**JAK2-negative acute monocytic leukemia with TET2 mutation in essential thrombocythemia with JAK2 mutation with literature review**

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**Abstract**

Essential thrombocythemia (ET) is an indolent myeloproliferative neoplasm (MPN) with a transformation to acute myeloid leukemia in <5% of patients. A 79-year-old man with JAK2V617F-positive ET exhibited leukocytosis with an increase in monoblastic cells, leading to a diagnosis of acute monoblastic and monocytic leukemia. Leukemic cells carried a TET2 mutation but not JAK2V617F mutation. We concluded that the TET2 mutation occurred in MPN-initiating cells and overcame JAK2-mutated cells. The absence of a JAK2 mutation in the leukemic cells in this case suggests the leukemia emerged from a JAK2-negative MPN cell clone carrying the TET2 mutation.

**Keywords:** Essential thrombocythemia, Acute monocytic leukemia, Myeloproliferative neoplasm, 10–11 Translocation 2 protein, Janus activating kinase 2

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1. **Introduction**

**BCR-ABL1**-negative myeloproliferative neoplasms (MPNs) constituting polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF) are progressive clonal diseases. These diseases share common pathologic and clinical features, including genetic abnormalities such as the JAK2V617F mutation, which is found in almost 99% of PV cases and 60% of patients with ET and PMF [1]. Although the reported median survival of ET is approximately 20 years, which is better than that of other MPNs, a propensity to develop leukemic transformation apart from thrombo-hemorrhagic complications is a major constraint of life expectancy in these disorders. Leukemic transformation 10 years after diagnosis is estimated to occur in 2.3–14.4% of PV, 10–20% of PMF, and 0.7–3% of ET cases [2]. Secondary AML in these MPNs occurs spontaneously, but an increased risk of leukemic transformation following treatment with some cytoreductive agents is also recognized [1].

The 10–11 translocation 2 (TET2) protein is a DNA methylation regulator enzyme that converts 5-methylcytosine (5-mc) to 5-hydroxymethylcytosine (5-hmc), and a loss-of-function mutation has been identified in a patient with myeloid neoplasms [3]. A TET2 mutation might contribute to the onset and progression of hematopoietic tumors in cooperation with other driver genes. We describe a patient in whom acute monocytic leukemia developed four years after being diagnosed with ET. Leukemic cells were derived from a JAK2-mutation-negative clone that might have differed from a JAK2-mutation-positive ET clone. We discuss the mechanism of leukemic clone emergence and review the literature regarding secondary leukemia in ET.

2. **Case presentation**

A 79-year-old man diagnosed with ET was found to carry the JAK2V617F mutation at age 75 in 2011. Laboratory tests revealed hemoglobin (Hb) 14.9 g/dL; platelets 1,191 × 10^9/L; white blood cells (WBC) 12.4 × 10^9/L with no immature cells; lactate dehydrogenase (LDH) 436 IU/L (normal, 142–246 IU/L); and the bone marrow was normocellular, with megakaryocyte hyperplasia but no significant fibrosis. The patient was treated with hydroxyurea and aspirin. He had a fever in January 2015 and presented with appetite loss and general fatigue in December 2014 and presented with appetite loss and general fatigue in January 2015. A physical examination revealed marked splenomegaly and no palpable superficial lymph nodes. He was covered with a maculopapular rash. Computed tomography revealed mild hepatomegaly and massive splenomegaly. Peripheral blood revealed Hb 10.1 g/dL, platelets 12.6 × 10^9/L, and WBC 53. × 10^9/L, with 3.5% of blasts containing prominent nucleoli and vacuoles in the basophilic cytoplasm. Biochemical findings revealed LDH 3,164 IU/L, lactate 367 μg/mL (normal, 5.0–10.2 μg/mL), uric acid 12.8 mg/dL, creatinine 1.42 mg/dL, aspartate transaminase 370 IU/L, alanine transaminase 103 IU/L, and alkaline phosphatase 120 IU/L. The patient was diagnosed with acute myeloid leukemia in <5% of patients.

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There were 16.0 × 10⁴/μL and 63.0/μL nucleated cells and megakaryocytes, respectively. Bone marrow examination showed hypercellular marrow with 81.4% monoblastic cells (Wright-Giemsa stain) (a) positive on peroxidase staining and positive on non-specific esterase staining (b), with NAF inhibition of esterase staining (c). Expression of p53 on tumor cells by immunohistochemistry using an antibody that recognizes both wild type and mutant forms of p53 protein (HISTOFINE, Nichirei Biosciences, Tokyo) was not found at the diagnosis of ET (d) but was detected in the nucleus of leukemic cells at the development of AML (e). A TET2 mutation (indicated by the arrow) but not JAK2 mutation (data not shown) was detected in leukemic cells at the development of AML, while the JAK2 mutation was found at the diagnosis of ET (data not shown) (f).
## Table 1
Reported cases of AML following ET after JAK2 era.

| Case | Report                       | Age at the onset of AML | Sex  | Therapy | AML subtype (FAB) | karyotype                        | JAK2V617F at the onset of ET | JAK2V617F at the onset of AML | TET2 onset AML | Survival |
|------|------------------------------|-------------------------|-------|---------|------------------|----------------------------------|------------------------------|------------------------------|----------------|----------|
| 1    | Jelinek J. et al. (2005) [6] | 52                      | M     | NA      | M7               | Complex del 9(q22)              | Positive                     | NA                          | NA              | 5M       |
| 2    | 51                           | F                        | NA    | M       | M7               | Complex                          | Positive                     | NA                          | NA              | 3M       |
| 3    | Au WY et al. (2006) [7]      | 59                      | F     | HU/splenectomy/HSCT | NA   | 46XX                | Positive                      | Negative                   | NA              | NA       |
| 4    | Au WY et al. (2007) [8]      | 64                      | M     | HU/splenectomy/HSCT | NA   | 46XY                | NA                           | Positive                   | NA              | NA       |
| 5    | Theocharides A. et al. (2007) | 91                      | M     | HU      | NA               | -Y                               | Positive                      | Negative                   | NA              | NA       |
| 6    | 54                           | F                        | no    | M       | M1               | t(10;16)                        | Positive                      | Negative                   | NA              | NA       |
| 7    | 71                           | M                        | HU    | M       | M6               | Complex del(5q),+7              | Positive                      | Negative                   | NA              | NA       |
| 8    | 72                           | F                        | HU    | M       | M4               | Complex del(5q)                 | Positive                      | Negative                   | NA              | NA       |
| 9    | 69                           | F                        | HU/Thal | NA    | M2               | Complex del(5q), del(20q), -5,-7, +8 | Positive                    | Positive                   | NA              | NA       |
| 10   | 78                           | F                        | NA    | M       | M2               | Complex del(20q), -5,-7, +8    | Positive                      | Positive                   | NA              | NA       |
| 11   | 74                           | F                        | HU/Pipo | NA    | NA               | NA                               | Positive                      | Positive                   | NA              | NA       |
| 12   | 74                           | M                        | HU    | M       | M4               | NA                               | Negative                      | Negative                   | NA              | NA       |
| 13   | 73                           | F                        | HU/32P/IFN/Ana | NA   | M2               | NA                               | Negative                      | Negative                   | NA              | NA       |
| 14   | 49                           | M                        | HU    | M       | M7               | Complex                          | Negative                      | Negative                   | NA              | NA       |
| 15   | 83                           | F                        | HU    | M       | M7               | Complex del(5q), -7             | Negative                      | Negative                   | NA              | NA       |
| 16   | 77                           | F                        | HU    | M       | M2               | NA                               | Negative                      | Negative                   | NA              | NA       |
| 17   | Hsiao H-H et al. (2008) [10] | 78                      | M     | HU      | NA               | 46XY                            | Positive                      | Negative                   | NA              | NA       |
| 18   | Beer PA et al. (2010) [11]   | 63                      | M     | HU      | NA               | inv(3),del(7)                   | Positive                      | Positive                   | NA              | NA       |
| 19   | 73                           | M                        | HU    | NA      | del(5),t(5;17)   | NA                               | Positive                      | Positive                   | NA              | NA       |
| 20   | 86                           | F                        | HU/BU | NA      | NA               | NA                               | Positive                      | Positive                   | NA              | NA       |
| 21   | Braun TP. et al. (2015) [12] | 83                      | F     | ruxolitinib | M3   | t(15;17)         | Positive                      | Positive                   | NA              | 18M      |
| 22   | Present case                 | 79                      | M     | HU      | M5b              | trisomy 8                       | Positive                      | Negative                   | Positive                   | 5M       |

HU: Hydroxyurea, 32P: phosphorus 32, IFN: interferon-α, Thal: thalidomide, Pipo: pipobroman, BU: busulphan, Ana: anagelide, HSCT: hematopoietic stem cell transplantation, NA: Not Available.
499 IU/L, and alkaline phosphatase 1806 IU/L. Bone marrow examination showed that 81.4% of monoblasts were positive for non-specific esterase with NaF inhibition (Fig. 1a–c). Flow cytometry revealed the expression of CD13 (88.9%), CD33 (77.5%), CD14 (81.4%), and CD36 (96.7%), as well as HLA-DR (91.3%) on leukemic cells. These findings were compatible with a diagnosis of acute monoblastic and monocytic leukemia according to the WHO classification 2016 and AML M5b according to the FAB classification. Cytogenetic analysis of bone marrow cells revealed a trisomy 8 abnormality in 12 of 20 divided cells. An immunohistochemical study of bone marrow that had been negative on autopsy. Bone marrow was occupied by leukemia cells without apoptosis. The patient died several months after being diagnosed with AML. Extramedullary transformation showed that 81.4% of monoblasts were positive for non-specific esterase with NaF inhibition (Fig. 1d and e). Although the JAK2V617F mutation was detected at the onset of ET, it was not evident in leukemic cells at the time of AML development. We further examined the TET2 mutation in bone marrow specimens and found a point mutation in TET2 isoform 1 (codon 4671 from G to T; Fig. 1f). The patient was started on induction chemotherapy with idarubicin (6 mg/m², 2 consecutive days) and cytarabine (50 mg/m², 5 consecutive days). Two courses of this treatment normalized the JAK2 mutation and that aberrant TP53 expression and progression to a leukemic clone. Model 3 indicates that a JAK2-allelic subclone was derived from a JAK2+ ET clone that acquired the TET2 mutation as a second hit (JAK2 first/JAK2 clone) and then developed into leukemic clones with additional abnormalities, including aberrant TP53 expression. Model 4 proposes that a TET2+ clone emerged independently of the JAK2+ ET clone and acquired aberrant TP53 expression and caused de novo AML.

3. Discussion

Both intrinsic disease-related and extrinsic therapy-related factors are involved in leukemic transformation. Employing certain cytotoxic agents such as 32P and alkylators leads to increased risk of leukemic transformation in MPN. However, controversy remains over the leukemogenic risk of hydroxyurea, an anti-metabolite agent frequently used in treating the chronic phase of MPNs [1]. Bjorkholm et al examined 162 patients with MPNs, including ET with AML/myelodysplastic syndrome, and they found that hydroxyurea monotherapy was not significantly associated with an increased risk of leukemic transformation [4]. In contrast, the observational EXEL study, which involved 3649 ET patients treated with hydroxyurea and/or anagrelide and included 67 secondary AML cases, reported a high incidence ratio for AML in hydroxyurea-treated patients [5]. Given these results, it cannot be completely ruled out that the hydroxyurea used as a prior therapy in this case contributed to AML transformation. We searched the English-language literature and found case reports that described 22 patients with AML secondary to ET since 2005, including the present patient (Table 1) [6–12]. Although various AML subtypes have been identified according to the FAB classification, only our patient had the M5 subtype. At the time of ET diagnosis, 16 of 22 patients reported in the literature were positive for the JAK2V617F mutation (referred to hereafter as JAK2), and only 7 of these patients were JAK2 positive at the time of AML development, suggesting that a JAK2-negative leukemic clone was derived from MPN progenitor cells without a JAK2 mutation [13]. While four patients had complex karyotypic abnormalities, trisomy 8 was found only in our patient.

Among the recently reported variety of genetic mutations in addition to JAK2, a mutation involved in epigenetic regulation is thought to contribute to both disease progression and leukemic transformation. TET2 is a major epigenetic regulator, and a loss-of-function mutation is found in 10–20% of chronic MPN cases and 13–32% cases of leukemic transformation of MPN [14,15]. Lundberg et al. suggested that the TET2 mutation precedes the JAK2 mutation and that aberrant TET2 and TP53 play roles in the development of leukemia [16]. Consistent with this hypothesis, the TET2 mutation and p53 expression were found in leukemic cells of the current patient, indicating that these genetic aberrations played an important role in the pathogenesis of the leukemic transformation in this patient.

Fig. 2 shows four models that might explain the origin of the JAK2-negative TET2-positive (JAK2−/TET2+) AML clone seen in our case. Model 1 involves the derivation of a JAK2+ ET clone from a TET2+ clone (TET2 first/JAK2), with the remaining JAK2-TET2+ ET clone developing into leukemic cells with additional genetic changes, including TP53. Model 2 indicates that a JAK2-allelic subclone emerged from a TET2 first/JAK2 clone, accompanied by aberrant TP53 expression and progression to a leukemic clone. Model 3 indicates that a JAK2-allelic subclone was derived from a JAK2+ ET clone that acquired the TET2 mutation as a second hit (JAK2 first/JAK2) and then developed into leukemic clones with additional abnormalities, including aberrant TP53 expression. Model 4 proposes that a TET2+ clone emerged independently of the JAK2+ ET clone and acquired aberrant TP53 expression and caused de novo AML.
independently, as in our model 4, cannot be excluded as an explanation for the situation of the present patient [11]. We could not find any other description of acute monocytic leukemia accompanied by a TET2 mutation arising from ET in the English-language literature. Considering that hematopoietic progenitor cells are prone to differentiate into myelomonocytic cells by inhibiting TET2 expression [18], the TET2 mutation might have functioned in the development of acute monocytic leukemia in our patient.

An effective treatment for AML secondary to MPN including ET has not been established, and thus, the prognosis is very poor, as evidenced by our patient. However, azacitidine has reportedly induced complete remission in a patient with leukemic transformation of MPN [19], suggesting that a regimen incorporating azacitidine should be assessed to establish an effective therapy against AML with epigenetic mutations secondary to MPNs such as ET.

Declaration of Competing Interest

The authors declare no conflict of interest.

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