



T315I-mutated myeloid sarcoma

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ARTICLE INFO

Keyword:

T315I mutation
Myeloid sarcoma
Chronic myelogenous leukemia

ABSTRACT

Myeloid Sarcoma (MS) is diagnosed by an extramedullary proliferation of immature granulocytic cells. Its association with chronic myeloid leukemia (CML) is rare. CML is characterized by *BCR-ABL1* gene rearrangement and therapies with tyrosine kinase inhibitors (TKI) are very effective. However, TKI resistance may occur secondary to the development of *ABL1* mutations. T315I is a common mutation that accounts for ~20% clinical resistance to TKIs. We report the first case of a patient with T315I mutated myeloid sarcoma that occurred after complete cytogenetic response with dasatinib of a chronic phase CML. The patient was successfully treated with induction chemotherapy and ponatinib.

1. Introduction

Myeloid sarcoma (MS) is a rare clinical scenario and is diagnosed by an extramedullary proliferation of blasts that disrupt the normal architecture of tissue in which it is found. MS is most commonly found concurrently with or following a diagnosis with acute myeloid leukemia, or as an isolated leukemic tumor. MS may also occur with a myeloproliferative neoplasm [1].

Chronic myeloid leukemia (CML) is a rare myeloproliferative disorder characterized by the presence of the chromosomal translocation t(9:22)(q34;q11) of *BCR-ABL1* fusion gene. Since the 20th century, first-line treatment with *BCR-ABL1* tyrosine kinase inhibitors (TKIs) revolutionized the treatment of CML by achieving long remission periods [2]. However, resistance has emerged. Threonine-to-isoleucine exchange at amino acid position 315 (T315I) can lead to resistance to most TKIs, and is only sensitive to ponatinib [3].

Despite marked reduction in the incidence of CML blast phase (BP) after introduction of TKI therapy, the transformation to BP still carries a very poor prognosis with a median survival of only 12 months. Extramedullary BP accounts for approximately 30% of cases with CML-BP with a predominance in myeloid immunophenotype. In the largest cohort of CMP-BP by Jain et al., myeloid immunophenotype and prior TKI therapy confers a shorter overall survival of 15% in 5 years. Treatment strategies that consist of the combination of TKI with chemotherapy followed by stem cell transplant (SCT) had a superior outcome compared to TKI alone [4].

To our knowledge, a T315I mutated MS has not yet been reported.

Here, we report a case of T315I-mutated MS that occurred after pre-existing chronic phase CML had a complete cytogenetic response in BM with Dasatinib treatment. The patient was successfully treated with induction chemotherapy and ponatinib.

2. Case report

Twenty-one year old African American male presented to an urgent care center in July 2017 with acute abdominal pain and a significant leukocytosis to $70 \times 10^3/\mu\text{L}$ (normal range: $4.6\text{--}10.2 \times 10^3/\mu\text{L}$) with 1% myeloblast, 3% promyelocytes, 5% metamyelocyte, and 75% neutrophils. Bone marrow (BM) biopsy report showed chronic phase CML with Philadelphia chromosome positive cells containing 46,XY,t(8:17)(p21;q25),t(9:22)(q34;q11.2) by karyotype in 20/20 cells. He was started on imatinib 400 mg daily. At 7 months, patient was evaluated at Moffitt Cancer Center. Fluorescence in situ hybridization (FISH) was performed and positive for *BCR/ABL1* gene rearrangement in 55% interphased cells identified. *BCR/ABL1* major (p210) fusion transcript was 296% International Scale (IS) by reverse transcriptase-polymerase chain reaction (RT-PCR) quantification analysis, while p190 transcripts were not detected. Repeat BM biopsy confirmed residual chronic phase CML without cytogenetic response with persistent t(9:22) and t(8:17) by karyotyping in 14/20 cells. He was switched to dasatinib 140 mg daily in March 2018. The BM biopsy at 3 months of Dasatinib therapy showed normal cytogenetics analysis, 46, XY[20] suggesting complete cytogenetic response (CCyR). No molecular response was achieved, as *BCR/ABL1* p210 RT-PCR on BM was 20.8% by IS. Repeat testing in

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<https://doi.org/10.1016/j.lrr.2019.100184>

Received 14 April 2019; Received in revised form 24 July 2019; Accepted 14 August 2019

Available online 17 August 2019

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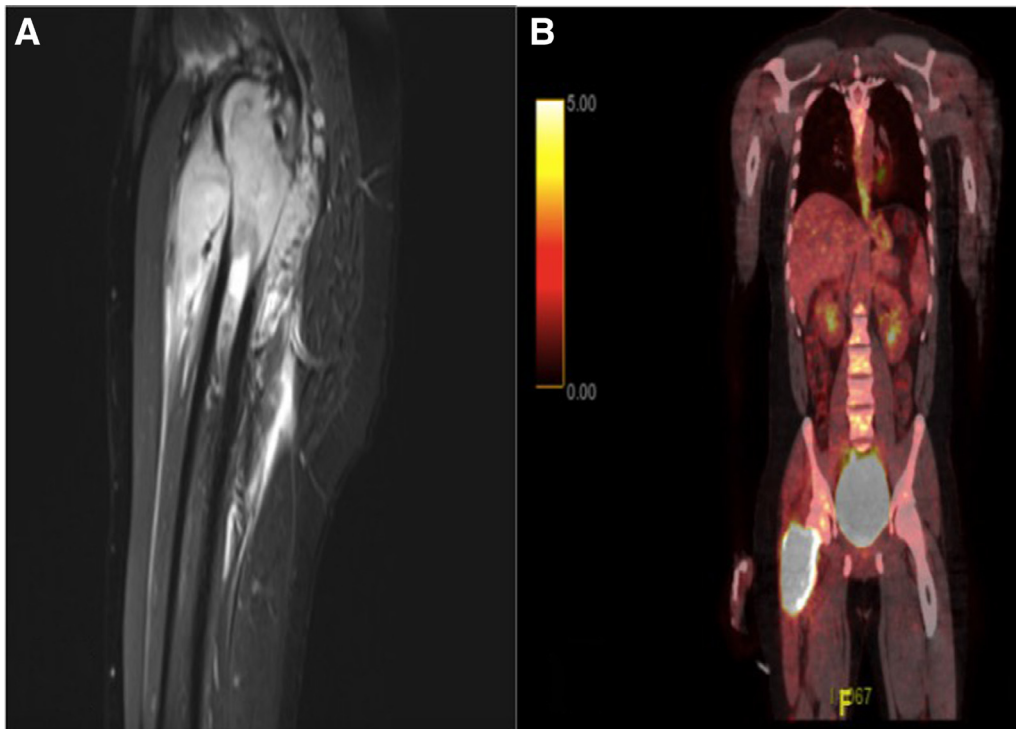


Fig. 1. A) MRI of large hyperintense mass in proximal femur with osseous involvement and satellite metastatic nodules. B) Whole Body PET/CT showed a large destructive proximal right femur lesion with avid uptake with SUV maximum 13.3.

blood at 4.5 months of Dasatinib therapy showed PCR of 9.7% by IS.

Five months after the initiation of Dasatinib therapy, he started to notice progressively worsening right hip pain. MRI of the right femur showed a 7.8 cm × 6.6 cm mass involving the proximal femur with bony destruction and extra-osseous extension to the adjacent musculature with satellite masses. Positron emission tomography-computed tomography (PET-CT) confirmed the mass with avid uptake with a maximum standardized uptake values (SUVs) of 13.3 (Fig. 1). The bone biopsy revealed diffuse infiltrate of large, atypical immature cells with eosinophilic cytoplasm, fine chromatin and occasional nucleoli, CD34+ and CD33+ immature myeloid cells, and *BCR-ABL1* gene rearrangement by FISH (24.5% nuclei by FISH) (Fig. 2). Next generation sequencing (NGS) using a commercial panel (Foundation One Heme) on the bone biopsy revealed an unexpected T315I mutation in a p190 *BCR-ABL1* fusion transcript with mutation allele frequency of 31.5% as well as *ETV6-BCR* fusion. The restaging BM biopsy showed a normocellular marrow with essential normal trilineage hematopoiesis (Fig. 2). Cytogenetics analysis was normal, 46, XY[20] and FISH analysis for *BCR/ABL1* gene rearrangement was negative. *BCR-ABL1* kinase domain mutation analysis on BM was also negative (level of detection at 0.1% IS). Discordantly, *BCR-ABL1* p210 transcripts per RT-PCR in peripheral blood remained 10.5% by IS. No central nerve system involvement was noted on cerebral spinal fluid studies. The patient was diagnosed with a blast phase of CML with MS and proceeded with induction chemotherapy consisting of 5-day cytarabine 2000 mg/m² and cladribine 5 mg/m² (CLA) followed by 2 cycles of 3-day consolidation therapy. Ponatinib 45 mg daily was also started on day 9 of CLA induction therapy. Four months after, a PET/CT showed a complete metabolic resolution of the right femur mass. The *BCR-ABL1* p210 transcripts per RT-PCR were also significantly reduced (0.2% by IS). Clinical response has been achieved based on imaging. He remained on maintenance Ponatinib 45 mg daily while awaiting an allogeneic stem cell transplant.

3. Discussion

T315I mutation is a single base substitution act>att resulting in a threonine-to-isoleucine exchange at amino acid position 315 on *BCR-ABL1* kinase domain. This threonine controls the access of the inhibitors to the active site, acting as a gate keeper, so its substitution to isoleucine leads to the lack of activity by the tyrosine kinase inhibitors [5]. T315I mutation has been well reported in CML and B-cell acute lymphoblastic leukemia. It mediates resistance to Imatinib, Dasatinib, Nilotinib and Bosutinib, and only retains sensitivity to Ponatinib [6]. This mutation accounts for about 20% total burden of clinical resistance in imatinib naïve patients with CML [7].

In our report, the patient with chronic phase CML was treated with Dasatinib after Imatinib failure with eventual complete cytogenetic response in the bone marrow. Despite this finding, the patient was later diagnosed with an isolated T315I mutated myeloid sarcoma of his femur. Cobin et al. has proposed that T315I mutation may already exist in patients prior to therapy. In the absence of therapy, it confers no growth advantage. When under selective pressure from TKIs therapy, it emerged as a predominant clone [8]. Furthermore, in our patient the detection of p190 transcript instead of p210 transcript (present in primary bone marrow diagnosis) in myeloid sarcoma also raises the possibility of p190 transcript emerging as a secondary event. Kurt et al. described a phenomenon with the acquisition of secondary Philadelphia (Ph) chromosome during therapy for acute leukemia and myelodysplastic syndrome. Interestingly, most cases are associated with p190 transcript subtype, and some cases received prior TKI therapies [9]. In our case, MS cells with p190 transcript Ph chromosome by NGS likely emerge after TKI therapy, and subsequently or simultaneously acquired T315I mutation, leading to resistance. However, it cannot be ascertained, because no ultra-sensitive sequencing was performed to evaluate the presence of very low T315 allele frequency at time of diagnosis. The question of emergence or selection in this case remains to be answered in future studies.

In addition, the *ETV6/BCR* fusion could also be clinically relevant here. *ETV6* encodes an ETS family transcription factors required for

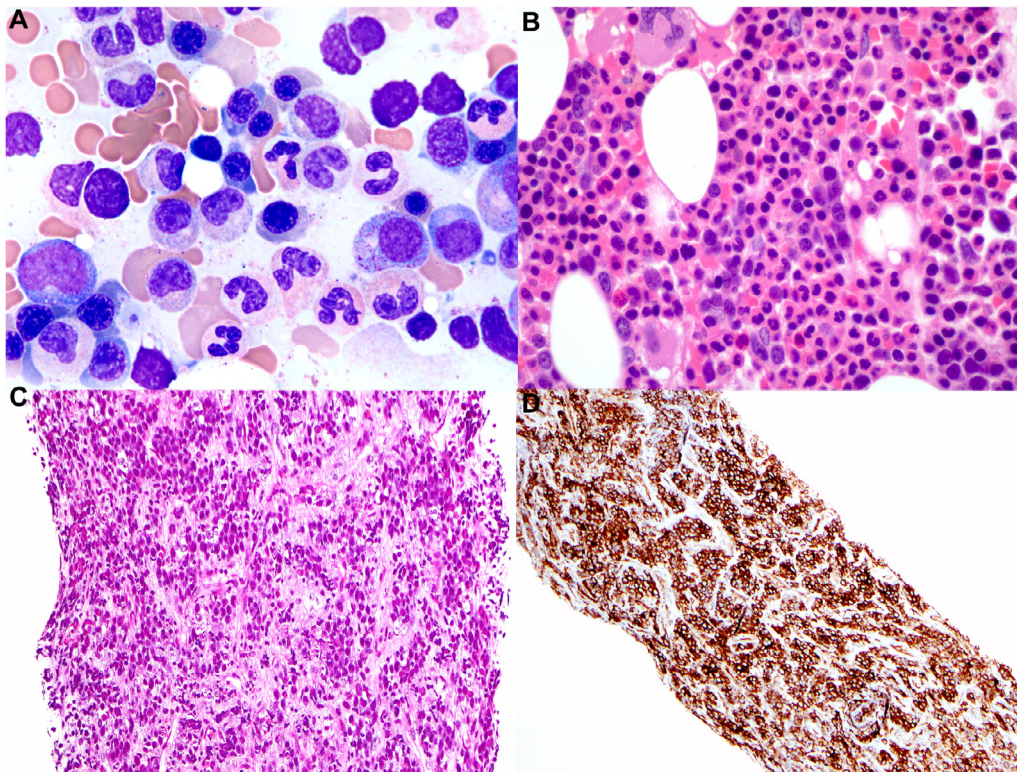


Fig. 2. A. Bone marrow aspirate shows adequate cellularity with complete myeloid maturation and essentially normal myeloid to erythroid ratio, consistent with hematologic remission, post treatment for chronic myeloid leukemia (Wright-Giemsa, x1000) and B. the bone marrow core shows normal trilineage hematopoiesis with adequate M:E ratio (H&E, x600). Core biopsy of femur. C) showed immature precursors with oval to irregular nuclei, fine chromatin, eosinophilic cytoplasm and inconspicuous nucleoli associated with fibrosis and scattered eosinophils (H&E, x100). D) immunohistochemical stain highlights the immature precursors/blasts to be CD34 positive (immunoperoxidase, x100).

hematopoiesis. ETV6 alterations result in loss of the N-terminal pointed oligomerization domain and/or the ETS DNA-binding domain, which were predicted to be inactivating [10]. In our case, its fusion with BCR gene could be an additional parameter for the extramedullary presentation of blast phase CML.

The successful treatment with CLA chemotherapy/ponatinib in this case also provides evidence that the combination of TKI and chemotherapy can effectively induce remission in CML-BP and MS, as previously reported [4]. However, innovative treatment strategies are needed to address molecular complexity of CML-BP and MS to improve its poor overall prognosis.

To our best knowledge, this is the first case report that describes the T315I mutated MS as a clonal and unique form of progression in a patient with CP-CML with otherwise adequate disease control by Dasatinib. This case also showed the importance of potential pre-existing or evolving clones leading to resistance and/or development of CML-BP, as well as the significance of an adequate TKI selection and its combination with chemotherapy in treating CML-BP and MS.

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