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RESEARCH

# Treatment of Refractory Hairy Cell Leukemia with a BRAF-inhibitor: Lessons to be Learnt

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**Abstract** Hairy cell leukemia is a rare chronic lymphoproliferative disorder with indolent but progressive clinical course. Patients require treatment when they have significant cytopenia or recurrent infections. The gold standard treatment are purine nucleoside analogues (cladribine and pentostatine), with these agents the rate of complete remission can approach even 95 %. The differential diagnosis between classical hairy cell leukemia and other, rare splenic lymphomas that can mimic this disease might be really challenging. Splenic lymphoma with villous lymphocytes and other new, provisional WHO entities share some, but not all immunophenotypical features with hairy cell leukemia. The correct diagnosis is of an extreme importance as these entities require different treatment. Thus further investigation in the pathogenesis of hairy cell leukemia is required in order to solve this challenge. Discovery of the *BRAF* V600E mutation as a disease-defining genetic event in hairy cell leukemia can be helpful in both differential diagnosis and treatment of this disease. We report the case of three hairy cell leukemia patients, whose diagnosis or treatment was based on this newly discovered somatic mutation, but the treatment results and side effects were individual.

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## Introduction

Hairy cell leukemia (HCL) is an indolent mature B-cell non-Hodgkin-lymphoma accounting for 2 % of adult leukemias. The diagnosis is often incidental, the asymptomatic patients present usually with cytopenias (leukopenia with monocytopenia, anaemia and/or thrombocytopenia). The peripheral blood smear shows lymphoid cells with typical cytoplasmic projections. Histological investigation of the bone marrow is crucial (honey-comb bone marrow), however based on just morphological examinations classical HCL is not distinguishable from HCL-like disorders. In these cases, immunophenotyping and immunohistochemistry are the backbones of diagnosis (CD11c-CD20-CD25-CD103-CD125 positivity, Annexin A1, TBet, TRAP, HBME-1 positivity) [1,2].

The treatment of classical hairy cell leukaemia shows a remarkable evolution: starting from the risky splenectomy of immunodeficient patients, later in the mid-1980s interferons replaced surgical intervention. A few years later purine nucleoside analogues showed promising activity, and currently they represent the best treatment alternatives [3]. In contrast to other splenic lymphomas classical HCL has a response rate of 90 %, with patients achieving complete or at least partial remission, the disease becomes asymptomatic for years or decades, and life expectancy of the patients will not differ from the healthy population. In some cases, however, distinguishing HCL from other splenic lymphomas with villous lymphocytes (hairy cell leukemia variant type, splenical marginal zone lymphoma, prolymphocytic leukemia, splenic red pulp lymphoma) is really difficult. In 2011, a disease-defining genetic event was discovered, namely the somatic

point mutation of *BRAF* in classical HCL [4]. *BRAF* is one of the most frequently mutated genes in human cancers, playing a critical role in cell proliferation, differentiation and survival [5]. These results were confirmed by several other groups [6–15]. All of them identified the *BRAF* V600E mutation in virtually all classical HCL samples as a recurrent genetic event. Different diagnostic methods can be used in the routine practice to detect the mutation, some of them are able to detect the mutation with a variant allele frequency as low as 0.1 %. Since May 2012, seven case reports have been published about refractory HCL patients who were successfully treated with BRAF inhibitors, achieving partial or complete remission [16–22]. Patients were heavily pretreated, and reacted to the *BRAF* inhibitor quite rapidly (in 2–3 weeks), and had no severe, life quality worsening side effects (Table 1.). The first reported patient is still in remission at 8 months after treatment, with no evidence of minimal residual disease in the bone marrow [23]. Unfortunately, long term follow up data and observations are still missing.

One of our case reports represent – to the best of our knowledge-the longest follow up of a successfully treated HCL patient with vemurafenib.

## Case Report 1

A 40-year-old male patient experienced fatigue, loss of weight in May 2012, and consulted his GP in June 2012 because of persistent fever. The medical examination demonstrated mild hepatomegaly, therefore he was referred to a gastroenterological examination. Blood count showed pancytopenia (white blood count (WBC) 0.86 G/L, absolute neutrophil count (ANC) 94/μL, haemoglobin (Hgb) 40 g/L, platelets (Plt) 22 G/L). The cause of the high grade fever was a severe bacterial lymphadenitis in the right inguinal region caused by a coagulase negative Staphylococcus species. In the peripheral blood smear the proportion of the hairy cells was 10 %, the abdominal ultrasound examination revealed hepatosplenomegaly (spleen size 68×203 mm). The bone marrow biopsy showed a dominantly diffuse, but partially interstitial lymphocytosis with intensive CD20, intermediate CD11c and weak Annexin A1 positivity. In the bone marrow aspirate, 90 % of the lymphocytes showed intermediate CD103 positivity, but only 10 % was intensively CD103 positive (Fig. 1). The diagnosis was not equivocal, the pathologist suggested either splenic marginal zone lymphoma or hairy cell leukemia. Thus, the presence of a mature B-cell lymphoproliferative clonal disease was certain, and the young, severely neutropenic patient needed urgent treatment. In that situation rituximab was the only drug thought to be effective in the case of either of the two B-cell lymphoproliferative disorders. The patient received rituximab in June 2012 (weekly 375 mg/m$^2$ iv infusion for 4 weeks). In the meantime, pyrosequencing of the DNA isolated from the bone marrow aspirate identified that 19 % of the lymphocytes carried the *BRAF* V600E mutation, confirming the diagnosis of classical hairy cell leukemia (Fig. 2). Following the immunotherapy the blood counts did not improve, the patient needed platelet and red blood cell transfusions, and even G-CSF treatment. In August 2012, he underwent cladribine treatment (0.14 mg/kg/d subcutaneous 5-times), which also proved to be ineffective. The control bone marrow biopsy performed in September 2012 revealed massive, diffuse infiltration (70–80 %) of hairy cell leukemia, with intensive CD20 and partial Annexin A1 positivity (Fig. 3). In October 2012, the patient still had pancytopenia thus we decided to start interferon-alpha treatment (3 MU subcutaneous, 3 times per week), but

**Table 1** Comparison of different studies using vemurafenib treatment for classical hairy cell leukemia patients

| | Nr.of treatment agents prior to vemurafenib | Dose of vemurafenib | Treatment duration (days) | Side effects | Response to Treatment |
|---|---|---|---|---|---|
| Die S et al. (NEJM 2012) | 3 | 240 mg BID and dose escalation | 56 | not reported | CR |
| Follows GA et al. (BJH 2012) | 4 | 240 mg BID | 58 | hyperbilirubinaemy, seborrheatic keratosis | PR |
| Peyrade F et al. (Hematologica 2013) | 3 | 240 mg BID | 56 | not reported | CR |
| Munoz J et al. (JCO 2013) | 5 | 480 mg BID | 21 | arthralgia | PR |
| Maurer H et al. (Ann Hematol 2013) | 3 | 960 mg BID | 54 | not reported | CR |
| Urnova ES et al. (Ter Arkh 2013) | 3 | 480 mg BID | 60 | toxicoderma, orchitis | CR |
| Samuel J et al. (NEJM 2014) | 3 | 240 mg BID | not reported (at least 60 days) | alopecia | CR |
| Present case report #1 | 3 | 240 mg BID | 58 | none | PR |
| Present case report #2 | 3 | 240 mg BID | 14 | athralgia | minor response |
| Present case report #3 | 3 | 240 mg BID, reduced to 240 mg | 60 | toxicoderma | minor response |

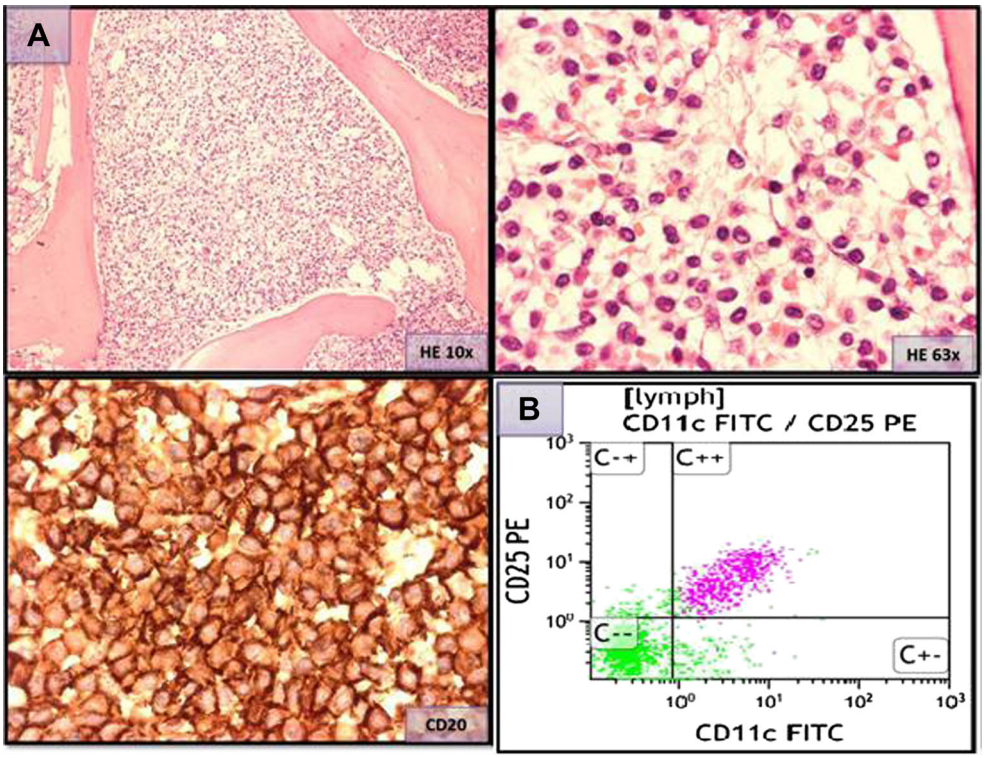


**Fig. 1** **a**: Treatment-naive bone marrow showed a dominantly diffuse, but interstitially lymphocytosis with intensive CD20 and weak Annexin A1 positivity. **b**: Flow-cytometry shows CD11c-CD25 positive lymphocytes

after the second injection severe weakness, and fever presented, which are well-known side-effects of the immunotherapy, but intolerable for the patient. According to the favourable data on vemurafenib treatment in resistant HCL, in January 2013 low dose vemurafenib treatment was started (240 mg BID) with the permission of the National Institute for Quality and Organizational Development in Healthcare and Medicines. Blood count improvement was already seen in the second week of the treatment and the patient became transfusion-independent. Vemurafenib treatment lasted for 58 days. An end-of-treatment bone marrow biopsy performed on day 59, showed significant reduction of leukemic infiltration from 80 to 15–20 %, *BRAF* was wild type, which can be explained by the heterogeneity of the tumour cells (Fig. 4). Reduction of the leukemic bone marrow infiltrate was also confirmed by pre-and post-treatment magnetic resonance imaging of the femurs (Fig. 5). The patient achieved a partial remission lasting already more than 12 months and has nearly normal blood counts with a significantly better quality of life. (Fig. 6).

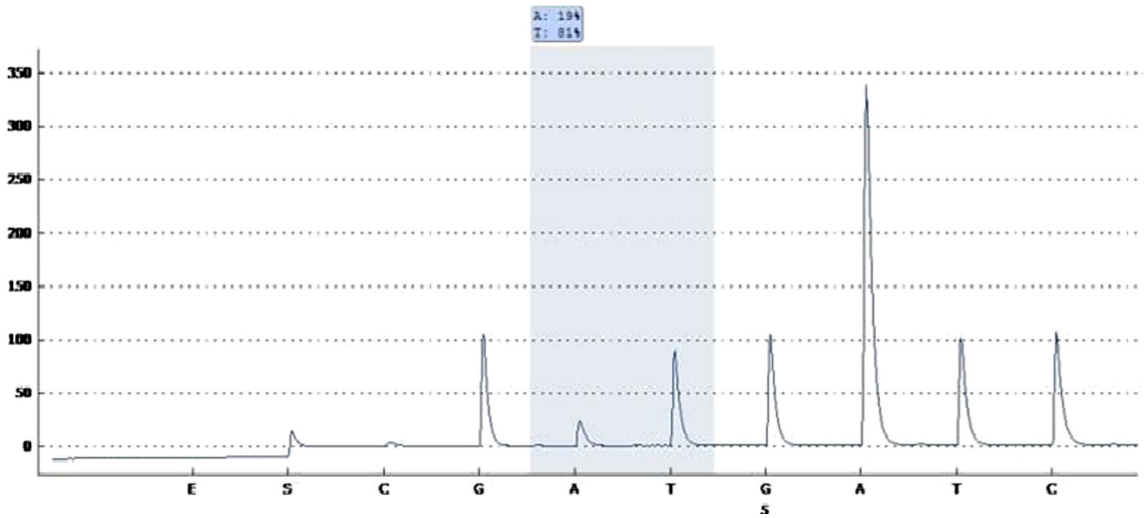


**Fig. 2** Pyrogram from the original bone marrow aspirate: 19 % of DNA isolated from the bone marrow carries *BRAF* V600E mutation

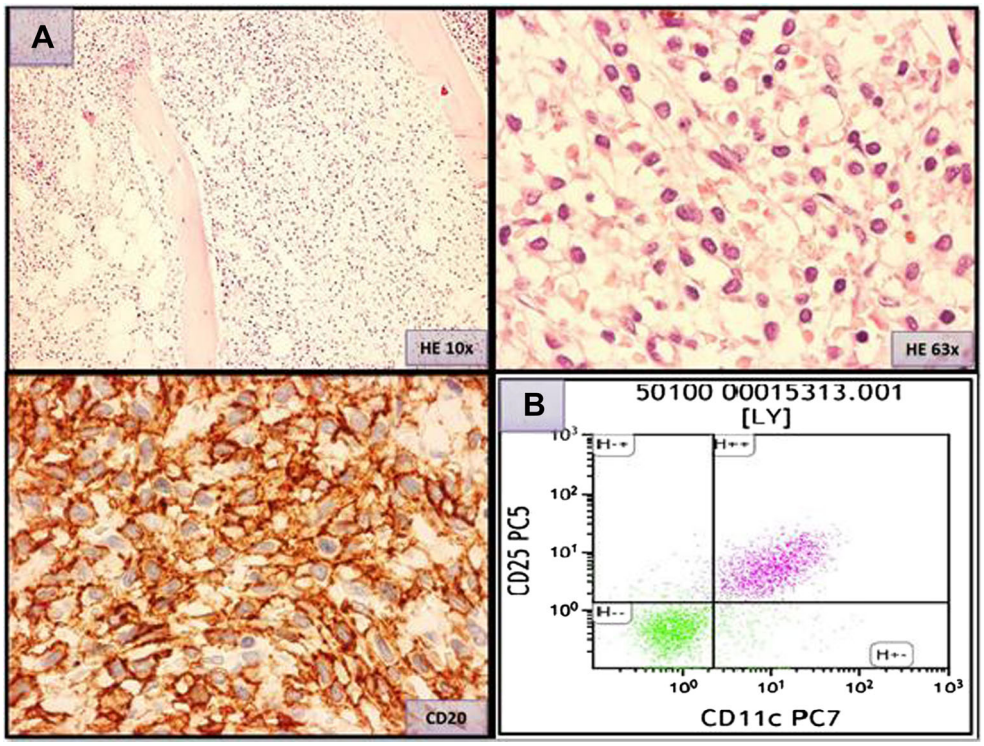


**Fig. 3** **a**: Bone marrow biopsy and flow-cytometry after rituximab and cladribine treatments show diffuse lymphoid infiltration, with the same immunophenotypic and immunohistochemical pattern. **b**: Flow-cytometry shows persistent CD11c-CD25 positive lymphocytes

Case Report 2

The pancytopenic, male patient was diagnosed with hairy cell leukemia at the age of 45 years in 1998. During the subsequent years, he was treated with multiple modalities/agents: interferon-alpha (for 3 months in 1998, 9 months in 2004–2005 and 13 months in 2006–2007), cladribine (0,14 mg/kg sc. for 5 days in 1998, the treatment caused severe toxicoderma), rituximab (375 mg/m$^2$ iv. for 4 weeks in 2008, and 2012, each infusion caused cytokine-release

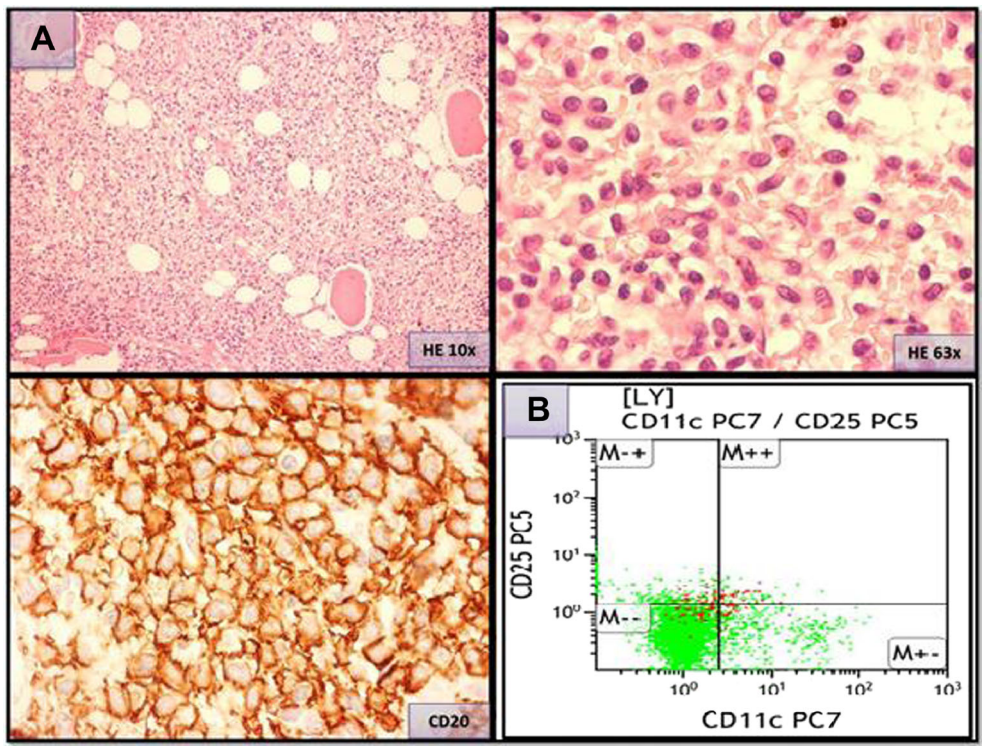


**Fig. 4** **a**: Bone marrow biopsy and **b**: flow-cytometry after vemurafenib treatment. A mild 20–30 % lymphoid infiltration persists, but flow-cytometry shows significant reduction of leukemic cells

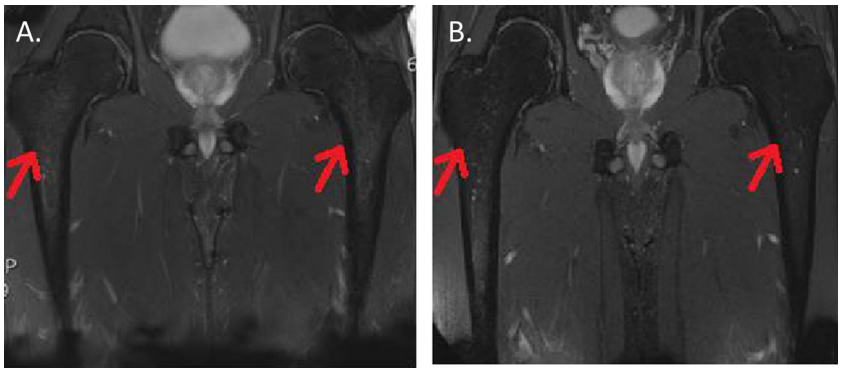


**Fig. 5** Pre-treatment coronal T2 fat-supressed MRI of pelvis and both femurs (Panel A.). Moderately and diffusely increased T2 fat suppressed signal in the proximal diaphysis of the both femur bone marrow: abnormal bone marrow. Post-treatment MRI 109 days after completing therapy showed complete recovery of the bone marrow signal (Panel B.)

syndrome). In 2013, he was reporting fatigue and had worsening pancytopenia (ANC 294/uL, Hgb 87 g/L, Thrcy 44 G/L). Bone marrow biopsy showed massive interstitial lymphoid infiltration, immunphenotypic analysis of the bone marrow aspirate showed B-cells with intensive CD20 and moderate CD19-CD11c-CD103 coexpression. Molecular analysis (PCR-based DNA pyrosequencing) revealed *BRAF* V600E mutation with a mutation load of 21 %. In July 2013, the patient was treated with vemurafenib 240 mg by mouth twice daily for 2 weeks. Therapy was stopped because of grade 3 arthralgias. In addition, mild jaundice with dominantly indirect hyperbilirubinaemy developed, without any other signs of hemolysis. Adverse effects abated promptly after discontinuation of vemurafenib. Four months after starting the treatment, the patient's absolute neutrophil count increased from 0,8 to 2,5 G/L, accompanied by reduction of anaemia and thrombocytopenia (Fig. 7).

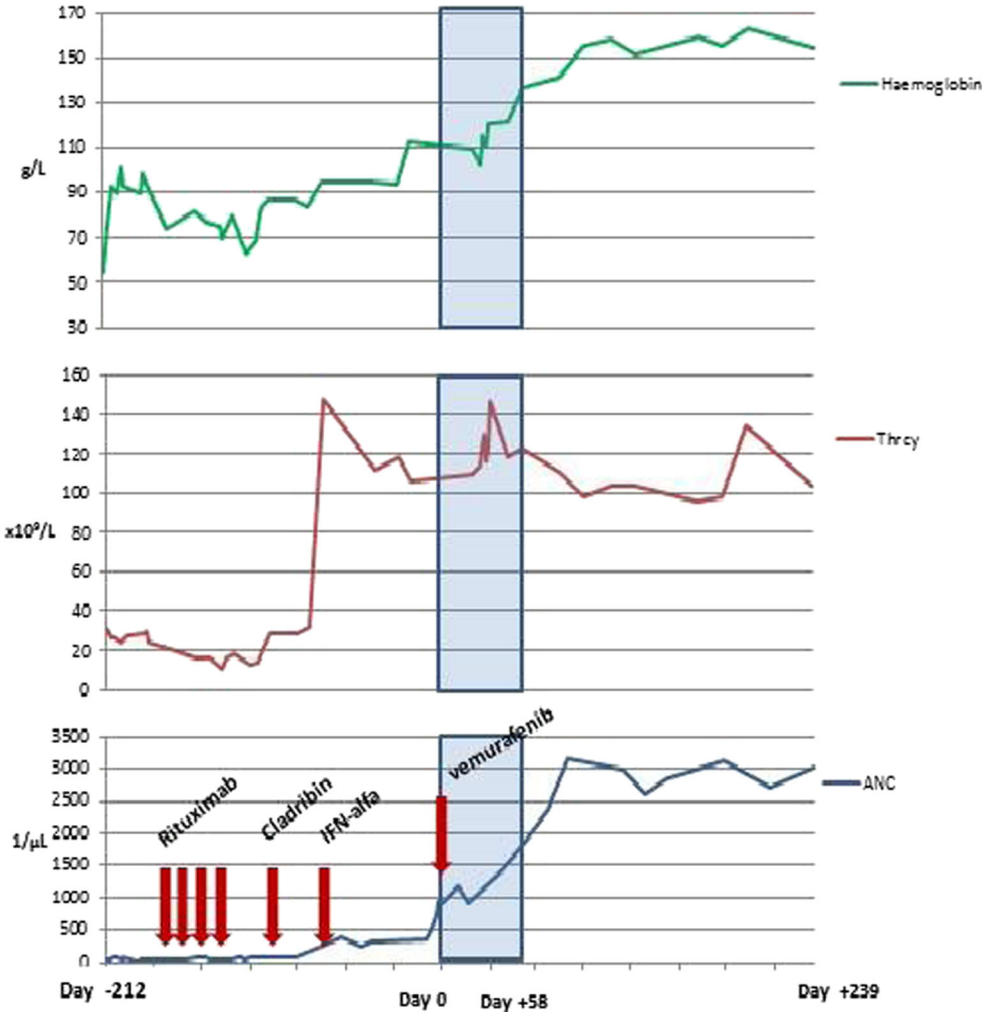


**Fig. 6** Haemoglobin, thrombocyte and absolute neutrophil counts during disease course of Patient 1. Red arrow: start of treatment. Thrcy: platelet count, ANC: absolute neutrophil counts

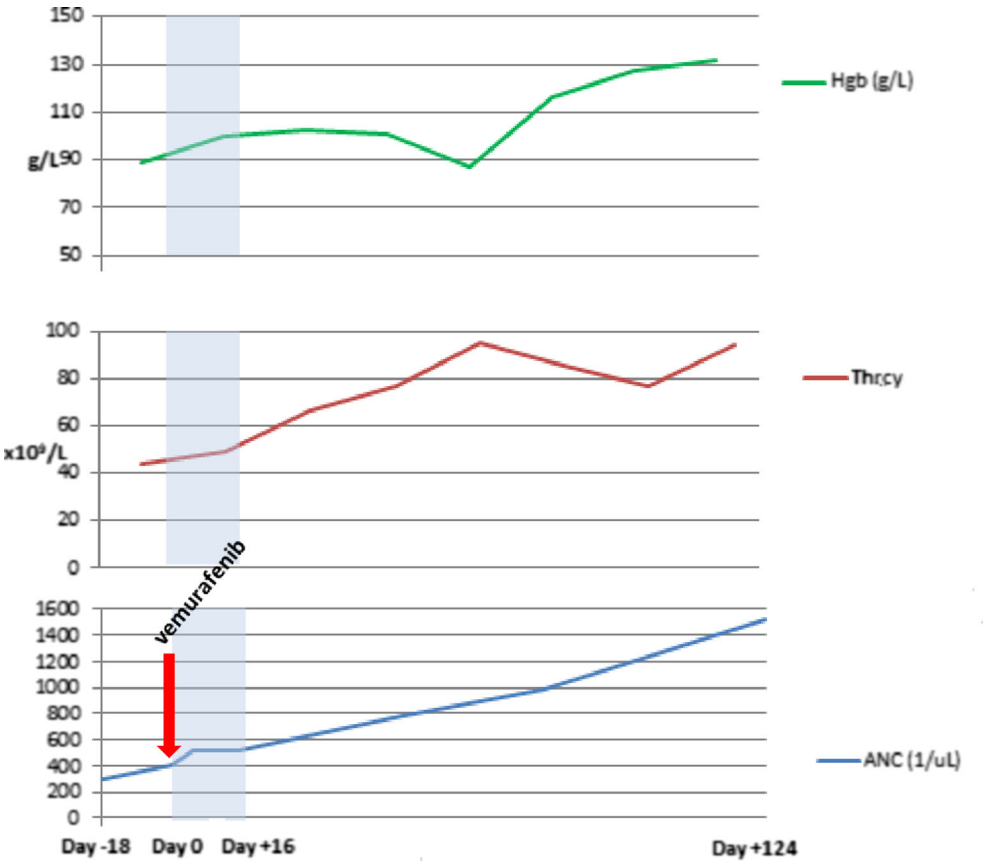


**Fig. 7** Haemoglobin, thrombocyte and absolute neutrophil counts during disease course of Patient 2. Hgb: haemoglobin, Thrcy: platelet count, ANC: absolute neutrophil counts

Case Report 3

A 66-year-old male patient with severe anaemia was diagnosed with classical hairy cell leukemia in 2006. He was treated with interferon-alpha for a year, and achieved partial remission. This period lasted only for a year, therefore he was re-treated with interferon-alpha in 2008, with same outcome. In 2010, a second relapse happened, the patient underwent cladribine therapy, resulting in good partial remission. In June 2012, the patient relapsed for the third time, at this point, the bone marrow biopsy showed 50–60 % infiltration of HCL, with the presence of *BRAF* V600E mutation with a mutant allele frequency of 25 %. In May 2013, neutropenia and cumulating infections indicated rituximab administration (375 mg/m$^2$ iv. for 4 weeks), however the haemogram did not show any significant change. As ultimum refugium, vemurafenib treatment was started in September 2013 with a daily dose of 240 mg bid. Cytopenias showed slight but immediate improvement but at treatment day 25 toxicoderma presented. In parallel with the reduction of vemurafenib dose to daily 240 mg and the use of local steroids the phenomenon showed rapid regression. On day 60, the pancytopenia ceased. Nevertheless, the control bone marrow biopsy showed 50 % lymphocytic infiltration with no *BRAF* V600E mutation (Fig. 8).

## Discussion

The pathogenesis and the genetic background of classical hairy cell leukemia remained unclear until 2011, nonetheless it was one of the best manageable indolent non-Hodgkin lymphoma since the era of purine nucleoside analogues.

The somatic point mutation *BRAF* V600E is present in virtually all patients with classical hairy cell leukemia. BRAF is a key serine-threonine protein kinase component of the Ras-Raf-MEK-ERK intracellular signaling pathway which is a highly conservative signal transduction pathway transducing extracellular signals to the nucleus in order to regulate the expression of genes affecting cell proliferation, differentiation and survival [24]. After description of the *BRAF* V600E mutation as the basic genetic event in HCL, application of specific targeted therapy became possible in the clinical practice. Nowadays, both first and second line *BRAF* inhibitors are available in the treatment of metastatic malignant melanoma; the most prevalent are vemurafenib (PLX-4032) and dabrafenib (GSK-2118436). In malignant melanoma the most important limiting factor of the single-agent *BRAF*-targeted therapy is the relatively short duration of the antitumor effect. In clinical trials most of the patients progress after 6–7 months

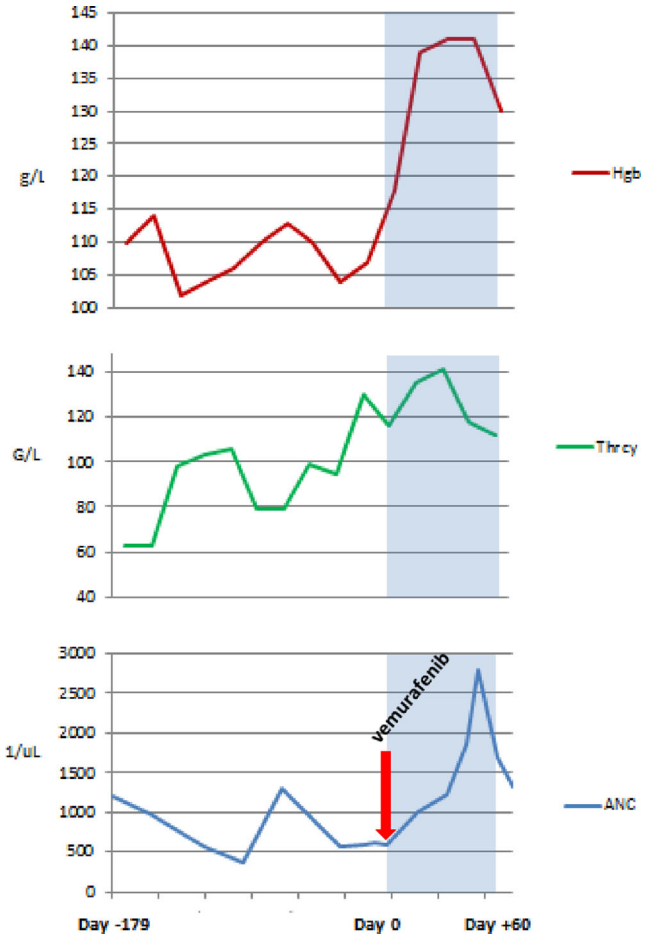


**Fig. 8** Haemoglobin, thrombocyte and absolute neutrophil counts during disease course of Patient 2. Hgb: haemoglobin, Thrcy: platelet count, ANC: absolute neutrophil counts

and only a minority of patients is still on therapy after 2 years [24].

Our experience confirms that *BRAF* inhibition can be successfully applied in the treatment of resistant HCL, resulting in at least non-symptomatic, stable disease. This promising treatment modality points out the importance of *BRAF* V600E mutation testing in every HCL patient, with a view of identifying the therapeutic target. The mutation may also serve as a marker used for differential diagnosis or minimal residual disease.

The remarkable advantages of this treatment are the relatively fast response (in comparison with purine nucleoside analogues), the excellent oral bioavailability, and the absence of hematologic toxicity in this usually heavily-pretreated, cytopenic population. Side effects are manageable, neither our nor other study group has reported life-threatening side effects. The reported treatment duration is usually 2 months, but even a 2 weeks long therapy results in improved peripheral blood counts. Our patients took 240 mg vemurafenib BID, which dose is far below the dose used in malignant melanoma (960 mg BID). Long term follow up data will be needed to evaluate the duration of the remission and the late effects of this targeted treatment, however we are able to report, for the first time, about the longest (more than one year) very good partial response. Since very few data are available concerning the use of vemurafenib in HCL, further cases are needed to confirm this demonstrated efficiency. A phase II study would be of considerable interest to determine the dosage and the duration of response in HCL cases. Despite the very promising results of the present and previous reports, this step is likely inevitable for the clinical development of vemurafenib treatment for HCL. In malignant melanoma, the remissions achieved with BRAF inhibitor treatment are relatively short-lived due to the rapid formation of resistant clones. Thus, another important issue is whether the remission achieved in an indolent lymphoma with the use of vemurafenib can be reinduced by cotargeting MEK inhibitors.

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