Whether CAR-T cell bridging to allo-HSCT increases the incidence of graft-versus-host disease in patients with high-risk chronic lymphocytic leukemia

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Research article

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Abstract

Background The TP53 aberration[both del(17p) and TP53 mutations], a high-risk prognostic factor in patients with chronic lymphocytic leukemia (CLL)/small lymphocytic lymphoma (SLL), is closely related to drug-resistance, rapid disease progression, and short-term survival. Although novel agents, such as ibrutinib, idelalisib, as well as venetoclax, have shown good responses, allogenic hematopoietic stem cell transplantation (allo-HSCT) has been considered the only approach offered with curative intent.

Case presentation We reported a case of young high-risk CLL/SLL patient with Ritcher's transformation received ibrutinib plus CAR-T cells after the BCL-2 inhibitor venetoclax resistance, and then bridging to allo-HSCT when she had a complete remission (CR). Unfortunately, the patients died on day + 99 after transplantation due to intestinal grade IV acute graft-versus-host-disease (aGVHD) which could not be reversed by intensified immunosuppressive agents, including high-dose glucocorticoid, anti-CD25 monoclonal antibody basiliximab, and small-molecular inhibitors ruxolitinib, ibrutinib, as well as fecal microbiota transplantation (FMT).

Conclusion TP53 aberrations in CLL/SLL patients are prone to large cell transformation, and have a poor prognosis, especially in venetoclax-resistant patients. Ibrutinib combined with CAR-T cells offered a treatment option, but whether bridging allo-HSCT following CAR-T cells increased severe aGVHD remained unclear.

Background

Chronic lymphocytic leukemia (CLL)/Small lymphocytic lymphoma (SLL) is a clonal, mature B-cell proliferative tumor with high heterogeneity that mainly occurs in elderly people in Western countries, and it is characterized by the accumulation of lymphocytes in the peripheral blood, bone marrow, spleen, and lymph nodes. The TP53 aberrations [both del(17p) and TP53 mutations] were listed as absolute high-risk indicators according to NCCN guidelines for CLL and the CLL-IPI index [1–3]. In recent years, the availability of novel agents, such as BTK inhibitor ibrutinib [4], PI3K inhibitor duvelisib [5], and BCL-2 inhibitor venetoclax [6], have shown significant success in high-risk CLL patients. In addition, chimeric antigen receptor T-cell (CAR-T) targeting CD19 antigen has achieved good responses in relapse or refractory (R/R) CD19-positive tumors. However, CLL remains incurable, especially in patients with TP53 aberrations. Allogenic hematopoietic stem cell transplantation (allo-HSCT) has been considered the treatment choice for high-risk patients, and is the only approach offered with curative intent [7]. Here, we report the case of a young, high-risk CLL/SLL patient with del(17p) and Ritcher's transformation who received ibrutinib plus CAR-T cells after resistance to venetoclax and then was bridged to haploidentical HSCT (haplo-HSCT) when she had a complete remission (CR).

Case Report
A 33-year-old woman presented at the Affiliated Cancer Hospital of Zhengzhou University (Zhengzhou, China) on 7 March 2018. She was diagnosed with stage IIIA SLL with unmutated IGHV genes, a normal karyotype (46, XX), and without TP53 aberrations in March 2016, and then received a series of chemotherapy at the local hospital. In February 2018, the patient felt weak and fatigued, and had white blood cells (WBCs) \(63.6 \times 10^9/L\), lymphocytes (LYMs) \(43.8 \times 10^9/L\), hemoglobin (HGB) 64 g/L, and platelets \(45 \times 10^9/L\). Computed tomographic scanning revealed multiple enlarged lymph nodes in the cervical, axillary, and abdominal area (Figs. 1a, 1b and 2a), the largest of which was approximately 10.6 cm × 7.3 cm × 9.2 cm in size (Fig. 1b). She also had splenomegaly (approximately 13 cm below the costal margin) (Fig. 2b). Flow cytometry (FCM) showed that the abnormal rate of mature lymphocytes in peripheral blood was 98.5%, and the rate of 17p deletion detected by fluorescence in situ hybridization (FISH) was 96%. Her lactate dehydrogenase (LDH) level was 261 U/L, and \(\beta_2\)-M level was 3.6 mg/L. This patient was diagnosed as CLL/SLL (CLL international prognostic index 9 points, extremely high risk; Rai stage IV; Binet stage C) [1]. She started to take venetoclax with weekly dose escalation on May 9, 2018. After two months of venetoclax, her tumor burden reduced 37% compared with pretreatment assessment by contrast CT scan (Figs. 1a-d, 2a, and 2b), and her HGB and PLT counts returned to nearly normal. However, her multiple lymph nodes rapidly enlarged again after three months, especially in the neck accompanied by hoarseness and superior vena cava compression syndrome. The tumor burden was increased by 31.6% within one week. In addition, multiple new extracellular lesions appeared in the subcutaneous tissue of the left lower extremity, and HGB and PLT rapidly decreased again, suggesting venetoclax resistance. What's worse, large cells were found by bone marrow aspiration, indicating Richter's transformation. Hence, the patient received an emergency ibrutinib monotherapy (420 mg/day), and obtained partial remission (PR) two months later.

To prevent ibrutinib from developing rapid resistance, 100 mL of peripheral blood (PB) was collected for anti-CD19 CAR-T cell therapy after the patient provided consent. A FC preconditioning regimen (fludarabine 30 mg/m\(^2\)/day d1-3 and cyclophosphamide 0.6 g/m\(^2\)/day d1-2) was administered on November 3, 2018. A total dose of \(2 \times 10^6\) CAR-positive T-cells/kg were infused five days later. The patient developed cytokine release syndrome (CRS) level 1 (CAR-T cell copy number and cytokine levels are shown in Figs. 2c and 2d). She achieved CR with minimal residual disease (MRD) negative in the PB and bone marrow measured by FCM four weeks after infusion. The patient was del (17p) negative by FISH and mutation negative by second-generation sequencing. The multiple, enlarged lymph nodes found in the neck, axilla, and groin were significantly reduced or disappeared, but the spleen remained approximately 2.1 cm below the costal margin (Fig. 2b).

To prevent the rapid recurrence of disease due to large cell transformation, a salvage haplo-HSCT from her father was performed in the absence of an HLA-identical donor. The reduced intensity conditioning (RIC) regimen consisted of fludarabine (30 mg/m\(^2\)/day) on days − 6 to -2, busulfan (3.2 mg/kg/day) on days − 6 to -3, cyclophosphamide (0.5 g/m\(^2\)/day) on days − 6 to -3, and 8 mg/kg thymoglobuline for 4 days. The GVHD prophylaxis regimen was comprised of cyclosporine, mycophenolate mofetil (MMF), and a short course of methotrexate. The donor's peripheral blood stem cells were infused on January 20,
2019. The total number of MNCs was $11.13 \times 10^8$/kg, and the total number of CD34+ cells was $3.84 \times 10^6$/kg. The patient suffered from oral mucositis on day 7 after transplantation (+7 day). Both neutrophil and platelet successfully engrafted on day +12. A rash appeared on both hands on day +15 and 0.4 mg/kg/day methylprednisolone was prescribed. The patient developed nausea, vomiting, and diarrhea on day +21, rapid diarrhea deterioration within two days (watery, grey-green, stool volume up to 2,000 ml per day, approximately 20–30 times), frequent urination, urgency, pain in urination, blood in urine, and a rash gradually covered her whole body. The patient was diagnosed with intestinal grade IV acute graft-versus-host-disease (aGVHD), skin grade II aGVHD, hemorrhagic cystitis, and a urinary tract infection. Combined antibiotics were given for infection. A series of intensified immunosuppressive agents were administered for aGVHD treatment, including methylprednisolone (1.6 mg/kg/day), three doses of the anti-CD25 monoclonal antibody basiliximab (20 mg/time on days +30, +33, and +39, respectively), low dose of ruxolitinib (5 mg twice daily from days +34 to +60), ibrutinib (420 mg/day continuously), and two times of fecal microbiota transplantation (FMT) (on days +45 and +46, respectively) (Fig. 2e). The skin aGVHD soon disappeared, however, the diarrhea symptoms only achieved transient remission and continued to deteriorate. The patient ultimately died on April 28, 2019.

**Discussion**

This report presented a young high-risk CLL/SLL patient with 17p deletion who developed drug-resistance and large cell transformation after taking venotoclax for three months, received ibrutinib combined with CAR-T cells and obtained CR with MRD negative, and then allo-HSCT was performed. However, intestinal grade IV aGVHD occurred and could not be reversed by intensified immunosuppressive agents, and the patient ultimately died +99 day post-transplantation.

CLL patients with TP53 aberrations have poor response to immunochemotherapy and short-term survival [2]. Small-molecule targeted drugs have shown significant success [4–6], but still can not completely overcome the poor prognosis, particularly patients with large cell transformation [8]. Recently, it was found that ibrutinib could regulate T cell immunity and significantly increase the implantation of CAR-T cells and enhance their targeted cytotoxicity in CLL patients [9, 10]. The 33-year-old patient with 17p deletion in this study developed resistance to BCL-2 inhibitor three months later, followed by large cell transformation and rapid disease progression. As a result, she took ibrutinib monotherapy as an emergency treatment, then received ibrutinib combined with CAR-T cell therapy two months later, and achieved MRD-negative CR after one month of combination therapy. Allo-HSCT may be the best choice for the long-term survival in young, high-risk CLL patients, and the only way to cure the disease, as shown in a previous report with 63% OS at 2 years and 55% at 5 years [11]. Therefore, she underwent haplo-HSCT, but unfortunately died of severe intestinal aGVHD, which deserves our in-depth consideration and discussion. It is not clear whether young, high-risk CLL patients with TP53 aberrations or large cell transformation still require bridging allo-HSCT after CAR-T cell therapy, which may be different from those with acute lymphoblastic leukemia (ALL) [12]. In addition, whether allo-HSCT followed by CAR-T cells increases the occurrence of aGVHD or whether preconditioning regimens and GVHD prophylaxis for
CLL patients needs to be changed, remained unknown. Shadman M et al. [13] reported the safety of allo-HSCT after CAR T-cell therapy in ALL and CLL patients. Brudno et al. also provided evidence that CAR-T cells do not increase GVHD, because aGVHD takes a median of four weeks to develop after transplantation, whereas the persistence of CAR19 T cells was generally limited to fewer than four weeks [14]. Therefore, the interval between CAR-T cell infusion and transplantation should be more than 4 weeks in order to prevent the occurrence of GVHD to the maximum extent.

GVHD is the most common complication of allo-HSCT, particularly in high-risk CLL patients. Pretreatment schemes are associated with GVHD, and RIC regimen has a lower incidence of aGVHD than myeloablative conditioning [15]. In addition, incompatible HLA type, donor gender, and age are also risk factors for aGVHD [16]. This patient received haplo-HSCT from her 63-year-old father with RIC regimen, which may be the cause of her severe aGVHD. Intestinal aGVHD is the major cause of non-relapse mortality in allo-HSCT recipients [17]. Although there are well-developed therapeutic strategies for aGVHD, including glucocorticoids as first-line therapy and new options such as the JAK1/2 inhibitor, anti-CD25 monoclonal antibody, mesenchymal stem cells (MSCs), and others [18], severe aGVHD is also a life-threatening complication. The patient in this study received intensified immunosuppressive agents after the failure of glucocorticoids, including basiliximab, ruxolitinib, and ibrutinib, and even combined with FMTs, which still could not reverse the severe intestinal aGVHD.

In conclusion, this study reported that ibrutinib combined with CAR-T cells can be used as a treatment option for high-risk CLL patients with TP53 aberrations and/or large cell transformation, particularly after the failure of BCL-2 inhibitors. Here, the challenge is that whether bridging allo-HSCT is needed in the era of new drugs, and whether bridging allo-HSCT following CAR-T cell therapy will increase the occurrence of severe aGVHD. More clinical trials are needed to verify the benefits and limitations of this strategy for high-risk CLL patients.

Abbreviations

CLL/SLL: Chronic lymphocytic leukemia/small lymphocytic lymphoma (SLL); allo-HSCT: Allogeneic hematopoietic stem cell transplantation; CAR-T cells: Chimeric antigen receptor T cells; CR: Complete remission; aGVHD: Acute graft-versus-host disease; β2-M: β2 microglobulin; HLA: Human leukocyte antigen; MRD: Minimal residual disease; OS: Overall survival; CRS: Cytokine-release syndrome; FCM: Flow cytometry; FMT: fecal microbiota transplantation;

Declarations

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Availability of data and materials

Not applicable.

Authors’ contributions

JJG and QW collected the patient information, analyzed the data, designed the figures, and wrote the manuscript. JJG and QW contributed equally to this work. YS, HA, and YWF participated in the management of patients before and after HSCT. LH, XDW, and YPS participated in the analysis and interpretation of the data before and after CAR-T cell infusion. QSY participated in the whole management of patients, analyzed the data, and wrote and revised the manuscript.

Ethics approval and consent to participate

The ethics committee of the Affiliated Tumor Hospital of Zhengzhou University has approved this study.

Consent for publication

Written informed consent was obtained from the patient.

Conflict of interest

The authors declare that they have no conflict of interest. This manuscript has been read and approved by all authors and is not under consideration for publication elsewhere.

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Figures
Figure 1

Changes in tumor lesions in the neck/abdominal areas at different treatment stages. a and b Before the treatment of BCL-2 inhibitor venetoclax.; c and d The third month of treatment for venetoclax; e and f Drug resistance and disease progression after 4 months of venetoclax treatment (before treatment of BTK inhibitor ibrutinib). g and h Seventeen days after ibrutinib combined with CAR-T cells.
Figure 2

Clinical parameters and the anti-GVHD drugs used for the high-risk CLL patient treated with ibrutinib plus CAR-T cells and bridged haplo-HSCT. a and b Changes in tumor lesions in the neck/abdominal areas and spleen at different treatment stages. c and d Changes in the copies of CAR DNA and cytokine levels following CAR-T cell infusion by PCR and immunological assay. e Adjustment in anti-GVHD drugs and change in disease condition after transplant.