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Eosinophilia characterized by a rare CCT6B mutation and responsive to tyrosine kinase inhibition: Case report and literature review

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ABSTRACT

Hypereosinophilic syndrome is a rare disorder arising from neoplastic, or idiopathic causes. The availability of NGS panels has increasingly identified rare mutations as underlying pathogenic events and have led to reclassification of cases of idiopathic hypereosinophilic syndrome as chronic eosinophilic leukemia(CEL). In this report, we describe a case of a young man with hypereosinophilia whose disease initially did not fit the WHO criteria for CEL but harbored a rare mutation in *CCT6B* gene. We report our experience in successfully treating this patient with multiple tyrosine kinase inhibitors and provide literature review of this rare entity including potential treatment strategies.

Introduction

Idiopathic HyperEosinophilic Syndrome (IHES) is characterized by eosinophil overproduction in the bone marrow without any known cause but with evidence of associated organ or tissue damage due to eosinophilic hyperactivation [1]. On the other hand, chronic eosinophilic leukemia, not otherwise specified (CEL-NOS) is a myeloproliferative neoplasm with eosinophilic expansion and activation, but devoid of putative molecular genetic alterations (i.e. negative for BCR-ABL fusion, PDGFRA/B- or FGFR1-rearrangements) [1]. The key criteria for diagnosis of CEL-NOS are outlined in Table 1 [1].

IHES is a diagnosis of exclusion when secondary, clonal and neoplastic causes have been excluded. IHES can be reclassified to CEL-NOS using next-generation sequencing (NGS) if putative clonal events are identified [2]. Authors in one series reported presence of myeloid somatic mutations (e.g. *ASXL1, DNMT3A, JAK2* and others) in 14/51 patients with IHES [2]. These findings suggest that in conjunction with other diagnostic features, determining clonality via NGS may be critical in distinguishing between IHES and CEL-NOS in patients with hypereosinophilia. This distinction has prognostic significance as patients with IHES with mutations exhibited inferior prognosis as compared to IHES without mutations, but comparable to CEL-NOS. Increasing identification of myeloid-associated mutations are expected to increase the overall incidence of CEL-NOS, due to growing NGS adoption.

We report a case of a young man with hypereosinophilia whose disease harbored a rare splice site mutation in the *CCT6B* gene, at a variant allele frequency of 47.3%. We provide a brief review of the available medical literature, significance of this large *CCT6B* clone and provide treatment strategies that may be helpful in treating individuals with this rare disease and mutation.

2. Case report

A 36-year-old man presented to our leukemia clinic for a second opinion in February 2017 for worsening leukocytosis, recurrent episodes of hives and night sweats. Past medical history included well-controlled ulcerative colitis and type 2 diabetes mellitus. Upon presentation, his white blood cell (WBC) count was 92.52×10^9/L, hemoglobin 9.6 g/dL, platelet count 343×10^9/L, with 66% eosinophils. A thorough work-up that included comprehensive metabolic panel with uric acid, lactate dehydrogenase, liver function tests, vitamin B12 levels, peripheral blood smear review [without evidence of other blood count abnormalities (eg, dysplasia, monocytosis, circulating blasts)], and infectious work-up negative for strongyloides and parasitic infections. In addition, patient had no offending medication exposure and no metabolic or lymphomatous etiologies, that excluded secondary causes of hypereosinophilia.

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Table 1
Diagnostic criteria of Chronic Eosinophilic Leukemia- Not Otherwise Specified (CEL-NOS) based on the 2017 Revised WHO Classification1.

Eosinophilia (eosinophil count of $\geq 1.5x10^9$/L).
An increase of blast count in peripheral blood and bone marrow myeloblasts < 20%.
Clonality of eosinophils verified by detection of clonal cytogenetic or molecular genetic abnormality.
Clonality of eosinophils verified by detection of clonal cytogenetic or molecular genetic abnormality.
No rearrangement of PDGFRA, PDGFRB, or FGFR1; no PCM1/JAK2, ETV6/JAK2, or BCR/JAK2 fusion gene.
No CBFB rearrangements and other diagnostic features of acute myeloid leukemia (AML).

* Abbreviations: CML = Chronic myelogenous leukemia, CMML = Chronic myelomonocytic leukemia, ET = Essential thrombocythemia, CNL = Chronic neutrophilic leukemia, PMF = Primary myelofibrosis, PV = Polycythemia Vera.

Additional laboratory testing was not considered at the time based on patient's history, symptoms, and findings on physical examination. He, however, was offered a bone marrow biopsy, which he refused and chose to seek care with local oncologist, closer to home. He was started on hydroxyurea for cytoreduction. A few days later, he was hospitalized with respiratory failure requiring intubation, confusion and leukocytosis (WBC 99.4 $\times$ 10^9/L with 61% eosinophils) necessitating leukapheresis. He had cardiac enzyme elevation, troponin that peaked at 18.7 ng/mL (normal, < 0.4 ng/mL), EKGs revealing sinus tachycardia and echocardiogram that showed borderline enlarged left ventricle size and thickness. CT chest suggested atypical pneumonitis. Overall, his presentation was thought to be consistent with eosinophilic myocarditis and worsening breathing due to eosinophilic pneumonitis.

After recovering from this hospitalization, he reestablished care with our institute and was instructed to resume hydroxyurea (500 mg daily), for a WBC count threshold of $\geq$50 $\times$ 10^9/L. Two weeks later, he underwent bone marrow biopsy and aspirate, [WBC count was normal (5.40 $\times$ 10^9/L) at the time of marrow] which demonstrated normocellular marrow for age, progressive trilineage hematopoiesis, increased marrow eosinophilia and 1% blasts. Cytogenetic studies revealed a normal male karyotype and FISH analysis negative for PDGFRA/B, FGFR1, PCM1-JAK2/ FIP1L1-PDGFRA rearrangements or BCR-ABL translocation. T-cell immunophenotype testing by flow cytometry was unrevealing for any abnormal. Comprehensive NGS panel (FoundationOneHeme®) evaluating 406 known cancer gene mutations revealed a rare *CCT6B* gene mutation (reported previously in rare reports as benign variant) but raising the possibility of a cryptic genetic alteration (Protein Effect: Splice Site 615–2A > *G*; VAF = 47.3%) classifying our patient as CEL NOS.

Based on the 2016 WHO Classification of eosinophilic disorders, the presentation was consistent with either CEL-NOS or IHES [1]. We favored the diagnosis of CEL-NOS over IHES due to the presence of this rare genomic abnormality in a young individual and presence of factors portending inferior prognosis e.g. presence of cardiac disease and WBC count of 100 $\times$ 10^9 / L [1,3].

2.1. Therapeutic challenges and sequence of therapy

Based on the above findings, an empiric trial of imatinib was initiated at a dose of 200 mg per day. A bone marrow biopsy after 6 months showed complete remission with 5% marrow eosinophil count. A dose reduction by 50% had to be instituted after six months of therapy, due to generalized arthralgias and dermatitis. Dose-reduction resulted in eventual rise in eosinophils indicating ineffectiveness at lower doses (Fig. 1).Therapy was then changed to dasatinib 140 mg daily based on reports demonstrating effectiveness of dasatinib in blocking growth and survival of eosinophils [4]. The patient continued on dasatinib with excellent response (Fig. 1) and remission for 9 months, until he was hospitalized for worsening dyspnea. CT chest revealed pleural effusions, and echocardiogram suggested early tamponade. Dasatinib was discontinued and high dose steroids were initiated.

Patient was then started on nilotinib, a second-generation tyrosine kinase inhibitor (TKI) developed to overcome imatinib-resistance in

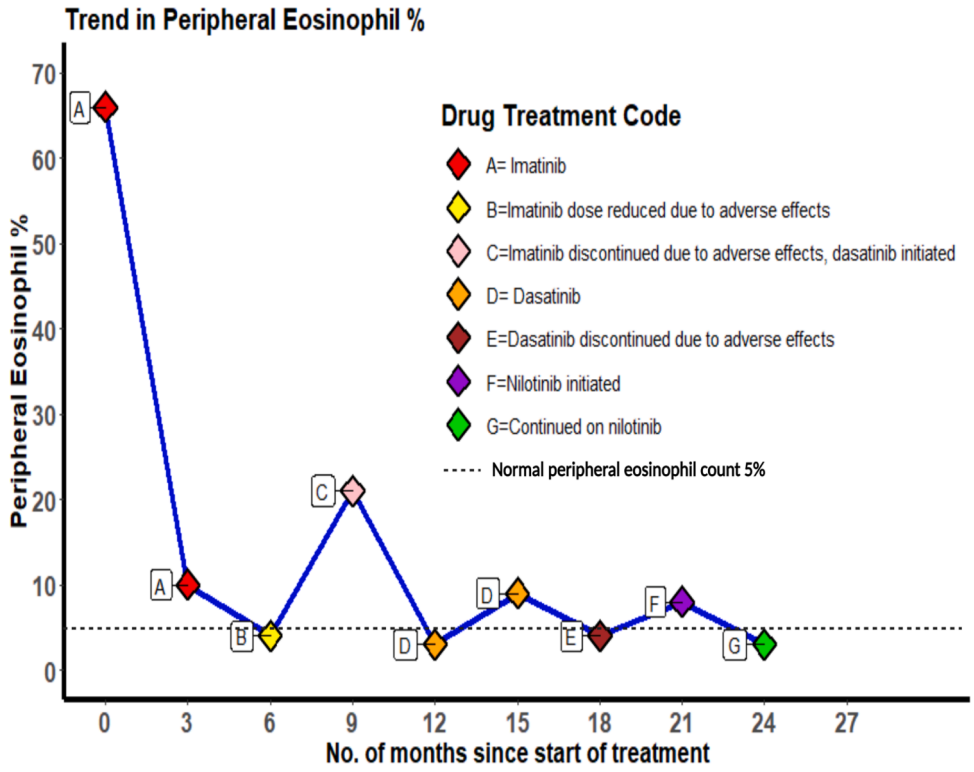

Fig. 1. Clinical response to tyrosine kinase inhibitor therapy as shown by peripheral eosinophil percentage over time with treatment.

chronic myeloid leukemia. Nilotinib had been used to treat individuals with FIP1L1-PDGFRA (F/P) mutation as well those with negative F/P status [5]. This is in line with the findings of Phase 2 study that demonstrated promising results with nilotinib in IHES/CEL patients irrespective of F/P mutation status [6].

The patient now has been on nilotinib 400 mg twice daily for 2.5 years (and TKI therapy for 48 months) with no cardiovascular events attributed to the hypereosinophilia or disease related symptoms and has normal eosinophil levels. On the most recent visit, the patient presented in good health, with a hemoglobin value of 14.2 g/dL, hematocrit of 40.4%, WBC 7.39 $\times$ 10^9/L; (61% segmented neutrophils, 1% basophils, 2% eosinophils, 20% lymphocytes and 11% monocytes) and platelet count 252 $\times$ 10^9/L.

3. Discussion

The diagnosis of IHES is made following the exclusion of other factors that may lead to increased eosinophil count. After excluding secondary causes, the WHO classification delineates two broad categories - F/P rearrangement positive and negative hypereosinophilias [1]. The wide use of NGS and resultant discovery of newer less described somatic mutations pose a diagnostic quandary, making diagnosis in F/P negative patients challenging. Our patient had no other cytogenetic abnormality or molecular aberration, except for the rare *CCT6B* gene mutation at VAF ~50%, which was perhaps the differentiating clone between WHO defined categories of CEL-NOS and IHES. Clinically, *CCT6B* mutation has only been implicated in Burkitt's Lymphoma [7]. Here, we describe a case of CEL with no known driver genes but with a rare mutation in *CCT6B* gene, successfully treated with TKI therapy. This mutation would have been otherwise missed had we obtained a limited myeloid specific panel instead of a broad NGS panel such as FoundationOneHeme® [8]. Since this panel evaluates somatic mutations and most cancer-associated somatic mutations are heterozygous i.e. they affect only one allele, a VAF 47.3% in our patient's case implies that majority of cells carried the somatic *CCT6B* mutation. The large clone size likely represents a pathogenic variant. To strengthen this statement, assessing VAF on the post treatment blood specimens would have been ideal and would have also helped establish TKIs effect on *CCT6B* clone size, however, this could not be performed due to logistical reasons.

The treatment options and clinical outcomes for patients with CEL-NOS vary from treatment of underlying etiology to immunosuppression to targeted therapy. Targeted therapy with TKIs is indicated for patients with putative rearrangements [1]. When in doubt between CEL-NOS and IHES, hydroxyurea is preferred as a first line option. In our patient, use of hydroxyurea did not halt development of organ/tissue damage, and imatinib was subsequently initiated with good response. Our patient had a complete response to several TKIs (Fig. 1), with clinical benefit affirming that a subset of population with unknown mutational status may respond favorably to different therapies. As such, a broad hematological malignancy NGS panel in all patients with hyperosinophilia (after exclusion of secondary causes) can provide meaningful information to select first line therapy. There have been reports of unique molecular alterations in patients with eosinophilia, sensitive to TKI therapy[9–11] and a few highlighting resistances to TKI therapy [12]. Our case represents a molecular event that conferred TKI sensitivity, however, more research is needed to establish this finding.

Not surprising, NGS is now part of diagnostic work up in the recently published 2019 WHO guidelines update [13]. As data about newer somatic mutations continues to evolve, introduction of targeted therapy in the management of these patients will be an important step. At the same time, the growing knowledge about presence of somatic mutations in vasious eosinophilias especially IHES[14] will need careful interpretation and expertise as some of these events may represent clonal hematopoiesis of indeterminate potential (CHIP) in healthy individuals, which at present is of uncertain significance [15].

In summary, we report a rare mutation [*CCT6B,* not a known CHIP mutation] in a patient with hyperosinophilia responding exquisitely to multiple TKIs. Despite lack of defined molecular rearrangements, patient responded to TKIs and continues to maintain normal eosinophil count and excellent quality of life. Identification of this rare mutation as well as incidental discovery of other mutations with widespread use of NGS will have future implications in management of this rare disease. Future studies should include broad genomic panels that include this mutation to evaluate its pathogenicity.

Declaration of Competing Interest

E.W. serves on advisory board for Abbvie, Astellas, BMS/Celgene, Genentech, Jazz, Kite Pharmaceuticals, Macrogenics, Pfizer, PTC Therapeutics, Stemline, Takeda, BMS (Celgene). E.W. holds speaker role for Stemline, Pfizer, Dava Oncology and serves on data monitoring committees for Abbvie, Rafael Pharmaceuticals. Other authors disclose no conflicts of interest. All Authors listed have seen and approved the manuscript being submitted. The article is the Authors' original work. The article has not been submitted for publication nor has it been published in whole or in part elsewhere. The corresponding author bears full responsibility for the submission on behalf of all co-Authors, and all Authors listed on the title page have contributed significantly to the work.

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