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Outcomes of Locally Produced Donor-Derived Anti-CD19 Chimeric Antigen Receptor T-Cells in Relapsed or Refractory B-Cell Acute Lymphoblastic Leukemia Patients

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Short Report

Keywords: Donor-derived anti-CD-19 CAR T-cells, Relapsed/Refractory B-cell acute lymphoblastic leukemia

Posted Date: May 30th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9568776/v1

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Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at Cancer Chemotherapy and Pharmacology on September 17th, 2026. See the published version at https://doi.org/10.1007/s00280-026-04952-3.

Abstract

Donor-derived anti-CD19 chimeric antigen receptor T-cell (CAR T-cell) therapy has emerged as one of the most promising treatments for relapsed/refractory (R/R) B-cell acute lymphoblastic leukemia (B-ALL). In this retrospective study, we evaluated the safety and efficacy of donor-derived anti-CD19 CAR T cells manufactured at our local GMP-compliant production facility. Eleven patients with R/R B-ALL received donor-derived anti-CD19 CAR T-cell therapy. Seven of the eleven patients had previously undergone allogeneic hematopoietic stem cell transplantation (allo-HSCT). All eleven patients achieved minimal residual disease (MRD) negativity by day 28 post-infusion. Three patients developed grade 1 cytokine release syndrome (CRS), and one patient developed grade 2 CRS. No case of immune effector cell-associated neurotoxicity syndrome (ICANS) was observed. The patient who experienced grade 2 CRS also developed grade 2 graft-versus-host disease (GVHD. At the median follow-up time of 47 months (range, 6-104 months), eight patients remained alive and MRD-negative. Three patients died: one from bone marrow (BM) relapse, one from concurrent BM and central nervous system (CNS) relapses, and one from candidemia. The 5-year overall survival and event-free survival rates were both 70%. In this retrospective study, donor-derived anti-CD19 CAR T-cells produced in a GMP-compliant environment demonstrated a favorable safety profile and high efficacy in treating patients with R/R B-ALL. Larger studies are warranted to confirm these findings.

Full Text

With the combination of chemotherapy and allogeneic hematopoietic stem cell transplantation (allo-HSCT), children with B-cell acute lymphoblastic leukemia (B-cell ALL) have an excellent prognosis, with up to 90% of the patients surviving beyond 5 years from diagnosis. (1, 2) However, relapsed or refractory (R/R) disease becomes less likely to respond to conventional chemotherapy, resulting in a drastically reduced 5-year survival rate of approximately 10%. (3) As a result, development of novel therapies is being done to solve this problem.

Over the past decade, chimeric antigen receptor T-cells (CAR T-cells) targeting CD19, one of the well-known B-cell surface antigens, have emerged as a novel treatment for R/R B-ALL. (4) In several trials, anti-CD19 CAR T-cells have demonstrated exceptional antitumor activity, achieving a complete remission (CR) rate of 80%. (5,6) However, the majority of these studies were based on autologous CAR T-cells, which were difficult to produce. Most patients who are candidates for CAR T-cell therapy have typically undergone multiple chemotherapy regimens, which can compromise the quantity and functional characteristics of the harvested T cells, resulting in inferior transduction efficiency and, in some cases, manufacturing failure. (6, 7)

Donor-derived T-cells have therefore emerged as an important alternative source for CAR T-cell production. Recent studies have shown that T-cells harvested from healthy allogeneic donors yield superior quantity and quality, making them more amenable to CAR modification. (8,9) Furthermore, recent evidence suggested that donor-derived CAR T-cells achieve satisfactory clinical outcomes comparable to those of autologous origin. (10, 11)

Despite these encouraging results, questions remain regarding the durability of response and safety profile of the donor-derived CAR T-cells, especially cytokine release syndrome (CRS) and graft-versus-host disease (GVHD). (12) This study aimed to evaluate the manufacturing outcome, clinical efficacy, and safety of donor-derived CAR anti-CD19 T-cells produced at our facility for the treatment of patients with R/R B-ALL.

The production processes for anti-CD19 CAR T-cells have been described in previous studies. (13,14) All anti-CD19 CAR T-cells administered in this study were manufactured in a fully GMP-certified environment using a single-use closed system. Allogeneic donors underwent a single standard leukapheresis at the hospital, and the harvested cell product was cryopreserved in a CryoMACS freezing bag (Miltenyi, USA). The cells were subsequently thawed, washed, and T cells were isolated and activated using TransACT (Miltenyi, USA). Activated cells were transduced with a self-inactivating minimal lentiviral vector (LVV) containing the anti-CD19 CAR transgene (VIVE Biotech, Spain) using a static culturing method. Transduced cells were then expanded and harvested. Final products underwent complete release testing for sterility, potency, purity, and safety before release. In vitro potency was assessed using a cytotoxicity assay as previously described. (13,14)

The CAR viral copy number was determined using droplet digital PCR (*ddPCR). (13,14)* Final products were tested for mycoplasma using a detection kit (Lonza, Switzerland), for microbial contamination by light microscope, and for sterility using Bactec (Becton Dickinson, Canada).

Patients with R/R B-ALL who received anti-CD19 CAR T-cells were recorded from June 2017 through October 2025. **(Table 1)** Inclusion criteria were: R/R B-ALL with CD19-positive leukemic cells, and an available allogeneic donor for peripheral blood mononuclear cell (PBMC) collection. Exclusion criteria were: (i) refusal by the patient or parents, (ii) ongoing severe infection uncontrolled with antibiotics, and (iii) R/R disease with a full-blown disease status. For patients who had previously undergone allo-HSCT, the same donor was selected for donor-derived T-cell harvest. For patients who had not received allo-HSCT, donors were selected based on human leukocyte antigen (HLA) typing, including HLA-matched related, HLA-matched unrelated, and HLA-haploidentical donors. Minimal residual disease (MRD) was evaluated using a 14-color panel with 27 leukemia-associated markers, adapted from previously reported methods. (15)

The T-cell products achieved a median viability of 85.4 % (range, 76.7-97.3%) and a median transduction efficiency of 49% (range, 21-100%), both exceeding the release criteria of ≥70% viability and ≥ 20% transduction efficiency. *The median in vitro cytotoxicity test (effector:target; 20:1) was 51.4% (range, 24.1-71.1%), surpassing the release criteria of ≥ 20% **(Table 2)**. All anti-CD19 CAR T-cell products tested negative for mycoplasma and bacterial contamination.*

Eleven patients with R/R B-ALL received anti-CD19 CAR T-cell infusion. The majority were classified as high-risk (HR) or very high-risk (VHR) per NCI criteria at initial diagnosis **(Table 1).** All patients previously received 3 to 6 protocols or regimens (median = 4) with protocol changes guided by treatment response assessed via morphological evaluation and MRD studies by flow cytometry. Prior to CAR T-cell therapy, patients were categorized into three groups: (i) complete remission (CR) post-transplant (n=4), (ii) R/R disease post-transplant (n=3), and (iii) R/R disease without prior transplant (n=4) (Table 1). All patients received chemotherapy to achieve CR with MRD-negative status before donor-derived anti-CD19 CAR T-cell infusion, except one patient (patient # 03), who had bone marrow (BM) relapse before infusion.

The anti-CD19 CAR T-cell dose ranged from 0.5 to 4.5 × 10^6 cells/kg (median = 1.7× 10^6 cells/kg). The median follow-up time was 47 months (range, 6-104 months). All 11 patients achieved CR, with MRD negativity on day +28 post-infusion.

At the data cutoff date, two patients had experienced disease relapse. One patient developed BM relapse on day +67 and died on day +283; the other developed concurrent BM and central nervous system (CNS) relapse on day +177 and died on day +745. One patient died from candidemia on day +133.

Cytokine release syndrome (CRS) was graded per the ASTCT consensus criteria, with interleukin-6 (IL-6) levels used as a laboratory indicator. (16) Three patients developed grade 1 CRS, with peak IL-6 levels of 91, 164, and 631 pg/mL. One patient developed grade 2 CRS with a peak IL-6 level of 1191 pg/mL. No cases of immune effector cell-associated neurotoxicity syndrome (ICANS) were observed. (17)

Four of the 11 patients who had not undergone HSCT prior to CAR T-cell therapy (patients # 6, #7, #8, #9) subsequently proceeded to HSCT using the same donor.

The 5-year overall survival (OS) and event-free survival (EFS) rates were both 70% (95% CI: 32–89%) **(Figure 1).**

With ddPCR, pharmacokinetic studies of anti-CD19 CAR T-cells were confirmed in the peripheral blood of all patients. CAR T-cells level gradually decreased over time, with peak level observed at d+14 post-infusion **(Figure 2 and Table 1)**. Noteably, patients #3, #6, and #12, who experienced grade 1-2 CRS, demonstrated markedly elevated CAR expression levels of 5,777, 1,259, and 414 copies/10^4 cells, respectively.

This study demonstrates that our facility can successfully manufacture donor-derived anti-CD19 CAR T-cells that meet essential quality standards and release criteria for compassionate use at the standard dosage. Furthermore, treatment with these locally produced CAR T-cells yields satisfactory clinical outcomes with a favorable safety profile in patients with R/R B-ALL.

The patient population in this study represented a heavily pretreated cohort; the majority were classified as HR or VHR B-ALL per NCI criteria. (18,19) Patients had received multiple treatment protocols or regimens to achieve MRD-negative status before CAR T-cell infusion, and several had relapsed following allo-HSCT. Despite this challenging clinical profile, donor-derived anti-CD19 CAR T-cells achieved a 5-year OS and EFS of 70%, comparable to the prior studies of both donor-derived and autologous CAR T-cell products.(10,11,20)

Safety remains a critical consideration in donor-derived CAR T-cell therapy, with previous studies reporting risks of CRS, ICANS, and GVHD. (20) In this study, these complications were infrequent and generally mild: only three patients developed grade 1 CRS, and one patient developed grade 2 CRS. No cases of ICANS or severe GVHD were observed, findings consistent with prior reports. (20) Notably, patients who experienced CRS episodes demonstrated markedly elevated peripheral blood CAR T-cell levels, suggesting a potential association between CAR T-cell expansion and CRS severity. Further investigation in a larger population is warranted to elucidate the relationship between circulating CAR T-cell levels and CRS incidence and severity.

CAR T-cell kinetics in this study followed a pattern consistent with the existing literature. Anti-CD19 CAR T-cells were confirmed to persist and expand in the peripheral blood of all patients, with peak level observed at day +14 post-infusion. Levels subsequently declined gradually and became undetectable by approximately month +6. These findings are concordant with previous studies reporting peak CAR T-cell levels around d+10, followed by a continuous decline with loss of detectability by month +6. (21,22) The characteristic triphasic pattern of expansion, contraction, and persistence observed in our products further supports the comparability of our locally manufactured CAR T-cells with products from established facilities.

Several limitations of this study should be acknowledged. The retrospective design, small sample size, and single-center setting limit the generalizability of the result. Additionally, the heterogeneity in patient characteristics, including varying disease status and prior treatment history at the time of CAR T-cell infusion, may confound the interpretation of the outcomes. Prospective, multicenter studies with larger cohorts are needed to validate these results and further characterize the long-term safety and durability of donor-derived anti-CD19 CAR T-cell therapy.

This study demonstrated that donor-derived CD19 CAR T-cell manufactured in a GMP-compliant local facility are both safe and effective for the treatment of R/R B-ALL, achieving a 5-years OS and EFS of 70% with a low incidence of CRS and no cases of ICANS or severe GVHD. These finding support the feasibility of local CAR T-cell production that meet global quality standards, offering a potential pathway to improve accessibility and affordability of this transformative therapy for patients in low and middle-income countries. Larger prospective, multicenter studies are needed to confirm these results and to further optimize manufacturing processes and clinical protocols for broader implementation.

Declarations

Funding

This study was supported by the Fundamental Fund from Thailand Science Research and Innovation (FRB650007/0185) to Dr. Usanarat Anurathapan and Senior Research Fund to Dr. Suradej Hongeng

Conflict of interest

There is no conflict of interest for all authors.

Ethical statment

This study was approved by the Ethical Clearance Committee on Human Rights Related to Research Involving Human Subjects, Faculty of Medicine Ramathibodi Hospital, Mahidol University (COA. MURA2023/504). All patients and parents who participated in this study provided their consents.

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Tables

Table 1 Patients characteristics

Case No	Age (yrs)	ALL risk classification at diagnosis	Patient Status Prior to CAR T-cell	Donor for CAR T-cell	MRD prior to CAR T-cell	CAR T-cell dose (x 10^6 cells/kg)	Peak of CAR expression (copies/10,000 cells) and (day)	Last day of CAR monitoring (day)	CRS grade	Peak level of IL-6 (pg/mL)	MRD last F/U	Status at last F/U
1	12	HR	remission post transplant	MSD	neg	1	362 (28)	2735	none	3	neg	alive
2	12	HR	remission post transplant	HaploD	neg	0.5	1078 (90)	2195	none	9.5	neg	alive
3	11	HR	refractory post transplant MSD and haplo	HaploD	3.69%	0.8	5777 (3)	745	1	164	CNS and BM relapse	dead
4	12	SR	relapse post transplant	MUD	neg	4.5	61 (28)	1695	none	10.5	neg	alive
5	10	VHR	remission post transplant	MSD	neg	0.5	44 (2)	1606	none	7.3	neg	alive
6	20	VHR	Refractory	MSD	neg	0.8	1259 (28)	283	1	91	BM relapse	dead
7	12	HR	Relapse	MSD	neg	1.7	583 (5)	1292	none	8.9	neg	alive
8	3	HR	Refractory	HaploD	0.03%	2	168 (7)	1173	none	34.8	neg	alive
9	51	VHR	relapse	MSD	neg	1.8	39 (7)	113	1	631	neg	dead
10	8	HR	remission post transplant	HaploD	neg	2.2	204 (1)	793	none	8.8	neg	alive
11	12	HR	relapse post transplant	HaploD	neg	3	414 (7)	90	2	1191	neg	alive

ALL; acute lymphoblastic leukemia, BM; bone marrow, CAR; chimeric antigen receptor, CNS; central nervous system, CRS; cytokine release syndrome, F/U; follow up, IL-6; interleukine-6, haploD; haploidentical donor, HR; high-risk; MRD; minimal residual disease, MSD; matched sibling donor, MUD; matched unrelated donor, SR; standard risk, VHR; very high-risk

Table 2 Anti CD19 CAR T cells characteristics of each donor

Case No	Viability (%)	Transudction efficiency of CAR expression (%)	Potency (% lysis at 20:1 (effctor:target))
1	97.3	21.0	38.9
2	85.6	22.8	39.0
3	80.0	65.9	53.4
4	83.8	83.2	62.0
5	83.8	63.4	46.0
6	76.7	65.7	51.4
7	85.4	49.0	54.7
8	85.2	34.0	68.2
9	89.9	36.8	34.5
10	87.2	26.5	71.1
11	90.4	100.0	24.1

Figures

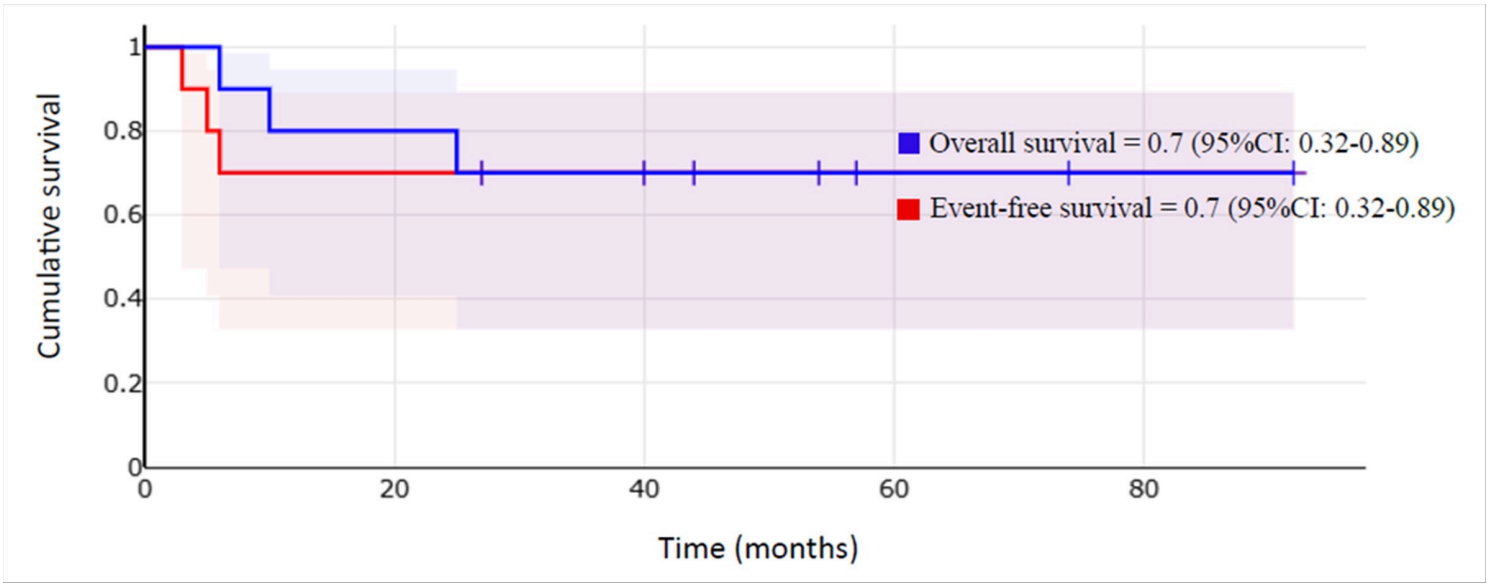


Figure 1

Overall and event-free survivals

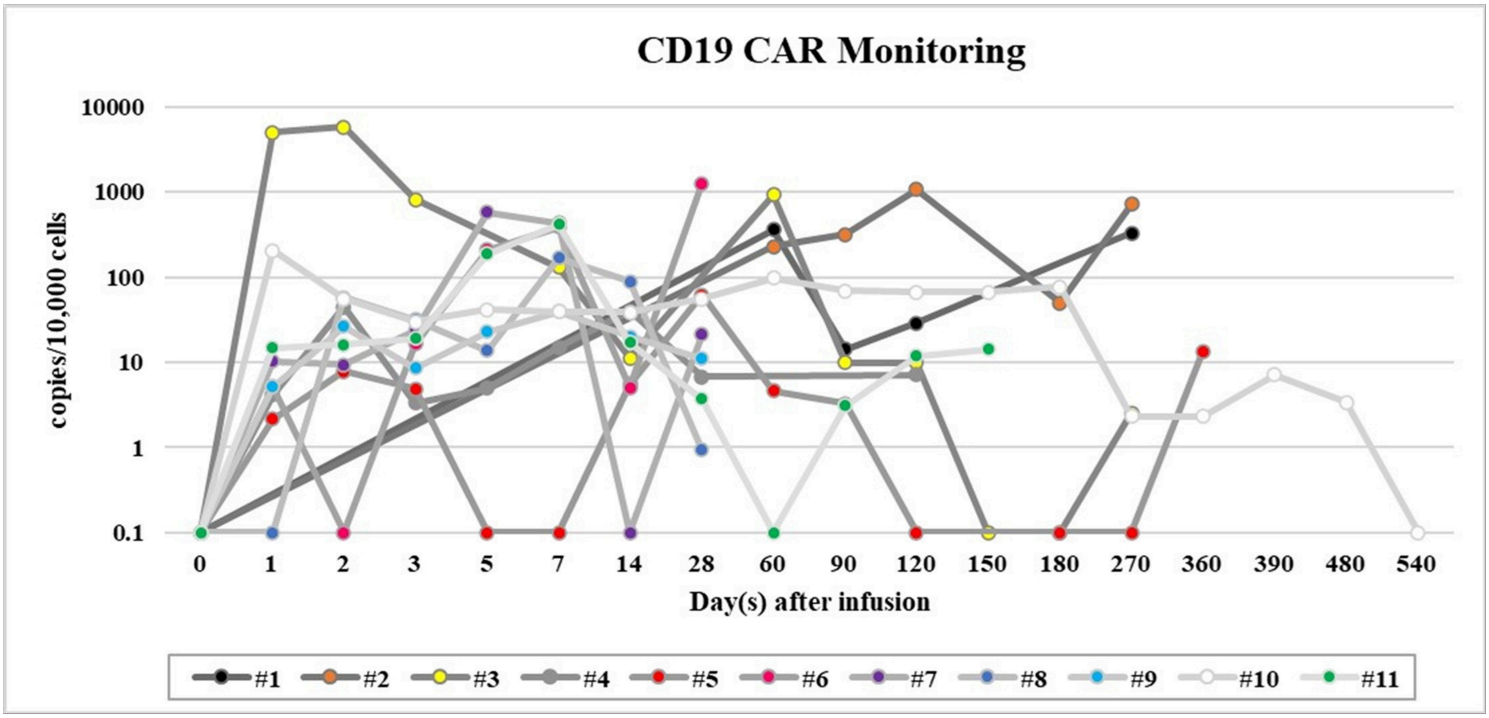


Figure 2

Pharmacokinetic study of CAR CD19 expression of each patient by ddPCR