Case Report: Temporal Heterogeneity of *ALK* Activating Mutations in Sequential ALK TKI–Treated Non–Small-Cell Lung Cancer Revealed Using NGS-Based Liquid Biopsy

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Clinical Practice Points

- Mutation profiling using liquid biopsy has become a promising approach for monitoring tumor genomic evolution when tissue biopsy is not available.
- Longitudinal monitoring of circulating tumor DNA mutation profiles along the treatment course of different tyrosine kinase inhibitors (TKIs) revealed temporal heterogeneity of anaplastic lymphoma kinase (*ALK*)-activating mutations, which could provide guidance for sequential use of ALK TKIs in *ALK*-positive non–small-cell lung cancer (NSCLC) patients.
- Although sequential TKI therapy showed clinical benefit to *ALK*-positive NSCLC patients, it could accelerate the stepwise accumulation of *ALK* resistance mutations, which might be very difficult to overcome. The selection for optimal first-line TKI is critical for patients to achieve better overall clinical outcome.

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Introduction

With the development of targeted drugs and next-generation sequencing (NGS) technology, the treatment paradigm of non–small-cell lung cancer (NSCLC) has evolved into oncogene-targeted precision medicine.[1] Another revolutionary advance in treating NSCLC is biomarker profiling using liquid biopsy.[2] High throughput NGS-based liquid biopsy testing overcomes spatial heterogeneity beyond a single tissue biopsy, provides us with more comprehensive genomic information, and has become a promising alternative for dynamically monitoring tumor genomic evolution and guiding decision-making for treatment strategy in a minimally invasive manner.

In NSCLC, chromosome rearrangements in anaplastic lymphoma kinase (*ALK*) have been one of the earliest well characterized oncogenic alterations, which cause a constitutional activation of the ALK kinase domain and accounted for 3% (8 in 305) to 7% (5 in 75) of NSCLCs.[2] Crizotinib is the first oral ALK tyrosine kinase inhibitor (TKI) approved for treating *ALK*-positive NSCLCs.[2] However, almost all treated patients inevitably developed resistance within 12 months and showed disease progression. Second-generation ALK TKIs, including ceritinib,[3] alectinib,[4] and brigatinib,[5,6] have received approval from the US Food and Drug Administration for the treatment of *ALK*-rearranged NSCLC to overcome the acquired resistance of crizotinib-pretreated *ALK*-positive patients. Brigatinib has been reported to overcome crizotinib-resistant *ALK* G1202R mutation in preclinical models[7] and active in vitro against many other ALK kinase domain mutations including C1156Y, I1171S/T, V1180L, L1196M, L1152R/P, E1210K, and G1269A.[8,9] The third-generation ALK TKIs lorlatinib, entrectinib, and ensartinib also showed promising data in terms of clinical activity and safety, and harbors the highest on-target potency with the widest spectrum of activity toward crizotinib resistance mutations.[10,11]

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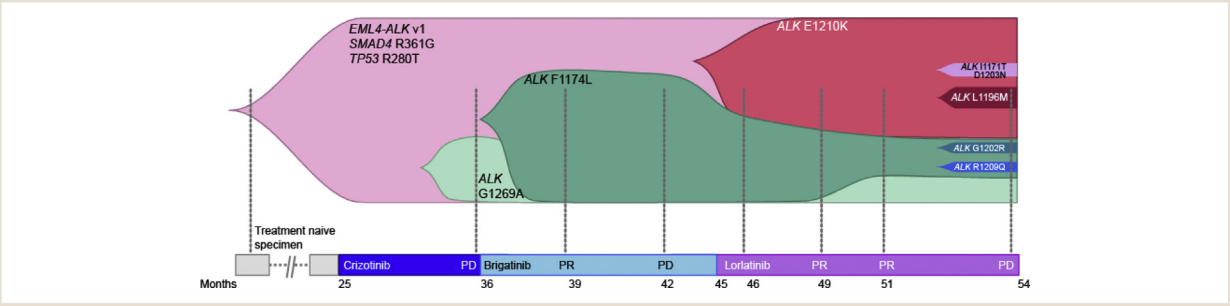
Figure 1 *ALK*-Activating Mutations Detected Along the Disease Course



Abbreviation: ALK = *anaplastic lymphoma kinase.*

Case

A 71-year-old male patient was diagnosed with stage IV lung adenocarcinoma in August 2013 and the primary tumor sample was subjected for targeted NGS for identification of driver mutations. This patient was confirmed ALK positive using immunohistochemistry and NGS, and was receiving sequential TKI treatments (Figure 1). Mutation profiles of circulating tumor DNA (ctDNA) from 7 sequential plasma samples during the treatment course of crizotinib, brigatinib, and lorlatinib were closely monitored using NGS targeting of 422 cancer-relevant genes. The dynamic mutation profiles during the disease course are represented in Table 1 and Figure 1.

Because echinoderm microtubule associated protein like 4-anaplastic lymphoma kinase (*EML4-ALK*) fusion (E13:A20) was detected in the primary tumor sample at a mutant allele frequency (MAF) of 5%, the patient was treated with crizotinib in August 2015 and reached a progression-free survival (PFS) of 11 months when *ALK* G1269A (1.0%) was identified in the patient's ctDNA from plasma upon progression. As a result, brigatinib, a second-generation ALK TKI overcoming G1269A-driven resistance, was applied in August 2016 at a dose of 90 mg once daily. Two months later, the patient achieved stable disease. A dramatic decrease of G1269A to an undetectable level was observed in the ctDNA sample and the level of *EML4-ALK* fusion also dramatically decreased to 0.6%. At the same time, a newly acquired *ALK* mutation F1174L started to develop (1%) and became the dominant clone (6.8%) with bone and liver metastasis that developed in the patient in January 2017. The disease progressed after 9 months of brigatinib treatment when an *ALK* mutation E1210K emerged (7%), and an elevated F1174L (8.7%) mutation, and *EML4-ALK* fusion (8.5%). The patient was then switched to lorlatinib (100 mg once daily) in April 2017 and achieved a partial response 3 months later. *EML4-ALK* fusion decreased to a MAF of 1.1% 4 months after lorlatinib treatment, but quickly went back to 13.8% when the tumor progressed in December 2017. *ALK* mutation E1210K (18.8%) quickly overtook F1174L (5%) as the dominant clone with the accumulation of more concomitant mutations with relative lower MAFs toward the end of the treatment with a PFS of 9 months. The concomitant *ALK* mutations include L1196M (1.5%), G1202R (0.4%), D1203N (2.3%), I1171T (2.6%), and R1209Q (0.4%). Brigatinib-sensitive G1269A also came back at 4.6% after 6 months of lorlatinib treatment.

Other gene mutations detected in the plasma samples during the treatment were mothers against decapentaplegic homolog 4 (*SMAD4*) mutation R361G, tumor protein 53 (*TP53*) mutation R280T, mesenchymal to epithelial transition gene (*MET*) W1251Q, and c-ros oncogene 1 (*ROS1*) A2335S. *SMAD4* mutation R361G was found at a MAF of 14% in the primary tumor sample whereas *TP53* mutation R280T was not detected. R361G gradually decreased when the patient underwent crizotinib treatment and R280T was detected at similar frequency as R361G at the same time. The MAF of R361G went back to 16.9% at the end of brigatinib treatment with R280T also increased up to 17.2%. Both mutations decreased again when lorlatinib treatment started and reached peak level (R361G, 32.8%; R280T 35.7%) when the patient progressed during lorlatinib treatment. *MET* W1251Q was developed when the patient started lorlatinib treatment and increased up to 21.0% in a progressive disease (PD) sample. *ROS1* A2335S was only detected in a PD sample (5.5%) at the end of lorlatinib treatment.

Discussion

Acquired resistant mechanism against crizotinib in lung cancer patients can be characterized into 3 categories: *ALK* secondary mutations, *ALK* amplification, and *ALK* nondominant resistance. Among them, *ALK* secondary mutations account for nearly 45% of acquired crizotinib-resistant cases.[12] In this case report, we analyzed the potential resistance *ALK* mutation during sequential use of ALK TKIs (crizotinib, brigatinib, and lorlatinib) in a stage IV lung cancer patient.

Most *ALK* resistance mutations we report in this patient were also reported by other groups, including G1269R,[13] F1174L,[14] E1210K,[9,15] L1196M,[16] G1202R,[9] D1203N,[17] and F1171T.[18] The gatekeeper L1196M is the most common and well characterized crizotinib mutation with the ability to impair the bind between crizotinib and the adenosine triphosphate (ATP) pocket of the target *EML4-ALK* fusion protein, similar to T790M epidermal growth factor receptor (*EGFR*)-positive NSCLC.[16] The mutation G1269A is the second most frequent acquired mutation at the ATP binding site, which interferes with binding of crizotinib to block the

Table 1 Genetic Alterations Detected in ctDNA Samples of the Patient Along the Disease Course

Gene	Variation Detected	Diagnose Date (0 mo)	TKI Treatment						
			Crizotinib (24-35 mo)	Brigatinib (36-44 mo)			Lorlatinib (44-52 mo)		
			Disease Evaluation						
		At Diagnosis (0 mo)	PD (35 mo)	SD (38 mo)	SD (41 mo)	PD (44 mo)	PR (47 mo)	PR (49 mo)	PD (52 mo)
ALK	EML4:exon 13-ALK:exon20	5.0%	4.0%	0.6%	2.4%	8.5%	1.1%	0.9%	13.8%
ALK	G1269A	-	1.0%	-	-	-	-	0.6%	4.6%
ALK	F1174L	-	-	1.0%	6.8%	8.7%	2.6%	2.8%	5.0%
ALK	E1210K	-	-	-	-	7.0%	0.1%	2.0%	18.8%
ALK	L1196M	-	-	-	-	-	-	-	1.5%
ALK	G1202R	-	-	-	-	-	-	-	0.4%
ALK	D1203N	-	-	-	-	-	-	-	2.3%
ALK	I1171T	-	-	-	-	-	-	-	2.6%
ALK	R1209Q	-	-	-	-	-	-	-	0.4%
SMAD4	R361G	14.0%	3.0%	1.0%	4.5%	16.9%	1.0%	4.0%	32.8%
TP53	R280T	-	3.0%	1.0%	6.2%	17.2%	0.8%	4.6%	35.7%
MET	W1251Q	-	-	-	-	-	1.1%	2.3%	21.0%
ROS1	A2335S	-	-	-	-	-	-	-	5.5%

Mutant allele frequencies are indicated. - Indicates not detected.

tyrosine kinase domain of target fusion protein.[13] Resistant *ALK* mutant G1202Rs are often found in ceritinib-treated relapsed tumors[15] and G1202R together with E1210K were also reported as potential mechanisms of clinical resistance to brigatinib.[9] *ALK* mutation F1174L affected crizotinib resistance to cancers harboring *EML4-ALK* translocations and preclinical studies suggested that higher doses of crizotinib could overcome the F1174L-mediated resistance.[14] Another 2 *ALK* mutations V1180L and I1171T were also reported to be alectinib-resistant in addition to G1202R.[18,19] All of these *ALK* resistance mutations were only detected in the ctDNA samples because tissue biopsy samples could not be acquired during the treatment course. Dual resistance mechanisms of *ALK* resistance mutation G1202R in ctDNA and small-cell lung cancer transformation resistance mechanisms in tissue samples has been reported during treatment with ALK inhibitors,[20] therefore, we could not rule out the possibility that there might be other resistance mechanisms present in the tissue samples because of the lack of tumor biopsy samples for histology analysis.

Although the sequential TKI treatment from first- to second- to third-generation ALK TKIs showed improved clinical outcomes, acquired resistance mutation inevitably developed and reached a point where no ALK TKI can be used to overcome. In this patient, classic crizotinib-resistant mutation G1269A, mutation F1174L, and mutation E1210K were 3 dominant acquired *ALK* resistance mutations that developed along course of the sequential TKI treatment. Brigatinib and lorlatinib were used after crizotinib to overcome the acquired resistance, however, at the time of this study, brigatinib and lorlatinib were not approved for use in China, so the 2 TKIs used in this case were purchased from India which might have some effect on the efficiency of TKI treatment in this patient.

Clonal analysis revealed that the *ALK* mutant clone F1174L emerged during brigatinib treatment and gave rise to 2 resistant mutant subclones *ALK* G1202R and R1209Q. The other *ALK* mutant clone E1210K emerged during lorlatinib treatment and gave rise to 1 compound resistant mutant subclone *ALK* I1171T/D1203N and 1 resistant mutant subclone *ALK* L1196M (Figure 1). Numerous *ALK* resistance mutations accumulated at the end of crizotinib-brigatinib-lorlatinib treatment, which highlighted that sequential TKI therapy accelerated the stepwise accumulation of *ALK* resistance mutations, which might be very difficult to overcome when established. Thus, the selection for optimal first-line TKI is very important for patients to achieve better clinical outcome. Yoda et al reported similar results in *ALK*-positive lung cancer patients and they suggested that lorlatinib might be used as the upfront treatment to completely suppress or at least significantly delay on-target resistance.[21]

The *TP53* gene mutation has been reported in approximately 50% of lung cancer patients[22] and ranges from 22% (2 in 9) to 41% (84 in 205) in lung adenocarcinoma patients.[23,24] Furthermore, nondisruptive *TP53* mutations are considered independent markers of shorter overall survival in patients with advanced NSCLC regardless of *EGFR* and kirsten rat sarcoma viral oncogene homolog mutation status.[25] In *ALK*-rearrangement NSCLC patients, the *TP53* mutant was also reported to be associated with poor overall survival and shorter PFS during crizotinib treatment.[26] In this case, *TP53* mutant R280T was detected in the patient ctDNA samples along the treatment course and eventually increased up to 35.7% at end of sequential TKI treatment. However, PFS for crizotinib treatment in this case seemed normal compared with PFS reported by another group, which was 10.9 months.[27] The PFS for brigatinib treatment was shorter compared with the reported 12.9 months of PFS in crizotinib-refractory *ALK*-positive NSCLC patients.[28] As for lorlatinib, the PFS was also shorter compared with the reported 16 months of PFS in a

single patient who underwent sequential crizotinib-brigatinib-lorlatinib treatment.[21]

Conclusion

Herein, we report a case with temporal heterogeneity of *ALK*-activating mutations along the treatment course of different TKIs via NGS-based liquid biopsy. The patient underwent crizotinib, brigatinib, and lorlatinib treatment and developed different *ALK* resistance mutations. In conclusion, although sequential TKI therapy showed clinical benefit to an *ALK*-positive NSCLC patient, it could also accelerate the stepwise accumulation of *ALK* resistance mutations, which might be very difficult to overcome when established. As a result, the selection for optimal first-line TKI is very important for patients to achieve overall better clinical outcomes.

Disclosure

Yang W. Shao, Xue Wu, and Ruoying Yu are shareholders or employees of Geneseeq Technology Inc. The remaining authors have stated that they have no conflicts of interest.

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