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Letter to the editor

# Successful treatment of relapsed acute B-cell lymphoblastic leukemia with CD20/CD22 bispecific chimeric antigen receptor T-cell therapy

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*Dear editors,*

Treatment of refractory/relapsed acute B-cell lymphoblastic leukemia is still challenging nowadays [1]. Although chimeric antigen receptor T cells targeting CD19 (CART19) hopefully provide an additional CR for most those refractory/relapsed patients, durable remission is difficult to achieve due to relapse as the follow up prolongs or nonresponse in a lower proportion [2]. Once relapse occurs after CART19, the prognosis is extremely poor due to limited alternative therapeutic option. Increasing attempts have contributed to improve the efficacy of CART19, including combination of different antigen targets [3, 4]. Targeting dual antigens in a single CAR structure is a promising strategy for optimizing CART therapy with a potential low relapse risk on account for preventing antigen escape [5].

Here, for the first time we uncovered a case of a relapsed B-ALL patient whose blast cells expressed low CD19 with robust CD22 and dim CD20, received a novel CD20-CD22 bispecific CART product and achieved durable CR. The outcome indicates the comparable potency of CART cells targeting alternative combination of antigen targets without CD19 for relapsed B-ALL.

A 26-year-old female who presented with ecchymosis in right upper limb for 2 weeks and a sudden apopsychia 6 months ago was diagnosed as B-ALL in September 2019. Blood sample analysis showed leukocytosis ($24.4 \times 10^9$/L), erythrocytopenia ($2.42 \times 10^{12}$/L), thrombocytopenia ($45 \times 10^9$/L), and lower hemoglobin (70 g/L). 73% type I blasts was detected by bone marrow aspiration (BMA) and ALL was considered in morphology. Flow cytometry showed 77.38% of the nucleated cells to be lymphoblasts with the immunophenotypic features as $CD19^+$ $CD34^+$ $CD22^+$ $CD200^+$ $CD58^+$ $cyCD79a^+$ $nTdT^+$. The karyotype was 46XX and no positive fusion gene or mutation was detected. The patient's treatment was initiated a single cycle of VICP (vindesine 4 mg d1, d8; idarubicin 10 mg d1, d8; cyclophosphamide 1.1g d1, Dexamethasone 10 mg qd) in September 2019 and achived CR with MRD <0.01%. A second cycle therapy of CAM (cyclophosphamide 1.1g d1, 0.8g d8; cytosine arabinoside 150 mg d1-3, d8-10; 6-MP 100 mg d1-7) was administrated in October 2019. Unfortunately, the disease relapsed in November 2019 and the BMA showed 10% lymphoblasts. So the treatment was calibrated to BCL2 inhibitor (venetoclax, 400 mg qd) with prednison 50 mg qd. However, the disease still progressed with increased lymphoblasts of 37% two weeks later and BCL2 inhibitor was withdrawn the next week. Bone marrow reexamine showed 40.3% of lymphoblasts in Jan 2020. Thus, the patient was recruited in clinical trial of bispecific CART therapy (ChiCTR-1800017669). Considering the characteristics of MRD phenotype with low expression of CD19 and $CD22^+$ $CD20^{dim}$ at relapse (Fig. 1A) , the patient was personalized with a novel combination targeting CD20 and CD22. The patient was infused with CD20/CD22 bispecific CART cells at the dose of $3.21 \times 10^6$/kg in Jan 2020 after conditioning chemotherapy of FC (fludarabine 48 mg d1-3, cyclophosphamide 0.8 mg d 2–3). After CART infusion, the patient developed a rapid fever which lasted within a week and the peak temperature reached 40.3 °C at day6. Typical peaks of increased CART associated cytokines (IFN-γ, IL-6, IL-10) in plasma was also detected (Fig. 1C), considering for a grade III of cytokine release syndrome according to Penn grading scale. The fever was soon eliminated after tocilizumab (8 mg/kg) and supportive treatment (Fig. 1B). There was no obvious neurotoxicity or other systemic symptoms complained. Meanwhile, CART cells expanded and reached 90% of total $CD3^+$ cells in peripheral blood during CRS period (Fig. 1D). The CART component was largely CD8 positive after a week expansion (Fig. 1E). Two weeks after CD20/CD22 CART infusion, the bone marrow evaluation showed CR and flow cytometry showed minimal residual disease ( MRD ) < 0.01% (Fig. 1F). The state of complete remission sustained for 2 months.

CART cells targeting CD19 have demonstrated remarkable response in B-ALL. Besides CD19, other pan-B cell markers such as CD20 and CD22 has been proposed as potential target for B-ALL and increasing trials have indicated similar safety and efficacy [6]. Sequential treatment with CD20-CART or CD22-CART was proposed effective to induce remission after failure of CART19 therapy [7]. Moreover, Bispecific CART targeting CD19-CD20 or CD19-CD22 is expected responsible and provides an effective safeguard against antigen escape by malignant B cells, thus contributing to the durability of remission [8, 9]. So, reasonable combination of these targets for CART cell manufacture would benefit the clinical outcome for B-ALL treatment, especially in consideration for the relapse due to antigen loss. Nevertheless, it's unclear whether CD19 is indispensable for CART administration in B-ALL treatment,

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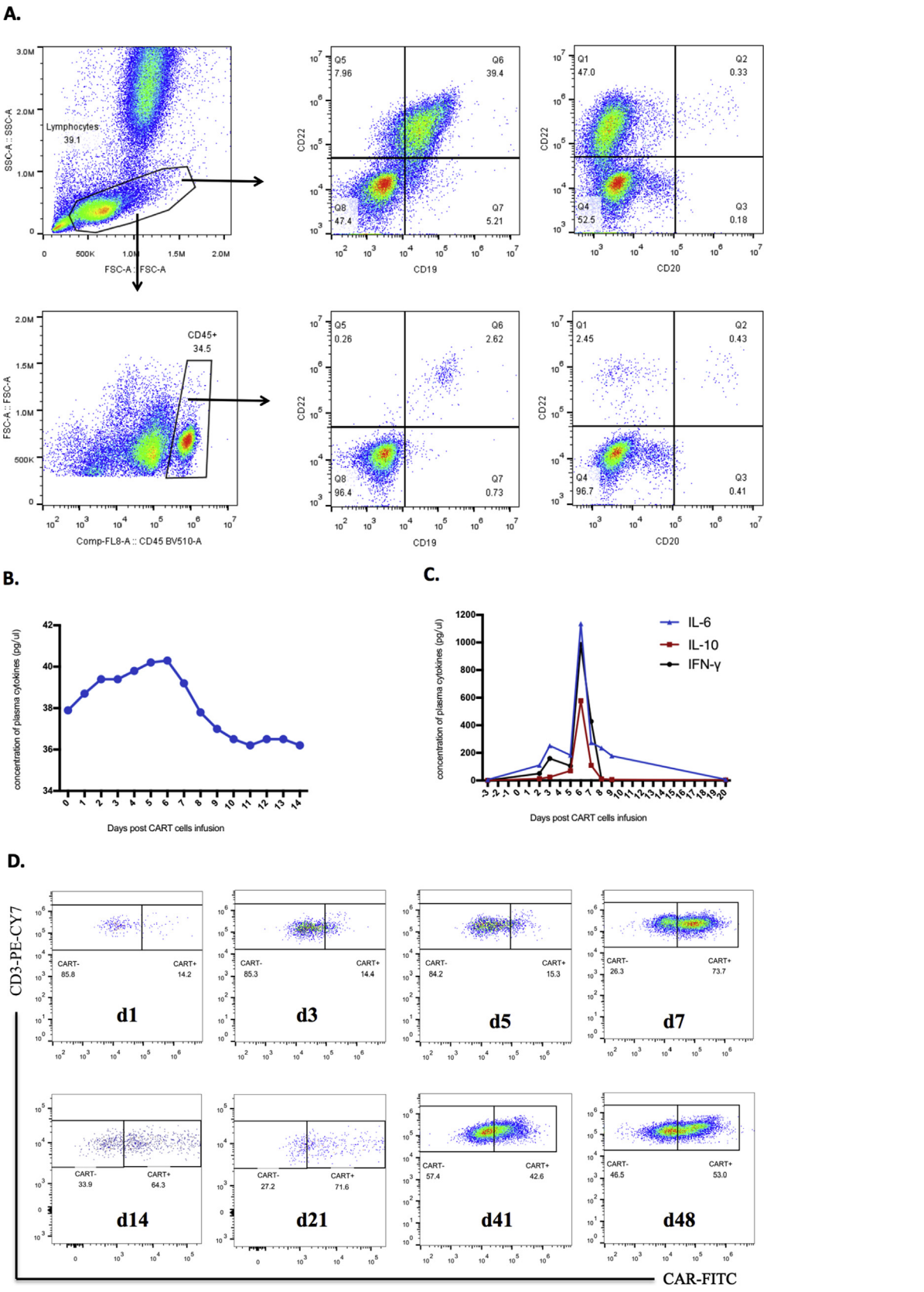


**Fig. 1.** **A.** The phenotype of lymphoblasts at the time of relapse after chemotherapy and before CD20/CD22 CART infusion. **B.** The body temperature after CART infusion. **C.** The CRS related cytokines in plasma after CART infusion. **D.** Dynamic of CART cells expansion after infusion. The CART cells were gated as $CD3^{+}CAR^{+}$(Biotin-SP-AffiniPure F (ab)2 Fragment Goat Anti-Human IgG, Jackson immunoresearch, 109-066-006; anti-CD3-PE-CY7, Biolegend, 300,420). **E.** The changes of $CD8^{+}$ and $CD4^{+}$ ratio of CART cells after infusion. **F.** The blasts burden in bone marrow (detected by flow cytometry), and the percentage of CART cells during the chemotherapy and CART cell treatment. **G.** Comparison of targeted antigens before and post CD20/CD22 CART relapse. There was no antigen loss or diminished expression of CD20, CD22, CD19.

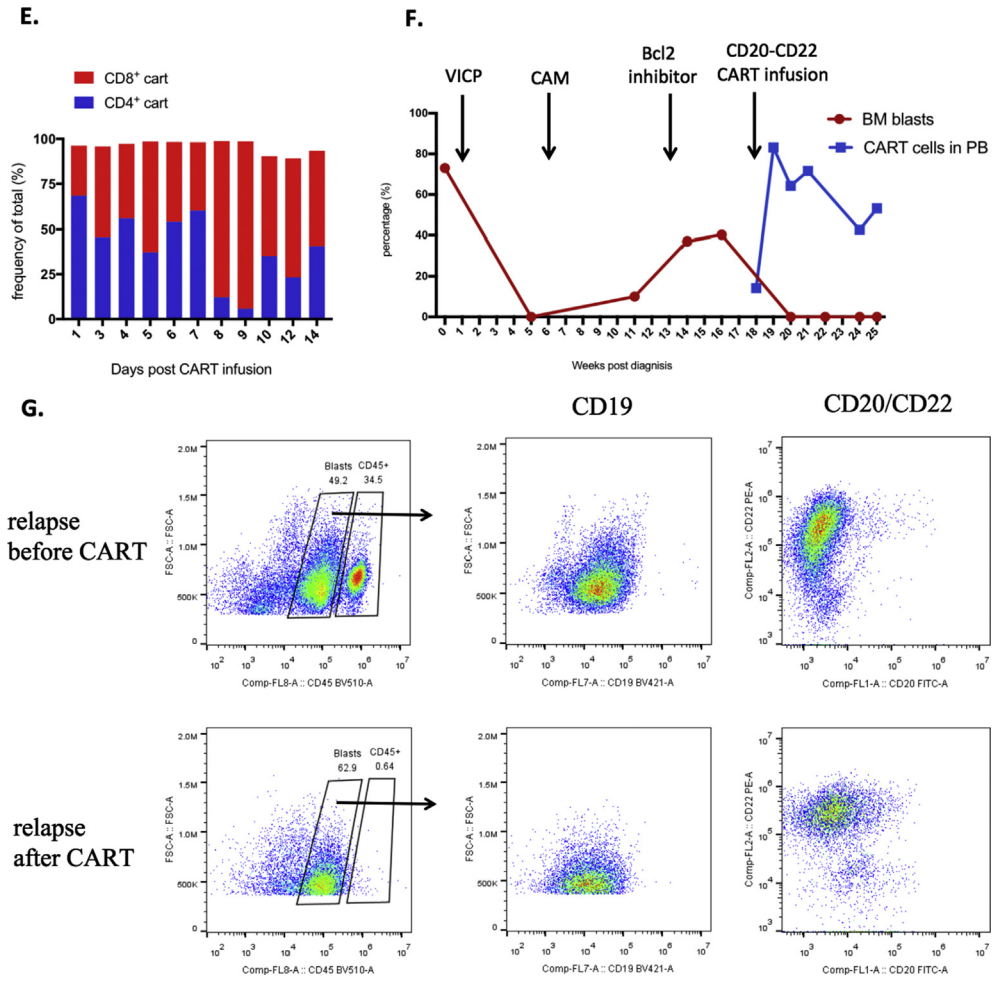


**Fig. 1.** (*continued*).

especially for those B-ALL patients whose CD19 expression on malignant cells is not high enough. In the present case, Although the karyotype and the mutation detection was both normal, the disease soon progressed after sequential treatment of chemotherapy combined with BCL-2 inhibitor, suggesting a poor prognosis. Despite of the limited CD20 expression in the patient's ALL cells, we choose CD20 and CD22 as the targets for the preparation of the dual CART because of the available target candidates for ALL was still restricted (referring to CD19, CD20, CD22 and CD52). We observed the successful elimination of refractory/relapsed acute B cell lymphoblasts by the novel CD20/CD22 bispecific CART product. Bispecific CART products targeting dual antigens help to diminish the antigen loss relapse. We noticed that an antigen-positive relapse occurred two months after CD20/CD22 CART therapy. There was no antigen loss or diminished expression of CD20, CD22 and CD19 in the relapsed blast cells after CD20/CD22 CART treatment (Fig. 1G). The patient was infused later on with a dual CD19/CD22 universal CART product in April 2020 and kept CR so far.

Collectively, the novel CD20/CD22 CART targeting relapsed $CD19^{low}$ acute B-cell lymphoblastic leukemia is comparable effective. It was the first report of such a combination of antigen target for CART products. This case suggests the CART product should be personalized as possible for relapsed B-ALL patients, especially those lacking CD19 expression.

## Ethical approval

All procedures performed in the study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with approved by the Medical ethics committee of the First Affifiliated Hospital, Zhejiang University School of Medicine.

## Informed consent

Written informed consent was obtained from the patient for being included in the study.

## Declaration of competing interest

The authors declare that they have no conflict of interest.

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