Case Report

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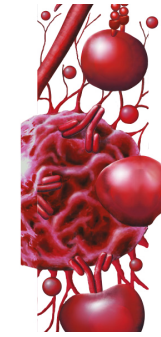

Role of allogeneic natural killer T cells in the treatment of a patient with gefitinib-sensitive lung adenocarcinoma

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Gefitinib has shown good efficacy in patients with *EGFR* mutation-positive non-small-cell lung cancer, but acquired resistance is inevitable. Here we report a patient with an advanced lung adenocarcinoma with the *EGFR* mutation who achieved surgical opportunity and long-term survival following treatment with chemotherapy and bevacizumab, followed by sequential gefitinib combined with allogeneic haploidentical $CD8^+$ $CD56^+$ natural killer T cells. Our case provides a potential effective strategy for delaying acquired gefitinib resistance and extending progression-free survival among patients with non-small-cell lung cancer who harbor common *EGFR* mutations.

Plain language summary: As one of the EGFR tyrosine kinase inhibitors used clinically, gefitinib has achieved good efficacy in patients with *EGFR* mutation-positive non-small-cell lung cancer (NSCLC), though eventual drug resistance is inevitable. Currently, the efficacy of anti-PD-1/anti-PD-L1 immunotherapy has not been demonstrated in NSCLC because of the low expression of PD-1 in this disease. Thus new strategies for NSCLC treatment need to be further explored. Here we report a patient with advanced lung adenocarcinoma with the *EGFR* mutation, who was treated with chemotherapy and bevacizumab and sequential gefitinib combined with allogeneic haploidentical natural killer T cells, who achieved a surgical opportunity and long-term survival. To delay the time to resistance to gefitinib, a combination of allogeneic haploidentical $CD8^+$ $CD56^+$ natural killer T cells and gefitinib may offer a viable treatment option for patients with *EGFR* mutation-positive NSCLC.

First draft submitted: 11 July 2022; Accepted for publication: 12 September 2022; Published online: 28 September 2022

Keywords: cellular immunotherapy • epidermal growth factor receptor • gefitinib • natural killer T cells • non-small-cell lung cancer • tyrosine kinase inhibitor

Gefitinib, a first-generation EGFR tyrosine kinase inhibitor (TKI), has demonstrated significant efficacy against advanced *EGFR* mutation-positive non-small-cell lung cancer (NSCLC) [1]. However, acquired resistance has always been an Achilles' heel in EGFR TKI treatment. Thus, new strategies for treating advanced NSCLC require further exploration. $CD8^+$ $CD56^+$ natural killer T (NKT) cells form a natural and unique lymphocyte subset in humans that presents characteristics of both T and NK cells and exerts cytotoxicity to tumor cells in a granzyme B-dependent manner. Moreover, NKT cells have shown efficient expansion *in vitro*, indicating their potential usage in tumor immunotherapy [2]. Here we report the case of a patient with advanced lung adenocarcinoma who was treated with chemotherapy and bevacizumab, followed by sequential gefitinib and allogeneic haploidentical NKT cell therapy, to obtain a surgical opportunity and achieve long-term survival.

Future Medicine

Case report

A 34-year-old male was referred to the People's Hospital of Peking University in September 2017 with wheezing and chest tightness. Chest computed tomography (CT) revealed a right hilar mass (22 × 18 mm), mediastinal lymph node enlargement (maximum short diameter 16 mm), right pleural effusion (effusion depth 5.2 cm) and three small nodules in the left lung (Figure 1Aa). Endobronchial ultrasound-guided transbronchial needle aspiration of the tumor was performed, followed by an immunohistochemical examination that indicated poorly differentiated adenocarcinoma, and the patient was diagnosed with T4N3M1a stage IV. After seven cycles of treatment (3 weeks per cycle) with pemetrexed 1 g + cisplatin 140 mg + bevacizumab 600 mg, chest CT in February 2018 showed a right lower lung nodule (14 × 11 mm), mediastinal lymph node enlargement (largest short diameter 6 mm) and localized thickening in the right pleura (Figure 1Ab). Treatment efficacy was evaluated as a partial response, and the wheezing and chest tightness symptoms improved significantly. During chemotherapy, an *EGFR* exon 19 deletion was found via genetic testing, so the patient was treated with gefitinib (250 mg, orally, once daily) starting in March 2018. At follow-up 8 weeks later, the chest CT revealed right lower lung nodules (14 × 8 mm), no evident enlarged lymph nodes in the mediastinum, and local thickening of the right pleura (Figure 1Ac). Efficacy was evaluated as stable disease (SD). Subsequently, the patient was enrolled in a clinical trial of gefitinib combined with allogeneic haploidentical $CD8^+$ $CD56^+$ NKT cell therapy (registration no. ChiCTR-IIR-17013471) [3]. In July 2019, after three cell treatment cycles (4 weeks per cycle and two cell transfusions per week), PET-CT demonstrated a right lower lung nodule shadow (12 × 8 mm) with a slight increase in ^{18}F-flurodeoxyglucose metabolism, revealing the possibility of active tumor tissue (Figure 1Ad). The curative effect led to SD after re-evaluation, and adverse events included grade 1 abnormal liver function. In August 2019 the patient underwent a thoracoscopic wedge resection of the right lower lobe tumor. The pathological results showed that the size of tumor was 12 × 8 mm, with focal residual invasive adenocarcinoma, right pleural fibrous tissue hyperplasia with vitreous degeneration, no definite malignant lesions and no lymph node metastasis. The descending stage of the patient was considered T1N0M0 stage IA. In January 2020, due to the COVID-19 pandemic, the patient had to discontinue the NKT cell transfusions, but the gefitinib treatment was continued. Thereafter, no metastasis was seen in the brain, but multiple signs of small metastatic tumors were found in the lung (Figure 1Ae), with a concomitant increase in carcinoembryonic antigen levels (72.0 ng/ml) in March 2020, indicating progressive disease. NKT cell transfusion was then resumed, and the curative effect was evaluated as SD in June 2020 (Figure 1Af). At the latest visit (July 2021), the patient felt well overall, had no adverse reactions and was still evaluated as SD under the Response Evaluation Criteria In Solid Tumors (Figure 1Ag). The entire treatment course and clinical outcomes are summarized in Figure 1B. Overall disease burden is described in Figure 1C. To date, the patient continues to receive gefitinib combined with allogeneic haploidentical NKT cells as maintenance therapy. A timeline of serological markers and treatment response evolution is presented in Figure 2.

Discussion

EGFR TKIs have become the standard treatment for advanced NSCLC with *EGFR*-sensitive mutations, but most patients exhibit disease progression within about 1 year [3]. To further extend progression-free survival (PFS) and overall survival as well as to improve the quality of life of patients with advanced lung cancer, a number of clinical trials combining chemotherapy, antiangiogenic agents and immunotherapy are underway [4–7]. Indeed, not only increased levels of PD-1 [8,9] but also increased levels of other adhesion molecules, such as intracellular adhesion molecule 1, were found in EGFR TKI-resistant cancer cells [10]. These data indicate that killer immune cells can be designed as target cells for combination with EGFR TKI treatment in these diseases [10,11]. Our study utilized cultured $CD8^+$ NKT cells with sufficient cytotoxicity for adoptive therapy, combined with gefitinib, to treat advanced *EGFR*-mutated NSCLC. It was designed as a prospective, randomized, open-label, controlled phase I/II parallel-group trial [12]. Primary data in the clinical trials have shown that NKT cell transfusion therapy is a safe and effective treatment for this disease (unpublished data). Here, the patient was treated with chemotherapy combined with bevacizumab, then gefitinib combined with allogeneic haploidentical NKT cells, resulting in a PFS of at least 29 months and a successful right lung tumor resection with tumor downstaging.

NKT cells are a heterogeneous population of cells obtained by coculturing human peripheral blood mononuclear cells with multiple cytokines *in vitro* for a period of time. This combines the powerful and specific anti-tumor activity of T lymphocytes with the non-MHC-restricted tumor-killing advantages of NK cells [13]. Studies have confirmed that NKT cells exert cytotoxicity against a variety of malignancies, including leukemia and solid tumors [2,14–16]. Our patient showed a prolonged PFS of 29 months. The reasons for the favorable response were

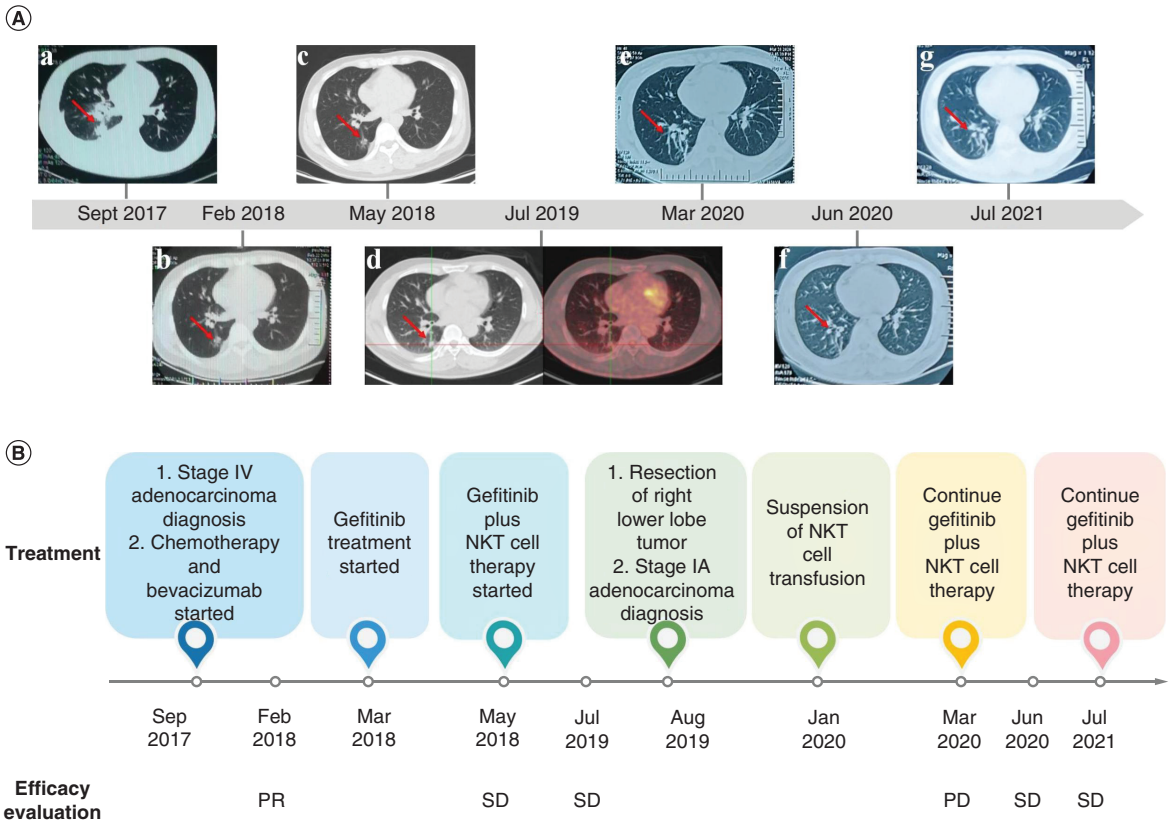

Figure 1. Clinical treatment course and assessments of the main lesions during treatment of a patient with advanced lung adenocarcinoma with an *EGFR* mutation. (A) Primary tumor changes on the computed tomography scan: **(a)** Primary tumor located on the right hilar and pleural effusion; **(b)** Partial disease response after 5 months of chemotherapy combined with bevacizumab treatment; **(c)** Stable disease after 2 months of gefitinib treatment; **(d)** Stable disease after 14 months of gefitinib combined with allogeneic haploidentical NKT cells treatment; **(e)** Follow-up 6 months after surgery and progressive disease after suspension of NKT cell therapy for 2 months; **(f)** Follow-up nearly 1 year after surgical intervention; **(g)** Follow-up nearly 2 years after surgical intervention. **(B)** Timeline of the entire treatment course. **(C)** The volume of the tumor was calculated as: long diameter × short diameter × short diameter × 0.5.
NTK: Natural killer T cells; PD: Progressive disease; PR: Partial response; SD: Stable disease.

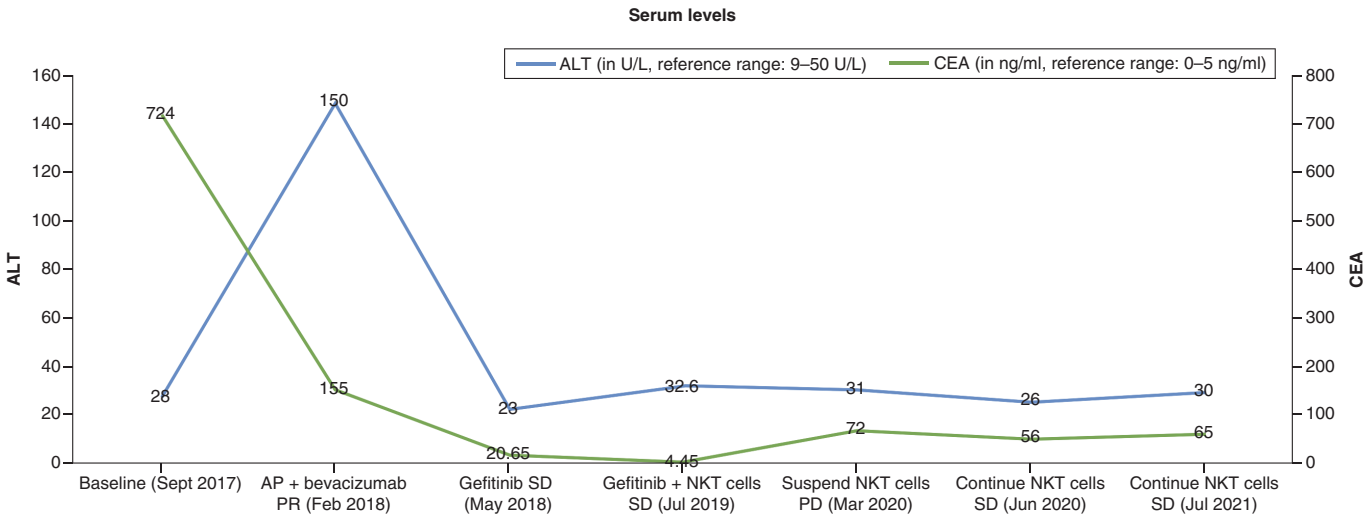

Figure 2. Timeline of serological markers (carcinoembryonic antigen and alanine aminotransferase) and treatment response evolution.
PD: Progressive disease; PR: Partial response; SD: Stable disease.

as follows. First, the patient had responded well to chemotherapy and antiangiogenic therapy with bevacizumab. As the first monoclonal antibody against angiogenesis, bevacizumab has become an important part of first-line treatment for advanced NSCLC [17]. This agent improves the efficacy of chemotherapy, EGFR TKIs and immune checkpoint inhibitors. However, the optimal combination scheme still needs to be determined by new randomized controlled trials. Second, after targeted gefitinib therapy, the tumor load of the patient decreased to a low point. What's more, the tumor-associated immunosuppression environment in patients was dramatically modified [18,19]. Thus the adoptive transferred NKT cells are more liable to keep their cytotoxicity to tumor cells, including newly produced EGFR TKI-resistant tumor cells. In particular, the transferred allogeneic lymphocytes are easily activated through tumor antigen and MHC class I/II molecules from the recipient, which promotes alloreactivity and boosts immunity [20]. Thus adoptive allogeneic NKT cells have much stronger antitumor activity than autologous cells from patients with advanced tumors. Notably, the data showed that disease progression in the patient occurred after NKT cell transfusion was suspended in March 2020, but was again reversed to SD after cell transfer therapy was resumed and maintained. Thus, we believe that NKT cell therapy had a vital role in improving the patient's condition, although the mechanism remains unclear. Indeed, allogeneic haploidentical NKT cell immunotherapy and gefitinib showed synergistic effects in this trial, which effectively reduced the tumor size for surgical treatment and played an important role in removing the residual microscopic metastases after the operation, prevented cancer spread and recurrence and improved patient immunity (unpublished data).

Simultaneously, owing to its mechanism of action, allogeneic haploidentical NKT cell transfusion may induce inflammatory side effects, also known as immune-related adverse events (irAEs) [21,22], such as potentially life-threatening pneumonitis. In our case, the patient did not present any serious irAEs (no evident clinical manifestation or abnormal liver function) and showed SD for 14 months after gefitinib combined with allogeneic haploidentical $CD8^+$ $CD56^+$ NKT cell therapy was commenced in May 2018. In fact, our preclinical study and primary clinical safety trial showed that $CD8^+$ $CD56^+$ NKT cell transfusion caused no evident adverse reactions or cytotoxicity in recipients (unpublished data). The difference between irAEs in immune checkpoint blockade treatment and NKT cell immunotherapy largely lies in their different mechanisms. Further studies on the overlapping toxicity of NKT cell immunotherapy in combination with other treatment options are still needed in the future.

This study has several limitations. First, this patient did not undergo next-generation sequencing testing to confirm disease progression at the time of relapse or after initiation of NKT treatment, so their mutated gene and *EGFR* transcript burden could not be fully analyzed. Second, gefitinib, erlotinib and osimertinib are currently used as first-line treatment drugs for *EGFR* mutation-positive advanced NSCLC, according to the National Comprehensive Cancer Network [23] and Chinese Society of Clinical Oncology [24] guidelines. However, this study was implemented in July 2017, so gefitinib was the only drug in the protocol design. Thus we have no data to show the synergistic effects between osimertinib/erlotinib and NKT cell therapy, which remains to be further explored in the future.

Conclusion

Different treatment methods lead to different dominant tumor subclones, so diverse therapy strategies provide patients with durable clinical benefits [25]. Chemotherapy combined with bevacizumab, followed by sequential therapy with gefitinib combined with allogeneic haploidentical NKT cells, significantly delayed the time of molecular-targeted drug resistance in this patient while enhancing the antitumor immune response and providing conditions for surgical treatment, yielding a better clinical outcome. Determining the optimal combination therapy regimen, managing adverse events and screening for possible patient groups that would benefit from this approach are important topics for clinical practitioners and basic researchers in the future.

Executive summary

- As one of the EGFR tyrosine kinase inhibitors used clinically, gefitinib has achieved good efficacy in patients with *EGFR* mutation-positive non-small-cell lung cancer, but eventual drug resistance is inevitable.
- $CD8^+$ natural killer T cells have shown cytotoxicity on cancer cells in animal models and preclinical studies.
- We report a young patient who received sequential chemotherapy combined with bevacizumab and gefitinib combined with allogeneic haploidentical $CD8^+$ natural killer T cells.
- The treatment significantly postponed resistance to gefitinib and enhanced the antitumor immune response, which provided conditions for surgical treatment and achieved a better clinical result.

Author contributions

S Xia and D Chen were responsible for initiating, applying, organizing, funding and monitoring this clinical study; M Zhang provided key technical support for the preparation of NKT cells; C Mao supervised the conduct of the study; W Yu drafted the manuscript, S Xia revised the manuscript; X Yuan and F Ye enrolled participants, assigned participants to interventions and coordinated with other members; J Li played an important role in the diagnosis and treatment of diseases. All authors read and approved the final manuscript.

Financial & competing interests disclosure

This work was supported by the Jiangsu Provincial Key Research and Development Program (grant no. BE2017696) and the National Natural Science Foundation, China (grant no. 81871234 and 82172763). The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Data sharing disclosure

The authors certify that this manuscript reports original clinical trial data. Deidentified, individual data that underlie the results reported in this article (text, tables, figures and appendices), along with the study protocol will be available indefinitely for anyone who wants access to them.

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