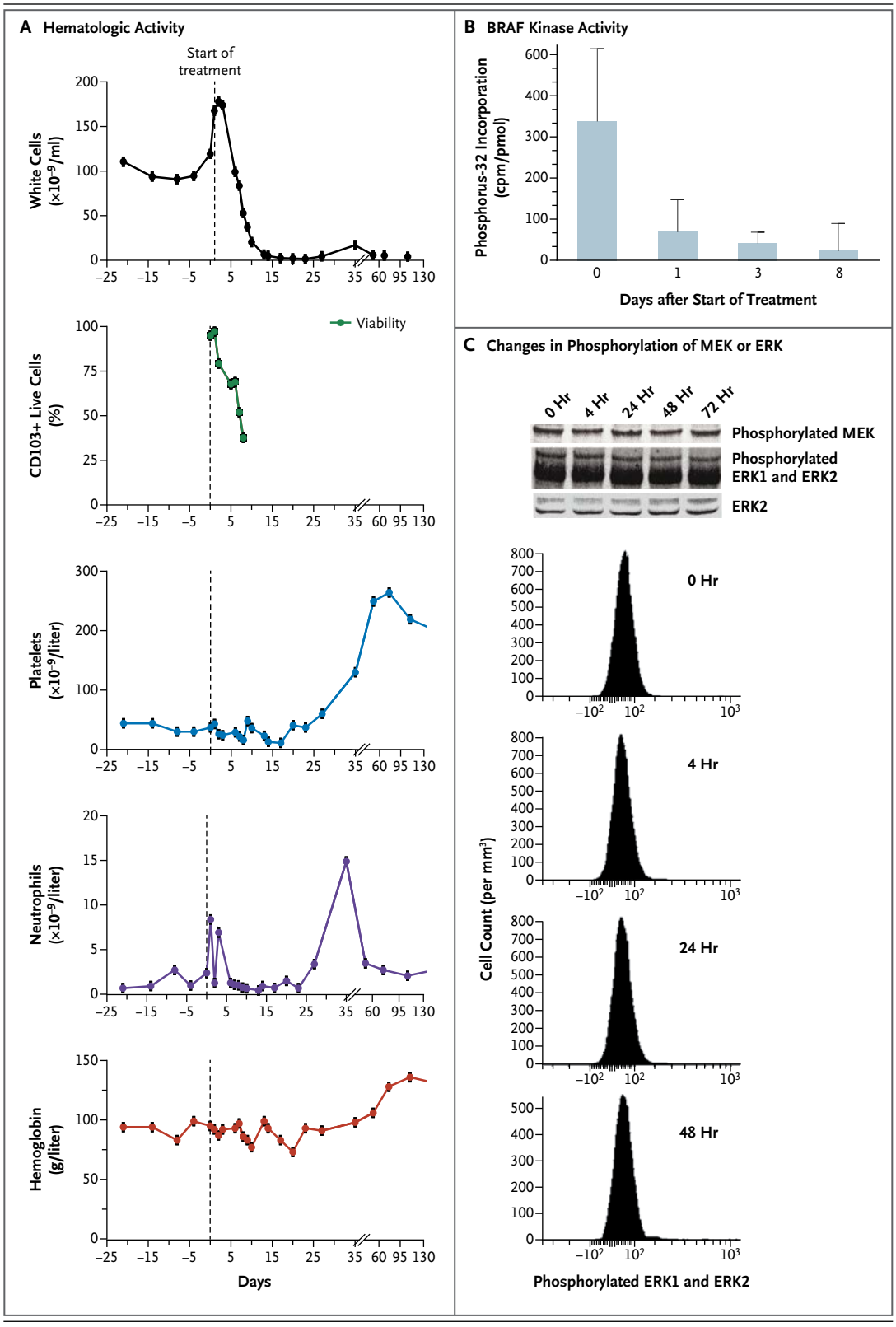

Disclosure forms provided by the authors are available with the full text of this letter at NEJM.org.

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CORRECTIONS

Acute High-Altitude Illnesses (October 24, 2013;369:1664-7). In the list of authors for the letter from Richalet et al. (page 1665), the second author's surname should have been Canouï-Poitrine, rather than Canou-Poitrine. The article is correct at NEJM.org.

Targeted versus Universal Decolonization to Prevent ICU Infection (June 13, 2013;368:2255-65). In the second paragraph of the Outcomes subsection of Results (page 2259), the end of the penultimate sentence should have read, ". . . P=0.03)," rather than ". . . P=0.04)." Also, in the first paragraph of the Discussion (page 2261), the final sentence should have ended, ". . . and 99 patients would need to undergo decolonization . . . ," rather than ". . . and 54 patients . . . ." The article is correct at NEJM.org.

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