CASE REPORT

Durable Molecular Remission in a Lymphoid BP-CML Patient Harboring T315I Mutation Treated with Anti-CD19 CAR-T Therapy

This article was published in the following Dove Press journal:
OncoTargets and Therapy

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Abstract: Despite the prominent effects of BCR-ABL tyrosine kinase inhibitors (TKI) therapy in patients with chronic phase-chronic myeloid leukemia (CP-CML) and thus low incidence of blastic transformation, blast phase (BP)-CML remains a major therapeutic challenge in the TKI era. The "gatekeeper" mutation T315I in BCR-ABL1 kinase, which often coupled with a poor prognosis, is quite common and resistant to all TKIs except for ponatinib. The occurrence of T315I mutation in BP-CML makes the situation more complex. Anti-CD19 chimeric antigen receptor T cell (CAR-T) technology is a new immunotherapy which has significantly improved the efficacy of B cell hematologic malignances. Here we report a lymphoid BP-CML patient harboring T315I mutation who achieved complete molecular remission and returned to chronic phase by anti-CD19 CAR-T therapy. Our study provides a new therapeutic strategy for patients in BP-CML.

Keywords: anti-CD19 CAR-T, BP-CML, BCR-ABL1, T315I

Introduction

Chronic myeloid leukemia (CML), accounting for 15–20% of all leukemia cases in adults, is a malignant clonal hematological disease that makes the myeloid neoplastic cells proliferation out of control.[1] It is characterized by the formation of a fusion gene, *BCR-ABL1*, by *ABL1* gene on chromosome 9 and *BCR* gene on chromosome 22, which results in the expression of oncoprotein BCR-ABL1. CML has a natural history of 3 distinct stages: chronic phase (CP), accelerated phase (AP), and blast phase (BP). The final transformation of CML can result in myeloblastic (50%) or lymphoblastic (25%) phenotypes, with the remaining 25% comprising bi-phenotypic or undifferentiated blasts.[2,3]

The biologic basis of the progression from chronic phase through accelerated phase to blast crisis is poorly understood. It is now generally accepted that it is the consequence of continued BCR-ABL activity leading to genetic instability, DNA damage, and impaired DNA repair.[4,5] This progression usually leads to patient death in 3 years.[6] Reports show the median overall survival and failure-free survival of BP-CML was 12 months and 5 months, respectively.[7] Treatment with TKIs has reduced the rate of progression to BP and improved survival in blast crisis (BC) modestly. However, the efficacy of TKI monotherapy in BP-CML is quite unsatisfactory, probably due to an inability to eliminate the leukemic clone[8] and rapid onset of *BCR-ABL*1 mutations which are resistant to TKI monotherapy.[9,10]

Furthermore, even if favorable responses can be achieved with a combination therapy of TKI and conventional chemotherapy in some patients,[11] their responses are usually transitory. The combination treatment may bring more side effects and possibly not feasible for elderly or unfit patients. In most cases, TKIs alone or in combination with conventional chemotherapy provide a possible bridge to bone marrow transplant. Nowadays, allogeneic bone marrow transplant remains the only curative option for CML patients, but only in a minority of eligible patients with a compatible donor. Thus, there is yet to be an ideal method for managing patients in this phase. Further studies are urgently needed to improve therapeutic efficacy and long-term outcome.

Since its first report in 2006, CAR-T, as a new immunotherapy, has been widely used for various diseases, especially in the treatment of hematological malignances, in which CAR-T has achieved unprecedented success.[12–14] The most successful results were mainly from the anti-CD19 CAR-T therapy, which significantly improved the clinical efficacy of B-cell malignances.[15,16] As a result, it was named "the top ten scientific breakthrough in 2013" by Science along with the "Immune Monitoring Point Blocking Antibody Therapy". CAR-T cells could bypass the normal activation pathway, transcend the restriction of MHC molecules, partially overcome the immune escape of cancer cells, and more efficiently kill cancer cells. At the same time, as CAR-T cells have high affinity for specific tumor antigens, they can kill tumor cells expressing these antigens efficiently. Compared with conventional chemotherapy regimens, CAR-T cells enhance the killing ability of specific targets and reduce treatment-related toxicity. Therefore, it is of great significance to explore the efficacy and safety of anti-CD19 CAR-T cell therapy for lymphoid BP-CML patients.

In this paper, we report a lymphoid BP-CML patient harboring T315I mutation in BCR-ABL1 who returned to chronic phase and achieved complete molecular remission with anti-CD19 CAR-T therapy. As far as we know, this is the first case of a T315I-bearing lymphoid BP-CML patient displaying satisfactory response to anti-CD19 CAR-T therapy.

Case Report

A 60-year-old male patient was found to have leukocytosis in a routine physical examination in 2015. He did not attach much importance to it. However, on November 13, 2016, he was admitted to our hospital complaining of limb pain and being emaciated for half a year. Blood tests showed a high white blood cell count of 130×10^9/L, while the platelet count was 123×10^9/L and hemoglobin level was 109 g/L. Spleen size was normal. Bone marrow examination and flow cytometry suggested chronic myelogenous leukemia in chronic phase. Cytogenetics revealed Philadelphia chromosome was positive. FISH analysis detected that the BCR-ABL1 expression was 95%. He was thus diagnosed with CP-CML, with low risk according to the Sokal score 0.78.

The patient was given imatinib (400 mg/d) starting from January 13, 2017, but resistance occurred quickly after half a year. Gene sequencing showed Y253H mutation in the ABL1 kinase domain (Figure 1). As a result, dasatinib (100mg/d) was given instead. On February 23, 2018, bone marrow examination revealed a blast crisis, with 55% of leukemic blasts that were CD19+/CD10+/CD34+/CD22 +/CD79α+/CD3-/CD56-/CD16-/CD13-/CD33±. The total percentage of cells expressing CD19 was 57%. No additional chromosomal alterations were identified. Moreover, T315I mutation was identified in Sanger sequencing (Figure 1). The patient was then given induction chemotherapy with daunorubicin, L-asparaginase, prednisone, and cyclophosphamide (DVCLP) regimen in combination with dasatinib (100mg/d) for two courses of treatment on March 5 and April 23, 2018, respectively. It was shown that the BCR-ABL1 level decreased from 50.76% (IS) to 4.12% (IS) after chemotherapy in combination with dasatinib, then increased to 10.82% (IS) 3 months later (Figure 2).

Subsequently, on July 7, 2018, the patient received an infusion of anti-CD19 CAR-T cells that had been activated ex vivo with anti-CD3/CD28 antibody-coated beads and transduced with a lentiviral vector containing the anti-CD19 CAR transgene. The total dose was 1.6×10^6 CAR-positive T-cells/kg, given over 3 consecutive days. Meanwhile, the patient was not given dasatinib during the CAR-T therapy since he was resistant to dasatinib. No immediate infusion-related toxicity was observed, but he developed rigor and fever (38°C) by day +10, with C-reactive protein (CRP 2.65 mg/L), cytokine levels (Figure 3), and ferritin (960 ng/mL) increasing significantly. Then, the patient was given an intravenous infusion of 320 mg tocilizumab. The patient's body temperature dropped to a normal level in a few hours. Within 60 days after the infusion of CAR-T cells, no visceral toxicity and no cytokine release syndrome (CRS) above 3 degrees (NCI-CTCAE standard) were observed (Figure 3). BCR-ABL1 was monitored every 3 months after CAR-T treatment. Unexpectedly, BCR-ABL1 increased from 10.82% (IS) to
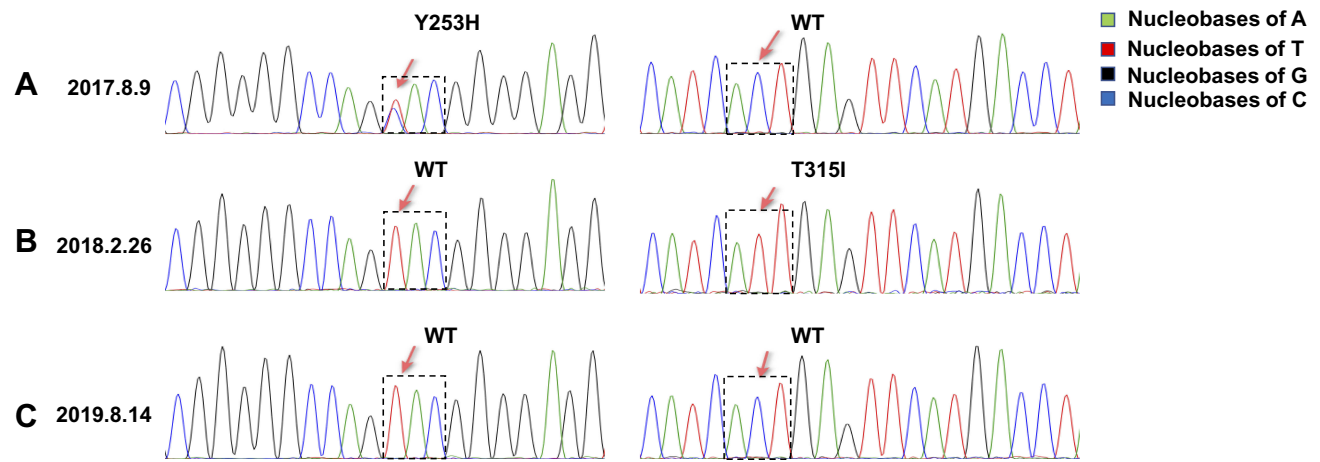
Figure 1 Y253H and T315I mutation in the *ABL1* kinase domain were detected by PCR-direct sequencing before and after anti-CD19 CAR-T treatment. (**A**) Y253H mutation in the *ABL1* kinase domain was identified in the patient after imatinib treatment for half a year. (**B**) T315I mutation was identified about half a year after switching from imatinib to dasatinib, while Y253H was undetectable. (**C**) No mutations were identified after chemotherapy followed by anti-CD19 CAR-T therapy. Colors green, red, black and blue represent nucleobases of A, T, G and C, respectively.
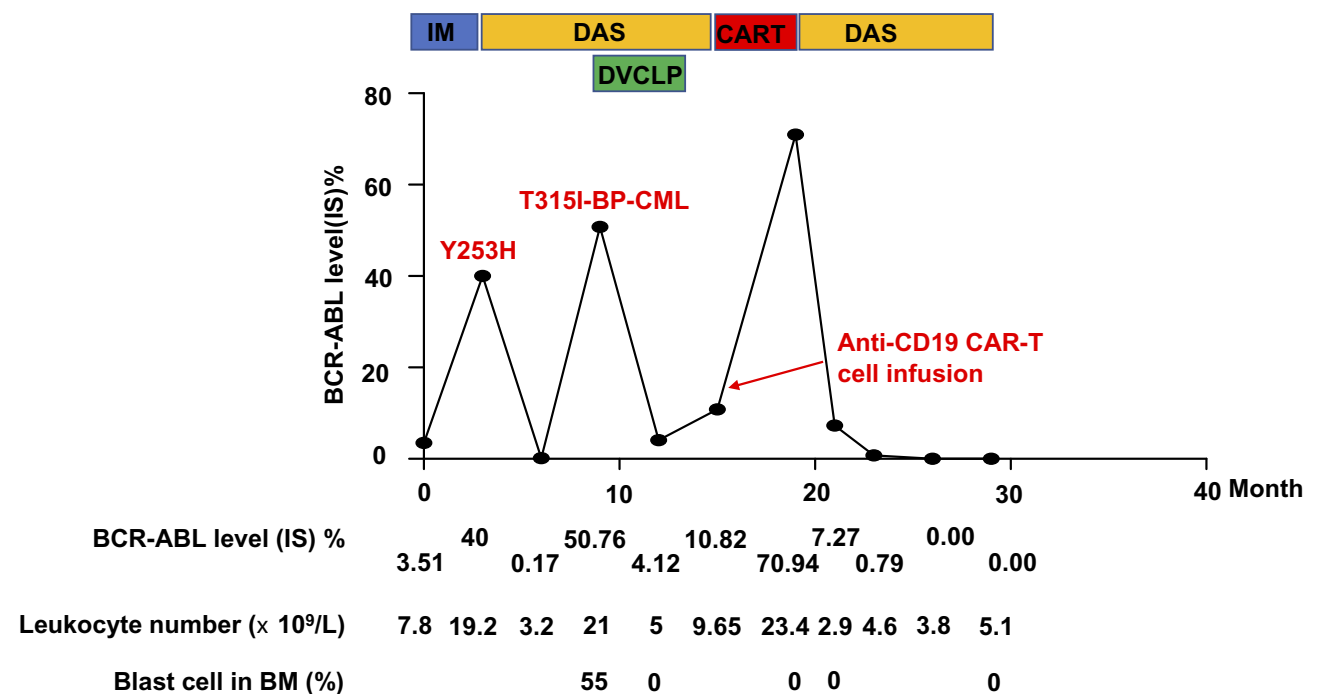

Figure 2 *BCR-ABL1* expression level, leukocyte number and the percentage of blast cells in bone marrow before and after anti-CD19 CAR-T treatment since April 24, 2017.

70.94% (IS). Since no other treatment option was available, the patient was given dasatinib (150mg/d) again to determine his sensitivity to dasatinib after CAR-T therapy. To our surprise, it decreased from 70.94% (IS) to 7.27% (IS). By August 27, 2019, the *BCR-ABL*1 level was still at the level of 0 (Figure 2). Moreover, Sanger sequencing on August 14, 2019 detected no *BCR-ABL1* kinase mutation in the patient (Figure 1). No additional chromosomal alterations were identified on September 11, 2019.

Discussion

TKI resistance occurs in more than 25% of CML patients, which is the main reason for disease progression and shortened survival (13). It is related to a variety of mechanisms, among which point mutations in *ABL1* kinase region that make TKI unable to bind BCR-ABL1 are the most common reason.[17–19] To date, more than 100 emergent mutations have been reported to be related to various degrees of resistance to imatinib.[20] Among them,
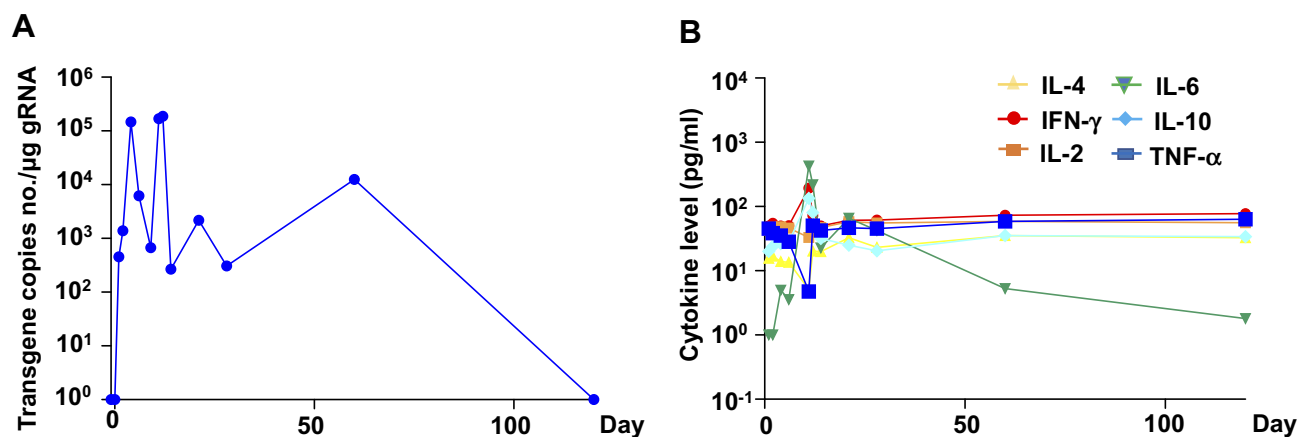
Figure 3 Patient responses after infusion. (**A**) After infusion, the number of CAR copies in the peripheral blood continued to increase and reached the highest value on day 10. The number of CAR copies remained high even after administration 320 mg tocilizumab. No CAR copies were detected 120 days after infusion. (**B**) Serum cytokine levels increased after anti-CD19 CAR-T cell infusion.

T315I and P-loop mutations are associated with the highest level of imatinib resistance and the worst clinical outcome.[21–26] Furthermore, recent studies have shown that about half of the non-responsive patients do not appear to carry *ABL1* kinase mutations.[27] The involvement of somatic mutations in other genes, such as *DNMT3A*,[28] *ASXL1*[28] and *RUNX1*[29–32] may be associated with TKI resistance and progression to advanced stages in CML. Y253H mutation, which developed half a year after imatinib treatment in this case, is located on the P-loop of *BCR-ABL1*. Although it was cleared after the dasatinib treatment, T315I mutation developed subsequently and the patient entered blast crisis phase soon after that. It is possible that Y253H in the P-loop and T315I mutation were the key reason for the patient's rapid entrance into blast phase.[33–36] However, since exon sequencing was not performed in this case, there is still a great possibility that other genetic variations led to the rapid disease progression in this patient.

Prior to the TKI era, blast phase was unavoidable. Today, the occurrence of blast phase is about 1% annually,[37] for which the suggested treatment objective is to either push the disease progression back into the chronic phase or to provide alleviation for the patient. In BP-CML, dasatinib or ponatinib coupled with chemotherapy was suggested to increase the chances of survival and response. However, a study conducted with 477 CML patients in blast phase demonstrated that coupling TKI treatment with intensive chemotherapy yielded the most successful results of 5-year survival rate only of 30%.[7] In this case, *BCR-ABL1* level decreased significantly after treatment with a combination of dasatinib and chemotherapy. However,

BCR-ABL1 level increased again soon after suggesting that chemotherapy could not overcome the resistance to dasatinib. After anti-CD19 CAR-T cell infusion, the level of *BCR-ABL1* continued to surge to 70.94% (IS). However, it decreased significantly from 70.94% (IS) to 0% (IS) after taking dasatinib again with no mutation detected. It seems that anti-CD19 CAR-T therapy cleared T315I mutation by eliminating CD19+ cell clones, which made the patient resensitize to the dasatinib.

It is well known that there are at least two groups of cell clones in BP-CML, one in chronic phase and the other in blast phase.[38] We speculate that the cell clones harboring T315I mutation happen to be the CD19-expressing clones in blast crisis, which were attacked by anti-CD19 CAR-T cells, resulting in the clearance of T315I mutation. Meanwhile, clearance of clones in blast crisis led to the dominant growth of clones in the chronic phase, which led to the large expansion of *BCR-ABL1* expressing cells. This is probably the reason why *BCR-ABL1* increased significantly after CAR-T treatment but decreased prominently after adding dasatinib.

CRS is the most common and serious side effect in CAR-T therapy. It was observed in our patient and was quickly managed. Studies have shown that patients with CRS are more likely to benefit from CAR-T therapy than those without CRS.[39–41] The tumor loading before CAR-T cell infusion and the expansion of CAR-T cells in vivo are considered to be closely related to the clinical efficacy and severity of CRS[42]. In this case, dasatinib combined with chemotherapy was given before CAR-T therapy which was helpful to reduce disease burden before infusion. The early use of tocilizumab to inhibit IL-6 also contributed to controlling CRS.

Conclusion

BP-CML with T315I mutation is still challenging even in the TKI era. Combination of anti-CD19 CAR-T therapy with dasatinib successfully cleared the T315I mutation and achieved complete molecular remission in this patient. Therapy regimens to reduce tumor burden and early use of tocilizumab are useful to control CRS without affecting efficacy.

Future Perspective

Immunological abnormality has been described in CML patients at diagnosis and worsens at disease progression.[43] Immunological control may contribute to the achievement of deep molecular response (DMR) and treatment-free remission (TFR).[44] CAR-T cell therapy is a new cell immunotherapy. Patients successfully treated with anti-CD19 CARs often have profound B cell aplasia[14] with some preservation of plasma cells and prior humoral immunity.[45] Furthermore, dasatinib was reported to be associated with beneficial immunomodulatory effects such as increased LGL lymphocytosis and decreased Tregs.[46–48] Whether the immunomodulatory effect of dasatinib could be changed after CAR-T therapy needs further research.

Informed Consent

The authors state that they have obtained verbal and written informed consent from the patient for the inclusion of their medical and treatment history within this case report.

Ethical Conduct of Research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Disclosure

The authors report no conflicts of interest in this work.

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