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

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Background

ALK Tyrosine Kinase Inhibitors (ALK TKIs) have demonstrated 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.

Material and methods

We performed a retrospective multicentric study aiming to determine the frequency and clinical relevance of ALK alterations detected by targeted Next-Generation Sequencing (t-NGS) in patients with advanced ALK-rearranged NSCLC following progression on an ALK TKI. 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 one repeat biopsy at progression on an ALK TKI. 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 two ALK TKIs. Five out of seven patients who received a different ALK TKI following detection of an ALK mutation achieved an objective response. All the patients who received a TKI presumed to act on the detected ALK mutant achieved disease control.

Conclusions

Targeted NGS is suitable for detecting ALK resistance mutations in ALK-rearranged NSCLC patients in routine practice. It may help select the best treatment at progression on an ALK TKI.

1. Introduction

ALK rearrangements are found in approximately 4% of non-squamous 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 demonstrated superiority over chemotherapy against untreated advanced ALK-rearranged NSCLC patients, and alectinib has demonstrated superiority over crizotinib in the same context [4–6]. Several TKIs have proved active following crizotinib failure, 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 two main types: i) on-target mechanisms, including mutation of the ALK kinase domain and amplification of the ALK-rearranged gene and ii) off-target mechanisms, including activation of an alternative signaling pathway such as EGFR or KIT and pathological transformation [11].

ALK mutations are the main cause of resistance to ALK TKIs. With high-throughput methods of detection [11], they are found in 20-50% of tumor samples obtained at progression on 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 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 may help in choosing the most appropriate treatment after progression on that ALK TKI, particularly since several ALK

inhibitors are now available for use in this context. Here we show that targeted 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.

2. Material and methods

2.1. 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 progression on an ALK TKI as part of the routine procedure. Both tissue biopsies and circulating tumor DNA samples were considered. Clinical and molecular data were collected retrospectively at each center. The investigators used RECIST1.1 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 the data reported in this study were extracted from a database approved by the French National Data Protection Authority (CNIL). This non-interventional 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.

2.2. Detection of ALK mutations

Targeted NGS (t-NGS) analysis was conducted with the NGS commercial panel “Ion Ampliseq Colon and Lung Research Panel V2” at two 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 two laboratories between February 2012 and February 2017. DNA was extracted with the QIAamp FFPE

tissue kit (Qiagen) 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) on a Xenius XC spectrofluorometer (Safas 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 (Table S1). Emulsion PCR, enrichment, and chip loading were performed on an Ion Chef instrument with the Ion PI™ Hi-Q™ Chef Kit or the Ion 540™ Kit-Chef. Samples were sequenced with Ion P1 chips on Ion Proton System or Ion 540 chips on the Ion S5XL system (Thermofisher Scientific). Data were analyzed with the Torrent suite software v.5.2.2 (Thermofisher Scientific). For the ALK, EGFR, and KRAS genes, manual inspection of sequences was done on an integrative genomic viewer (IGV).

2.3. Statistical analysis

Quantitative variables are expressed as means (standard deviation, SD) in the case of a normal distribution or as medians (interquartile ranges) otherwise. Categorical variables are expressed as numbers (percentages). The normality of distributions was assessed with 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 two-tailed α-level of 0.05. Data were analyzed with the SAS software version 9.4 (SAS Institute, Cary, NC).

3. Results

The study included 23 patients with advanced ALK-positive NSCLC having undergone at least one biopsy at progression on 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). Sixteen patients (70%) had received platinum-based chemotherapy prior to ALK TKI treatment. Twenty-two patients (96%) had received crizotinib as the first ALK TKI. A second-generation ALK TKI (alectinib, ceritinib, or brigatinib) had been given to one patient (4%) as first-line treatment and to seventeen patients (74%) following crizotinib failure. Three patients having 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 (Table S2). The samples studied were from 13 of the 23 patients (57%) having progressed on a first TKI, 12 of the 15 patients (80%) having progressed on a second TKI, and 3 of the 7 patients (43%) having progressed on a TKI given as a third TKI or further. The studied samples also included five biopsies performed at progression on therapies other than ALK TKI.

The results of targeted NGS applied to these samples are as follows. ALK mutations were detected in eight patients (35%). They included four G1202R mutations and one C1156Y, one V1180L, one I1171N, and one L1196M mutation. In one of the patients where 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 three in circulating free DNA. For 3 patients, liquid and tissue biopsies were performed at the same time. The results were consistent in one patient.

An ALK amplification with no concomitant ALK mutation was detected by FISH in one biopsy (4.34 %) (Figure 1). In addition to an ALK mutation, seven patients had a TP53 mutation (A138P, R175H, C238R, C277G, R248Q, R337C, R337P) and one had a MET mutation in exon 18 (c.3686_3686 + 3del). No mutation was identified in any other oncogene tested with this NGS panel..

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

Among patients on the first ALK TKI, the objective response rate was 74% and the disease control rate was 91%. By the end of data collection, twelve patients (52%) had died. Median overall survival from the start of the first ALK TKI treatment was 37.28 months (95% CI; 19.58-NR). We did not observed any difference in OS between patients with or without TP53 mutations. Among the eight patients in whom an ALK mutation was identified, seven (87%) received a new ALK TKI after the mutation was found (**Figure 3**). In these seven patients the 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 seven patients with an ALK mutation who received a subsequent ALK TKI, six received a TKI presumed on the basis of *in vitro* data to act against the identified mutant [11]. Among these six patients, five achieved an objective response and one showed stable disease. To the remaining patient alectinib was given despite detection of a G1202R mutation, as 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 displayed progressive disease at first assessment **(Figure 3).**

4. Discussion

The present study has 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 out of 23 patients (39%) with advanced ALK-positive NSCLC. Gainor *et al*. report 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*. having shown that they arise more frequently following second- or third-generation TKI treatment (54-71%) than after crizotinib (20% of patients) [11]. Meanwhile two second-generation TKIs, ceritinib and alectinib, have demonstrated 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 progression on second-generation TKIs given as first-line therapy, it is reasonable to speculate that they will exceed the 20% observed after crizotinib failure. Data further suggest that the ALK mutation rate also increases when several ALK TKIs are given sequentially. Solomon *et al.* report ALK mutation rates of 16.6% (14/84) following first-line ALK TKI treatment and 29.2% (31/106) after at least two ALK TKIs [26]. We have likewise observed a 15% ALK mutation rate after failure of a first ALK TKI and a 33% ALK mutation rate after failure of two ALK TKIs. Whether this is due to the number of ALK TKIs administered or to the use of next-generation TKIs remains to be determined.

All these findings and the fact that 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 having progressed after ALK TKI treatment is becoming crucial to determining the optimal therapeutic strategy. *In vitro*, different ALK mutations have been found 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 two patients, for example, the I1171N mutation has been found 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 six 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 displayed progressive disease at first assessment. Interestingly, two patients harboring a G1202R mutation achieved a partial response on brigatinib, 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 progression on an ALK TKI. Yet besides the above-mentioned case reports, data from prospective trials suggest that patients harboring an ALK mutation are more likely to respond to subsequent ALK TKIs. In patients receiving the third-generation TKI lorlatinib after failure of two or three prior ALK TKIs, Shaw *et al.* report objective response rates of 26% in patients with no ALK mutation and 61% in patients with at least one 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. Here, the presence of an ALK mutation may 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 may be due to an ALK-pathway-independent mechanism that is not sensitive to ALK inhibition. Various ALK-independent resistance mechanisms have been described, including activation of EGFR, IGF1R, and SRC, mutation of *KRAS* or *PIK3CA*, amplification of *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 on crizotinib with no evidence of an *ALK* mutation have been shown to benefit from second-generation ALK TKIs [39], but whether their resistance was due to an ALK-

independent mechanism, to an ALK-dependent mechanism not involving an ALK mutation, or to an ALK mutation that escaped detection because of sensitivity issues remains unknown.

Sequential treatment with several ALK TKIs may select resistant clones harboring multiple (i.e. compound) ALK mutations. In a recent study, Yoda *et al.* found a 35% compound mutation rate in patients treated with lorlatinib [40]; in our set, two patients had progressed on lorlatinib and a compound mutation was detected at the time of progression in a patient known to harbor a G1202R mutation. *In vitro*, compound mutations have been found to confer resistance to most ALK TKIs, including lorlatinib [40]. Yet 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 on crizotinib. It is necessary to further determine the sensitivity of compound mutants to ALK TKIs [16].

Finally, other molecular alterations may impact patients outcome. Recent findings have shown that TP53 mutations or ALK variants may be associated with shorter PFS 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.

Conclusions:

In summary, our findings demonstrate 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 may help selecting the best subsequent treatment

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Legends

Table 1

Baseline characteristics

Figure 1

Distribution of mechanisms of resistance to ALK TKIs

** One patient with a G1202R mutation was found, following 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 one patient. Treatments received before the first ALK TKI are not represented.

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 based on *in vitro* data by Gainor et al[11].

Tables

Table 1

	n = 23
Age, median (years) (range)	57 (17-70)
Sex, n (%) - Male - Female	 12 (52%) 11 (48%)
Adenocarcinoma, n (%)	23 (100%)
Stage at diagnosis, n (%) - Stage III - Stage IV	 2 (9%) 21 (91%)
Never Smokers, n (%)	19 (83%)
Number of lines of treatment before first ALK TKI, n (%) 0 1 2 or more	 7 (30%) 13 (57%) 3 (13%)
Type of ALK TKI received (n, %) 1st generation 2nd generation 3rd generation	 22 (96%) 17 (74%) 3 (13%)

Figure 1

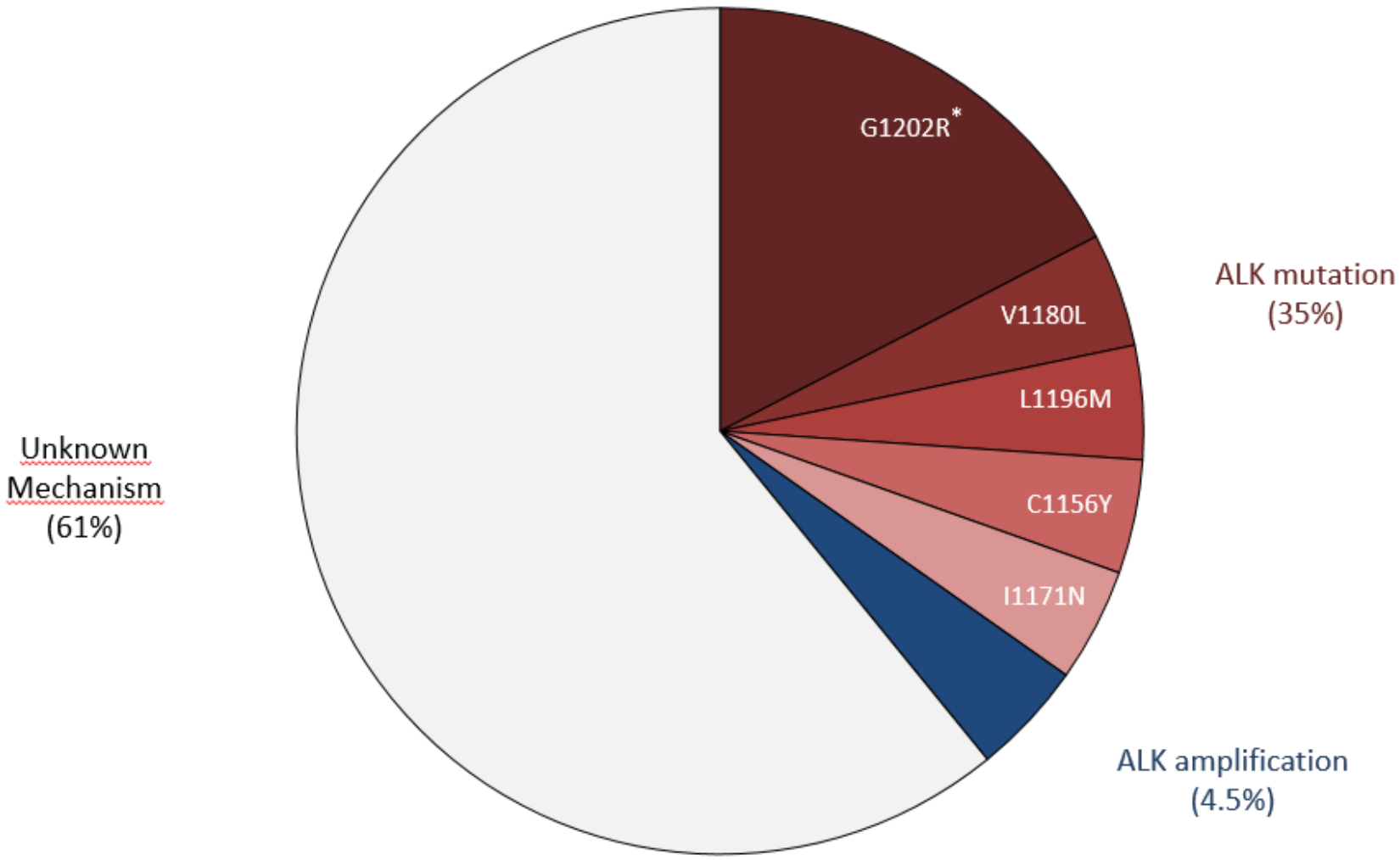
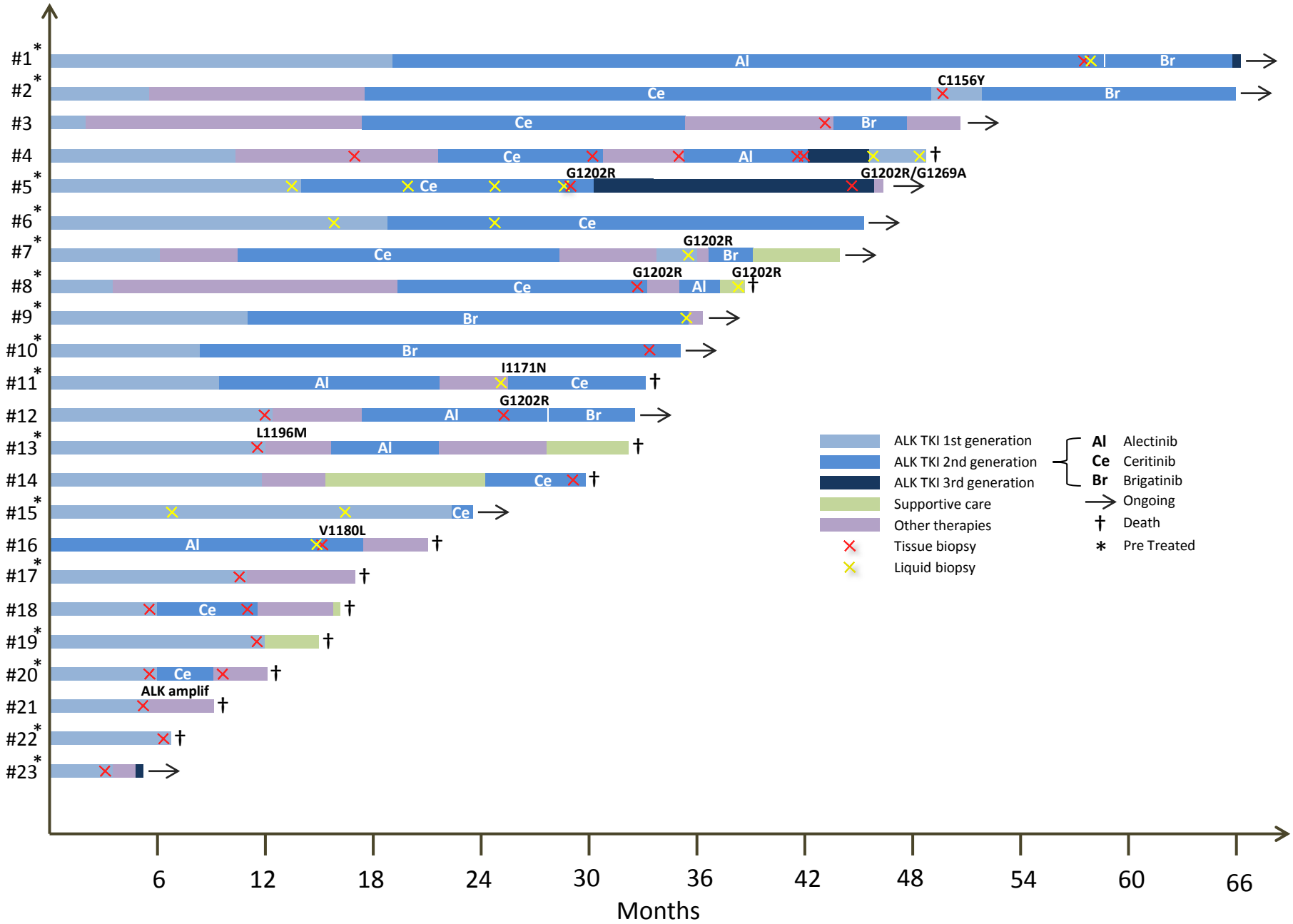

G1202R*
V1180L
L1196M
C1156Y
I1171N
ALK mutation
(35%)
Unknown
Mechanism
(61%)
ALK amplification
(4.5%)

Figure 2


#1*
#2*
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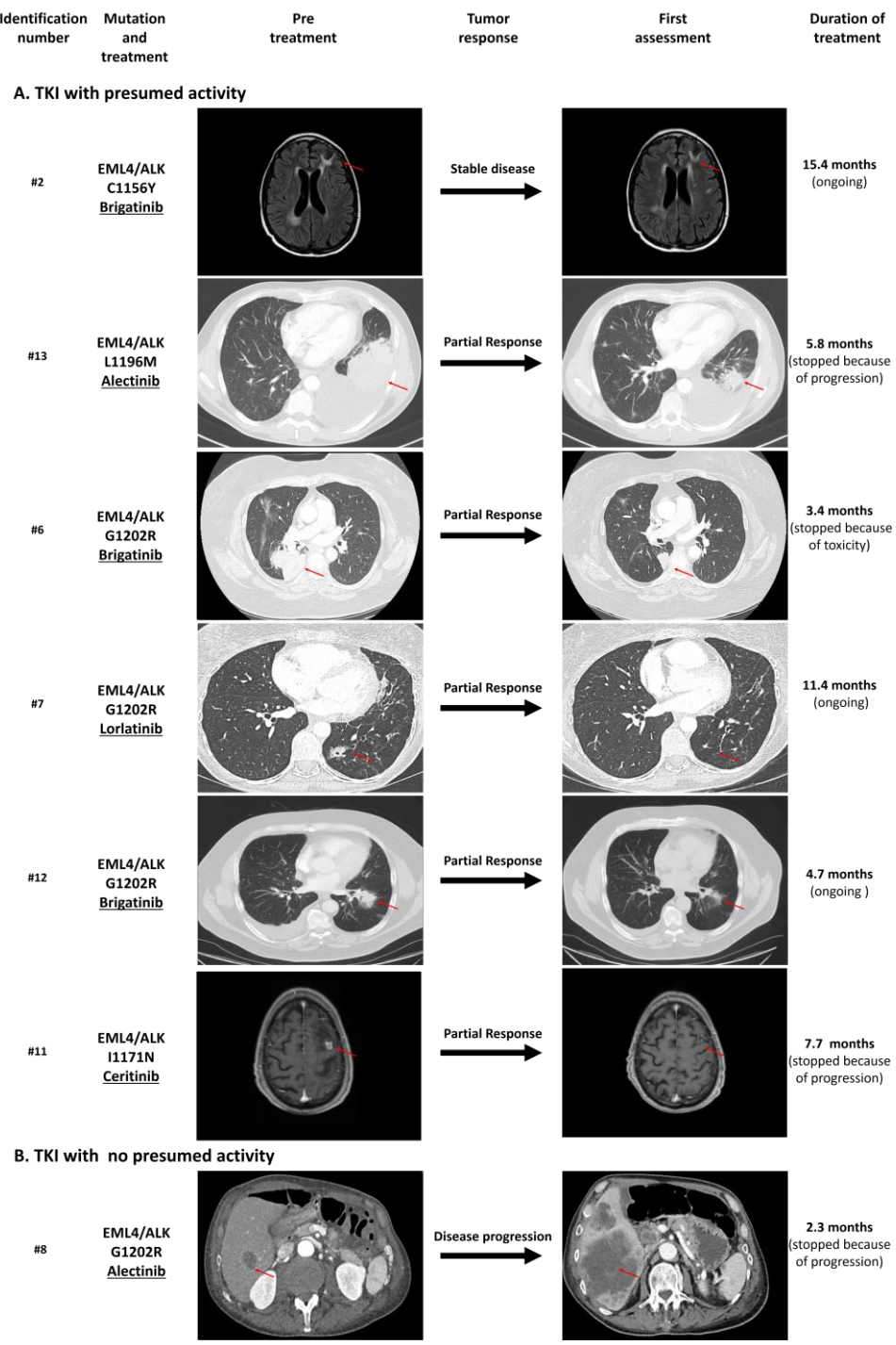
#14
#15*
#16
#17*
#18
#19*
#20*
#21
#22*
#23*
Al
Br
Ce
C1156Y
G1202R
G1202R/G1269A
I1171N
L1196M
V1180L
ALK amplif
†
6
12
18
24
30
36
42
48
54
60
66
Months
ALK TKI 1st generation
ALK TKI 2nd generation
ALK TKI 3rd generation
Supportive care
Other therapies
Tissue biopsy
Liquid biopsy
Al Alectinib
Ce Ceritinib
Br Brigatinib
Ongoing
† Death
* Pre Treated

Figure 3


Identification number
Mutation and treatment
Pre treatment
Tumor response
First assessment
Duration of treatment
A. TKI with presumed activity
#2
EML4/ALK C1156Y Brigatinib
Stable disease
15.4 months (ongoing)
#13
EML4/ALK L1196M Alectinib
Partial Response
5.8 months (stopped because of progression)
#6
EML4/ALK G1202R Brigatinib
Partial Response
3.4 months (stopped because of toxicity)
#7
EML4/ALK G1202R Lorlatinib
Partial Response
11.4 months (ongoing)
#12
EML4/ALK G1202R Brigatinib
Partial Response
4.7 months (ongoing)
#11
EML4/ALK I1171N Ceritinib
Partial Response
7.7 months (stopped because of progression)
B. TKI with no presumed activity
#8
EML4/ALK G1202R Alectinib
Disease progression
2.3 months (stopped because of progression)

Tables

Table 1

	n = 23
Age, median (years) (range)	57 (17-70)
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Adenocarcinoma, n (%)	23 (100%)
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