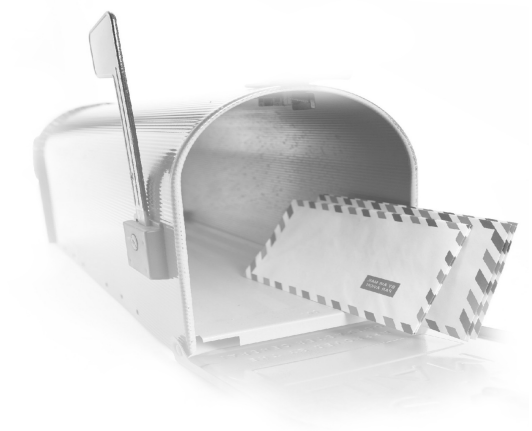

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Letters to the Editor

Acute myeloid leukemia with t(4;12)(q12;p13): report of 2 cases

TO THE EDITOR: Acute myeloid leukemia (AML) with t(4;12) is rare, but its unique morphologic and clinical characteristics have been described in several reports [1-8]. Most cases show CD7 expression, low or absent myeloperoxidase activity, basophilia, unique blast morphology, dysplastic features, and poor prognosis [1-8]. Harada *et al.* reported that the incidence among adults is 0.6% [1, 2]. *ETV6*, at 12p13, and *CHIC2*, at 4q12, have been reported to be involved in AML [5, 8]. Here, we report 2 cases of AML with t(4;12)(q12;p13) which showed the common characteristics of AML with t(4;12).

Case 1

A 53-year-old male underwent a bone marrow (BM) examination following detection of circulating blasts. His initial complete blood count was hemoglobin 12.4 g/dL, white blood cell count 44.57×10^9/L (differential count: myeloblasts 99%, atypical lymphocytes 1%), and platelets 120×10^9/L. BM aspirates revealed 84% myeloblasts and dysmegakaryopoiesis, such as multinucleation and nuclear hypolobation. Myeloblasts were large and showed a fine and blocky chromatin pattern (Fig. 1A). Flow cytometric analysis revealed CD34, CD13, CD33, CD117, HLA-DR, and CD7 expression but was negative for myeloperoxidase. The karyotype was 46,XY,t(4;12)(q12;p13),del(5)(q22q35) in 20 metaphases (Fig. 1B). FISH analysis using a Vysis LSI 4q12 tricolor probe (Abbott Molecular, Des Plaines, IL, USA) showed 1 tri-color fusion, 1 orange/green fusion, and 1 aqua signal in 89% of cells. These results confirmed that the break point is at 4q12 and suggested that *PDGFRA* or *CHIC2* may be involved (Fig. 1C). FISH analysis using a Vysis LSI ETV6/RUNX1 ES dual-color translocation probe (Abbott Molecular) showed 3 green signals in metaphase cells, suggesting an *ETV6* gene rearrangement (Fig. 1D). The patient did not achieve complete remission after standard induction chemotherapy (idarubicin and cytarabin). Post-induction, 24% of all marrow nucleated cells were myeloblasts and the karyotype was 46,XY,t(4;12)(q12;p13), del(5)(q22q35)[2]/46,XY[2]. After reinduction using high-dose cytarabin and daunorubicin, blasts were not increased and a normal karyotype was detected (46,XY[17]). The patient underwent allogenic hematopoietic stem cell transplantation from an unrelated matched donor and has been in complete remission for 52 months.

Case 2

A 74-year-old male with a history of acute myocardial infarction presented at a local clinic complaining of melena. He was referred to the tertiary hospital following detection of myeloblasts on a peripheral blood smear. His initial complete blood count was hemoglobin 7.7 g/dL, white blood cell count 20.91×10^9/L (differential count: myeloblasts 45%, atypical lymphocytes 19%, and basophils 3%), and platelets 252×10^9/L. In BM aspirates, approximately 50% of all marrow nucleated cells were myeloblasts, which were large with a blocky chromatin pattern with prominent nucleoli (Fig. 2A). Basophils (3.8%) were also detected. Erythroid precursors showed minimal dysplastic features, such as multinuclearity, and myeloid precursors showed hypogranulation. Megakaryocytes were increased in number and showed dysplastic features, such as micromegakaryocytes and nuclear hypolobation. Flow cytometric analysis showed positivity for CD34, CD13, CD33, CD117, CD64, TdT, HLA-DR, aberrant CD7, and CD56 expression and was negative for myeloperoxidase. Cytogenetic analysis showed a karyotype of 46,XY,t(4;12)(q12;p13)[13] (Fig. 2B). FISH analysis using an XL FIP1L1/CHIC2/PDGFRA probe (Metasystems, Altlussheim, Germany) showed 2 orange/green fusions and 1 green signal in 82% of cells, indicating a translocation involving a breakpoint at 4q12 (Fig. 2C). An LSI ETV6/RUNX1 ES dual-color translocation probe (Abbott Molecular) showed 3 green signals, suggesting an *ETV6* gene rearrangement in metaphase cells (Fig. 2D).

The patient received 3 courses of decitabine single therapy, based on his age and comorbidities. Following the therapy, blasts were still detected and the karyotype was 46,XY,t(4;12)(q12;p13)[37]/46,sl,del(7)(q22)[3]. A subclone harboring a 7q deletion was found to have evolved. Blasts and trilineage dysplasia remained after 14 courses of decita-

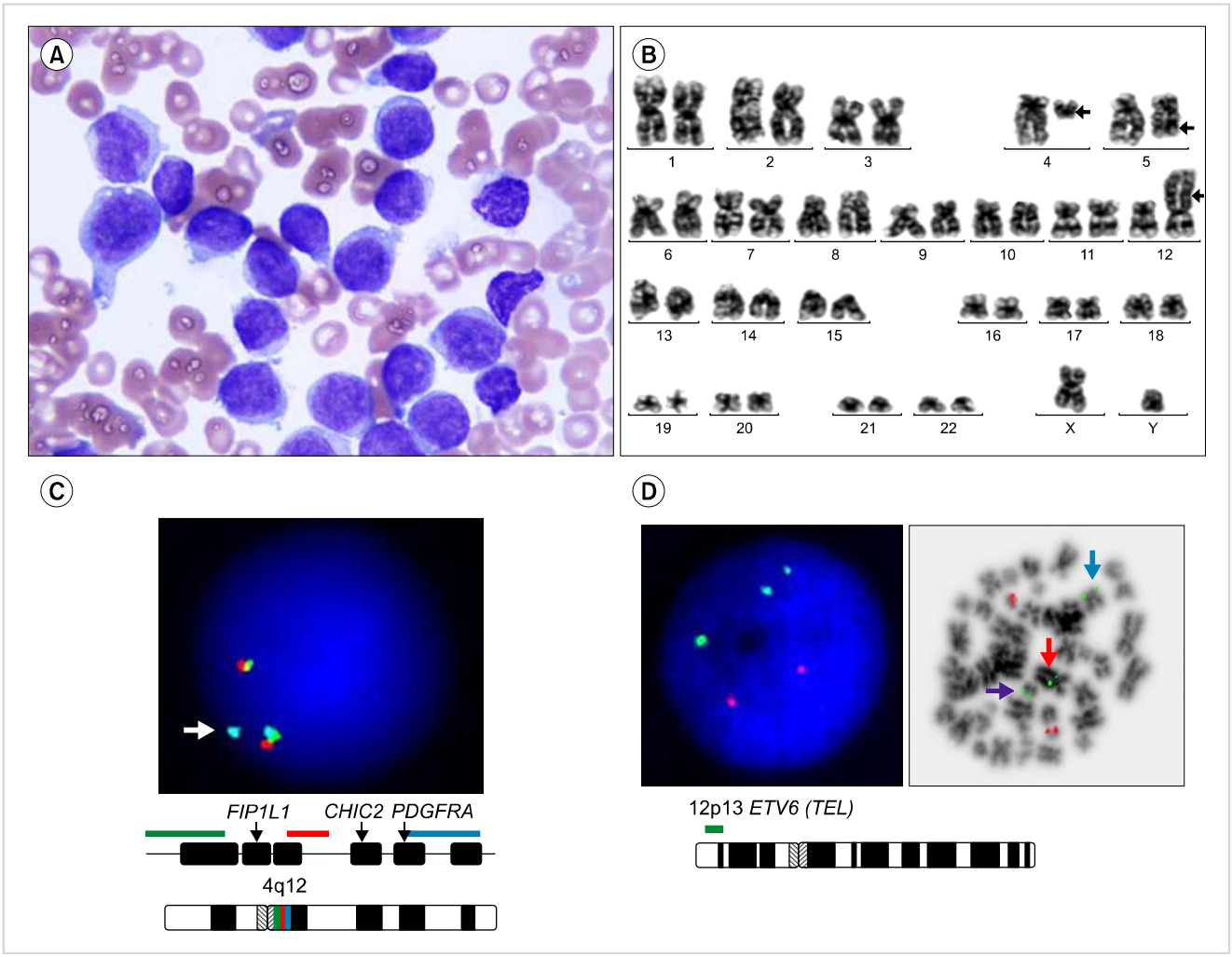

Fig. 1. Case 1 **(A)** Bone marrow aspirates from patient 1. Large, round nucleus with a blocky chromatin pattern in agranular cytoplasm (Wright stain, ×1,000). **(B)** Karyotype of patient 1: 46,XY,t(4;12)(q12;p13),del(5)(q22q35)[20]. **(C)** FISH analysis using a Vysis LSI 4q12 tricolor probe showed cells with 1 tri-color fusion, 1 orange/green fusion, and 1 aqua signal (arrow). **(D)** FISH analysis using a Vysis LSI ETV6/RUNX1 ES probe showed 3 green and 2 orange signals in interphase and metaphase cells, revealing an *ETV6* rearrangement [purple arrow: *ETV6* signal on der(4)t(4;12), blue arrow: *ETV6* signal on native chromosome 12, red arrow: *ETV6* signal on der(12)t(4;12)].

bine treatment. The patient then received induction chemotherapy with idarubicin and cytarabine, though BM aspirates still showed 50% blasts and dysplastic features. FISH analysis using an XL del(7)(q22q31) tri-color probe (Metasystems) was performed with the initial and follow-up BM to confirm the clonal evolution of del(7q). The initial sample showed no deletion signals (Fig. 2E); however, the follow-up sample showed deletion signals for 7q22-7q31 (Fig. 2F).

Discussion

t(4;12)(q11-q13;p12-p13) was previously described as a recurrent translocation in different types of acute leukemia. Eighteen cases of AML have been reported, along with 3 cases of acute lymphoblastic leukemia in children [9]. The cases that presented as acute myeloid leukemia showed unique blasts, with "pseudo-lymphoid" or "mature lymphoid" morphology, and with a blocky chromatin pattern resembling that of lymphocytes. Dysplastic features are also frequently noted. The 2 cases presented here both showed the morphology described above.

The translocation presented as either a single chromosomal abnormality or combined with other abnormalities (Table 1) [1, 3-8, 10-15]. The karyotype of the first patient was 46,XY, t(4;12)(q12;p13),del(5)(q22q35), and del(5q) is a common chromosomal abnormality in myelodysplastic syndrome and in AML with myelodysplasia-related changes. Basophilia was not observed in this case. Other common characteristics, such as CD7 expression, negative myeloperoxidase activity, and failure of the first induction chemotherapy were observed in the first case. As in previous reports, intensive treatment or hematopoietic stem cell transplantation were curative [5, 7]. In the second case, t(4;12) presented as a single abnormality, which showed development of a subclone with a 7q deletion as the disease

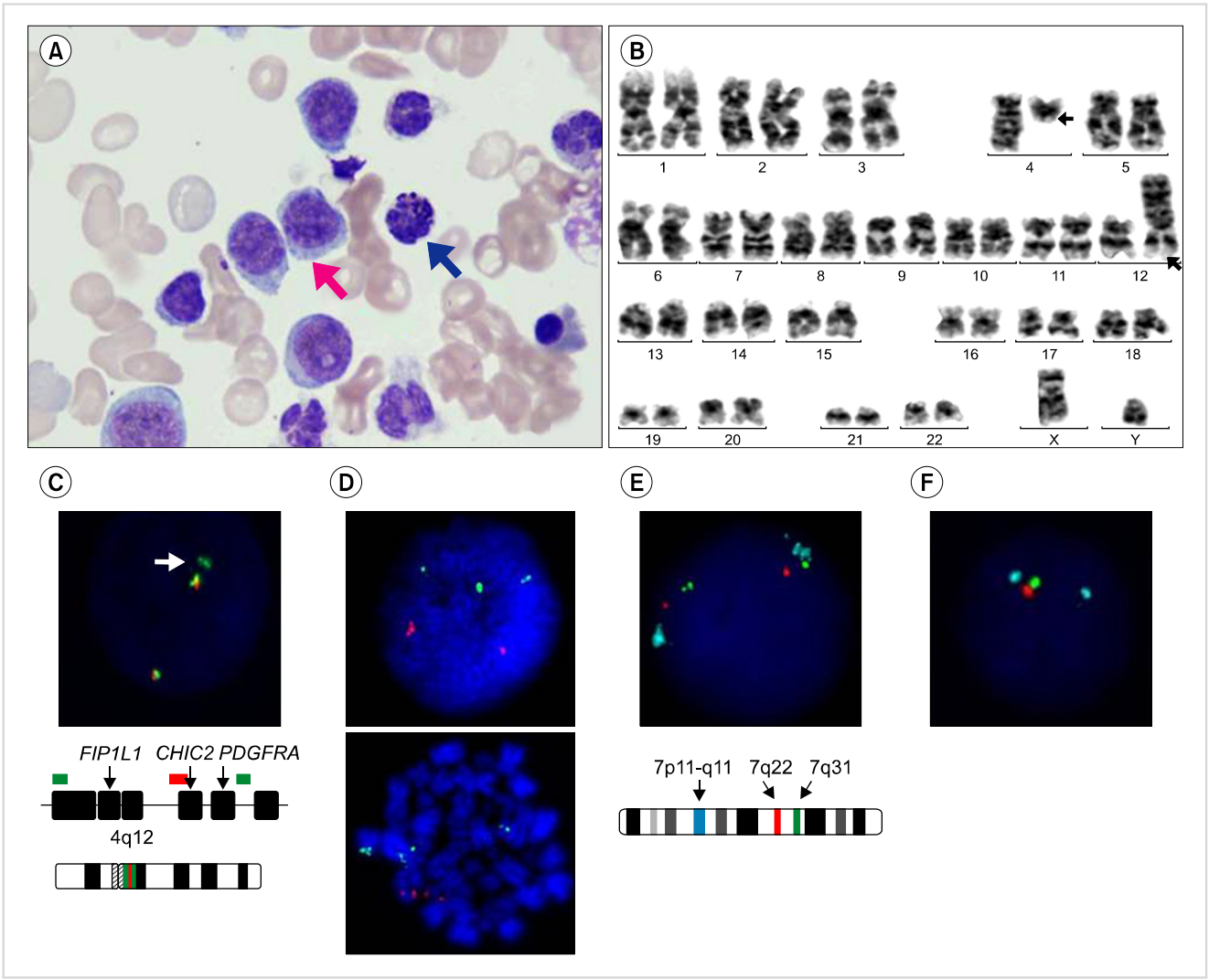

Fig. 2. Case 2 **(A)** Bone marrow aspirates from patient 2. Large, round nucleus with a blocky chromatin pattern with a prominent nucleolus (red arrow). Basophil (blue arrow) (Wright stain, ×1,000). **(B)** Karyotype of patient 2: 46,XY,t(4;12)(q12;p13)[13]. **(C)** FISH analysis using an XL FIP1L1/CHIC2/PDGFRA probe showed 2 orange/green fusions and 1 green signal (arrow). **(D)** FISH analysis using a Vysis LSI ETV6/RUNX1 ES probe showed 3 green and 2 orange signals in interphase and metaphase cells, revealing an *ETV6* rearrangement. **(E)** FISH analysis using an XL del(7)(q22q31) tri-color probe showed 2 aqua, 2 green, and 2 orange signals at the initial diagnosis. **(F)** FISH analysis using an XL del(7)(q22q31) tri-color probe showed 2 aqua, 1 green, and 1 orange signal at the follow-up, showing deletion signals for 7q22-7q31.

progressed. Several previously described features, such as CD7 expression, absence of myeloperoxidase activity, and basophilia were present. Despite administration of decitabine therapy, the usual treatment for myelodysplastic syndromes and AML for elderly patients, and additional induction chemotherapy, this patient has not entered remission.

ETV6 (also known as *TEL*) is involved in several malignancies, including pre-B cell ALL with t(12;21)(p13;q22) and CMML with t(5;12)(q33;p13) [8]. A previous study mapped the breakpoints of patients with t(4;12) as *ETV6* at 12p13 and *CHIC2* at 4q12, resulting in the formation of *CHIC2-ETV6* fusion transcripts [5] and the involvement of *ETV6* [8]. The *CHIC2-ETV6* fusion transcript has been suggested to be involved in the leukemogenic process [5]. FISH analysis of these cases also suggested rearrangements of *ETV6* and *CHIC2*.

In summary, the 2 cases discussed here shared most of the common characteristics of AML with t(4;12)(q11-q13;p12-p13), as well as some different features. When myeloblasts show a distinctive blocky chromatin pattern or "mature lymphoid" morphology, t(4;12) should be suspected and the appropriate evaluation should follow. Intensive chemotherapy or early hematopoietic stem cell transplantation should be considered. Further studies of this rare but recurrent cytogenetic abnormality will guide proper diagnosis and management.

Table 1. Patients with t(4;12) in acute myeloid leukemia.

Patient	Age (y)/ Gender	Diagnosis	Karyotype	Immunophenotype	Remission (mo)	Survival (mo)	Reference
1	59/M	AML/M7	46,XY,t(4;12)(q13;p13)	NG	NR	2.5	[10]
2	74/F	AML/M1	47,XX,+11/46,XY,t(4;12)(q13;p13), del(10)(q11)	NG	NR	2.5	[11]
3	61/F	AML	43,XXq-,-5,-21,8p-,9p-,t(2;3)(p23;q14), t(4;17)(p12;q11),t dic(4;12) (p12;?p12)	CD7+,CD9+,CD13+,CD33+	NG	NG	[12]
4	47/F	AML/M4	46,XX,t(4;12)(q11;p13),-7,+8	NG	NR	3	[13]
5	14/F	AML/M1	46,XX,inv(3)(p25q21),t(4;12)(q12;p13), del(6)(q13q21),+mar/47,idem,+15	CD7-,CD10+,CD19+,CD33+,CD13+, HLA-DR+	CR 8	?	[14]
6	66/F	AML/M1	46,XX,t(4;12)(q12;p13)/46,XY,idem, i(17)(q10)	NG	NR	6	[15]
7	61/M	AML/M0	46,XY,t(4;12)(q11-12;p13)	CD7+,CD13+,CD34+,HLA-DR+	CR 19	44	[1]
8	43/M	AML/M0	46,XY,t(4;12)(q11-12;p13)	CD7+,CD13+,CD34+,HLA-DR+	CR >48	>51	[1]
9	49/M	AML/M2	46,XY,t(4;12)(q11-12;p13)	CD7+,CD13+,CD34+,HLA-DR+,c-kit+	CR 6	14	[1]
10	82/M	AML/M1	46,XY,t(4;12)(q11;p13)	CD7-,CD13+,CD34+,HLA-DR+, c-kit+, CD11a+, CD56+	NG	NG	[3]
11	70/M	AML/M0	46,XY,t(4;12)(q12;p13)	CD7+,CD13+,CD33+,CD34+,HLA-DR+ ,TDT+	NR	5	[4]
12	76/F	Myeloid/ NK cell leukemia	46,XX,t(4;12)(q12;p13)	CD2+,CD5+,CD7+,CD13+,CD56+, CD33+,MPO+	CR	8	[5]
13	70/M	AML/M0	46,XY,t(4;12)(q11;p13)[13]/47,XY,t(4;12)(q11;p13)del(1)(p11p35)[18]	CD7+,CD13+,CD34+,HLA-DR+	Death at induction	-	[5]
14	81/M	AML/M0	46,XY,t(4;12)(q12;p13)[7]/46,XY,t(4;12)(q11;p13),del(5)(q13q33),add(11)(p15)[13]	CD7+,CD13+,CD33+,CD34+	NR	3	[5]
15	54/M	AML/M0 Relapse	46,XY,t(4;12)(q11;p13) 46,XY,t(4;12)(q11;p13)[3]/46,XY[12]	CD7+,CD13+,CD33+,CD34+	CR CR(BMT)	26 24	[5]
16	33/M	AML	46,XY,t(4;12)(q11;p13)[20]	CD7+,CD13+,CD33+,CD34+,CD56+, CD117+,HLA-DR+	Death at second induction	NG	[6]
17	18/F	AML with MRC	46,XY,t(4;12)(q12;p13)[19]/46,XX[1]	CD7+,CD13+,CD15+,CD33+,CD34, CD71,HLA-DR+,CD117+, MPO-	CR(BMT)	>12	[7]
18	64/M	AML	46,XY,t(4;12)(q12;p13)[18]/ 46,XY,t(4;12)(q12;p13),del(5q)[2]	ND	NG	NG	[8]
19	54/M	AML with MRC	46,XY,t(4;12)(q12;p13)del(5)(q22q35)[20]	CD7+,CD13+,CD33+,CD34+, HLA-DR+	CR(BMT)	>52	Case 1
20	75/M	AML with maturation	46,XY,t(4;12)(q12;p13)[13][a] 46,XY,t(4;12)(q12;p13)[37]/46,XY,t(4;12), del(7)(q22)[3][b]	CD7+,CD13+,CD16+56,CD33+, CD34+,TDT+,HLA-DR+,c-kit+	NR	>25	Case 2

[a]The karyotype of patient 2 at diagnosis; [b]The karyotype of patient 2 after 3 courses of decitabine therapy.
Abbreviations: AML, acute myeloid leukemia; AUL, acute unclassified leukemia; Tx, treatment; NK, Natural killer; MRC, myelodysplasia-related changes; NG, not given; ND, not done; NR, no remission; CR, complete remission; BMT, bone marrow transplantation.

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Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

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Primary acquired chronic pure red cell aplasia refractory to standard treatments: remission with rituximab

TO THE EDITOR: Pure red cell aplasia (PRCA) is a rare syndrome caused by erythropoietic hypoplasia in the absence of leukocytopenia and thrombocytopenia. It is characterized by severe normocytic and reticulocytopenic anemia, with a normally cellular bone marrow (BM) but devoid of erythroblasts [1]. The acquired form of PRCA is a chronic illness that is often diagnosed in conjunction with a variety of diseases [1], such as lymphoproliferative disorders [2], viral infections, autoimmune hemolytic anemia (AIHA) [3], rheumatologic disorders [4], and allogeneic stem cell transplantation [5]. However, this disorder is rarely diagnosed as an idiopathic condition. Acquired PRCA is managed as an immunologically mediated disease, using immunosuppressive therapy (IST) with corticosteroids and cyclosporine A (CSA) as the treatments of first choice [1]. As alternative and salvage treatment, rituximab has been reported to be highly effective [2-5]; however, to the best of our knowledge, no case of idiopathic PRCA managed with this agent has been reported. A 63-year-old woman was diagnosed in June 2003 as having PRCA after the discovery of isolated normocytic and reticulocytopenic anemia, the course of which had been insidious and progressive. All other possible underlying causes of erythroblastopenia were ruled out by appropriate investigations (Table 1); other laboratory and radiological evaluations revealed no abnormal findings. The patient had required transfusions of almost 2 units of packed red blood cells (RBC) every 2 to 3 weeks. Once the diagnosis was made, she was started on CSA plus corticosteroids, and soon achieved full recovery from BM erythropoiesis and attained normalization of peripheral blood counts. The patient no longer required transfusions. This was considered complete remission (CR) of PRCA. Therefore, the dosage of CSA was gradually reduced and discontinued. However, there was a progressive loss of response, and CSA was resumed in February 2007 due to a full relapse. The patient achieved a second CR, and the dosage of CSA was carefully tapered. However, the patient experienced progressive chronic renal failure (CRF) in January 2011, which fully resolved after discontinuation of CSA. PRCA recurred soon after, and the patient again required frequent RBC transfusions. When she required approximately 4 RBC units per month, steroids were retried, but without any benefit. Azathioprine was tried without any response. By May 2013, the need for transfusions had reached about 6 RBC units/month, and direct and indirect Coombs blood compatibility

Table 1. Laboratory findings at the PRCA diagnosis.

	Results
Hemoglobin	4.4 g/dL
WBC	4,770/μL
Platelets	227×10^3/μL
MCV	93 fL
MCH	33.2 pg
Reticulocyte	0.04%
Albumin	4.8 g/dL
ALT	31 U/L
AST	35 U/L
Total bilirubin	1.1 mg/dL
Direct bilirubin	0.5 mg/dL
Azotemia	25 mg/dL
Creatinine	0.9 mg/dL
Glucose	92 mg/dL
LDH	195 U/L
PT	13 sec (12.6–15.7)
aPTT	28 sec (26–35)
Fibrinogen	350 mg/mL (220–498)
Direct and indirect antiglobulin tests	Negative

Abbreviations: WBC, white blood cell; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDH, lactate dehydrogenases; PT, prothrombin time; aPTT, activated partial thromboplastin time.