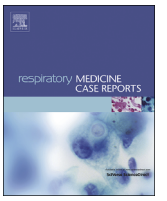
Respiratory Medicine Case Reports 42 (2023) 101804

Contents lists available at ScienceDirect

Respiratory Medicine Case Reports

journal homepage: www.elsevier.com/locate/rmcr



Case Report

A case of response to combination treatment with autologous immunotherapy and bevacizumab in advanced non-small cell lung cancer

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ARTICLE INFO

Keywords:
Non-small cell lung cancer (NSCLC)
Programmed cell death ligand - 1 (PD-L1) expression
Autologous natural killer cells
Bevacizumab

ABSTRACT

Natural killer (NK) cells have developed as a potent tool in cancer immunotherapy. Especially, patients who have failed in the first-line or maintenance treatment received a good response with immunotherapy in association with other approaches. We report the case of a 61-year-old male patient with programmed cell death ligand - 1(PD-L1) expression in advanced non-small cell lung cancer (NSCLC) (stage IV). Even though the patient was treated with standard therapy using keytruda, he still appeared with new lesions. Therefore, the patient was treated in combination with autologous NK cells therapy, gemcitabine, bevacizumab. NK cells were expanded from peripheral blood mononuclear cells (PBMCs) of the patient, and after that, they were transferred back to the patient. After 6 infusions of autologous NK cells in combination with gemcitabine, bevacizumab, the patient decreased significantly the size of primary, metastatic lesions and had a marked improvement in the quality of life. Besides, during combination therapy, no side effects have been reported and there was no toxicity observed in the hematopoietic system, liver as well as kidneys. Our case suggests that this treatment regimen is a potential treatment approach for advanced NSCLC with PD-L1 expression.

1. Introduction

NSCLC is supposed to be the most popular form of lung cancer, which is one of the leading causes of death worldwide and is partly responsible for high health care expenses [1,2]. Avastin®, the reference bevacizumab, is a recombinant humanized immunoglobulin G1 monoclonal antibody that precisely sticks to human vascular endothelial growth factor (VEGF). This alignment prohibits its interaction with VEGF receptors (VEGFRs) on the surface of endothelial cells and leads to limit angiogenesis. As a result, bevacizumab can diminish existing tumor size and prevent tumor growth by stimulating regression of abnormal tumor vasculature along with averting the formation of new tumor blood vessels [3]. Bevacizumab is approved by the US Food and Drug Administration (FDA) for the treatment of several cancers, including metastatic non-squamous NSCLC [4]. According to various international guidelines, the combination of bevacizumab with chemotherapy is used as a first-line and maintenance treatment in advanced NSCLC [5]. Moreover, recent

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https://doi.org/10.1016/j.rmcr.2022.101804
Received 19 June 2022; Received in revised form 26 December 2022; Accepted 28 December 2022
Available online 29 December 2022

studies have shown that maintenance treatment or disease progression is based on bevacizumab in association with new molecular therapies or immunotherapy [6,7].

Immune cell therapy has been attractive for a long time, and this remedy used in some developed countries has given extremely positive initial results. In this therapy, immune cells are isolated from the patient's peripheral blood, cultured in a specific medium to expand the number of one or more kinds of cells, and the whole of these expanded cells are transfused back into patient's body in the final step. Many different types of immune cells can be targeted by this therapy, including NK cells [8,9]. Recently, NK cells have admitted to playing a crucial role in tumor immunosurveillance by destroying infected or transformed cells without the need for prior sensitization. NK cells can distinguish between normal cells and abnormal cells in the body based on an abnormal expression level of the major histocompatibility complex (MHC) class I on the surface cells. Cancer cells are killed by NK cells through several mechanisms, for instance, the release of perforin and granzymes causing cell apoptosis, the alignment to death receptors, and the secretion of different effectors [10]. With the current knowledge of NK cells biology and functions, NK cells have developed as a potent tool in cancer immunotherapy. Especially, patients who have failed in the first-line or maintenance treatment received a good response with immunotherapy in association with other approaches [11].

Herein, we report the case of a patient with PD-L1 (+25%), with a significant response to a combinational regimen of bevacizumab, gemcitabine, autologous NK cell immunotherapy, following disease progression on the standard treatment of PD-L1-positive.

2. Case report

In May 2019, a 60-year-old male patient with no smoking history and symptoms of cough, backache and fatigue was diagnosed as NSCLC. A computed tomography (CT) showed a primary tumor, measuring 25 × 31 mm in upper lobe of left lung, invading mediastinal tissue, surrounding the aorta (Fig. 1A). The mediastinum appeared several lymph nodes with the largest size being 5 × 15 mm while the upper lobe of right lung indicated two nodes with the size of 5 mm and 7 mm respectively. CT-guided percutaneous biopsy of the nodule and adenocarcinoma was confirmed by histopathological examination (PHE). The pathological stage was T4N2M1a (stage IV). Tumor markers presented a slight increase for CA125 (55U/ml) and normal limits for CA199 and CEA. Molecular analysis revealed no mutation with EGFR. Because the lesions were large and invaded mediastinum, surgery could not be performed.

From June 2019 to December 2019, the patient received the first-line treatment which was 3 cycles of carboplatin 650mg (AUC5) plus paclitaxel 300 mg (175mg/m2) in combination with radiotherapy at the third cycle. He achieved a partial response in which there were an improvement in both clinical and paraclinical symptoms. The patient's Positron Emission Tomography (PET)/CT scan showed a reduction in the size of the primary tumor (12 × 7 mm) (Fig. 1B) and the tumor marker values returned to normal in December 2019.

Nevertheless, the disease progressed locally in May 2020. PET/CT scan showed that the primary tumor had a size of 28 × 29 mm (Fig. 2A), maximum Standardized uptake value (SUV_{max}) 11.2, adhesion to mediastinal pleura and aorta. Besides, there was an appearance of the left supraclavicular lymph node having a size of 9 × 6 mm (Fig. 2B) and a few small nodes in the left hilum having a size under 10 mm.

He showed positive PD-L1 expression (25%) and was indicated to receive second-line treatment with 4 cycles of alimta (500 mgx2), carboplatin (150 mgx2, 450 mgx1), keytruda (4ml x2). However, his condition was not stably performed on the PET/CT scan in September 2020. Primary tumor witnessed an increase to 26 × 35 mm (SUV_{max}: 22.6) (Fig. 3A). Moreover, there were new appearances such as right supraclavicular lymph nodes, lymph nodes in the anterior mediastinum and side of the tracheal, and a few lymph nodes in the hilum on both sides. The patient's treatment regimen was changed due to the progressive disease. In October 2020, the patient was hospitalized to transmit autologous NK cells with 6 infusions in treatment combination with gemcitabine/avastin. On admission, the patient was afebrile with a pulse rate of 79 beats per minute, and blood pressure of 110/70 mmHg. He experienced a dry cough as well as dull left chest pain. Paraclinical results including hematology and blood biochemistry were normal, except for white blood cells (2.81 G/L, below normal).

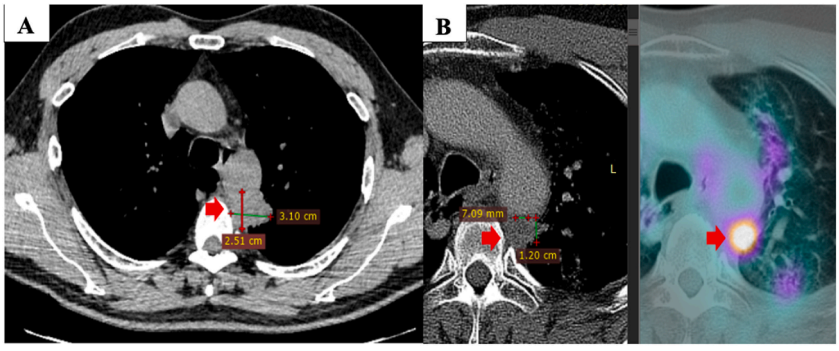

Fig. 1. CT scan examination of the primary tumor at the first time of diagnosis (A). PET/CT scan examination of the primary tumor after the first-line treatment (3 cycles of carboplatin 650mg (AUC5) plus paclitaxel 300 mg (175mg/m^2) in combination with radiotherapy at the third cycle) (B).

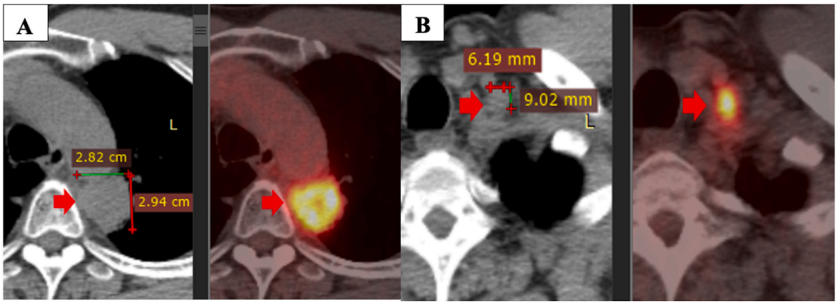

Fig. 2. Disease progressed after one year of the first-line treatment: primary tumor (A), left supraclavicular lymph node (B).

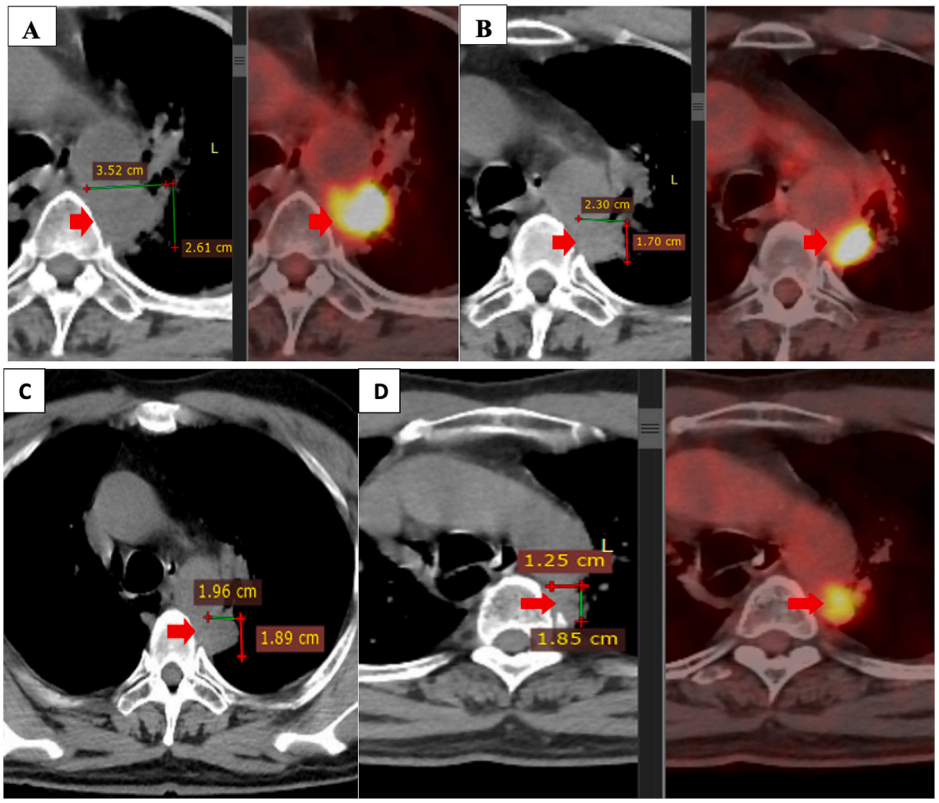

Fig. 3. Primary tumor before and after combination treatment. The primary tumor progressed after the second-line treatment (4 cycles of alimta, carboplatin, keytruda) (A). The primary tumor decreased at the end of treatment in combination with autologous NK cell therapy, gemcitabine, bevacizumab (B), after 3 months (C), after 6 months (D).

Autologous NK cell immunotherapy: The patient was conducted to take 20 ml of peripheral venous blood and extracted immune cells by a density-changing centrifugation method using Ficoll. Immune cells were then cultured, activated, and proliferated in AIM-V (Therapeutic Grade – Thermo Fisher) supplemented with cytokines IL-2, IL-12, and IL-18 with appropriate concentration. After 21 days of culture, the cells were harvested, counted, and analyzed the phenotype of the obtained cell mass by flow cytometry method on Novocyte Flow Cytometer (ACEA Biosciences, Inc). Depending on surface markers can determine proportion of each type of immune cells, which helps to evaluate cells after culture: lymphocytes ($CD45^+$): B lymphocytes ($CD45^+CD19^+$), T lymphocytes ($CD45^+CD3^+CD16^+CD56^-$), NK cells ($CD45^+CD3^-$ $CD16^+CD56^+$), NK-T cells ($CD45^+$ $CD3^+$ $CD16^+CD56^+$). Safety assessment of the obtained cell mass was performed through bacteriological examination of the culture medium, mycoplasma detection, endotoxin detection. The number of obtained cells after 21 days of active and proliferating culture reached over 5×10^8 living cells, in which the percentage of NK cells accounted for more than 60%, all cell samples were safe because of having no bacteria, mycoplasma, and endotoxin in the cell mass after culture. After the NK cell mass was collected, it would be transferred back to the patient's body by intravenous route.

The patient received 6 infusion courses of autologous NK cell mass within 4.5 months, once every 3 weeks (Fig. 4). During that time, he was indicated in combination treatment with gemcitabine (1800mg) on day 1, day 8 (2 cycles for every 3 weeks) plus avastin

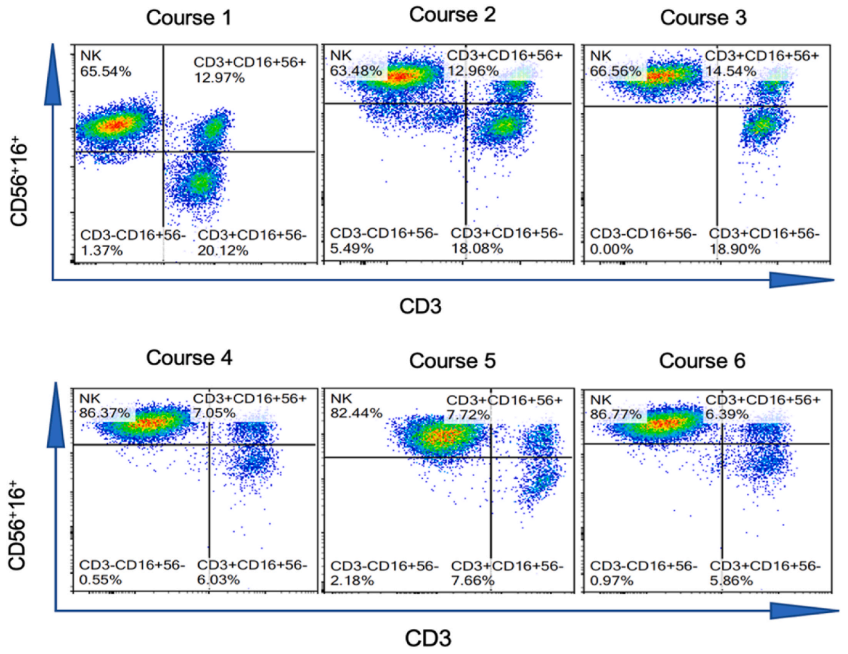

Fig. 4. Immunophenotypes monitoring for NK cell treatments. The patient received cell treatments starting from October 2020. The patient received 6 treatment courses (1 infusion per treatment course) of NK cell treatments for 4.5 months. An infusion was performed every 3 weeks for 6 infusions.

(400 mg, 2 cycles every 3 weeks). Outcomes after combination treatment for 4.5 months: The symptoms of dry cough and chest pain were gone while the patient was eating better, and the patient's quality of life was significantly improved (Fig. 5). No significant side effects were observed during the use of combination therapy, and liver and kidney function assessments were within normal limits (Fig. 6). The PET/CT scan performed in March 2021 and September 2021 showed that the size of the primary tumor (Fig. 3B, 3D) decreased significantly compared to pre-treatment with combination therapy. Besides, mediastinal lymph nodes and supraclavicular lymph nodes on both sides were not found on this film.

3. Discussion

We describe a patient with NSCLC (stage IV), PD-1 (+) 25% whose disease progressed on 4 combinational cycles of alimta, carboplatin, and keytruda. Then, he changed his regimen with autologous immunotherapy and bevacizumab, gemcitabine, which brought a good response to paraclinical and clinical symptoms as well as the quality of life.

Bevacizumab is a humanized monoclonal antibody that acts on VEGF. They inhibit the growth of preexisting blood vessels and prevent the formation of new ones. Sandler's study showed that the group of NSCLC patients treated with the combination of bevacizumab and chemotherapy increased overall survival (OS) and progression-free survival (PFS) more than the group receiving chemotherapy alone [12]. Moreover, a combination of bevacizumab and atezolizumab was indicated to have a promising antitumor activity with good tolerability for patients with metastatic NSCLC whose disease had progressed after atezolizumab monotherapy [13]. In NSCLC patients with EGFR (Epidermal Growth Factor Receptor) T790M mutation, concomitant use of bevacizumab and osimertinib may have more effective therapy than osimertinib alone. The overall response rate was higher in patients who underwent

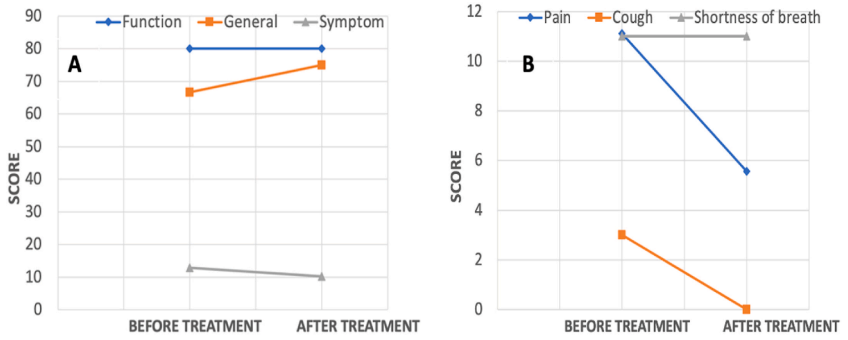

Fig. 5. Evaluation of the patient's quality of life before and after treatment. A. The quality of life: The patient's general was better and symptoms decreased dramatically. B. Functional symptoms: pain and cough of the patient went down significantly after 6 infusions.

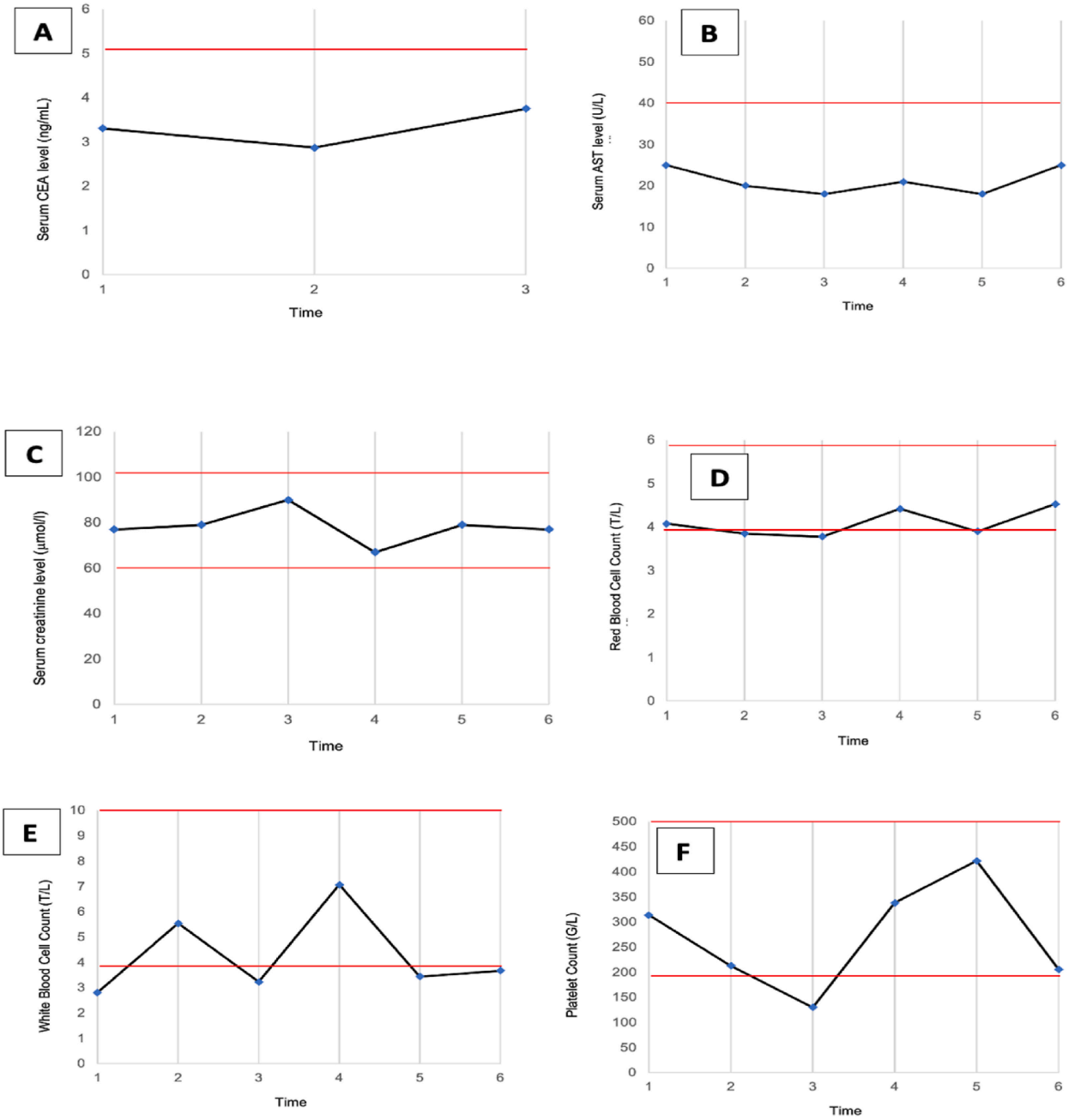

Fig. 6. Blood biochemical examination. Most of biochemical markers: CEA, AST, Creatinin maintained at normal lever before and after NK cell treatments. Read blood cells, white blood cells, and platelet were a bit lower than normal at some times during treatment. A. CEA, B. AST, C. Creatinine, D. Red blood cells, E. White blood cells, F. Platelet.

osimertinib plus bevacizumab than in the osimertinib-alone group, and the OS and PFS were also dramatically improved in the combination group [14].

In recent decades, there have been significant advances in the field of immunology as well as the application of immunotherapies in cancer treatment. Among the emerging new treatments, autologous NK cell therapy is seen as a promising candidate for the next important steps for cancer treatment in general and lung cancer in particular. Many studies have elucidated the important role of NK cells in supporting the entire immune system and the relationship of NK cells to several diseases in the human body. Most NK cells express the CD16 (FcγRIII) marker on the surface, which is capable of binding to the Fc region of IgG1, IgG3 and causing ADCC (antibody-dependent cellular cytotoxicity). Therefore, NK cells are transfused into the patient's body, they play an extremely important role, not only causing target cell toxicity through antibodies, but they are also capable of mobilizing other immune cells together to participate in the response to cancer treatment [15]. Most notably, recent studies have demonstrated that NK cells can identify and

destroy cancer stem cells. This is especially important because therapy using autologous NK cells can suppress the proliferation of cancer cells and thus reduce the risk of recurrence and metastasis [16]. According to Mao'study, advanced NSCLC patients with PD-L1 (+) given pembrolizumab plus NK cells had longer survival than did patients given pembrolizumab alone. Moreover, the patients in the group treated with multiple courses of NK cell infusion had better OS than those who received a single course of NK cell infusion [17]. Besides, for advanced lung adenocarcinoma with EGFR mutations, the level of serum CEA and CA125 values were lower in the NK cell therapy group than that of the non-NK treatment group while the response rate in the group that received autologous NK cells was significantly higher than that in the control one [18].

Our patient has undergone traditional treatment methods such as chemoradiotherapy at the right time of diagnosis. After that time, the disease progressed and he was found to be PDL-1 positive. Even though the patient was treated with standard therapy using keytruda, he still appeared with new lesions. Therefore, the patient was treated in combination with autologous NK cell therapy, gemcitabine, bevacizumab. After going through combination therapy, the patient gave extremely positive initial results. Clinical and paraclinical results have shown that combination treatment in this patient was safe and effective. The patient was monitored during and after the infusion in all infusions, so far, no side effects have been reported such as fever, chills, rash, high/low blood pressure, vomiting/soreness, diarrhea, joint pain, or muscle pain. In addition, the paraclinical indicators assessed after each infusion were still within normal limits, there was no toxicity observed in the hematopoietic system, liver as well as kidneys. Furthermore, before combination therapy, the patient presented with dull chest pain, dry cough, and poor appetite. However, after treatment, the above functional symptoms disappeared or significantly decreased and the assessment score of quality of life performed after the end of the treatment course also increased dramatically. Specifically, the patient no longer had chest pain, cough symptoms decreased, appetite increased, and the overall health status assessment score increased. The difference in PET/CT scan results before and after combination treatment also explained the improvement in our patient. The primary tumor was reduced by almost half compared to the original size, mediastinal was disappeared and supraclavicular lymph nodes had a significant reduction in SUV_{max}. Therefore, with the combination of treatment among autologous NK cell immunotherapy, gemcitabine, and bevacizumab on this patient, the patient gave unexpected results. This is one of the first clinical cases included in our phase I clinical trial. Hopefully, this trial will contribute to providing important evidence for researchers as well as clinicians about new cancer treatments in the future.

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Funding

This research received grant from National Project “Application of autologous gamma delta (γδ) T and natural killer (NK) cells for treatment of lung cancer” [number grant KC.10.26/16-20] in the preparation of data.

Declaration of competing interest

We have no conflicts to declare.

Acknowledgements

Thuy Mau Thi Nguyen was funded by Vingroup JSC and supported by the Master, PhD Scholarship Programme of Vingroup Innovation Foundation (VINIF), Institute of Big Data, code: VINIF.2021.TS.070.

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