Successful treatment of second-time CAR-T 19 therapy after failure of first-time CAR-T 19 and ibrutinib therapy in relapsed mantle cell lymphoma

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Abstract

Background. A patient with relapsed mantle cell lymphoma (MCL) showed stable disease after receiving ibrutinib therapy as a salvage therapy, after the failure of his first chimeric antigen receptor (CAR)-T 19 cell therapy.

Objectives. The combined effects of CAR-T 19 cells from the patient and ibrutinib on JeKo-1 cell were explored in vitro and in vivo.

Materials and methods. The expression of programmed death-1 (PD-1) receptor on $CD3^+$ T cells in the peripheral blood decreased from 82.95% in the first CAR-T 19 cell therapy to approx. 40% after 14 months of ibrutinib therapy. When the disease progressed again during the ibrutinib therapy, the patient was enrolled into the same clinical trial of CAR-T 19 cell therapy.

Results. The efficacy of CAR-T 19 cells increased after the ibrutinib therapy. The mRNA expression level of PD-1 in CAR-T 19 cells after ibrutinib therapy was lower than in CAR-T 19 cells before the ibrutinib therapy. Nevertheless, CAR-T 19 cell therapy combined with ibrutinib had no synergistic effect in a short term in vitro and in the JeKo-1 cell mouse model.

Conclusions. We expect our results to provide evidence for the combination treatment of ibrutinib for MCL or even other types of B-cell lymphomas. Moreover, the improvement in CAR-T 19 cell function was based on long-term ibrutinib therapy.

Key words: chimeric antigen receptor, immunotherapy, lymphoma, clinical chimeric antigen receptor T cell trials, ibrutinib

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Background

Relapsed/refractory (R/R) B-cell non-Hodgkin's lymphoma (B-NHL) is usually characterized by poor prognosis.[1,2] Chimeric antigen receptor (CAR) T-cell therapy has shown to be more effective in patients with R/R B-cell acute lymphoblastic leukemia (B-ALL)[3–9] and R/R B-NHL.[10–13] However, patients with R/R B-NHL have lower complete remission (CR) rate of CD19 CAR T–cell (CAR-T 19) therapy than those with R/R B-ALL.[14,15]

Ibrutinib, as an inhibitor of Bruton's tyrosine kinase (BTK), has been approved by the US Food and Drug Administration (FDA) for the treatment of relapsed chronic lymphocytic leukemia (CLL)[16] and mantle cell lymphoma (MCL).[17] This drug has been extensively administered for the treatment of various B-cell lymphomas.[18–20] Bruton's tyrosine kinase expressed on B cells acts as a cytoplasmic tyrosine kinase in the B-cell receptor signaling pathway.[21,22] Ibrutinib blocks BTK activation in different pathways to inhibit B-cell receptor signaling in B-cell lymphomas.[23–25]

The combination of ibrutinib and CAR-T 19 cells has shown excellent results in preclinical and clinical studies.[26] The CAR-T 19 cell therapy has been proven to be highly effective in CLL patients who have previously taken ibrutinib.[27] This drug can enhance the engraftment and efficacy of CAR-T 19 cells in CLL patients.[28] Other clinical reports have indicated a promising antitumor activity of CAR-T 19 cells in CLL patients who are resistant to ibrutinib therapy.[29] Ibrutinib combined with CAR-T 19 cells has been studied in an MCL cell line.[30] The efficacies of CAR-T 19 cells on the MCL cell line in vitro and in vivo were enhanced by this drug. In this study, a patient with relapsed MCL obtained CR with his second CAR-T 19 cell therapy after the failure of the first CAR-T 19 cell therapy and 14 months of ibrutinib therapy. The combination of CAR-T 19 cells from the patient and ibrutinib on JeKo-1 cell was explored in vitro and in vivo.

Objectives

The aim of the study was to observe whether a long-term ibrutinib therapy could enhance the efficacy of CAR-T 19 cell therapy in the treatment of R/R MCL in vitro, in mice, and clinically.

Materials and methods

Medical history

A 58-year-old man experienced right epigastric pain for 3 days in September 2014. Abdominal computed tomography (CT) revealed the presence of multiple low-density shadows in the spleen and lymph nodes of the splenic portal area/retroperitoneum (Fig. 1A). The biopsy of the hilum of the lymph node and immunohistochemical examination confirmed the diagnosis of MCL (Fig. 1B). The cyclin D1 level was 72.5%. The CR was obtained after 6 cycles of R-CHOP combination chemotherapy. No autologous hematopoietic stem cell transplantation or other maintenance therapy was initiated due to lung injury caused by heart stent surgery and rituximab.

In February 2017, the patient was again admitted to our hospital owing to lymph node enlargement in the right neck. There were no immunoglobulin H (IgH) and T-cell receptor gene rearrangements (Fig. 1C). Neck CT revealed that multiple cervical lymph nodes were enlarged (Fig. 2A). Cervical lymph node biopsy confirmed the previous diagnosis of MCL (Fig. 1D). The cyclin D1 level was 69.2%. Owing to the complications, the patient could not receive further combination chemotherapy. Subsequently, he participated in a clinical trial of autologous CAR-T 19 cells, expressing murine anti-CD19 scFv and 4-1BB-CD3ζ costimulatory activation domain (ChiCTR-ONN-16009862). The patient provided informed consent prior to the enrollment. This clinical trial was conducted under the approval of the Medical Ethics Committee of Tianjin First Central Hospital, China (approval No. 2015002X). Lymphodepleting chemotherapy with fludarabine (30 mg/m^2) and cyclophosphamide (400 mg/m^2) was given to the patient from the day –4 to day –2. On day 0, the patient was infused with autologous CAR-T 19 cells (2×10^7 cells/kg). The patient had high fever, chills and headache in the first 3 days. At day 7 of the treatment, the cervical lymph node enlargement suddenly increased with laryngeal edema and obstructive dyspnea (Fig. 2B). Therefore, we were forced to terminate CAR-T 19 cell therapy; the laryngeal edema and obstructive dyspnea were treated using dexamethasone.

Two weeks after the failure of CAR-T 19 cells therapy, ibrutinib therapy was administered as the salvage therapy. No increase was found in the percentage of anti-CAR-T 19 cells and gene expression level of anti-CD19 CAR. After ibrutinib therapy for 2 months, the size of the cervical lymph node decreased. Meanwhile, the programmed death-1 (PD-1) expression in peripheral blood CD3$^+$ T cells was 82.95%. In the following 2 months, no increase was observed in the percentage of anti-CAR-T 19 cells as well as gene expression level of anti-CD19 CAR in the peripheral blood. Therefore, the patient continued ibrutinib therapy for approx. 14 months with a stable disease condition.

Successful second CAR-T 19 cell therapy

After ibrutinib treatment for 14 months, cervical lymph node enlargement was observed again (Fig. 2C). Nevertheless, the PD-1 expression decreased to 42.5% at this time. As a result, the patient was once again enrolled in the same clinical trial (ChiCTR-ONN-16009862). He received the same dose of lymphodepleting chemotherapy and autologous CAR-T 19 cells as the 1st CAR-T 19 cell

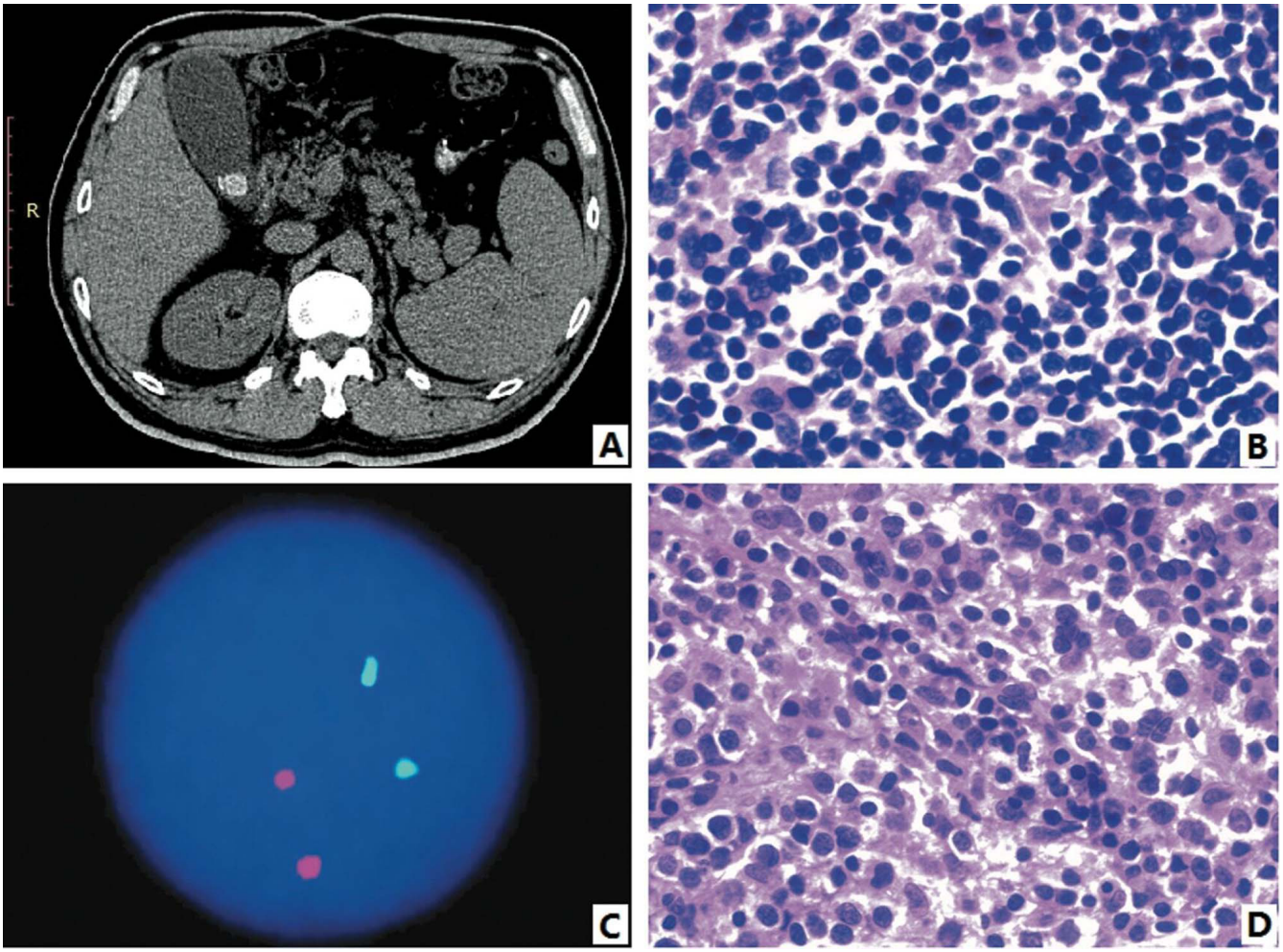

Fig. 1. Diagnosis of mantle cell lymphoma. A. Abdominal computed tomography (CT) in May 2014; B. Result of the pathological biopsy in May 2014; C. Immunoglobulin H (IgH) and T-cell receptor gene rearrangements were negative; D. Result of the pathological biopsy in February 2017

therapy. The level of interleukin-6 (IL-6) was measured using electrochemiluminescence analysis. Flow cytometry was used to detect the level of PD-1 in $CD3^+$ T cells and the percentage of CAR-T 19 cells in peripheral blood. The gene expression level of CAR-T 19 was determined using quantitative polymerase chain reaction (qPCR).

CAR-T 19 cell proliferation, cytotoxicity and mRNA expression level of PD-1

The CAR-T 19 cells from the patient's $CD3^+$ T cells were divided into various groups: the CAR-T 19 cell group derived from the patient before ibrutinib therapy with or without ibrutinib and CAR-T 19 cell group after ibrutinib therapy with or without ibrutinib. Ibrutinib (4 μg/mL/day) was added to the CAR-T 19 cell culture system on the first day. Cell Counting Kit-8 (Dojindo Molecular Technologies, Kumamoto, Japan) was used to detect the CAR-T 19 cell proliferation at 0 h, 24 h and 48 h. The transfection efficiency of CAR-T 19 cells was analyzed using flow cytometry. The JeKo-1 cell line was bought from the American Type Culture Collection (ATCC; Manassas, USA). Each group of CAR-T 19 and JeKo-1 cells was cocultured in a 1:1 ratio for 48 h without adding cytokines. The lactate dehydrogenase cytotoxicity test kit was used for the measurement of cytotoxicity at 0 h, 24 h and 48 h; the detection was performed at a wavelength of 490 nm. The enzyme-linked immunosorbent assay (ELISA) kit (Becton Dickinson Biosciences, Franklin Lakes, USA) was applied to determine the release of tumor necrosis factor alpha (TNF-α) and interferon gamma (IFN-γ). The absorbance (A) at 0 h, 12 h, 24 h, and 48 h was measured at 450 nm. The PD-1 mRNA expression in CAR-T 19 cells was determined with reverse transcription (RT)-qPCR. The PD-1 expression, normalized to that of GAPDH and relative to a calibrator, was calculated using the $2^{-\Delta\Delta Ct}$ method. All experiments were repeated 3 times to calculate the mean values.

Comparison of the efficacy of CAR-T cell therapy before and after ibrutinib therapy in mice

Each procedure was performed under the approval of the Animal Care and Use Committee of the Ethics

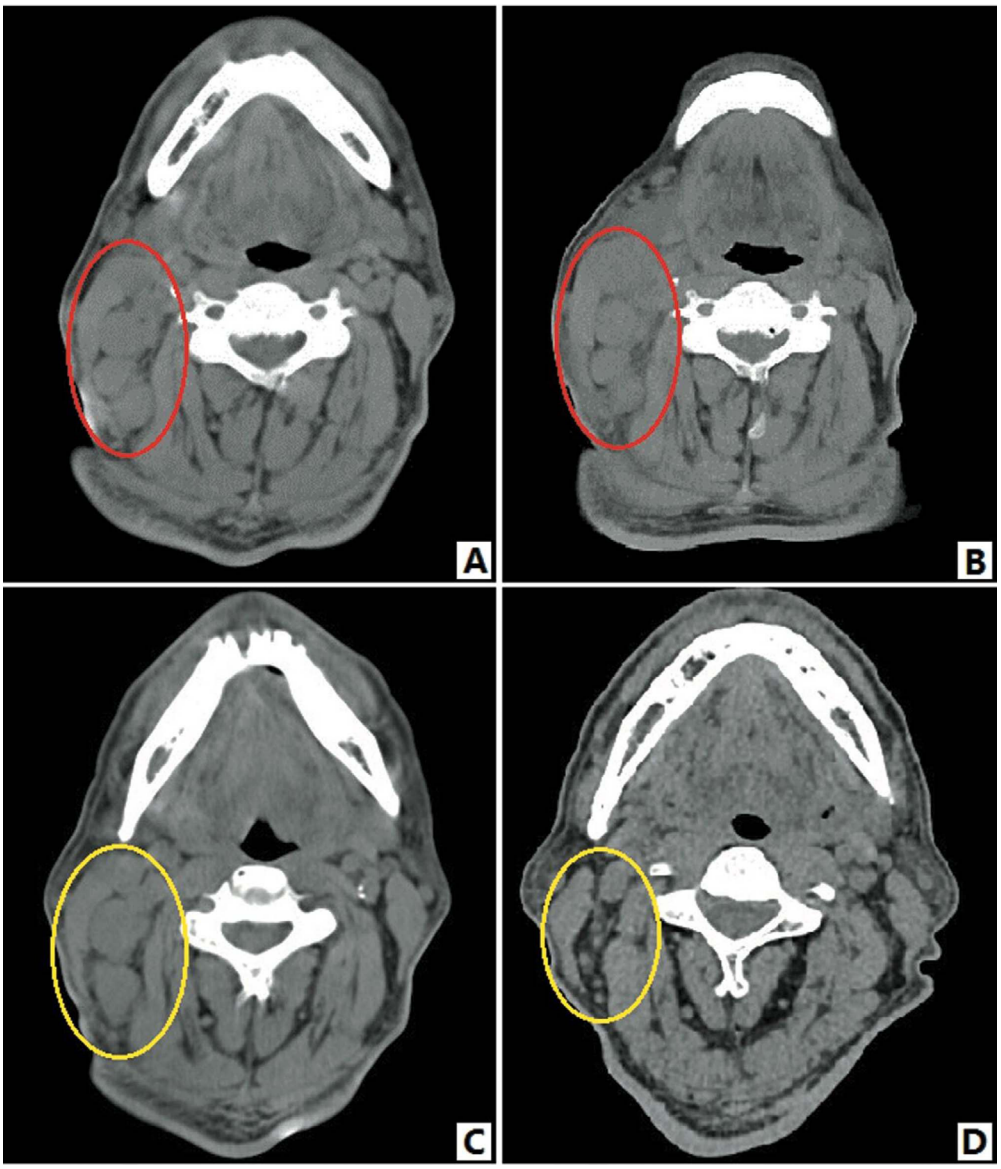

Fig. 2. Changes in the lymph nodes in the 2 chimeric antigen receptor (CAR)-T 19 cell therapies. A. Neck computed tomography (CT) before the first therapy; B. Enlargement of cervical lymph node after the 1st therapy; C. The cervical lymph node increased in size again after ibrutinib therapy; D. The size of the cervical lymph node returned to normal after the 2nd CAR-T 19 cell therapy

Committee of Nankai University, Tianjin, China (approval No. 2017-000012). In a lymphoma animal mode, 6–8 weeks old female mice (CAnN.Cg-Foxn1^{nu}/CrlVR) bought from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China), were injected with 1×10^7 JeKo-1 cells through caudal vein. After 10 days, the successful establishment of the model was confirmed with flow cytometry of the peripheral blood of mice. The mice of lymphoma model were randomly divided into different groups and treated with CAR-T 19 cells (1×10^7/kg), with or without ibrutinib. The ibrutinib monotherapy group was used as the control group. On the first day of treatment, CAR-T 19 cells were administered through caudal vein. Mice were given the clinically recommended dose of ibrutinib (8 mg/kg/day) via oral gavage. Each group consisted of 5 mice. The proportion of JeKo-1 and CAR-T 19 cells in mice was determined with flow cytometry.

Statistical analysis

Data are expressed as mean ± standard error (SE), along with the number of repeated experiments. All data were independently analyzed using the t-test. Statistically significant difference was defined as $p < 0.05$.

Results

Results of the second CAR-T 19 cell therapy

We obtained the agreement from the patient for using their specimens and data in this study. The patient had high fever, chills and headache during the first 5 days following the 2nd CAR-T 19 cell therapy. The highest serum IL-6 level was 275.6 pg/mL on day 4 of the 2nd treatment, compared with 15.2 pg/mL on the same day of the 1st treatment

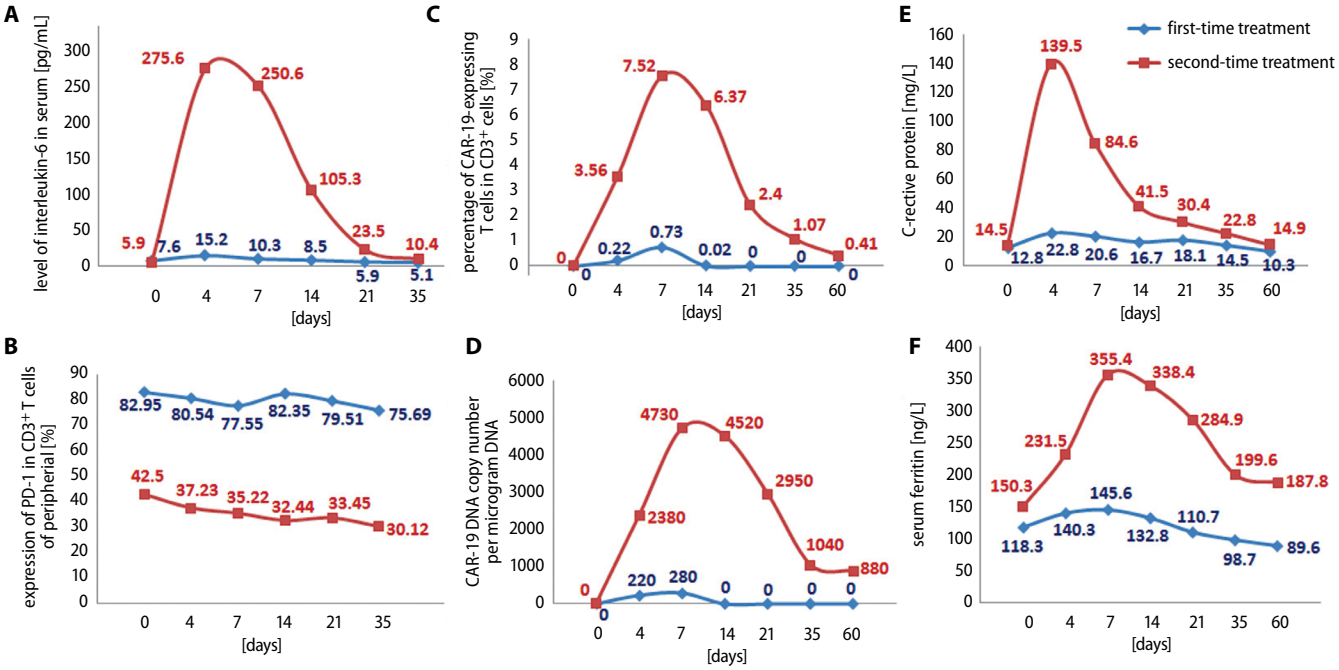

Fig. 3. Comparison of the 2 chimeric antigen receptor (CAR)-T 19 cell therapies. A. The highest serum interleukin-6 (IL-6) level was 275.6 pg/mL in the 2nd therapy; B. The programmed death-1 (PD-1) expression decreased from approx. 80% in the 1st therapy to approx. 40% in the 2nd therapy; C. The highest percentage of CAR-T 19 cells was 7.52% in the 2nd therapy; D. The highest copy number of CAR-T 19 DNA was 4730 copies/µg DNA in the 2nd therapy; E. The highest C-reactive protein (CRP) level was 139.5 mg/mL in the 2nd therapy; F. The highest ferritin level was 355.4 ng/mL in the 2nd therapy

(Fig. 3A). The expression level of PD-1 was maintained at >80% in the 1st treatment, in the 2nd treatment, it was maintained at approx. 40% (Fig. 3B).

On day 7 of the 2nd treatment, the percentage of CAR-T 19 cells was the highest (7.52%) in CD3+ T cells (Fig. 3C). Compared with the highest CAR-T 19 DNA value of 280 copies/µg DNA obtained in the 1st treatment, the value was 4730 copies/µg DNA in the 2nd treatment (Fig. 3D). The changes in serum ferritin and C-reactive protein (CRP) levels were similar to those in IL-6 levels (Fig. 3E,F).

In the 2nd treatment, the size of the cervical lymph node was significantly reduced with grade II CRS[31] after the 2nd CAR-T 19 cell therapy (Fig. 2D). The patient achieved CR after 2 months; CR has been maintained for 15 months, up to the present.

Results in vitro

No significant difference of the transfection rate was found in the 4 groups ($p = 0.6572$) (Fig. 4A). There was also no difference of the proliferation in the 4 groups at 24 h or 48 h ($p_{24h} = 0.3660$ and $p_{48h} = 0.2061$) (Fig. 4B). Ibrutinib therapy for 14 months and the addition of ibrutinib into the culture system did not affect the proliferation and transfection of CAR-T 19 cells. The CAR-T 19 cells derived from T cells before or after ibrutinib therapy showed almost no change in JeKo-1 cell cytotoxicity, regardless of whether ibrutinib was present or not in the culture system. Ibrutinib alone was used as the control group (before ibrutinib therapy: $p_{24h} = 0.8770$ and $p_{48h} = 0.4923$; after ibrutinib therapy: $p_{24h} = 0.5871$ and $p_{48h} = 0.6969$). The CAR-T 19 cells after ibrutinib therapy showed significant JeKo-1 cell activity regardless of whether ibrutinib was present or not in the culture system (without ibrutinib in the culture: $p_{24h} = 0.0000$ and $p_{48h} = 0.0000$; with ibrutinib in the culture: $p_{24h} = 0.0000$ and $p_{48h} = 0.0000$) (Fig. 4C). There was no difference in the IFN-γ release profile of all groups ($p_{24h} = 0.4006$ and $p_{48h} = 0.5819$) (Fig. 4D). Similarly to the results of proliferation and transfection, ibrutinib therapy and the addition of ibrutinib into the culture system did not affect the IFN-γ release. After ibrutinib therapy, PD-1 mRNA expression in CAR-T 19 cells was lower than before ibrutinib therapy (without ibrutinib in the culture: $p = 0.0013$; with ibrutinib in the culture: $p = 0.0052$). The mRNA expression level of PD-1 exhibited no difference in the CAR-T 19 cell culture systems with or without ibrutinib (before ibrutinib therapy: $p = 0.2104$; after ibrutinib therapy: $p = 0.1036$) (Fig. 5).

Results in mice

In the animal experiments, the percentage of JeKo-1 cells decreased in the CAR-T 19 cell group after ibrutinib therapy with ibrutinib compared with that before ibrutinib therapy with ibrutinib on 14 days and 28 days after therapy ($p_{14days} = 0.0000$ and $p_{28days} = 0.0000$). The percentage of JeKo-1 cells decreased in the CAR-T 19 cell group after ibrutinib therapy compared with that before ibrutinib therapy on 14 days after therapy ($p14_{days} = 0.0000$).

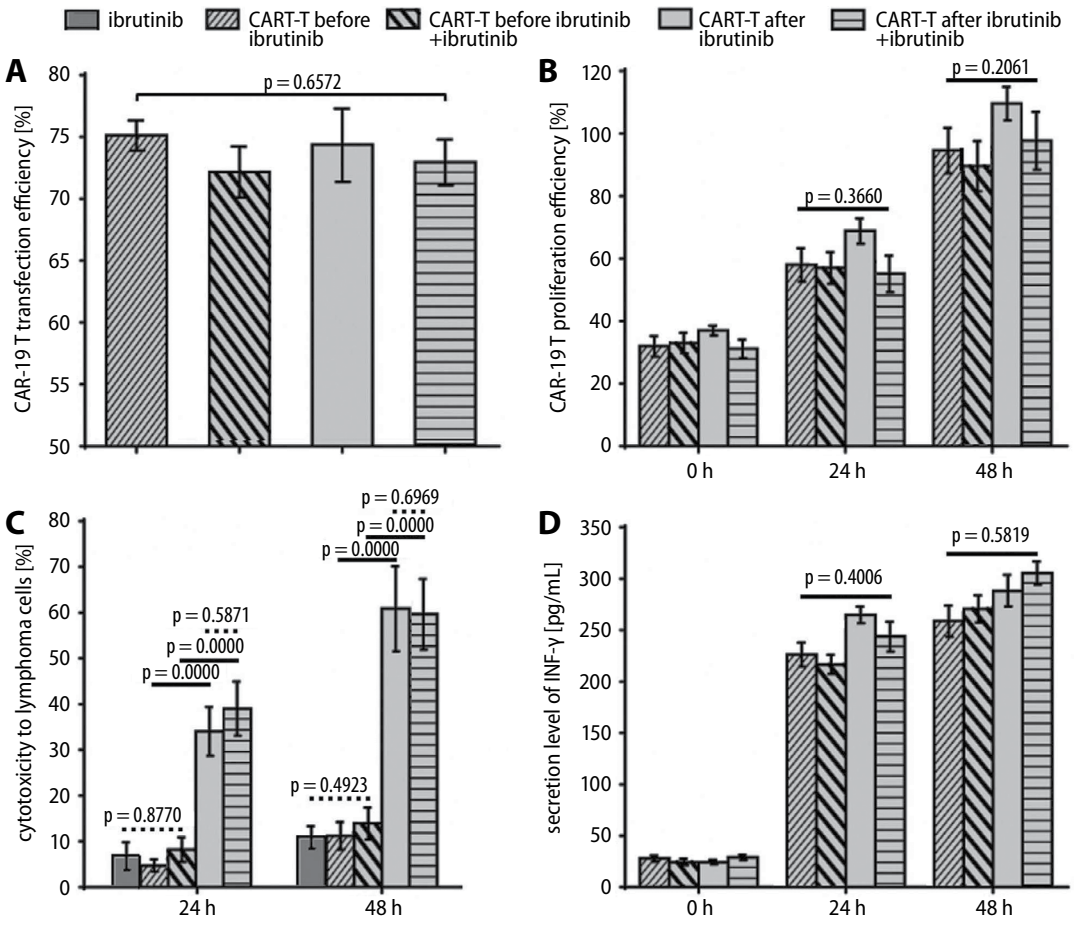

Fig. 4. Cytotoxicity of all chimeric antigen receptor (CAR)-T 19 cell groups to lymphoma cells. A,B. No significant difference was found in the transfection and proliferation of cells in all groups; C. The CAR-T 19 cells derived from T cells before or after ibrutinib therapy showed almost no change in JeKo-1 cell cytotoxicity regardless of whether ibrutinib was present or not. The CAR-T 19 cells after ibrutinib therapy showed significant JeKo-1 cell activity; D. There was no difference in the interferon gamma (IFN-γ) release profile in all groups

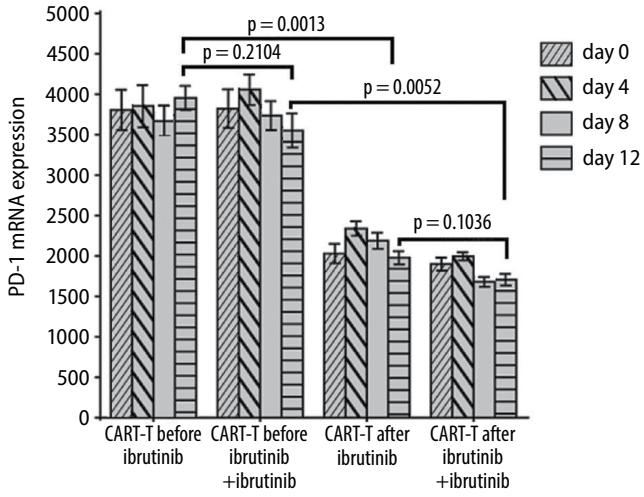

Fig. 5. The programmed death-1 (PD-1) mRNA expression in chimeric antigen receptor (CAR)-T 19 cells. The PD-1 mRNA expression in CAR-T 19 cells after ibrutinib therapy was lower than that before ibrutinib therapy. However, it showed no difference in the before or after ibrutinib therapy groups, regardless of whether ibrutinib was present or not in the system

Nevertheless, no difference was found in the two CAR-T 19 cell groups after ibrutinib therapy at 14 days or 28 days after CAR-T 19 cell therapy (p_{14days} = 0.1459 and p_{28days} = 0.4005). The proportion of JeKo-1 cells in the group of CAR-T 19 cells rapidly increased before ibrutinib therapy, resulting in the immediate death of the mice after 14 days, compared with that in the group treated with ibrutinib (p_{14days} = 0.0021). Both ibrutinib and CAR-T groups exhibited no difference, which was combined with ibrutinib before ibrutinib therapy (p_{14days} = 0.4292 and p_{28days} = 0.3175). The percentage of JeKo-1 cells in the CAR-T 19 cell groups after ibrutinib therapy combined with ibrutinib was lower than in the ibrutinib group (p_{14days} = 0.0000 and p_{28days} = 0.0000) (Fig. 6A). The proportion of CAR-T 19 cells in the CAR-T 19 cell groups after ibrutinib therapy with ibrutinib (p_{14days} = 0.0000) or without ibrutinib (p_{14days} = 0.0000) was higher than before ibrutinib therapy, but no difference was found in the two CAR-T 19 cell groups after ibrutinib therapy (p_{14days} = 0.1922 and p_{28days} = 0.4490). Besides, the proportion in the CAR-T 19 cell group exhibited no difference before ibrutinib therapy with or without ibrutinib (p_{14days} = 0.3514) (Fig. 6B).

Discussion

In our study, the patient with relapsed MCL showed stable disease during his 14-month ibrutinib treatment after the 1st therapy failed. When his condition worsened again

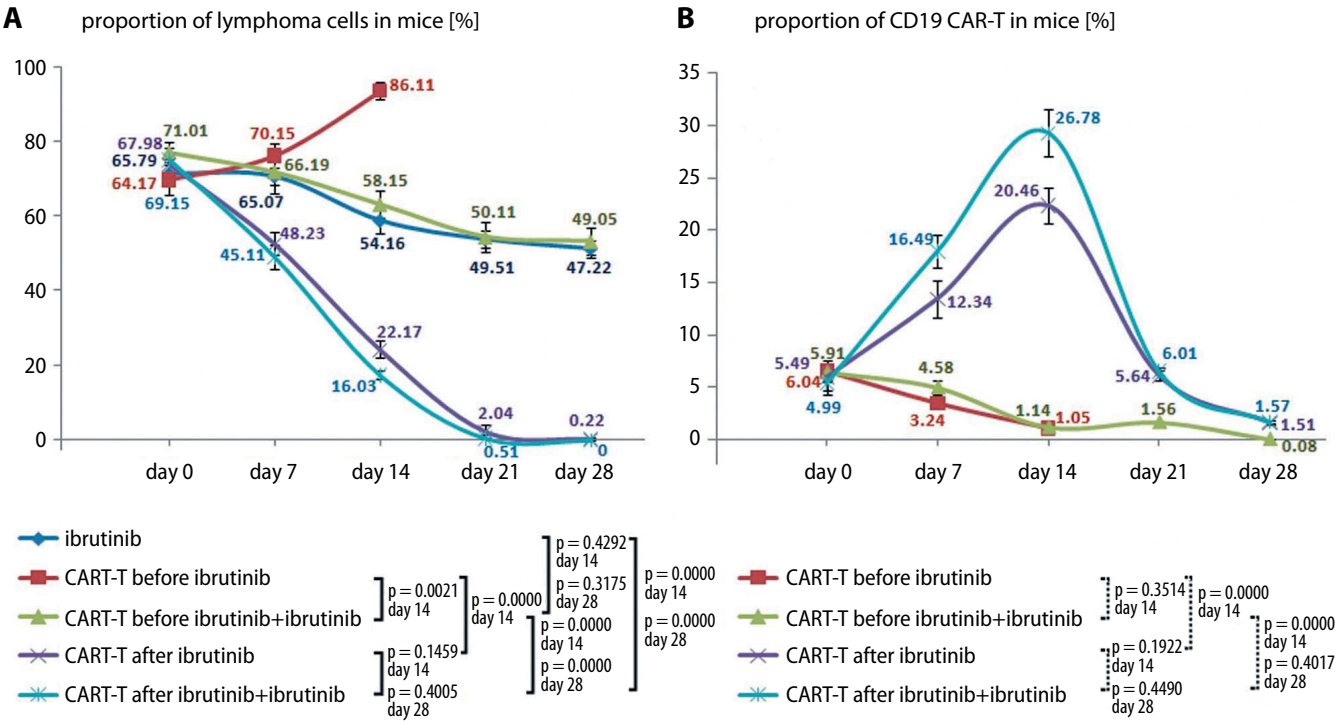

Fig. 6. Proportion of lymphoma and chimeric antigen receptor (CAR)-T 19 cells in mice. A. Regardless of whether ibrutinib was administered at the same time or not, the percentage of JeKo-1 cells in mice significantly decreased in the group of CAR-T 19 cell after ibrutinib treatment. The proportion of JeKo-1 cells in mice rapidly increased in the group of CAR-T 19 cell before ibrutinib treatment, leading to the immediate death of the mice after 14 days; B. The proportion of CAR-T 19 cells showed an inverse relationship with the proportion of JeKo-1 cells

during ibrutinib treatment, he received the 2nd CAR-T 19 cell therapy to achieve CR. The reason for the failure of the 1st CAR-T 19 cell therapy was unclear. It was observed that PD-1 expression was 82.95% in $CD3^+$ T cells before the first CAR-T 19 therapy. Interestingly, it decreased to approx. 40% after the 14-month ibrutinib therapy. This research investigated the efficacy of CAR-T 19 cell therapy before and after ibrutinib therapy in vitro and in mice.

Ibrutinib functions as a BTK inhibitor to downregulate NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) signaling, thus controlling the homing and migration of tumor cells. Blocking the interaction between macrophages and tumor cells is an important way to decrease the chemoattraction of tumor cells to the bone marrow microenvironment.[32–35] In one study, ibrutinib therapy with CLL for >5 months increased the proliferation and efficacy of CAR-T 19 cells.[28] A long-term ibrutinib therapy for patients with CLL could result in overcoming the immunosuppression of the primary disease, repairing the functional defects of autologous T cells, and decreasing the PD-1 expression in T cells. This effect of ibrutinib therapy is achieved through BTK-dependent and BTK-independent mechanisms.[36]

Another research has shown that ibrutinib modulates the tumor microenvironment of patients with CLL through the downregulation of the expression level of programmed death ligand-1 in CLL cells after 3 months of ibrutinib therapy.[37] Therefore, although the resistance to ibrutinib might occur during the treatment,[38,39] the CAR-T 19 cell therapy was highly effective in CLL patients after the failure of ibrutinib therapy.

Does long-term ibrutinib therapy improve T-cell function in patients with CLL and other types of B-cell lymphomas? The combination of ibrutinib improved the therapeutic effect of CAR-T 19 cell therapy on MCL cell line in vitro and in vivo.[30] In our study, the patient with relapsed MCL achieved CR with the 2nd CAR-T 19 cell therapy after 14-month ibrutinib therapy.

Limitations

This study has some limitations. The study did not investigate whether the mechanism of improving the T-cell function in MCL patients following long-term ibrutinib therapy is consistent with that in patients with CLL, and whether long-term ibrutinib therapy improves T-cell function in other types of B-cell lymphomas, which will be explored in the further studies.

Conclusions

In our study, we demonstrated that the efficacy of CAR-T 19 cell therapy was enhanced after 14-month ibrutinib therapy in vitro, in mice and in the clinical trial in which a patient was enrolled. However, there was no

synergistic effect of a short period of combination of CAR-T 19 cells and ibrutinib in vitro and in mice. The results of our study support the conclusion that long-term ibrutinib therapy could enhance the efficacy of the CAR-T 19 cell therapy. These results in MCL cell lines and the patient with MCL are similar to those of previous studies on CLL cell lines and patients with CLL.[28]

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References

1. Wang M, Fowler N, Wagner-Bartak N, et al. Oral lenalidomide with rituximab in relapsed or refractory diffuse large cell, follicular and transformed lymphoma: A phase II clinical trial. *Leukemia*. 2013;27(9):1902–1909. doi:10.1038/leu.2013.95
2. Gisselbrecht C, Glass B, Mounier N, et al. Salvage regimens with autologous transplantation for relapsed large B-cell lymphoma in the rituximab era. *J Clin Oncol*. 2010;28(27):4184–4190. doi:10.1200/jco.2010.28.1618
3. Zhao Z, Chen Y, Francisco NM, Zhang Y, Wu M. The application of CAR-T cell therapy in hematological malignancies: Advantages and challenges. *Acta Pharm Sin B*. 2018;8(4):539–551. doi:10.1016/j.apsb.2018.03.001
4. Chow VA, Shadman M, Gopal AK. Translating anti-CD19 CAR T-cell therapy into clinical practice for relapsed/refractory diffuse large B-cell lymphoma. *Blood*. 2018;132(8):777–781. doi:10.1182/blood-2018-04-839217
5. Davila ML, Riviere I, Wang X, et al. Efficacy and toxicity management of 19-28z CAR T cell therapy in B cell acute lymphoblastic leukemia. *Sci Transl Med*. 2014;6(224):224ra25. doi:10.1126/scitranslmed.3008226
6. Kebriaei P, Huls H, Singh H, et al. Adoptive therapy using Sleeping Beauty gene transfer system and artificial antigen presenting cells to manufacture T cells expressing CD19-specific chimeric antigen receptor. *Blood*. 2014;124(21):311. doi:10.1182/blood.V124.21.311.311
7. Turtle CJ, Hanafi LA, Berger C, et al. Immunotherapy of non-Hodgkin's lymphoma with a defined ratio of $CD8^+$ and $CD4^+$ CD19-specific chimeric antigen receptor-modified T cells. *Sci Transl Med*. 2016;8(355):355ra116. doi:10.1126/scitranslmed.aaf8621
8. Lee DW, Kochenderfer JN, Stetler-Stevenson M, et al. 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(9967):517–528. doi:10.1016/s0140-6736(14)61403-3
9. Kochenderfer JN, Dudley ME, Kassim SH, et al. Chemotherapy-refractory diffuse large B-cell lymphoma and indolent B-cell malignancies can be effectively treated with autologous T cells expressing an anti-CD19 chimeric antigen receptor. *J Clin Oncol*. 2015;33(6):540–549. doi:10.1200/jco.2014.56.2025
10. Porter DL, Levine BL, Kalos M, Bagg A, June CH. Chimeric antigen receptor-modified T cells in chronic lymphoid leukemia. *N Engl J Med*. 2011;365(8):725–733. doi:10.1056/NEJMoa1103849
11. Porter DL, Hwang WT, Frey NV, et al. Chimeric antigen receptor T cells persist and induce sustained remissions in relapsed refractory chronic lymphocytic leukemia. *Sci Transl Med*. 2015;7(303):303ra139. doi:10.1126/scitranslmed.aac5415
12. Neelapu SS, Locke FL, Bartlett NL, et al. Axicabtagene ciloleucel CAR T-Cell therapy in refractory large B-cell lymphoma. *N Engl J Med*. 2017;377(26):2531–2544. doi:10.1056/NEJMoa1707447
13. Maude SL, Laetsch TW, Buechner J, et al. Tisagenlecleucel in children and young adults with B-cell lymphoblastic leukemia. *N Engl J Med*. 2018;378(5):439–448. doi:10.1056/NEJMoa1709866
14. Schuster SJ, Bishop MR, Tam CS, et al. Tisagenlecleucel in adult relapsed or refractory diffuse large B-cell lymphoma. *N Engl J Med*. 2019;380(1):45–56. doi:10.1056/NEJMoa1804980
15. Ogba N, Arwood NM, Bartlett NL, et al. Chimeric antigen receptor T-cell therapy. *J Natl Compr Canc Netw*. 2018;16(9):1092–1106. doi:10.6004/jnccn.2018.0073
16. Byrd JC, Furman RR, Coutre SE, et al. Targeting BTK with ibrutinib in relapsed chronic lymphocytic leukemia. *N Engl J Med*. 2013;369(1):32–42. doi:10.1056/NEJMoa1215637
17. Wang ML, Rule S, Martin P, et al. Targeting BTK with ibrutinib in relapsed or refractory mantle-cell lymphoma. *N Engl J Med*. 2013;369(6):507–516. doi:10.1056/NEJMoa1306220
18. Rule S, Jurczak W, Jerkeman M, et al. Ibrutinib versus temsirolimus: 3-year follow-up of patients with previously treated mantle cell lymphoma from the phase 3, international, randomized, open-label RAY study. *Leukemia*. 2018;32(8):1799–1803. doi:10.1038/s41375-018-0023-2
19. Buggy JJ, Elias L. Bruton tyrosine kinase (BTK) and its role in B-cell malignancy. *Int Rev Immunol*. 2012;31(2):119–132. doi:10.3109/08830185.2012.664797
20. Schliffke S, Akyüz N, Ford CT, et al. Clinical response to ibrutinib is accompanied by normalization of the T-cell environment in CLL-related autoimmune cytopenia. *Leukemia*. 2016;30(11):2232–2234. doi:10.1038/leu.2016.157
21. Mohammad DK, Nore BF, Hussain A, Gustafsson MO, Mohamed AJ, Smith CI. Dual phosphorylation of Btk by Akt/protein kinase b provides docking for 14-3-3ζ, regulates shuttling, and attenuates both tonic and induced signaling in B cells. *Mol Cell Biol*. 2013;33(16):3214–3226. doi:10.1128/mcb.00247-13
22. Ponader S, Burger JA. Bruton's tyrosine kinase: From X-linked agammaglobulinemia toward targeted therapy for B-cell malignancies. *J Clin Oncol*. 2014;32(17):1830–1839. doi:10.1200/jco.2013.53.1046
23. Advani RH, Buggy JJ, Sharman JP, et al. Bruton tyrosine kinase inhibitor ibrutinib (PCI-32765) has significant activity in patients with relapsed/refractory B-cell malignancies. *J Clin Oncol*. 2013;31(1):88–94. doi:10.1200/jco.2012.42.7906
24. Herman SE, Mustafa RZ, Gyamfi JA, et al. Ibrutinib inhibits BCR and NF-κB signaling and reduces tumor proliferation in tissue-resident cells of patients with CLL. *Blood*. 2014;123(21):3286–3295. doi:10.1182/blood-2014-02-548610
25. Burger JA, Buggy JJ. Bruton tyrosine kinase inhibitor ibrutinib (PCI-32765). *Leuk Lymphoma*. 2013;54(11):2385–2391. doi:10.3109/10428194.2013.777837
26. Sadelain M, Rivière I, Riddell S. Therapeutic T cell engineering. *Nature*. 2017;545(7655):423–431. doi:10.1038/nature22395
27. Turtle CJ, Hay KA, Hanafi LA, et al. Durable molecular remissions in chronic lymphocytic leukemia treated with CD19-specific chimeric antigen receptor-modified T cells after failure of ibrutinib. *J Clin Oncol*. 2017;35(26):3010–3020. doi:10.1200/jco.2017.72.8519
28. Fraietta JA, Beckwith KA, Patel PR, et al. Ibrutinib enhances chimeric antigen receptor T-cell engraftment and efficacy in leukemia. *Blood*. 2016;127(9):1117–1127. doi:10.1182/blood-2015-11-679134
29. Romero D. Haematological cancer: After ibrutinib, CAR T cells induce responses. *Nat Rev Clin Oncol*. 2017;14(10):588. doi:10.1038/nrclinonc.2017.124
30. Ruella M, Kenderian SS, Shestova O, et al. The addition of the BTK inhibitor ibrutinib to anti-CD19 chimeric antigen receptor T cells (CART19) improves responses against mantle cell lymphoma. *Clin Cancer Res*. 2016;22(11):2684–2696. doi:10.1158/1078-0432.Ccr-15-1527
31. Brudno JN, Kochenderfer JN. Toxicities of chimeric antigen receptor T cells: Recognition and management. *Blood*. 2016;127(26):3321–3330. doi:10.1182/blood-2016-04-703751
32. Saleh LM, Wang W, Herman SE, et al. Ibrutinib downregulates a subset of miRNA leading to upregulation of tumor suppressors and inhibition of cell proliferation in chronic lymphocytic leukemia. *Leukemia*. 2017;31(2):340–349. doi:10.1038/leu.2016.181
33. Chang BY, Francesco M, De Rooij MF, et al. Egress of CD19(+)CD5(+) cells into peripheral blood following treatment with the Bruton tyrosine kinase inhibitor ibrutinib in mantle cell lymphoma patients. *Blood*. 2013;122(14):2412–2424. doi:10.1182/blood-2013-02-482125
34. Cheng S, Ma J, Guo A, et al. BTK inhibition targets in vivo CLL proliferation through its effects on B-cell receptor signaling activity. *Leukemia*. 2014;28(3):649–657. doi:10.1038/leu.2013.358

35. Ponader S, Chen SS, Buggy JJ, et al. The Bruton tyrosine kinase inhibitor PCI-32765 thwarts chronic lymphocytic leukemia cell survival and tissue homing in vitro and in vivo. *Blood*. 2012;119(5):1182–1189. doi:10.1182/blood-2011-10-386417
36. Long M, Beckwith K, Do P, et al. Ibrutinib treatment improves T cell number and function in CLL patients. *J Clin Invest*. 2017;127(8): 3052–3064. doi:10.1172/jci89756
37. Kondo K, Shaim H, Thompson PA, et al. Ibrutinib modulates the immunosuppressive CLL microenvironment through STAT3-mediated suppression of regulatory B-cell function and inhibition of the PD-1/PD-L1 pathway. *Leukemia*. 2018;32(4):960–970. doi:10.1038/leu.2017.304
38. Hamasy A, Wang Q, Blomberg KE, et al. Substitution scanning identifies a novel, catalytically active ibrutinib-resistant BTK cysteine 481 to threonine (C481T) variant. *Leukemia*. 2017;31(1):177–185. doi:10.1038/leu.2016.153
39. Jones D, Woyach JA, Zhao W, et al. PLCG2 C2 domain mutations co-occur with BTK and PLCG2 resistance mutations in chronic lymphocytic leukemia undergoing ibrutinib treatment. *Leukemia*. 2017;31(7): 1645–1647. doi:10.1038/leu.2017.110