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To the editor:

Absence of *BRAF*-V600E in the human cell lines BONNA-12, ESKOL, HAIR-M, and HC-1 questions their origin from hairy cell leukemia

Hairy cell leukemia (HCL) shows distinct clinicopathologic, immunophenotypic, and gene expression features.[1-3] We previously identified the *BRAF*-V600E mutation as the disease-defining genetic event in HCL.[4] This mutation is present in virtually all cases of HCL but rarely in other B-cell lymphomas, remains stable over time (being consistently detectable at relapse), and leads to the constitutive activation of the mitogen–activated protein kinase (MAPK) pathway[4] that is potentially druggable with *BRAF*-V600E inhibitors. These findings have been confirmed in 2 studies recently published in this journal.[5,6]

Functional assays in HCL have been hampered by the scarcity of leukemic cells available for analysis because of frequent

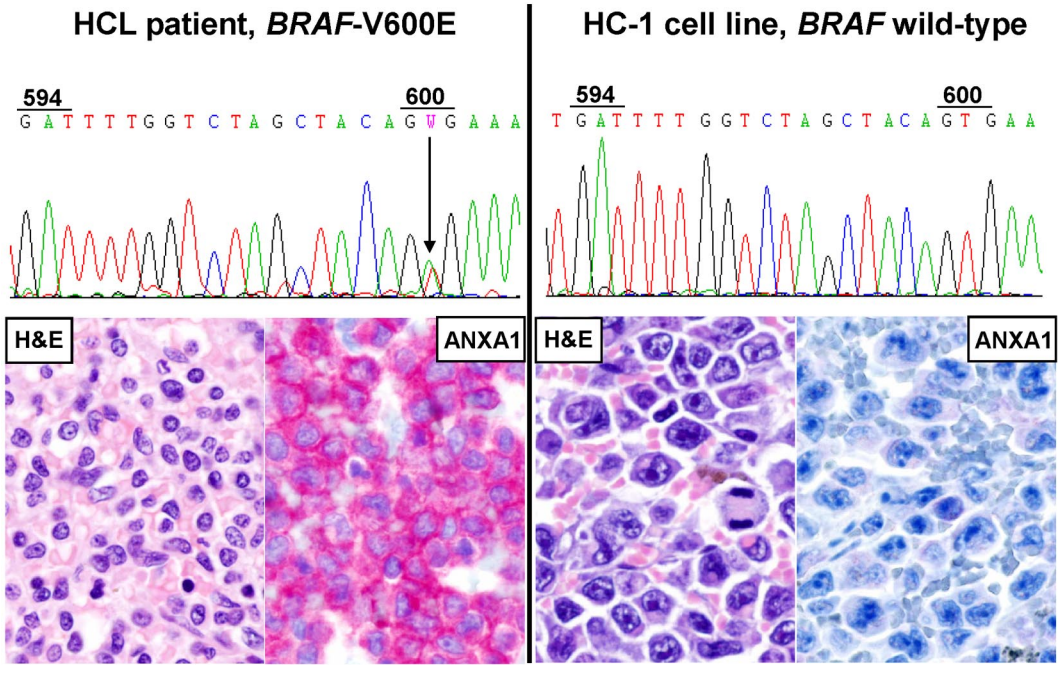

Figure 1. Human cell lines of putative HCL origin lack the *BRAF*-V600E mutation and key phenotypic features of HCL. Direct DNA Sanger sequencing of *BRAF*-exon15[4] in the HC-1 cell line (right chromatogram) shows the absence of the T-to-A point mutation at codon 600 leading to the V600E amino acid replacement, which is instead present heterozygously in primary leukemic cells MACS-purified from the peripheral blood of an HCL patient (left chromatogram). Both HC-1 cells and patient's leukemic cells display a wild-type codon 594 (GAT), as opposed to HAIR-M cells harboring a clonal heterozygous T-to-A point mutation at this codon (not shown) that leads to the D594E amino acid replacement. HC-1 cells xenografted in an NSG mouse show diffuse infiltration of the spleen by large B-cell lymphoma-like cells (right H&E staining) that are negative for annexin-1 (ANXA1; right ANXA1 immunostaining[1]). Conversely, the splenectomy specimen of the HCL patient is infiltrated by small mature-looking lymphoid cells with wide pale cytoplasm (left H&E staining) strongly expressing annexin-1 (left ANXA1-staining). All micrographs were collected using an Olympus B61 microscope (equipped with an Olympus UPlanApo 40×/0.8 NA objective and with an Olympus E330-ADU1.2x camera) and were acquired and processed using Olympus cell^B imaging software.

pancytopenia and/or *punctio sicca* at marrow aspiration. Moreover, no animal models of HCL are available.[3] To overcome these problems, cell lines have been established from HCL patients[7-10] and used for functional studies. However, there is no definitive evidence that they are of authentic HCL origin.

To clarify this issue, we searched for *BRAF*-V600E (the genetic hallmark of HCL) in the human HCL cell lines BONNA-12, ESKOL, HAIR-M, and HC-1 obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ). None of them carried *BRAF*-V600E (Figure 1). However, HAIR-M carried a clonal heterozygous T-to-A transversion at position 1782 of the *BRAF* coding sequence (not shown), leading to the replacement of aspartic acid with glutammic acid (D594E). This variant has been previously reported in only 1 case (a primary cutaneous melanoma[11]) of $>$ 19 000 *BRAF*-mutated cancer samples listed in COSMIC-v56 (Catalog of Somatic Mutations In Cancer). Although this missense variant (D594E) is not described as a germ line polymorphism in single-nucleotide polymorphism (SNP) databases (dbSNP135 and 1000-genomes), its somatic origin in that melanoma patient was not evaluated.[11] Thus the functional consequences of *BRAF*-D594E in the HAIR-M cell line remain unclear. Taken together, these results cast doubts on the HCL origin of the BONNA-12, ESKOL, HAIR-M, and HC-1 cell lines.

Doubts were also reinforced by the morphologic appearance of these cell lines (similar to lymphoblastoid cells), their EBV-positivity (except for HAIR-M),[7-10] and their lack of the typical HCL immunophenotype (coexpression of annexin-1/CD25/CD11c/CD103; not shown). Similarly, ESKOL and HC-1 cells xenotransplanted in severely immunodeficient NSG mice showed the morphology of diffuse large B-cell lymphoma and were annexin-1 negative (Figure 1).

Because these cell lines do not represent a reliable HCL model either in vitro or in vivo, we sought to engraft in NSG mice MACS-purified ($>$ 90% pure) patients' hairy cells. No engraftment was observed in a mouse that was injected intravenously with 3.8 million HCL cells and died 1 year later. Engraftment was not achieved even in another mouse that died 10 months after injection of 15 million leukemic cells from a different HCL patient harboring a hemizygous/homozygous *BRAF*-V600E mutation and presenting with markedly high hairy cell count (WBC 39000/mmc). Conversely, as few as 1.2 million ESKOL cells and 0.98 million HC-1 cells were enough to kill the animals as early as 3 weeks after intravenous injection. These results suggest that primary HCL cells appear difficult to engraft even in NSG mice.

Altogether our findings call for the development of conditional knock-in mice expressing *BRAF*-V600E in specific mature B-cell subsets as appropriate models for studying HCL.

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Contribution: E.T. designed the study, analyzed data, and wrote the manuscript; A.P., B.B., V.P., F.S., M.P.M. and A.T. performed research and analyzed data; H.G.D. contributed vital reagents; and B.F. led the project, supervised the study, and wrote the manuscript.

Conflict-of-interest disclosure: B.F. and E.T. applied for a patent on the clinical use of the *BRAF*-V600E mutant in HCL. The remaining authors declare no competing financial interests.

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