Dear editor,

Blast phase of chronic myeloid leukemia (BP-CML) was characterized by the presence of more than 20% blasts in the peripheral blood or the bone marrow in the progression of CML according to World Health Organization definition. Lymphoid blast phase CML (LB-CML) occurs in approximately 20%–30% of patients with BP-CML [1]. Tyrosine kinase inhibitors (TKIs) have demonstrated clinical activity in BP-CML, but responses are of short duration. Moreover, the emergence of mutations in the BCR-ABL kinase domain, especially T315I (the threonine-to-isoleucine mutation at position 315) confers complete resistance to all currently available TKIs except ponatinib [2]. On account of limited therapeutic approaches, the outcome of patients with BP-CML harboring T315I mutations remains very poor.

Chimeric antigen receptor T (CAR-T) cells targeting CD19 have emerged as a highly effective therapy in patients with refractory/relapsed acute lymphocytic leukemia [3,4]. The cytotoxicity of CAR-T cells depends on the antigen recognition by chimeric antigen receptors and subsequent activation. In principle, CAR-T cells targeting CD19 represent a promising strategy to eliminate CD19 receptors and subsequent activation. In principle, CAR-T cells tar-

A 56-year-old man was diagnosed as chronic-phase Ph+ CML in January 2013. Bone marrow conventional cytogenetic analysis demonstrated a t(9; 22) (q34; q11) in 20/20 metaphases analyzed. The patient was treated with hydroxyurea consecutively in order to control leukocytosis. His condition has been kept stable until the latest follow-up. Demonstrated a t(9; 22) (q34; q11) in 20/20 metaphases analyzed. The patient was treated with hydroxyurea consecutively in order to control leukocytosis. His condition has been kept stable until the latest follow-up. The cytogenetic analysis showed 88% of lymphoblasts and the BCR-ABL/ABL ratio was 112.65%. Meanwhile the T315I mutation was detectable by QuantStudioTM 3D Digital system (life technologies), accounting for 63.5% of total BCR-ABL transcripts. Dasatinib was immediately discontinued, and a combined chemotherapy was employed until obtaining the authorization for CD19-targeted CAR-T cells clinical trial (ChiCTR–OCC–15007008). In July 2018, the patient was infused with autologous CAR-T cells at the dose of 5 × 10^6/kg after conditioning chemotherapy (Fludarabine 30mg/m2/day at d1–3 consecutively and Cyclophosphamide 750mg/m2/day at d2,3). The preparation of CAR-T cells and the method for CAR-T cells detection by flow cytometry has been described previously [6]. A grade 3 of Cytokine Release Syndrome (CRS) associated with CAR-T cells expansion was observed and managed by Tocilizumab combined with methylprednisolone and supportive treatment (Fig. 1C,D). But no significant neurotoxicity was observed. The flow cytometry detecting phenotype of lymphoblasts showed minimal residual disease (MRD) < 0.01%, and T315I mutation disappeared in bone marrow 2 weeks after CAR-T cells infusion (Fig. 1F). While BCR-ABL transcripts were still detectable with a ratio of 15.26%, and increased to 46.8% 4 weeks later without T315I mutation and lymphoblasts progression. In consideration of residual Ph+ leukemic clones without T315I mutation, the patient was again treated with dasatinib 70 mg daily. Three months later, the BCR-ABL/ABL ratio decreased to 0.15% with T315I mutation still undetectable, and no additional mutation was detectable (Fig. 1E). Fourteen months after CAR-T cell infusion at the latest follow-up, the patient still keeps complete remission.

For patients with LB-CML, Ph+ ALL (acute lymphoblastic leukemia) or CML treated by TKIs consecutively, relapse frequently occurs associated with the mutations in BCR-ABL kinase domain, which correlate with a significant poor prognosis especially with T315I mutation. Since the resistance of T315I mutation applies to first and second generation TKIs, alternative therapy was limited. More seriously, the emergence of the compound mutations (the presence of 2 or more mutations in the same molecule) confers broad resistance to all kinds of approved TKIs including ponatinib [7], which proposed the greatest challenge to clinicians. Nowadays, the flow cytometry showed a percentage of 55.09% lymphoblasts in the bone marrow with the expression of CD34,CD38,HLA-DR,CD10,CD19 and CD22 (Fig. 1A,B). The BCR-ABL/ABL ratio was 101.01% without mutations in BCR-ABL kinase domain. Dasatinib was initially given at a dose of 70 mg daily, and changed into 50 mg daily for maintenance. In March 2018, the bone marrow examination showed 88% of lymphoblasts and the BCR-ABL/ABL ratio was 112.65%. Meanwhile the T315I mutation was detectable by QuantStudioTM 3D Digital system (life technologies), accounting for 63.5% of total BCR-ABL transcripts. Dasatinib was immediately discontinued, and a combined chemotherapy was employed until obtaining the authorization for CD19-targeted CAR-T cells clinical trial (ChiCTR–OCC–15007008). In July 2018, the patient was infused with autologous CAR-T cells at the dose of 5 × 10^6/kg after conditioning chemotherapy (Fludarabine 30mg/m2/day at d1–3 consecutively and Cyclophosphamide 750mg/m2/day at d2,3). The preparation of CAR-T cells and the method for CAR-T cells detection by flow cytometry has been described previously [6]. A grade 3 of Cytokine Release Syndrome (CRS) associated with CAR-T cells expansion was observed and managed by Tocilizumab combined with methylprednisolone and supportive treatment (Fig. 1C,D). But no significant neurotoxicity was observed. The flow cytometry detecting phenotype of lymphoblasts showed minimal residual disease (MRD) < 0.01%, and T315I mutation disappeared in bone marrow 2 weeks after CAR-T cells infusion (Fig. 1F). While BCR-ABL transcripts were still detectable with a ratio of 26%, and increased to 46.8% 4 weeks later without T315I mutation and lymphoblasts progression. In consideration of residual Ph+ leukemic clones without T315I mutation, the patient was again treated with dasatinib 70 mg daily. Three months later, the BCR-ABL/ABL ratio decreased to 0.15% with T315I mutation still undetectable, and no additional mutation was detectable (Fig. 1E). Fourteen months after CAR-T cell infusion at the latest follow-up, the patient still keeps complete remission.

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Successful treatment of T315I BCR-ABL mutated lymphoid blast phase chronic myeloid leukemia with chimeric antigen receptor T cell therapy followed by dasatinib
Fig. 1. A. The phenotype of lymphoblasts at the time of diagnosis. B. The bone marrow morphological feature before and after CAR-T cells infusion. C. The dynamics of CAR-T cells after infusion. D. The changes of cytokines in plasma after CAR-T cells infusion. E. T315I mutation disappeared in bone marrow 2 weeks after CAR-T cells infusion. While BCR-ABL transcripts were still detected with a ratio of 26%, and increased to 46% 4 weeks later. When dasatinib was given again, the BCR-ABL/ABL ratio decreased to 0.15% with T315I mutation still undetectable. F. The detection of T315I mutation before and after CAR-T cells infusion.
with the introduction of CAR-T cells, the new approach regardless of gene mutations in leukemic cells represents an effective and feasible strategy to abrogate the mutated clones, and an allogeneic hematopoietic stem cell transplant (HSCT) procedure could be carried out subsequently. CAR-T cells emerged as a promising approach to promote the survival rate of these patients.

In the present case, we observed a complete disappearance of T315I mutated transcripts and lymphoblastic cells almost undetectable by flow cytometry after CAR-T cells infusion. While BCR-ABL fusion gene existed persistently and increased gradually without detectable T315I mutation and progressive lymphoblasts. Then a favorable prognosis was achieved after dasatinib administration. This suggests that the T315I mutated clones existed in lymphoblastic cells and CD19 negative leukemic cells with BCR-ABL fusion gene remained as an existence of CML. Anastasi J et al. [5] observed that CML presenting in lymphoblast reverted to chronic phase CML after treatment, and proposed the notion that LB-CML and Ph+ ALL are distinct clinically as well as biologically. The same phenomenon occurred in this patient. Lymphoblasts were eliminated by CD19-targeted CAR-T cells but the CML clones without CD19 expression escaped from the cytotoxicity of CAR-T cells. TKIs such as dasatinib can suppress the cytotoxic function of CAR-T cells [8], but it may benefit patient with LB-CML involving multilineage disease when lymphoblasts have been eliminated by CAR-T cells, and provide the opportunity for allogeneic HSCT.

In conclusion, the leukemic population in LB-CML may present a heterogeneous characteristic. Specially we observed the T315I mutated cells existed in CD19 positive lymphoblasts while the non-mutated leukemic cells remained as an existence of CML and all of which were eliminated by CD19-targeted CAR-T cells followed by dasatinib administration. This special clinical case reminds us to consider the phenomenon of multilineage disease in patients with LB-CML when using CAR-T cells therapy, and the necessity of combined treatment.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent

Informed consent was obtained from the patient for being included in the study.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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