Dear Editor,

Differentiation of classic hairy cell leukemia (HCL-c) from HCL-variant (HCL-v) or splenic marginal zone lymphoma (SMZL) is important owing to their different treatment strategies and prognostic implications. Recently, testing for \textit{BRAF} V600E mutations was suggested as an important diagnostic option for HCL-considering that it was exclusively detected in almost all cases [1]. The \textit{BRAF} V600E mutation has been reported to be absent in most cases of immunoglobulin variable heavy chain rearrangements 4-34 (IGHV4-34)-positive HCL-c, HCL-v, and SMZL [2]. However, it was recently reported that high prevalence of \textit{MAP2K1} mutation is observed in IGHV-34-positive HCL-c (5/7, 71.4%) [3].

We investigated the presence of \textit{BRAF} V600E and \textit{MAP2K1} mutations in four HCL-c, two HCL-v, and four SMZL cases involving the bone marrow that were diagnosed between June 2005 and June 2014 at our hospital. HCL and SMZL was diagnosed in accordance with the 2008 WHO classification of tumors of hematopoietic and lymphoid tissues [4]. HCL-c was defined as the expression of Annexin A1, CD20, CD22, CD103, and CD25. HCL-v was defined as the negative expression of CD25 and Annexin A1 [4]. Real-time PCR was performed by using the Real Q \textit{BRAF} V600E Detection Kits (BioSe-woom Inc., Seoul, Korea) on the 7500 Fast Real-Time System (Applied Biosystems, Foster City, CA, USA), according to the manufacturer's instructions [5]. Mutant enrichment 3'-modified oligonucleotide (MEMO)-PCR and sequencing analysis for the \textit{BRAF} V600E mutation were performed as previously described [6]. We designed the sequencing primers for \textit{MAP2K}: exon 2 (forward) 5'-TTCTCTGGTGACAGTATTGACTTG-3', (reverse) 5'-CCCTGAGAAATAATCCAATTACC-3'; exon 3 (forward) 5'-CATCCCTTCCTCCCTCTTCTC-3', (reverse) 5'-CTCTTAAGGC-CATTGGCTCA-3'. Sequencing was performed by using the Big-Dye Terminator Cycle Sequencing Ready Reaction Kit on the ABI Prism 3130 Genetic Analyzer (Applied Biosystems). The DNA extracted from bone marrow aspirate slide was used for sequencing analysis.

We detected the \textit{BRAF} V600E mutation in all HCL-c cases ei-
Table 1. Basic characteristics and results of BRAF V600E and MAP2K1 mutation analyses

| No. | Age (yr) | Sex | Diagnosis | Hb (g/dL) | WBC (×10^9/L) | PLT (×10^9/L) | BRAF V600E | MAP2K1 | CD25 | CD11c | CD103 A1 | Annexin A1 | Treatment | F/U (yr) | Clinical status |
|-----|----------|-----|-----------|-----------|---------------|---------------|-------------|---------|------|-------|---------|-----------|-----------|-----------|---------|-----------------|
| 1   | 48       | F   | HCL-c     | 7.7       | 1.79          | 46            | +           | -       | +    | NA    | +        | Cladribine | 3.5       | alive     |
| 2   | 75       | F   | HCL-c F/U*| 7.6       | 1.18          | 20            | +           | -       | NA   | NA    | NA      | Cladribine | 11        | dead      |
| 3   | 50       | M   | HCL-c     | 9.2       | 4.96          | 30            | +           | -       | +    | +     | +        | Cladribine | 2.9       | alive     |
| 4   | 27       | M   | HCL-c F/U | 9.7       | 1.32          | 19            | +           | NA      | +    | +     | +        | Cladribine | 2.5       | alive     |
| 5   | 40       | M   | HCL-v     | 15.9      | 12.71         | 148           | -           | -       | -    | +     | +        | Cladribine | 6         | alive     |
| 6   | 75       | M   | HCL-v     | 14.6      | 26.30         | 131           | -           | NA      | -    | +     | -        | R-CVP     | 0.4       | alive     |
| 7   | 30       | F   | SMZL      | 6.6       | 1.60          | 52            | -           | NA      | NA   | NA    | NA      | Fluoranthine R-CHOP | 2 | alive     |
| 8   | 74       | M   | SMZL      | 10.5      | 2.00          | 74            | -           | -       | NA   | NA    | NA      | Gastrectomy | 2.5       | dead      |
| 9   | 75       | M   | SMZL      | 10.6      | 8.48          | 142           | -           | -       | NA   | NA    | NA      | Splenectomy R-CVP | 2.6       | alive     |
| 10  | 62       | F   | SMZL      | 11.1      | 16.76         | 473           | -           | NA      | NA   | NA    | NA      | Splenectomy R-CVP | 2 | alive     |

*At initial diagnosis; Hb-WBC-PLT: 5.2 g/dL-1.01×10^9/L-36×10^9/L; †At initial diagnosis; Hb-WBC-PLT: 9.7 g/dL-1.32×10^9/L-19×10^9/L.

Abbreviations: WBC, white blood cell; PLT, platelet; F/U, follow-up; HCL, hairy cell leukemia; SMZL, splenic marginal zone lymphoma; NA, not available; R-CHOP, Rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone; R-CVP, rituximab with cyclophosphamide, vincristine, and prednisone.

Furthermore by real-time PCR or by the MEMO-sequencing method (Table 1). All SMZL and HCL-v cases were negative for BRAF V600E on both real-time PCR and MEMO-sequencing analyses. The MAP2K1 mutation analysis of three HCL-c cases, one HCL-v case, and three SMZL cases revealed negative results.

The most common types of BRAF mutation involve exon 15, and the substitution from valine to glutamate at the 600th amino acid (V600E) constitutes >90% of all reported cases of mutation. BRAF mutations in hematological malignancy are relatively rare [1, 7, 8]. In 2011, Tiacci et al. [1] reported that nearly all cases of HCL-c harbored the BRAF V600E mutation, although this mutation was not detected in any other B cell lymphomas including SMZL; this finding is in agreement with that of other studies [8-10]. Presently, BRAF V600E mutation analysis is considered to be the most useful diagnostic tool for differentiating HCL from related lymphomas. We also confirmed the presence of BRAF V600E in all HCL-c cases, but not in HCL-v or SMZL cases.

Recently, MAP2K1 mutation was identified in a subset of HCL patients: 6/15 of IGHV-34-negative HCL-v, 4/9 of IGHV-34-positive HCL-v, and 5/7 of IGHV-34-positive HCL-c cases [3]. MAP2K1 encodes mitogen-activated protein kinase kinase 1, which is a component of the MAP kinase signal transduction pathway. Somatic mutations were detected in HCL-v and IGHV-34-positive HCL clusters in exons 2 and 3, which encode the N-terminal autoregulatory domain [3]. We detected negative results in all cases, which may be attributed to the small sample size and the low incidence of MAP2K1 mutation.

In conclusion, we analyzed BRAF V600E and MAP2K1 mutations in a small series of HCL-c, HCL-v, and SMZL cases. The BRAF V600E mutation was detected in all cases of HCL-c, but in none of the HCL-v or SMZL cases. This observation is consistent with that of a previous study, confirming the diagnostic utility of BRAF testing in HCL. Considering the rarity of HCL cases, further studies are needed for drawing conclusive observations from a sufficiently large sample size.

Authors’ Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

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