ORIGINAL ARTICLE

Brigatinib in Patients With Alectinib-Refractory *ALK*-Positive NSCLC

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ABSTRACT

Introduction: The second-generation anaplastic lymphoma kinase (ALK) inhibitor alectinib recently showed superior efficacy compared to the first-generation ALK inhibitor crizotinib in advanced *ALK*-rearranged NSCLC, establishing alectinib as the new standard first-line therapy. Brigatinib, another second-generation ALK inhibitor, has shown substantial activity in patients with crizotinib-refractory *ALK*-positive NSCLC; however, its activity in the alectinib-refractory setting is unknown.

Methods: A multicenter, retrospective study was performed at three institutions. Patients were eligible if they had advanced, alectinib-refractory *ALK*-positive NSCLC and were treated with brigatinib. Medical records were reviewed to determine clinical outcomes.

Results: Twenty-two patients were eligible for this study. Confirmed objective responses to brigatinib were observed in 3 of 18 patients (17%) with measurable disease. Nine patients (50%) had stable disease on brigatinib. The median progression-free survival was 4.4 months (95% confidence interval [CI]: 1.8–5.6 months) with a median duration of treatment of 5.7 months (95% CI: 1.8–6.2 months). Among 9 patients in this study who underwent post-alectinib/pre-brigatinib biopsies, 5 had an *ALK* I1171X or V1180L resistance mutation; of these, 1 had a confirmed partial response and 3 had stable disease on brigatinib. One patient had an *ALK* G1202R mutation in a post-alectinib/pre-brigatinib biopsy, and had progressive disease as the best overall response to brigatinib.

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Conclusions: Brigatinib has limited clinical activity in alectinib-refractory *ALK*-positive NSCLC. Additional studies are needed to establish biomarkers of response to brigatinib and to identify effective therapeutic options for alectinib-resistant *ALK*-positive NSCLC patients.

Keywords: Alectinib; Brigatinib; ALK; NSCLC; Resistance

Introduction

Anaplastic lymphoma kinase (*ALK*) gene rearrangements lead to expression of potent oncogenic fusion proteins and are found in approximately 5% of NSCLC.[1-4] Advanced NSCLC harboring an *ALK* rearrangement (*ALK*-positive NSCLC) can be effectively treated with small-molecule tyrosine kinase inhibitors (TKIs) that target ALK. Until recently, the standard first-line therapy for patients with advanced *ALK*-positive NSCLC was crizotinib, with an objective response rate (ORR) of 74% and a median progression-free survival (PFS) of 10.9 months.[5] As responses to crizotinib are often short-lived due to acquired resistance, numerous next-generation ALK TKIs have been developed including second-generation TKIs such as ceritinib, alectinib, and brigatinib, and the third-generation TKI lorlatinib.[6-14] These next-generation ALK TKIs are more potent and central nervous system (CNS)–penetrant compared to crizotinib and retain variable activity against different crizotinib-resistant *ALK* mutations.[4,15]

Brigatinib is a second-generation ALK inhibitor that was developed to overcome resistance to crizotinib. In pre-clinical studies, brigatinib potently inhibited the majority of crizotinib-resistant *ALK* mutations, including the most common gatekeeper mutation, L1196M.[16] In a multicenter phase II study, brigatinib was highly active in patients with crizotinib-refractory *ALK*-positive NSCLC. Among 222 patients receiving one of two dosing regimens of brigatinib (90 mg once daily versus 180 mg once daily with a 7-day lead-in at 90 mg), the ORRs were 45% and 54%, with a median PFS of 9.2 months and 16.7 months, respectively.[13,17] These findings led to accelerated approval of brigatinib by the United States Food and Drug Administration for the treatment of crizotinib-refractory *ALK*-positive NSCLC.

Recent randomized trials have established a new role for second-generation TKIs, specifically alectinib and ceritinib, as first-line therapy for advanced *ALK*-positive NSCLC.[18,19] For example, the global randomized phase III ALEX trial showed that alectinib was significantly superior to crizotinib in terms of efficacy and toxicity in untreated *ALK*-positive NSCLC.[18] Alectinib has since received approval for the initial treatment of patients with *ALK*-positive NSCLC and has now been widely adopted as the standard of care in this setting. Nonetheless, for patients receiving alectinib either as first- or second-line therapy, resistance invariably develops, and the optimal treatment after alectinib has not been established. In particular, whether other second-generation TKIs may be effective after alectinib is unknown.

Pre-clinical studies suggest that brigatinib could represent a potentially effective treatment option for alectinib-refractory patients. Brigatinib has been shown to harbor significant activity against certain alectinib-resistant *ALK* mutations such as I1171N, I1171S, and V1180L.[16] However, the degree of its pre-clinical and clinical activity against the highly recalcitrant solvent front mutation, *ALK* G1202R, has not been as well defined.[15,16] *ALK* G1202R is the most common resistance mutation after failure of alectinib.[15] This mutation has also been detected in repeat tumor biopsy specimens obtained from patients progressing on brigatinib suggesting suboptimal inhibitory activity against G1202R.[15] Thus, the activity of brigatinib in alectinib-resistant patients may be impacted by the presence of specific *ALK* resistance mutations.

Here, based on a multicenter, retrospective analysis of 22 *ALK*-positive patients treated with alectinib followed by brigatinib, we report the efficacy and safety of brigatinib in the setting of alectinib resistance.

Materials and Methods

Study Population

Patients were identified at three participating institutions: Massachusetts General Hospital (MGH; n = 11), Memorial Sloan Kettering Cancer Center (n = 6), and University of California–Irvine (n = 5). All patients had advanced NSCLC with an *ALK* rearrangement identified by local molecular profiling (e.g., fluorescent in situ hybridization, immunohistochemistry, DNA-based next-generation sequencing [NGS], or targeted RNA sequencing). Patients had to have received alectinib (as any line of systemic therapy) with progression of disease before receiving brigatinib. Brigatinib was prescribed either commercially or on an expanded access protocol. This study was approved by the Institutional Review Board at each participating institution.

Data Collection

Medical records were retrospectively reviewed, and data were extracted on clinical, pathologic, and molecular features as well as treatment histories. Overall and intracranial responses to therapy were determined using the Response Evaluation Criteria in Solid Tumors version 1.1 based on investigator assessment. PFS was measured from the time of brigatinib or alectinib treatment

initiation to clinical/radiographic progression or death. Patients without documented disease progression were censored on the date of last follow-up. Duration of treatment was measured from the time of brigatinib or alectinib initiation to the date that the drug was discontinued, or — if continuing on brigatinib at the time of data analysis — censored on the date of last follow-up. All data were updated as of April 15, 2018.

ALK Mutation Genotyping

A subset of the patients included in this study underwent repeat tumor or liquid biopsies at the time of progression on alectinib and before starting brigatinib, under Institutional Review Board – approved protocols. Five patients underwent a tumor biopsy of the progressing lesion followed by targeted NGS using the commercially available FoundationOne platform (n = 2; Foundation Medicine, Inc., Cambridge, Massachusetts) or the MGH SNaPshot NGS platform (n = 3), as previously described.[20] Four patients underwent a liquid biopsy using either the commercially available Guardant360 cell-free DNA (Guardant Health, Inc., Redwood City, California) or FoundationACT platform (Foundation Medicine, Inc.). Additionally, five patients underwent a tumor (FoundationOne, n = 2), liquid (Guardant360, n = 1; FoundationACT, n = 1), or both tumor and liquid (MGH SNaPshot NGS and Guardant360, n = 1) biopsy after progression on brigatinib.

Statistical Analysis

PFS and duration of treatment endpoints were estimated using the Kaplan-Meier method. 95% confidence intervals (CIs) were calculated using the log-log transformation. Data analysis was performed using SAS 9.4 (SAS Institute, Cary North Carolina).

Results

Patient Characteristics

We identified 22 patients with advanced *ALK*-positive NSCLC who were treated with alectinib followed by brigatinib. The baseline clinicopathologic features of these patients are summarized in Table 1. The median age was 55 years (range, 22 to 76 years), and 59% were women. The majority of patients were never smokers (77%) with adenocarcinoma histology (86%). Most (n = 19; 86%) patients received brigatinib as the immediate next line of therapy following alectinib. The median number of intervening lines of therapy between alectinib and brigatinib was 0 (range, 0 to 5). At the time of starting brigatinib, five patients (23%) had received one prior ALK TKI (alectinib); 13 (59%) had received two prior ALK TKIs (crizotinib and alectinib); and four (18%) had received three prior ALK TKIs (crizotinib, ceritinib, and alectinib) (Table 1).

Table 1. Baseline Clinical and Pathologic Features of Patients Enrolled in the Study

Characteristic	All Patients (N = 22)
Age at diagnosis, y	
Median	55
Range	22-76
Sex	
Male	9 (41)
Female	13 (59)
Race	
White	18 (82)
Asian	3 (14)
Unknown	1 (5)
Smoking history	
Never	17 (77)
Light (≤10 pack-years)	3 (14)
Heavy (>10 pack-years)	2 (9)
Histology	
Adenocarcinoma	19 (86)
Other	1 (5)
Not specified	2 (9)
Stage at diagnosis[a]	
Stage I-III	7 (32)
Stage IV	15 (68)
Brain metastases at diagnosis	
Present	8 (36)
Absent	12 (55)
Not assessed[b]	2 (9)
Brain metastases at the start of brigatinib therapy	
Present	18 (82)
Absent	4 (18)
Lines of systemic therapy before alectinib	
0	3 (14)
1	12 (55)
2	6 (27)
≥3	1 (5)
Intervening lines of therapy between alectinib and brigatinib	
0	19 (86)
1	2 (9)
5	1 (6)
Number of ALK TKIs before brigatinib	
1	5 (23)
2	13 (59)
3	4 (18)

Values are n (%) unless otherwise noted.
[a]Staging based on the American Joint Committee on Cancer TNM seventh edition.
[b]No baseline magnetic resonance image of brain or computed tomography scan of head with contrast obtained at the time of advanced disease diagnosis.
TKI, tyrosine kinase inhibitor.

Outcomes on Alectinib

Three patients received alectinib as first-line therapy, whereas the remainder received alectinib as second-line therapy or beyond. All patients discontinued alectinib

because of disease progression (intracranial, n = 7; extracranial, n = 9; both intra- and extracranial, n = 6). The median PFS on alectinib was 10.4 months (95% CI: 5.4–13.5 months), and median duration of alectinib treatment was 12.4 months (95% CI: 9.6–17.1 months).

Of note, two patients had undergone dose reduction(s) of alectinib. In one patient, the alectinib dose was decreased to 450 mg twice a day after 4 months of therapy due to cumulative fatigue, myalgia, and creatine phosphokinase (CPK) elevation; this patient experienced disease progression 6 months later. The second patient received alectinib 300 mg twice a day because of marked fatigue, which developed after 7 months on the standard dose. His intra- and extracranial disease progressed shortly thereafter. The alectinib dose was then gradually re-escalated to 600 mg twice a day without significant response, and alectinib was therefore discontinued.

Outcomes of Brigatinib Treatment Post-Alectinib

Eighteen of 22 patients had measurable disease at baseline and underwent at least one set of imaging for tumor response evaluation. A confirmed partial response (PR) per Response Evaluation Criteria in Solid Tumors version 1.1 was observed in three patients (17%). Nine patients (50%) experienced stable disease (SD) on brigatinib (Fig. 1*A*), of which one was an unconfirmed PR. Three additional patients had nonmeasurable disease at baseline but were evaluable for tumor response. Of these, two (67%) had non–complete response/non–progressive disease and one (33%) had progressive disease (PD) as their best overall response. Additionally, among 4 of 22 patients who had measurable intracranial disease at baseline, 1 patient (25%) had an unconfirmed intracranial PR but experienced PD extracranially, leading to the termination of brigatinib treatment at 1.7 months. Three patients (75%) had PD as the best intracranial response.

The median PFS on brigatinib was 4.4 months (95% CI: 1.8–5.6 months), with 5 of 22 patients censored (Fig. 1*B*). Of the 17 patients who experienced disease progression on brigatinib, 7 had CNS-only progression. The median duration of treatment was 5.7 months (95% CI: 1.8–6.2 months), with 5 patients continuing on brigatinib at the time of data cutoff (Fig. 1*C*).

All patients started brigatinib at the lead-in dose of 90 mg once daily. The median interval from the start of brigatinib 90 mg daily to the start of 180 mg daily dosing was 7 days (range, 6 to 47 days). One patient continued brigatinib 90 mg daily without dose escalation due to CPK elevation (grade 3 per the Common Terminology Criteria for Adverse Events [CTCAE] version 4.03) which had persisted from prior alectinib therapy. Another patient started brigatinib 90 mg daily and developed drug-related grade 3 pneumonitis within 2 days (Supplemental Figure) requiring permanent discontinuation of brigatinib. There was no escalation of brigatinib dosing beyond 180 mg daily.

The most common (>10%) treatment-related CTCAE grade 1/2 adverse events on brigatinib in this study included CPK elevation (27%), increase in alanine aminotransferase and/or aspartate aminotransferase (18%), diarrhea (14%), and fatigue (14%) (Table 2). One

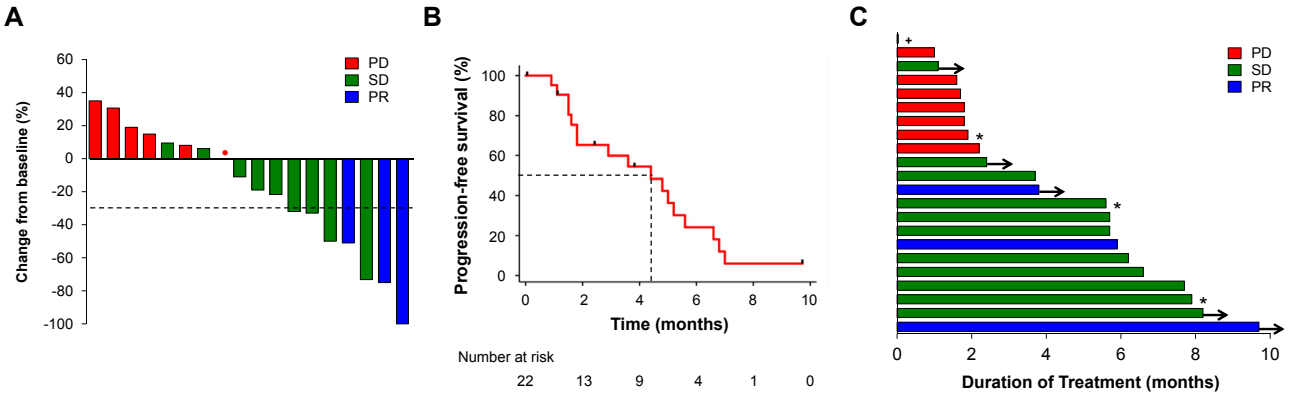

Figure 1. Brigatinib activity in alectinib-refractory *ALK*-positive NSCLC. (*A*) Best confirmed tumor responses of 18 *ALK*-positive patients who received brigatinib and had baseline measurable disease. All patients received and progressed on prior alectinib. The *bars* show best percent change in the target tumor burden from baseline. The *dotted horizontal line* shows the 30% threshold for partial response. The *red dot* indicates a patient who had best overall change from baseline of 0%, with new lesions. (*B*) Progression-free survival (PFS) on brigatinib for 22 patients. Vertical tick marks on the PFS curve indicate censoring of data. *Dotted lines* show the median PFS. (*C*) Swimmer plots showing the duration of brigatinib treatment for each patient in the study cohort. *Arrows* indicate patients continuing on brigatinib at the time of data cutoff. An asterisk (*) indicates patients with evaluable but non-measurable disease. A plus symbol (+) indicates a patient who required early permanent discontinuation of brigatinib due to pneumonitis and was therefore not evaluable for response to brigatinib. PD, progressive disease; SD, stable disease; PR, partial response.

Table 2. Treatment-Related Adverse Events in All Patients (N = 22)

	Grade 1-2[a]	Grade 3[a]
CPK increased	6 (27)	1 (5)
AST/ALT increased	4 (18)	1 (5)
Diarrhea	3 (14)	0
Fatigue	3 (14)	0
Myalgia	2 (9)	0
Lipase increased	2 (9)	0
Amylase increased	2 (9)	0
Constipation	1 (5)	0
Pneumonitis	1 (5)	1 (5)
Rash	1 (5)	0
Nausea	1 (5)	0
Dyspnea	1 (5)	0
Cough	1 (5)	0
Fever	1 (5)	0
Mucositis	0	1 (5)

Values shown are n (%).
[a]Grading per the Common Terminology Criteria for Adverse Events version 4.0.
CPK, creatine phosphokinase; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

patient experienced CTCAE grade 3 pneumonitis as noted above, and one patient required two dose reductions for grade 3 mucositis. Two patients underwent a dose interruption without subsequent dose reductions. One patient experienced grade 2 pneumonitis after 4 days of brigatinib therapy at the 90 mg daily dosing (Supplemental Figure) requiring a drug hold for 4 days. She tolerated the re-challenge of brigatinib 90 mg daily, and was able to escalate to 180 mg daily 6 weeks later without recrudescence of pneumonitis. The second patient experienced grade 3 liver function test abnormalities attributed to brigatinib. She held brigatinib for 3 weeks with improvement in the liver function tests and subsequently resumed the drug with gradual re-escalation to the full dose (180 mg daily) without recurrence of this adverse event.

Molecular Characteristics of Post-Alectinib/Pre-Brigatinib Specimens

Nine patients underwent a repeat biopsy (tumor, n = 5; liquid, n = 4) at the time of progression on alectinib before switching to brigatinib. *ALK* resistance mutations were identified in six (67%) cases. The spectrum of resistance mutations included *ALK* I1171N (n = 2), V1180L (n = 2), I1171T (n = 1), and G1202R (n = 1). Data on the individual response and duration of treatment based on *ALK* resistance mutation for those patients who underwent a post-alectinib/pre-brigatinib biopsy are shown in Figure 2. Among five patients with an I1171N/T or V1180L mutation in the post-alectinib/pre-brigatinib biopsy, one achieved a confirmed PR (shown in Fig. 3*A*) and three had SD as the best overall

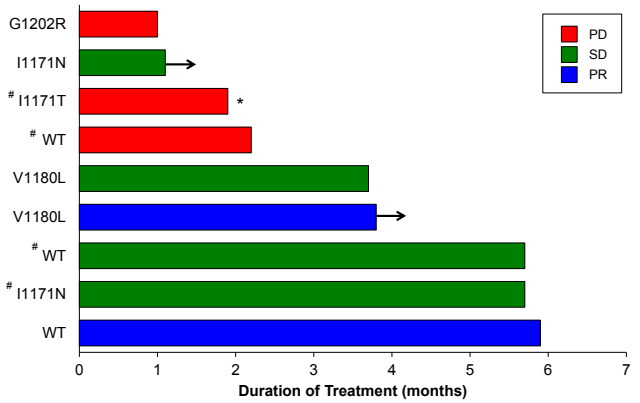

Figure 2. Individual duration of brigatinib treatment in patients with post-alectinib/pre-brigatinib biopsies. Patients who achieved a confirmed partial response (PR) are represented in *blue*; those with stable disease (SD) are represented in *green*; and those with progressive disease (PD) are in *red*. One patient (marked with an asterisk [*]) had evaluable but non-measurable disease. WT indicates wild-type *ALK* (no *ALK* mutation identified in the resistant specimen). # indicates testing by liquid (rather than tumor) biopsy; all cases not marked with # underwent a tumor biopsy. *Arrows* indicate patients still receiving brigatinib at the time of data cutoff.

response. The one patient with a known G1202R mutation had PD on the first tumor re-assessment (Fig. 3*B*). Among three patients who did not have an *ALK* resistance detected on biopsy (tumor, n = 1; liquid, n = 2), one patient each had a PR, SD, and PD.

Clinical Outcomes After Brigatinib

Five of the 17 patients in this cohort who experienced disease progression on brigatinib underwent a tumor or liquid biopsy at the time of progression; three of these patients also had a paired pre-brigatinib/post-alectinib biopsy (Supplemental Table). In one patient who had PD as the best overall response to brigatinib, the *ALK* G1202R mutation was detected on liquid biopsy at the time of progression (Fig. 3*C*). This patient had not undergone a pre-brigatinib biopsy; thus, we were unable to determine whether this mutation emerged in the setting of alectinib (i.e., before brigatinib exposure) or de novo during the course of brigatinib treatment. Another patient with an *ALK* V1180L mutation in the post-alectinib/pre-brigatinib tumor biopsy had SD on brigatinib, but experienced extracranial disease progression after 3.6 months of treatment. A liquid biopsy at the time of progression on brigatinib revealed an *ALK* G1202R resistance mutation (allele fraction [AF] of 7.9%) in addition to L1196M (AF 0.5%) and V1180L (AF 0.04%) mutations (Supplemental Table). Both of these patients with *ALK* G1202R in the post-brigatinib biopsy (in addition to the patient mentioned above who had PD on brigatinib with a pre-brigatinib *ALK* G1202R) were

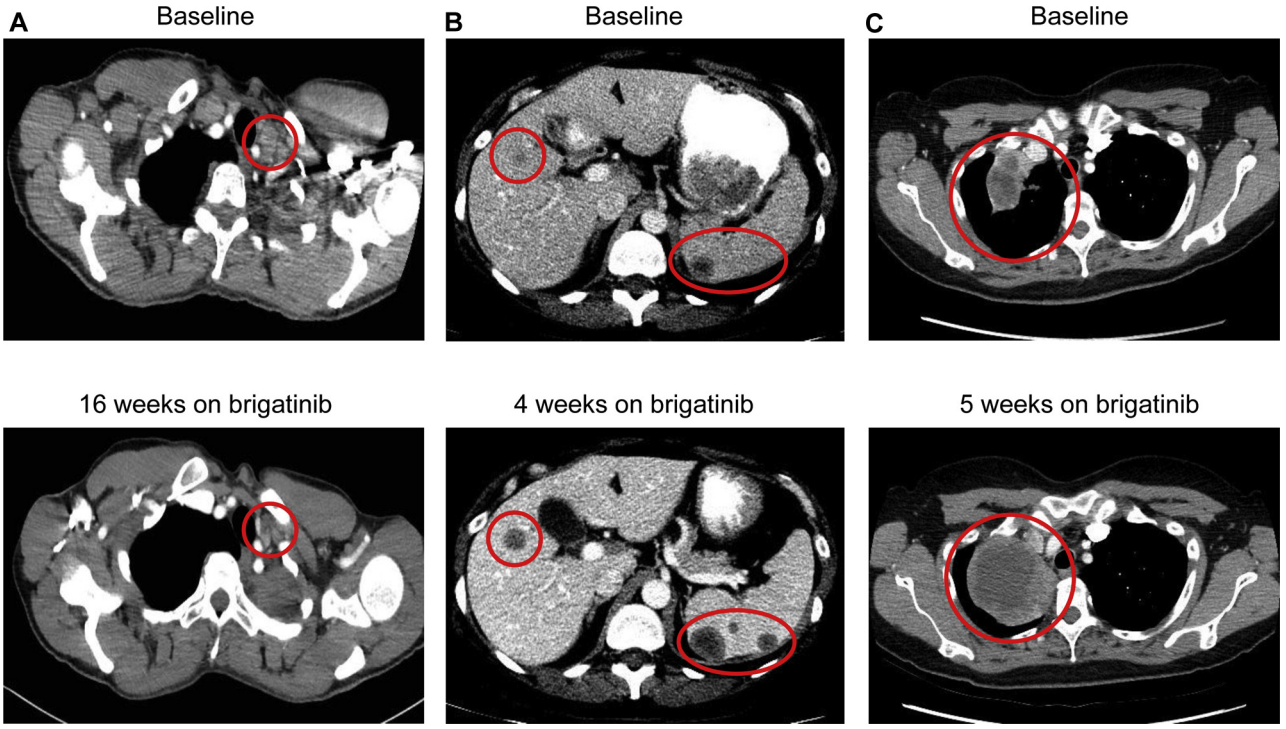

Figure 3. Examples of tumor responses to brigatinib in *ALK*-positive cases with *ALK* resistance mutations. (*A*) Confirmed response of a supraclavicular lymph node to brigatinib in a patient with a V1180L resistance mutation detected in the post-alectinib/pre-brigatinib biopsy. This patient remained on treatment at the time of data cutoff. (*B*) Progressive disease with enlarging and new hepatic and splenic metastases after 1 month of treatment in a patient with *ALK* G1202R detected in the post-alectinib/pre-brigatinib biopsy. (*C*) Progressive disease with an enlarging right lung mass in a patient who did not undergo a post-alectinib/pre-brigatinib biopsy. This patient experienced disease relapse after approximately 1 month of therapy, and had a post-brigatinib liquid biopsy revealing a G1202R mutation (allele frequency of 3.2%).

subsequently treated with lorlatinib and responded, confirming that their tumors did remain ALK-dependent.

A third patient was found to have *ALK* D1203N, also a solvent front resistance mutation, after experiencing disease progression on brigatinib. This patient's post-alectinib/pre-brigatinib liquid biopsy had revealed an *ALK* fusion without kinase domain mutations (Supplemental Table). In the remaining two patients, post-brigatinib biopsies revealed an *ALK* L1196M mutation (no pre-brigatinib biopsy), and MET proto-oncogene, receptor tyrosine kinase gene (*MET)* amplification (by NGS) along with *ALK* I1171N (which was present in the post-alectinib/pre-brigatinib biopsy), respectively.

Discussion

In this multicenter retrospective analysis, we evaluated a cohort of patients with alectinib-refractory *ALK*-positive NSCLC treated with brigatinib. We found that brigatinib shows limited clinical activity in this context, with an ORR of 17% and median PFS of 4.4 months. Brigatinib was generally well tolerated by patients with a safety profile largely consistent with what has been reported.[12,13]

The standard treatment approach to advanced *ALK*-positive NSCLC continues to evolve. Although crizotinib was established as the standard first-line therapy in 2014, more recent studies have evaluated (or are evaluating) the role of more potent and CNS-penetrant next-generation ALK TKIs such as ceritinib, alectinib, and brigatinib as first-line therapy.[5,18,19,21] Most notably, in the global randomized phase III ALEX trial, alectinib was significantly superior to crizotinib in treatment-naïve *ALK*-positive NSCLC, showing a 53% reduction in the risk of cancer progression or death (median PFS not reached for alectinib versus 11.1 months for crizotinib).[18] These data have now effectively established alectinib as the standard initial treatment for patients diagnosed with advanced *ALK*-positive lung cancer.

As a result of this shift from first- to second-generation ALK TKIs as initial therapy, new questions have emerged. Perhaps most urgently as alectinib moves into the front-line setting, what are the most effective treatment options for patients who develop resistance to alectinib, and is there still a role for sequential ALK TKIs? Previous work has shown that brigatinib is highly effective in crizotinib-refractory *ALK*-positive NSCLC, with an ORR of 45% to 54% and median PFS of 9.2 to 16.7 months.[12,13,17] To the best of our knowledge, however, no study has yet evaluated the clinical activity

of brigatinib in the alectinib-refractory setting. Our analysis provides the first insight into this question, helping inform how to conceptualize the sequential treatment approach for *ALK*-positive patients whose disease progresses on alectinib as either the initial or later-line ALK TKI. Importantly, in this study, the efficacy of brigatinib in alectinib-refractory disease was substantially lower than what has been reported in the crizotinib-refractory context.[12,13] This finding may not be entirely surprising given the comparable ALK-inhibitory potencies and excellent CNS penetration of brigatinib and alectinib.[15,16] Nevertheless, the potential role for brigatinib in certain alectinib-refractory settings should not be underemphasized. Indeed, we observed confirmed responses in 3 (17%) of 18 patients with baseline measurable disease who previously progressed on alectinib, with stable disease in an additional nine (50%) patients. Moreover, 6 (27%) of 22 patients had a duration of brigatinib treatment lasting longer than 6 months, with 1 patient continuing on brigatinib at almost 10 months at the time of manuscript submission.

We speculate that the presence of specific *ALK* resistance mutations may influence responses of alectinib-refractory tumors to brigatinib. Prior studies have shown that although *ALK* resistance mutations are present in only ~20% of crizotinib-refractory tumors, they are significantly more common (in ~50% to 60%) following second-generation ALK TKIs.[15] Of note, each second-generation ALK TKI gives rise to a distinct spectrum of resistance mutations. In the case of alectinib, the most common resistance mutations include G1202R (identified in ~30% of cases), I1171N, and V1180L. In pre-clinical models, brigatinib retains activity against I1171X and V1180L, although it is less potent against G1202R.[15,16] In this study, of the seven of 22 evaluable patients who had progressive disease as the best overall response to brigatinib, one had a known G1202R mutation in the post-alectinib/pre-brigatinib biopsy, and another was found to have G1202R in a post-brigatinib biopsy (and did not have a pre-brigatinib biopsy). Of the 14 patients who had disease control on brigatinib, 6 underwent a post-alectinib/pre-brigatinib biopsy, of which four had a known I1171N (n = 2) or V1180L (n = 2) mutation. Collectively, our findings begin to suggest that brigatinib may represent a viable therapeutic option in a small subset of alectinib-refractory patients with tumors harboring I1171X or V1180L, but may not be as effective otherwise, including in those with G1202R (Fig. 4). Given the small number of patients and the limited pre-brigatinib biopsy data in this cohort, larger studies are required to establish the activity of brigatinib in alectinib-refractory NSCLC based on *ALK* mutations.

The potential role of *ALK* resistance mutations as a molecular biomarker of response to ALK inhibitors is

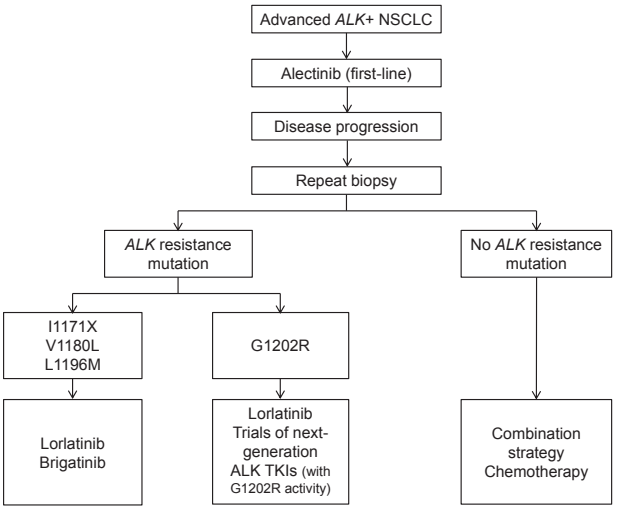

Figure 4. Proposed sequential treatment approach to *ALK*-positive patients with acquired resistance to alectinib. This schema is based on the available pre-clinical and clinical data. Once patients experience disease progression on first-line alectinib, repeat biopsies should be pursued, if feasible, to determine the *ALK* resistance mutation status. Cases with an *ALK* resistance mutation can be treated with sequential lorlatinib therapy. In a specific subset of cases with an I1171X, V1180L, or L1196M mutation, brigatinib may serve as an additional potential option; ceritinib could also be considered in this setting, although not preferred given its lower central nervous system activity. In the absence of an *ALK* resistance mutation, patients may be treated with chemotherapy or combination strategies.

becoming increasingly appreciated, underscoring the importance of pursuing repeat biopsies in patients progressing on alectinib to inform the choice of next-line therapy. This was recently highlighted in the phase I trial of lorlatinib. Lorlatinib previously showed potent pre-clinical activity against all single *ALK* resistance mutations including I1171X, V1180, and notably, G1202R.[14,15] This finding was recapitulated in the phase I study with tumor regression in all patients whose tumors harbored *ALK* resistance mutations including G1202R. In contrast, no tumor regression was observed in patients whose tumors lacked *ALK* resistance mutations and were presumably ALK-independent.[22] In the subsequent phase II study, lorlatinib has shown activity in patients who failed prior second-generation ALK TKIs including alectinib with a confirmed ORR of 39%.[23] Altogether, the growing body of data support a sequential TKI approach to *ALK*-positive NSCLC wherein the initial treatment with alectinib should be followed by a repeat biopsy at the time of disease relapse if feasible, with the selection of subsequent therapy tailored to the presence or absence of specific *ALK* resistance mutations (Fig. 4).

Our study has several notable limitations. First, this was a retrospective analysis with a relatively small number of patients and lacking a comparator cohort.

Although we performed a multicenter analysis to identify more patients eligible for the study, all participating centers were highly specialized academic institutions, incurring the possibility of a referral bias. The duration of follow-up was also limited. Another important limitation of this analysis is the small number of patients who had post-alectinib/pre-brigatinib biopsies, rendering it challenging to draw robust conclusions regarding the efficacy of brigatinib on the basis of *ALK* resistance mutations. Finally, a majority of patients in this study received alectinib as a second- or greater-line therapy for *ALK*-positive NSCLC. We cannot exclude the possibility that the exposure to additional TKIs (e.g., crizotinib or ceritinib) before alectinib may have resulted in a lower efficacy of brigatinib than what may be observed in patients who receive alectinib as the first and only TKI before brigatinib. It is theoretically conceivable that sequential TKI treatment with multiple ALK inhibitors including alectinib may have resulted in more complex resistance mechanisms than would have emerged with alectinib only as the prior therapy. Ultimately, larger prospective studies in *ALK*-positive patients — ideally those treated with alectinib as the first and only prior ALK TKI — will be needed to confirm and extend our findings. A phase II study investigating the activity of brigatinib in patients whose disease has progressed on prior next-generation ALK TKIs is ongoing (ClinicalTrials.gov identifier NCT02706626).

In conclusion, this study provides the first insight into the clinical activity of brigatinib in alectinib-refractory, *ALK*-positive NSCLC. We found that the overall efficacy of brigatinib in this setting was limited. Responses were noted in 17% of patients, highlighting the potential utility of brigatinib in a small subset of alectinib-resistant patients. These findings help refine a tailored sequential treatment approach to *ALK*-positive NSCLC in patients who relapse on alectinib.

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Supplementary Data

Note: To access the supplementary material accompanying this article, visit the online version of the *Journal of Thoracic Oncology* at www.jto.org and at https://doi.org/10.1016/j.jtho.2018.06.005.

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