Long-term survival with targeted therapy in an advanced non-small cell lung cancer patient based on genetic profiling

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Abstract: Non-small cell lung cancer (NSCLC) is a profoundly devastating disease that is the leading cause of cancer-related death worldwide. With the rapid development of next-generation sequencing (NGS), which has supplied the ability to decode tumors at the DNA level, so that targeted therapy plays a crucial role in improving NSCLC survival. We first reported a 32-year-old Chinese female patient received the ninth-line treatment, who was initially diagnosed with advanced NSCLC with *EGFR* 19 deletion. The patient had a satisfactory clinical response to initial gefitinib treatment. Subsequently, an *EGFR* T790M mutation was detected from plasma-derived circulating tumor DNA (ctDNA) by ddPCR after disease progression, while NGS did not. Osimertinib was still tried but had no therapeutic effect. Then the disease even progressed on the administration of chemotherapy and gefitinib in succession. Rebiopsy for NGS detection was performed, and gefitinib plus anlotinib/vemurafenib were tried. And then, gefitinib plus crizotinib were administrated for *MET* amplification after the third biopsy. Furthermore, chemotherapy combined with immunotherapy was performed due to the PD-L1 positive expression. Up to now, osimertinib treatment was undertaken to base on an *EGFR* exon 20 T790M mutation using NGS-based genotyping in cerebrospinal fluid (CSF) ctDNA. Tumor genome dynamic monitoring can identify tumor driving genes and drug resistance mechanisms to guide tumor treatment. This study found that the total survival time of advanced NSCLC patients was more than four years after chemoradiotherapy and targeted therapy, indicating the significance of dynamic monitoring of gene alterations for cancer treatment.

Keywords: Non-small cell lung cancer (NSCLC); epidermal growth factor receptor (EGFR); next-generation sequencing (NGS); progression; biopsy

Introduction

The discovery of epidermal growth factor receptor (*EGFR*) mutations and their sensitivity to *EGFR* inhibitors have dramatically altered the treatment of patients with non-small cell lung cancer (NSCLC) (1,2). Furthermore, multi-generations tyrosine kinase inhibitors (TKI) against *EGFR*-driven mutations have produced significant effects on NSCLC patients (3-5). Targeted therapy though, is similar to chemotherapy and is facing problems of resistance. The underlying mechanisms of acquired resistance (AR) to

EGFR-TKIs are also complex. As such, there may be added factors other than *EGFR* mutations that contribute to its disease progression (6-8). Thus further research needs to be carried out to find new targets. The administration of these targeted drugs, dynamic monitoring of genomic profiles during treatment, and the promotion of targeted therapy all depend on the extensive application of next-generation sequencing (NGS) in tissue biopsy or liquid biopsy.

In this report, we regularly used tissue biopsies or liquid biopsies to monitor a patient with EGFR-exon19del positive NSCLC who has at present achieved overall

survival for up to 48 months through dynamic monitoring of genomic profile during cancer processes.

Case presentation

Patient management is described in *Figure 1A*. A 32-year-old woman with no prior history of smoking was referred to another hospital complaining of cough accompanied with numbness of the right upper limb and face, mild headache, and dizziness in November 2015. The systematic evaluation indicated that a stage IV T3N0M1 poorly differentiated NSCLC accompanying with bone metastasis and intracranial metastasis were involved. The examination of *EGFR* mutations by amplification refractory mutation system demonstrated *EGFR* with exon 19 deletion mutations.

Beginning on December 1, 2015, this patient orally received gefitinib treatment plus whole-brain radiotherapy PTV 30 Gy/10 F. 4mg zoledronic acid intravenous drip regularly was used for bone treatment. On June 18, 2016, a chest and abdomen computed tomography (CT) scan showed a significant reduction in primary lesions (*Figure 1B*). Efficacy was assessed as partial remission (PR). And brain magnetic resonance imaging (MRI) showed stable disease (SD) in metastases (*Figure 1C*). After a year of continued gefitinib treatment, chest-abdomen CT showed progressive disease (PD) (*Figure 1B*). Blood NGS testing was subsequently performed, but no *EGFR* driving genes and other positive driving genes were found, while ddPCR indicated *EGFR* 20 exon T790M mutation (*Table 1*). Thus, osimertinib targeted therapy was selected for two months, but no significant effect.

The patient was moved to our unit on 31 August 2017 after undergoing the above treatment. A treatment regimen of pemetrexed/ cisplatin with bevacizumab was firstly administered in 4 cycles. The partial regression treatment effect was obtained (*Figure 1B*), and the brain MRI showed a stable lesion (*Figure 1C*). However, the disease progressed after two more cycles of chemotherapy. Re-challenge with gefitinib was selected since April 2018. Unfortunately, the disease progressed again after a brief remission (*Figure 1B*). Therefore, a rebiopsy was used to guide further treatment. And gefitinib plus anlotinib was performed as the next treatment. The lesion was diminished but enlarged again. Then the targeted therapy with gefitinib plus vemurafenib was started while targeted NGS found *EGFR* exon 19 p.E746_S752 delinsI (1.54%) and *BRAF* V600E mutation (2.09%) (*Table 1*). The size

of the primary lung tumor was increased after a month of treatment. Subsequently, a third biopsy was performed on March 27, 2019. And NGS revealed *EGFR* exon 19 p.E746_S752 delins I (39.97%), *MET* amplification (n=5.18) and *EGFR* amplification (n=4.3). So the treatment regimen was switched to gefitinib plus crizotinib. However, the patient complained of dizziness, the right eye was diplopia, and the right eyelid was drooping by May 2019. Challenge with Paclitaxel /carboplatin plus pembrolizumab was selected for 2 cycles followed. Surprisingly, there was an obvious improvement in tumor lesions (*Figure 1B*), no obvious abnormality in the right eyelid, no obvious headache or dizziness, and diplopia disappeared. Undesirably, the patient developed hyperthyroidism with palpitations, irritability, and prominent eyes. The PS of the patient worsens from 1 to 2 with obvious poor appetite, weight loss, slight headache, and weakness again. On Augest10, 2019, the NGS-based genotyping was performed on cerebrospinal fluid (CSF) derived circulating tumor DNA (ctDNA). *EGFR* exon 19 p.E746_S752 delinsI (65.14%), *EGFR* exon 20 p.T790M (44.51%) and *RICTOR* amplification (n=3.66) were observed. And the patient is currently receiving treatment with osimertinib.

Discussion

In the past decade, considerable progress has been made in the treatment of advanced NSCLC, especially molecular targeted therapy. Regardless of the technique used, the determination of tumor molecular status has become the standard for NSCLC treatment. The *EGFR* signaling pathway plays a crucial role in the NSCLC development and progression, and *EGFR* mutation sensitization is found in approximately 10–15% of white patients and up to 50% of Asian NSCLC patients (9,10). Here, we report an advanced NSCLC Chinese patient with *EGFR* 19 deficiency who was initially treated with gefitinib orally and responded to PR. Numerous studies (11,12) have shown that first and second-generation *EGFR* TKIs such as erlotinib, gefitinib, or afatinib were recommended for patients with advanced NSCLC who have *EGFR* mutation activation (exon 19 deletion and 21 L858R point mutation). However, NSCLC invariably produces AR to first-line inhibitors. Most potential AR mechanisms have been identified, including secondary mutations in *EGFR* (e.g., *EGFR*-T790M), activation of bypass signaling pathways (such as *BRAF*, *MET*, *PIK3CA* signaling pathway), and histological transformation to small cell lung cancer (13). To our
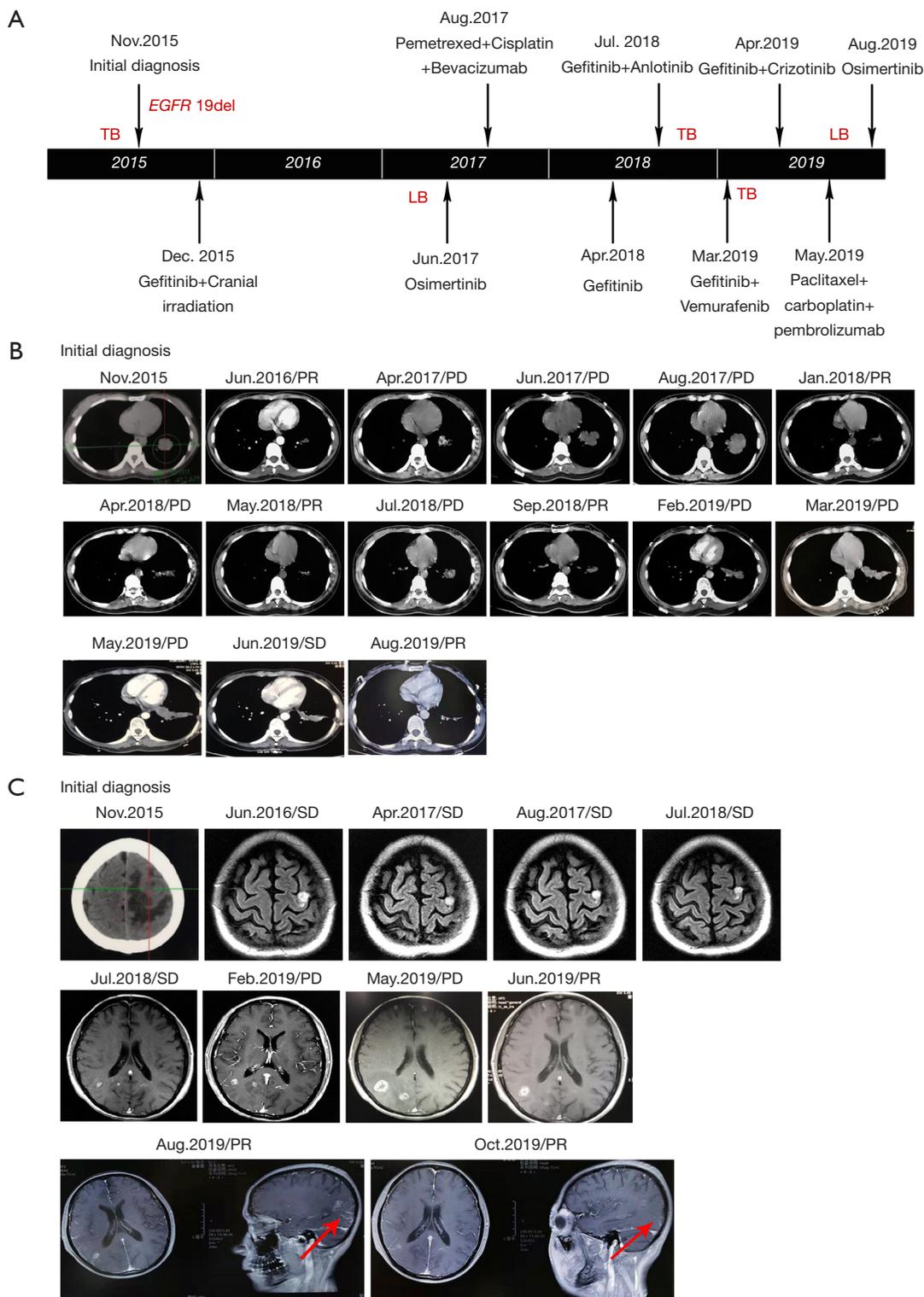
Figure 1 Timeline of patient's therapy and the effect of therapy. (A) Genomic testing and targeted treatments; (B) chest CT scanning of a primary lung tumor; (C) MRI of brain metastasis. EGFR, epidermal growth factor receptor; PR, partial remission; PD, progressive disease; SD, stable disease; CT, computed tomography; MRI, magnetic resonance imaging.

Table 1 Results of immunohistochemical staining and molecular profiling analysis on patient's tumor tissue or liquid biopsy

Immunohistochemistry	Status
First biopsy	
CK5/6, P63, P40, TTF1, NapsinA, CK7, MCK, Ki67 (50%)	Positive
Vimentin, CGA, CD56, Syn	Negative
Second biopsy	
AE1/AE3, CK5, P63, TTF1, NapsinA, CK7, Ki67 (70%), PD-L1 (90%)	Positive
P40, CD56, Syn	Negative
Third biopsy	
AE1/AE3, CK5, P63, TTF1, NapsinA, CK7, Ki67 (50%), PD-L1 (55%)	Positive
P40, CD56, Syn	Negative
Molecular profiling	
Initial diagnosis	
EGFR exon 19 del	
Firstly	
No drivers (NGS)	Mutated
EGFR exon 20 p.T790M (ddPCR)	
Secondly	
EGFR exon 19 p.E746_S752 delinsI (1.54%), BRAF p. V600E (2.09%)	Mutated
MSI	Stable
TMB	11.6 mut/mb
Thirdly	
EGFR exon 19 p.E746_S752 delinsI (65.14%), MET amplification (n=5.18), EGFR amplification (n=4.3), TP53 p.p278-D281delPGRD, PMS2 p.K651N	Mutated/copy number variation
MSI	Stable
TMB	2.54 mut/mb
Fourthly	
EGFR exon 19 p.E746_S752 delinsI (39.97%), *EGFR* exon 20 p.T790M (44.51%), *RICTOR* amplification (n=3.66), *TP53* p.p278-D281delPGRD, *MSH3* p.S408F, *PRKCI* p.V492I, *TSHR* p.V448A	Mutated/copy number variation
ALK, BRAF, ERBB2, KRAS, MET, NTRK1, NTRK2, NTRK3, RET, ROS1	Wild type

knowledge, this report is the first to show a patient received the ninth-line therapy who underwent multiple biopsies, including tissue biopsy and liquid biopsy, and then targeted therapy based on corresponding different AR mechanisms to achieve an overall survival currently up to 48 months.

A previous Phase II Study had indicated that gefitinib re-challenge was effective as an option after the first-line *EGFR*-TKI treatment and second-line chemotherapy, especially for the T790M-negative patients (14). Here, a similar treatment regimen was performed, while progression continued again after patients received first line gefitinib and third-line chemotherapy, although *EGFR* T790M mutation was detected by ddPCR not in blood NGS before. And there was also no response to second line osimertinib,

suggesting that it was likely to be a false positive mutation, and blood NGS results seem more reliable. Compared with the traditional detection methods, the sensitivity of NGS has been significantly improved with a reported detection sensitivity of 0.1–1.0%. And it can detect multiple genes simultaneously, including point mutation and insertion/deletion, copy number variation and chromosome rearrangement (15). Thus, more comprehensive and precise AR mechanisms have been discovered. In this study, an alternative arrangement of resistance such as *BRAF* V600E mutation, *MET* amplification, and *EGFR* T790M mutation was detected. Noteworthily, a *RICTOR* amplification was found finally in the CSF using NGS test, which occurs in 13% (132/1,016) of patients with lung cancers from the

TCGA database. And another independent series revealed that 8% (85/1,070) of lung cancer patients with *RICTOR* amplification. Of these, 26% (22/85) of these patients had alterations in *EGFR* (16-19).

Interestingly, *RICTOR* amplification is associated with sensitivity to *mTOR*1/2 inhibitors. The index lung cancer patient was stable after receiving treatment with *mTOR*1/2 inhibitors (20), whether it can provide a reference for the next treatment plan for the patient in our study.

Besides, as of October 2016, the FDA approved the first line pembrolizumab for PD-L1 high-expression tumor patients with a score of ≥50%. At present, the examination of tumor PD-L1 level was also considered the standard of care for advanced metastatic NSCLC. Interestingly, our case receiving multiple rebiopsies showed dynamism in PD-L1 expression. Similar to previous cases, suggesting that PD-L1 expression may also be affected by *EGFR*-TKIs, just as T790M status was affected by *EGFR*-TKI exposure (21,22). Our results from multiple biopsies also varied. It may due to that PD-L1 expression is spatially heterogeneous (23), or its expression can also be regulated by *EGFR*-TKIs treatment (24,25). Therefore, multiple biopsies may be desirable to examine PD-L1 expression as a predictor of immunotherapy or explore AR mechanisms such as T790M status. In this case report, the first two tissue biopsies of NGS did not reveal the T790M mutation, whereas the recent CSF NGS test showed a T790M mutation. The missing of T790M mutation in tumor tissue may be due to limited tissue available from the lung as well as inter- and intra-tumor heterogeneity, where the tissue biopsy does not provide a comprehensive understanding of the genetic heterogeneity of the entire tumor or metastatic disease (26). Of course, the progression of alternative resistance mechanisms may also lead to such results. Meanwhile, the T790M mutation detected in liquid biopsy may be due to the nature of the metastatic disease and the diversity of ctDNA population, which represents the overall mixed heterogeneity of the disease rather than being confined to a single small tissue sample (26). On that basis, we were able to make an informed clinical decision to start using osimertinib, and patients responded.

In summary, this case gave us some important hints for early NSCLC diagnosis and treatment. Firstly, biomarker analysis should be regularly considered part of the resistance mechanism and initial molecular diagnosis, particularly with NGS technique. Secondly, liquid biopsy, including CSF liquid biopsy, could be a promising method for the diagnosis of resistance mechanisms with metastatic disease.

Finally, the dynamic monitoring of the tumor genome by multiple biopsies can name tumor driving genes and drug resistance mechanisms to guide tumor treatment.

Acknowledgments

Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/tlcr.2020.01.21). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The Research Ethics Committee approved this study of Shanxi Provincial Cancer Hospital. The written informed consent was obtained from the patient for specimen collection, genetic testing, and her information used for research.

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