CASE REPORT

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4SCAR-GD2-modified T-cell therapy in neuroblastoma with *MYCN* amplification: A case report with over 4-year follow-up data

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ABSTRACT

Introduction: Neuroblastoma (NB) is the most common extracranial solid tumor among children. The 5-year event-free survival rate for high-risk (HR) NB is still poor, especially for patients with advanced NB with *MYCN* gene amplification. Chimeric antigen receptor T (CAR-T) cell therapy is a new treatment for HR-NB.

Case presentation: A 55-month-old boy with stage IV HR-NB received 4th-generation CAR-T cells that target disialoganglioside GD2, as consolidation maintenance treatment after intensive chemotherapy, surgery, and autologous stem-cell transplantation. As of February 2019, his CAR-T follow-up time was 37.5 months, indicating prolonged survival. Cranial MRI and ultrasound showed no mass; ^{123}I-metaiodobenzylguanidine (^{123}I-MIBG) scan was negative.

Conclusion: GD2-CAR-T cells may be an effective treatment option for NB patients with *MYCN* amplification.

KEYWORDS

Neuroblastoma, GD2, CAR-T, *MYCN* amplification, Bone marrow, Metastasis, Encephalic metastasis, Long term survival

INTRODUCTION

Neuroblastoma (NB) is the most common extracranial solid tumor among children. Although multidisciplinary approaches have improved 5-year event-free survival (EFS) rates for patients in low- or intermediate-risk groups, EFS for high-risk (HR) NB is still poor.[1-5] Chimeric antigen receptor T (CAR-T) cell therapy is a new treatment for HR-NB.[6-7] Here, we report on a 55-month-old boy with *MYCN*-amplified NB, who received 4SCAR-GD2-modified T cells targeting NB as a consolidated maintenance treatment after chemotherapy, combined radiotherapy (RT) and autologous hematopoietic stem cell transplantation (ASCT), and was followed up for more than 4 years.

CASE REPORT

Ethical approval was obtained from the Ethics Committee of Beijing Children's Hospital and written informed consent was obtained from the child's guardians.

A 55-month-old boy was admitted in September 2014 for fever and abdominal mass. A computed tomography (CT) scan showed a 7.0 cm × 4.4 cm × 10 cm mass with sporadic calcification in his left retroperitoneum. His serum neuron-specific enolase (NSE) level was > 370 ng/mL, his lactase dehydrogenase (LDH) was 1224 U/L, and his urinary vanillylmandelic acid (VMA) was 11.58 μmol/L. Cranial magnetic resonance imaging (MRI) showed soft

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tissue masses in both the right occipital tempus and left forehead (Figure 1A). Positron-emission tomography-computed tomography (PET-CT) scan showed hypermetabolic lesions. A bone scan showed multiple bone lesions. NB cells (54%–58.5%) were also found in bone marrow. Immunohistochemical testing showed positive for synaptophysin, chromogranin A, 70% Ki-67, and S-100 and negative for N-myc, CD68, cytokeratin, and leukocyte common antigen. FISH showed *MYCN* gene amplification (10–12 copies). This boy was therefore diagnosed with stage IV HR-NB.

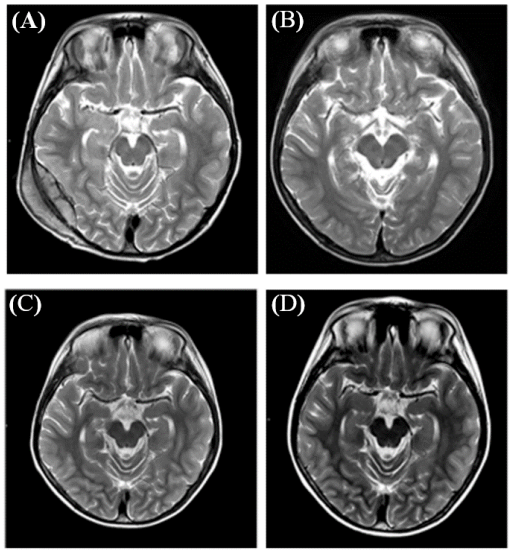

FIGURE 1 Cranial magnetic resonance imaging (MRI) of the child with neuroblastoma (NB). (A) MRI in September 2014 (in admission). Protrusions on right occipital brain indicate skulls destruction. Soft tissue signal shadow was seen inside and outside the cranial plate and rain tissue was compressed. (B) MRI in January 2016 (before GD2-CAR-T cells therapy). The signal intensity of greater wing of sphenoid bone and sella sphenoid bone was inhomogeneous and the local signal was slightly longer T1 and longer T2. The occipital cistern, and the cerebellar sulcus on both sides of the cerebral hemispherehad widened. The skull of the right occipital part is slightly thick. (C) MRI in May 2016 (18 weeks after undergoing GD2-CAR-T cells therapy). No abnormal signal was found in brain parenchyma. T2 signal of the right occipital region is more obvious than that of the front, and the signal of the sellar bone is still uneven, compared with Figure 1B. (D) MRI in February 2019 (37.5 months after CAR-T cells therapy). The abnormal signals of right occipitotemporal, sphenoid wing and sella turcica were better than before, compared with Figure 1C. CAR, chimeric antigen receptor.

According to the BCH-HR-NB-2007 protocol (Hong Kong NB-N6 protocol),[1,2] the treatment schedule is 7 cycles of chemotherapy, using a regimen of cyclophosphamide, adriamycin and vincristine for Cycles 1, 2, 4 and 6; and a regimen of cisplatin and etoposide (VP16) for Cycles 3 and 5. After the second course, his tumor volume had reduced 74% (from 63.3 cm^3 to 16.5 cm^3). After the fourth course, his NSE level was 15.5 ng/mL and urinary VMA was 1.95 μmol/L. The mass behind his peritoneum was 2.6 cm × 1.8 cm × 3.4 cm, and it was removed completely by surgery in December 2014. Pathological biopsy confirmed it to be poorly differentiated NB. After the 7th course, the ^{123}I-metaiodobenzylguanidine (^{123}I-MIBG) scan was negative. He received ASCT in May 2015, and received isotretinoin (160 $mg \cdot m^{-2} \cdot d^{-1}$) for Days 1–14 and Days 29–42 of a 42-day cycle, as maintenance therapy after ASCT. He also received intermittent RT to skull and abdomen from July 2015 to October 2015.

After ASCT, only the cranial MRI showed abnormal signals in the original site. Tumor markers were normal, and the patient had stable disease (SD). He received maintenance chemotherapy and local RT for 9 months after the ASCT, after which no progression was seen in his cranial MRI, and disease status was still SD. However, although the patient's tumor markers were normal and bone marrow minimal residue disease (MRD) was negative, his cranial MRI still showed abnormal signals (Figure 1B) at a routine examination after 6 months of RT and maintenance isotretinoin use.

As consolidation therapy, we decided to use 4th-generation 4SCAR-GD2-modified T cells, which target disialoganglioside GD2 (a well-characterized tumor antigen in NB) and incorporate multiple costimulatory molecules ($GD2^+/CD37^+/CD28^+/CD137^+/CD27^-$/iCasp9). They are associated with better anti-tumor efficacy than with 3rd-generation CAR-T, and incorporate a suicide gene, namely the inducible caspase 9 (*iCasp9*), which may improve safety and decrease the likelihood of cytokine release syndrome (CRS), a potentially life-threatening systemic inflammatory response observed following administration of antibodies and adoptive T cell therapy, without impairing the efficacy of CAR. Lymphocytes were collected (5.2×10^9) in December 2015 and pretreated in January 2016. Lymphocyte pretreatment protocol was cyclophosphamide 250 $mg \cdot m^{-2} \cdot d^{-1}$ for 3 days and fludarabine 25 $mg \cdot m^{-2} \cdot d^{-1}$ for 3 days. On the third day after the end of the pretreatment regimen, the patient was infused with 7.7×10^7 CAR-T cells (3.5×10^6/kg). We used PCR on peripheral blood specimens to detect CAR-T cells and found that the cells peaked at 6.36% on Day 14 after infusion, and remained a long time in the body (0.57% at Day 28 after infusion).

The target of CAR-T cells was GD2, which is detected semiquantitatively by immunohistochemical testing of paraffin sections, and divided into five grades, including negative and + to 4 +.[6-8] The patient's GD2 expression was 2.5 +. Functions of B cells and T cells may be modified by combining antibody genes in B cells with surface receptor genes in T cells, using lentivirus as a carrier, to create a chimeric gene. This would modify T cells to become target T cells. All T cells could be transformed into target T cells instantaneously by this method. Finally, the CAR-T transition efficiency was 20.77%.

This patient's main adverse effect (AE) was grade 2 CRS,[9] which presented as low blood pressure during the

24–48 h after infusion, fever during Days 11–14; and rash, hypoalbuminemia and edema during Days 11–17. All these symptoms were thought to be related to his CAR-T cell level. Eighteen weeks after the infusion, cranial MRI (Figure 1C) showed significant abnormal signals in his right occipital tempus; at the same time, 3% CAR-T cells were detected in his bone marrow.

After the CAR-T cells therapy, the patient was tested for tumor biological factors, including NSE, urinary VMA and homovanillic acid (HVA), on Days 0, 7, 14, 21 and 28, Week 6, Months 2, 3 and 6, and every 6 months thereafter. We also examined his bone marrow on Days 0 and 28, Week 6, Months 3 and 6, and every 6 months thereafter, and performed B-ultrasound or CT for any solid tumors at Week 6, Months 3 and 6, and every 6 months thereafter.

Regular examinations have shown the child to be in stable condition. Although the prognosis of NB with *MYCN* amplification is poor, this patient has SD all along. His total follow-up time was 53.5 months overall, and 37.5 months after his CAR-T treatment, as of February 2019. His height was 135 cm, and weight was 31 kg. Laboratory tests showed the serum NSE level was 17.5 ng/mL, LDH was 223 U/L, urinanry VMA was 3.85 μmol/L, and urinanry HVA was 0.40 μmol/L. Tumor cells were not found in his bone marrow, and marrow MRD was negative (PCR amplification of *PHOX2B*). Cranial MRI and ultrasound found no abdomen, neck or mediastinum mass (Figure 1D). ^{123}I-MIBG scintigraphy was also negative.

DISCUSSION

Neuroblastoma is the most common extracranial solid tumor of childhood. NB is often metastatic at the time of diagnosis, which indicates poor prognosis. Although patients in low-risk and middle-risk groups have better prognoses through multidisciplinary treatment, the 5-year survival rate for HR patients is less than 50%. *MYCN* amplification or multiple bone or bone marrow metastases are associated with poor prognosis.[4,5]

CAR-T cells are a type of immunological therapy that combines the specificity of antibodies and lethality of T cells.[6-8] GD2-CAR-T cell is reportedly safe and effective in anti-tumor therapy. This therapy has been the subject of a multi-center clinical trial by a cooperative study group in China,[9] in which patients with HR-NB received 4SCAR-GD2-CAR-T (GD2 expression was ≥ grade 2 + in 71% of patients) and followed-up for 1 year; 24% of patients had no AEs, 76% had grade 1–2 AEs (including fever, rash and peripheral nerve pain), but none had AEs grade 3 or higher, which indicates that CAR-T is safe. At 1 year after infusion, 38% of patients had SD and 15% had partial responses; the 1-year overall survival rate was 74%. A single-center study from the Hematology and Oncology Center of Beijing Children's Hospital summarized the CAR-T treatment of 13 patients with HR-NB (refractory or recurrent). All patients had grade 1–2 CRS. The total effective rate was 66.6% for 1 year among 13 patients. It also proved CAR-T to be safe and effective.[9,10]

NB with *MYCN* gene amplification has a poor prognosis.[11] The 16 children with *MYCN*-amplified HR-NB we recently summarized had a median progression-free survival of 8.25 months and median overall survival of 10.75 months, which indicates that these children critically need new treatment methods.

In this case, conventional multidisciplinary treatment was unsatisfactory. To find a relatively safe, effective therapy for this patient, we choose 4SCAR-GD2-modified T cells as a consolidation of his maintenance treatment, after using a multidisciplinary approach of chemotherapy with RT and ASCT. He has survived for more than four years. We conclude that GD2-CAR-T maybe a potential safe and effective method to treat NB with *MYCN* amplification. Further large-sample studies are needed.

CONSENT FOR PUBLICATION

Consent was obtained from the patient's guardians.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

REFERENCES

1. Ma X, Jin M, Zhang D, Wang H, Qin H, Qin M, et al. Clinical features and efficacy of recent treatment analysis of multimodality treatment of 91 children with neuroblastoma. Chin J Appl Clin Pediatr. 2013;28:178-182. (in Chinese)
2. Monclair T, Brodeur GM, Ambros PF, Brisse HJ, Cecchetto G, Holmes K, et al. The International Neuroblastoma Risk Group (INRG) staging system: An INRG task force report. J Clin Oncol. 2009;27:298-303.
3. Park JR, Bagatell R, Cohn SL, Pearson AD, Villablanca JG, Berthold F, et al. Revisions to the International Neuroblastoma Response Criteria: A consensus statement from the National Cancer Institute Clinical Trials Planning Meeting. J Clin Oncol. 2017;35:2580-2587.
4. Yue ZX, Huang C, Gao C, Xing TY, Liu SG, Li XJ, et al. *MYCN* amplification predicts poor prognosis based on interphase fluorescence in situ hybridization analysis of bone marrow cells in bone marrow metastases of neuroblastoma. Cancer Cell Int. 2017;17:43
5. Hao T, Li S, Li X, Jin M, Huang C, Wang X, et al. Clinical features, treatment and prognosis of neuroblastoma with bone metastasis in children. Chin J Appl Clin Pediatr. 2017;32:128-186. (in Chinese)
6. Louis CU, Savoldo B, Dotti G, Pule M, Yvon E, Myers GD, et al. Antitumor activity and long-term fate of chimeric antigen receptor-positive T cells in patients with neuroblastoma. Blood. 2011;118:6050-6056.
7. Porter DL, Hwang WT, Frey NV, Lacey SF, Shaw PA, Loren AW, et al. Chimeric antigen receptor T cells persist and induce sustained remissions in relapsed refractory chronic

lymphocytic leukemia. Sci Transl Med. 2015;7:303ra139.
8. Heczey A, Louis CU, Savoldo B, Dakhova O, Durett A, Grilley B, et al. CAR T cells administered in combination with lymphodepletion and PD-1 inhibition to patients with neuroblastoma. Mol Ther. 2017;25:2214-2224
9. Yang L, Ma X, Liu Y, Zhao W, Yu L, Qin M, et al. Chimeric antigen receptor 4SCAR-GD2-Modified T cells targeting high-risk neuroblastoma: A phase II multi-center trial in China. Blood. 2017;130:3335.
10. Xia AL, Wang XC, Lu YJ, Lu XJ, Sun B. Chimeric-antigen receptor T (CAR-T) cell therapy for solid tumors: challenges and opportunities. Oncotarget. 2017;8:90521-90531.
11. Campbell K, Gastier-Foster JM, Mann M, Naranjo AH, Van Ryn C, Bagatell R, et al. Association of *MYCN* copy number with clinical features, tumor biology, and outcomes in neuroblastoma: a report from the Children's Oncology Group. Cancer. 2017;123:4224-4235.

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