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Treatment of testicular relapse of B-cell acute lymphoblastic leukemia with CD19-specific chimeric antigen receptor T cells

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Abstract

BACKGROUND: Irradiation has been a standard treatment for testicular relapse but is associated with severe hypogonadism. Because CD19-specific CAR-T cells can eradicate leukemic blasts in cerebrospinal fluid, a pharmacologic sanctuary site, we tested the efficacy of this therapy in seven boys with isolated testicular relapse of B-acute lymphoblastic leukemia.

METHODS: CD19 specific CAR T cells were generated with the use of autologous T cells transduced with a lentiviral vector to express a CAR molecule containing anti-CD19 scFv derived from the HI19α murine monoclonal antibody, human CD8α hinge, and human 4-1BB (CD137) and CD3ζ costimulatory signaling transmembrane domains. After the conditioning regimen consisted of intravenous fludarabine and intravenous cyclophosphamide, seven patients with a median age of nine years (range, two to ten years) with isolated testicular relapse received a single infusion of CD19 CAR-T cells at a total dose of 5×10^6 all T cells per kilogram.

RESULTS: All seven patients achieved complete remission with normal testes. Six patients remained in second remission for 5 to 23 months (median, 14 months), and one patient subsequently relapsed in the bone marrow. The probability of event-free survival for all patients at

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AUTHOR CONTRIBUTIONS

Xiaofan Zhu, and Jianxiang Wang designed the research; Xiaojuan Chen, Ying Wang, Min Ruan, Zhanqi Li, Fang Liu, Shuchun Wang, Yumei Chen, Lipeng Liu, Jun J Yang, Xiaofan Zhu, Jianxiang Wang, and Ching-Hon Pui conducted, and analyzed the study; Xiaojuan Chen, Ying Wang, Xiaofan Zhu, Jianxiang Wang, and Ching-Hon Pui wrote and edited the manuscript; and all authors reviewed and edited the final version of the manuscript.

12 months of follow-up was 83.3%±15.2% (SE). The treatment was well tolerated with grade 1 cytokine-release syndrome developing in five patients.

CONCLUSION: These results suggest that CAR-T cell therapy is a treatment option for patients with testicular relapse.

Keywords

acute lymphoblastic leukemia; testicular relapse; CD19 CAR-T cell therapy

Introduction

The testes have long been considered a pharmacologic sanctuary site in the treatment of acute lymphoblastic leukemia (ALL). Contemporary treatment including high-dose methotrexate has not only substantially reduced the risk of testicular relapse[1] but can also eradicate overt testicular leukemia at diagnosis, without the need for local irradiation.[2] Nonetheless, irradiation remains a component of standard treatment for testicular relapse at many centers. Because of the significant late effects associated with testicular irradiation, including sterility, and the requirement for testosterone replacement treatment for most patients of pubertal age or older,[3] the Dutch Study Group used intensive chemotherapy only to treat patients with off-therapy isolated testicular relapse, with all five patients attaining second remissions of 1 to 15 years (median, 4 years).[4] Encouraged by this finding, the Children's Oncology Group conducted a larger study using intensive chemotherapy alone to treat 28 B-ALL patients with late-onset (initial remission duration ≥18 months) isolated testicular relapse who had normal testicular size or a negative biopsy for testicular leukemia at the end of remission induction.[5] However, of these 28 patients, six subsequently developed a testicular recurrence, two hematological relapse, one a second neoplasm and one death in remission, resulting in a 5-year event-free survival rate of only 60.7%. Hence, the evaluation of other treatment modalities is needed to identify alternative treatments for testicular relapse. Given the ability of CD19-specific chimeric antigen receptor modified T (CAR-T) cells to eradicate leukemic blasts in cerebrospinal fluid of some patients with relapsed CD19+ B-ALL,[6,7] we undertook a study to assess the safety and efficacy of CD19-specific CAR-T cell therapy for B-ALL patients with isolated testicular relapse.

Patients and Methods

Between September 2017 and March 2019, seven children less than 18 years of age with CD19+ B-ALL, who developed isolated testicular relapse after treatment including high-dose methotrexate on the CCLG-2008 and CCCG-2015 protocols,[8,9] were enrolled in the XH-CAR-T-003 study (Chinese Clinical Trial Registry ChiCTR1900025419) for patients with relapsed or refractory hematologic malignancies. The study design and methods were in compliance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee and Institutional Review Board of the Chinese Academy of Medical Sciences & Peking Union Medical College. Written informed parental consent was obtained for all patients.

CD19-specific CAR-T preparations were generated with the use of autologous T cells transduced with a lentiviral vector to express a CAR molecule containing anti-CD19 scFv derived from the HI19α murine monoclonal antibody, human CD8α hinge, and human 4-1BB (CD137) and CD3ζ costimulatory signaling transmembrane domains (CNCT19, Juventas Cell Therapy).[10] The conditioning regimen consisted of intravenous fludarabine 25 to 30 mg/m^2 per day on days −4, −3, and −2 and intravenous cyclophosphamide 350 mg/m^2 per day on days −4 and −2. All patients received a single infusion of CD19 CAR-T cells at a total dose of 5×10^6 all T cells per kilogram. No additional antileukemic treatment was given after CAR T-cell therapy.

After infusion, CAR-T cell levels in peripheral blood were determined at regular intervals. Serum immunoglobulin and cytokines including interleukin-1 beta, interleukin-2 receptor, interleukin-6, interleukin-8, interleukin-10 and tumor necrosis factor were also measured monthly. Testicular volume was measured by the b-mode ultrasonography at relapse and monthly after CAR-T cell therapy and was calculated as π / 6 × length × height × width.[11] Cytokine-release syndrome was graded according to the revised grading system of Lee et al, [12] and other toxicities were graded using the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) v4.03. For the time-to-event analysis, the Kaplan-Meier method was used to estimate the event-free distribution after CAR-T cell infusion. The statistical test was performed with SAS software (version 9.4).

Results

The seven boys in this study had a median age of 9 years (range, 2 to 10). They developed testicular relapse at a median time of 48 months (range, 7 to 60) after the initial diagnosis of ALL (Table 1). At relapse, one patient (no. 6) had minimal residual disease of 0.01% in the bone marrow. Before CD19 CAR-T cell therapy, five patients received two to four courses of high-dose methotrexate (5 g/m^2). Testicular size decreased in four of the five patients after high-dose methotrexate treatment and returned to normal in all seven patients after CAR-T therapy (Table 1). However, patient 7, who had a slight increase in testicular size after 2 courses of high-dose methotrexate and a normal size one month after CAR-T cell infusion, developed hematologic relapse 6 months after CAR-T cell therapy with 82.9% blasts in the bone marrow that expressed CD19, CD38, CD123, cCD79a, CD34, CD117, CD10, CD33, and cCD22. This patient had normal testicular size at the time of hematologic relapse. The other six patients remained in second remission for 5 to 23 months (median, 14 months). The estimated 12-month event-free survival rate for the entire cohort was 83.3%±15.2% (SE) (Figure 1).

After infusion, CD19 CAR-T cells were readily detected in peripheral blood by flow cytometry. The transduction efficiency was 33%, 25%, 39%, 69.6%, 60.5%, 74.5% and 78.3% for patients 1 to 7, respectively. The highest ratios of CAR-T cells to CD3+ T cells in patients 1 to 7 after infusion were 20%, 43.9%, 29.4%, 40.7%, 10.1%, 19.8%, and 20.7%, respectively (median, 20.7%). This ratio generally peaked between days 14 and 21, with the exception of patient 1, whose peak occurred on day 4 (Figure 2).

Levels of the interleukin-2 receptor and interleukin-6 increased in six and five patients, respectively, while changes in the levels of interleukin-1-beta, interleukin-8, interleukin-10 and tumor necrosis factor-α were unremarkable (Table 2). None of patients had serious side effects after CAR-T cell infusion nor required blood transfusions. Grade 1 cytokine-release syndrome developed in five patients between days 8 and 12 after infusion, but not in patients 2 and 7.

Discussion

CAR-T cell treatment was well tolerated in this study. The cytokine-release syndrome was relatively mild in the five patients who developed this complication, while adverse neurologic events did not occur in any of the patients. Importantly, six patients remained in remission for a median of 14 months. In the patient who relapsed in the bone marrow 6 months after the infusion, CAR-T cells were not measured beyond day 120 post infusion; the expression of CD19 on the blasts at the time of the second relapse suggested that his CAR-T cells might have been lost. However, the size of his previously involved testis remained normal, suggesting a lasting therapeutic effect of CAR T-cell therapy.

Our results substantiate recent anecdotal reports that CAR-T cells can eradicate extramedullary leukemia in patients with ALL. In a case report of a 24-year-old male with isolated testicular relapse after haploidentical hematopoietic cell transplantation for Philadelphia chromosome-positive ALL, allogeneic CD19-specific CAR-T cell therapy eradicated testicular leukemia on day 28 and he remained in second remission 153 days after the treatment.[13] In another study of 10 patients who had two to four previous relapses and extramedullary involvement of various sites within 0 to 9 months before CAR-T cell infusion, one had progressive leukemia and three had medullary relapse with CD19+ leukemia after CAR-T cell treatment; the remaining six patients remained alive in remission for 3 to 16 months.[14]

This study suggests that CD19-specific CAR-T cell therapy is a reasonable therapeutic option for testicular relapse in children with B-ALL by virtue of its safety and efficacy. Additional studies of larger number of patients are needed to confirm our results and to determine if CAR T-cell therapy is also effective for patients with combined relapse. Conceivably, the development of dual CD19/CD22-specific CAR-T therapy would further improve treatment outcome in these patients.

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Disclosure

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Clinical Practice Points

- CD19-specific CAR-T cell therapy is a reasonable therapeutic option for testicular relapse in patients with B-ALL.
- CD19-specific CAR-T cell may replace radiotherapy for the treatment of extramedullary disease in B-ALL.

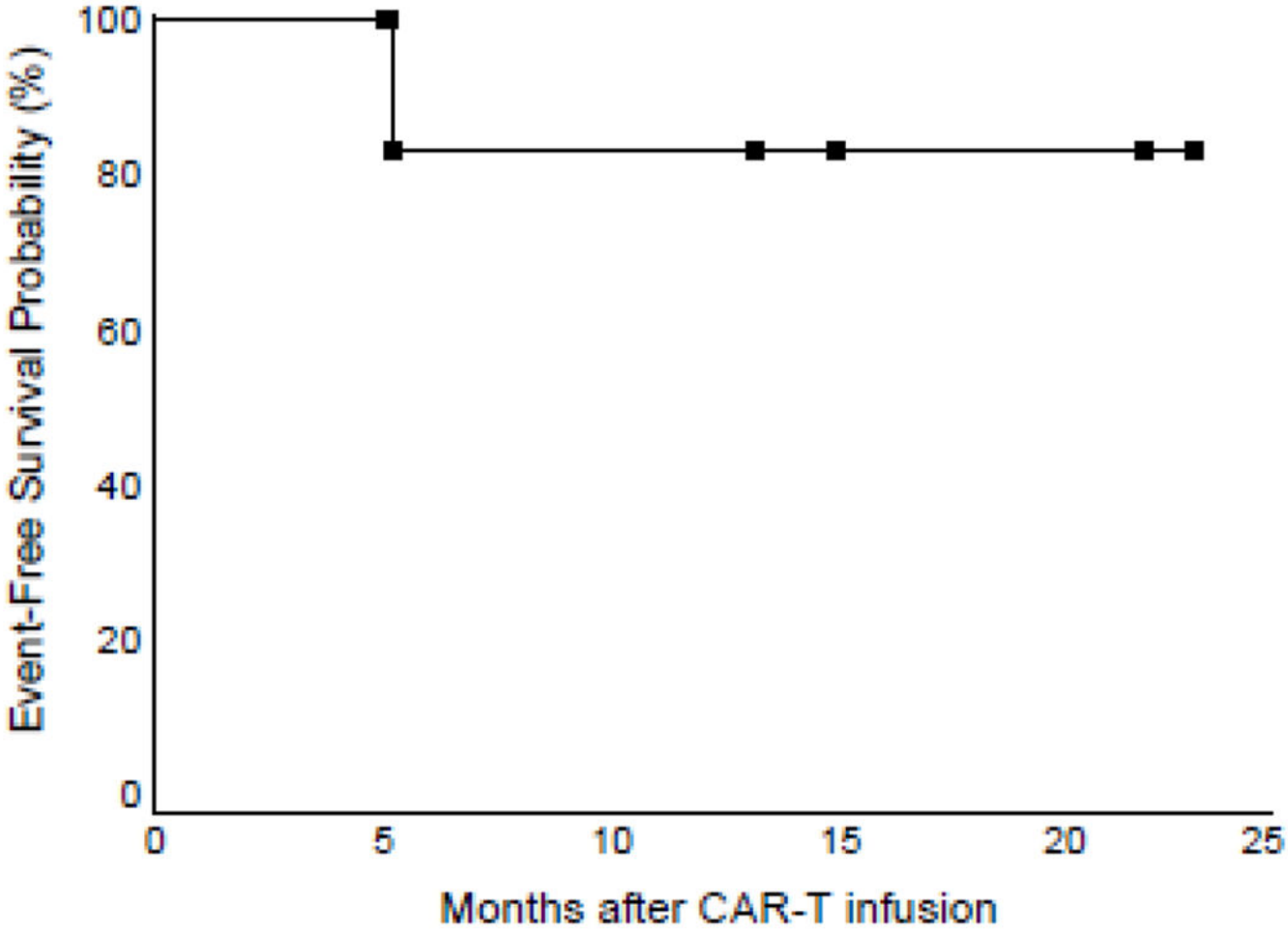

Figure 1.
Event-free survival probability after CAR-T cell therapy. Black squares indicate patients still at risk of relapse.

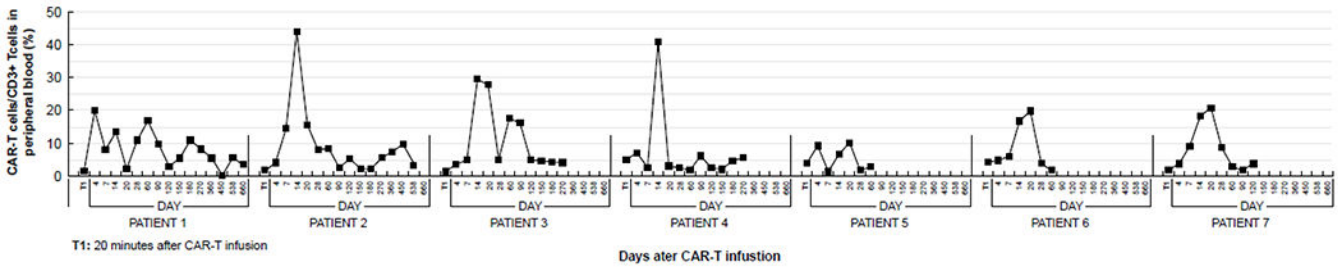

Figure 2.

The ratio of CAR-T cells to CD3+ T cells in peripheral blood before and after CAR T infusion according to time.

Table 1

Patient characteristics and treatment outcome

Case No	Age at relapse (year)	Time from initial diagnosis to testicular relapse (month)	Site of relapse	Treatment before CAR-T cells	Testicular size (cm^3) At relapse	After HDMTX	After CAR-T cells	Outcome (month)
1	10	48	left testis	HDMTX×4	14.88	9.96	6.85	CR (23+)
2	10	48	left testis	HDMTX×4	31.62	10.72	6.62	CR (22+)
3	10	51	left testis	None	29.17	None	3.23	CR (15+)
4	5	39	left testis	None	29.72	None	3.4	CR (13+)
5	9	60	left testis	HDMTX×2	6.55	5.62	4.86	CR (5+)
6*	2	7	left testis	HDMTX×4	15.55	5.53	1.88	CR (5+)
7	5	39	left testis	HDMTX×2	6.72	8.28	2.41	marrow relapse (6)

*Minimal residual disease of 0.01% in bone marrow at relapse

HDMTX, high-dose methotrexate; CR, complete remission

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Table 2

Sequential levels of cytokines before and after CAR-T-cell therapy

Time point	D0	D7	D14	D21	D28	D0	D7	D14	D21	D28	D0	D7	D14	D21	D28
	IL-1ß (U/ml)					IL-2R (U/ml)					IL-6 (pg/ml)				
Case1	<5	<5	<5	ND	ND	710	1111	1104	ND	ND	<5.9	9.79	<2	ND	ND
Case2	<5	<5	<5	ND	ND	1153	1277	1563	ND	ND	2.56	3.13	2.72	ND	ND
Case3	<5	<5	<5	6.28	12.6	722	1059	1724	950	509	4.08	4.42	3.38	26	106
Case4	<5	<5	<5	<5	<5	439	587	1406	839	517	2.81	2.14	2.81	20.5	6.49
Case5	<5	<5	<5	<5	<5	918	1028	1847	685	597	6.09	3.45	<2	<2	<2
Case6	ND	ND	ND	ND	ND	930	238	1500	4970	2596	11.6	<2	2.98	20.3	<2
Case7	<5	<5	<5	8.62	13.4	692	756	727	804	903	3.18	2.88	40.9	19.1	<2
	IL-8 (pg/ml)					IL-10 (pg/ml)					TNF-α (pg/ml)				
Case1	<62	11.5	10	ND	ND	<9.1	78	<5	ND	ND	ND	ND	ND	ND	ND
Case2	9.87	14.3	9.05	ND	ND	<5	<5	10.1	ND	ND	ND	ND	ND	ND	ND
Case3	25.8	8.64	10.7	430	944	<5	22.5	67.1	6.7	<5	113	46.7	14.1	111	411
Case4	13.4	6.86	5.61	438	5.27	<5	13	32.2	<5	5.31	23	69.3	10.6	93.3	13.4
Case5	23.4	53.8	26.5	71.5	15.2	<5	<5	<5	19.9	<5	<4	9.08	7.04	<4	12.6
Case6	442	8.96	34.1	22.6	13.3	6.93	<5	94	144	14.2	20.5	128	11.1	25.2	12.3
Case7	53.5	149	581	619	120	<5	<5	5.69	5.33	6.62	ND	ND	ND	ND	ND

ND, not done