


Cytokine Release Syndrome After Modified CAR-NK Therapy in an Advanced Non-small Cell Lung Cancer Patient: A Case Report

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Abstract

Use of chimeric antigen receptors (CARs), as an immune cell therapy, has generated excellent clinical outcomes against hematologic tumors in recent years. Among them, the CAR-NK (natural killer) therapy has shown better efficacy, and less toxicity, than chimeric antigen receptor T-cell (CAR-T) therapy. In our phase II clinical trials, administering chimeric costimulatory converting receptor (CCCR)-NK92 cells on advanced non-small cell lung cancer patients proved efficacious in cell and animal experiments. However, we observed occurrence of cytokine release syndrome (CRS), a rare and unexpected side effect, never reported before during CAR-NK therapy. Here, we provide a detailed report of the patient's case, emphasize on the need to pay attention to CRS in NK cell therapy, and suggest improvements that will minimize potential toxicity.

Keywords

immune cell therapy, chimeric antigen receptor, cytokine release syndrome, CAR-NK

Introduction

Immune cell therapy, in which immune cells are used to fight tumors, is a novel approach for treating cancer¹. Chimeric antigen receptor T-cell (CAR-T) therapy is one of the most commonly used cell therapies that uses CARs-engineered T cells for cancer therapy². Its targeting, lethality, and persistence makes it the most convincing breakthrough in immune cell therapy. According to the 2019 New York Cancer Institute (CRI) analysis of global cancer cell treatment results, there were 1011 cell therapies worldwide, as of May 2019, of which 568 (56.2%) were CAR-T (the global landscape of cancer cell therapy)³. However, the CAR-T technology has been associated with numerous limitations, including difficulty to collect enough lymphocytes, especially from heavily pretreated or lymphopenic patients, as well as time-consuming and costly manufacture of CAR-T cells⁴. In addition, CAR-T therapy has been shown to cause substantial morbidities, which are mainly associated with a serious side effect called cytokine release syndrome (CRS) caused by abundant cytokine release⁵.

Natural killer (NK) cells are innate immune effector cells that are capable of killing tumor cells and producing cytokines without previous stimulation⁶. The CAR-NK therapy does not cause CRS, due to a different spectrum of the

secreted cytokines, and is therefore considered safer than CAR-T^{7,8}. The NK92, a cell line established from non-Hodgkin's lymphoma, has potential therapeutic effects due to its characteristics of activated NK cells⁹. Consequently, it has been extensively studied in the management of hematologic and solid tumors¹⁰. In our research group, we sought to produce an efficient, industrially produced NK cell therapy technology. Therefore, we designed a novel chimeric costimulatory converting receptor (CCCR), comprising mainly the extracellular domain of PD1, transmembrane and cytoplasmic domains of NKG2D, and the cytoplasmic

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domain of 41BB. This NK-tailored CCCR was able to switch the negative PD1 signal to an activating signal and hence reversed the immune suppressive effects of PD1. We first adopted quantitative real-time polymerase chain reaction (PCR) (qPCR) technology to synthesize an artificially synthesized chimeric costimulatory convert receptor coding gene *PD1-NKG2D-41BB*; we then obtained recombinant vector *PD1-NKG2D-41BB-pCDH* by using lentivirus as vector; and then, the lentivirus-packaged recombinant vector was transfected into NK-92 cells to obtain stably expressed CCCR-NK92 cell. Next, we detected the expression of PD-1 and the cell proliferation rate in CCCR-NK92 cells, and verified the killing effect of CCCR-NK92 cells on lung cancer cell lines *in vitro*. Finally, animal experiments were carried out in the human lung cancer NOG mouse subcutaneous xenografts model. The tumor volumes of the mice treated with CCCR-NK92 cells were significantly smaller than those of the control, and no significant toxic or side effects were observed in the experimental mouse¹¹. Thereafter, we designed a phase II clinical trial to evaluate efficacy and safety of the CCCR-NK92 cell therapy. Two patients were enrolled into the clinical trial; one patient developed CRS after CCCR-NK92 cell therapy. Here, we provide a detailed report of this case and emphasize on the need to pay attention to CRS during NK cell therapy. To our knowledge, this is the first report describing CRS after NK cell therapy.

Case Presentation

A 69-year-old female patient was histopathologically diagnosed with non-small cell lung cancer (NSCLC) on February 2, 2017, in the First Affiliated Hospital of Xinxiang Medical University. Image results showed intracranial metastases, whereas genetic tests revealed Exon-19 19-del mutant and T790 mutation, as well as PD-L1 expression $\geq 1\%$ in the lung tissue. She was first given gefitinib for 3 months. However, she was switched to Osimertinib for 10 months due to disease progression. After that, she received head radiotherapy on July 13, 2018, due to intracranial metastases. She was administered with targeted therapeutic drugs and local radiation after 3 months, and then evaluated on September 27, 2018. She refused chemotherapy for fear of side effects. Considering that her condition was suitable, she was enrolled in the clinical trial comprising CCCR-NK92 cells for NSCLC. However, multistage treatment of late recurrence and metastasis failed (NCT03656705) in November 2018. The CCCR-NK92 cells were intravenously injected, twice a week (d0/d3), for a 7-day treatment cycle. The first cycle comprised 1×10^7 CCCR-NK92 cells, as the beginning dose, which was increased to 2×10^7 at the second injection. The dose was increased during the second and third cycles, with 5×10^7 and 1×10^8 cells per injection, administered, respectively, according to her tolerance levels. During those treatment cycles, the patient experienced high fever, which was subsequently controlled to a normal range

after symptomatic treatment. A chest computed tomography (CT), performed after this three-cycle treatment, revealed that the lesion was stable.

After the third injection, of the second cycle, we observed a series of unexpected symptoms. Specifically, the patient developed hypotensive shock, coupled with high fever, hemoptysis, myalgia, liver damage, and abnormal blood coagulation (Fig. 1 and Table 1). We found neither evidence of infection in blood cultures nor signs of tumor lysis. Multidisciplinary consultations suggested occurrence of CRS. Consequently, we immediately performed anti-infection, continuous pumping of norepinephrine to maintain blood pressure, methylprednisolone shock, transfusion of clotting factor with cryoprecipitation, and symptomatic treatment. We also reported the severe adverse effect (SAE) to the ethics committee at our hospital. We exposed the patient to an initial dose of 80 mg/d methylprednisolone on January 4, 2019; gradually increased this dose to a maximum 180 mg/d on January 9, 2019; and then gradually reduced the dose. Finally, we administered a low-dose oral prednisone tapering and then stopped the medication on March 16, 2019. Telephone follow-up was terminated SAE.

Results

Analysis of serum cytokine levels, following occurrence of severe symptoms, showed that the expression of interferon- γ and tumor necrosis factor α (TNF- α) reached a peak at eighth treatment (Fig. 1). The CT images showed that cell therapy had some efficacy in treating the tumor. Specifically, the left lung encapsulated intrathoracic fluid reduced from 46.22 to 33.59 mm (Fig. 2A, C), whereas lymph nodes of the anterior tracheal-posterior vena cava interstitial area decreased from 11.3 to 8.55 mm (Fig. 2B, D). The CT image revealed that the right pulmonary nodule was significantly larger than the baseline one (Fig. 3). Although the patient's condition gradually stabilized, after a series of symptomatic treatments, she opted out of this clinical trial considering the possibility of serious side effects (evaluation reference Recist 1.1).

Ethics and Knowledge

With an institutional review board (IRB) approval from the First Affiliated Hospital of Xinxiang Medical University (Xinxiang, China), a phase II clinical trial (NCT03656705) was initiated to evaluate the safety and effectiveness of CCCR-NK92 cell therapy with NSCLC. The clinical study was performed in accordance with the principles of the Declaration of Helsinki. Each patient had received formal informed consent before entering the study.

Discussion

The CRS, caused by cytokine release from target and immune effector cells, is one of the most common life-threatening

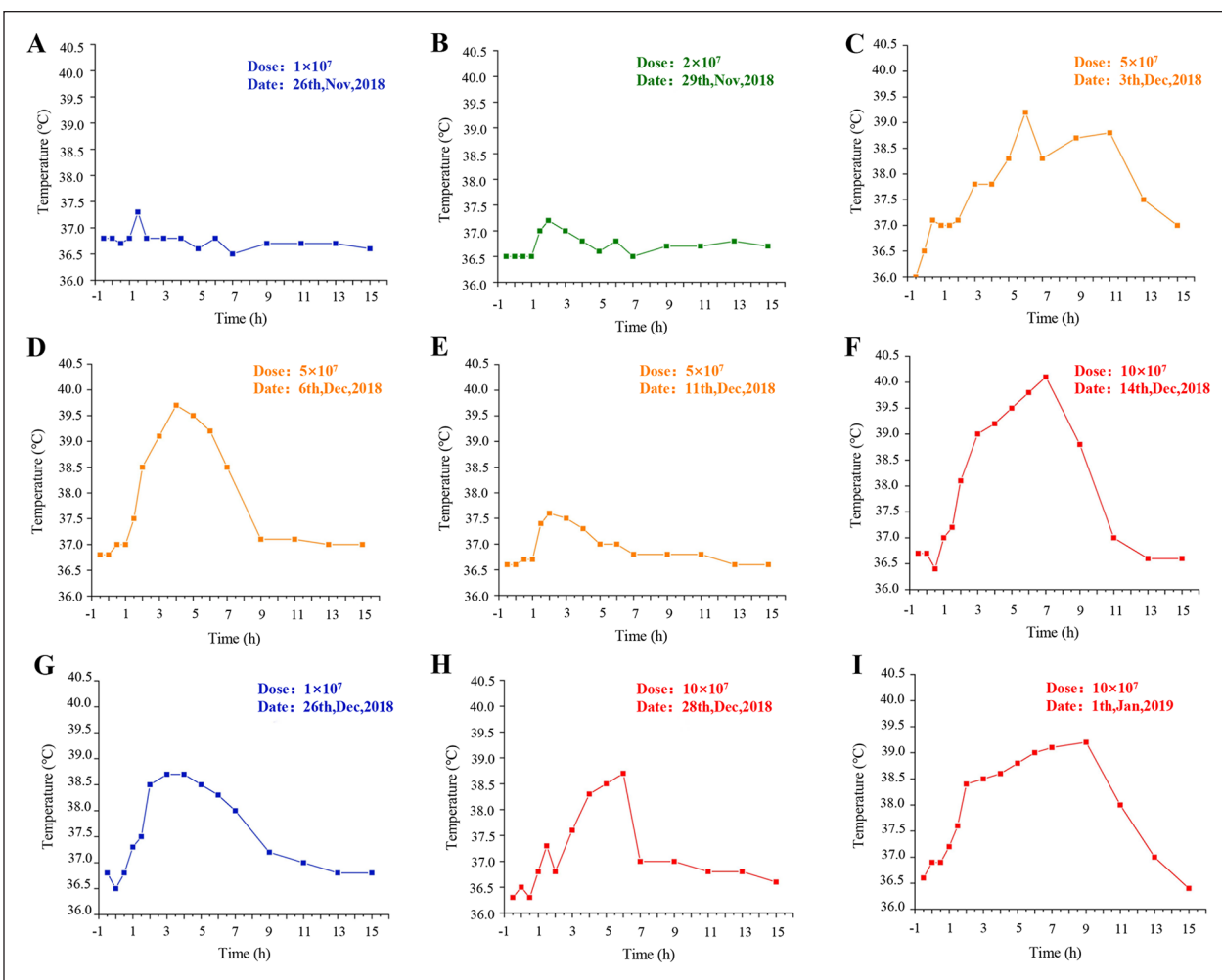


Figure 1. Profiles of the patient's body temperature after chimeric costimulatory converting receptor-NK92 cell injection. (A–C). The body temperature curve at first cycle of cell therapy. (D–F). The body temperature curve at second cycle of cell therapy. (G–I). The body temperature curve at third cycle of cell therapy.

Table 1. Patient Blood Test Results After CCCR-NK92 Cell Therapy.

Date	LDH (U/l) (109–245 U/l)	ALT (U/l) (7–40 U/l)	AST (U/l) (13–35 U/l)	FBG (mg/dl) (200–400 mg/dl)	D-D E-(μ g/ml) (0–1 μ g/ml)	PLT ($10^9/l$) (125–350 $\times 10^9/l$)	WBC ($10^9/l$) (3.5–9.5 $\times 10^9/l$)
2018/12/25	407	8	13	426	11.1	306	6.6
2019/01/03	1155	—	—	308	27	122	3.9
2019/01/05	321	82	95	97.9	43.7	118	6
2019/01/07	287	124	254	53.8	3.3	78	8.2
2019/01/11	241	59	53	122.1	3	314	7.6

ALT: alanine aminotransferase; AST: aspartate aminotransferase; CCCR: chimeric costimulatory converting receptor; D-D: D-Dimer; FBG: fibrinogen; LDH: lactate dehydrogenase; PLT: platelets; WBC: white blood cells.

toxicities associated with cellular immunotherapy¹². To date, the exact mechanism of CRS is not fully understood. The NK cells reportedly exert nonspecific tumor cell killing effects that are not restricted by human leukocyte antigen (HLA). This mainly occurs through the surface cytotoxic receptors that trigger release of perforin and granzyme. To date, a

series of CAR-modified NK and CAR-expressing NK-92 cells that target a wide range of cancer antigens have been used in cancer therapy¹³. Functionally, NK cells produce cytokines, like interferon (IFN)- γ , interleukin (IL)-3, and TNF- α , that differ from the proinflammatory cytokines produced by T cells that initiate CRS¹⁴. Theoretically, CRS

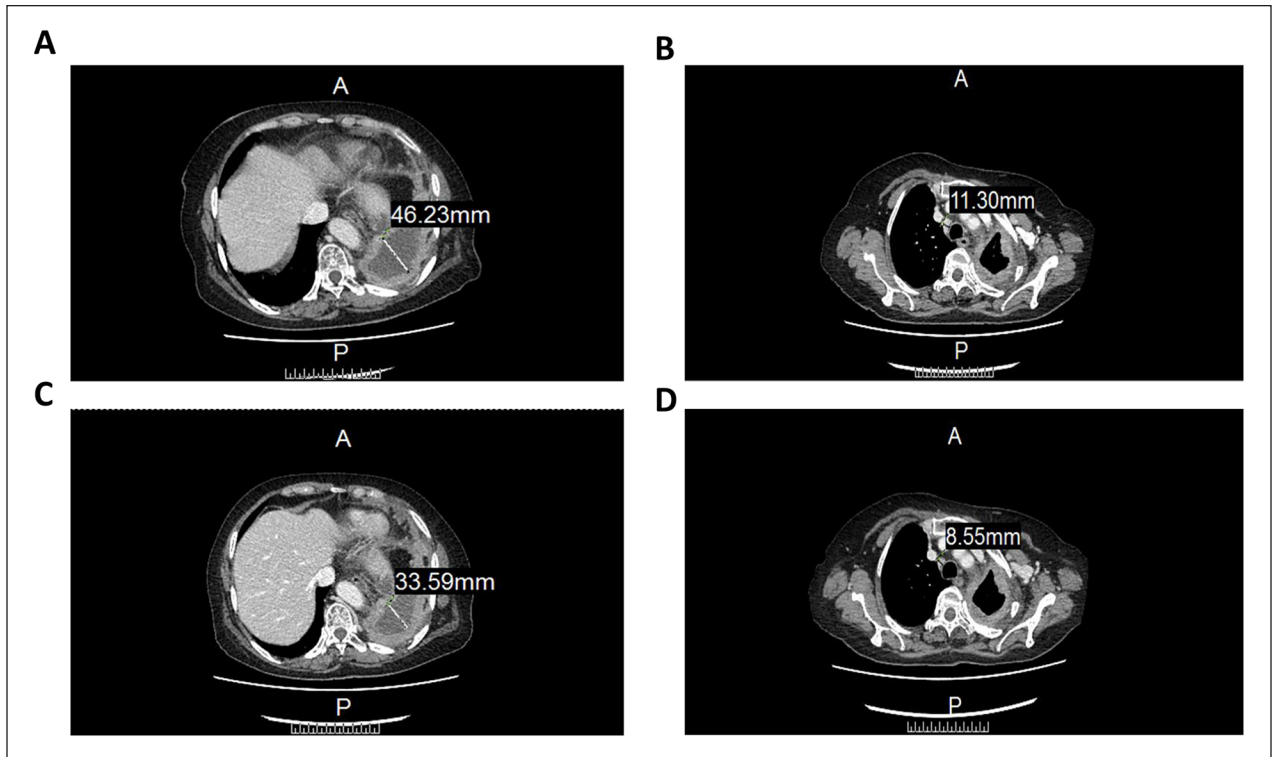


Figure 2. A CT image of mediastinal window before and after CCCR-NK92 cell therapy. (A) CT image before CCCR-NK92 cell therapy. (B) CT images after one cycle of CCCR-NK92 cell treatment. (C) CT image after one cycle of CCCR-NK92 cell treatment. (D) CT images after two cycles of cell treatment. CCCR: chimeric costimulatory converting receptor; CT: computed tomography.

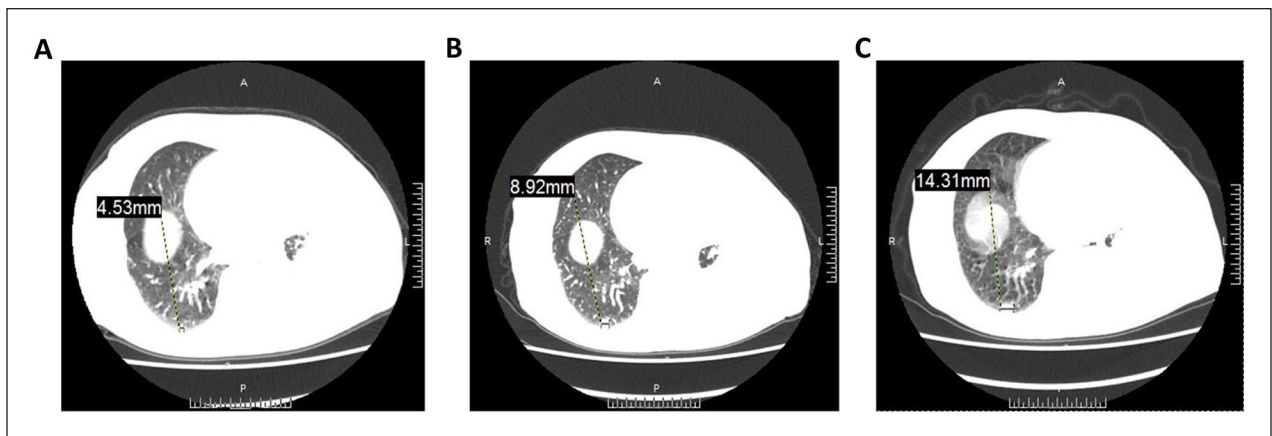


Figure 3. A CT image of lung window before and after CCCR-NK92 cell therapy. (A) CT image before CCCR-NK92 cell therapy. (B) CT images after one cycle of CCCR-NK92 cell treatment. (C) CT images after two cycles of CCCR-NK92 cell treatment. CCCR: chimeric costimulatory converting receptor; CT: computed tomography.

rarely occurs during CAR-NK cell therapy⁴. However, our patient manifested the CRS side effect on the 13th day of treatment. Specifically, she showed a 10-fold upregulation of IFN- γ and TNF- α (Fig. 4) and this was accompanied by a series of side effects such as persistent fever, persistent hypotension, increased inflammatory indexes, electrolyte

disturbance, and abnormal blood coagulation function. Generally, an interaction between immune effector cells with tumor cells causes a release of inflammatory factors that subsequently activate the immune cells and exacerbate the anti-tumor effect. However, the high level of cytokines triggers a simultaneous occurrence of a series of side effects.

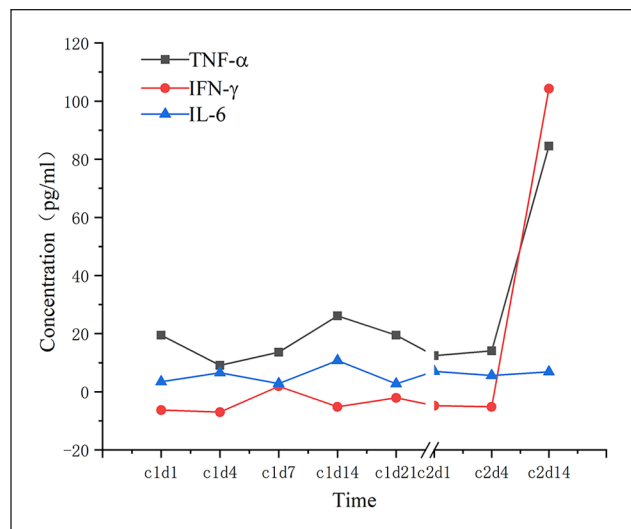


Figure 4. Cytokine levels in the patient's serum after chimeric costimulatory converting receptor-NK92 cell therapy. IL-6, IFN- γ , and TNF- α were tested on 1, 4, 7, 14, and 21 day of each treatment cycle. IFN- γ : interferon gamma; IL-6: interleukin 6; TNF- α : tumor necrosis factor α .

There are two possible causes for the release of large amounts of cytokines. First, NK cells have nonspecific tumor cell killing effects that are not restricted by human leukocyte antigen (HLA). It mainly through the surface cytotoxic receptors to trigger the release of perforin and granzyme, producing cytotoxic factors and tumor necrosis factor and other cytokines, but not IL-2, IL-6 and other cytokines which could cause cytokine storms. However, based on the strategic consideration of constructing CCCR-NK92 cells, the construct of CCCR includes *PD-1-NKG2D-41BB*, which is expected to convert the inhibitory signaling from PD-1 into activating signaling when killing tumor cells; this design may lead to NK cell overactivation, resulting in overproduction of cytokines, such as IFN- γ and TNF- α .

In addition, the immune system in patient may recognize CCCR-NK92 cells as foreign substances and produce an immune response to it, and the release of inflammatory factors is related to the activation of the body's immune system. We will test this hypothesis in future clinical experiments. We can reinfuse CCCR-NK92 cells after cell therapy to observe whether the infused cells are quickly cleared by the body's immune system. If the CCCR-NK92 cells are quickly cleared, it is confirmed that the body has a rejection reaction to foreign cells. The release of cytokines can be considered to be caused by the body's autoimmune reaction. In addition, we can also extract the serum of patients after cell treatment and detect the presence of antibodies to CCCRNK-92 cells in the serum by flow cytometry, which can also prove the hypothesis if there are antibodies in the serum.

Managing CRS-related toxicity is a critical consideration during application of CAR-NK therapy. First, it is important

to monitor biomarkers used for predicting the risk of CRS, such as cytokines, and their changes during the CAR-NK therapy. Early intervention, associated with close monitoring of these biomarkers, can prevent the patient from developing a deadly syndrome. Second, clinicians should strive to reduce the tumor burden prior CAR-NK cell infusion. Previous studies have associated higher levels of tumor burden with elevated side effects in immune cell therapy¹⁵. Therefore, applying chemotherapy prior to immune cell therapy can increase its efficacy and reduce side effects such as CRS¹⁶. Third, it is important to reduce CAR-NK cell dosage in patients, owing to the fact that it plays a key role in determining the intensity and kinetics of CRS^{17,18}. In this case, clinicians should ensure that a patient's condition is stable before increasing cell dose. This will minimize the chances of CRS.

Overall, it is important to consider potential occurrence of toxicity during CAR-NK treatment. Consequently, clinicians should pay more attention to CRS in CAR-NK therapy and other immune therapies.

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Ethical Approval

Ethical approval to report this case was obtained from the Ethics Committee of the First Affiliated Hospital of Xinxiang Medical University.

Statement of Human and Animal Rights

All procedures in this study were conducted in accordance with the protocol approved by the Ethics Committee of the First Affiliated Hospital of Xinxiang Medical University. This article does not contain any studies with animal subjects.

Statement of Informed Consent

Written informed consent was obtained from the patient(s) for their anonymized information to be published in this article.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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