case reports

Hairy Cell Leukemia Masquerading as CD5+ Lymphoproliferative Disease: The Importance of *BRAF* V600E Testing in Diagnosis and Treatment

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INTRODUCTION

Hairy cell leukemia (HCL) is a rare B-cell neoplasm typically of middle-aged men. Pancytopenia, particularly monocytopenia, splenomegaly, and hairy-appearing leukemic B cells in the bone marrow (BM) represent the classical triad of findings.[1] In contrast, chronic lymphocytic leukemia (CLL) is usually diagnosed on the basis of a constellation of findings including lymphocytosis and the presence of a monotonous population of small lymphocytes with clumped chromatin and scant cytoplasm in the peripheral blood smear.[1] CLL cells generally have bright expression of CD5 and CD23, with dim CD20 and monotypic surface immunoglobulin, whereas HCL cells exhibit bright expression of CD19, CD20, CD22, and monotypic surface immunoglobulin with expression of CD103, CD25, CD11c, annexin A1, DBA.44, and cyclin D1 (usually weak). Variant immunophenotypes of classical HCL are well-documented; however, CD5+ is extremely rare (< 2%) and CD23 expression is generally reported to be negative with several reports showing atypical and heterogeneous expression in a small subset of cases (6%).[2,3] Dual, uniformly bright expression of CD5 and CD23 has not been previously described in HCL, which may pose a diagnostic challenge, especially in the absence of immediate molecular data. Diagnosis of HCL has traditionally depended upon morphologic phenotyping and immunophenotyping, which allow for distinction from HCL-like disorders. A correct diagnosis is vital because purine analogs, such as cladribine or pentostatin, are very effective in HCL, with complete remission rates of around 75%,[4] but generally less effective in other lymphomas. The *BRAF* V600E point mutation, substituting thymine with adenine at position 1799 on exon 15, has been identified as an HCL-defining genetic mutation,[5] with presence in the entire tumor cell clone in virtually all patients with HCL.[6] Additionally, mutant BRAF-targeted agents, such as vemurafenib, have been shown to be effective in relapsed, refractory HCL and in situations where critically ill patients cannot be treated with purine analogs.[7,8] Given the high sensitivity and specificity of *BRAF* V600E testing for genetics-based diagnosis of HCL,[9,10] early testing for *BRAF* V600E will allow both diagnostic accuracy and appropriate treatment regimens.

Herein, we describe a 68-year-old female with HCL who was treated for a CD5+ B-cell lymphoproliferative disorder prior to definitive diagnosis because of phenotypically aberrant findings. We provide evidence of true classical HCL diagnosis as well as *BRAF* V600E mutational status and treatment implications.

CASE REPORT

A 68-year-old female presented in March 2019 with marked pancytopenia, WBC count 0.92 K/μL, hemoglobin (Hgb) 8.3 g/dL, platelets (PLT) 61 K/μL, mean corpuscular volume 104.8 fL, and absolute neutrophil count (ANC) 0.27 K/μL and monocyte count 5.4%. Physical examination was remarkable for cellulitic rash over the lower extremity and associated edema. No palpable lymphadenopathy nor splenomegaly was identified. Computed tomography imaging also showed no evidence of splenomegaly. A BM biopsy was performed on March 13, 2019, and although there were atypical features such as bright CD20+ and LEF1–, the patient was diagnosed with a CD5+ B-cell lymphoproliferative disease, not otherwise specified. On the basis of these findings, the patient was started on rituximab and bendamustine on March 22, 2019. However, she developed severe pancytopenia, with WBC 0.4 K/μL, Hgb 8.0 g/dL, PLT 41 K/μL, and ANC 0 K/μL. Her course was complicated by a severe drug rash, neutropenic septic shock, anasarca, and bilateral lower extremity deep vein thrombosis. At this time, she was transferred to Memorial Sloan Kettering Cancer Center on April 6, 2019, for further management and molecular results from the original BM biopsy were reported (March 25, 2019).

Workup at our institution was initiated and peripheral blood flow cytometry revealed an abnormal B-cell population with immunophenotype summarized in Figure 1. A subsequent BM biopsy was performed on April 30, 2019, along with repeat cytogenetic and molecular studies.

Author affiliations and support information (if applicable) appear at the end of this article.

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METHODS

This study was performed in accordance with the Declaration of Helsinki. The patient provided informed consent, and the study has been approved by the Memorial Sloan Kettering Cancer Center institutional review board. The patient consented to publication of this case report.

Immunohistochemical (IHC) stains were performed as per departmental protocol. Somatic mutation profiling and copy number variant analysis were done using a target next-generation sequencing (NGS)–based assay with a panel of 400 genes relevant to hematologic malignancies.[11] IGH clonal rearrangement studies were performed using a commercially available NGS-based assay, LymphoTrack (Invivoscribe Inc, San Diego, CA). Clonal calling and analyses were performed as described previously.[12]

GENETIC TESTING

Cytogenetic Analysis

Pathologic examination of the March 2019 BM biopsy revealed extensive involvement (> 95%) by a CD5+ B-cell lymphoma (Fig 1). Immunophenotyping of these populations by IHC staining and flow cytometry demonstrated CD20+; PAX5 (dim)+; CD5+; CD23+; CD79a+; kappa; CD200+; FMC7+; CD10–; LEF1–; annexin A1–; CD25–; CD103–; and negative for cyclin D1, Sox11 BCL2, BCL6, and CMYC. Cytogenetic testing revealed normal karyotype 46, XX [20] and normal fluorescent in situ hybridization results using probes for MALT1, MYB, ATM, CCND1, IGH, CEP 12, 13q, and TP53.

Repeat pathologic examination after R-Benda treatment showed persistent HCL, in peripheral blood and BM. In light of previous findings, BRAF V600E immunohistochemistry was performed and positive, along with persistent positivity for CD5 and CD23 (Fig 2).

Multiplex Polymerase Chain Reaction Analysis

Multiplex polymerase chain reaction analysis detected a *BRAF* mutation in exon 15, p.V600E or p.V600D or p.V600E2.

IGH Clonal Rearrangement Analysis

NGS-based assay was performed on the in-house BM sample, which exhibited a single clonal sequence with mutated (5.8%) *IGHV* mutation status and V-J segment usage of V3-7-J4.

Somatic Mutation Profiling

Targeted NGS assay was performed with germline variant filtering using DNA from the patient's fingernails and revealed a BRAF (NM_004333) exon15 p.V600E (c. 1799T>A) with a mutational allele frequency of 13% (Fig 2).

TREATMENT AND FOLLOW-UP

On the basis of these results, the patient was diagnosed with classical HCL. Because of her severe pancytopenia and ongoing infection issues from the R-Benda treatment, it was decided that she should be treated with the *BRAF* V600E–targeted agent vemurafenib, rather than purine analogs. She started receiving vemurafenib 240 mg twice a day on May 30, 2019. She developed a generalized drug rash secondary to vemurafenib leading to dose reduction to 240 mg daily from June 11, 2020, to July 12, 2020. Her rash was also treated with prednisone, and she resumed vemurafenib 240 mg twice a day on July 13, 2020, and escalated to 480 mg twice a day from August 1, 2019, to March 13, 2020, with prednisone 20 mg and a prolonged taper, completing nine cycles. Treatment with vemurafenib correlated with resolution of circulating hairy cells in the peripheral blood and an improvement of her hematologic parameters, WBC 6.6 K/μL, Hgb 13.1 g/dL, PLT 140 K/μL, mean corpuscular volume 100 fL, and ANC 4.8 K/μL (Fig 3).

DISCUSSION

Classical HCL is characterized by its distinct morphologic appearance and fairly specific immunophenotype. To our knowledge, this is the first case report showing strong and uniform expression of CD5 and CD23, typical for CLL in a case that is morphologically and clinically consistent with HCL. Such initial findings were misleading, and as expected, treatment for CLL, rituximab and bendamustine, had minimal effect. Many studies have shown that upwards of 95%-100% of classical HCL cases are associated with a mutation of the BRAF serine/threonine protein kinase, *BRAF* V600E, a key driver mutation in HCL.[3,5] The *BRAF* V600E mutation induces constitutive activation of BRAF through autophosphorylation, which then phosphorylates and activates MEK1 and MEK2 downstream of the MERK-ERK signaling pathway, leading to increased expression of cyclin D1 and decreased expression of p27—both distinctive features of HCL.[13] *BRAF*-V600E has been identified as the HCL-defining genetic lesion for its ubiquitous presence in all stages of the disease, including clinically atypical HCL, such as those without splenomegaly or with leukocytosis.[9] Although BRAF mutational identification is included as part of the diagnostic criteria, it is not currently required, likely because of its absence in one subset of classical HCL associated with IgVH4-34 usage.[14] Given the atypical features on the initial diagnostic workup in this case (such as diffusely positive CD20 and LEF1 negativity), testing for the BRAF V600E mutation should have been considered early in the diagnostic workup. Although this mutation has been described in other B-cell malignancies, including splenic marginal-zone lymphoma, B-cell chronic lymphoproliferative disorders, B-prolymphocytic leukemia, and CLL, these cases have been very rare when compared with its near-ubiquitous presence in HCL.[15,16] BRAF mutations occur at a low frequency in CLL, about 2%-5%, and are associated with worse prognosis; however, the majority of CLL cases do not involve the V600E domain responsible for constitutive BRAF activation and mutations are instead found around the activation segment of the kinase domain.[17,18] A positive test would have raised the suspicion

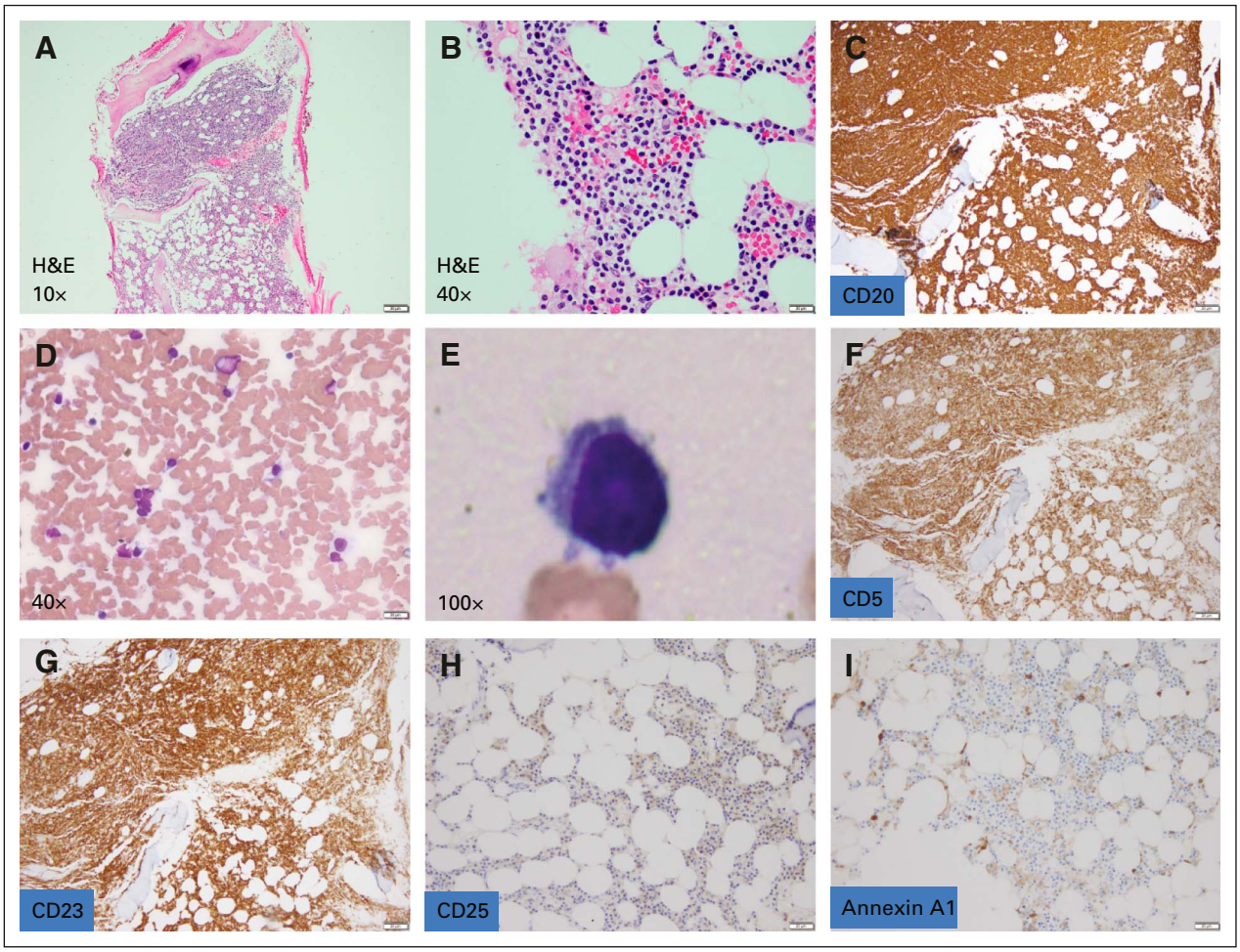

FIG 1. Initial pathologic examination of BM biopsy. (A) H&E-stained sections show a hypercellular BM. (B) On high power, the cells show a characteristic fried egg appearance. (C) They are CD20 diffusely positive. (D) Aspirate smears were paucicellular and pauci-spicular. (E) Peripheral blood showed occasional circulating hairy cells. (F and G) The BM showed the CD20+ cells were diffusely and strongly CD5+ and CD23+ (H and I) while being negative for CD25 and annexin A1. BM, bone marrow; H&E, hematoxylin and eosin.

for HCL and prompted further immunophenotypic workup or closer reinspection of morphology.

Notably the presence of the *BRAF* V600E mutation included a targeted inhibitor into therapeutic consideration for this patient. BRAF inhibitors induce MEK and ERK dephosphorylation in primary HCL cells,[6] and several case reports have shown efficacy of low-dose vemurafenib in relapsed or refractory patients with HCL.[19] Conversely, in vitro studies showed that inhibition of the MEK-ERK signaling using mutant BRAF inhibitors showed no effect on the viability of CLL cells.[18,20] This patient in particular was treated with vemurafenib, rather than first-line HCL treatment with purine analogs, because of severe pancytopenia and ongoing infection issues secondary to the CLL treatment she initially received. Vemurafenib, a BRAF inhibitor, has been shown to have efficacious results in relapsed or refractory patients with HCL and in patients with severe cytopenias preventing continued frontline purine analog therapy.[7,21] Although vemurafenib is usually reserved for relapsed or refractory treatment and is not approved for this indication, it is being tested in a phase II clinical trial in combination with obinutuzumab (anti-CD20 antibody) as frontline therapy in patients with previously untreated classical HCL (NCT03410875).

Various simple, inexpensive tests for *BRAF* V600E detection, such as an allele-specific polymerase chain reaction qualitative assay followed by gel electrophoresis or *BRAF* V600E mutation–specific antibody VE1, have also been described in literature.[6,22,23] In our case, although the diagnosis was initially misguided because of atypical immunophenotype, HCL phenotype was ultimately corroborated by both IHC staining and flow cytometry with uniform and bright expression, particularly with BRAF mutation identification. This highlights the importance of morphologic evaluation in conjunction with clinical parameters even in the setting of new and improved testing methodology. *BRAF* V600E genetic testing should be

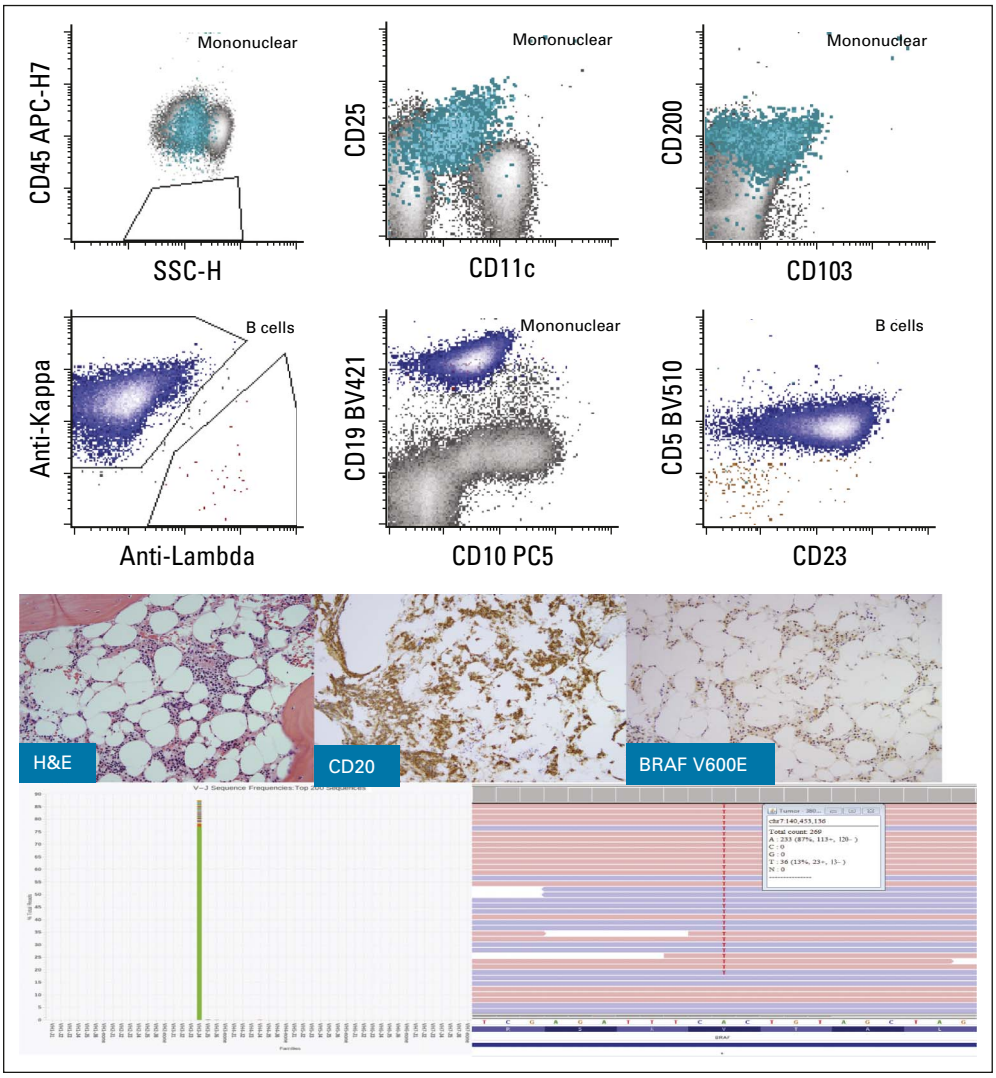

FIG 2. In-house pathologic examination. Flow cytometric analysis of the peripheral blood shows the neoplastic B cells (blue) that appear in the monocyte gate are positive for CD25 and CD11c (partial), CD200 (bright), and CD103 (small subset). They are monotypic for kappa light chain and persistently express CD5 and CD23. H&E shows a hypocellular bone marrow with an extensive CD20 infiltrate that expresses BRAF V600E by immunohistochemistry. LymphoTrack (see Methods) shows a dominant sequence (266 bp in length) with V3-7 J4 usage comprising 91.6% reads. Integrated Mutation Profiling of Actionable Cancer Targets testing shows a *BRAF* V600E mutation with overall coverage 269× comprising 36 of 269 (13%) corresponding with the mutant allele frequency. bp, base pair; H&E, hematoxylin and eosin; SSC-H, side scatter height.

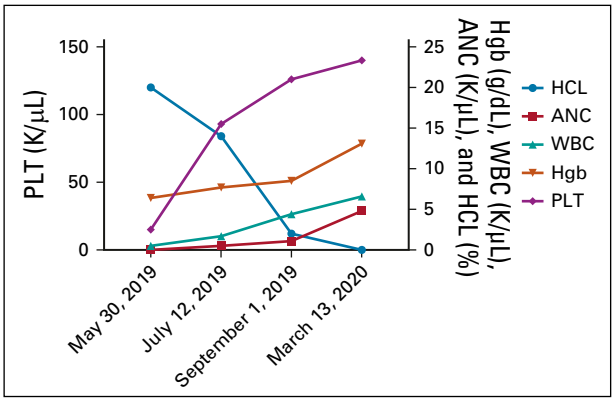

FIG 3. Vemurafenib initiated on May 30, 2019, once *BRAF* V600E identified with improving cell lines and notable decrease of HCL. Despite dose reduction of vemurafenib to 240 mg daily from twice daily, the patient continued to exhibit resolution of circulating hairy cells and significant improvement in her hematologic markers. ANC, absolute neutrophil count; HCL, hairy cell leukemia; Hgb, hemoglobin; PLT, platelets.

widely available to aid in the diagnosis of HCL and especially considered in cases where there is diagnostic ambiguity. This could ultimately prevent incorrect diagnosis and treatment, which would improve overall outcomes in HCL remission and survival rates.

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