

Case report

A case of chronic lymphocytic leukemia complicated by autoimmune hemolytic anemia due to ibrutinib treatment

Takaharu Suzuki,^{1,2)} Shukuko Miyakoshi,^{1,2)} Ayako Nanba,¹⁾ Takayoshi Uchiyama,³⁾
Keisuke Kawamoto,¹⁾ Sadao Aoki^{2,3)}

Ibrutinib (IBR) covalently binds to the active site of Bruton's tyrosine kinase (BTK) and is used for the treatment of relapsed/refractory chronic lymphocytic leukemia (CLL). Approximately 5-10% of CLL is complicated by autoimmune cytopenia (AIC), such as autoimmune hemolytic anemia (AIHA). Several cases of AIC have reportedly demonstrated improvement during IBR treatment. However, in our case, the patient developed AIHA during oral IBR treatment. As AIHA is exacerbated by the increased number of CLL cells in the peripheral blood, it may have developed because of disease progression rather than IBR use. This phenomenon may also be attributed to the production of autoantibodies due to increased number of CD5+ B cells. In this case, withdrawal of IBR and administration of rituximab improved hemolysis. If AIHA develops during treatment, its etiology must be examined to confirm the effects of treatment.

Keywords: Chronic lymphocytic leukemia, Autoimmune hemolytic anemia, Ibrutinib

INTRODUCTION

Chronic lymphocytic leukemia (CLL) is the most common type of leukemia in adults in Europe and the United States.¹ However, its incidence is lower in East Asia, including Japan.² Therapeutic agents for the treatment of CLL have been developed, including the introduction of alkylating agents (chlorambucil, and cyclophosphamide) in 1960, purine analogs (fludarabine, pentostatin, and cladribine) in 1980, and combination therapy of purine analogs and alkylating agents in 2000.³ Chemoimmunotherapy (CIT) that uses employing anti-CD20 antibody in addition to chemotherapy has become common in recent years.³ Furthermore, since 2010, new molecular targeted drugs that can be orally administered have been developed.³ Ibrutinib (IBR) covalently binds to the active site of Bruton's tyrosine kinase (BTK) and exerts antitumor effects via the inhibition of survival/proliferation signal transduction,⁴ and is used for the treatment of relapsed/refractory CLL.

Approximately 5-10% of CLL cases are complicated by autoimmune cytopenia (AIC) such as autoimmune hemolytic anemia (AIHA).⁵ AIHA occurs in any risk classification and is not associated with prognostic factors.⁵ IBR is effective for the treatment of CLL complicated by AIHA.⁶ However,

in this report, we describe a case of CLL that became complicated by AIHA during IBR treatment.

CASE REPORT

A 75-year-old Japanese woman was diagnosed with CLL (Rai classification: 0, and Binet classification: A) 10 years ago and was followed-up at our hospital. The patient presented with progressive lymphadenopathy, splenomegaly, peripheral blood lymphocytosis, and thrombocytopenia 4 years and 1 month ago, for which fludarabine treatment was started. Although partial response to fludarabine was observed, she repeatedly relapsed. Partial response was achieved with ofatumumab or bendamustine and rituximab (BR) therapy. Due to relapse, the patient was admitted to our hospital to initiate IBR treatment. The clinical course during hospitalization is shown in Fig. 1. Blood test results upon admission revealed anemia, thrombocytopenia, peripheral blood lymphocyte proliferation, and an increased lactate dehydrogenase (LDH) level (Table. 1). As her reticulocyte count was low and direct bilirubin was within the normal range, hemolysis was excluded. Computed tomography (CT) demonstrated intraperitoneal lymph node swelling and splenomegaly (Fig. 2A). On the bone marrow examination,

Received: April 5, 2018. Revised: May 30, 2018. Accepted: June 19, 2018. J-STAGE Advance Published: July 14, 2018
DOI:10.3960/jslrt.18012

¹⁾Department of Hematology, Endocrinology and Metabolism, Faculty of Medicine, Niigata University, Niigata, Japan, ²⁾Niigata Minami Hospital, Niigata, Japan, ³⁾Department of Pathophysiology, Niigata University of Pharmacy and Applied Life Sciences, Niigata, Japan

Corresponding author: Sadao Aoki, 265-1, Higashijima, Akiha-ku, Niigata, Niigata 956-8603, Japan. E-mail: saoki@nupals.ac.jp

Copyright © 2018 The Japanese Society for Lymphoreticular Tissue Research


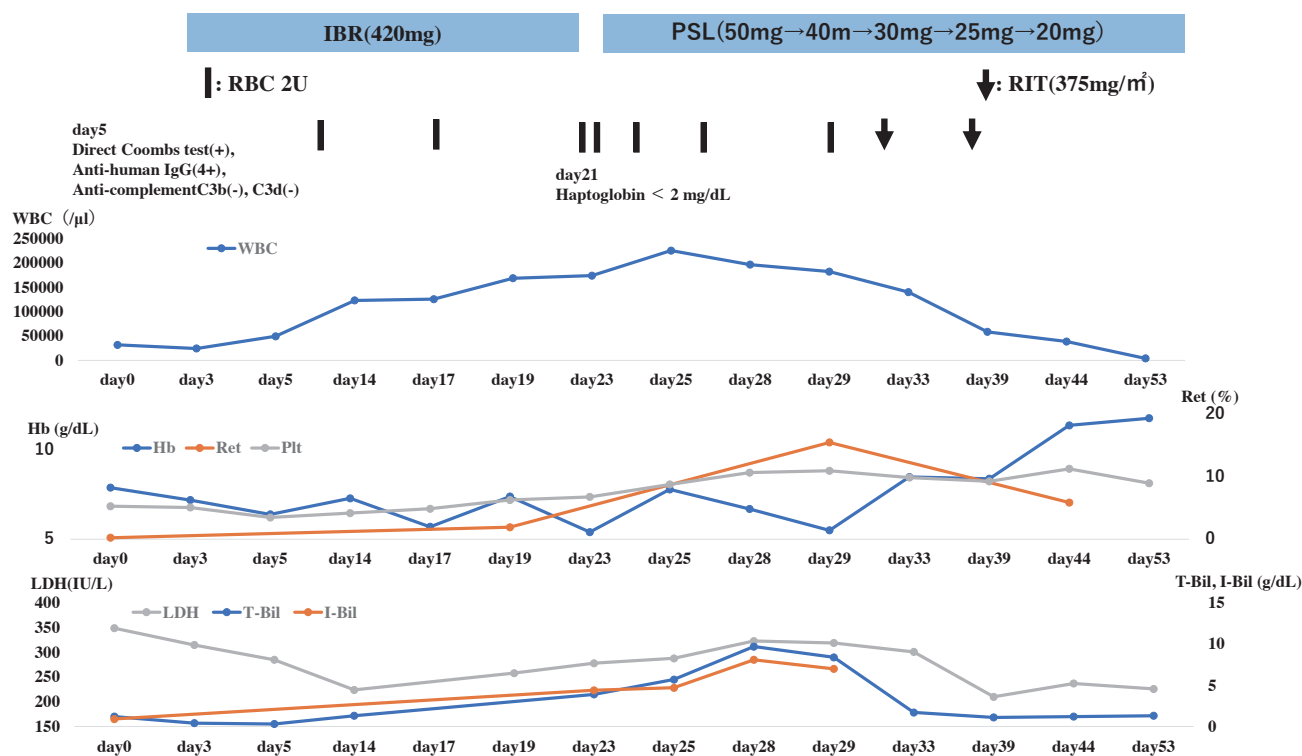
 This work is licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International License.

Table 1. Laboratory findings on admission

Peripheral blood		Chemistry		Seroimmunological test	
WBC	31870 / μ L	TP	5.9 g/dL	IgG	5339 IU/mL
Seg	5.0 %	Alb	4.3 g/dL	IgA	176 mg/dL
Eo	0.5 %	BUN	19.9 g/dL	IgM	< 10 mg/dL
Bas	0.0 %	Cre	0.89 g/dL	Soluble IL-2R	5339U/L
Lym	94.5 %	T-Bil	1.2 g/dL	CRP	< 0.30 IU/mL
Mono	0.0 %	D-Bil	0.3 g/dL		
RBC	241 $\times 10^4$ / μ L	ALP	162 g/dL		
Ret	0.25 %	AST	39 IU/L		
Hb	7.9 g/dL	ALT	29 IU/L		
Ht	23.1 %	LDH	349 IU/L		
MCV	95.9 %	γ -GTP	16 IU/L		
Plt	5.3 $\times 10^4$ / μ L	Amy	72 IU/L		
		Na	148 mEq/L		
		K	4.4 mEq/L		
		Cl	107 mEq/L		
		Ca	8.8 mg/dL		

**Fig. 1.** Clinical course

On day 3, ibrutinib (IBR; 420 mg/day) was started. On day 5, the following results were obtained: direct Coombs test, +; anti-human IgG, 4+; and anti-complement C3b and C3d. The patient had no symptoms, and was thus followed-up. On day 8, she exhibited palpitations, dyspnea upon walking, and dizziness. She received RBC transfusion on days 8, 15, 20, 21, 22, 24, and 27. IBR treatment was continued, but the number of WBC increased. On day 23, IBR treatment was discontinued, and on day 23, PSL was started. We used rituximab for AIHA on days 31 and 38. AIHA was improved, and the number of WBC decreased. On day 59, she was discharged from the hospital.

95% heteromorphic lymphocytes were observed, and numerous round lymphocytes with a smooth nuclear membrane and fine granular chromatin were noted (Fig. 3A). On flow cytometry analysis of peripheral blood using CD19 gating, CLL cells were positive for CD5 and CD23, weakly positive for CD20, and negative for CD22 (Fig. 3B). The Matutes score⁷ was 4, consistent with CLL. Mutation TP53 was not

investigated because the remaining specimen was inadequate, but 17p13 deletion was negative on fluorescence in situ hybridization.

Based on these results, IBR treatment (420 mg/body) was started. However, after initiating IBR treatment, peripheral blood lymphocyte proliferation (highest count at 165169/ μ L on day 19) was observed. However, treatment was continued

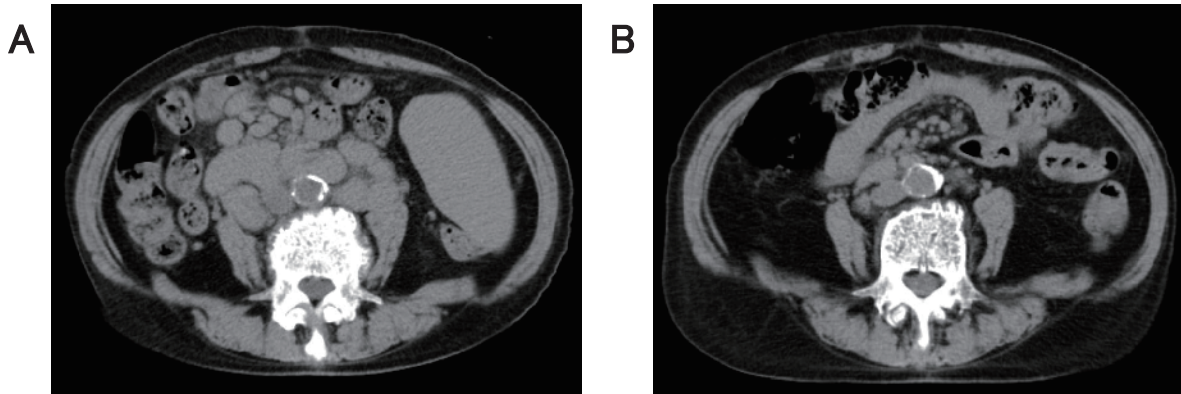


Fig. 2. Intra-abdominal CT scan

A) On day 2, CT was carried out, showing swelling of intraperitoneal lymph nodes and splenomegaly.

B) After discharge, repeat CT was performed, revealing decreased swelling of the intraperitoneal lymph nodes and spleen.

because there was no overt organopathy. On day 3, the hemoglobin (Hb) level was 6.4 g/dL. The test results were as follows: direct Coombs test, +; anti-human IgG, 4+; and anti-complement C3b and C3d. As no subjective symptom of anemia was observed, follow-up observation was performed. On day 5, her Hb level was 6.6 g/dL, and she presented with palpitations, dyspnea upon walking, and dizziness. One hundred milligrams of hydrocortisone was administered, and 2 units of red blood cells (RBC) were transfused. On day 21, her haptoglobin level decreased to < 2 mg/dL. The following laboratory test results indicated hemolysis: RBC level, $194 \times 10^4/\mu\text{L}$; Ret, 3.79%; LDH level, 248 IU/L; and T-Bil, 4.5 mg/dL. The patient was thus diagnosed with AIHA. As IBR was suspected as a cause of AIHA, it was discontinued on day 21. Prednisolone (PSL) (50 mg/day) was started on day 23. Sustained hemolysis was prolonged, and two units of RBC were transfused on days 15, 20, 21, 22, 24, and 27. However, PSL was not effective and was discontinued. We administered rituximab (RIT) as a single agent on days 31 and 38. After RIT treatment, peripheral blood lymphocyte proliferation and hemolysis improved, and palpitations and dyspnea disappeared. Her Hb level increased, and T-Bil and LDH levels were within the normal range (Fig. 1). Considering AIHA to have improved, the patient was discharged on day 59, but CT revealed swelling in the lymph nodes and spleen (Fig. 2B).

DISCUSSION

Approximately 5-10% of CLL cases are complicated by AIC, which may be developed in any risk classification.⁵ Approximately 90% of AIC-complicated CLL cases are due to high affinity IgG antibodies against erythrocytes/-platelets that are produced by non-malignant B cells.⁸ Several studies have demonstrated that IBR treatment improves AIC.^{9,10} Molica reported that AIHA was suppressed during IBR treatment and worsened as IBR was withdrawn when pneumonia developed.¹¹ Rider found that AIHA developed at initial IBR treatment, but did not develop during re-treatment.¹² In our case, the patient did not previously develop AIHA, and onset

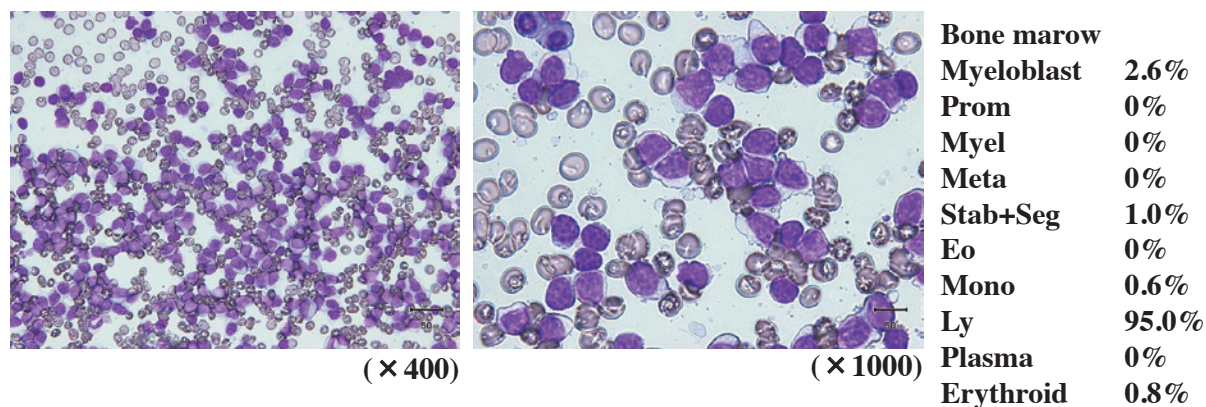
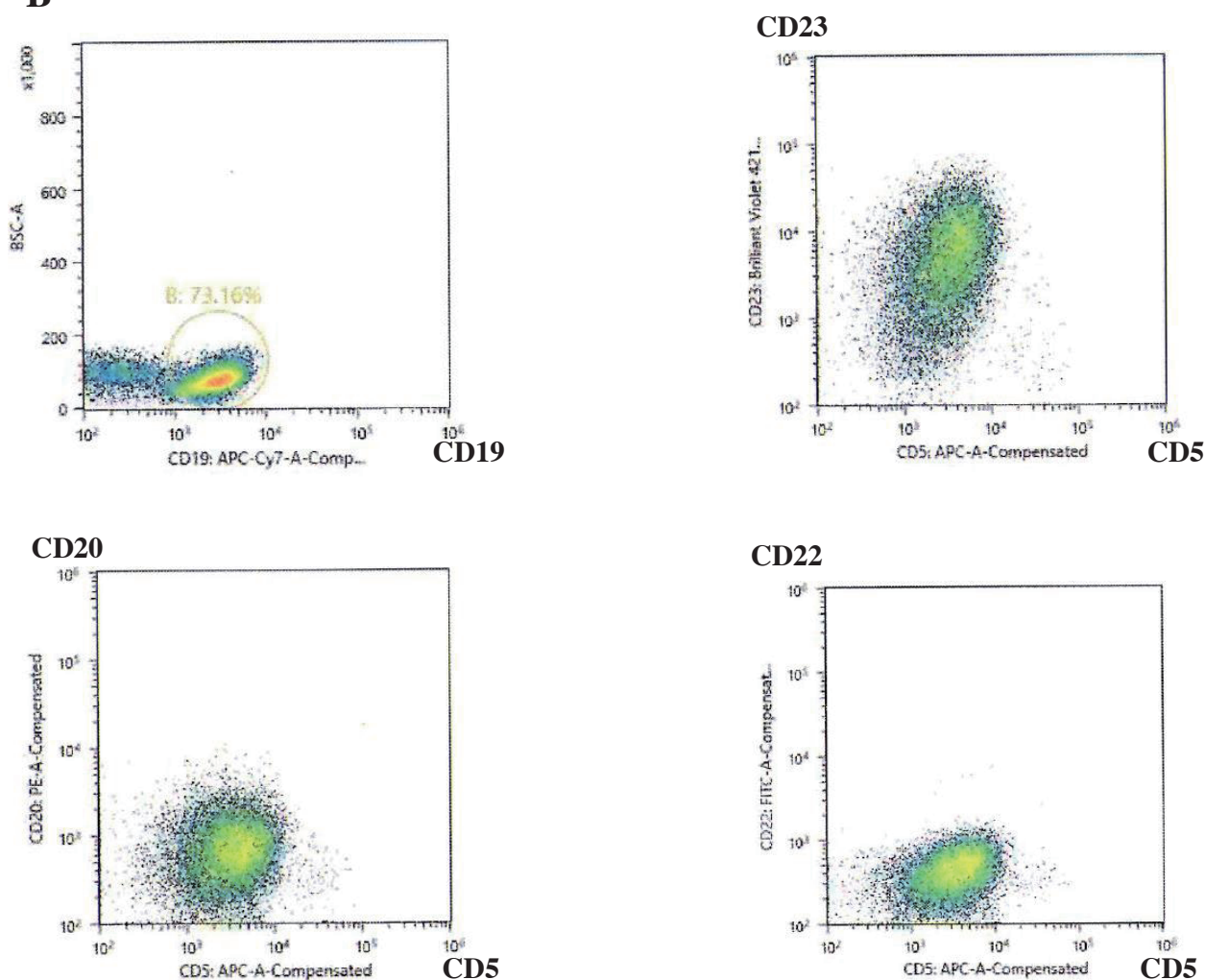
was observed during IBR treatment. Therefore, these reports differ from our case. Although the cause of AIHA during IBR treatment is unknown, the following possibilities have been considered:

First, IBR itself causes AIHA. Fludarabine causes AIHA during CLL treatment.¹³ This drug causes autoimmune tolerance due to the destruction of regulatory T cells.¹⁴ Therefore, AIHA may be induced by the administration of fludarabine.¹⁴ A small number (1.28%) of AIHA cases with AIHA attributed to the use of IBR has been reported.⁶ Although IBR has immunological activity, pharmacological effects similar to those of fludarabine have not been observed by IBR. Thus, the probability of IBR causing AIHA is low.

Second, a rapid increase in lymphocytes due to IBR is involved. As shown in Fig. 1, white blood cells (WBC) in the peripheral blood significantly increased after administration of IBR, exacerbating AIHA. In general, after IBR treatment, CLL cells are released from lymph nodes,^{15,16} which causes transient lymphocytosis. These lymphocytes then undergo apoptosis. If the disease is not PD, follow-up is considered sufficient. In this case, AIHA was observed 4 weeks after IBR treatment. Moreover, AIHA was exacerbated by the increase in the peripheral blood lymphocyte count. Thus, lymphocytes may promote autoantibody production.

Third, the efficacy of IBR was delayed. Administration of IBR to patients with CLL and diabetes reportedly decreases insulin and GAD antibody levels.¹⁷ As such, IBR may have suppressed autoantibody production. The delayed effects of inhibiting autoantibody production by IBR can worsen the condition of patients with AIHA.

We believe that the most likely reason for why AIHA was not controlled in this case was the amount of time required for the effects of IBR to develop. Based on RIT treatment, lymphocytes in the peripheral blood rapidly decreased and AIHA was also improved. RIT may have more immediate effects than IBR. Rituximab is considered to be effective for AIHA.¹⁸ In conclusion, when AIHA develops during IBR treatment, RIT should be administered immediately.

A**B****Fig. 3.**

A) On day 1, bone marrow aspiration was performed. Numerous round lymphocytes with a smooth nuclear membrane and fine granular chromatin were observed.

B) On flow cytometry, CLL cells were positive for CD5 and CD23, weakly positive for CD20, and negative for CD22. The Matutes score was 4.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

- 1 Scarfo L, Ferreri AJ, Ghia P. Chronic lymphocytic leukaemia. *Critical reviews in oncology/hematology* 2016; 104 : 169-182.
- 2 Ruchlemer R, Polliack A. Geography, ethnicity and “roots” in chronic lymphocytic leukemia. *Leuk Lymphoma*. 2013; 54 : 1142-1150.
- 3 Rai KR, Jain P. Chronic lymphocytic leukemia (CLL)-Then and now. *Am J Hematol*. 2016; 91 : 330-340.
- 4 Dubovsky JA, Beckwith KA, Natarajan G, *et al.* Ibrutinib is an irreversible molecular inhibitor of ITK driving a Th1-selective pressure in T lymphocytes. *Blood*. 2013; 122 : 2539-2549.
- 5 Zent CS, Kay NE. Autoimmune complications in chronic lymphocytic leukaemia (CLL). *Best Pract Res Clin Haematol*. 2010; 23 : 47-59.
- 6 Rogers KA, Ruppert AS, Bingman A, *et al.* Incidence and description of autoimmune cytopenias during treatment with ibrutinib for chronic lymphocytic leukemia. *Leukemia*. 2016; 30 : 346-350.
- 7 Matutes E, Owusu-Ankomah K, Morilla R, *et al.* The immunological profile of B-cell disorders and proposal of a scoring system for the diagnosis of CLL. *Leukemia*. 1994; 8 : 1640-1645.
- 8 Visco C, Barcellini W, Maura F, *et al.* Autoimmune cytopenias in chronic lymphocytic leukemia. *Am J Hematol*. 2014; 89 : 1055-1062.
- 9 Manda S, Dunbar N, Marx-Wood CR, Danilov AV. Ibrutinib is an effective treatment of autoimmune haemolytic anaemia in chronic lymphocytic leukaemia. *Br J Haematol*. 2015; 170 : 734-736.
- 10 Galinier A, Delwail V, Puyade M. Ibrutinib is effective in the treatment of autoimmune haemolytic anaemia in mantle cell lymphoma. *Case Rep Oncol*. 2017; 10 : 127-129.
- 11 Molica S, Polliack A. Autoimmune hemolytic anemia (AIHA) associated with chronic lymphocytic leukemia in the current era of targeted therapy. *Leuk Res*. 2016; 50 : 31-36.
- 12 Rider TG, Grace RJ, Newman JA. Autoimmune haemolytic anaemia occurring during ibrutinib therapy for chronic lymphocytic leukaemia. *Br J Haematol*. 2016; 173 : 326-327.
- 13 Myint H, Copplesstone JA, Orchard J, *et al.* Fludarabine-related autoimmune haemolytic anaemia in patients with chronic lymphocytic leukaemia. *Br J Haematol*. 1995; 91 : 341-344.
- 14 Beyer M, Kochanek M, Darabi K, *et al.* Reduced frequencies and suppressive function of CD4+CD25hi regulatory T cells in patients with chronic lymphocytic leukemia after therapy with fludarabine. *Blood*. 2005; 106 : 2018-2025.
- 15 Burger JA. Nurture versus nature: the microenvironment in chronic lymphocytic leukemia. *Hematology Am Soc Hematol Educ Program*. 2011; 2011 : 96-103.
- 16 Woyach JA, Smucker K, Smith LL, *et al.* Prolonged lymphocytosis during ibrutinib therapy is associated with distinct molecular characteristics and does not indicate a suboptimal response to therapy. *Blood*. 2014; 123 : 1810-1817.
- 17 Skrabbs C, Pickl WF, Perkmann T, Jager U, Gessl A. Rapid decline in insulin antibodies and glutamic acid decarboxylase autoantibodies with ibrutinib therapy of chronic lymphocytic leukaemia. *J Clin Pharm Ther*. 2018; 43 : 145-149.
- 18 Penalver FJ, Alvarez-Larran A, Diez-Martin JL, *et al.* Rituximab is an effective and safe therapeutic alternative in adults with refractory and severe autoimmune hemolytic anemia. *Ann Hematol*. 2010; 89 : 1073-1080.