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A BRAF Negative Classic Hairy Cell Leukemia Patient in Long Lasting Complete Remission after Rituximab and Pentostatin

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To the Editor,
BRAF gene is mutated (V600E) in more than 95% of classic hairy cell leukemia (HCLc)(1-3), but cases have been reported of *BRAF* negativity in HCLc patients (4-7). It has also been suggested to analyze mutations exon 15 and 11 in case of BRAF negativity (7). Mutations in MAP2K1 were identified in wild type BRAF gene (8-9). Few data are available about these patients. We report here a case of HCL that had long lasting reponse to rituximab and pentostatin treatment. A 51 year old woman was referred for lymphocytosis and fatigue. Physical examination revealed a splenomegaly 5 cm below the costal margin. Laboratory findings confirmed lymphocytosis with WBC 17.64 x 10^3/µl, Hb 11.4 g/dl, platelets 187 x 10^3/µl. Blood smear revealed 32% of cells with hairy features. Immunophenotype of peripheral blood showed a 45% cells CD5-, CD19+, CD20+, CD11c+, FMC7+,CD25+, CD103+, CD123+, lambda restricted. Bone marrow aspirate was dry tap and bone marrow biopsy confirmed HC infiltration >90%, TRAP+, DBA44+/-, ANXA1+. CT scan confirmed splenomegaly. IGHV status was mutated and showed a 96.88 homology with IGHV3-7*01 usage. Mutation analysis of *TP53* performed by polymerase chain reaction and DNA direct sequencing exons 2 through 10 revealed a wild type status. Allele specific-PCR for *BRAF* V600E, T599I, V600M, K601E at exon 15 and G464E, G464V, G466R, G466A, G466V, G466E, G469R, G469A, G469V, G469E,V471F at exon 11 did not detect mutations. PCR and direct DNA Sanger sequencing of both exons 15 and 11 did not reveal mutations. A diagnosis of HCLc BRAF negative was made. Due to the presence of fatigue in a young woman with disease related anemia, she was treated with Cladribine (CD) 10 mg total dose daily for 5 days subcutaneously but splenomegaly was still present 2 cm below the costal margin four months later, and HC were still 50% at bone marrow biopsy. After 10 months she developed a severe neutropenia (WBC 2.1x10^3/µl, neutrophils

4%, HC 25%, Hb 10.5g/dl, PLT 142x10^3/µl). R 375 mg/m^2 IV and P at 4 mg/m^2 every 14 days were administered for a total of eight times. Normalization of blood counts and absence of HC resulted at bone marrow biopsy and flow cytometry four months after. CT scan showed normal spleen diameters. At last follow up (78 months after therapy) bone marrow aspirate and biopsy still confirm a CR. Hematologic values were normal: WBC 5.1x10^3/µl, neutrophils 56%, Hb 13.5g/dl, PLT 182x10^3/µl. Splenic diffuse non Hodgkin lymphoma could be excluded by the presence of TRAP+ ANXA1+ and CD123+cells. Few BRAF negative cHCL patients have been reported (Table 1): 11/53 pretreated patients with cHCL in one study, without data related to response (4). Another reported 2 patients BRAF negative at exon 15, responsive one to CD and the other to splenectomy (5). One study reported 1 patient negative at exon 15 and responsive to CD (6); another reported 3 patients negative at exon 15, two of which showed a mutation at exon 11 (7) and another responsive to CD was reported (10). More cases need to be studied.

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HAIRY CELL LEUKEMIA FOUNDATION

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Table 1. BRAF wild-type HCLc cases reported in current literature

	Exon 15	*Exon 11*	*IGHV*
Xi et al. Blood 2012	11/53 (21%)	Not studied	5/11 IGHV 4-34^{+}
Schnittger et al. Blood 2012	2/117 (1,7%)	Not studied	2/2 IGHV 4-34^{-}
Langabeer et al. Case Rep Hematol. 2013	11. 1	Unmutated	Unknown
Tschernitz et al. Brit J Haematol. 2014	12. 3/24 (12,5%)	2/3 mutated	Unknown
Hossain et al. Leuk Res Rep. 2017	13. 1	Not studied	Wild-type
Gozzetti et al.	14. 1	Unmutated	IGHV 4-34^{-}