Characteristics of anti-CLL1 based CAR-T therapy for children with relapsed or refractory acute myeloid leukemia: the multi-center efficacy and safety interim analysis

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Abstract

C-type lectin like molecule-1 (CLL1) is preferentially expressed on acute myeloid leukemia (AML) stem cells and AML blasts, and can be considered as AML-associated antigen. Anti-CLL1-based CAR-T cells exhibited effective tumor killing capacity *in vitro* and in AML-bearing mouse model. In this report, eight children with relapsed or refractory AML (R/R-AML) were recruited for a phase 1/2 clinical trial of autologous anti-CLL1 CAR-T cell immunotherapy. The objectives of this clinical trial were to evaluate the safety and the anti-AML responses after CLL1-CAR-T cell treatment, with long-term prognosis within those patients who did not receive allogeneic hematopoietic stem cells transplantation (allo-HSCT) as an additional aim. These R/R-AML patients received one dose of autologous CLL1-CAR-T cells after lymphodepletion conditioning. Grade 3–4 hematologic adverse events were observed post CAR-T cell infusion. Meanwhile, grade 1–2 cytokine release syndrome (CRS) was observed but without any lethal events. 4 out of 8 AML patients achieved incomplete remission (CRi) and minimal residual disease (MRD) negativity, 2 patients with CRi but MRD positivity, and 2 patients with decreased AML burden and CLL1 positive AML blast clearance. These results suggested that anti-CLL1-based CAR-T cell immunotherapy can be considered as a well-tolerated and effective option for treating children with R/R-AML.

Introduction

Outcomes of children with acute myeloid leukemia (AML) remain lagged behind that of children with acute lymphoblastic leukemia (ALL), with 5-year disease-free survival (DFS) varying between 33.3% and 79.5% worldwide, as reported by the CONCORD-2 study[1]. Though 50% – 70% of children with primary AML can be cured with conventional intensive therapy[2,3], the cure rate is not improved much by the introduction of novel agents[4]. The relative poor prognosis for childhood AML has been mostly attributed to less available targeted agents or therapies for those relapsed/refractory AML (R/R-AML) patients. Up to now, allogeneic hematopoietic stem cell transplantation (allo-HSCT) is generally considered as the best chance of cure for patients with high-risk- or R/R- AML[5].

Impressively, the prognosis for patients with R/R-AML can be greatly enhanced if they are in a negative minimal residual disease (MRD) state even without receiving allo-HSCT[6]. Therefore, novel therapies are highly needed for further improvement in the R/R-AML therapy. Several novel agents have been currently introduced into pediatric R/R-AML therapies (i.e., sorafenib, gilterinib, gemtuzumab ozogamicin (GO), and venetoclax), however the responses remain unsatisfied, with ~ 50% CR/CRi (complete remission/complete remission with incomplete hematologic recovery) rate[7–10]. The development of immunotherapy has given the clinicians with new strategy for treating patients with hematological malignancies. In this regard, autologous chimeric antigen receptor (CAR) T cell therapy has been increasingly accepted as a highly effective regimen for relapsed/refractory hematological malignancies[11–13]. For example, anti-CD19 CAR-T cell immunotherapies are reported to be highly effective in R/R B-cell ALL (B-ALL) patients, with CR rates ranging from 70 to 94%[14–16], although near one third responding patients eventually experienced relapse due to multiple mechanisms[17]. However, the potential of CAR-T cell therapy in R/R-AML remains undetermined.

C-type lectin-like molecule-1 (CLL1) is identified as a novel AML stem cell associated antigen[18]. Intriguingly, CLL1 is highly expressed on AML leukemia stem cells (LSCs), majority of AML blasts and normal myeloid cells, but not on normal hematopoietic stem cells (HSCs) and lymphoid cells, suggesting that targeting CLL1 can be a novel AML therapy while not affecting normal hematopoiesis and lymphocyte-directed immune function. Several studies have successfully developed novel CLL1-directed therapies (i.e., antibody-based and cellular therapies) with definite efficacy on human AML with promising *ex vivo* and *in vivo* evidences[19–24]. Liu and Zhang[25] have independently and successfully treated two secondary AML patients with anti-CLL1 based CAR-T cells, highlighting the potential of CAR-T cell therapy in R/R-AML.

To this end, we recruited 8 children with R/R-AML for a phase 1/2 clinical trial with autologous anti-CLL1 based CAR-T cell therapy to test its safety and efficacy among two independent institutions.

Materials And Methods

Eligibility, ethics approval, and treatment schema

This study was approved by the Institutional Review Board of Guangzhou Women and Children's Medical Center (2020-23) and Nanfang-Chunfu Children's Institute of Hematology and Oncology, Dongguan Taixin Hospital (TXEC-2019-005). This clinical trial was registered at Chinese Clinical Trial Registry (www.chictr.org.cn) with registration number ChiCTR1900027684 and www.clinicaltrials.gov (NCT03222674). Children with R/R AML patients who met criteria for this clinical trial were enrolled. Informed consent according to institutional guidelines and the Declaration of Helsinki was obtained from the parents or guardians. All enrolled patients received one lymphodepleting regimen (cyclophosphamide, 500 - 900 mg/m^2/day, day -4 to day -1; fludarabine, 25 - 30 mg/m^2/day, day -2 to day -1 depending on the AML burden)[25], prior to CAR-T cells infusion. A single dose (0.35-1×10^6/kg) of anti-CLL1 based CAR-T cells was infused through a peripherally inserted central venous catheter (PICC). The treatment response and CAR-T cell-related toxicities were systemically evaluated. Bone marrow morphologic and flow cytometric assessments for treatment response were performed every month for the first three months after CAR-T cell therapy, and every three months thereafter if not followed by allo-HSCT. Patients achieving CR were transferred for further allo-HSCT if their socioeconomic status and donor status allowed.

Generation of clinical-grade CLL1 CAR-T cells

All GMP viral vector production practices were following the regulatory guidelines. Lentiviral vector supernatant for the CLL1-CAR was produced by transient transfection of 293T cells (Takeda) with the corresponding CAR plasmid and 3 packaging plasmids: pLP1, pLP2 and pLP/VSVG and the medium was changed 4 hours after transfection. Forty-eight hours later, the cell supernatant was pooled and filtered with a 0.45μm filter, followed by Benzonase treatment (Merck) for 16 hours. Then, the harvest was passed through a Mustang Q ion-exchange capsule (Pall, Ann Arbor, MI). The Mustang Q membrane was washed using 50mM Tris-HCl, pH 8.0 with 750mM NaCl and then eluted in fractions using 50mM Tris-HCL, pH 8.0 with 1.5 M NaCl and diluted with phosphate buffer pH 7.2. The elution was further concentrated approximately 10- fold by 300 KD TFF column. The final concentrate was formulated with human serum albumin (HSA) to 2%, filtered with a 0.22 um filter, aliquoted to 2ml cryotubes, quick frozen on dry-ice, and stored at −80℃.

Patients' T cells from peripheral blood mononuclear cells (PBMCs) were enriched by CD3 magnetic beads (Miltenyi), and stimulated by anti-CD3/CD28 beads (Dynabeads, Human T Activator CD3/CD28, Life Technologies) at a 1:3 (beads: T cells) ratio, and then cultured in H3 medium (Takara) with 4% Human AB serum and 10 ng/mL recombinant human IL7 and IL15 (Miltenyi). Cells were exposed to lentivirus containing supernatant on days 2 and 3 with multiplicity of infection (MOI) of five, on Retronectin-coated non-tissue culture plates (Takara/Clonetech). Beads were magnetically removed on day 4 or 5, and cells were further expanded for 3-5 days in H3 media containing 10 ng/mL recombinant human IL7 and IL15 until use *in vitro* or *in vivo*. The cells were harvested and cryo-preserved. Once the standard operating procedure was completed, the cell product was shipped for clinical application under the clinical trial guideline.

The *ex vivo* and *in vivo* AML targeting capacity of anti-CLL1 based CAR-T cell therapy

In vitro cytotoxic assays were performed by co-culturing 50,000 CAR-T cells with 50,000 AML cells in complete media in a 96 well plate. The supernatant was collected after 24h co-incubation. Human interferon gamma (IFNγ) from cell culture supernatant were measured by enzyme-linked immunosorbent assay (ELISA) development kit (4A Biotech, Beijing) according to the manufacturer's instructions.

Severe immune-deficient B-NDG mice were purchased from Jiangsu Biocytogen Co., Ltd. (Nantong, China), and anesthetized with 3% isoflurane (Minrad International, Buffalo, NY, USA) in an incubation chamber. Anesthesia (David Kopf Instruments, Tujunga, CA, USA) was maintained at 2% isoflurane delivered through a nose adaptor. Half million AML HL60-luc cells were injected into 6-10 week-old B-NDG mice using a blunt-end needle through tail vein. Leukemia occurrence was serially monitored by bioluminescence *in vivo* imaging on an IVIS spectrum instrument (Caliper Life Science) and quantified with Living Image software (PerkinElmer, Waltham, MA, USA). With the establishment of HL60-luc AML B-NDG mice model, mice were randomized

and treated with 3 million or 10 million CLL1-CAR-T cells or an equivalent number of non-CAR T cells (matched for total T cell dose) intravenously by tail vein injection. Living Image software was used to analyze the IVIS data.

Monitoring cytokine release

Novus Biologicals human IL-6 ELISA kit was used to determine the IL6 levels in patients' plasma.

Treatment response evaluation

Bone marrow (BM) specimens were longitudinally collected prior to and after CLL1-CAR-T cell infusion for experiments. The National Comprehensive Cancer Network (NCCN) AML Clinical Practice Guidelines Version 3.2021 were used to evaluate the treatment response [26].

Safety and tolerability

The common terminology criteria for adverse events (CTCAE) 5.0 criteria was applied to systemically evaluate the safety and tolerability of anti-CLL1 CAR-T cells in this study. All patients were managed using guidelines from Mahadeo and Neelapu[27,28].

Statistical analyses

The safety, tolerability, side effects, and clinical response data of patients who received anti-CLL1 CAR-T cells were pooled for analysis using SAS® Version 9.2 or higher, and descriptive statistics were used to summarize the data.

Results

We initiated a single-arm, Phase 1/2 clinical trial to test the feasibility and safety of the autologous T cells, expressing a CLL1-targeted CAR with 4-1BB and CD3z endoplasmic domains, in patients with refractory/relapsed (R/R) AML. The human CLL1 CAR-T extracellular scFv was derived from a high-affinity CLL1 monoclonal antibody (clone 27H4, **Supplementary Fig. 1A**), which was generated by hybridoma technology. Membrane protein array was applied to confirm the CLL1 binding specificity of clone 27H4 as shown in **Supplementary Fig. 1B**. Then we proceeded to construct the second generation of CAR using the variable region derived from clone 27H4 targeting CCL1 (**Supplementary Fig. 2A**). The design, development and generation of clinical grade anti-CLL1 based CAR-T cells were shown in **Supplementary Fig. 2B**. Firstly, we tested their anti-tumor efficacy by co-culturing anti-CLL1 CAR-T cells with Raji-CLL1 (B lymphoblastoid cell line ectopically expressing CLL1), or HL60 (CLL1-expressing AML cell line). After co-culturing, a significant IFN-γ release was observed when CLL1-CAR-T cells co-cultured with Raji-CLL1, or HL60 cells, but not with the parental Raji cells which do not express CLL1 (**Supplementary Fig. 3**), these results demonstrating the targeting capacity of the CLL1-CAR-T cells. Next, we investigated the tumor killing efficacy using human AML-bearing B-NDG mouse model. An increasing dose of anti-CLL1 CAR-T cells (3×10^6 and 1×10^7) were infused into HL60-luc AML-bearing B-NDG mice and the leukemic burden was evaluated by *in vivo* imaging methods. As shown in Fig. 1A-B, the AML burden was significantly reduced in CLL1-CAR-T cells-treated group, when compared to their control groups (vehicle and T-mock). Moreover, infusion of CLL1-CAR-T cells, but not the mock T cells, significantly prolonged the survival of the AML-bearing mice, confirming the tumor killing potential of the CLL1-CAR-T cells *in vivo* (Fig. 1C).

To initiate the Phase 1/2 clinical trial, 8 children with R/R-AML were enrolled; 6 were male; median age was 12 (range 8–16) years (Table 1). According to the clinical trial protocol, lymphodepletion conditioning was applied on days − 4, −3, − 2 and − 1 (cyclophosphamide, 500–900 mg/m^2/day) and days − 2 and − 1 (fludarabine, 25–30 mg/m^2/day) followed by anti-CLL1 based CAR-T cell infusion on day 0, except patient 2 without lymphodepletion conditioning. The general time schedule of CLL1-CAR-T clinical trial was shown in Fig. 2A. The therapeutic responses at 1, 2, 3, 6, 9 and 12 months after infusion were assessed by bone marrow morphology and multiple flow cytometry analysis. Detailed patient characteristics and individual CAR-T product information were summarized in Table 1. The CLL1 expression on AML blasts were determined by flow cytometry using anti-CLL1 antibody. The positivity of CLL1 in AML blasts ranged from 65–96% among these patients (Table 1). Patients

1, 3, 5–8 received 1 million CLL1-CAR-T cells per kilogram, patient 2 received 0.35 million CLL1-CAR-T cells per kilogram, and patient 4 received 0.8 million CLL1-CAR-T cells per kilogram (Table 1).

Table 1
Patient information

Patient #	Sex/ Age	AML subtype	Mutation	Prior Treatment	CLL1% in Tumor Cells	CAR-T Product	Product CAR%	CAR-T Dosage
1	Male/ 16Y	AML-M2	RUNX1, FLT-ITD+, NRAS+, U2AF1+	Relapse after HSCT	96%	CLL1	43.2%	1×10^6/kg
2	Male/ 11Y	AML-M5 + JMML	FLT3+	Relapse after HSCT	95%	CLL1	12%	0.35×10^6/kg
3	Male/ 13Y	AML-M2	AML1-ETO fusion, WT1 high expression	5 chemo	86%	CLL1	31.3%	1×10^6/kg
4	Female/ 12Y	AML-M2	AML1-ETO fusion, c-KIT mutation	4 chemo	91%	CLL1	42.9%	0.8×10^6/kg
5	Male/ 8Y	AML-M6	N/A	2 chemo	95%	CLL1	40.8%	1×10^6/kg
6	Female/ 13Y	AML-M2	NUP98DDX10+, KRAS/ETV6/GATA2/W1 /SETD2/MYCN/KDM5C	5 chemo+ Venetoclex	75%	CLL1	39.5%	1×10^6/kg
7	Male/ 12Y	AML-M2A	PHF6, IDH1, TET2	2 chemo	65%	CLL1	67.6%	1×10^6/kg
8	Male/ 9Y	AML-M5	N/A	4 chemo	96%	CLL1	60.7%	1×10^6/kg

In “Prior Treatment”, # chemo indicates the number of course of chemotherapy has been taken on the patient.

Adverse events (AEs) and clinical outcomes were monitored from the starting point of lymphodepletion until 60 days after CAR-T cell infusion according to the Common Terminology Criteria for Adverse Events (CTCAE), version 5.0. As summarized in Table 2, all CLL-1 based CAR-T cells treated patients experienced grade 1–2 CRS in the first month. Satisfyingly, no immune effector cell-associated neurotoxicity syndrome (ICANS) was recorded among these patients. To further confirm the CRS/ICANS, we had continuously monitored the pro-inflammatory cytokine IL-6 release. As shown in **Supplementary Fig. 4**, the serum IL-6 levels were significantly increased in the first month post CAR-T infusion, which was consistent with the occurrence of CRS. To identify the kinetics of CAR-T cells in patients, we performed flow cytometry analysis to monitor the CAR^+ T cell expansion. The CAR-T cells were efficiently expanded in the first month after infusion, with the average peak time varied from day 10 to 12, and sustained *in vivo* for varied time in different individuals (**Supplementary Fig. 5**). In addition to CRS, we also found that all patients experienced grade 3–4 pancytopenia and absence of monocytes and granulocytes with varied duration (with the shortest for 7 days, and longest for 49 days) following CAR-T infusion. Among these 8 patients, the longest absence of monocytes was 7 weeks in Patient 5. Next, the toxicity profiles of the CAR-T cell infusion were evaluated according to the criteria of CTCAE 5.0. Notably, treatment of CLL1-CAR-T cells had no observable toxicity on all the organs we examined in these 8 patients (Table 2). From this point, we concluded that the anti-CLL1 based CAR-T therapy was well tolerated, with the similar toxicity profiles as previously reported using CLL1-CAR-T cells[24,25].

Table 2
Patient safety information

Patient #	Product	CRS	Myeloablation	Cardiac	Respiratory	Renal	Liver	Gastrointestine	Dermatology
1	CLL1	Grade 1	Yes	No	No	No	No	No	No
2	CLL1	Grade 1	Yes	No	No	No	No	No	No
3	CLL1	Grade 2	Yes	No	No	No	No	No	No
4	CLL1	Grade 2	Yes	No	No	No	No	No	No
5	CLL1	Grade 2	Yes	No	No	No	No	No	No
6	CLL1	Grade 1	Yes	No	No	No	No	No	No
7	CLL1	Grade 1	Yes	No	No	No	No	No	No
8	CLL1	Grade 1	Yes	No	No	No	No	No	No

We next assessed the efficacy profiles in these 8 patients with R/R-AML after CLL1-CAR-T treatment. As shown in Fig. 2B and summarized in Table 3, morphologic CR (CRm) and MRD negativity were achieved in 4 patients (patient 1, 2, 5, and 8), CRm and MRD positivity were achieved in 2 patients (patient 3 and 7), and partial remission (PR) was achieved in patient 4, and patient 6 remained stable disease (SD) until 1 month after CLL1-CAR-T therapy. To define the long-term effect of anti-CLL1 based CAR-T therapy, we performed a long-term follow-up study among these patients. Among these R/R AML patients, 6 patients (patient 1, 2, 3, 4, 7 and 8) completed allo-HSCT (Fig. 2B and Table 3) after CAR-T treatment. The patient 2 relapsed two months post HSCT and died of GVHD. The patient 4 relapsed six months post HSCT and died of PD. The remaining 4 patients (patient 1, 3, 7 and 8) were still alive and remained complete remission, with the longest 26 months' follow-up study. The patient 5 remained CR for twelve months without receiving allo-HSCT before relapse. For the patient with stable disease (patient 6) after CAR-T infusion, the percentage of AML blasts decreased from 91.3–70.6% two weeks after CAR-T cell therapy, while the remaining AML blasts were CLL1 negative and CD33 positive (**Supplementary Fig. 6**). The patient 6 only survived for three months after CAR-T treatment and succumbed to disease progression and lung infection. To summarize the interim analysis of the phase 1/2 clinical trial, the anti-CLL1 CAR-T cells displayed an ideal targeting capacity for treating the pediatric R/R AML patients, and should be considered as an alternative strategy for the treatment of AML in the future.

Table 3
Patients' responses to anti-CLL1-CAR T cells

Patient #	Sex/ Age	AML subtype	AML baseline prior to pre-conditioning	Product	CAR-T Dosage	Best response within 1 month	Following HSCT	Current status
1	Male/ 16Y	AML-M2	62.3%	CLL1	1×10^6/kg	CR/MRD-	Yes	CR/MRD-
2	Male/ 11Y	AML-M5 + JMML	92.4%	CLL1	0.35×10^6/kg	CR/MRD-	Yes	Death (GVHD)
3	Male/ 13Y	AML-M2	21.4%	CLL1	1×10^6/kg	CR/MRD+	Yes	CR/MRD-
4	Female/ 12Y	AML-M2	27.7%	CLL1	0.8×10^6/kg	PR	Yes	Death (PD)
5	Male/ 8Y	AML-M6	14.2%	CLL1	1×10^6/kg	CR/MRD-	No	Relapsed
6	Female/ 13Y	AML-M2	91.3%	CLL1	1×10^6/kg	SD	No	Death (Lung infection)
7	Male/ 12Y	AML-M2A	30%	CLL1	1×10^6/kg	CR/MRD+	Yes	CR/MRD-
8	Male/ 9Y	AML-M5	69.5%	CLL1	1×10^6/kg	CR/MRD-	Yes	CR/MRD-

Discussions

The application of CD19-directed CAR-T cell therapy has achieved great success for the cure of relapsed or refractory B-cell malignancies (i.e., B-cell acute lymphoblastic leukemia, B-cell lymphoma), also ignited the hope of the treatment of other hematological malignancies. However, the translation of CAR-T cells into AML treatment remains lagged behind, which resulting in immoderate reliance on intensified chemotherapy and allo-HSCT. Here, we reported the safety and efficacy profiles of anti-CLL1-based CAR-T cell therapy in eight children with R/R-AML from two independent medical centers.

Up to now, CAR-T cells targeting CLL1, CD13, CD33, TIM3, NKG2D, CD123, CD7, NPM1, and FLT3 have been extensively studied and shown to effectively eradicate AML cells *in vitro* and *in vivo*[29]. However, the number of successful case reports using CAR-T cells in treating AML patients is limited[30]. For example, NKG2D-targeted CAR-T has been reported to induce CR in one AML patient[31]. Nevertheless, this successful case cannot be replicated later in a large cohort of AML patients. In a dose-escalation Phase 1 clinical trial, five AML patients treated with high-dose of CD123-CAR-T cells from Mustang Bio Inc, two patients achieved CR and the other three remained SD. It has been reported that CLL1-CD33 compound CAR-T cells treated 9 AML patients, 7 of them reached CR. In addition, two successful case reports have utilized CLL1 as single target of CAR-T therapy, suggesting the promising potential of targeting CLL1 in AML treatment[24 25]. Here, our data further support the efficacy of anti-CLL1-based CAR-T cells in R/R-AML treatment. 7 out of 8 enrolled patients responded to the treatment with only grade1-2 CRS. The CR rate was 75%, PR rate was 12.5% and SD rate was 12.5%. The most severe adverse events associated with this treatment was myeloablation. Meanwhile, no other systemic toxicities were recorded in this study cohort. Together, our findings highlighted the fact that the effective and safe characteristics of anti-CLL1-based CAR-T cell therapy in children with R/R-AML.

Several studies have shown that the un-biased tumor associated antigens (TAAs) targeting by CAR-T cells also damaged antigen-expressed healthy tissues (albeit at low levels). For example, treatment with anti-CD123, CD33 CAR-T cells generated a potent anti-AML efficacy while causes severe/prolonged myelosuppression[34]. It has been reported that CLL1 is preferentially highly expressed on AML-stem cells but not on normal HSC, which make it a promising target for AML immunotherapy, although it is also expressed on granulocytes and monocytes[35]. In this study, we did observe severe myelosuppression in all responding cases. Among these patients, patient 5 experienced prolonged myelosuppression, which could not be simply explained by prior lymphodepletion therapy, indicating a higher susceptibility to intracellular bacteria and parasitic infection. For safety, to monitor intracellular bacteria and parasitic infection will be needed for anti-CLL1-based CAR-T therapy. To reduce the risk of infection, we do recommend patients to bridge allo-HSCT once CR was achieved. In addition, we observed that patient 6 poorly responded to anti-CLL1 based CAR-T cells therapy, however, the CLL1 positive AML cells were completely abolished while the remaining AML blasts were CLL1 negative. For the patients with similar outcomes as patient 6, combination of a second CAR-T target depending on the AML immunophenotypic characteristics, such as CD33, CD123, Lewis Y, or CD38, might be helpful in the future to improve the CAR-T cell efficacy. How to balance the risk and benefit when using the CAR-T therapy for R/R-AML treatment remains an important issue in the future. To this end, our results suggested that patients with more than 90% of CLL1 positivity on AML blasts may benefit from single CLL1-CAR-T treatment. However, for patients with less than 90% of CLL1 positivity on AML blasts, an additional CAR to target a different tumor antigen may be beneficial to achieve complete deletion of AML cells.

In summary, our findings demonstrate a very encouraging outcome with a safe and manageable profile and high targeting efficacy for the use of anti-CLL1-based CAR-T cells in the treatment of children with R/R-AML. This is the first report of multi-center based clinical trial for application of anti-CLL1 based CAR-T cells in R/R-AML.

Declarations

Competing Interests statement

GL, ZZ, WD, YZ and ML are employees of Guangzhou Bio-Gene Technology Co., Ltd., while other authors have nothing to disclose.

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Author Contributions

The study was conceived by HZ and CL, designed by HZ, CL, and ML, supervised by HZ, CL, and ML. HZ, CB, ZP, GL and CL performed the research. HZ, CB, ZP, YH, ZH, and KP recruited the patients and collected clinical data. Data was conducted and interpreted by HZ, CB, ZP, GL, ZZ, WD, ML and CL. HZ, CL, YZ and ML wrote the manuscript. All authors approved the final version for publication.

Competing Interests

GL, ZZ, WD, YZ and ML are employees of Guangzhou Bio-Gene Technology Co., Ltd., who have potential interest, while other authors have nothing to disclose.

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Figures

Figure 1

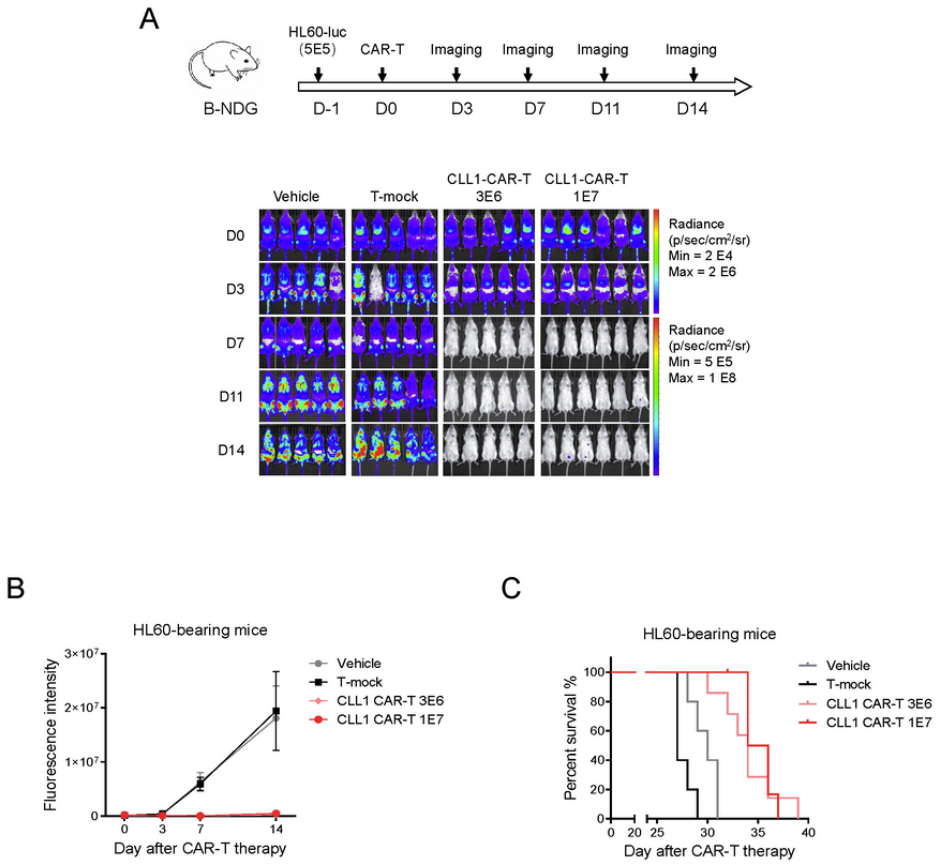

Figure 1

CLL1-CAR-T showed strong anti-tumor activity in pre-clinical mouse model. (A) B-NDG mice were i.v. injected with 5 x10^5 HL60-luciferase cells. After 2 days, the mice were injected intravenously with 3 x10^6 or 1 x10^7 CLL1-CAR T cells. The BLIs indicated the mouse tumor burden at different time points. (B-C) Tumor burden and survival analysis of mice treated with CLL1-CAR-T cells. (n = 5 mice per group).

Figure 2

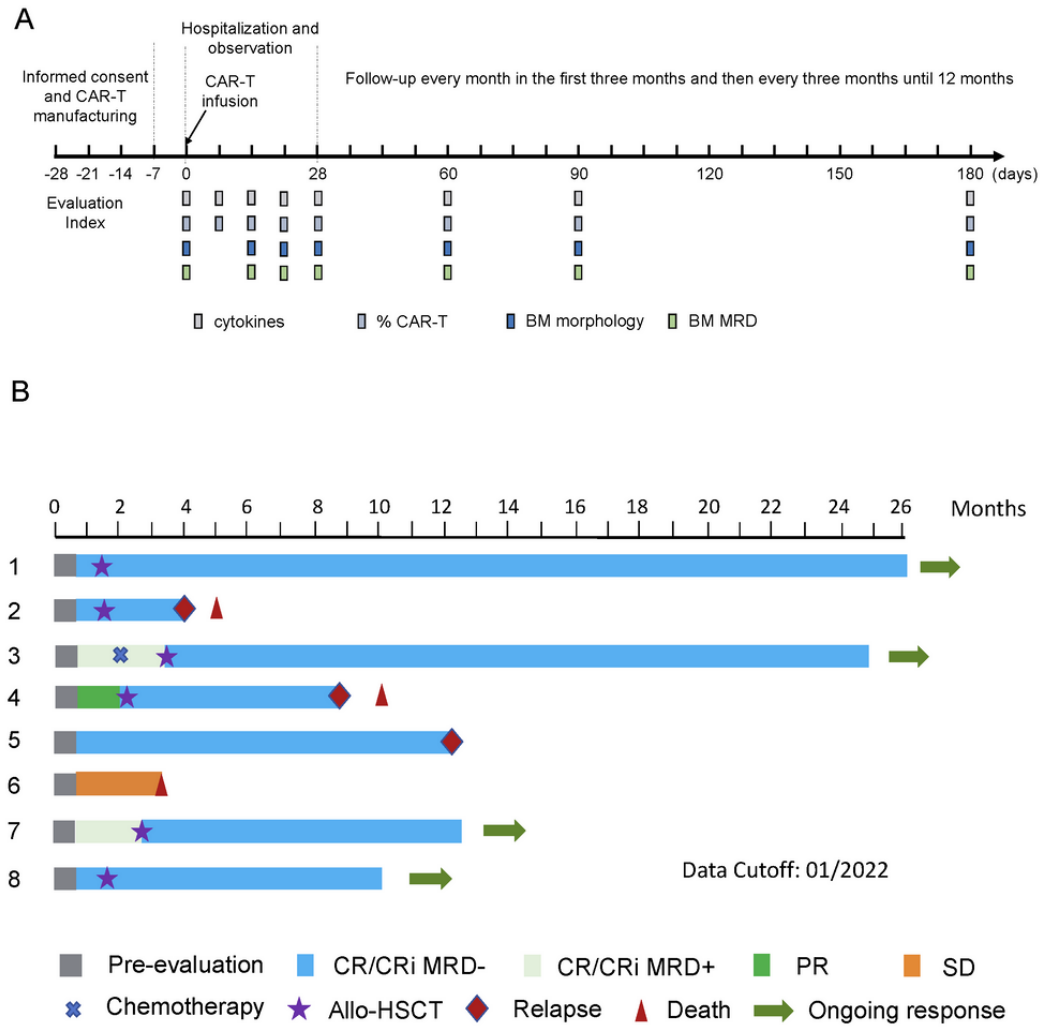

Figure 2

CLL1 CAR-T induced complete remission in AML patients. (A) Schematic of the Phase I/II clinical trial design. (B) Duration of the response and overall survival after the infusion of anti-CLL1 based CAR-T cells. 6 out of 8 patients received allo-HSCT after CAR-T cell infusion. Patient 2 died from GVHD after second HSCT. Patient 4 relapsed and died of PD post HSCT. For the other 2 patients who did not receive allo-HSCT, patient 5 relapsed after twelve months remission, patient 6 died from uncontrolled lung infection. CR/CRi: complete response/incomplete response; MRD: minimal residual disease; PR: partial response; SD: stable disease.

Supplementary Files

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