



Emergence of t(3;21)(q26.2;q22) during eltrombopag treatment in a patient with relapsed aplastic anemia who received chemotherapy for angioimmunoblastic T-cell lymphoma

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ABSTRACT

A 65-year-old man with nonsevere aplastic anemia received rabbit anti-thymocyte globulin and cyclosporine and partially responded. Six months after the initiation of treatment, he was diagnosed with stage IV angioimmunoblastic T-cell lymphoma and received chemotherapy. PET/CT scan analysis indicated a complete response. However, he showed sustained myelosuppression and was diagnosed with relapse of aplastic anemia. He did not respond to cyclosporine, eltrombopag or methenolone. Fifteen months after eltrombopag administration, he developed MDS with t(3;21)(q26.2;q22). Patients should be monitored carefully for the emergence of not only -7/del(7q) but also 3q26 abnormalities, including t(3;21)(q26.2;q22), during and after eltrombopag treatment.

1. Introduction

Eltrombopag (EPAG), a thrombopoietin-receptor agonist, has improved the treatment outcomes of patients with aplastic anemia. Hematological response to EPAG as a single treatment was observed in approximately 40% of patients with refractory aplastic anemia [1, 2]. In addition, immunosuppressive therapy (IST) with anti-thymocyte globulin and cyclosporine in combination with EPAG for previously untreated severe aplastic anemia showed a higher overall response rate than IST alone (94% vs 66%) [3].

Patients with aplastic anemia are at risk for developing clonal cytogenetic abnormalities after IST, which could lead to progression to myelodysplastic syndromes (MDS) or acute myeloid leukemia (AML). The incidence of MDS or AML after IST is 2–4% at 5–6 years and approximately 15% at 10 years [4]. There have also been concerns about the emergence of chromosomal abnormalities after EPAG administration in patients with aplastic anemia, unlike those with immune thrombocytopenia. Clonal cytogenetic abnormalities are observed in 8% of patients newly treated with a combination of EPAG and IST at 2 years [3]. In that study, chromosome 7 cytogenetic abnormalities were

detected at 3–6 months in 4% of patients after the combination treatment. Cytogenetic abnormalities of unclear significance such as del(13q), or, +6 and +15, were observed in one each patient as well. Other studies also reported that approximately 13% of patients with refractory aplastic anemia treated with EPAG alone developed cytogenetic abnormalities at 3 months after initiation [5]. Patients with aplastic anemia who developed chromosome 7 abnormalities or complex karyotypes, compared to trisomy 8, had a higher risk of progression to MDS or AML [6]. The emergence of chromosomal abnormalities associated with poor prognosis in a short period is a serious concern after EPAG administration.

Here, we report a patient with a history of lymphoma who rapidly progressed to MDS/AML with t(3;21)(q26.2;q22) after EPAG treatment for relapsed aplastic anemia.

2. Case presentation

A 65-year-old man with pancytopenia (hemoglobin, 82 g/L; polymorphonuclear cells, $0.9 \times 10^9/L$; platelets, $39 \times 10^9/L$; reticulocytes, 1.1%) was referred to a hospital without any clinical symptoms. He was

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transfusion dependent and received 2 units of red blood cell and 30 units of platelet transfusion for one month after the initiation of immunosuppressive therapy. The bone marrow biopsy revealed a hypocellular and fatty marrow with a cellularity of 17%. No megakaryocytes were observed. Neither dysplastic cells in the granulocytic lineage nor other morphological bone marrow abnormalities were noticed. The cytogenetic analysis showed a normal karyotype. The presence of paroxysmal nocturnal hemoglobinuria-type red blood cells or granulocytes was not assessed. The patient was diagnosed with nonsevere aplastic anemia according to the modified Camitta criteria [7] and received rabbit anti-thymocyte globulin (ATG) at a dose of 2.5 mg/kg for 5 days and cyclosporine at a dose of 6 mg/kg/day for 6 months by monitoring the serum concentration. He achieved a partial response at 3 months after the treatment according to the response criteria established by Camitta [8]. At four months after the treatment, he presented an enlarged left neck lymph node without B symptoms and visited Dokkyo Medical University Hospital. A left neck lymph node biopsy was performed 6 months after the diagnosis of aplastic anemia and revealed angioimmunoblastic T-cell lymphoma (AITL). Immunohistochemical and *in situ* hybridization analyses showed that lymphoma cells were positive for CD 3, CD 5, and EBV-encoded small RNA (EBER). An FDG-PET/CT scan showed increased uptake in the abdominal lymph nodes, the right adrenal gland, and the sternum. The bone marrow aspiration revealed that nucleated cell and megakaryocyte counts were $4.3 \times 10^4/\mu\text{L}$ and $15/\mu\text{L}$, respectively, and myeloid/erythroid (M/E) ratio was 0.99 with 1% of blasts and 16.4% of lymphocytes but without lymphoma cell infiltration. The bone marrow biopsy showed that the bone marrow cellularity was 11% without dysplastic cells. G-banding analysis revealed 45, X, -Y in 4 cells, 46, XY, t(10;14)(p15;q24) in 1 cell, and 46, XY in 15 out of 20 metaphases analyzed. The patient was diagnosed with stage IVA AITL. He was classified as high-risk according to the Prognostic Index for AITL (PIAI) [9]. He then received a THP-COP regimen consisting of 500 mg/m² of cyclophosphamide, 30 mg/m² of pirarubicin, 1 mg/m² of vincristine on day 1 and 30 mg/m² of prednisolone from day 1 to day 5 at monthly intervals. The dose of cyclophosphamide was reduced to 75% due to kidney dysfunction. Cyclosporine for the treatment of aplastic anemia was administered for a total of 9 months and was ceased upon the diagnosis of AITL. The patient discontinued the THP-COP regimen after five cycles due to severe myelosuppression. He achieved a complete response for AITL as determined by the PET/CT scan results. He had prolonged pancytopenia for two months after the last cycle of chemotherapy (polymorphonuclear cells, $0.6 \times 10^9/\text{L}$; platelets, $11 \times 10^9/\text{L}$; reticulocytes, 0.7%). He received 6 units of red blood cell transfusion and 20 units of platelet transfusion a month. The bone marrow aspiration showed 0.8% of blasts. The bone marrow biopsy showed hypocellularity with a cellularity of 10% and no dysplasia was observed. G-banding analysis revealed 45, X, -Y in 3 cells and 46, XY in 17 out of 20 metaphases analyzed. The possibility of MDS was ruled out based on a lack of chromosomal abnormalities and dysplasia. He was diagnosed with relapse of aplastic anemia 9 months after the diagnosis of AITL. He was treated with cyclosporine (6 mg/kg/day) for 18 months, EPAG (100 mg/day) for 15 months and methenolone (20 mg/day) for 3 months without significant hematologic improvements. Although he remained transfusion-dependent during EPAG administration, white blood cell count was rising gradually as shown in Fig. 2. As a result, EPAG therapy was continued for 15 months. He received 8 units of red blood cell transfusion and 40 units of platelet transfusion a month. Fifteen months after the EPAG administration, which was 17 months after the relapse of aplastic anemia, a bone marrow biopsy revealed that bone marrow cellularity was 17% with 2.2% of blast and trilineage dysplasia (Fig. 1A). No blasts were observed in the peripheral blood. Flow cytometric analysis showed that the blasts expressed CD13, CD33, CD34, and glycophorin A. G-banding analysis revealed 45, X, -Y, t(3;21)(q26.2;q22), add(7)(q11.2) in all 20 of the metaphases analyzed (Fig. 1B). Spectral karyotyping (SKY) identified add (7) (q11.2) in G-banding as der (7) t (1; 7) (q23; q11.2) (data not shown). Nested

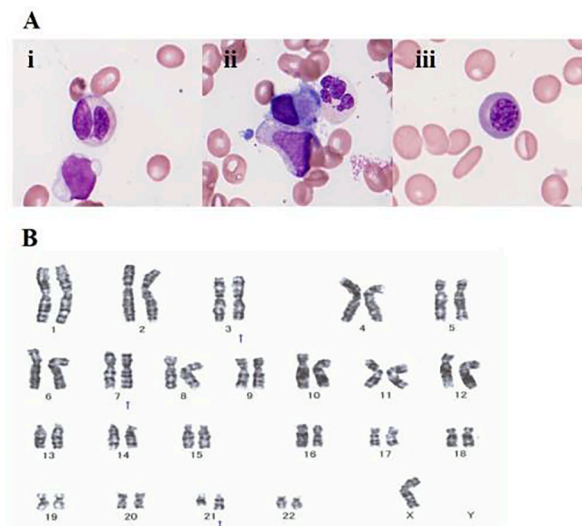


Fig. 1. Morphological and cytogenetic analyses of bone marrow cells at the time point of MDS development. (A) Bone marrow smear illustrating i) pseudo-Pelger anomaly ii) micromegakaryocyte and iii) megaloblastic change. (B) G-banding analysis: the arrows indicate t(3;21)(q26.2;q22) and add(7)(q11.2).

reverse transcription-polymerase chain reaction (RT-PCR) analysis performed using *RUNX1* and *MECOM* primers as previously described [10] revealed the *RUNX1-MECOM* fusion transcript. Sequencing of the PCR product indicated that exon 8 of *RUNX1* was fused to exon 2 of *MECOM* (data not shown). Based on these findings, the patient was diagnosed with therapy-related MDS and MDS with multilineage dysplasia according to the 2017 WHO classification [11]. He was classified as high-risk according to the Revised International Prognostic Scoring System [12]. He then received six cycles of azacitidine therapy. Azacitidine (75 mg/m²/day) was administered for 7 or 5 days per one cycle. However, no hematological improvements were observed, and he progressed to AML 8 months after the initiation of azacitidine therapy. Blasts in the peripheral blood were 5%. The bone marrow's cellularity was 33% with 23.4% of blasts that were positive for CD13, CD33, CD34, CD41 and glycophorin A. G-banding analysis revealed 45, X, -Y, t(3;21)(q26.2;q22.1), der(7)t(1;7)(q23;q11.2) in 10 cells and 46, idem, +8 in 9 cells out of 20 metaphases analyzed. He received an induction therapy with 40 mg/m²/day of daunorubicin for 3 days and 200 mg/m²/day of enocitabine for 8 days. Because blasts in the peripheral blood and the bone marrow were 8% and 8.2% after the induction therapy, we considered it as a failure. He subsequently received a MEC reinduction therapy with 6 mg/m²/day of mitoxantrone, 40 mg/m²/day of etoposide and 1 mg/m²/day of cytarabine for 6 days. The dose of etoposide was reduced to 50% due to liver dysfunction. After the reinduction therapy, the bone marrow aspiration showed 70.2% of blasts. He died 7 months after the diagnosis of leukemia due to respiratory failure caused by infectious pneumonia or leukemic infiltration. The clinical course of the patient is summarized in Fig. 2. In summary, this patient with aplastic anemia received immunosuppressive therapy with ATG + cyclosporine, followed by the diagnosis of AITL six months later. Five cycles of THP-COP therapy resulted in relapse of aplastic anemia nine months after the diagnosis. Fifteen months after the initiation of eltrombopag administration for relapsed aplastic anemia, he developed MDS with t(3;21), leading to transformation of AML eight months later.

We performed targeted-capture sequencing of bone marrow samples at the time of aplastic anemia relapse and at the time of progression to MDS and AML for a panel of common driver genes implicated in myeloid malignancies [13]. The analysis was approved by the Institutional Review Boards of Clinical Research at Dokkyo Medical University and Kyoto University and performed in accordance with the Declaration of Helsinki. Written informed consent was obtained. At relapse of aplastic

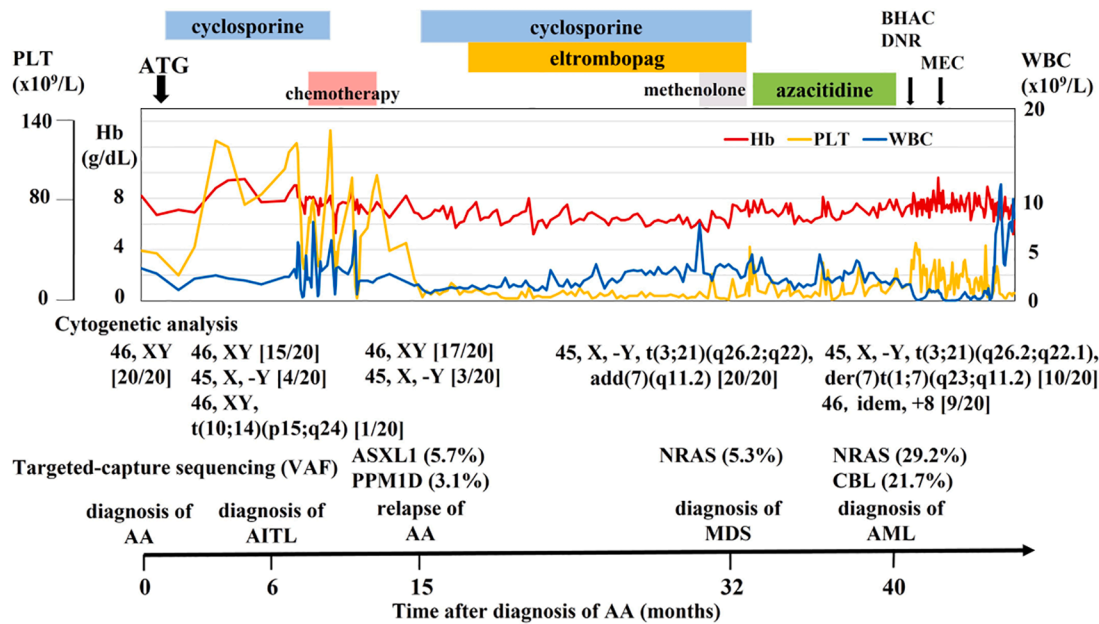


Fig. 2. Clinical course and results of cytogenetic and targeted-capture sequencing analyses of bone marrow cells. The patient with aplastic anemia received immunosuppressive therapy with ATG + cyclosporine, followed by the diagnosis of AITL six months later. Five cycles of THP-COP therapy resulted in relapse of aplastic anemia nine months after the diagnosis. Fifteen months after eltrombopag administration for relapsed aplastic anemia, he developed MDS with t(3;21), leading to transformation of AML eight months later. ATG, anti-thymocyte globulin; BHAC, enocitabine; DNR, daunorubicin; MEC, mitoxantrone, etoposide, cytarabine; VAF, variant allele frequency.

anemia, mutations in *ASXL1* Q432X and *PPM1D* T416fs were identified at variant allele frequencies (VAFs) of 5.7% and 3.1%, respectively. However, at the development of MDS, these mutations completely disappeared, and the *NRAS* G12D mutation was identified at a VAF of 5.3%. Furthermore, clones with *NRAS* G12D (VAF of 29.2%) and *CBL* C384R (VAF of 21.7%) mutations became dominant at the progression to AML.

3. Discussion

3q26 chromosomal abnormalities such as t(3;3)(q21;q26) and inv(3)(q21q26) is one of characteristic abnormalities in megakaryoblastic leukemia, and leukemic cells with these chromosomal abnormalities may be expanded by thrombopoietin and thrombopoietin-receptor agonists. Actually, the emergence of 3q26 chromosomal abnormalities such as t(3;3)(q21;q26) and inv(3)(q21q26) has been reported in two patients after EPAG treatment [3,5]. This is the third case of 3q26 abnormalities emerging after EPAG treatment. The t(3;21)(q26.2;q22) translocation observed in this case is a member of 3q26 abnormalities, and is frequently observed in patients with myeloid leukemia of the megakaryoblastic origin and therapy-related MDS/AML [10,14]. This translocation generates *RUNX1-MECOM* overexpressing MECOM, similar to other 3q26 chromosomal abnormalities. In this case, chemotherapy for AITL might have induced t(3;21), but it was unclear when t(3;21) emerged. To clarify the timing of its emergence, we performed RT-PCR using bone marrow sample at the time of aplastic anemia relapse after chemotherapy, but the *RUNX1-MECOM* fusion transcript was not amplified. However, considering that bone marrow was completely occupied by the cells with t(3;21) at 15 months after EPAG treatment, the administration of EPAG might have at least rapidly expanded the pre-existing cells with t(3;21). This patient also had add(7q), which had a pathogenetic significance comparable to that of del(7q). It is interesting to note that other 3q26 abnormalities, such as t(3;3) and inv(3), are usually associated with -7/del(7q) and could collaborate to transform hematopoietic stem cells/megakaryoblastic progenitors.

We performed targeted-capture sequencing at three time points during the clinical course: at relapse of aplastic anemia, at diagnosis of

MDS and at progression to AML. These results were useful for understanding changes in the clonal structure of bone marrow cells. At the time of aplastic anemia relapse, *ASXL1*- and *PPM1D*-mutated clones were identified in a minor portion of bone marrow cells. However, at the diagnosis of MDS, these mutations-carrying cells were not detected. Instead, all the cells possessed t(3;21) and some of these cells had an *NRAS* G12D mutation. Further studies are required to clarify how *RUNX1-MECOM* and -7/del(7q) or *NRAS* mutation could collaborate to induce leukemia.

4. Conclusion

The 3q26 abnormalities are rarely observed after IST but are reported in a few cases after EPAG treatment. Therefore, the 3q26 abnormalities can be considered as one of characteristic chromosomal abnormalities after EPAG treatment. EPAG might stimulate pre-existing clones with 3q26 abnormalities. During EPAG treatment, patients, especially with a history of chemotherapy, should be monitored for the emergence of these chromosomal abnormalities every 6 months by cytogenetic and fluorescence *in situ* hybridization (FISH) analyses on the bone marrow cells. Patients with 3q26 abnormalities, especially in combination with -7/del(7q), are considered to be at high risk for developing MDS and AML, even when observed in a few metaphases.

Author contributions

Fumi Nakamura: Writing - Original Draft, **Yuka Nakamura:** Visualization, **Yasuhito Nannya:** Formal analysis, Data Curation, Writing - Review & Editing, **Honoka Arai:** Resources, **Kei Shimbo:** Resources, **Yuko Nakamura:** Resources, **Sachiko Seo:** Writing - Review & Editing, **Ko Sasaki:** Writing - Review & Editing, **Motoshi Ichikawa:** Writing - Review & Editing, **Seishi Ogawa:** Supervision, **Kinuko Mitani:** Supervision, Writing - Review & Editing

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Declaration of Competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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