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ORIGINAL REPORT

# Impact of *EML4-ALK* Variant on Resistance Mechanisms and Clinical Outcomes in *ALK*-Positive Lung Cancer

*Jessica J. Lin, Viola W. Zhu, Satoshi Yoda, Beow Y. Yeap, Alexa B. Schrock, Ibiayi Dagogo-Jack, Nicholas A. Jessop, Ginger Y. Jiang, Long P. Le, Kyle Gowen, Philip J. Stephens, Jeffrey S. Ross, Siraj M. Ali, Vincent A. Miller, Melissa L. Johnson, Christine M. Lovly, Aaron N. Hata, Justin F. Gainor, Anthony J. Iafrate, Alice T. Shaw, and Sai-Hong Ignatius Ou*

Author affiliations and support information (if applicable) appear at the end of this article.

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A.T.S. and S.-H.I.O. contributed equally to this work.

Corresponding author: Alice T. Shaw, MD, PhD, Massachusetts General Hospital Cancer Center, Department of Thoracic Oncology, 32 Fruit St, Boston, MA 02114; e-mail: ashaw1@partners.org.



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## ABSTRACT

### Purpose

Advanced anaplastic lymphoma kinase (*ALK*) fusion-positive non–small-cell lung cancers (NSCLCs) are effectively treated with ALK tyrosine kinase inhibitors (TKIs). However, clinical outcomes in these patients vary, and the benefit of TKIs is limited as a result of acquired resistance. Emerging data suggest that the *ALK* fusion variant may affect clinical outcome, but the molecular basis for this association is unknown.

### Patients and Methods

We identified 129 patients with *ALK*-positive NSCLC with known *ALK* variants. *ALK* resistance mutations and clinical outcomes on ALK TKIs were retrospectively evaluated according to *ALK* variant. A Foundation Medicine data set of 577 patients with *ALK*-positive NSCLC was also examined.

### Results

The most frequent *ALK* variants were *EML4-ALK* variant 1 in 55 patients (43%) and variant 3 in 51 patients (40%). We analyzed 77 tumor biopsy specimens from patients with variants 1 and 3 who had progressed on an ALK TKI. *ALK* resistance mutations were significantly more common in variant 3 than in variant 1 (57% *v* 30%; $P = .023$). In particular, *ALK* G1202R was more common in variant 3 than in variant 1 (32% *v* 0%; $P < .001$). Analysis of the Foundation Medicine database revealed similar associations of variant 3 with *ALK* resistance mutation and with G1202R ($P = .010$ and .015, respectively). Among patients treated with the third-generation ALK TKI lorlatinib, variant 3 was associated with a significantly longer progression-free survival than variant 1 (hazard ratio, 0.31; 95% CI, 0.12 to 0.79; $P = .011$).

### Conclusion

Specific *ALK* variants may be associated with the development of *ALK* resistance mutations, particularly G1202R, and provide a molecular link between variant and clinical outcome. *ALK* variant thus represents a potentially important factor in the selection of next-generation ALK inhibitors.

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## INTRODUCTION

Anaplastic lymphoma kinase (*ALK*) gene rearrangements encode driver fusion oncoproteins and are found in approximately 5% of non–small-cell lung cancers (NSCLCs).[1] Since crizotinib,[2-5] multiple second-generation (eg, ceritinib, alectinib, brigatinib)[6-14] and third-generation (eg, lorlatinib)[15] ALK tyrosine kinase inhibitors (TKIs) have been developed for patients with *ALK*-positive NSCLC, all with higher potency and greater CNS penetration than crizotinib. Although these ALK inhibitors have dramatically expanded the therapeutic landscape of *ALK*-positive NSCLC, clinical outcomes in patients can vary widely, and the biologic mechanisms that underpin such heterogeneous outcomes are unknown. Moreover, at some point, essentially all patients experience a relapse while receiving TKI therapy as a result of acquired drug resistance.[1,4,5] The elucidation of resistance mechanisms in patients has proven critical in efforts to rationally select subsequent therapies.[1,16]

Emerging data indicate that *ALK* fusion variants may have biologic and clinical implications in *ALK*-positive lung cancer. The predominant *ALK* fusion partner in NSCLC is the

ASSOCIATED CONTENT

See accompanying article on page 1257

Data Supplements

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echinoderm microtubule–associated protein-like 4 (*EML4*) gene.[17,18] Among > 15 *EML4-ALK* variants identified to date, the most common are variant 1 (v1; exon 13 of *EML4* fused to exon 20 of *ALK* [E13;A20]) and v3a/b (exon 6a/b of *EML4* fused to exon 20 of *ALK* [E6a/b;A20]).[17,19-22] All variants retain the entire tyrosine kinase domain of ALK and the N-terminal coiled-coil region of EML4, which is necessary and sufficient for the dimerization and constitutive activation of ALK.[17] Different variants may have different protein stabilities, which affects sensitivity to crizotinib in vitro.[23-26]

Recent studies have suggested differential responses to crizotinib according to *ALK* variant in patients.[27,28] For example, longer responses to crizotinib were reported with v1 compared with non-v1[27] or with non-v3 compared with v3,[28] yet two other studies found no difference in clinical response to crizotinib on the basis of *ALK* variant, which highlights the need for additional investigation.[29,30] Moreover, the potential effect of *ALK* variants on the efficacy of next-generation ALK TKIs or the development of resistance mechanisms, which can influence responses to subsequent therapies, has not been examined. We evaluated the frequency and spectrum of *ALK* resistance mutations according to fusion variant in patients with *ALK*-positive NSCLC with acquired TKI resistance and clinical outcomes of these patients who received various generations of ALK inhibitors.

## PATIENTS AND METHODS

### Study Population

Between January 2008 and January 2017, 129 patients with *ALK*-positive NSCLC and known *ALK* variant were identified at the Massachusetts General Hospital (MGH; n = 113) and University of California, Irvine (n = 16; Data Supplement). The study was approved by the institutional review boards (IRBs) at each site.

In addition, a separate group of 577 patients with *ALK*-positive NSCLC and known *ALK* variant identified during routine clinical care from August 2012 to December 2016 with FoundationOne next-generation sequencing (NGS) assays at Foundation Medicine were analyzed for the frequency and distribution of *ALK* resistance mutations. Approval for the study of this cohort was obtained from the Western IRB (protocol no. 20152817).

### Data Collection

For the 129 patients included in the main study cohort, data on clinicopathologic features and treatment histories were extracted from medical records. Progression-free survival (PFS) and overall survival (OS) outcomes were measured as detailed in the Data Supplement. Data were updated as of November 15, 2017.

### Identification of ALK Variant

*ALK* fusion variants were detected by using the MGH fusion panel, which uses targeted RNA sequencing with anchored multiplex polymerase chain reaction (PCR) to detect fusion transcripts that involve known oncogenes, including *ALK*[31]; the FoundationOne platform[32]; targeted NGS platforms at outside institutions as previously described[33,34]; or reverse transcription PCR (commercial or as previously described[35]) and Sanger sequencing of cDNA (Data Supplement).

### Genotyping for ALK Resistance Mutation

Postprogression tumor biopsy specimens were analyzed for the presence of *ALK* resistance mutations under an IRB-approved tissue collection protocol. Methodologies to detect *ALK* resistance mutations included the MGH SNaPshot NGS platform (which uses anchored PCR to detect single-nucleotide variants and insertions/deletions in cancer-related genes, including *ALK*)[31], the FoundationOne NGS[32], and the OncoPanel NGS.[33] A subset of specimens underwent Sanger sequencing of cDNA for the entire ALK domain,[36] and one specimen underwent whole-exome sequencing as previously reported.[16]

### Statistical Analysis

Detailed statistical methods are provided in the Data Supplement. PFS and OS curves were estimated using the Kaplan-Meier method, and the Cox proportional hazards regression model was used to estimate the hazard ratio (HR) to express PFS and OS differences between variant groups. All statistical analyses were performed with SAS 9.4 software (SAS Institute, Cary, NC).

## RESULTS

### Patient Characteristics

Baseline patient characteristics are listed in Table 1. The majority of patients received crizotinib (121 [94%] of 129)—67 (52%) as first-line, 31 (24%) as second-line, and 23 (18%) as third-line or higher-line treatment. Over the course of their disease, 45 patients (35%) received two ALK inhibitors, and 56 (43%) received three or more ALK inhibitors.

Among the 129 patients, 123 (95%) had an *EML4-ALK* fusion. The most frequent *EML4-ALK* variants were v1 in 55 patients (43%), and v3 in 51 patients (40%; Fig 1). No differences were found in clinicopathologic features between patients with v1 and v3 (Table 1). The remaining *EML4-ALK* fusions consisted of v2 (6% [E20;A20]), v5′ (4% [E18;A20]), v5 (2% [E2;A20]), and v7 (1% [E14;A20]). Among non–*EML4-ALK* fusions detected in six patients (5%), the fusion partner genes included *HIP1* (n = 3),[37-39] *KIF5B* (n = 1),[40] *PRKAR1A* (n = 1),[41] and *MTA3* (n = 1; not previously reported). Comparable baseline characteristics also were observed across these variant groups (Data Supplement).

### ALK Resistance Mutations by Variant

To determine whether the *ALK* variant affects the development of molecular mechanisms of resistance, we identified patients in the study cohort who underwent a repeat biopsy after progression on a first- or second-generation ALK inhibitor. Seventy-seven patients (60%) had a postprogression tumor biopsy. Of these, 12 had two repeat biopsies, and two underwent three serial biopsies (Data Supplement). A total of 93 tumor biopsies were performed. The Data Supplement shows the distribution of post-TKI biopsies according to *ALK* fusion variant and timing of biopsy.

Because the number of patients with non-v1 and/or -v3 was small (Data Supplement; Fig 1), the most common variant groups, v1 and v3, were selected for additional analysis. Overall, 33 v1 and 44 v3 repeat biopsies were performed after progression on an ALK inhibitor. *ALK* resistance mutations were identified in 10 patients with v1 (30%) compared with 25 with v3 (57%; $P = .023$; Fig 2). Of note, the *ALK* G1202R solvent-front mutation, which causes steric interference with drug binding[16,36,42] and confers high-level resistance to first- and second-generation ALK inhibitors,[16] was detected in zero (0%) of 33 patients with v1 versus 14 (32%) of 44 with v3 ($P < .001$).

Our group has previously shown that *ALK* resistance mutations as a whole and *ALK* G1202R specifically occur more frequently after second-generation ALK TKIs (50% to 60% and 20%

**Table 1.** Clinical Characteristics According to *ALK* Variant

| Characteristic | All, No. (%) | Patients, No. (%) | | *P* |
|---|---|---|---|---|
| | | Variant 1 | Variant 3 | |
| No. of patients | 129 | 55 | 51 | |
| Median age, years (range) | 52 (22-78) | 55 (22-78) | 51 (31-76) | .559 |
| Sex | | | | .846 |
| Male | 65 (50) | 30 (55) | 26 (51) | |
| Female | 64 (50) | 25 (45) | 25 (49) | |
| Smoking history | | | | .739 |
| Never | 99 (77) | 42 (76) | 41 (80) | |
| Light (< 10 pack-years) | 15 (12) | 6 (11) | 6 (12) | |
| Heavy (≥ 10 pack-years) | 15 (12) | 7 (13) | 4 (8) | |
| Race/ethnicity | | | | .169 |
| Asian | 22 (17) | 7 (13) | 14 (27) | |
| White | 97 (75) | 43 (78) | 33 (65) | |
| Other | 10 (8) | 5 (9) | 4 (8) | |
| Pathology | | | | 1.000 |
| Adenocarcinoma | 126 (98) | 53 (96) | 50 (98) | |
| Squamous | 1 (0.8) | 1 (2) | 0 | |
| Not otherwise specified | 2 (2) | 1 (2) | 1 (2) | |
| Stage at diagnosis* | | | | .678 |
| I | 4 (3) | 3 (5) | 1 (2) | |
| II | 12 (9) | 6 (11) | 4 (8) | |
| III | 13 (10) | 4 (7) | 6 (12) | |
| IV | 100 (78) | 42 (76) | 40 (78) | |
| Extrathoracic metastases at diagnosis | | | | .837 |
| Present | 85 (66) | 36 (65) | 35 (69) | |
| Absent | 44 (34) | 19 (35) | 16 (31) | |
| CNS metastases at diagnosis† | | | | 1.000 |
| Present | 26 (26) | 9 (22) | 10 (24) | |
| Absent | 75 (74) | 31 (78) | 32 (76) | |
| Number of ALK TKIs‡ | | | | .279 |
| One | 28 (22) | 15 (27) | 8 (16) | |
| Two | 45 (35) | 20 (36) | 18 (35) | |
| Three or more | 56 (43) | 20 (36) | 25 (49) | |
| ALK TKI treatment§ | | | | |
| Crizotinib | 121 (94) | 51 (93) | 49 (96) | .680 |
| Ceritinib | 54 (42) | 22 (40) | 22 (43) | .844 |
| Alectinib | 70 (54) | 27 (49) | 30 (59) | .336 |
| Brigatinib | 14 (11) | 5 (9) | 6 (12) | .755 |
| Lorlatinib | 39 (30) | 13 (24) | 17 (33) | .289 |

Abbreviation: TKI, tyrosine kinase inhibitor.
*Initial stage at diagnosis according to American Joint Commission on Cancer TNM staging (7th edition).
†Only patients with documented brain magnetic resonance imaging or head computed tomography scan with contrast at initial diagnosis were included in this analysis.
‡Number of distinct ALK TKIs received by the patient during the course of the disease.
§Exposure to the specified ALK TKI during the course of the disease.

to 40%, respectively) compared with crizotinib (20% to 30% and 2%, respectively).[16] To address imbalances in postcrizotinib versus post–second-generation ALK TKI samples that could potentially confound the comparison of resistance mutations in v1 versus v3, we focused on the distribution of *ALK* mutations in biopsy specimens obtained after progression on a second-generation ALK inhibitor. *ALK* resistance mutations were more common in v3 (21 [66%] of 32) than v1 (eight [42%] of 19), although this difference was not statistically significant ($P = .145$). *ALK* G1202R was significantly more common in v3 (14 [44%] of 32) than in v1 (zero [0%] of 19; $P = .001$; Fig 3). Of note, no statistically significant difference was found between v1 and v3 in the prebiopsy second-generation ALK inhibitor administered, although more v3 than v1 specimens were postalectinib biopsies. Similarly, the cumulative number of prior TKIs was not significantly different (Data Supplement). The sequencing methods used to detect an *ALK* resistance mutation for v1 and v3 are shown in the Data Supplement.

Among postcrizotinib tumor biopsy specimens, *ALK* resistance mutations were detected in two (14%) of 14 samples of v1 and four (33%) of 12 samples of v3 ($P = .365$; Fig 4). None of the samples harbored an *ALK* G1202R mutation. The overall lower frequency of *ALK* resistance mutations (23%; $P = .007$) and specifically of *ALK* G1202R (0%; $P = .002$) after crizotinib versus a second-generation ALK inhibitor (57% and 27%, respectively) is consistent with our previous report.[16]

### *Analysis of 577* ALK*-Positive NSCLCs*

To validate the associations between variant and *ALK* resistance mutations, we subsequently examined a database of *ALK*-positive NSCLCs with known *ALK* variants sequenced at Foundation Medicine. Among 577 patients with *ALK*-positive NSCLC, the most common variants were *EML4-ALK* v3 (n = 186 [32%]) and v1 (n = 182 [32%]) followed by v2 (n = 47 [8%]), other

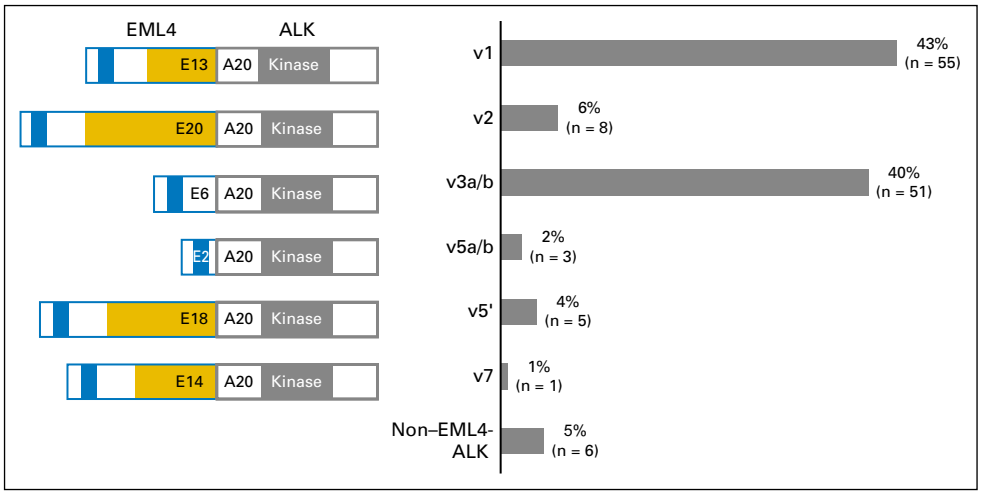



**Fig 1.** Frequency of *ALK* variants in the study cohort (N = 129). Schematic key: blue, coiled-coil region of EML4; gold, tandem atypical propeller EML domain of EML4; gray, tyrosine kinase domain of ALK. Note that v3 and v5 exist as isoforms (v3a and v3b and v5a and v5b, respectively) generated by alternative splicing.[24] A, *ALK* exon; E, *EML4* exon; v, variant.

*EML4-ALK* variants (n = 69 [12%]), and non–*EML4-ALK* variants (n = 93 [16%]; Data Supplement). A total of 624 tumor tissue biopsy specimens were taken from these patients (v3, n = 201; v1, n = 199; v2, n = 49; other *EML4-ALK*, n = 74; non–*EML4-ALK*, n = 101). Although the frequencies of *EML4-ALK* v1 and v3 were almost identical, *ALK* resistance mutations (n = 30) and specifically *ALK* G1202R (n = 9) were significantly more common for v3 than for v1 (*ALK* mutation, 8% *v* 2% [*P* = .010]; G1202R, 3.5% *v* 0% [*P* = .015]; Data Supplement). Of note, clinical information, including prebiopsy treatment history, was not available for the patients included in the Foundation Medicine data set, and therefore, the distribution of patients who were treatment naive, or treated with crizotinib or next-generation ALK inhibitors, is not known. Nevertheless, these findings collectively support the notion that *EML4-ALK* v3 is associated with the development of *ALK* resistance mutations and, in particular, the highly refractory G1202R mutation.

### *Clinical Outcomes on ALK TKIs With v1 and v3*

We next evaluated the effect of *ALK* variants on clinical responses to various ALK inhibitors. Again, we focused this analysis on the most common variants v1 and v3. Treatment histories of patients in the v1 and v3 cohorts were overall well balanced (Table 1). The median OS from the time of diagnosis of advanced disease was 5.0 years and 3.6 years for v1 and v3 cohorts, respectively (HR, 1.16; 95% CI, 0.67 to 2.01; *P* = .584; Data Supplement), similar to the previously reported OS for patients with *ALK*-positive NSCLC who received sequential ALK TKIs.[43] Of note, this OS analysis is relatively immature, with only 52 deaths (49%) at the time of data cutoff.

Among 99 patients treated with crizotinib as the first ALK inhibitor, no significant difference was found in PFS in patients with *EML4-ALK* v1 (n = 51) versus v3 (n = 48; HR, 1.30; 95% CI, 0.85 to 1.98; *P* = .229; Fig 5A). To address whether these results were confounded by prior lines of chemotherapy, we also examined PFS among 55 patients who received crizotinib as first-line therapy. Again, no difference in PFS between v1 (n = 27) and v3 (n = 28) were found (HR, 1.61; 95% CI, 0.84 to 2.75; *P* = .163; Fig 5B). Similarly, no significant difference was found in PFS after second-generation ALK TKIs (ie, ceritinib, alectinib, brigatinib) given as the second ALK inhibitor after crizotinib among 77 patients with v1 (n = 37) versus v3 (n = 40; HR, 1.45; 95% CI, 0.88 to 2.38; *P* = .141; Fig 5C). For patients in each cohort who experienced disease progression, the pattern of progression (ie, CNS only, both intra- and extracranially, or extracranially only) was not different between v1 and v3 (Data Supplement).

In an exploratory analysis of 12 patients with v1 and 17 with v3 who received the third-generation ALK TKI lorlatinib after experiencing treatment failure with both crizotinib and at least one second-generation ALK inhibitor, v3 was associated with significantly longer PFS than v1 (median, 11.0 *v* 3.3 months; HR, 0.31; 95% CI, 0.12 to 0.79; *P* = .011; Fig 5D). By univariable analysis, no other baseline features, including sex, age, race/ethnicity, smoking history, initial stage at diagnosis, presence of extrathoracic disease, and presence of CNS metastasis at the diagnosis of advanced disease, was significantly associated with PFS on lorlatinib. Lorlatinib has previously been shown to retain potent activity against all known crizotinib-resistant *ALK* mutations, including G1202R,

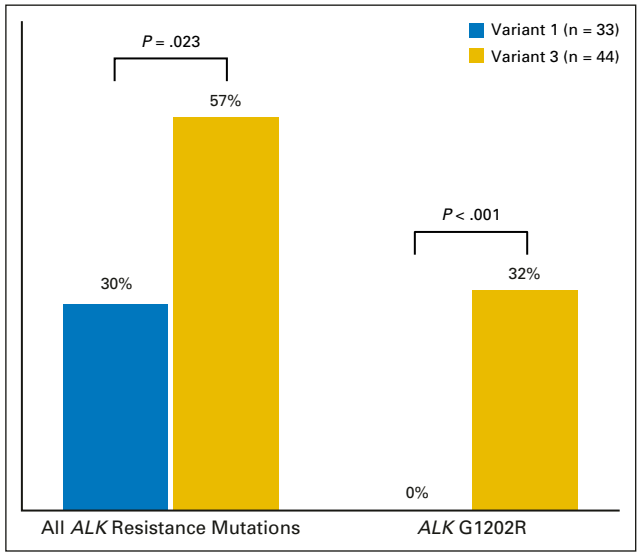
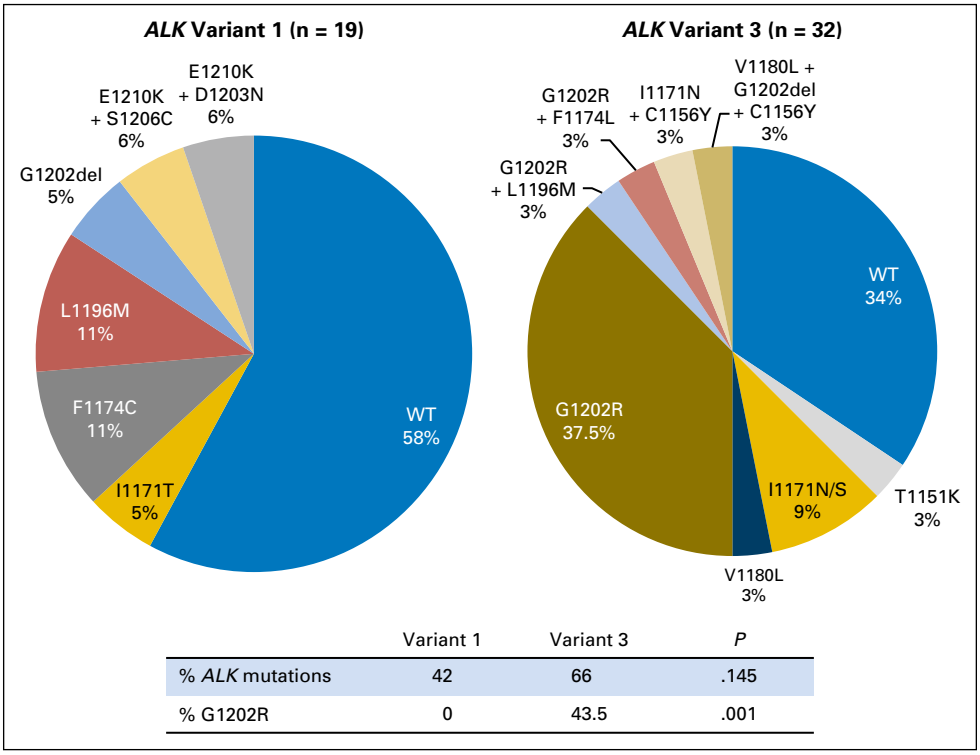



**Fig 2.** *ALK* resistance mutations in tumor biopsy specimens obtained after progression on an ALK inhibitor according to *EML4-ALK* variant.



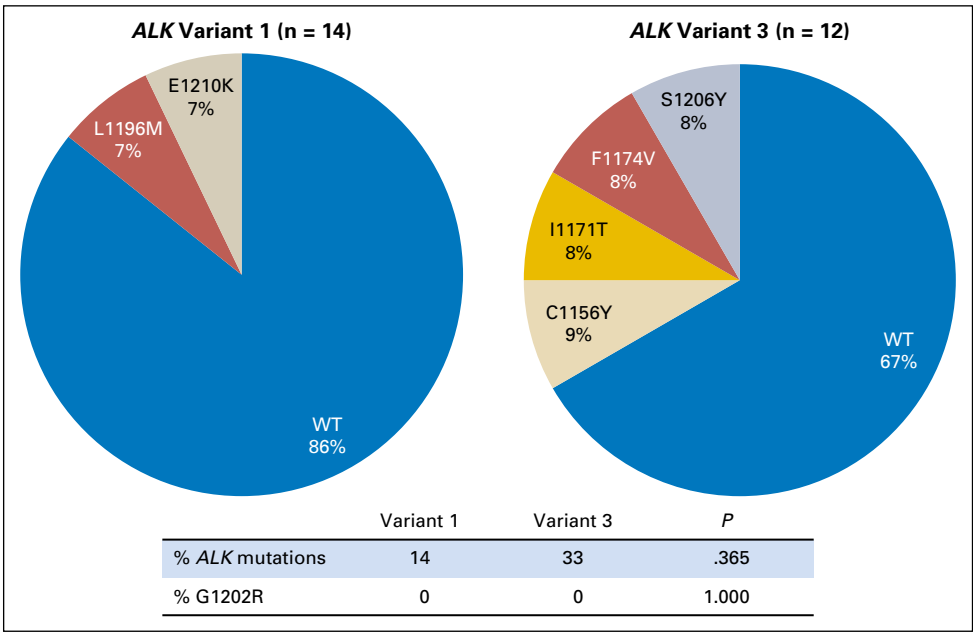

| | Variant 1 | Variant 3 | *P* |
|---|---|---|---|
| % *ALK* mutations | 42 | 66 | .145 |
| % G1202R | 0 | 43.5 | .001 |

**Fig 3.** Distribution of *ALK* resistance mutations in tumor biopsy specimens obtained after disease progression on a second-generation ALK inhibitor by *EML4-ALK* variant. WT, wild-type (nonmutated) *ALK*.

in vitro[16,44] and has demonstrated activity in patients with *ALK*-positive NSCLC previously treated with two or more ALK inhibitors, including those with *ALK* G1202R.[15] In the current cohort, six patients with v1 and 15 with v3 who received lorlatinib underwent a prelorlatinib tumor biopsy, and an *ALK* resistance mutation was present in one v1 (17%; G1269A) and 13 v3 (87%) samples, respectively (Data Supplement). Of the 13 v3 samples with a prelorlatinib *ALK* mutation, 12 harbored *ALK* G1202R. Finally, no difference in PFS was noted in patients with v1 versus v3 who received pemetrexed (HR, 1.11; 95% CI, 0.60 to 2.07; $P = .742$) or platinum and pemetrexed combination (HR, 0.84; 95% CI, 0.40 to 1.78; $P = .649$; Data Supplement).

## DISCUSSION

To our knowledge, we present the largest analysis to date to examine the clinical effect of *ALK* variants in *ALK*-positive NSCLC




| | Variant 1 | Variant 3 | *P* |
|---|---|---|---|
| % *ALK* mutations | 14 | 33 | .365 |
| % G1202R | 0 | 0 | 1.000 |

**Fig 4.** Distribution of *ALK* resistance mutations in tumor biopsy specimens obtained after disease progression on crizotinib by *EML4-ALK* variant. WT, wild-type *ALK*.

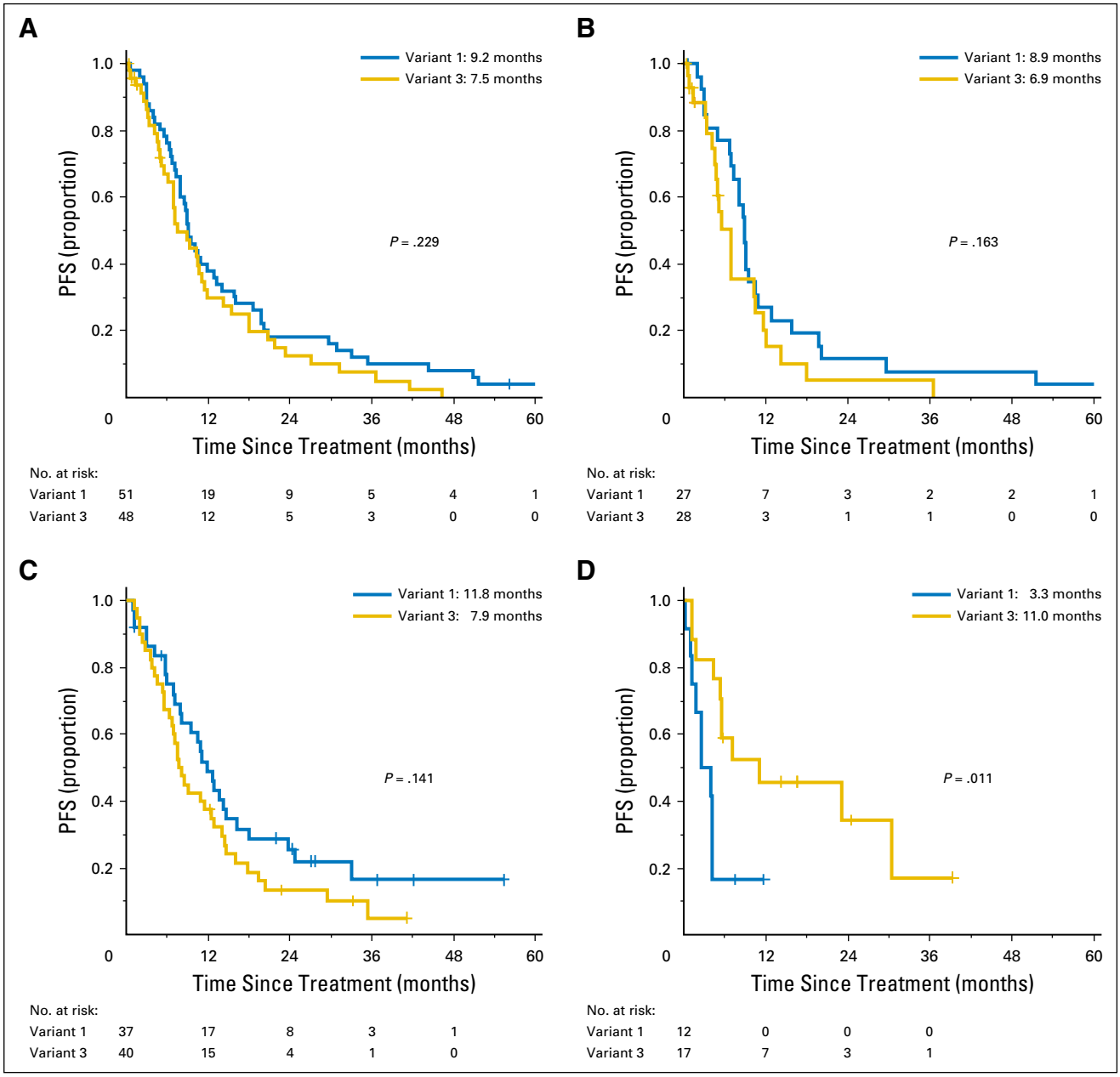



**Fig 5.** Kaplan-Meier curves for progression-free survival (PFS) for (A) crizotinib as first ALK tyrosine kinase inhibitor (TKI) in patients with *EML4-ALK* variant 1 (v1; n = 51) versus v3 (n = 48), (B) crizotinib as first-line therapy in v1 (n = 27) versus v3 (n = 28), (C) second-generation ALK TKI administered as the second ALK inhibitor after crizotinib in v1 (n = 37) versus v3 (n = 40), and (D) lorlatinib administered after crizotinib and at least one second-generation ALK TKI in v1 (n = 12) and v3 (n = 17).

and the first study to evaluate *ALK* resistance mutations according to *EML4-ALK* variant. We found that *EML4-ALK* v3 was significantly associated with the development of *ALK* resistance mutations, particularly *ALK* G1202R, which suggests that patients with v3 may be more likely to acquire distinct secondary *ALK* resistance mutations as they undergo treatment with ALK TKIs.

Previously, we demonstrated the unique spectrum of activity of each currently available ALK inhibitor against different *ALK* resistance mutations.[16] Of note, the solvent-front *ALK* G1202R mutation conferred high-level resistance to all first- and second-generation ALK inhibitors but retained sensitivity to lorlatinib.[16] The strong association between v3 and *ALK* G1202R suggests that the establishment of *ALK* variant status may have important therapeutic implications. Indeed, we observed a significantly longer PFS among patients with *EML4-ALK* v3 versus v1 who received lorlatinib. A priori knowledge of the *ALK* variant status thus could help to select patients more likely to achieve a durable response to lorlatinib. We speculate that the greater propensity of v3 to develop *ALK* mutations, particularly G1202R, likely underlies these different outcomes. Supportive of this notion, the activity of lorlatinib was found to correlate tightly with the presence of an *ALK* resistance mutation (which suggests continued ALK dependency) in a small series of ceritinib-resistant patient-derived cell lines.[16] Several ongoing and completed studies of second- and

third-generation ALK TKIs, including the phase III study of first-line lorlatinib versus crizotinib (ClinicalTrials.gov identifier: NCT03052608), are retrospectively evaluating the association between efficacy and *ALK* variant and/or preexisting *ALK* resistance mutations and may further enhance our understanding of the molecular variables that contribute to heterogeneous clinical outcomes.

We also examined the effect of *ALK* variants on clinical responses to first- and second-generation ALK inhibitors. In this study, no statistically significant difference in PFS was observed among patients with v1 versus v3 treated with crizotinib or a second-generation ALK TKI. However, the median PFS in all contexts was numerically shorter for v3 than for v1, which suggests that larger patient cohorts may be needed to achieve sufficient power to detect a statistically significant difference. In light of the Alectinib Versus Crizotinib in Untreated ALK-Positive NSCLC study that demonstrated superiority of front-line alectinib over crizotinib,[12] continuing the investigation of the effect of specific *ALK* variants on resistance patterns and clinical outcomes in patients treated with first-line alectinib will be of particular interest.

Four previously published studies evaluated clinical responses to ALK TKIs according to *ALK* variant. All were centered on crizotinib, and conflicting findings have been reported (eg, PFS longer for patients with v1 *v* non-v1,[27] longer for those with non-v3 *v* v3,[28] not different on the basis of variant[29,30]). Several limitations may account for the discordant findings, including small sample size, lack of distinction in crizotinib line of therapy, and clustering of variants into groups that may not be biologically or clinically relevant. To address these limitations, we analyzed a larger cohort of patients with a known *ALK* variant, and focused on the most common variants v1 and v3 (to avoid the confounding effect of arbitrary variant clustering) and on crizotinib administered as the first ALK TKI or as first-line therapy (to minimize the effect of treatment lines).

Nonetheless, the current study had limitations. First, it was a retrospective study and still limited in the number of patients. Variant analysis in larger, prospective studies will be needed to validate and expand on our findings. Second, this study focused on the most common *EML4-ALK* variants v1 and v3, and therefore, the potential effect of non-v1/v3 variants on ALK TKI resistance mechanisms and clinical outcomes remains unknown. Moreover, the effect of *ALK* variants on OS of patients with *ALK*-positive NSCLC requires more investigation because the OS data in this study were not mature. Of note, as shown in the Data Supplement, some differences were found in the sequencing methods used to detect the *ALK* resistance mutations in v1 and v3 post-TKI biopsies. To our knowledge, the particular methods used in this study are not limited in the ability to detect specific *ALK* fusion variants or resistance mutations such as G1202R.

Future studies will need to explore the mechanisms that underlie the differential association between *ALK* variant and resistance mutations. Prior studies have shown that *ALK* variants retain varying portions of the EML4 tandem atypical propeller EML (TAPE) domain, which results in differential fusion protein stability in vitro.[25,26] In particular, shorter *EML4-ALK* variants (eg, v3) that lack the entire TAPE domain have been found to be more stable than longer variants (eg, v1) that retain a partial TAPE domain.[23-26] In theory, *ALK*-rearranged tumor cells with the more stable v3 could be more ALK addicted, which necessitates the development of potent on-target *ALK* mutations (eg, G1202R) to mediate acquired resistance. Alternatively, an assessment of whether certain variants such as v3 are more structurally vulnerable to developing particular *ALK* resistance mutations and whether mutations confer differential levels of resistance depending on the variant would be interesting.

In summary, the findings suggest that *EML4-ALK* v3 is associated with a significantly higher incidence of *ALK* resistance mutations, particularly G1202R, and provide a potential molecular link between variant and clinical outcome. Thus, *ALK* variant status may represent an important emerging factor in guiding the treatment strategy for *ALK*-positive NSCLC.

## AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at jco.org.

## AUTHOR CONTRIBUTIONS

**Conception and design:** Jessica J. Lin, Jeffrey S. Ross, Alice T. Shaw, Sai-Hong Ignatius Ou
**Financial support:** Alice T. Shaw
**Provision of study materials or patients:** Jessica J. Lin, Alexa B. Schrock, Alice T. Shaw, Sai-Hong Ignatius Ou
**Collection and assembly of data:** Jessica J. Lin, Viola W. Zhu, Satoshi Yoda, Alexa B. Schrock, Nicholas A. Jessop, Ginger Y. Jiang, Long P. Le, Jeffrey S. Ross, Alice T. Shaw, Sai-Hong Ignatius Ou
**Data analysis and interpretation:** Jessica J. Lin, Satoshi Yoda, Beow Y. Yeap, Alexa B. Schrock, Ibiayi Dagogo-Jack, Long P. Le, Kyle Gowen, Philip J. Stephens, Jeffrey S. Ross, Siraj M. Ali, Vincent A. Miller, Melissa L. Johnson, Christine M. Lovly, Aaron N. Hata, Justin F. Gainor, Anthony J. Iafrate, Alice T. Shaw, Sai-Hong Ignatius Ou
**Manuscript writing:** All authors
**Final approval of manuscript:** All authors
**Accountable for all aspects of the work:** All authors

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### *Affiliations*

**Jessica J. Lin**, **Satoshi Yoda**, **Beow Y. Yeap**, **Ibiayi Dagogo-Jack**, **Nicholas A. Jessop**, **Ginger Y. Jiang**, **Long P. Le**, **Aaron N. Hata**, **Justin F. Gainor**, **Anthony J. Iafrate**, and **Alice T. Shaw**, Massachusetts General Hospital, Boston; **Alexa B. Schrock**, **Kyle Gowen**, **Philip J. Stephens**, **Jeffrey S. Ross**, **Siraj M. Ali**, and **Vincent A. Miller**, Foundation Medicine, Cambridge, MA; **Viola W. Zhu** and **Sai-Hong Ignatius Ou**, Chao Family Comprehensive Cancer Center, University of California Irvine School of Medicine, Orange, CA; **Melissa L. Johnson**, Sarah Cannon Research Institute; and **Christine M. Lovly**, Vanderbilt-Ingram Cancer Center, Nashville, TN.

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**Impact of *EML4-ALK* Variant on Resistance Mechanisms and Clinical Outcomes in *ALK*-Positive Lung Cancer**

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**Jessica J. Lin**
**Honoraria:** Chugai Pharmaceutical
**Consulting or Advisory Role:** Boehringer Ingelheim

**Viola W. Zhu**
**Stock or Other Ownership:** TP Therapeutics (I)
**Honoraria:** AstraZeneca
**Consulting or Advisory Role:** AstraZeneca, Bayer AG, TP Therapeutics, Biocept
**Speakers' Bureau:** AstraZeneca, Roche, Genentech, Takeda Pharmaceuticals

**Satoshi Yoda**
No relationship to disclose

**Beow Y. Yeap**
**Stock or Other Ownership:** SISCAPA Assay Technologies (I)
**Consulting or Advisory Role:** Abcodia (I)

**Alexa B. Schrock**
**Employment:** Foundation Medicine
**Stock or Other Ownership:** Foundation Medicine

**Ibiayi Dagogo-Jack**
**Honoraria:** Foundation Medicine
**Consulting or Advisory Role:** Boehringer Ingelheim

**Nicholas A. Jessop**
No relationship to disclose

**Ginger Y. Jiang**
**Employment:** Novartis (I)

**Long P. Le**
**Stock or Other Ownership:** ArcherDx
**Consulting or Advisory Role:** ArcherDx
**Patents, Royalties, Other Intellectual Property:** Anchored multiplex polymerase chain reaction patent licensed to ArcherDx

**Kyle Gowen**
**Employment:** Foundation Medicine
**Stock or Other Ownership:** Foundation Medicine
**Travel, Accommodations, Expenses:** Foundation Medicine

**Philip J. Stephens**
**Employment:** Foundation Medicine
**Leadership:** Foundation Medicine
**Stock or Other Ownership:** Foundation Medicine

**Jeffrey S. Ross**
**Employment:** Foundation Medicine
**Leadership:** Foundation Medicine
**Stock or Other Ownership:** Foundation Medicine

**Siraj M. Ali**
**Employment:** Foundation Medicine
**Stock or Other Ownership:** Exelixis, Otonomy, OncoSec Medical, Genocea Biosciences
**Consulting or Advisory Role:** Incysus
**Patents, Royalties, Other Intellectual Property:** Patents through Foundation Medicine, and patents through Seres Health on microbiomes in non-neoplastic disease (I)

**Vincent A. Miller**
**Employment:** Foundation Medicine
**Leadership:** Foundation Medicine
**Stock or Other Ownership:** Foundation Medicine
**Consulting or Advisory Role:** Revolution Medicine
**Patents, Royalties, Other Intellectual Property:** Receives periodic royalties related to T790M patent awarded to Memorial Sloan Kettering Cancer Center

**Melissa L. Johnson**
**Consulting or Advisory Role:** Astellas Pharma (I), Otsuka (I), Genentech (Inst), Roche (Inst), Celgene (Inst), Boehringer Ingelheim (Inst), AbbVie (Inst)
**Research Funding:** OncoMed Pharmaceuticals (Inst), BerGenBio (Inst), Eli Lilly (Inst), EMD Serono (Inst), Kadmon Holdings (Inst), Janssen Pharmaceuticals (Inst), Mirati Therapeutics (Inst), Genmab (Inst), Pfizer (Inst), AstraZeneca (Inst), Genentech (Inst), Roche (Inst), Stemcentrx (Inst), Novartis (Inst), Checkpoint Therapeutics (Inst), Array BioPharma (Inst), Regeneron Pharmaceuticals (Inst)

**Christine M. Lovly**
**Honoraria:** Novartis, Sequenom, QIAGEN, Pfizer, National Comprehensive Cancer Network, Takeda Pharmaceuticals
**Consulting or Advisory Role:** ARIAD Pharmaceuticals, Clovis Oncology, Genoptix, Novartis, ARIAD Pharmaceuticals, Foundation Medicine, Cepheid
**Research Funding:** AstraZeneca, Novartis
**Travel, Accommodations, Expenses:** Pfizer, Takeda Pharmaceuticals, Roche, Cepheid

**Aaron N. Hata**
**Research Funding:** Amgen, Novartis (Inst), Relay Therapeutics

**Justin F. Gainor**
**Honoraria:** Merck, Incyte, ARIAD Pharmaceuticals, Pfizer, Novartis
**Consulting or Advisory Role:** Genentech, Bristol-Myers Squibb, Theravance Biopharma, Loxo Oncology, Takeda Pharmaceuticals
**Research Funding:** Merck (Inst), Novartis (Inst), Genetech (Inst), Bristol-Myers Squibb (Inst), Adaptimmune (Inst), AstraZeneca (Inst), ARIAD Pharmaceuticals (Inst), Jounce Therapeutics (Inst), Moderna Therapeutics (Inst)
**Travel, Accommodations, Expenses:** Affymetrix

**Anthony J. Iafrate**
**Stock or Other Ownership:** ArcherDx
**Consulting or Advisory Role:** Debiopharm Group, Constellation Pharmaceuticals, Chugai Pharmaceutical, Roche
**Research Funding:** Blueprint Medicines
**Patents, Royalties, Other Intellectual Property:** ArcherDx exclusive license to AMP technology

**Alice T. Shaw**
**Honoraria:** Pfizer, Novartis, Roche, Genentech, Foundation Medicine
**Consulting or Advisory Role:** Pfizer, Novartis, Genentech, Roche, ARIAD Pharmaceuticals, Ignyta, Blueprint Medicines, Daiichi Sankyo, EMD Serono, Taiho Pharmaceutical, KSQ Therapeutics, Natera
**Research Funding:** Pfizer, Novartis, Roche, Genentech

**Sai-Hong Ignatius Ou**
**Honoraria:** Novartis, Pfizer, Roche, Genentech, ARIAD Pharmaceuticals, Takeda Pharmaceuticals, AstraZeneca
**Consulting or Advisory Role:** ARIAD Pharmaceuticals, Takeda Pharmaceuticals, Pfizer, Novartis, AstraZeneca, Roche, Genentech
**Speakers' Bureau:** AstraZeneca, Genentech, Takeda Pharmaceuticals, ARIAD Pharmaceuticals
**Research Funding:** AstraZeneca (Inst), Pfizer (Inst), Roche (Inst), AstraZeneca (Inst), MedImmune (Inst), Clovis Oncology (Inst), ARIAD Pharmaceuticals (Inst), Ignyta (Inst), Peregrine Pharmaceuticals (Inst), GlaxoSmithKline (Inst), Astellas Pharma (Inst), Chugai Pharmaceutical (Inst), TP Therapeutics (Inst), Novartis (Inst), Blueprint Medicines (Inst)