Relevance of Detection of Mechanisms of Resistance to ALK Inhibitors in ALK-Rearranged NSCLC in Routine Practice

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Abstract

Anaplastic lymphome kinase (*ALK*) mutations are responsible for resistance to ALK tyrosine kinase inhibitors (TKIs) in ALK-rearranged non–small-cell lung cancer but their frequency and relevance has not been assessed in routine practice. In this retrospective multicenter study, the *ALK* mutation rate was 15% and 33% after failure of treatment with 1 and 2 TKIs, respectively. Most of the patients harboring an *ALK* mutation achieved an objective response with subsequent ALK TKI treatment.

Background: Anaplastic lymphoma kinase (ALK) tyrosine kinase inhibitors (TKIs) have shown efficacy in the treatment of ALK-rearranged non–small-cell lung cancer (NSCLC), but the disease eventually progresses in all patients. In many cases, resistance to ALK TKIs arises through *ALK* mutations. Although clinical and biological data suggest variations in TKI efficacy according to the mechanism of resistance, *ALK* mutations are still rarely investigated in routine practice. **Materials and Methods:** We performed a retrospective multicentric study with an aim to determine the frequency and clinical relevance of ALK alterations detected using targeted next-generation sequencing in patients with advanced ALK-rearranged NSCLC after progression during an ALK TKI treatment. Data on clinical, pathological, and molecular characteristics and patient outcomes were collected. **Results:** We identified 23 patients with advanced ALK-rearranged NSCLC who, between January 2012 and May 2017, had undergone at least 1 repeat biopsy at progression during an ALK TKI treatment. A resistance mechanism was identified in 9 of the 23 patients (39%). The anomalies involved included 9 *ALK* mutations in 8 patients and one *ALK* amplification. The *ALK* mutation rate was 15% after failure of a first ALK TKI and 33% after failure of 2 ALK TKI treatments. Five of 7 patients who received a different ALK TKI after detection of an *ALK* mutation achieved an objective response. All of the patients who received a TKI presumed to act on the detected ALK mutant achieved disease control. **Conclusion:** Targeted next-generation sequencing is suitable for detecting ALK resistance mutations in ALK-rearranged NSCLC patients in routine practice. It might help select the best treatment at the time of disease progression during treatment with an ALK TKI.

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Introduction

Anaplastic lymphome kinase (*ALK*) rearrangements are found in approximately 4% of nonsquamous non–small-cell lung cancer (NSCLC).[1] The fusion protein resulting from ALK rearrangements has a constitutively activated ALK kinase leading to activation of downstream signaling pathways involved in cell proliferation and cell survival.[2,3] The discovery of ALK rearrangements has led to the development of tyrosine kinase inhibitors (TKIs) targeting the ALK kinase. Crizotinib and ceritinib have shown superiority over chemotherapy in untreated advanced ALK-rearranged NSCLC patients, and alectinib has shown superiority over crizotinib in the same context.[4-6] Several TKIs have proved active after failure of crizotinib treatment, including ceritinib, alectinib, brigatinib, and lorlatinib.[7-10]

Mechanisms of resistance to ALK TKIs in ALK-rearranged NSCLC patients are partly known and are of 2 main types: (1) on-target mechanisms, including mutation of the ALK kinase domain and amplification of the ALK-rearranged gene; and (2) off-target mechanisms, including activation of an alternative signaling pathway such as epidermal growth factor receptor (EGFR) or stem cell growth factor receptor (SCFR/KIT) and pathological transformation.[11]

Anaplastic lymphome kinase mutations are the main cause of resistance to ALK TKIs. With high-throughput methods of detection,[11] they are found in 20% to 50% of tumor samples obtained at the time of progression during treatment with an ALK TKI. Numerous ALK kinase mutations can confer resistance to ALK TKIs in ALK-rearranged NSCLC,[12-19] and the frequency and type of *ALK* mutations detected at the time of progression depend on the type of ALK TKI received.[11] Thus, each ALK TKI has its own spectrum of activity against ALK kinase mutants,[20] and a given ALK kinase mutant is not resistant to all ALK TKIs. For example, the L1196M mutation is reported to confer resistance only to crizotinib, the C1156Y mutation is associated with resistance to crizotinib and ceritinib but remains sensitive to alectinib and brigatinib, and the G1202R mutation leads to resistance to most ALK TKIs but not to lorlatinib.[21-24]

Thus, identifying the mechanism of resistance to an ALK TKI might help in choosing the most appropriate treatment after disease progression during treatment with that ALK TKI, particularly because several ALK inhibitors are now available for use in this context. Herein we show that targeted next-generation sequencing (NGS) performed in routine practice is suitable and relevant for detecting *ALK* mutations in tumor tissue samples or circulating tumor DNA from ALK-positive NSCLC patients whose disease has progressed after treatment with an ALK TKI.

Materials and Methods

Study Design and Patients

In this observational, multicentric, retrospective study, we identified patients with advanced ALK-positive NSCLC from whom tumor samples had been obtained at the time of disease progression during treatment with an ALK TKI as part of the routine procedure. Tissue biopsies and circulating tumor DNA samples were considered. Clinical and molecular data were collected retrospectively at each center. The investigators used Response Evaluation Criteria In Solid Tumors 1.1 to evaluate responses to ALK TKIs. The objective response rate was defined as the rate of complete or partial response. The disease control rate was defined as the rate of complete or partial response or stable disease. Overall survival was calculated from the start of the first ALK TKI treatment received.

All of the data reported in this study were extracted from a database approved by the French National Data Protection Authority. This noninterventional study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. It was approved by a national ethics committee and by the French Advisory Committee on Information Processing Related to Research in the Field of Health. All included patients received information from their referring physician.

Detection of ALK *Mutations*

Targeted NGS analysis was conducted using the NGS commercial panel "Ion Ampliseq Colon and Lung Research Panel V2" (Thermo Fisher Scientific, Waltham, MA) at 2 molecular centers certified by the French National Cancer Institute. In the case of tissue samples, genomic DNA was extracted from formalin-fixed paraffin embedded (FFPE) samples received in the 2 laboratories between February 2012 and February 2017. DNA was extracted using the QIAamp FFPE tissue kit (Qiagen, Duesseldorf, Germany) according to the manufacturer's instructions. In the case of blood samples, cell-free DNA was extracted from plasma with the QIAamp Circulating Nucleic Acid Kit (Qiagen) according to the manufacturer's instructions. Extracted DNA was quantified with the Quant-it PicoGreent dsDNA Assay Kit (Thermofisher Scientific) using a Xenius XC spectrofluorometer (Safas Monaco, Monaco). AmpliSeq libraries were prepared with the Ion AmpliSeq Library Kit 2.0 Ion Xpress barcode adapters kit, primers (Ion AmpliSeq custom panel or Colon and Lung V2; Thermofisher Scientific), and 10 ng or less of each DNA sample according to the manufacturer's instructions (see Supplemental Table 1 in the online version). Emulsion polymerase chain reaction, enrichment, and chip loading were performed using an Ion Chef instrument with the Ion PI Hi-Q Chef Kit or the Ion 540 Chef Kit (Thermofisher Scientific). Samples were sequenced with Ion P1 chips using an Ion Proton System or Ion 540 chips on the Ion S5XL system (Thermofisher Scientific). Data were analyzed using the Torrent suite software version 5.2.2 (Thermofisher Scientific). For the *ALK*, *EGFR*, and Kirsten rat sarcoma viral oncogene homolog (*KRAS*) genes, manual inspection of sequences was done using an integrative genomic viewer.

Statistical Analysis

Quantitative variables are expressed as mean (SD) in the case of a normal distribution or as median (interquartile range) otherwise. Categorical variables are expressed as numbers (percentages). The normality of distributions was assessed using histograms and the Shapiro–Wilk test. Survival rates were calculated from the date of introduction of the first ALK TKI until the date of death or last news and derived from Kaplan–Meier survival curves. Medians of survival were calculated with confidence intervals. Statistical testing was conducted at a 2-tailed α level of .05. Data were analyzed using SAS software version 9.4 (SAS Institute, Cary, NC).

Results

The study included 23 patients with advanced ALK-positive NSCLC who had undergone at least 1 biopsy at the time of disease

Table 1 Baseline Characteristics (n = 23)

Characteristic	Value
Median Age (Range), Years	57 (17-70)
Sex, n (%)	
Male	12 (52)
Female	11 (48)
Adenocarcinoma, n (%)	23 (100)
Stage at Diagnosis, n (%)	
III	2 (9)
IV	21 (91)
Never Smoker, n (%)	19 (83)
Number of Lines of Treatment Before First ALK TKI, n (%)	
0	7 (30)
1	13 (57)
2 or more	3 (13)
Type of ALK TKI Received, n (%)	
First generation	22 (96)
Second generation	17 (74)
Third generation	3 (13)

Abbreviations: ALK = anaplastic lymphome kinase; TKI = tyrosine kinase inhibitor.

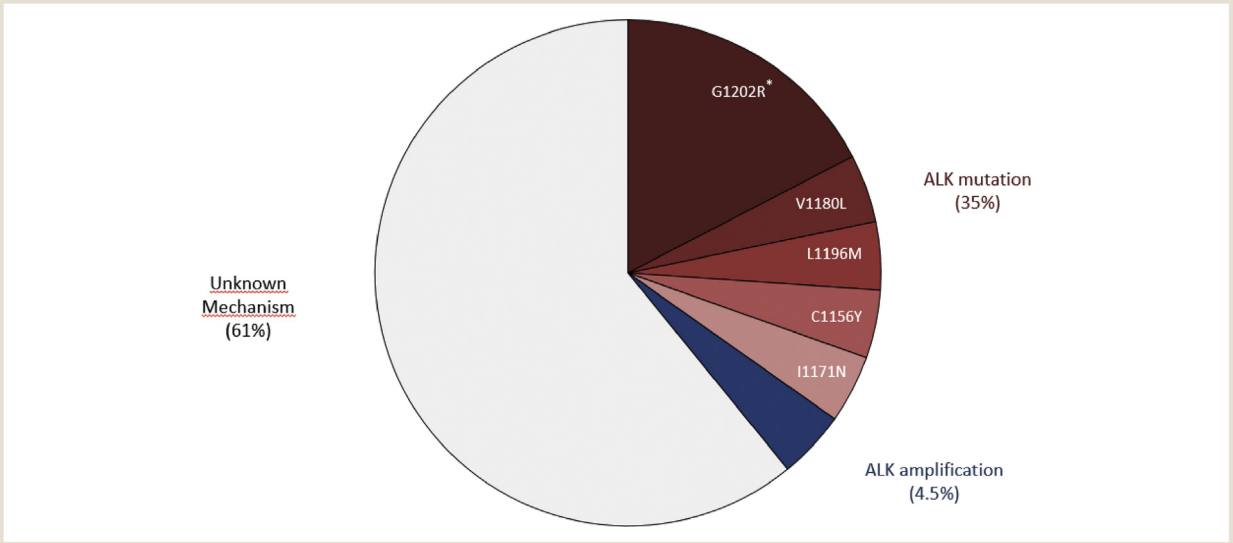
progression during treatment with an ALK TKI between January 2012 and May 2017 (Table 1). The patients were mostly never smokers (n = 19; 83%) and the median age was 57.0 years (range, 17-70 years). Sixteen patients (n = 16; 70%) had received platinum-based chemotherapy before ALK TKI treatment. Twenty two patients (n = 22; 96%) had received crizotinib as the first ALK TKI. A second-generation ALK TKI (alectinib, ceritinib, or brigatinib) had been given to 1 patient (4%) as first-line treatment and to 17 patients (74%) after failure of crizotinib treatment. Three patients who had first received crizotinib and then a second-generation ALK TKI had subsequently received a third-generation ALK TKI (lorlatinib).

The number of biopsies performed on the 23 patients totaled 42, including 26 tissue biopsies and 16 liquid biopsies (cell-free DNA samples). This corresponds to a mean of 1.83 biopsies per patient (range, 1-7). Main biopsy sites were lungs in 8 patients, pleural effusion in 5 patients, and liver in 4 patients (see Supplemental Table 2 in the online version). The samples studied were from 13 of the 23 patients (57%) who had disease progression during treatment with a first TKI, 12 of the 15 patients (80%) who had disease progression during treatment with a second TKI, and 3 of the 7 patients (43%) who had disease progression during treatment with a TKI given as a third TKI or further. The studied samples also included 5 biopsies performed at the time of disease progression during therapies other than ALK TKIs.

The results of targeted NGS applied to these samples are as follows. *ALK* mutations were detected in 8 patients (35%). They included 4 G1202R mutations and 1 C1156Y, 1 V1180L, 1 I1171N, and 1 L1196M mutation. In one of the patients in whom a G1202R mutation was detected initially, a G1202R/G1269A compound mutation was found in a later biopsy. Seven *ALK* mutations were detected in tissue biopsies and 3 in circulating free DNA. For 3 patients, liquid and tissue biopsies were performed at the same time. The results were consistent in 1 patient.

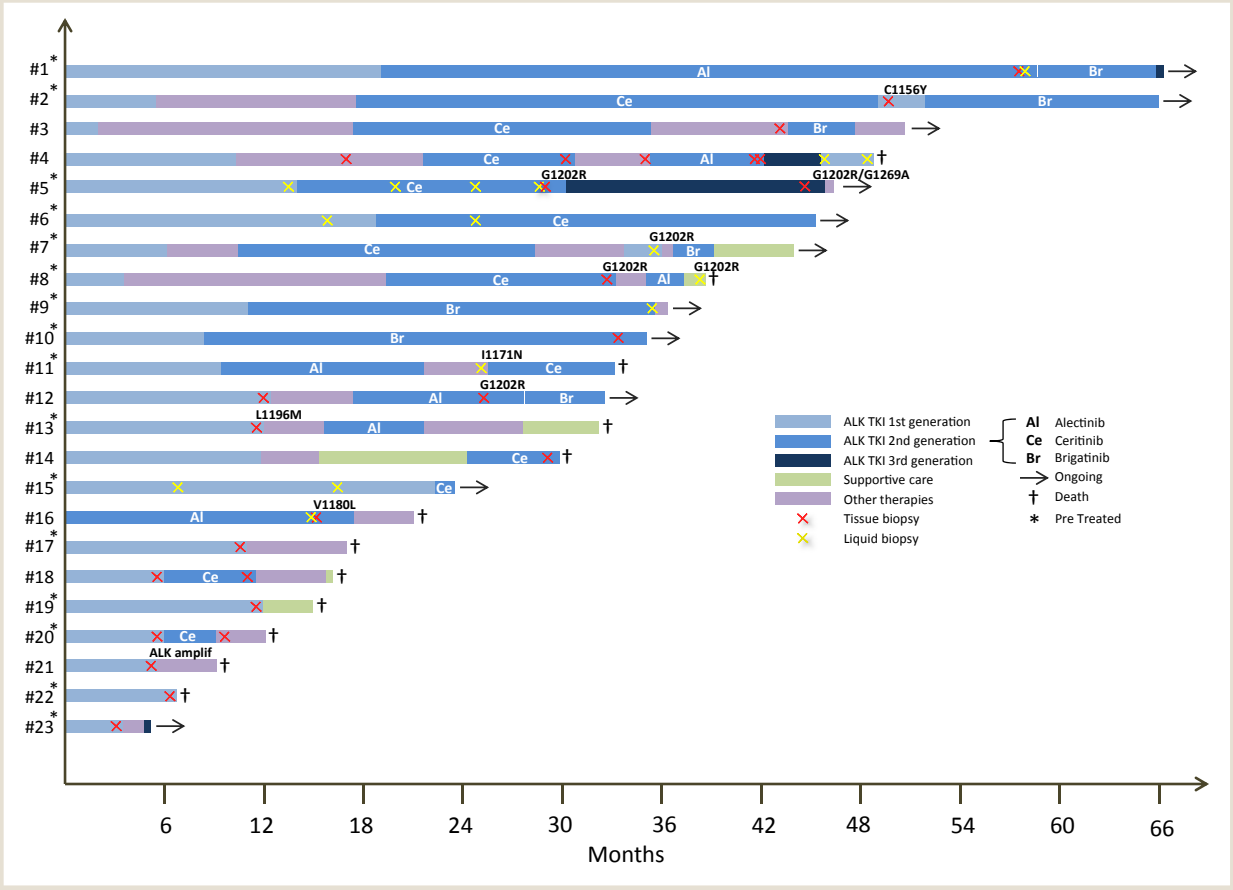
An ALK amplification with no concomitant *ALK* mutation was detected using fluorescence in situ hybridization in 1 biopsy (4.34 %; Figure 1). In addition to an *ALK* mutation, 7 patients had a TP53 mutation (A138P, R175H, C238R, C277G, R248Q, R337C, R337P) and 1 had a N-methyl-N′-nitroso-guanidine human osteosarcoma transforming gene mutation in exon 18 (c.3686_3686 + 3del). No mutation was identified in any other oncogene tested with this NGS panel.

Figure 1 Distribution of Mechanisms of Resistance to ALK TKIs



Abbreviations: ALK = anaplastic lymphome kinase; TKI = tyrosine kinase inhibitor. * One patient with a G1202R mutation was found, after treatment with lorlatinib, to harbor a G1202R/G1269A compound mutation.

Figure 2 Treatment of ALK-Positive NSCLC Patients. Swimming Plot Showing, for Each Patient, the Treatments Received and the Number, Type(s), and Time of Completion of the Biopsies Performed. Each Bar Represents 1 Patient. Treatments Received Before the First ALK TKI are Not Represented



Abbreviations: ALK = anaplastic lymphome kinase; NSCLC = non–small-cell lung cancer; TKI = tyrosine kinase inhibitor.

Among the 9 *ALK* mutations detected, one was found in a sample taken at the time of disease progression during crizotinib treatment as the first TKI. The 8 remaining mutations were identified in samples from patients exposed to a second- or third-generation TKI. The rate of detection of *ALK* mutations at the time of disease progression was 15% after a first TKI (2 of 13 patients tested), 33% after a second TKI (4 of 12 patients tested), and 66% after a third TKI or more (2 of 3 patients tested; Figure 2).

Among patients during treatment with the first ALK TKI, the objective response rate was 74% (17/23) and the disease control rate was 91% (21/23). By the end of data collection, 12 patients (52%) had died. Median overall survival (OS) from the start of the first ALK TKI treatment was 37.28 months (95% confidence interval, 19.58-not reached). We did not observe any difference in OS between patients with or without TP53 mutations. Among the 8 patients in whom an *ALK* mutation was identified, 7 (87%) received a new ALK TKI after the mutation was found (Figure 3).[11] In these 7 patients the objective response rate (ORR) with the new TKI was 71% (5/7). Among the patients in whom no *ALK* mutation was found, the ORR with the third ALK TKI was 33% (1/3).

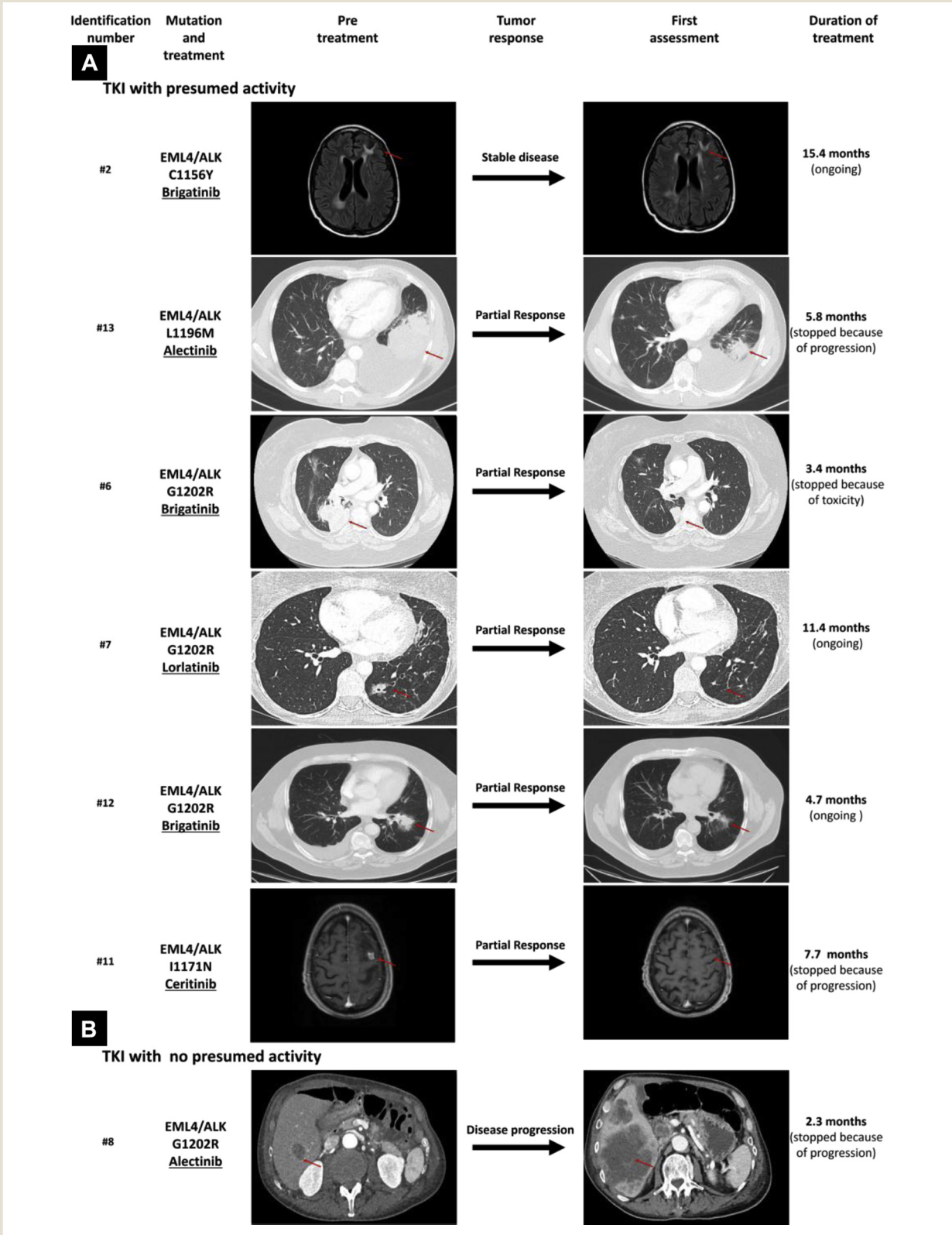
Among the 7 patients with an *ALK* mutation who received a subsequent ALK TKI, 6 received a TKI presumed on the basis of in vitro data to act against the identified mutant.[11] Among these 6 patients, 5 achieved an objective response and 1 showed stable disease. To the remaining patient alectinib was given despite detection of a G1202R mutation, because at the time alectinib was the only available next-generation TKI and data were still sparse regarding the activity of different ALK TKIs according to the *ALK* mutation present. This patient showed progressive disease at first assessment (Figure 3).

Discussion

The present study focused on targeted NGS, a technique now widely used in daily molecular testing. We show that its use in routine practice allowed identifying an ALK TKI resistance mechanism in 9 of 23 patients (39%) with advanced ALK-positive NSCLC. Gainor et al reported a similar rate of *ALK* mutations for other NGS panels.[11] Thus, targeted NGS appears suitable for detecting *ALK* mutations in routine practice.

It is anticipated that *ALK* mutations will be detected more frequently in the near future; Gainor et al have shown that they arise

Figure 3 Clinical Review of Patients Treated With a TKI After Identification of an *ALK* Mutation. Presumed Activity Was Defined by Sensitive or Intermediate IC50 on the Basis of in Vitro Data by Gainor et al[11]



Abbreviations: ALK = anaplastic lymphoma kinase; IC50 = half maximal inhibitory concentration; TKI = tyrosine kinase inhibitor.

more frequently after second- or third-generation TKI treatment (54%-71%) than after crizotinib (20% of patients).[11] Meanwhile 2 second-generation TKIs, ceritinib and alectinib, have shown efficacy as first-line treatment,[4,25] and ongoing clinical trials are currently evaluating next-generation TKIs (NCT03052608, NCT02737501, NCT02767804) in this context. Although no report so far has provided rates of *ALK* mutations after disease progression during treatment with second-generation TKIs given as first-line therapy, it is reasonable to speculate that they will exceed the 20% observed after failure of crizotinib treatment. Data further suggest that the *ALK* mutation rate also increases when several ALK TKIs are given sequentially. Solomon et al reported *ALK* mutation rates of 16.6% (14/84) after first-line ALK TKI treatment and 29.2% (31/106) after treatment with at least 2 ALK TKIs.[26] We have likewise observed a 15% *ALK* mutation rate after treatment failure of a first ALK TKI and a 33% *ALK* mutation rate after failure of 2 ALK TKI treatments. Whether this is because of the number of ALK TKIs administered or to the use of next-generation TKIs remains to be determined.

All of these findings, and because ALK-positive patients have been shown to benefit from receiving multiple TKIs,[8,27,28] suggest that the future will see an increase in the proportion of patients exposed to next-generation ALK TKIs, along with an increase in the rate of *ALK* mutations.

Detection of *ALK* mutations in patients with disease progression after ALK TKI treatment is becoming crucial to determining the optimal therapeutic strategy. In vitro, different *ALK* mutations have been reported to predict different sensitivities to a single ALK TKI, and ALK TKIs do not all share the same spectrum of activity against ALK mutants.[11,29] Similar findings have been reported in patients, although extrapolating from in vitro data to patients requires caution. In 2 patients, for example, the I1171N mutation was reported to drive resistance to alectinib and sensitivity to ceritinib.[30,31] In another patient, the L1152R mutation has on the contrary been reported to confer resistance to ceritinib and to predict a response to alectinib.[32] Consistently, we have found that all 6 patients who were treated with an ALK TKI presumed to act against the detected mutation achieved disease control, whereas the only patient who was treated with an ALK TKI not presumed to act effectively against the detected mutation showed progressive disease at first assessment. Interestingly, 2 patients harboring a G1202R mutation achieved a partial response during treatment with brigatinib, which is reported to exert intermediate activity on G1202R mutants in vitro.

To date, no clinical trial has assessed the clinical relevance of identifying *ALK* mutations at the time of disease progression during treatment with an ALK TKI. Besides the previously mentioned case reports, data from prospective trials suggest that patients harboring an *ALK* mutation are more likely to respond to subsequent treatments with ALK TKIs. In patients who receive the third-generation TKI lorlatinib after failure of treatment with 2 or 3 previous ALK TKIs, Shaw et al reported objective response rates of 26% in patients with no *ALK* mutation and 61% in patients with at least 1 *ALK* mutation.[33] Interestingly, we have found a similar trend in our set of patients treated in routine practice: we observed a 71% ORR with subsequent TKI treatment in patients known to have an *ALK* mutation and a 33% ORR in patients with no *ALK* mutation, after treatment with a third TKI; the presence of an *ALK* mutation might reveal sustained dependence on the ALK pathway and thus predict sensitivity to ALK inhibition,[26] and the type of *ALK* mutation should guide the choice of the ALK TKI to be used. Inversely, the absence of any *ALK* mutation suggests that resistance might be because of an ALK pathway-independent mechanism that is not sensitive to ALK inhibition. Various ALK-independent resistance mechanisms have been described, including activation of EGFR, insulin growth factor 1 receptor, and viral oncogene homolog, mutation of *KRAS* or phosphatidylinositol 3-kinase, amplification of stem cell growth factor receptor (*SCFR*/*cKIT*) and gene fusions.[12,13,18,34-37] In in vitro studies, cells having acquired resistance to an ALK TKI via an ALK-independent mechanism showed cross-resistance to other ALK TKIs, but sensitivity to ALK TKIs could be restored by combined treatment with another TKI.[34,37,38] Efficacy of ALK TKI in patients having acquired resistance to a given ALK TKI via an ALK-independent mechanism is still poorly known.[37] Patients having progressed during crizotinib treatment with no evidence of an *ALK* mutation have been shown to benefit from second-generation ALK TKIs,[39] but whether their resistance was because of an ALK-independent mechanism, an ALK-dependent mechanism not involving an *ALK* mutation, or an *ALK* mutation that escaped detection because of sensitivity issues remains unknown.

Sequential treatment with several ALK TKIs might select resistant clones harboring multiple (ie, compound) *ALK* mutations. In a recent study, Yoda et al reported a 35% compound mutation rate in patients treated with lorlatinib[40]; in our set, 2 patients had disease progression with lorlatinib treatment and a compound mutation was detected at the time of disease progression in a patient known to harbor a G1202R mutation. In vitro, compound mutations have been reported to confer resistance to most ALK TKIs, including lorlatinib.[40] However, a patient having acquired resistance to crizotinib, ceritinib, and lorlatinib successively and who was found to harbor a C1156Y/L1196M compound mutation achieved an objective response with crizotinib treatment. It is necessary to further determine the sensitivity of compound mutants to ALK TKIs.[16]

Finally, other molecular alterations might affect patient outcomes. Recent findings have shown that TP53 mutations or ALK variants might be associated with shorter progression-free survival and OS in ALK-positive NSCLC patients.[41,42] In our study, no difference in OS was found between patients with or without TP53 mutations, but the number of patients in each subgroup was too low to draw any conclusion from this result. ALK variants were not assessed in our study.

Conclusion

Our findings show that mechanisms of resistance to ALK TKIs can be detected in routine practice in a third of ALK-positive NSCLC patients. Identifying ALK resistance mutations might help in selecting the best subsequent treatment.

Clinical Practice Points

- Anaplastic lymphome kinase (*ALK*) mutations confer resistance to ALK TKIs in ALK-rearranged NSCLC. The frequency and type of *ALK* mutations that occur at progression depends on the type of ALK TKI received. These mutations are still rarely investigated in routine practice.
- Using targeted NGS, we identified 8 patients harboring an *ALK* mutation among 23 ALK-rearranged NSCLC patients whose

disease progressed during ALK TKI treatment. Our results suggest that targeted NGS is suitable for detecting ALK resistance mutations in ALK-rearranged NSCLC patients in routine practice.

- The *ALK* mutation rate was 15% after failure of the first ALK TKI and 33% after failure of treatment with 2 ALK TKIs. Furthermore, all of the patients who received a TKI presumed to act on the detected ALK mutant achieved disease control, suggesting that the identification of *ALK* mutation could help to select the best treatment at progression.

Disclosure

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Supplemental Data

Supplemental tables accompanying this article can be found in the online version at https://doi.org/10.1016/j.cllc.2019.02.013.

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Supplemental Data

Supplemental Table 1 List of Genes and Exons Tested in the Colon & Lung V2 (Thermofisher Scientific, Waltham, MA) Panel

Gene	Exon
AKT1	3
ALK	22, 23
BRAF	11
CTNNB1	3
DDR2	6, 9, 13-16, 18
EGFR	12, 18-21
ERBB2	19-21
ERBB4	3-4, 6-9, 15, 23
FBXW7	5, 8-11
FGFR1	4, 7
FGFR2	7, 9, 12
FGFR3	7, 9, 14, 16, 18
KRAS	2-4
MAP2K1	2
MET	2, 16
NOTCH1	1, 3, 6, 7, 8
SMAD4	3, 5-6, 8-12
STK11	1, 4-6, 8
TP53	2, 4-8, 10

Supplemental Table 2 Sites of Tissue Biopsy (n = 26)

Biopsy Site	n (%)
Lung	8 (30.8)
Pleural Effusion	5 (19.2)
Liver	4 (15.4)
Breast	2 (7.7)
Lymph Node	2 (7.7)
Brain	1 (3.8)
Cerebrospinal Fluid	1 (3.8)
Bone	1 (3.8)
Peritoneal	1 (3.8)
Pericardial Effusion	1 (3.8)