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LETTER TO THE EDITOR

Successful chimeric Ag receptor modified T cell therapy for isolated testicular relapse after hematopoietic cell transplantation in an acute lymphoblastic leukemia patient

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Primary disease relapse after allogeneic hematopoietic stem cell transplantation (allo-HSCT) is a major cause of treatment failure in patients with ALL.[1,2] Bone marrow (BM) relapse is the most common type of relapse; extramedullary relapse occurs less commonly but usually refers to a poor prognosis.[3] The testes, regarded as an immune sanctuary organ, are one of the most common sites of extramedullary relapse after allo-HSCT.[4] For patients with isolated testicular relapse (ITR), current treatment modalities include local irradiation, orchiectomy, systemic chemotherapy, or a combination of these strategies.[5,6] However, side effects of such combination therapy impeded its clinical application and disease control. Thus, an appropriate treatment strategy for ITR is still urgently needed. Recently, chimeric Ag receptor-modified T cells against CD19 (CART19s) have shown promise as a novel therapy for relapsed/refractory (R/R) ALL patients in our own and other clinical trials.[7–9] Most of these trials demonstrated data on BM relapse, as well as on tracing of CART19s to several extramedullary sites, including the liver, lymph nodes and the central nervous system (CNS). Although the blood-brain barrier (BBB) and blood-testis barrier (BTB), which consist primarily of a continuous endothelium with tight junctions,[10] commonly inhibit immune cell migration and protect CNS or testis from cytotoxic cell attacks, in our trial, we have reported for the first time that CART19s penetrated BBB and eliminated CNS lymphoma in a patient with R/R ALL.[11] Since BBB and BTB have similar anatomical structures and immunologic features, the consideration that CART19s might penetrate BTB and eradicate ITR as a potential therapy needs to be clarified.

A 24-year-old male patient with arthralgia and recurrent fever was diagnosed with ALL with BCR/ABL p210(+) in August 2013. The patient presented with an enlarged right testicle which was suspected for leukemia infiltration at diagnosis. After receiving an induction chemotherapy VICP (consisting of VCR, idarubicin, cyclophosphamide and prednisone) and imatinib mesylate, the patient achieved CR with negative minimal residual disease by flow cytometry and qPCR for BCR/ABL expression. The enlarged testis was restored to normal size. Spinal fluid examination was negative for leukemic involvement. He received a second course of VICP as a consolidation therapy. Then, a haploidentical allo-HSCT from his two-locus-HLA-mismatched elder sister was performed in November 2013 with the conditioning regimen of cytarabine, busulfan, cyclophosphamide,

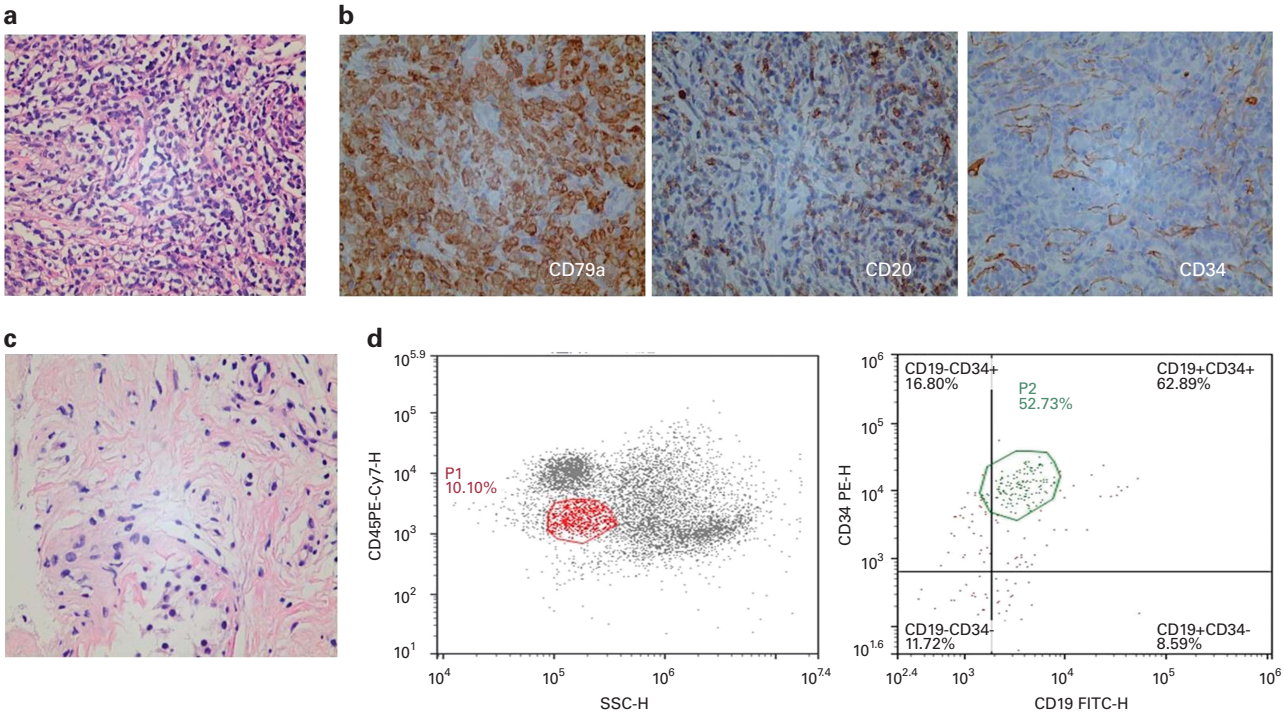

Figure 1. ITR diagnosis by pathology and flow cytometry. (**a**), Testicular biopsy demonstrating extensive and diffuse infiltrate of lymphoblasts by pathology (hematoxylin and eosin stain; ×200). (**b**), Immunohistochemical stain of testicular tissue showed positive CD79a, CD20 and CD34. (×200). (**c**), Testicular biopsy on 28 days after CART19 infusion showed complete elimination of extramedullary relapsed masses (hematoxylin and eosin stain; ×200). (**d**), Testicular biopsy demonstrating 52.73% of CD19+ leukemia cells.

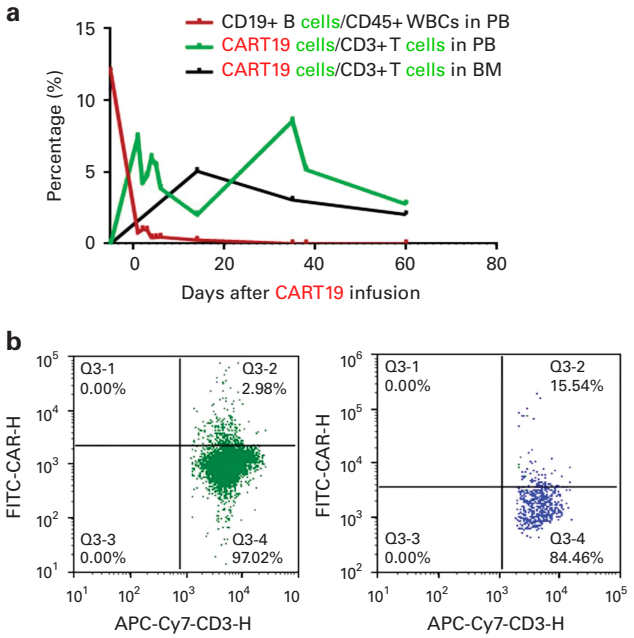

Figure 2. CART19s engrafted and expanded in PB, BM and testis tissue. (**a**), Variable levels of CART19 were detected in PB and BM at different time points after CART therapy. The percentage of CD19+ B cells in PB were also shown. (**b**), testicular biopsy 7 days after CART19 infusion showed 3.74% of aspirated cells were CD3+ T cells, and 15.54% of T cells expressing the anti-CD19 CAR, while 2.98% of T cells expressing the anti-CD19 CAR in PB as measured by flow cytometry with a CAR-specific Ab.

methyl-*N*-(2-chloroethyl)-*N*′-cyclohexyl-*N*-nitrosourea and anti-T-lymphocyteglobulin, as reported previously.[12] On day +13 after allo-HSCT, he had neutrophils >500/μL for three consecutive days. The platelet count reached 20 000/μL on day +14. A stage II acute GvHD of skin that developed on day 10 was controlled by steroids. Full donor chimerism was confirmed on day +29 by STR analysis. No chronic GvHD was observed. Imatinib 400 mg oral daily was initiated after allo-HSCT for total 16 months. 25 months after allo-HSCT, the patient presented a firm mass in his right testis. The right and left testes measured 4.7×2.0 cm and 4.4×1.6 cm with a mass of 2.3×1.5 cm in the right testis by ultrasonic scan. Testis aspiration was conducted and as speculated, the mass was an extramedullary relapse based on clinical manifestations and ultrasonic findings. The pathologic and immunophenotypic analysis of the aspirated tissues revealed a leukemic infiltration (Figure 1a). Leukemic cells in his testis were negative for CD3 and MPO, and positive for CD79a, CD20, CD34 and CD19 (Figure 1b, d), which is consistent with the extramedullary relapsed ALL. BCR/ABL chimeric oncogene was not tested in testicular tumor cells due to the inadequency of samples. Full chimerism was confirmed by STR in BM cells. BCR/ABL chimerism of BM cells by RT-qPCR was negative. There were no blast cells in the BM or cerebral spinal fluid. Based on these data, the patient was diagnosed as a case of ITR. The patient refused to take therapies including local irradiation, orchiectomy or systemic chemotherapy due to his concerns on pertinent toxicities and testes salvage. Then, the patient was recruited for CART19 clinical trial (Chictr.org N, ChiCTR-OCC-15007008) under informed consent. After recruitment, peripheral-blood mononuclear cells were collected by means of apheresis before administration of lymphocyte-depleting chemotherapy regimen FC (fludarabine 30 mg/m^2 day 1 to 3, cyclophosphamide 750 mg/m^2 day 3). CART19s were generated as previously reported.[11] The patient received an infusion of CD19 CART cells with a total dose of 2.1×10^7 CD3+ T cells per kg (4.7×10^6 CART cells per kg) over a period of 3 consecutive days.

After CART19 infusion, CART19 engrafted and expanded in peripheral blood (PB) and BM. On day 14 after CART infusion, ratios of CART19 cells in CD3+ T cells were about 2.01% and 5.05% in PB and BM, respectively. During the 2-month follow-up, CART19 /CD3+ T cell percentage maintained over 2% both in PB and BM (Figure 2a). The body temperature was normal during CART19 therapy and the follow-ups. No abnormal symptoms and signs occurred. A serial evaluation of serum cytokines showed normal levels including interferon-γ (IFN-γ), interleukin-6 (IL-6), interleukin-10 (IL-10), TNF-α, interleukin-4 (IL-4) and interleukin-17 (IL-17). The number of CD19+ B cells in PB was monitored regularly by flow cytometry. Peripheral B cells were eliminated since day 10 after CART19 infusion and B-cell aplasia sustained till date (Figure 2a). Although with hypogammaglobulinemia, the patient refused to receive any Ig supplementation and had no infection complications.

The patient was followed up consistently and remained in CR for 153 days. During the follow-up, the patient had no CART-associated complications. CART19s still showed significant anti-leukemia activities. Testis mass size was evaluated weekly by ultrasound. As shown in Supplementary Table 1, the testis mass shrank gradually. On day 28 after CART19 infusion, the mass had almost disappeared. A fine-needle aspiration was performed on the testis mass 7 days after CART19 infusion; 3.74% of aspirated cells were CD3+ T cells, and 15.54% of T cells expressing the anti-CD19 CAR, while 2.98% of T cells expressing the anti-CD19 CAR in PB as measured by flow cytometry with a CAR-specific Ab (Figure 2b). A fine-needle aspiration was performed on the testis mass again 28 days after CART19 infusion and no leukemia cell was detected (Figure 1c). The patient was considered to have achieved CR from ITR.

In order to clarify effects of CART19s on serum testosterone and sexual function, the patient had serum testosterone level measured by ELISA and filled out a questionnaire, the Sexual Health Inventory for Men (SHIM), 2 months after CART19 treatment. The serum testosterone was 221 ng/dL, within the normal range, and his SHIM score was 22, indicating a normal sexual function. Of note, the patient has lost his fertility confirmed by semen analysis after his HSCT.

In the current study, we demonstrated that CAR-positive T cells penetrated BTB and migrated to the extramedullary relapsed site in testis tissues. CART19s might prevent systemic relapse and exert robust cytotoxicities against relapsed leukemia cells involved in testis without obvious toxicities to normal testes function, which help the patient maintain a high quality of life. This instructive case provides new insights to a novel therapeutic modality different from conventional strategies.

According to current therapeutic modalities, local therapy including monolateral or bilateral testicular irradiation or orchiectomy is necessary for ITR, but these therapies could lead to infertility and abrogate sex hormone production. Although our patient did not have any complications from CART19s, it is worth bearing in mind that CART therapy could be associated with severe and sometimes fatal side effects such as cytokine release syndrome, serious neurotoxicity, or on-target off tumor effect. There has been extensive ongoing research aiming to minimize the side effects. Moreover, local therapy alone is insufficient for long-term survival as without systemic therapy. BM relapse would almost inevitably occur following ITR. Previous reports found that children with testicular recurrence, who were locally treated only, developed a BM recurrence within a few months or spread to abdominal lymph nodes, adding to the assumption that testicular leukemia must be considered part of the generalized disease.[13,14] Thus a systemic treatment should be considered a necessity. However, systemic chemotherapy might interrupt the effect of graft versus leukemia resulting in a subsequent BM relapse.[15]

Since systemic chemotherapy might affect the quality of life, such a treatment option still remains a debate. In this study, we used CART19 for ITR therapy for the first time. CART19 cells traffic to testes and eradicate leukemia cells, and persisted in PB and BM contributing to the prevention of BM relapse. During the therapy procedure, no CART-associated toxicities such as cytokine release syndrome, infection and pancytopenia were observed, indicating a safety profile. We also found the patient kept normal sex hormone production and normal sex function after CART19 therapy. Two months after CART19 therapy, the patient maintained MRD in BM and ITR still kept CR, indicating that CART19 therapy played dual roles in local and systemic disease control without toxicities. CARTs migration to BTB indicates their potential roles on treating testes leukemia, lymphomas and primary testes cancers which are refractory to routine therapies. The underlying mechanisms yet need further investigations.

In all, our unique case provides a novel therapeutic option for ITR. The quality of life in this case was much preferred to those reported with any other treatment. Additionally, this study brings new insights into the biological characteristics of CART19s. The long-term therapeutic efficacy needs further observation for ITR patients. Still, more clinical trials are warranted to consolidate this modality for future applications.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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