LETTER TO EDITOR

https://doi.org/10.15407/exp-oncology.2025.01.122

S.E. LANGABEER [1, *], G. CROTTY [2], C.L. BACON [3]

[1] Cancer Molecular Diagnostics, Trinity Translational Medicine Institute, St. James's Hospital, Dublin, Ireland

[2] Department of Haematology, Midlands Regional Hospital, Tullamore, Ireland

[3] Department of Haematology, St. James's Hospital, Dublin, Ireland

* Correspondence: Email: slangabeer@stjames.ie

ATYPICAL *BRAF* MUTATIONS IN HAIRY CELL LEUKEMIA: DIAGNOSTIC AND THERAPEUTIC CONSIDERATIONS

To the Editor

Classical hairy cell leukemia (HCL) is an uncommon lymphoid malignancy where the leukemic B-cells harbor the *BRAF* V600E mutation [*BRAF* p.(Val600Glu), c.1799T>A, and ref. seq. NM_004333.4] in nearly all cases. This mutation leads to constitutive activation of the RAF-MEK-ERK signaling pathway. Chemotherapy with cladribine or pentostatin, alone or combined with rituximab, is a highly effective standard care for HCL patients [1]. However, a significant proportion of patients relapse or become refractory and require further, alternative lines of treatment [2]. Identification of the *BRAF* V600E mutation is paramount, particularly in such relapsed and refractory cases, as targeted therapy with specific BRAF inhibitors is effective in achieving high response rates [1]. In those rare cases of HCL without the c.1799T>A mutation, alternative mutations and mechanisms of BRAF activation have been sporadically described [3].

At a center for hematological malignancy molecular diagnostics, screening for *BRAF* mutations in potential cases of HCL has been facilitated over the last five years by a next-generation sequencing (NGS) approach that covers all coding exons of *BRAF* with a detection sensitivity of 2% variant alleles. Two atypical *BRAF* variants have been detected in patients with classical HCL (Figure).

Case #1: This patient who has been previously reported [4], presented more than forty years ago and received multiple lines of treatment including splenectomy, chlorambucil, cladribine, and cladribine/rituximab. Allele-specific PCR and Sanger sequencing of *BRAF* exons 11 and 15 at the third relapse demonstrated no V600E mutation. Of note, isolated B cells from the patient at that time treated with the BRAF inhibitor PLX4720 showed no decrease in ERK phosphorylation [4]. At a subsequent hematological relapse, NGS identified a *BRAF* c.1802A>C; p.(Lys601Thr) (K601T) mutation at a variant allele frequency (VAF) of 2.0%. The patient underwent a trial of the BRAF inhibitor Vemurafenib, but no hematological or clinical response was observed.

Case #2: A patient presented with classical HCL laboratory features apart from the lack of CD103

Citation: Langabeer SE, Crotty G, Bacon CL. Atypical BRAF mutations in hairy cell leukemia: diagnostic and thera-peutic considerations. *Exp Oncol.* 2025; 47(1): 122-123. https://doi.org/10.15407/exp-oncology.2025.01.122

BRAF codon	599 600 601
BRAF wild type	…GGT CTA GCT ACA GTG AAA TCT CGA TGG…
BRAF c.1799T>A canonical	…GGT CTA GCT ACA GAG AAA TCT CGA TGG…
BRAF c.1802A>C	…GGT CTA GCT ACA GTG ACA TCT CGA TGG…
BRAF c.1797_1799delinsGGA	…GGT CTA GCT ACG GAG AAA TCT CGA TGG…

Atypical *BRAF* mutations detected in a cohort of HCL patients. (Mutated nucleotides are underlined)

expression. At the presentation, NGS detected a non-canonical c.1797_1799delinsGGA mutation with a VAF of 9.4% though still predicted to result in a *BRAF* p.(Val600Glu) (V600E) amino acid change. The patient is planned to commence cladribine.

These cases raise two important issues. Firstly, identification of the *BRAF* V600E is imperative in relapsed or refractory HCL patients in order to proceed with therapy with a *BRAF* inhibitor. It is currently unknown whether patients with any atypical *BRAF* mutation or rearrangement will respond to *BRAF* inhibition. Considering the patient with the K601T mutation, given no decrease in ERK activation in malignant B cells, no clinical response to inhibitor, and that in solid tumors, this mutation is designated a Class II mutation with a moderate kinase activity [5], there remains concern for the use of a *BRAF* inhibitor in HCL patients without canonical V600E. Secondly, the methodological approach to mutation detection must consider coverage of all of *BRAF* exon 15 and all other *BRAF* exons [3]. The optimal approach must also possess a sensitivity to detect low VAFs as difficult aspiration due to disease-associated fibrosis can result in an unrepresentative degree of bone marrow infiltration: this would exclude less sensitive techniques such as Sanger sequencing [6]. Attention should also be given to allele-specific primer annealing sequences for real-time or droplet digital PCR used for diagnostic and measurable residual disease monitoring [7], which may lead to a false-negative result due to a non-canonical *BRAF* V600E mutation such as described herein.

REFERENCES

1. Falini B, Tiacci E. Hairy-cell leukemia. *N Engl J Med*. 2024;391(14):1328-1341. https://doi.org/10.1056/NEJMra2406376
2. Robak T, Robak P. Refractory and relapsed hairy-cell leukemia (HCL): casting light on promising experimental drugs in clinical trials. *Expert Opin Investig Drugs*. 2023;32(4):311-324. https://doi.org/10.1080/13543784.2023.2193323
3. Maitre E, Macro M, Troussard X. Hairy cell leukaemia with unusual BRAF mutations. *J Cell Mol Med*. 2023;27(17):2626-2630. https://doi.org/10.1111/jcmm.17890
4. Langabeer SE, O'Brien D, McElligott AM, et al. BRAF V600E-negative hairy cell leukemia. *Case Rep Hematol*. 2013;2013:513049. https://doi.org/10.1155/2013/513049
5. Wang W, Lian B, Xu C, et al. Expert consensus on the diagnosis and treatment of solid tumours with BRAF mutations. *Innovation (Camb)*. 2024;5(6):100661. https://doi.org/10.1016/j.xinn.2024.100661
6. So CC, Chan A, Chung LP, et al. A comparison of high resolution melting, allele-specific priming and Sanger sequencing for the detection of BRAF V600E mutation in hairy cell leukaemia from different haematological specimens. *Pathology*. 2014;46(6):533-537. https://doi.org/10.1097/PAT. 0000000000000151
7. Broccoli A, Terragna C, Nanni L, et al. Droplet digital polymerase chain reaction for the assessment of disease burden in hairy cell leukemia. *Hematol Oncol*. 2022;40(1):57-62. https://doi.org/10.1002/hon.2932

Submitted: November 25, 2024