



Letter to the editor

Ruxolitinib treatment for acute gastrointestinal graft-versus-host disease caused by donor-derived CD19-Chimeric antigen receptor T-Cell infusion in a patient with B-ALL relapsed after Allo-HSCT

Keywords:

Acute lymphoblastic leukemia
Chimeric antigen receptor
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1. Background

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) has remarkably improved the dismal prognosis of many aggressive hematological malignancies. However, post-transplant relapse remains a major challenge [1]. As there are limited treatment options for the patients relapsed after allo-HSCT, these populations often have poor prognosis. Donor lymphocyte infusions (DLIs) are routinely used as a preemptive measure or therapeutic modality for recurrence. The engraftment of donor T cells is able to eradicate tumor cells and mediate antitumor activity mainly through graft-versus-lymphoma (GVL) effect. However, DLI has met with limited success as these allogeneic lymphocytes can also target normal tissues, thus leads to high risk of clinically significant GVHD [2]. For this reason, new therapeutic interventions are urgently needed for relapsed B-cell malignancies.

CD19-chimeric antigen receptor (CAR19) T-cell therapy has emerged as a promising immunotherapy for relapsed and/or refractory B-cell malignancies. It has been considered both as an ideal bridge leading to a transition to allo-HSCT [3], and as a potential salvage therapy for progressive malignancy [4]. However, this therapeutic modality is not yet fully mastered owing to the risk of acute GVHD (aGVHD) or chronic GVHD (cGVHD) after introducing of CAR19 T cells, which is the Achilles heel of CAR-T therapy. CAR19 T-cell immunotherapy mainly exerts antitumor activity through the GVL effect mediated by adoptively transferred T cells. A subset of allo-reactive T cells do not specifically target tumor cells but also mediate allogeneic immune responses against normal recipient tissues, which may potentially leading to fatal complication of GVHD.

Currently, available treatment approaches for GVHD has ranged from steroids to many other immune-suppressants, immunotoxins

and monoclonal antibodies, among which steroids constitute a mainstay of therapy [5]. However, only about 50–70% patients respond to the first-line treatment with steroids, leaving remaining patients in need for a secondary treatment. Until now, there is no consensus in treatment of steroid-refractory GVHD (SR-GVHD) due to the absence of prospective clinical trials for various second-line agents. Recently, a novel agent, ruxolitinib, has been investigated for treatment of SR-GVHD [6]. Ruxolitinib is an orally administrated selective Janus kinase (JAK) 1/2 inhibitor that has been approved to improve SR-GVHD in murine models and humans [7,8]. We here report a case of relapsed B-ALL after allo-HSCT. After CAR19 T cells infusion, patient achieved remission but developed severe gastrointestinal aGVHD manifesting as persistent diarrhea. Our report shows an impressive experience of using ruxolitinib as salvage treatment for steroid-refractory aGVHD.

2. Case presentation

A 24-year-old female was diagnosed with acute lymphoblastic leukemia (ALL) during December 2014. The patient was successfully treated with chemotherapy followed by allo-HSCT with mobilized peripheral blood stem cells from a sibling donor. Transient remission was achieved after allo-HSCT. However, the original disease relapsed with extramedullary involvement 13 months after allo-HSCT. It was confirmed by PET/CT scan (Fig. 1, a), which demonstrated bulky extramedullary infiltration in multiple organs. The bone marrow examination was performed and showed 22% lymphoblasts. Immunophenotype tested by flow cytometry showed CD19 expression on the surface of malignant cells. After receiving one course of salvage chemotherapy (constituted of mitoxantrone, VP-16, ifosfamide, vindesine, dexamethasone), the patient subsequently underwent 2 sessions of DLIs in August, September of 2017; however, she failed to achieve remission. The patient was later referred to our center in September 27th, 2017. In view of the patient's clinical status with highly aggressive disease, she was diagnosed with relapsed and refractory B-cell ALL and underwent a CAR19 T cells infusion ($2 \times 10^6/\text{kg}$) generated by effector cells obtained from original allogeneic donor following lymphodepleting chemotherapy consisting of fludarabine and cytosine arabinoside. The donor and patient provided written informed consent for research protocols (ChiCTR-ORN-16008948) approved by Research Ethics Committee of the First Affiliated Hospital, College of Medicine, Zhejiang University. The patient experienced low-grade fever from day 1 after CAR19 T-cell infusion until day 22, with significant elevation of various inflammatory cytokines in

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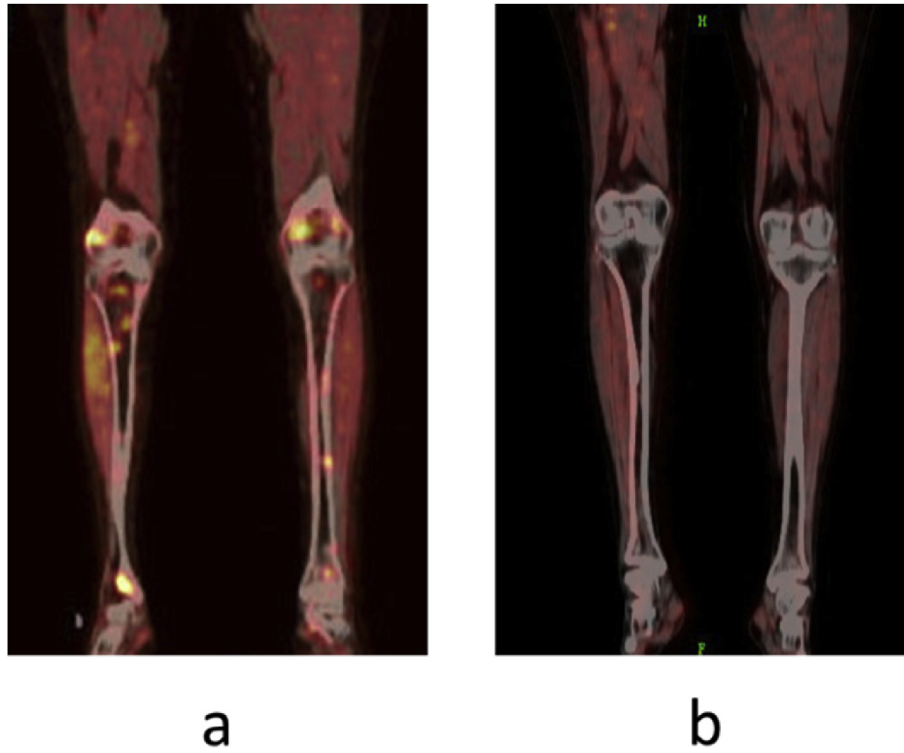


Fig. 1. Positron emission tomography scans before and after CAR19 T-cell therapy. (a) Left panel shows extramedullary infiltration previous to CAR19 T-cell infusion. (b) Follow-up PET/CT performed on 5 months after CAR19 T-cell infusion showed a dramatic regression of malignancies with nearly complete resolution of previously seen metabolically active lesions.

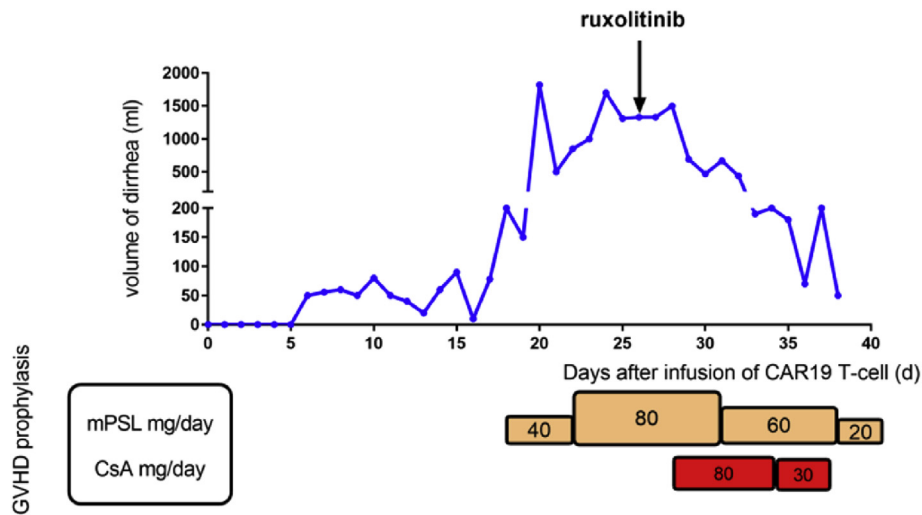


Fig. 2. Clinical process of the patient.

the first weeks after cell infusions (Fig. 3), which was consistent with a cytokine release storm.

On day 6 after CAR19 T cells infusion, the patient developed mild, watery diarrhea (<500 ml/day). The blood and stool cultures tested negative, ruled out gastrointestinal (GI) bacterial, viral or parasitic infections. The symptoms became aggravated progressively, manifesting as rapidly increasing volume of diarrhea (Fig. 2). The incidence of aGVHD was suspected and a typical steroid regimen was promptly started. Methylprednisolone was employed at a starting dose of 1 mg/kg/day with a dose increase to 2 mg/kg/day 4 days later, combined with additional cyclosporine at a starting dose of 40 mg twice per day. However, patient conditions

gradually deteriorated over the following days with diarrhea reaching up to 1–2L/day, accompanied by intensive abdominal cramp. Increased severity of diarrhea showed that she didn't response to steroid therapy, thus a diagnosis of SR-aGVHD grade 3 was established according to the NIH criteria. Consequently, a second-line therapy with ruxolitinib was given on day 26 at the dose of 5 mg BID. On day 35, cytomegalovirus DNA in blood tested positive (1.63×10^4 copies/ml), so we administered ganciclovir as antiviral therapy at the dose of 300 mg q12hr. Stools volume progressively and rapidly decreased to less than 500 mL one week after initiation of ruxolitinib treatment. Twenty days after initiation of ruxolitinib, response to treatment was observed with a marked decrease in the

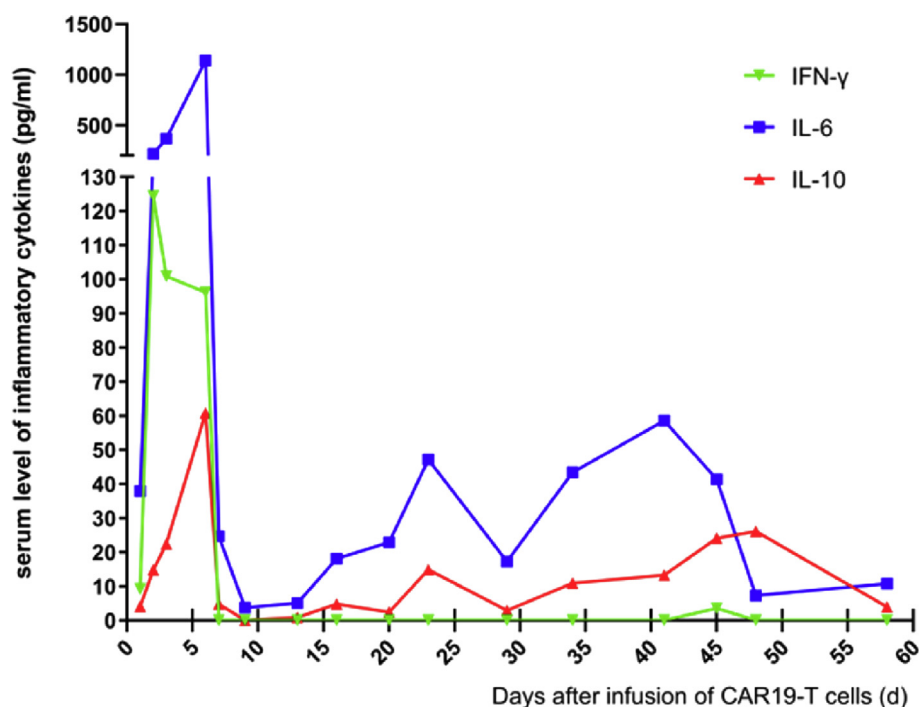


Fig. 3. Serum levels of inflammatory cytokines measured after CAR19 T-cell infusion. Serum levels of various inflammatory cytokines were measured in serum samples from day 1 (before the start of Ruxolitinib) to day 58. Patient's serum IL-6 level rapidly increased and reached its peak at 1142.61 pg/ml on day 5. Remarkable reduction in serum levels of inflammatory cytokines was observed after initiation of ruxolitinib. IFN- γ decreased from 124.41 pg/mL at day 2–3.58 pg/mL at day 45, while IL-10 decreased from 60.82 pg/mL to 3.97 pg/mL.

frequency and volume of diarrhea (Fig. 2) and substantial improvement of performance status. On day 45, the patient stopped taking ruxolitinib. Bone marrow re-examination was performed on day 49 and revealed complete remission in the hematopoietic system. On day 51, the patient was discharged. Until 8 months after CAR19 T cells infusion, the patient was doing well and remained in CR, without signs of GVHD or relapse. Post-therapy PET scan performed in March 2018 was negative for residual disease (Fig. 1 b). She remained in a durable complete remission until she had evidence of disease progression 10 months after CAR19 T cells infusion.

3. Discussion

Here, we reported a case of a patient with relapsed B-cell malignancies after allo-HSCT and had extramedullary leukemia involvement. The patient was resistant to salvage chemotherapy, and subsequent treatment with standard DLIs was unsatisfactory. Regression of malignancies was observed after infusion of donor-derived CAR19 T cells, accompanied by manageable CRS toxicities. The clinical outcomes demonstrated unparalleled antitumor activity of CAR19 T-cell immunotherapy in treating relapse/refractory B-cell ALL. Of note, the patient we reported received CAR19 T cells obtained from donor origin. Generally, the source of T cell is of patient origin. But the option of donor-derived effector T cells for patients who relapse after allo-HSCT is of great importance as these patients are less likely to have sufficient healthy T cells for CAR-T cells generation. There are several clinical trials investigating the effectiveness of donor-derived CAR-T cells infusion for treatment of relapsed ALL after allo-HSCT, in which it has shown to be safe and effective without significant GVHD effect [9–11]. Intriguingly and importantly, the patient we reported developed severe GI aGVHD after infusion of donor-derived CAR19 T cells. GVHD is induced by allo-reactive donor-derived effector T cells targeting against normal recipient

issues. In a systematic review of CAR-T cell therapy, it was documented that only 5 out of 72 (6.9%) of patients developed clinically significant GVHD after received donor-derived CAR-T cell therapy, raising the concern about the risk of incidence of GVHD associated with CAR-T therapy [12]. In this study, severe GI aGVHD was observed after infusion of donor-derived CAR19 T cells, and occurred almost simultaneously with CRS. Currently, there is a paucity of study in the literature addressing the rational explanation why donor-derived CAR T-cells induced aGVHD, and the underlying mechanism is not fully elucidated yet. Therefore, it is still unclear whether donor-derived CAR19 T cells caused aGVHD in this case. Fortunately, aGVHD was ameliorated rapidly and effectively with anti-GVHD therapy. Thus, we suggest that donor-derived CAR-T cell therapy could effectively salvage patients of relapsed B-cell malignancies after failure of allo-HSCT despite the risk of incidence of GVHD.

The patient we report had an episode of severe GI aGVHD in early phase post CAR19 T-cell therapy. Here, we report successful clinical experience using ruxolitinib as a second-line therapy to treat steroid-refractory aGVHD. Novel insight into the pathogenesis of GVHD offers the significant evidence that JAK1/2 signaling pathway has a strong correlation with tissue damage in GVHD. JAK1/2 is involved in the receptor-mediated signaling pathways under many kinds of inflammatory cytokines, which provides a rationale for blocking this pathway in order to inactivate acute or chronic GVHD. There is substantial evidence from literature to confirm the clinical activity of ruxolitinib in management of steroid-refractory GVHD: Choi et al. previously proved the preclinical efficacy of ruxolitinib to alleviate tissue damage in GVHD and meanwhile preserve the beneficial GVL effect in the murine model [13]; a retrospective multi-center survey in Euro and United States evaluated the promising therapeutic potential of ruxolitinib in management of acute or chronic steroid-refractory GVHD, as

evidenced by the overall response rate as high as nearly 80% [6]. Consistent with previous studies, the patient in our case obtained rapid remission within 4 weeks after first ruxolitinib dose, manifesting as reduction of diarrhea volume and serum levels of inflammatory cytokines. It suggests that ruxolitinib could induce durable, persistent response in patient with SR-aGVHD, with good tolerance and without significant adverse effects. Besides, CMV-reaction was observed during ruxolitinib treatment, and ganciclovir was given against CMV reactivation, indicating that infectious surveillance is necessary during ruxolitinib treatment. The activation of cytomegalovirus may associate with the side effects of ruxolitinib, as documented by previous studies. Furthermore, dose-dependent cytopenia, which was the most common adverse event of ruxolitinib treatment, were not occurred in our case.

Notably, the patient we presented has been heavily pre-treated with immune suppressive agents before initiation of ruxolitinib, including infliximab, CsA and a high dose of steroids. As we cannot exclude a potential effect of primary therapy, their contribution has to be taken into account when draw a definitive conclusion. Indeed, prolonged steroid therapy may cause additional side effects and lead to lethal complications, including increased risk of infections, osteopenia, and aseptic necrosis [14]. Overall, the addition of ruxolitinib as an add-on immunosuppression therapy in this case allowed for a reduction in steroid dosing from 80 mg per day to 30 mg per day, therefore reduced steroid exposure and protected the patient against steroid therapy related toxicity. As optimum dose and schedules of ruxolitinib in combination with steroids or other immunosuppressant remain to be determined, randomized prospective trial now required to fully compare ruxolitinib with best available treatment (BAT) and draw definitive conclusions [15].

Conflicts of interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.reth.2019.06.006>.

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