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JAK2 V617F mutation in plasma cell-free DNA preceding clinically overt myelofibrosis: Implications for early diagnosis

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ABSTRACT

A 52 year-old man with Erdheim-Chester Disease (ECD) (a non-Langerhans polyostotic sclerosing histiocytosis) had next-generation sequencing (NGS) performed as part of his diagnostic workup. In addition to the tissue *BRAF V600E* mutation that is found in over 50% of ECD cases, he was also found to have a *JAK2* V617F alteration in cell-free circulating tumor DNA (ctDNA) (liquid biopsy). The latter was thought to be an "incidental" finding, perhaps due to clonal hematopoiesis (though this usually occurs in older individuals), as his blood counts were normal and he had no splenomegaly. Approximately 13 months after the ctDNA test showing *JAK2* V617F, he developed anemia, thrombocytopenia, and splenomegaly. Marrow biopsy then showed megakaryocytic atypia and markedly increased marrow fibrosis, consistent with WHO grade 2 of 3 myelofibrosis. Therefore, the patient was determined to have ECD with a typical *BRAF* V600E mutation, as well as primary myelofibrosis, with the latter diagnosis manifesting clinically over one year after the *JAK2* V617F was first detected in ctDNA. He recently was started on the *JAK2* inhibitor ruxolitinib. This case demonstrates that genomic alterations detected by liquid biopsy for evaluation of specific malignancies already present may serve as an early harbinger of hematological disease.

Introduction

Next-generation sequencing (NGS) is being incorporated into the management of patients with cancer. In some rare tumors, such as Erdheim-Chester Disease (ECD) (a non-Langerhans histiocytosis), NGS can assist in confirming the diagnosis, as a majority cases have a mutation of *BRAF* V600E.[1,2] However, in some cases, NGS testing will report mutations that may be unexpected or appear to be irrelevant. When NGS testing is performed on blood-derived circulating tumor DNA (ctDNA), mutations in *TP53, IDH2, DNMT3A, TET2, ASXL1 and JAK2* may be found; these alterations are common in myelodysplastic syndrome or other myeloid disorders.[3] However, a subset of elderly individuals can harbor these alterations and be without any clinical signs of a hematologic disease, a condition now called clonal hematopoesis of indeterminate potential (CHIP).[4] CHIP is found in over 10% of patients that are 80 years old or more, but at much lesser frequencies in patients that are younger.[5] While CHIP is associated with a subsequent diagnosis of hematologic malignancy (or cardiovascular disease), the overall incidence is only about 0.5% to 1.0% per year for hematologic cancer.[6] Therefore, the clinical context can help in the assessment of NGS findings. Here we present a case in which a seemingly incidental mutation of *JAK2* was found on NGS of ctDNA, with development of clinically overt myelofibrosis (a disease classically associated with the the *JAK2*

V617F alteration) about 13 months after the blood test. The implications for early diagnosis and monitoring of hematologic and other malignancies are discussed.

Case presentation

A 52 year-old Caucasian gentleman presented initially with eye swelling and a retro-orbital mass histologically consisting of CD68-positive foamy histiocytes (Fig. 1A and B) in the background of chronic inflammation. He was also found to have sclerotic lesions of long bones, including tibia, fibula, and femur, with epiphyseal sparing. Collectively these findings were most consistent with a diagnosis of Erdheim-Chester disease (ECD), as reviewed by our ophthalmic pathologist (JHL).[7]

To further evaluate the nature of the foamy histiocytes, the orbital mass tissue was assessed by targeted NGS, using the clinical-grade Foundation One panel (315 genes) (https://www.foundationmedicine.com). The NGS showed a *BRAF* V600E that is commonly associated with ECD, and further supported the diagnosis.[2] Sequencing of the orbital mass also demonstrated *ASXL1* R693* and *U2AF1* Q157P mutations, which were felt to be incidental and, at the time, not clinically relevant (Table 1).

The patient was initially treated with vemurafenib and achieved disease stability. However, vemurafenib was poorly
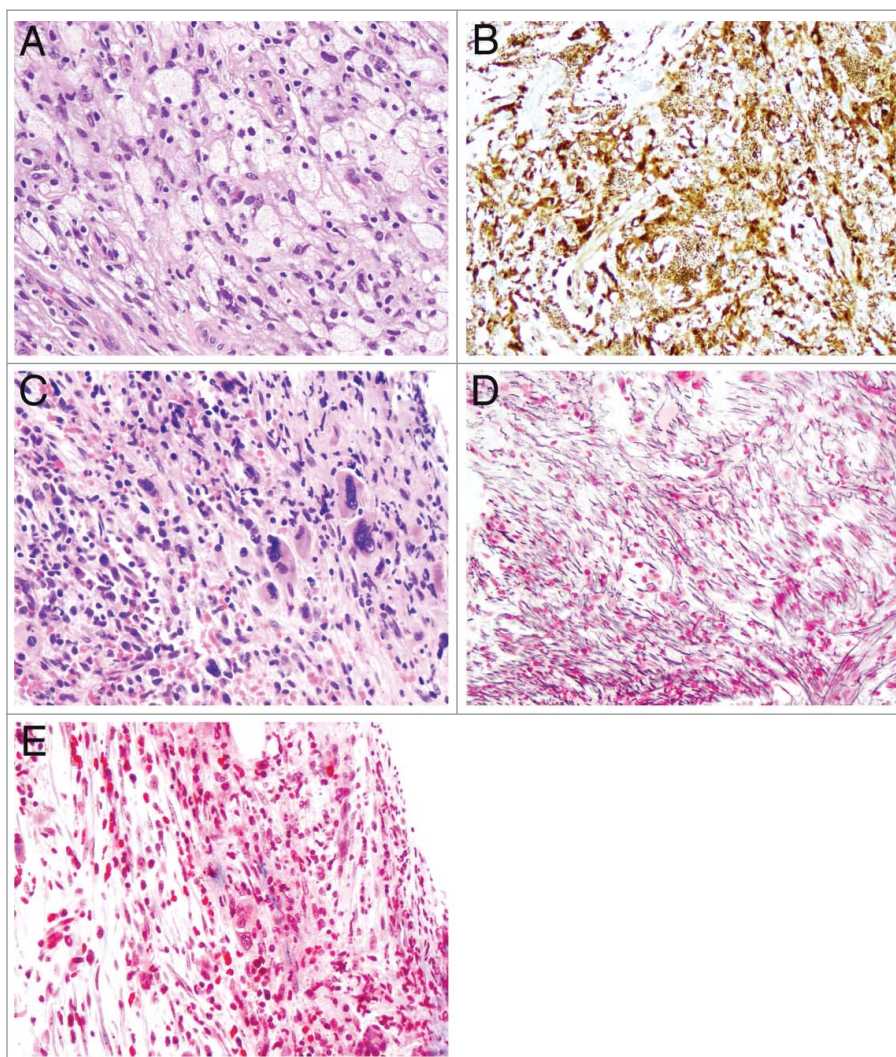
Figure 1. Microphotography of Erdheim Chester disease (ECD) and bone marrow fibrosis. A-B: There are numerous bland foamy histiocytes with abundant pale to clear cytoplasm (A) with immunoreactivity for CD68 (B) (original magnifications for A and B are 200x and 200x, respectively); C: The bone marrow core biopsy shows megakaryocytic hyperplasia, atypia and cellular streaming, indicative of fibrosis (original magnification 200x). D-E: Reticulin (D) and Trichrome (E) special stains show markedly increased reticulin fibrosis (D) and rare bundle of collagen (blue, E) (original magnifications of D and E are 200x and 200x, respectively).

tolerated due to arthralgias, rash, and visual changes, despite dose reduction. He was then treated with trametinib, but similarly had difficulty tolerating it due to conjunctival swelling and uveitis.

At that time, plasma was sent for assessment of ctDNA both as a biomarker of response and to identify other potential actionable alterations. We used the Guardant360 test (Guardant Health, Inc., http://www.guardanthealth.com/) (digital sequencing (73 genes) in a Clinical Laboratory Improvement Amendments (CLIA)-licensed, College of American Pathologist (CAP)-accredited, New York State Department of Health-approved clinical laboratory).[8] The BRAF V600E mutation was no longer detected, consistent with the fact that he had stabilized on BRAF and MEK inhibitors, which slow cell growth and turnover, leading to decreased shedding of abnormal ctDNA in the blood.[9,10] However, a mutation of JAK2 V617F was identified with a variant allele frequency (VAF) of 2.9%. In the absence of hematologic abnormalities, this anomaly was also interpreted as an incidental finding without clinical relevance.

He was then treated with pegylated interferon alfa-2b, with stability of ECD based on symptoms and radiographic findings. However, approximately 13 months after detection of the JAK2

alteration in ctDNA, he developed transfusion-dependent anemia (hemoglobin, 7 g/dL), thrombocytopenia (platelets 70–90^3/uL), and splenomegaly. A marrow core biopsy was performed and showed megakaryocytic atypia (Fig. 1C) and markedly increased marrow fibrosis by reticulin (Fig. 1D) and trichrome (Fig. 1E) stains, consistent with WHO grade 2 out of 3 myelofibrosis. There was no increase or aberrancy of myeloid blasts (not shown). Plasma was reassessed at this time by NGS of ctDNA and showed the following mutations: RIT1 M90V (VAF 4.0%), JAK2 V617F (3.2%), KRAS A59T (2.9%), and BRAF V600E (0.06%). (Table 1) Although KRAS and RIT1 have been associated with other malignancies, such as lung cancer, workup including a PET/CT scan did not reveal other malignancy.

Collectively, therefore, the patient is assessed to have ECD with a typical BRAF V600E mutation, as well as primary myelofibrosis, with a typical JAK2 V617F. The myelofibrosis was not clinically apparent when first detected. He recently started ruxolitinib therapy. A repeat marrow biopsy has not yet been assessed; however, he has had a clinical response, with a decrease in splenomegaly, from 16.1 cm craniocaudal

Table 1. Summary of molecular alterations and clinical findings.

Date of Assessment:	May 2014	December 2015	April 2017
Source for NGS: NGS results:	Orbital mass[1] *ASXL1* R693* *U2AF1* Q157P ***BRAF* V600E**	Blood (ctDNA)[2] ***JAK2* V617F (2.9%)**	Blood (ctDNA)[2] *RIT1* M90V (4.0%) ***JAK2* V617F (3.2%)** ***BRAF* V600E (0.06%)** *KRAS* A59T (2.9 %)
Intervention at the time of NGS assessment:	None	Vemurafenib followed by trametinib, starting in November 2014	Interferon alfa-2b, starting January 2016
Status of ECD		Stable disease	Stable disease
Status of myelofibrosis	Not clinically apparent: No anemia or thrombocytopenia	Not clinically apparent	WHO grade 2 myelofibrosis. Hgb 7.5, Platelet count 70

Bold: Associated with myelofibrosis. Other genomic alterations are potentially associated with ECD.
[1]315 gene NGS Foundation One.
[2]73 gene panel; Guardant.

dimension (4.1 cm greater than upper range of normal) to 13.9 cm (2.9 cm). He still remains dependent on packed red blood cell transfusions approximately monthly.

Discussion

This case illustrates an increasingly common scenario in which genomic evaluation of malignancy reveals unexpected findings, and it may be difficult to determine their significance or make sense of seemingly discrepant results. In this case, with the appropriate clinical context, *JAK2* V617F is very supportive of a diagnosis of a myeloproliferative neoplasm.[11] Similarly, mutations of *U2AF1, ASXL1,* and *RIT1* have been shown to occur commonly in myeloid neoplasms (Table 1).[12–15] The *U2AF1* and *ASXL1* genes found in tissue would not have been found in the ctDNA NGS targeted 73-gene panel because those genes are not covered. Why the *RIT1* M90V mutation was found in plasma but not tissue may reflect a tissue false negative due to spatial heterogeneity (present in a different location than the biopsied location) or temporal heterogeneity (mutation acquired after the tissue biopsy).

Seemingly incidental gene mutations may therefore represent very early stages of malignancy. The presence of asymptomatic premalignant states in hematologic cancers is well established. Monoclonal gammopathy of undetermined significance and monoclonal B-cell lymphocytosis very commonly precede the diagnoses of multiple myeloma and chronic lymphocytic leukemia, respectively. Similarly, in our patient, blood counts were normal and there was no splenomegaly at the time of initial ctDNA positivity for *JAK2 V617F*. Indeed, it was recently reported that *JAK2 V617F* mutated clones may be present at very low levels sometimes years prior to diagnosis.[16]

For those reasons, NGS may facilitate the detection of premalignant clones, and aid in determining prognosis. Indeed, the genes most commonly found to be mutated in healthy individuals include *DNMT3A, TET2,* and *ASXL1,* which have been shown to be early driver mutations that may result in a pre-leukemic state.[17,18] Similarly, *TP53* mutations have been identified at low levels several years before diagnosis of therapy-related acute myeloid leukemia (AML) and secondary myelodysplastic syndrome (MDS).[19] Monitoring for such mutations may be helpful in individuals at increased risk for developing secondary AML or MDS. Of relevance to our case is the recent finding

that there is a high prevalence (approximately 10%) of myeloid neoplasms in adults with ECD.[20]

On the other hand, clonal hematopoiesis may also be a benign condition, and this can confound the interpretation of NGS results. Blood cells of a proportion of healthy individuals without any clinically apparent hematologic abnormality can also be found to have such mutations, a condition called clonal hematopoiesis of indeterminate potential (CHIP).[21,22] This appears to be an aspect of aging, and such mutations can be found in greater than 10% of individuals over the age of 80; however, at age 52 (our patient's age), such mutations are uncommon.[6] Interestingly, CHIP is associated with a nearly two-fold risk of coronary heart disease, possibly due to increased inflammation.[22] CHIP is also associated with an increased relative risk for subsequent diagnosis of hematologic cancer, approximately ten times higher than the general population, though the absolute risk of hematologic cancer remains low, approximately 0.5-1% per year in persons with an incidental mutation.[4,6] Therefore, diagnosis of a hematologic malignancy is largely dependent on morphologic changes and clinical signs and symptoms.[23,24] As in this case, knowledge of mutations and their association with specific conditions may allow for a more streamlined diagnostic workup should blood count abnormalities develop in the future.

This case also illustrates the potential utility of monitoring ctDNA for genomic aberrations and early diagnosis. ctDNA is released to the circulation from cells undergoing cell death, and has been useful in aiding with the diagnosis of ECD, as well as other cancers, as an alternative or an adjunct to tissue biopsy.[8,25–31] The amount of mutant DNA may also correlate with disease burden or prognosis.[25–28,32] In this case, it appears that the variant allele fraction (VAF) of *BRAF* V600E correlated well with response to BRAF inhibitor treatment, becoming undetectable when on therapy, and then expanding at the most recent assessment to a low but detectable level when off vemurafenib and on interferon.

The clinician should be aware that genomic testing for established tumors may reveal CHIP mutations of hematopoietic origin. There are several relatively simple means of distinguishing a CHIP mutation from a somatic mutation due to a solid tumor or a disorder such as ECD. First of all, some of the genes that drive many hematologic malignancies are relatively uncommon in solid tumor malignancies and in conditions such as ECD.[33] Although one can find case reports of JAK2

mutations associated with cancers such as lung cancer, these may be related to tissue biopsies heavily infiltrated with lymphocytes. Notably drivers of lung cancer such as EGFR and ROS1 are not reported as drivers of hematologic conditions (although KRAS mutations may play a role in myelodysplastic syndromes).[34] Secondly, the VAF of the CHIP mutation is unlikely to change when the established malignancy is treated, unless the treatment would also be expected to impact the hematological disorder. For example, in this case, the *JAK2* V617F VAF in the liquid biopsy does not change over time (3.2% then 2.9%). A third potential differentiator is when the other somatic mutations cluster around one level of allele fraction, while the CHIP occurs at a much higher or lower VAF. This case was illustrated recently in a report by Zhang et al. where an IDH2 R140Q mutation was found at 19.6% VAF while the other somatic mutations clustered at VAFs two orders of magnitude lower, at 0.1%-0.2% VAF.[35]

Most importantly, our results suggest potential clinical utility of ctDNA analysis in early detection of malignant states. Indeed, our patient showed the *JAK2* V617F, a hallmark of myelofibrosis, in his ctDNA 13 months before any signs or symptoms suggestive of myelofibrosis. ctDNA may also be useful because dynamic changes in levels may indicate response to therapy, as seen in our patient and reported in the literature.[25] However, confounders to the interpretation of ctDNA for use as an early diagnostic tool exist, including the fact that benign lesions may harbor mutations often associated with malignancy[36] and that clonal hematopoiesis occurs in the elderly and does not always progress to malignancy.[5,6] Even so, as illustrated by the individual presented herein, ctDNA alterations can be a very early harbinger of cancer, indicating that further exploration of this modality for early diagnosis or prevention of hematological malignancy is needed, particular in high-risk individuals.

Declarations

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