Molecular Cancer

CORRESPONDENCE

Open Access

A case of response to combination treatment with TSA-DC-CTL immunotherapy and osimertinib in EGFR mutated advanced lung adenocarcinoma

Zhiyi Han[1†], Tao Li[2†], Heng Zhang[3], Kai Liang[3], Mingcong You[3], Mengdi Xu[3], Fan Bai[3] and Tongmei Zhang[4*]

Abstract

Background This study details a case of a patient with advanced lung adenocarcinoma harboring an exon 19 deletion in the EGFR gene.

Method A 46-year-old female patient was diagnosed with stage IVb left lung adenocarcinoma, with multiple bone and lymph node metastases. Following the identification of tumor-specific antigen peptides, the patient received a combination treatment of immunotherapy (TSA-DC-CTL) and oral osimertinib. Peripheral blood circulating immune cells and circulating tumor cells (CTCs) were monitored before and after treatment. PET-CT and CT scans were used to assess the tumor response to treatment.

Results A significant increase in total lymphocyte percentage and decrease in the number of CTCs in the patient was observed. Imaging studies showed a notable reduction in tumor metastases.

Conclusion This report demonstrates the safety and efficacy of TSA-DC-CTL cell immunotherapy combined with osimertinib in the treatment of a patient with advanced lung adenocarcinoma with an EGFR exon 19 deletions. This study describes a promising new treatment option for patients with advanced lung cancer with EGFR mutations.

Keywords Cellular immunotherapy, TSA-DC-CTL, EGFR gene mutation, Advanced lung cancer

Introduction

Primary lung cancer is the most prevalent malignant tumor in China. Based on the GLOBOCAN 2020 data, China contributes to 37.0% of the global lung cancer incidence and 39.8% of related deaths [1]. From pathological and therapeutic perspectives, lung cancer is categorized into non-small cell lung cancer (NSCLC) and small cell lung cancer, with NSCLC accounting for approximately 80–85% of cases, including histological subtypes such as adenocarcinoma and squamous cell carcinoma. The standard treatment options for patients with NSCLC include radiotherapy and chemotherapy. These approaches typically suppress tumor cell proliferation or induce cell

†Zhiyi Han and Tao Li contributed equally to this work.

*Correspondence:
Tongmei Zhang
tongmeibj@163.com
[1]Department of Surgery, Guang'anmen Hospital, China Academy of Chinese Medical Sciences, Beijing 100053, China
[2]Department of Medical Oncology, Affiliated Tumor Hospital of Nantong University & Nantong Tumor Hospital, Nantong 226361, China
[3]Healthy Cell Biotechnology LLC, Beijing 100036, China
[4]Department of Medical Oncology, Beijing Chest Hospital, Beijing Tuberculosis and Thoracic & Laboratory for Clinical Medicine, Capital Medical University, Beijing 101149, China

death in a non-specific manner, often leading to unsatisfactory outcomes. The five-year survival rate of patients remains low at only 15-20% [2, 3]. Targeted therapy and active immunotherapy offer personalized approaches to treatment. However, both treatments face challenges like drug resistance, which hinders sustained treatment response and long-term survival [4–6]. These issues underscore the necessity for better treatment strategies to bypass the limitations of existing treatments.

Cellular immunotherapy is a highly effective cancer treatment modality, representing the fourth major oncological intervention following surgery, radiotherapy, and chemotherapy [7]. The primary function of dendritic cells (DCs) is to act as antigen-presenting cells. DCs captures and processes antigens, presenting them on their surface via major histocompatibility complex (MHC) molecules to CD8 T cells, thereby activating T-cell immune responses [8]. The incomplete elimination of tumor cells by the immune system can lead to the persistence of "immune-edited" tumors. "Immune editing" is a crucial concept describing the interaction between tumors and the host immune system. This concept explains why the immune system, despite being able to recognize and attack tumor cells, sometimes fails to completely eradicate them. Immune editing consists of three main stages: elimination (early cancerous cells are identified and cleared by the immune system), equilibrium (the immune system limits further tumor expansion, but tumor cells still survive), and escape (the tumor gradually grows, exhibits clear clinical features, and establishes an immunosuppressive tumor microenvironment to evade immune destruction). During the immune editing process, tumor cells continuously evolve to escape immune surveillance [9]. Meanwhile, DCs form a rare immune cell population in tumors and lymphoid tissues, serving as central players in initiating antigen-specific immunity and tolerance. In tumor cell immunotherapy, DCs act as a bridge by presenting tumor-specific antigens (TSAs) to T cells and providing immunoregulatory signals through cell contact and cytokines, thus promoting either immunity or tolerance [10–12]. However, in many tumors, the function of DCs may be impaired, leading to reduced antigen-presenting capacity and immune evasion. Therefore, restoring or enhancing the function of DCs is a crucial strategy to improve the effectiveness of cancer immunotherapy [13]. The application of TSA-loaded DC vaccines in cancer treatment has been extensively studied in clinical trials [14]. Optimized DCs are used to activate specific cytotoxic T lymphocytes (CTLs) to effectively target and attack tumor cells, demonstrating significant potential in the process of immune editing [15]. This approach has shown promising clinical outcomes in patients with advanced solid tumors. Therefore, it is essential to further explore the combination of oncogene-targeted therapy with TSA-DC-CTL immunotherapy targeting tumor neoantigens as an effective treatment strategy for unresectable stage III NSCLC.

In this study, we report a case of advanced lung adenocarcinoma with EGFR exon 19 deletion who received TSA-induced in vitro expanded DC and CTL adoptive cellular immunotherapy combined with third-generation EGFR-TKI osimertinib in February 2022.

Method

The patient and the treatment plan

The patient, a 46-year-old female, began experiencing bone pain in March 2021. In October 2021, symptoms of hemoptysis and persistent bone pain emerged, and she showed no improvement until December of the same year. She sought medical attention on January 10, 2022, at an external hospital where a CT scan indicated a left lung mass. Subsequent PET-CT examination and biopsy for rapid pathology confirmed lung adenocarcinoma on February 7, 2022. PET-CT results revealed multiple bone and lymph node metastases, leading to a clinical diagnosis of stage IVb cancer. On February 11, 2022, the patient underwent NGS genetic testing, which detected an exon 19 deletion in the EGFR gene (EGFR E746_A750del mutation). Physical examination showed a Karnofsky Performance Status (KPS) score of 60, marked lethargy, significant chest tightness, and shortness of breath. The patient declined intravenous chemotherapy. After thorough communication with the patient and her family, a comprehensive treatment plan combining cellular immunotherapy (TSA-DC-CTL) with the targeted drug osimertinib was adopted. This research protocol was approved by the Ethics Committee of Jiangsu Cancer Hospital, and the patient signed a written informed consent form.

Tumor-specific antigen peptide detection

A 5 mL blood sample was collected from the patient, and blood cells were separated from plasma through centrifugation. The serum sample was treated with a protein precipitant (acetic anhydride) to enrich low-abundance proteins. The extracted proteins were digested with trypsin and a solid-phase extraction was used to purify and concentrate the peptides which were separated by high-performance liquid chromatography. The separated peptides were then subjected to mass spectrometry (MS) analysis.

Tumor marker detection was carried out in the UPLC/MSE mode, collecting sample spectra under the condition of a detection limit > 10 ppb to obtain primary spectra. The primary MS was set with a Match value ≥ 500, and the analysis filtered for target ions. The candidate target ions were further fragmented in secondary MS (MS/MS), generating a series of fragment ions. MS/MS

data were collected and analyzed using a software to identify the amino acid sequence of the peptides. These sequences were compared with a known database of tumor-specific antigen targets to identify and confirm the target peptide fragments.

Preparation of DC and TSA-DC-CTL vaccine

Preparation of DC vaccine

(1) Peripheral blood was drawn from the patient to obtain blood-free specific protein peptides, which served as TSAs for loading onto DCs. (2) Separation of peripheral blood mononuclear cells (PBMCs): About 45 mL of blood was drawn and layered over a lymphocyte separation medium, followed by centrifugation to separate PBMCs. The PBMCs were then resuspended in a serum-free DC culture medium. (3) Isolation, directed induction, and expansion of DCs: PBMCs were cultured for adhesion for 2–6 h to obtain DC precursors. Recombinant human IL-4 (rh-IL-4) and GM-CSF were added to induce and expand DC precursors for 7–9 days. Mixed tumor antigens were added to sensitize and activate DCs. PD-L1 molecules on the surface of DCs were blocked, and the DC vaccine was harvested. The patient received the first infusion of DCs loaded with TSAs (before the infusion, peripheral blood was drawn a second time to prepare cells for the second sequential treatment; similarly, blood was drawn before the second infusion to prepare cells for the third sequential treatment).

Preparation of TSA-DC-CTL vaccine

The TSA-loaded DC vaccine was used to induce and expand tumor antigen-specific CTLs in vitro. On day 8 of DC culture, a portion of the DCs was separated. These TSA-loaded DCs were co-cultured with T cells at a DC/T cell ratio of 1:20. Co-culture was stimulated with CD3 monoclonal antibodies pre-coated on plastic plates and expanded using IL-2. On days 14–16, the cells were infused into the patient via intravenous injection over three consecutive sequential sessions, with approximately $(0.5–1) \times 10^{10}$ CTL cells. The viability of CTL cells must exceed 90% to meet the requirements for therapeutic applications.

Total lymphocyte detection

Flow cytometry was used to detect the proportion of total lymphocytes. Whole blood samples from the patient were collected, and red blood cells were removed using hemolysin. Cells were stained using fluorescently labeled antibodies targeting lymphocyte surface markers. The stained cells were then analyzed using a flow cytometer. Data were collected and analyzed using a specialized software to determine the number and proportion of total lymphocytes among white blood cells.

CTC cell detection

Advanced immunofluorescence in situ hybridization technology was employed. Whole blood samples from the patient were collected by using a high-sensitivity, non-destructive negative CTC pre-treatment method (density gradient centrifugation) to maximize the retrieval of tumor cells from the blood. Tumor cells were enriched through an immunocapture method based on surface proteins. The enriched cells were then subjected to immunofluorescent staining to mark specific cell surface proteins, differentiating CTCs from other blood cells. Fluorescence in situ hybridization was performed on the stained cells by using fluorescent probes targeting specific nucleic acid sequences to identify the genetic characteristics of the tumor cells. A fluorescence microscope was used to observe and analyze the fluorescent signals of the cells, to comprehensively assess the status of the CTCs, including their nucleic acid and protein expression and cellular morphology. Images under the microscope were collected and quantitatively analyzed using specific software to determine the number of CTCs.

Results

Tumor-specific antigen peptide detection

Using peptide enrichment and purification combined with liquid chromatography-MS (LC-MS) analysis, four lung-derived tumor-specific antigen peptides were identified (designated as NS-12AP10, NS-17AP10, NS-19AP10, and NS-30AP10; Fig. 1A), revealing the presence of free tumor-specific antigens in the patient's peripheral blood serum. The monitoring data can guide the synthesis of specific antigen peptides to be used for DCs, thereby enabling the ex vivo induction and expansion of personalized DCs and CTLs.

Treatment process

The patient began oral administration of the third-generation targeted drug osimertinib (trade name: Tagrisso) at a dosage of one pill (80 mg) daily starting on February 22, 2022, which was marked as Day 0. She received injections of the DC vaccine on the 3rd day, 14–15th day, 23rd–24th day, 100th–101st day, 117th day, and 137th–138th day. Additionally, she was injected with TSA-DC-CTL on the 8th–9th day, 22nd–23rd day, 29–30th day, 108–109th day, 135–136th day, and 146–147th day (Fig. 1B-a). The patient completed two treatment cycles of TSA-DC-CTL therapy and continuously took osimertinib during this period of time.

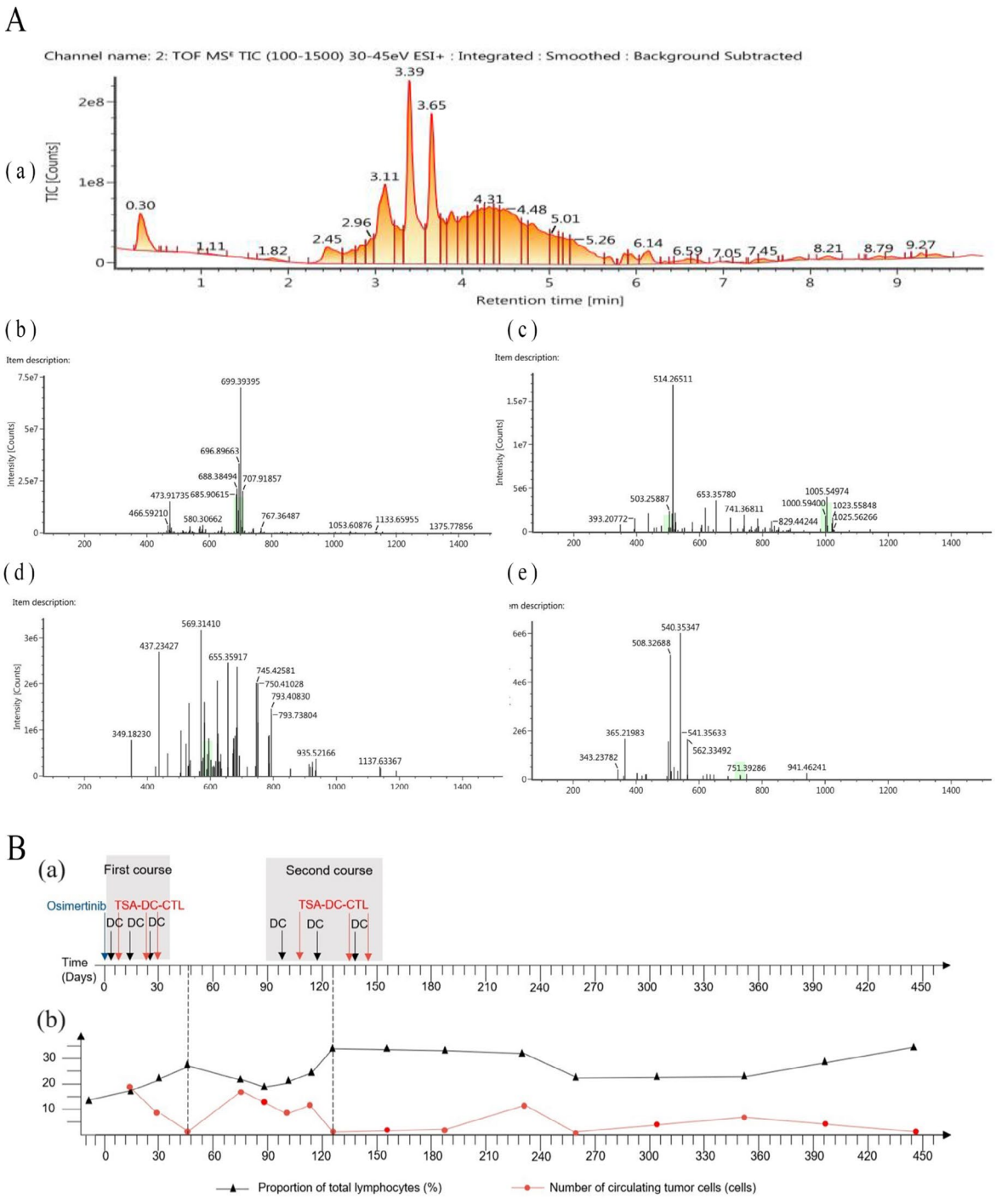

Fig. 1 Tumor-specific antigen peptide detection and TSA-DC-CTL vaccine combined with osimertinib treatment process. **A:** Mass spectrometry of tumor-specific antigen peptides. (**a**) Primary mass spectrum. The primary mass spectrum was set with a Match value ≥ 500. The analysis screened target parent ions, and four matching candidate parent ions were compared with the target mass spectrum library. (**b-e**) Secondary mass spectrum. The candidate target ions were individually subjected to secondary mass spectrometry fragmentation analysis to obtain secondary spectra. By comparing with a known database, the spectra were identified as tumor-specific antigen peptides NS-12AP10 (**b**), NS-17AP10 (**c**), NS-19AP10 (**d**), and NS-30AP10 (**e**), all of which are specific targets for NSCLC. **B:** (**a**) TSA-DC-CTL combined with osimertinib treatment. The TSA-DC-CTL vaccine administration began 3 days after the first dose of osimertinib. (**b**) Changes in peripheral blood circulating immune cells and circulating tumor cells (CTCs) during treatment (the reference range for the total lymphocyte percentage is 23.7–37.1%; the reference range for CTCs is 0–2 cells). The dashed lines represent the two peak values reached by the total lymphocyte percentage on Day 45 and Day 126, as well as the reduction of CTCs to 1 cell. TSA, tumor-specific antigen; DC, dendritic cell; CTL, cytotoxic T lymphocyte

Changes in observation indicators before and after TSA-DC-CTL combined with osimertinib treatment

Improvement in KPS score

After two treatment cycles of TSA-DC-CTL combined with osimertinib, the patient showed improved well-being, with significant alleviation of symptoms such as chest tightness, shortness of breath, cough, and sputum production. The patient also reported a slight relief in back pain. The KPS score improved from 60 before treatment to 80 post-treatment.

Peripheral blood circulating immune cells and CTC test results

The patient's total lymphocyte percentage showed a marked upward trend as the treatment progressed. Before the treatment, the total lymphocyte percentage was 13.3%, which was below the normal reference range (23.7–37.1%). After completing the first treatment cycle, the total lymphocyte percentage reached its first peak of 28.9% (Day 45); during the second treatment cycle, it reached a second peak of 33.0% (Day 126). The results are shown in Fig. 1B-b.

In terms of CTC test, 18 CTCs were detected in the patient's peripheral blood during the first treatment cycle. After completing the first treatment cycle, the number of peripheral blood CTCs decreased to 1 (Day 45). After completing the second treatment cycle, the number of peripheral blood CTCs was 2 (Day 188). The results are shown in Fig. 1B-b.

Preparation of TSA-DC-CTL

We performed quality control on the cultured CTL cells using flow cytometry. Total T cells were labeled with CD3 antibodies, and CTL cells were labeled with CD3 and CD8 antibodies. Quantitative results showed that CD3+T cells accounted for 98.62% of the total lymphocytes in the cultured samples, with CTL cells (CD3+CD8+T cells) accounting for up 90.45% (Fig. 2). The viability of CTL cells was greater than 90%, meeting the requirements for therapeutic applications.

DC and T cell morphology

Before receiving TSA-DC-CTL combined with osimertinib treatment (Day 0), under a 10× microscope, the patient's DCs appeared round or slightly irregular, indicating that they were resting or in the early stages of induction and differentiation. There were few T cell differentiation colonies, and no apparent signs of an immune response were observed. After TSA-DC-CTL combined with osimertinib treatment (Day 45 and Day 453), a significant increase in the number of DCs was noted. They exhibited enhanced elongation and mature morphology, with long protrusions crucial for interactions between DCs and T cells. T cell production was normal and the differentiation colonies increased. Observable dead lymphocytes and cell fragments can be seen, resulting from intense immune reactions leading to cell death. Active T cell responses are evident, characterized by cell clustering, indicating the activation of T cells and their engagement in antigen-specific attacks or interactions (Fig. 3).

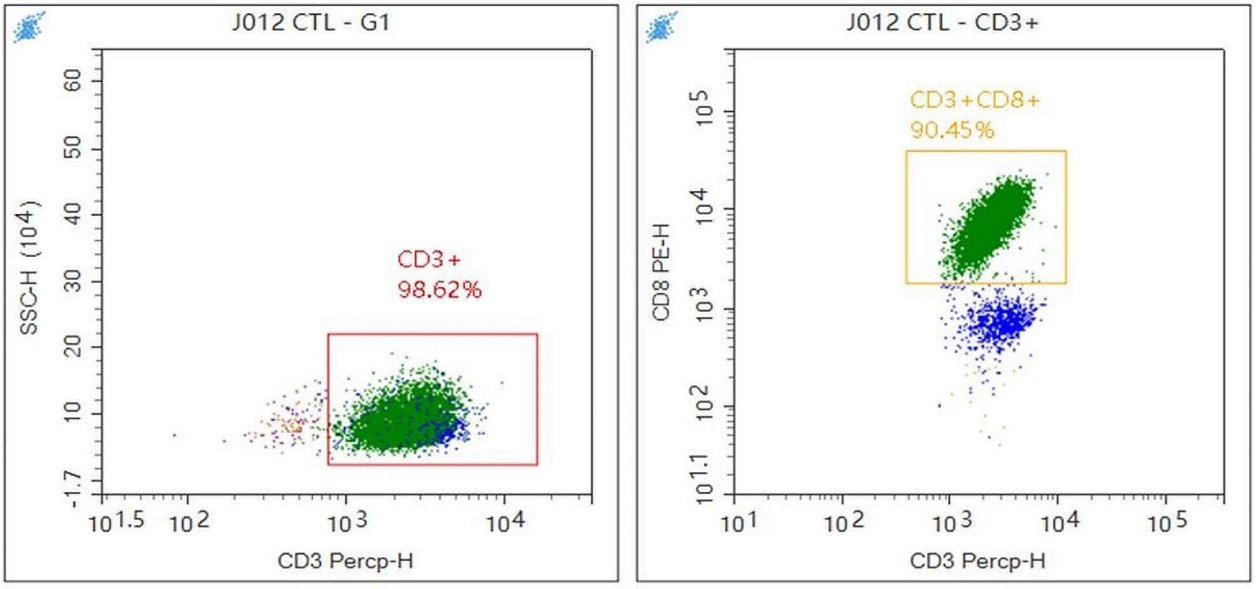

Fig. 2 Flow cytometry was used for quality control of CTL cells cultured in vitro

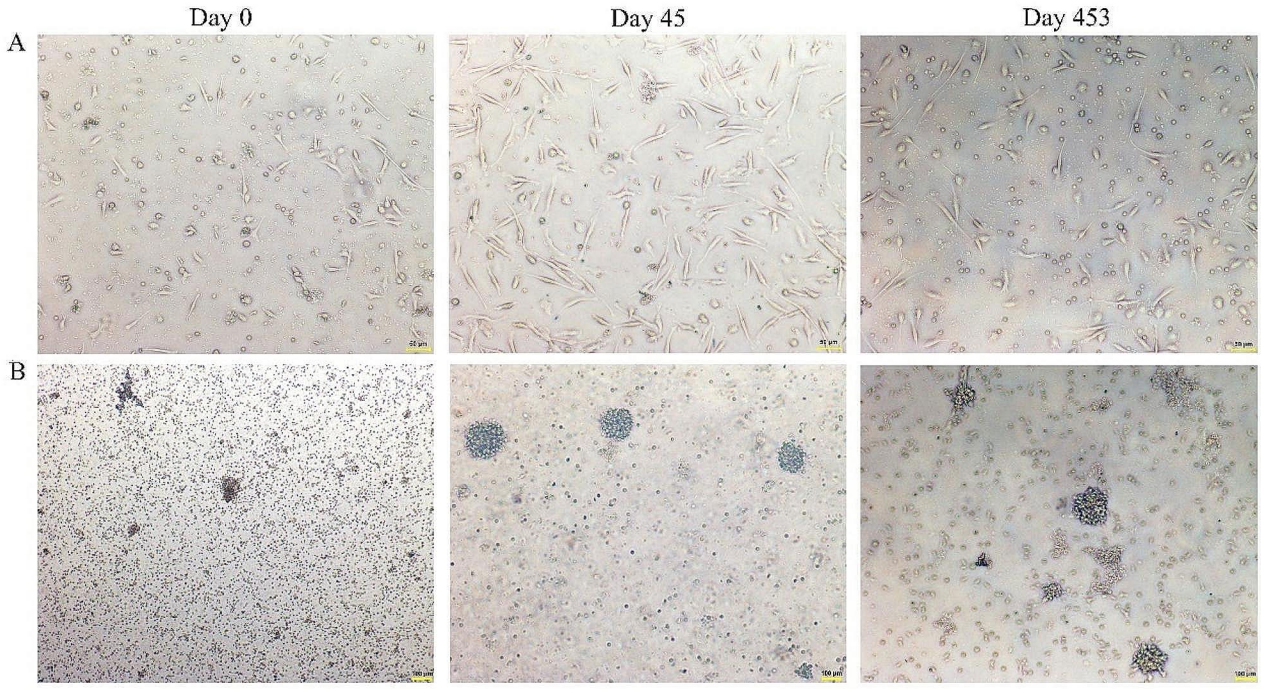

Fig. 3 TSA-DC-CTL vaccine plus osimertinib treatment mediated rapid regression of CTC cells and recovery of the total lymphocyte fraction. (**A**) Directional induction of DC (50 μm); (**B**) T cell immune response morphology (100 μm)

Post-combination therapy: disappearance of bone and lymph node metastatic lesions

The patient underwent a whole-body PET and CT scan after fasting for over 6 h and receiving an intravenous injection of a contrast agent. PET and CT images were displayed in multiple planes and frames. The initial whole-body PET-CT images (Day 0) showed multiple abnormal concentration foci in the C4 vertebra, right 8th rib, left ilium, left acetabulum, and right femoral head, with a maximum SUV (SUVmax) of 9.2. We found evidence of bone destruction in the right 8th rib. Multiple high-uptake lymph nodes were observed in the bilateral clavicular regions, mediastinum (zones 2R, 4R/L, 5, and 7), and left hilum. After completing two treatment cycles of TSA-DC-CTL combined with osimertinib (Day 59 and Day 190), increased bone density was noted in the C4 vertebra, right 8th rib, left ilium, left acetabulum, and right femoral head, with no abnormal radiotracer distribution observed. No increased glucose metabolism was found in the right clavicle, mediastinum, or left pulmonary hilum (Fig. 4A).

Significant reduction of left lung mass after combined therapy

The pretreatment CT images of the patient showed a tumor in the anterior basal segment of the left lower lobe of the lung, measuring approximately 3.2×2.4 cm (Fig. 4B-a). After completing the first cycle of TSA-DC-CTL combined with osimertinib treatment (Day 105), CT images revealed a reduction in the size of the tumor in the anterior basal segment of the left lower lobe to approximately 1.6×0.9 cm (Fig. 4B-b). After the completion of the second treatment cycle (Day 147), the tumor in the anterior basal segment of the left lower lobe further shrank to 1.0×0.9 cm (Fig. 4B-c).

Discussion

The scope of tumor immunotherapy is broad, encompassing active immunotherapy, cytokine immunotherapy, immune checkpoint inhibitor therapy, and immune cell therapy. Renowned international immunotherapy expert Rosenberg believes [16] that adoptive immune cell therapy has unique advantages compared with other immunotherapies that overly rely on the patient's innate immune functions. Cell-based immunotherapy primarily includes three categories: (1) Non-specific cell immunotherapy: this strategy is currently widely used in clinical applications, such as cytokine-induced killer cells (CIKs), DC-CIK, or natural killer (NK) cells [17–19]. However, their efficacy against recurrent and refractory tumors is limited. (2) Specific cell immunotherapy: this strategy utilizes non-personalized, common tumor antigens and unselected groups of tumor antigens to obtain DC, DC-CTL, and TIL for treatment. It has shown significant effectiveness in some patients with advanced-stage cancers [20, 21]. However, the intensity of the immune response is limited because of the non-specific nature of the antigens [22], and it can induce immune diseases

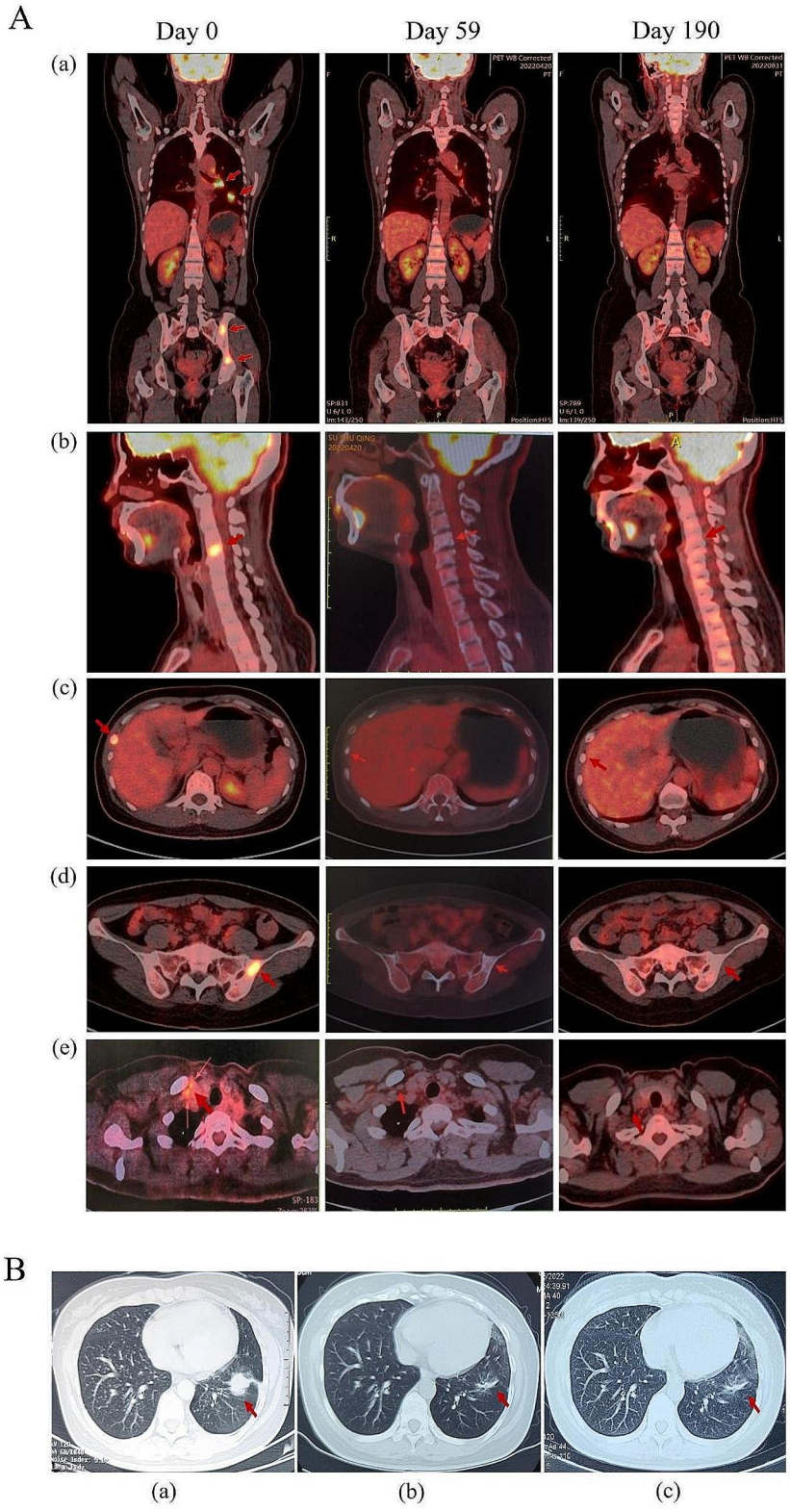

Fig. 4 PET-CT and CT were used to examine the regression of the lesions after treatment with TSA-DC-CTL vaccine combined with Osimertinib. **A:** Representative images of bone and lymph node metastases examined by PET-CT before and after treatment. **a:** The whole body picture. **b:** C4 vertebral body. **c:** Right 8 ribs. **d:** Left iliac crest. **e:** Right clavicle. **B:** Comparison of CT imaging of lung lesions. **A:** The patient did not start any treatment (Day 0); **B:** Patient after completion of the first course of TSA-DC-CTL plus osimertinib (Day 105). **C:** After completion of the second course of treatment (Day 147)

in patients, leading to adverse reactions such as allergies and fever. (3) Precision T cell immunotherapy: TSAs arising from specific gene mutations in tumors that can be presented on the surface of tumor cells, enhancing the binding capacity of T cells with MHC molecules. Precision cell immunotherapy entails the acquisition of TSAs that are specific to cancer cells, along with highly responsive precision T cells. These T cells are then expanded and utilized for precise immunotherapy in patients with tumors. This method has specific immune benefits, and T cells specifically recognize TSAs, avoiding damage to normal tissues [23, 24].

Robbins and colleagues developed a novel exome sequencing technique and an MHC molecule–antigen epitope affinity algorithm. They identified new antigens in patients with melanoma who showed progression after specific CTL therapy. These new antigens are capable of eliciting an effective T-cell response [25]. Tran and others discovered that tumor-infiltrating lymphocytes (TILs) from patients with metastatic cholangiocarcinoma contain CD4+helper T cells that recognize mutations in the ERBB2 interacting protein expressed by cancer cells. TILs, containing about 25% mutation-specific multifunctional T(H)1 cells, were transferred adoptively. Following the transfer, the patient experienced a decrease in the size of their target lesions, leading to an extension of the disease stabilization period [26].

The efficacy of TSA-DC-CTL immunotherapy in low-immunogenic solid tumors is still under exploration. For metastatic cancers with a high tumor burden and low KPS scores, if vaccine-induced epitope spreading fails to occur, the presence of epitope editing and clonal selection may result in suboptimal vaccine efficacy. In such cases, combination therapy may hold unique value. The patient in this study had advanced NSCLC with EGFR-sensitive mutations, and suffered from multiple vertebral and weight-bearing bone metastases and low KPS scores, making her unable to tolerate systemic chemotherapy. According to both domestic and international guidelines, the first-line preferred treatment option is EGFR TKI targeted therapy. Currently, first-line options include first, second, and third-generation EGFR TKIs. However, the development of resistance to EGFR TKI is inevitable. The third-generation EGFR TKI, osimertinib, can effectively and selectively inhibit both EGFR-TKI-sensitive mutations and the EGFR T790M resistance mutation, overcoming resistance issues that arise after treatment with the first- and second-generation EGFR TKIs. It is used to treat advanced or metastatic NSCLC [27–29]. Studies have reported that patients with metastatic NSCLC harboring EGFR mutations treated with first- and second-generation TKIs have a higher incidence of bone metastases and skeletal-related events [30, 31]. Osimertinib has demonstrated the longest overall survival (OS) for first-line monotherapy and has a favorable safety profile [32]. It has received the highest level of recommendation from four major authoritative guidelines both domestically and internationally. However, resistance to osimertinib is inevitable, with diverse mechanisms including EGFR pathway mutations, bypass activation, and histological transformation [33–35]. Preclinical studies have shown that a small number of resistant cells usually exist in tumors before the use of osimertinib. After using osimertinib, sensitive cells undergo apoptosis, while resistant cells survive and proliferate, leading to the development of resistance [36, 37]. Therefore, combination therapy to delay the onset of resistance is important, which is the rationale behind our choice to combine cell immunotherapy with EGFR TKI treatment.

Before undergoing cell immunotherapy, the patient was tested for tumor-associated specific protein biomarkers in serum at the peptide level via peptide enrichment purification combined with LC-MS. This method targeted 322 markers associated with 19 types of solid tumors. The results identified four lung-derived tumor-specific antigen peptides, all of them are specific targets for NSCLC. By analyzing the antigen information using molecular biology techniques, specific antigenic epitopes were identified, and peptides capable of binding with MHC class molecules and T-cell receptors were synthesized [38]. These peptides were co-cultured in vitro with DCs. The co-culture of DCs and T cells subsequently activated antigen-specific CTL cells.

During treatment with TSA-DC-CTL plus osimertinib, the patient did not experience any adverse reactions such as allergic reactions or fever, and the blood status remained stable. The KPS score improved significantly from 60 to 80. In cancer therapy, the change in the proportion of total lymphocytes is an important indicator for the patient's immune status and response to treatment. A low lymphocyte count (lymphopenia) is usually associated with poor prognosis, whereas an increase in lymphocyte count may indicate activation of the immune system and an enhanced antitumor response. This patient showed a notable upward trend in the total lymphocyte percentage throughout the treatment. It increased from 13.3% at the beginning of the treatment returned to the normal range of 33%. These results indicated a restoration of immune function and a positive response to the treatment. Additionally, morphological results of DCs and T cells showed that the number of DCs significantly increased after treatment, T cell production was normal, and differentiated colonies increased, indicating a normal immune response. These results reflected an improvement in the patient's general immune function.

The change in CTC count is widely used as a noninvasive biomarker to monitor the effectiveness of cancer treatment and the prognosis of patients [39, 40]. In this

study, the patient's mid-treatment CTC count decreased from 18 at the beginning of the treatment to 1. The reduction in peripheral blood CTCs may be associated with the specific immune targeted killing of tumor cells, reflecting a reduction in tumor burden. This phenomenon is generally indicative of a positive treatment outcome and is associated with a favorable clinical prognosis. The above results demonstrate that TSA-DC-CTL immunotherapy significantly impacts the patient's immune response. Osimertinib primarily inhibits tumor cell proliferation and promotes apoptosis by suppressing the EGFR signaling pathway. These immune-related changes are typically not a direct result of EGFR-TKI action. However, the reduction in tumor burden may indirectly enhance the overall immune status of the patient [41, 42]. A study in animal models have shown that EGFR-TKIs (Osimertinib) cause tumor shrinkage accompanied by CTL infiltration [43], indicating that EGFR-TKIs can influence the tumor immune microenvironment. EGFR-TKIs may increase CTL infiltration through several mechanisms. EGFR inhibition can reduce the production of immunosuppressive cytokines and growth factors by tumor cells, creating a more favorable environment for immune cell infiltration [44, 45]. Additionally, the apoptosis of tumor cells induced by EGFR-TKIs can release tumor antigens, which can be taken up by DCs and presented to CTLs, enhancing the immune response. TSA-DC-CTL immunotherapy involves using TSA-loaded dendritic cells to activate CTLs. When combined with Osimertinib, the increased presence of CTLs in the tumor microenvironment may enhance the efficacy of immunotherapy. This combination approach can potentially result in a more robust and targeted immune response against the tumor.

Regarding radiological assessment, post-treatment PET-CT scans of the patient exhibited a reduction or disappearance of glucose metabolism in multiple bone and lymph node metastatic lesions, in comparison to the pre-treatment scans. This indicates a positive response to the therapy after two treatment courses. This finding indicated a reduction or disappearance of metabolic activity in tumor cells, suggesting an improvement in the patient's condition. Chest CT scans revealed a significant reduction in the size of the lesion in the anterobasal segment of the left lower lobe of the lung. This reduction improved the patient's respiratory function and quality of life.

To summarize, the combined treatment in this case not only demonstrated the tumor growth inhibition effects of EGFR-TKI but also highlighted the unique role of TSA-DC-CTL immunotherapy in activating and enhancing the patient's immune system. This synergistic effect may be a crucial factor contributing to the significant improvement in the patient's clinical outcomes. While the safety and short- and long-term efficacy of TSA-DC-CTL as a first-line treatment warrant additional investigations, our study provides a reference for clinically managing patients with unresectable late-stage lung cancer with EGFR gene mutations. Although the results of this single-case study are promising, the presented data concerning a single case of contemporary combined treatment are not sufficient to attribute specific therapeutic values to the added component in a field with many individual variables of clinical outcome. Further research involving larger cohorts is necessary in the future to determine the generalizability of these findings and to better understand the specific contributions of TSA-DC-CTL immunotherapy when used in combination with Osimertinib.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12943-024-02070-3.

Supplementary Material 1

Acknowledgements
Not applicable.

Author contributions
Conception and design: ZH and TL; Provision of study materials or patient: KL and MX; Collection and assembly of data: HZ and MY; Data analysis and interpretation: FB and TZ; Manuscript writing: All authors; Final approval of manuscript: All authors.

Funding
This work was supported by Project A: Project number: Z2022036 of Jiangsu Provincial Health Commission and Special Key Project of Clinical Medicine of Nantong University (2022JZ012).

Data availability
Data is provided within the manuscript or supplementary information files.

Declarations

Ethics approval and consent to participate
This study was approved by the Medical Ethics Committee of Nantong Cancer Hospital (NO.2021015).

Consent for publication
The informed consent was taken from the patient.

Competing interests
The authors declare no competing interests.

Received: 7 March 2024 / Accepted: 22 July 2024
Published online: 09 August 2024

References

1. Sung H, Ferlay J, Siegel RL, et al. Global Cancer statistics 2020: GLOBOCAN estimates of incidence and Mortality Worldwide for 36 cancers in 185 Countries[J]. CA Cancer J Clin. 2021;71(3):209–49.
2. Spigel DR, Faivre-Finn C, Gray JE, et al. Five-year survival outcomes from the PACIFIC Trial: Durvalumab after Chemoradiotherapy in Stage III Non-small-cell Lung Cancer[J]. J Clin Oncol. 2022;40(12):1301–11.
3. Antonia SJ, Villegas A, Daniel D, et al. Overall survival with Durvalumab after Chemoradiotherapy in Stage III NSCLC[J]. N Engl J Med. 2018;379(24):2342–50.

4. Passaro A, Brahmer J, Antonia S, et al. Managing resistance to Immune checkpoint inhibitors in Lung Cancer: treatment and novel Strategies[J]. J Clin Oncol. 2022;40(6):598–610.
5. Lim ZF, Ma PC. Emerging insights of tumor heterogeneity and drug resistance mechanisms in lung cancer targeted therapy[J]. J Hematol Oncol. 2019;12(1):134.
6. Zhang KR, Zhang YF, Lei HM, et al. Targeting AKR1B1 inhibits glutathione de novo synthesis to overcome acquired resistance to EGFR-targeted therapy in lung cancer[J]. Sci Transl Med. 2021;13(614):eabg6428.
7. Zheng Y, Li Y, Feng J, et al. Cellular based immunotherapy for primary liver cancer. J Exp Clin Cancer Res. 2021;40(1):250.
8. Marciscano AE, Anandasabapathy N. The role of dendritic cells in cancer and anti-tumor immunity. Semin Immunol. 2021;52:101481.
9. Zapata L, Caravagna G, Williams MJ, et al. Immune selection determines tumor antigenicity and influences response to checkpoint inhibitors. Nat Genet. 2023;55(3):451–60.
10. Fu C, Ma T, Zhou L, et al. Dendritic cell-based vaccines against Cancer: challenges, advances and Future Opportunities[J]. Immunol Invest. 2022;51(8):2133–58.
11. Eisenbarth SC. Dendritic cell subsets in T cell programming: location dictates function[J]. Nat Rev Immunol. 2019;19(2):89–103.
12. He M, Roussak K, Ma F, et al. CD5 expression by dendritic cells directs T cell immunity and sustains immunotherapy responses. Science. 2023;379(6633):eabg2752.
13. Wculek SK, Cueto FJ, Mujal AM, et al. Dendritic cells in cancer immunology and immunotherapy[J]. Nat Rev Immunol. 2020;20(1):7–24.
14. Steinman RM. Decisions about dendritic cells: past, present, and future[J]. Annu Rev Immunol. 2012;30:1–22.
15. Son SW, Cho E, Cho H, et al. NANOG confers resistance to complement-dependent cytotoxicity in immune-edited tumor cells through up-regulating CD59. Sci Rep. 2022;12(1):8652.
16. Rosenberg SA, Restifo NP. Adoptive cell transfer as personalized immunotherapy for human cancer[J]. Science. 2015;348(6230):62–8.
17. Gang M, Marin ND, Wong P, et al. CAR-modified memory-like NK cells exhibit potent responses to NK-resistant lymphomas[J]. Blood. 2020;136(20):2308–18.
18. Sharma A, Schmidt-Wolf I, G H. 30 years of CIK cell therapy: recapitulating the key breakthroughs and future perspective[J]. J Exp Clin Cancer Res. 2021;40(1):388.
19. Sommaggio R, Cappuzzello E, Dalla Pieta A, et al. Adoptive cell therapy of triple negative breast cancer with redirected cytokine-induced killer cells[J]. Oncoimmunology. 2020;9(1):1777046.
20. Wang Y, Xiang Y, Xin VW, et al. Dendritic cell biology and its role in tumor immunotherapy[J]. J Hematol Oncol. 2020;13(1):107.
21. Ma H, Tan Y, Wen D, et al. DC-CTL targeting carbonic anhydrase IX gene combined with iAPA therapy in the treatment of renal cell carcinoma[J]. Hum Vaccin Immunother. 2021;17(11):4363–73.
22. Zhong G, Zhao W, Li Y, et al. MAGEA1 and hTERT peptide treatment improves the potency of the dendritic cell- cytotoxic T lymphocytes (DC-CTL) Immunotherapy in DAC treated Acute myeloid Leukemia[J]. J Cancer. 2022;13(4):1252–60.
23. Li J, Xiao Z, Wang D, et al. The screening, identification, design and clinical application of tumor-specific neoantigens for TCR-T cells[J]. Mol Cancer. 2023;22(1):141.
24. Shang S, Zhao Y, Qian K, et al. The role of neoantigens in tumor immunotherapy[J]. Biomed Pharmacother. 2022;151:113118.
25. Robbins PF, Lu YC, El-Gamil M, et al. Mining exomic sequencing data to identify mutated antigens recognized by adoptively transferred tumor-reactive T cells[J]. Nat Med. 2013;19(6):747–52.
26. Tran E, Turcotte S, Gros A, et al. Cancer immunotherapy based on mutation-specific CD4 + T cells in a patient with epithelial cancer[J]. Science. 2014;344(6184):641–5.
27. Ramalingam SS, Vansteenkiste J, Planchard D, et al. Overall survival with Osimertinib in untreated, EGFR-Mutated Advanced NSCLC[J]. N Engl J Med. 2020;382(1):41–50.
28. Wu YL, Tsuboi M, He J, et al. Osimertinib in Resected EGFR-Mutated non-small-cell lung Cancer[J]. N Engl J Med. 2020;383(18):1711–23.
29. Takeda M, Shimokawa M, Nakamura A, et al. A phase II study (WJOG12819L) to assess the efficacy of osimertinib in patients with EGFR mutation-positive NSCLC in whom systemic disease (T790M-negative) progressed after treatment with first- or second-generation EGFR TKIs and platinum-based chemotherapy[J]. Lung Cancer. 2023;177:44–50.
30. Li XY, Zhu XR, Zhang CC, et al. Analysis of progression patterns and failure sites of patients with metastatic lung adenocarcinoma with EGFR mutations receiving first-line treatment of tyrosine kinase inhibitors. Clin Lung Cancer. 2020;21(6):534–44.
31. Laganà M, Gurizzan C, Roca E, et al. High prevalence and early occurrence of skeletal complications in EGFR Mutated NSCLC patients with bone metastases. Front Oncol. 2020;10:588862.
32. Ramalingam SS, Vansteenkiste J, Planchard D, et al. Overall survival with Osimertinib in untreated, EGFR-Mutated Advanced NSCLC. N Engl J Med. 2020;382(1):41–50.
33. Sun R, Hou Z, Zhang Y, et al. Drug resistance mechanisms and progress in the treatment of EGFR-mutated lung adenocarcinoma[J]. Oncol Lett. 2022;24(5):408.
34. Li M, Qin J, Xie F, et al. L718Q/V mutation in exon 18 of EGFR mediates resistance to osimertinib: clinical features and treatment[J]. Discov Oncol. 2022;13(1):72.
35. Singh A, Mishra A. Investigation of molecular mechanism leading to gefitinib and osimertinib resistance against EGFR tyrosine kinase: molecular dynamics and binding free energy calculation[J]. J Biomol Struct Dyn. 2023;41(10):4534–48.
36. Di Noia V, D'aveni A, D'argento E, et al. Treating disease progression with osimertinib in EGFR-mutated non-small-cell lung cancer: novel targeted agents and combination strategies[J]. ESMO Open. 2021;6(6):100280.
37. Li F, Deng L, Jackson KR et al. Neoantigen vaccination induces clinical and immunologic responses in non-small cell lung cancer patients harboring EGFR mutations. J Immunother Cancer. 2021;9(7):e002531. Erratum in: J Immunother Cancer. 2021;9(9):1.
38. He J, Xiong X, Yang H, et al. Defined tumor antigen-specific T cells potentiate personalized TCR-T cell therapy and prediction of immunotherapy response[J]. Cell Res. 2022;32(6):530–42.
39. Lin D, Shen L, Luo M, et al. Circulating tumor cells: biology and clinical significance[J]. Signal Transduct Target Ther. 2021;6(1):404.
40. Feng Z, Wu J, Lu Y, et al. Circulating tumor cells in the early detection of human cancers[J]. Int J Biol Sci. 2022;18(8):3251–65.
41. Xiao X, Wu Y, Shen F et al. Osimertinib Improves the Immune Microenvironment of Lung Cancer by Downregulating PD-L1 Expression of Vascular Endothelial Cells and Enhances the Antitumor Effect of Bevacizumab. J Oncol. 2022, 2022:1531353.
42. Shan Y, Ni Q, Zhang Q, et al. Targeting tumor endothelial hyperglycolysis enhances immunotherapy through remodeling tumor microenvironment. Acta Pharm Sin B. 2022;12(4):1825–39.
43. Jia Y, Li X, Jiang T, et al. EGFR-targeted therapy alters the tumor microenvironment in EGFR-driven lung tumors: implications for combination therapies. Int J Cancer. 2019;145(5):1432–44.
44. Tan HY, Wang N, Lam W, et al. Targeting tumour microenvironment by tyrosine kinase inhibitor. Mol Cancer. 2018;17(1):43.
45. Okuyama K, Naruse T, Yanamoto S. Tumor microenvironmental modification by the current target therapy for head and neck squamous cell carcinoma. J Exp Clin Cancer Res. 2023;42(1):114.

Publisher's Note