Clinical Case Report

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Elevation of tumor mutation burden in ROS1-fusion lung adenocarcinoma resistant to crizotinib

A case report

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Abstract

Rationale: Although most of non-small cell lung cancer (NSCLC) patients with ROS1-fusions respond to crizotinb, acquired resistance eventually develop. The next-generations of ROS1 inhibitors have made some achievements, but the effects of immunotherapy have not been explored.

Patient concerns: A 44-year-old Chinese women presented with cough and dyspnea with a history of advanced lung adenocarcinoma.

Diagnosis: A PET/CT scan revealed primary tumors in bilateral lung lobes and multiple metastases in lymph nodes and bones. And ultrasound-guided left cervical lymph node biopsy revealed the pathological diagnosis was poor differentiated lung adenocarcinoma.

Interventions: The patients was started to be treated with 4 cycles of pemetrexed, carboplatin and bevacizumab, followed by one cycle of docetaxel, cisplatin and bevacizumab. As the ROS1-fusion was found by next generation sequencing, the patient received crizotinib treatment about 3 months.

Outcomes: After 5 cycles of chemotherapy, CT scans revealed increased size of bilateral lobe nodules indicative of progressive disease (PD). Then the patient received treatment of crizotinib and his progression-free survival reached 3 months. Due to uncontrollable disease progression, the patient expired.

Lessons: The genetic profile of NSCLC patients might be altered in various therapeutic processes. Thus, repeated genetic testing might be important at each progression. Moreover, immunotherapy might be a powerful weapon to overcome the resistance to Tyrosine kinase inhibitors (TKIs) in future.

Abbreviations: IHC = immunohistochemistry, MAF = mutation allele frequency, NGS = next generation sequencing, NSCLC = non-small-cell lung cancer, ORR = objective response rate, PD = progressive disease, PD-1 = programmed death-1, PD-L1 = programmed death-ligand 1, PR = partial remission, SD = stable disease, TKI = Tyrosine kinase inhibitors, TMB = tumor mutation burden.

Keywords: crizotinib, immune checkpoint inhibitor, ROS1-fusion, TMB

1. Introduction

ROS1 fusions define a unique molecular subgroup of non-small cell lung cancer (NSCLC), accounting for approximately 2% of patients with NSCLC.[1] While most of these patients respond to crizotinib, resistance to this therapy eventually develops. Therefore, novel strategies are urgently needed.

The second-generation ROS1 inhibitors have shown no obvious benefit in crizotinib-resistant NSCLC patients.[2,3] As the third-generation ROS1 inhibitor, lorlatinib showed a 50% objective response rate in ROS1-fusion NSCLC patients, irrespective of crizotinib resistance.[4] However, the role of immunotherapy in patients with crizotinib resistance has not been reported.

Herein, we reported a significant elevation of tumor mutation burden (TMB) in a ROS1-fusion NSCLC patient who progressed on crizotinib. As TMB is a recognized predictive biomarker for response to immune checkpoint blockade in NSCLC,[5] we speculated that PD-1 or PD-L1 antibodies might be a fine selection for this patient.

2. Case description

A 44-year-old Chinese female presented with discontinuous dry cough and enlargement of left cervical lymph nodes in November 2017. A PET/CT scan revealed that:

1) a tumor in the left upper lobe with multiple small metastases in bilateral lung lobes
2) metastases in C6 and T9 vertebra;
3) metastasis in bilateral parietal lobes, left frontal lobe and cerebellum; and
4) metastases in bilateral hilar and mediastinal lymph nodes, bilateral cervical and supraclavicular lymph nodes.

Then, ultrasound-guided left cervical lymph node biopsy revealed poorly differentiated lung adenocarcinoma, and immu-

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Tao Yang and Rui Xu These two authors were equally contributed to this article.

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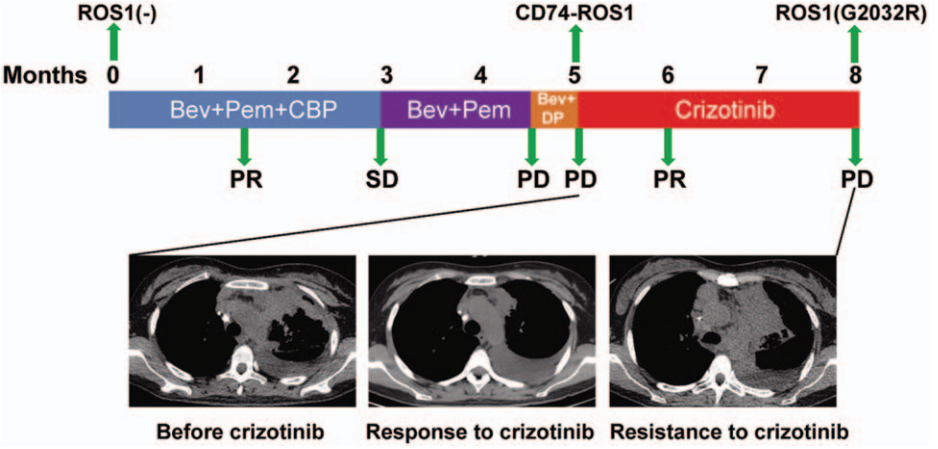

Figure 1. Timeline of therapy in months including treatment process, genomic testing, and disease status. Bev = Bevacizumab, C = carboplatin, D = Doxtaxel, P = cisplatin, PD = progressive disease, Pem = pemetrexed, PR = partial response, SD = stable disease.

nohistochemistry (IHC) indicated the following phenotype: CK5/6(+), TTF-1(+), NapsinA(+), Ki-67(30%+), P63(scattered+), P53 (−), P40(−), and ALK-L(−) (Ventana method). Thus, the staging of this patient was IVb stage (cT4N3M1c). EGFR mutations were not found in puncture biology of lymph node by genetic testing. Furthermore, a next-generation sequencing (NGS)-based ctDNA genetic testing on blood also showed no mutations or rearrangement of EGFR, ALK, ROS1 and BRAF. Thus, the patient was treated with 4 cycles of pemetrexed, carboplatin and bevacizumab. CT and MRI scans performed 2 cycles of treatment indicated a partial remission (PR), and subsequent scans performed after 4 cycles of treatment showed a stable disease (SD). After 2 cycles of maintained treatment of pemetrexed and bevacizumab, CT scans revealed increased size of the left upper lobe nodules and emerging pleural effusion indicative of progressive disease (PD) (See treatment timeline, Fig. 1).

Considering the resistance of first-line treatment, 1 cycle of docetaxel, cisplatin and bevacizumab was followed on April 12, 2018. Meanwhile, the second NGS-based ctDNA genetic testing was performed on blood sample. Surprisingly, 22 novel gene mutation sites were revealed, including CD74-ROS1 fusion, CDKN2A, TP53 and so on (Fig. 2A). Furthermore, TMB was also increased in this patient after chemotherapy (Fig. 2B). Then, a CT scan revealed increased size of bilateral lobe nodules indicative of PD again, and the patient was switched to crizotinib. Crizotinib (250 mg bid, oral) therapy was initiated on April 30, 2018. After 4 weeks of crizotinib treatment, repeat CT imaging revealed dramatic decrease and shrinkage of bilateral lobe nodules and mediastinal lymph nodes. Thus, the patient achieved a confirmed partial remission (PR) after 4 weeks of crizotinib treatment.

Over the next 2 months, the patient continued on crizotinib treatment. Then, a CT scan revealed increased size of the left upper lobe nodules and pleural effusion indicative of PD again. To determine the resistance mechanisms, the third NGS was performed on pleural fluid specimen and identified an acquired ROS1 G2032R (4.1% MAF) mutation and KIT (11.5% MAF) mutation (Fig. 2A). In particular, a significant elevation of TMB was observed in crizotinib-resistant sample compared with the pre-crizotinib one (21.0 Muts/Mb vs 5.8 Muts/Mb, Fig. 2B). Recently, TMB has been recognized as a predictive biomarker for response to immune checkpoint blockade in many tumors. Therefore, the patient was planned to be treated by the immune checkpoint inhibitor, but she developed respiratory failure and passed away on August 15, 2018.

3. Discussion

The discovery of driven genes in NSCLC, including EGFR mutations and ALK/ROS1 rearrangement, has launched a new anti-tumor era-molecular targeted therapy. However, the mutations profile in NSCLC might be altered in various therapeutic processes. Previous studies have shown that chemotherapy might lead to the mutual switch between EGFR wild and EGFR mutations,[6] but few studies reported the influence of chemotherapy on ROS1 alteration. Here, we identified a CD74-ROS1 fusion and a series of gene mutations caused by chemotherapy, which provided the opportunity of

Genes(MAF)	Ann acid	Pre-chemotherapy (blood)	Pre-crizotinib (blood)	Post-crizotinib (pleura fluid)
CD74-ROS1	fusion	ND	9.1%	28.8%
ROS1	p.G2032R	ND	ND	4.1%
CDNK2A	p.E10Lfs*4	ND	9.2%	31.7%
TP53	p.R342Efs*3	ND	13.1%	35.5%
KIT	-	ND	ND	11.5%

A

	TMB (Muts/Mb)
Pre-chemotherapy	0
Pre-crizotinib	5.8
Post-crizotinib	21.0

B

Figure 2. (A) Genomic alterations detected in samples from patient with ctDNA assays. (B) Alterations of TMB at each progression of treatment with ctDNA assays.

targeted therapy. These findings indicate that dynamic monitoring of gene mutations might be benefit for NSCLC patients.

As the first-generation of ROS1 inhibitor, crizotinib showed marked antitumor activity in patients with advanced ROS1-rearranged NSCLC (ORR 72%, mPFS 19.2m).[7] However, a resistant tumor eventually emerges. Extensive studies have explored the underlying mechanisms of crizotinib resistance, including ROS1 mutations,[8,9] KIT mutation,[10] BRAF mutation[11] and CDKN2A mutation.[12] In this report, we found a ROS G2032R mutation-induced resistance, and PFS was only 3.0 months. The second- and third-generations of ROS1 inhibitors have developed recently, but the ORR of crizotinib-resistant patients were not very satisfactory. Ceritinib showed no obvious benefit for crizotinib-pretreated patients.[2] Although 29% (2/7) of ORR was observed in crizotinib-pretreated patients in a phase 1 trial of lorlatinib,[4] more data remain to be verified in further studies.

In last decade, immunotherapy has shown a dramatic prospect in second- or even first-line treatment of many tumors, including NSCLC. Emerging evidences have proven that PD-L1 expression and TMB was lower in NSCLC patients with driven gene mutations (EGFR, ALK, ROS1, and BRAF) than those with wild-type.[13,14] As the most 2 important biomarkers for response to immunotherapy, the low level of PD-L1 and TMB might be the main reason for the failure of combined therapy of immune checkpoint inhibitor and Tyrosine kinase inhibitors (TKIs). However, few studies focused on the efficacy of immunotherapy on post-targeted therapy. Recently, a retrospective study reported that in EGFR-mutant NSCLC patients receiving first/second-generation EGFR TKIs, TMB was increased in post-progression samples compared to that in paired pre-treatment ones.[15] These findings suggest that immune checkpoint blockage might be a fine choice for NSCLC patients post-progression on EFGR TKIs. In this report, we also found a significant increase of TMB in a ROS1-fusion patient after crizotinib resistance, indicating the effectiveness of immunotherapy. Unfortunately, the tumor developed too rapid to use the PD-1/PD-L1 antibodies. A recent case that pembrolizumab treatment was effective in a ALK-positive NSCLC patient resistant to ALK-TKI treatment might further support our hypothesis.[16]

In conclusion, as more patients are treated with mutant-selective TKIs, the secondary mutations will lead to resistance, emphasizing the importance of repeated genomic profiling at each progression. Moreover, immunotherapy might be a powerful weapon to overcome the resistance to TKIs in the future.

Author contributions

The conception and design of the study, Hui Liu and Fang Li; acquisition and interpretation of data, Tao Yang and Bing Yan; writing, review, and revision of the manuscript: Tao Yang and Rui Xu.

Project administration: Hui Liu.
Resources: Fang Li.
Validation: Bing Yan.
Writing – original draft: Tao Yang, Rui Xu.
Writing – review & editing: Tao Yang.

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