

Case Report

Achievement of disease control with dasatinib after CAR T-cell therapy for relapsed Philadelphia chromosome-positive acute lymphoblastic leukemia: a case report and literature review

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Abstract: Historically, treatment outcomes for patients with Philadelphia-positive chromosome (Ph+) acute lymphoblastic leukemia (ALL) have been extremely poor. The introduction of tyrosine kinase inhibitor (TKI) and chimeric antigen receptor-engineered T (CAR T)-cell therapy mark crucial advancements in the treatment of Ph+ALL. In this case, we investigated a 42-year-old woman who was diagnosed with Ph+ALL and experienced multiple relapses. She was first treated with imatinib combined infusion chemotherapy and achieved complete remission (CR). She stayed disease free for 13 months until later diagnosed with central nervous system lymphoma (CNSL). Subsequently, she was treated with intrathecal injection, multi-agent chemotherapy along with dasatinib at 140 mg/d. Despite her initial recovery, she experienced her second relapse at a later stage during the infusion chemotherapy. She then received CAR T-cell therapy and responded well until a third relapse occurred 10 months later. She was again administered dasatinib at 140 mg/d and ever since then, no recurrence in peripheral blood, BM or CNS was observed. We suspect that a synergistic effect between CAR T-cell therapy and TKI exists in the treatment of Ph+ALL. Furthermore, we hypothesize that dasatinib may have in some way stimulated CAR T-cells proliferation in vivo, thereby restoring their function.

Keywords: Philadelphia chromosome positive, acute lymphoblastic leukemia, CAR T-cell therapy, dasatinib

Introduction

Philadelphia-positive chromosome acute lymphoblastic leukemia (Ph+ALL) is an aggressive heterogeneous group of hematologic malignancies. It is resulted from reciprocal translocation fusing the c-ABL1 tyrosine kinase gene on chromosome 9 and the BCR gene on chromosome 22 [1]. Generally, patients with Ph+ALL are more likely to develop central nervous system (CNS) diseases [2].

Prior to the tyrosine kinase inhibitor (TKI) era, patients with Ph+ALL suffer from dismal survival time. Imatinib, the earliest clinically approved TKI, substantially improved prognosis for Ph+ALL. Dasatinib, a second generation TKI that possesses a broader spectrum of multi-target kinase inhibition activity and overcomes imatinib's inability to penetrate the blood-brain-

barrier (BBB), yielded superior outcomes in patients resistant to imatinib or with CNS involvement. Nonetheless, in a substantial proportion of patients, TKI involved regimen alone does not guarantee prolonged remission without hematologic cell transplant (HCT).

Several immunotherapies have entered clinical phase in the past few years. Chimeric antigen receptor-engineered T (CAR T)-cell infusion, for instance, is one remarkable leap forward in the treatment of relapsed B-ALL [3-8]. Despite the overall satisfactory clinical outcomes, there are still several weaknesses such as the selection of CD19-negative clones, down-regulation of CD19 expression, lack of CD19 persistence or CD19 escape variants and limited T cell trafficking or immunosuppression of CAR T cells within the extramedullary tumor microenvironment [3-5, 7, 9, 10]. Up till now, no publications have

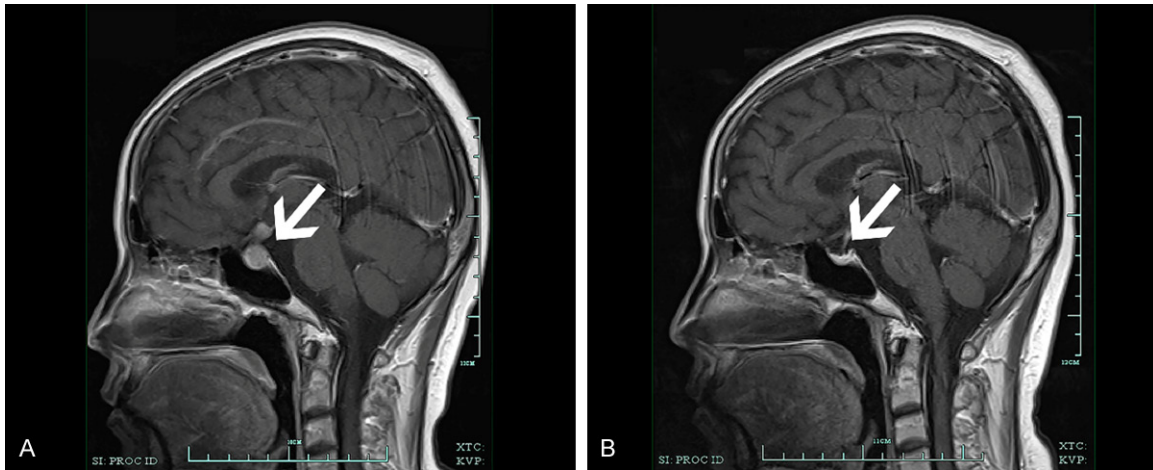


Figure 1. Magnetic resonance imaging of the pituitary images taken at the onset of the CNS blast crisis. A. Pituitary mass and a thickened pituitary stalk (white arrows). B. Significant regression of the brain lesion following treatment with dasatinib treatment. CNS, central nervous system.

explored the synergetic effect of CAR T-cell therapy and dasatinib combined regimen. The current case may provide some insights regarding this aspect.

Case presentation

A 42-year-old female was admitted to our hospital with sore throat and fatigue. Complete blood counts on admission showed the following: hemoglobin, 77 g/L; platelet count, $40 \times 10^9/L$; white blood cell count, $61.8 \times 10^9/L$ with 89% lymphoblast, and bone marrow (BM) sample showed 89% lymphoblast. The leukemia cells were positive for HLA-DR, CD10, CD13, CD19, CD34, CD38, CD123, cCD79a and TdT but negative for T-cell and myeloid cell markers using flow cytometry. Chromosomal analysis revealed normal female karyotype and PCR analysis of positive expression of the BCR/ABL (p210) fusion gene. She was diagnosed with Ph+ALL. We treated her with multi-agent induction chemotherapy, VDP regimen (vincristine, daunorubicin and prednisone) and hyper-CVAD A and B regimen (cyclophosphamide, vincristine, doxorubicin, dexamethasone, methotrexate and cytarabine) along with oral administration of imatinib capsules (400 mg/day). Complete remission (CR) was achieved on the 43th day of chemotherapy. However, 13 months after initial diagnosis, CNS relapse occurred during maintenance treatment with Hyper-CVAD A and B regimens. Subsequently, she underwent intrathecal injection (IT) (methotrexate, cytarabine and dexamethasone) twice per week, a series

of induction regimens (ifosfamide, methotrexate, doxorubicin, L-asparaginase and prednisone) and dasatinib intake at 140 mg/day. The patient achieved a second CR and continued with dasatinib only maintenance treatment, during which she stopped dasatinib for 3 weeks due to pancytopenia and resumed once the adverse reaction was gone.

The patient maintained disease free for 3 months until BM examination and lumbar puncture test revealed a relapse in both BM and CNS. Due to the lack of a HLA-matched donor, she did not receive hematologic cell transplant (HCT). Instead, three cycles of induction regimens with COATD (cyclophosphamide, cytarabine, teniposide and dexamethasone) were administered. She did not respond well and was enrolled in a clinical trial (ChiCTR.org number, ChiCTR-OCC-15007008).

On September 19, 2015, the patient received FC regimen (fludarabine and cyclophosphamide), followed by an infusion of CAR T cells. The total dose was 2.7×10^6 CAR T cells/kg, given over a period of 3 consecutive days. On day 11, the patient achieved a third CR, which maintained for 10 months. On day 320, she suffered a relapse with 7% lymphoblast in the BM, and a positive BCR-ABL p210 transcript. Pituitary magnetic resonance imaging (MRI) showed pituitary enlargement along with localized nodular thickening of the pituitary stalk (**Figure 1A**). She was diagnosed with central nervous system lymphoma (CNSL) and dasat-

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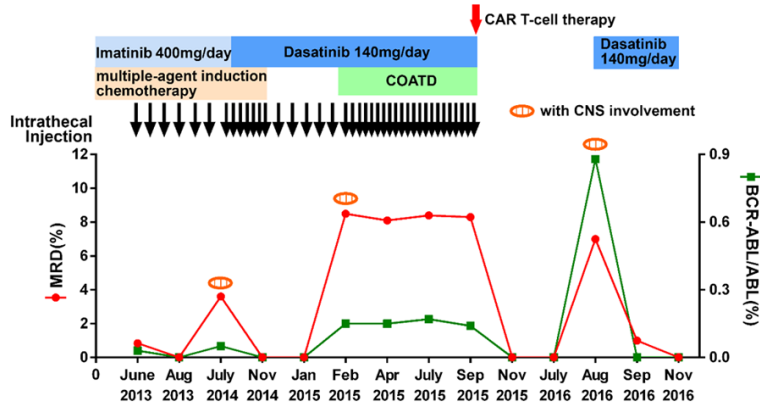


Figure 2. Clinical course of the patient with Ph+ALL undergoing a series of regimens involving imatinib, dasatinib and CAR T-cells/dasatinib in combination. The MRD and BCR-ABL/ABL transcript level responded towards each session of treatment. Ph+, Philadelphia-positive chromosome; ALL, acute lymphoblastic leukemia; CAR T, chimeric antigen receptor-engineered T; MRD, minimal residual disease.

inib at a dose of 140 mg/day was administered. 21 days later, leukemia cells were not found in the peripheral blood and BM and MRI showed depletion of the brain lesion (Figure 1B). She achieved a fourth CR again.

On October 2016, the patient developed fever, alternating constipation, diarrhea, and muscle weakness and numbness in both lower limbs. Cytokine detection revealed high level of interleukin (IL)-6 (23.20 pg/ml, 0-3.4), IL-10 (13.70 pg/ml, 0-9.1) and TNF- α (29.00 pg/ml, 0-8.1), which indicated grade 3 cytokine release syndrome (CRS). However, no signs of recurrence in peripheral blood, BM and CNS were found. Hormone treatment was provided but her condition worsened and severe pneumonia occurred. Subsequent broad-spectrum antibiotic and hormone treatment was ineffective and the patient died eventually. Her clinical course is shown in Figure 2.

Discussion

Imatinib was introduced as a first-line treatment for Ph+ALL back in 2001 and substantially improved therapeutic outcomes. However, it is suboptimal under certain conditions due to two major drawbacks. First, it is ineffective in dealing with cancer cells containing mutation within the BCR-ABL kinase domain (e.g., T315I) [11, 12]. Second, it is inadequate to penetrate the BBB [11, 13, 14]. On the other hand, dasatinib is a second-generation kinase inhibitor

with a wider spectrum. It inhibits SRC family kinase, which is involved in a signaling pathway in imatinib-resistant ALL [12]. It also penetrates the BBB more effectively and works against imatinib-resistant ABL kinase mutations [15]. Therefore, dasatinib is considered a superior alternative to imatinib. In our case, the patient initially achieved CR under imatinib integrated regimen but later suffered relapse in the CNS. We replaced imatinib with dasatinib and induced complete remission.

After the treatment, the patient remained disease free for 3 months until a second

relapse occurred. After that, she no longer responded to dasatinib-based chemotherapy and we thereby infused her with CD19-targeted CAR T-cells. Up till then, multiple studies have confirmed the efficacy of genetically modified T-cells against B-cell malignancies. Lee and colleagues [4] assessed the feasibility, response rate, and of toxicity and maximum tolerated dose of CD19 CAR T-cell therapy. Among 20 patients with B-ALL, 14 achieved CR and 12 achieved MRD-negative CR. Additionally, the rise of CD19 CAR T-cells count in CSF coincides with the disappearance of CNSL in 2 patients, which suggests CD19 CAR T-cell therapy as a favorable profile for CNSL [4].

Nonetheless, there are still cases in which patients experienced relapse after CAR T-cell therapy. Grupp and colleagues [8] reported 2 children with advanced B-ALL who achieved remission with the robust expansion of CD19 CAR T-cells in BM and cerebrospinal fluid (CSF), yet one patient had a clinical relapse due to the occurrence of CD19-blasts. Table 1 is a summary of clinical trials involving CAR T-cell therapy, including those failed to induce prolonged remissions.

Here, the patient remained healthy for 10 months after the CAR T-cell therapy before she again experienced BM and CNS relapse. Unfortunately, we are unable to retrieve detailed information regarding the proliferation level of CAR T-cells because the patient was taken care

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Table 1. A summary of parts of clinical trials involving CAR T-cell therapy

Reference	Patients (n)	CNS involvement	Infused CAR T cells (Ts/kg)	CAR transduction efficiency	Response	Identified CAR T cells persistence	Relapse-up (n)	Relapsed reason	Toxicities	Post CAR-T allo-SCT
Grupp SA [3]	2	NR	1.4×10 ⁶ or 1.2×10 ⁷	NR	MRD-CR: 2/2 (100%)	NR	1	CD19 escape variant	Fever and CRS: 100%	None
Lee DW [5]	20	2	1×10 ⁶ or 3×10 ⁶	Mean: 66%	CR: 14/20 (70%); MRD-CR: 12/20 (60%)	Up to 68 days	2; at 3 and 5 months	All CD19-negative	CRS: 30%; CNS: 30%	10/12 in MRD-CR
Davila ML [7]	16 (4 Ph+ALL)	2	3×10 ⁶	Mean: 24%; Range: 5-60%	CR: 14/16 (88%); MRD-CR: 12/14 (86%)	Up to 3 months	2	1/2: a low T-cell dose ^a	sCRS: 44%; CNS: 38%	7/10 in CR
Gardner R [8]	7	NR	2×10 ⁶ -1×10 ⁷	NR	CR: 7/7 (100%)	NR	2	CD19-negative	B cell aplasia	NR
Turtle CJ [9]	30 (29 evaluable patients)	2	2×10 ⁵ , 2×10 ⁶ or 2×10 ⁷	NR	CR: 27/29 (93%); MRD-CR: 25/27 (93%)	~300 days	9	2/9: CD19-negative ^b ; 7/9: loss of CAR-T cells	sCRS: 23%; CNS: 50%	13/25 in MRD-CR
Brudno JN [10]	5 ^c	NR	4.2×10 ⁶ -7.0×10 ⁶	NR	CR: 4/5 (80%); MRD-CR: 4/4 (100%)	NR	2 (1 Ph-; 1 Ph+)	NR	NR	NR

CAR T, chimeric antigen receptor T cell; ALL, acute lymphoblastic leukemia; CNS, central nervous system; allo-SCT, allogeneic stem cell transplant; CR, complete remission; MRD, minimal residual disease; CRS, cytokine release syndrome; sCRS, severe cytokine release syndrome; CTL019: CD19-directed chimeric antigen receptor; NR, not reported. a. May be due to limited T cell trafficking or immunosuppression of CAR T cells within the extramedullary tumor microenvironment. b. All patient had progressed after allo HSCT. c. One patient with a myeloid phenotype switched.

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of and examined in an independent third-party institution. We speculated that the relapse may be explained by several reasons: first, she has experienced multiple relapses prior to the CAR T-cell therapy, leading to a genetic background bearing more mutations in cancer stem cells. Second, a heavy leukemia burden of 89% was identified upon initial diagnosis. Third, the patient suffered extramedullary relapse, in which cancer cells resides in a microenvironment that enables them to grow via paracrine secretion of growth factors. It allows the cancer cells to evade CAR T-cell detection due to the local sufficiency of nutrients. Therefore, we hypothesize that CAR T-cell therapy yields inferior results in this particular case due to a complex genetic background, heavy disease burden and tricky relapse locations.

Generally speaking, to induce durable remissions, functional CAR T-cell need to maintain a therapeutic level long enough to eradicate malignant cells in the recipient; however, the inherently short half-life of T-cells made it very difficult to accomplish. In a recent study, Fraietta [16] found that long term ibrutinib therapy was capable of preventing T-cell down regulation in patients with CLL. Furthermore, notable T-cell expansions both ex vivo and in vivo were observed when using ibrutinib for 1 year prior to T-cell collection. There are also studies suggested that continual administration of ibrutinib diminish the expression of inhibitory receptors that cause T-cell exhaustions such as PD-1 [17] and CD200 on B-cells [18]. Since dasatinib is similar to ibrutinib in its molecular construction and anti-tumor mechanisms [19, 20], we assume that the patient in our case was able to achieve CR because dasatinib-shares ibrutinib's capability to lower the expression level of tumor-mediated suppressive receptors and/or T-cell inhibitory signaling, thereby enhancing CAR T-cell proliferation and function. Kim [21] confirmed our hypothesis in his research. He suggested that there were two ways dasatinib could mediate T-cell function: it reduces BCR/ABL transcription, a main inhibitor of T-cells, thereby restores T-cell numbers and it blocks SRC kinases, whose subtypes suppress the activation and proliferation of T and NK Cells. Similar findings were also reported in S. Mustjoki's [22] and Anna Kreutzman's [23] studies, suggesting a clinically relevant

and profound role of dasatinib in immunomodulation.

Studies involving CAR T-cell and dasatinib combined regimen in the treatment of relapsed Ph+ALL have yet to be reported. In this case, however, we are able to conclude that dasatinib enhances therapeutic outcomes in Ph+ALL patients with CNS relapse who previously received CAR T-cell infusion. To get a detailed understanding of the potential interaction between the two therapies, we need to perform clinical trials on a larger cohort of patients under controlled conditions. Further researches on guided clinical protocols with similar approaches may help to obtain favorable clinical outcome on relapse Ph+ALL.

Disclosure of conflict of interest

None.

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References

- [1] Rowley JD. Letter: a new consistent chromosomal abnormality in chronic myelogenous leukaemia identified by quinacrine fluorescence and giemsa staining. *Nature* 1973; 243: 290-293.
- [2] Lazarus HM, Richards SM, Chopra R, Litzow MR, Burnett AK, Wiernik PH, Franklin IM, Tallman MS, Cook L, Buck G, Durrant IJ, Rowe JM, Goldstone AH; Medical Research Council (MRC)/National Cancer Research Institute (NCRI) Adult Leukaemia Working Party of the United Kingdom and the Eastern Cooperative Oncology Group. Central nervous system involvement in adult acute lymphoblastic leukemia at diagnosis: results from the international ALL trial MRC UKALL XII/ECOG E2993. *Blood* 2006; 108: 465-472.
- [3] Brentjens RJ, Davila ML, Riviere I, Park J, Wang X, Cowell LG, Bartido S, Stefanski J, Taylor C, Olszewska M, Borquez-Ojeda O, Qu J, Wasielewska T, He Q, Bernal Y, Rijo IV, Hedvat C, Kobos R, Curran K, Steinherz P, Jurcic J, Rosenblatt T, Maslak P, Frattini M and Sadelain M. CD19-targeted T cells rapidly induce molecular remissions in adults with chemotherapy-refractory acute lymphoblastic leukemia. *Sci Transl Med* 2013; 5: 177ra38.

CAR T-cell therapy with dasatinib to acute lymphoblastic leukemia

- [4] Davila ML, Riviere I, Wang X, Bartido S, Park J, Curran K, Chung SS, Stefanski J, Borquez-Ojeda O, Olszewska M, Qu J, Wasielewska T, He Q, Fink M, Shinglot H, Youssif M, Satter M, Wang Y, Hosey J, Quintanilla H, Halton E, Bernal Y, Bouhassira DC, Arcila ME, Gonen M, Roboz GJ, Maslak P, Douer D, Frattini MG, Giralto S, Sadelain M and Brentjens R. Efficacy and toxicity management of 19-28z CAR T cell therapy in B cell acute lymphoblastic leukemia. *Sci Transl Med* 2014; 6: 224ra225.
- [5] Gardner R, Wu D, Cherian S, Fang M, Hanafi LA, Finney O, Smithers H, Jensen MC, Riddell SR, Maloney DG and Turtle CJ. Acquisition of a CD19-negative myeloid phenotype allows immune escape of MLL-rearranged B-ALL from CD19 CAR-T-cell therapy. *Blood* 2016; 127: 2406-2410.
- [6] Grupp SA, Kalos M, Barrett D, Aplenc R, Porter DL, Rheingold SR, Teachey DT, Chew A, Hauck B, Wright JF, Milone MC, Levine BL and June CH. Chimeric antigen receptor-modified T cells for acute lymphoid leukemia. *N Engl J Med* 2013; 368: 1509-1518.
- [7] Lee DW, Kochenderfer JN, Stetler-Stevenson M, Cui YK, Delbrook C, Feldman SA, Fry TJ, Orentas R, Sabatino M, Shah NN, Steinberg SM, Stroncek D, Tschernia N, Yuan C, Zhang H, Zhang L, Rosenberg SA, Wayne AS and Mackall CL. T cells expressing CD19 chimeric antigen receptors for acute lymphoblastic leukaemia in children and young adults: a phase 1 dose-escalation trial. *Lancet* 2015; 385: 517-528.
- [8] Maude SL, Frey N, Shaw PA, Aplenc R, Barrett DM, Bunin NJ, Chew A, Gonzalez VE, Zheng Z, Lacey SF, Mahnke YD, Melenhorst JJ, Rheingold SR, Shen A, Teachey DT, Levine BL, June CH, Porter DL and Grupp SA. Chimeric antigen receptor T cells for sustained remissions in leukemia. *N Engl J Med* 2014; 371: 1507-1517.
- [9] Brudno JN, Somerville RP, Shi V, Rose JJ, Halverson DC, Fowler DH, Gea-Banacloche JC, Pavletic SZ, Hickstein DD, Lu TL, Feldman SA, Iwamoto AT, Kurlander R, Maric I, Goy A, Hansen BG, Wilder JS, Blacklock-Schuver B, Hakim FT, Rosenberg SA, Gress RE and Kochenderfer JN. Allogeneic T cells that express an anti-CD19 chimeric antigen receptor induce remissions of B-cell malignancies that progress after allogeneic hematopoietic stem-cell transplantation without causing graft-versus-host disease. *J Clin Oncol* 2016; 34: 1112-1121.
- [10] Turtle CJ, Hanafi LA, Berger C, Gooley TA, Cherian S, Hudecek M, Sommermeyer D, Melville K, Pender B, Budiarto TM, Robinson E, Steevens NN, Chaney C, Soma L, Chen X, Yeung C, Wood B, Li D, Cao J, Heimfeld S, Jensen MC, Riddell SR and Maloney DG. CD19 CAR-T cells of defined CD4+:CD8+ composition in adult B cell ALL patients. *J Clin Invest* 2016; 126: 2123-2138.
- [11] Thomas DA. Philadelphia chromosome positive acute lymphocytic leukemia: a new era of challenges. *Hematology Am Soc Hematol Educ Program* 2007; 435-443.
- [12] von Bubnoff N, Peschel C and Duyster J. Resistance of Philadelphia-chromosome positive leukemia towards the kinase inhibitor imatinib (STI571, Glivec): a targeted oncoprotein strikes back. *Leukemia* 2003; 17: 829-838.
- [13] Ravandi F and Kebriaei P. Philadelphia chromosome-positive acute lymphoblastic leukemia. *Hematol Oncol Clin North Am* 2009; 23: 1043-1063, vi.
- [14] Takayama N, Sato N, O'Brien SG, Ikeda Y and Okamoto S. Imatinib mesylate has limited activity against the central nervous system involvement of Philadelphia chromosome-positive acute lymphoblastic leukaemia due to poor penetration into cerebrospinal fluid. *Br J Haematol* 2002; 119: 106-108.
- [15] O'Hare T, Walters DK, Stoffregen EP, Jia T, Manley PW, Mestan J, Cowan-Jacob SW, Lee FY, Heinrich MC, Deininger MW and Druker BJ. In vitro activity of Bcr-Abl inhibitors AMN107 and BMS-354825 against clinically relevant imatinib-resistant Abl kinase domain mutants. *Cancer Res* 2005; 65: 4500-4505.
- [16] Fraietta JA, Beckwith KA, Patel PR, Ruella M, Zheng Z, Barrett DM, Lacey SF, Melenhorst JJ, McGettigan SE, Cook DR, Zhang C, Xu J, Do P, Hulitt J, Kudchodkar SB, Cogdill AP, Gill S, Porter DL, Woyach JA, Long M, Johnson AJ, Maddocks K, Muthusamy N, Levine BL, June CH, Byrd JC and Maus MV. Ibrutinib enhances chimeric antigen receptor T-cell engraftment and efficacy in leukemia. *Blood* 2016; 127: 1117-1127.
- [17] Riches JC, Davies JK, McClanahan F, Fatah R, Iqbal S, Agrawal S, Ramsay AG and Gribben JG. T cells from CLL patients exhibit features of T-cell exhaustion but retain capacity for cytokine production. *Blood* 2013; 121: 1612-1621.
- [18] Pallasch CP, Ulbrich S, Brinker R, Hallek M, Uger RA and Wendtner CM. Disruption of T cell suppression in chronic lymphocytic leukemia by CD200 blockade. *Leuk Res* 2009; 33: 460-464.
- [19] Herman SE, Gordon AL, Hertlein E, Ramanunni A, Zhang X, Jaglowski S, Flynn J, Jones J, Blum KA, Buggy JJ, Hamdy A, Johnson AJ and Byrd JC. Bruton tyrosine kinase represents a promising therapeutic target for treatment of chronic lymphocytic leukemia and is effectively targeted by PCI-32765. *Blood* 2011; 117: 6287-6296.

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- [20] Woyach JA, Johnson AJ and Byrd JC. The B-cell receptor signaling pathway as a therapeutic target in CLL. *Blood* 2012; 120: 1175-1184.
- [21] Kim DH, Kamel-Reid S, Chang H, Sutherland R, Jung CW, Kim HJ, Lee JJ and Lipton JH. Natural killer or natural killer/T cell lineage large granular lymphocytosis associated with dasatinib therapy for Philadelphia chromosome positive leukemia. *Haematologica* 2009; 94: 135-139.
- [22] Mustjoki S, Ekblom M, Arstila TP, Dybedal I, Epling-Burnette PK, Guilhot F, Hjorth-Hansen H, Hoglund M, Kovanen P, Laurinolli T, Liesveld J, Paquette R, Pinilla-Ibarz J, Rauhala A, Shah N, Simonsson B, Sinisalo M, Steegmann JL, Stenke L and Porkka K. Clonal expansion of T/NK-cells during tyrosine kinase inhibitor dasatinib therapy. *Leukemia* 2009; 23: 1398-1405.
- [23] Kreutzman A, Juvonen V, Kairisto V, Ekblom M, Stenke L, Seggewiss R, Porkka K and Mustjoki S. Mono/oligoclonal T and NK cells are common in chronic myeloid leukemia patients at diagnosis and expand during dasatinib therapy. *Blood* 2010; 116: 772-782.