ORIGINAL ARTICLE

Analysis of Baseline Molecular Factors Associated With the Risk of Central Nervous System Progression Among Alectinib-Treated Patients With ALK–Positive NSCLC

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ABSTRACT

Introduction: Despite receiving alectinib therapy, patients with ALK-positive NSCLC remain at risk of central nervous system (CNS) progression. Our retrospective study aimed to identify baseline clinical and molecular factors associated with the risk of CNS progression in this patient subset.

Methods: We analyzed the clinical, molecular, and imaging data of 318 patients with ALK-positive advanced NSCLC who received alectinib as first-line (1L-alectinib) or second-line (2L-alectinib) therapy at baseline (1L, n = 183; 2L, n = 135) and at disease progression (1L, n = 80; 2L, n = 76).

Results: The incidence rates of CNS progression were 23.7% after 1L-alectinib treatment and 31.6% after 2L-alectinib treatment. Compared with patients who received 1L-alectinib, CNS progression was similar in patients who received 2L-alectinib ($p > 0.05$). Oligoprogression was detected in 55.0% (44 of 80) of patients who progressed after first-line alectinib, with the remaining 45.0% (36 of 80) having nonoligoprogression. Univariate and multivariate analyses and stepwise regression analyses consistently identified a higher likelihood of CNS progression among (1) patients who received 2L-alectinib than 1L-alectinib, (2) patients with non-3a/b variant *ALK* fusion than those with echinoderm microtubule-associated protein-like 4–*ALK* variant 3a/b, and (3) patients with programmed death ligand 1 (PD-L1) tumor proportion score (TPS) of 50% or higher than PD-L1 TPS of less than 50%.

Conclusions: Our study provided real-world evidence that patients who harbored PD-L1 TPS of 50% or higher were

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more likely to experience CNS progression during alectinib therapy. The association between CNS progression and breakpoint variants warrants further investigation. Our findings suggest that close monitoring and prompt intervention are crucial in prolonging the quality of life of this patient subset.

Keywords: Alectinib; CNS progression; Biomarker; ALK; Non–small cell lung cancer

Introduction

NSCLCs are associated with a high incidence of brain metastasis, with about 30% of patients detected with central nervous system (CNS) metastasis at initial diagnosis.[1–4] The incidence is even higher among NSCLCs harboring oncogenic gene rearrangements in anaplastic lymphoma kinase (ALK) as compared with other NSCLCs that lack oncogene drivers.[5–9] The likelihood of developing CNS metastasis rises during the disease course, with over 58% of surviving patients with ALK-positive lung cancer detected with CNS metastases at 3 years despite targeted therapy.[2,10] Hence, the National Comprehensive Cancer Network clinical practice guidelines for NSCLC management recommend brain imaging at the time of initial diagnosis and periodically during the treatment to monitor for CNS progression.[11] The incidence of CNS metastases has also increased among patients with advanced NSCLC due to advancements in diagnostic imaging modalities and improved systemic disease control, leading to improved detection and prolonged life span, respectively.[10] In a general context, the coexistence of CNS metastases is often associated with unfavorable prognosis and can contribute to heightened rates of morbidity and mortality.[12] In addition, CNS metastasis could contribute to neurocognitive impairment, which negatively impacts the overall quality of life experienced by the patient.[3,12,13]

Alectinib is a second-generation ALK tyrosine kinase inhibitor (TKI) and is currently one of the standard first-line treatment for patients with ALK-positive NSCLC. Compared with first-generation ALK TKI crizotinib, alectinib can effectively penetrate the CNS by reducing the efflux of P-glycoprotein-mediated blood-brain barrier transport.[14] Despite the extended clinical and survival benefits offered by second-generation ALK TKIs when utilized as the first-line treatment approach for patients with ALK-positive advanced NSCLC, the emergence of drug resistance and CNS progression remain significant challenges that demand attention.[15,16] Our retrospective study investigated baseline clinical and molecular factors potentially associated with the risk of CNS progression during first-line or second-line alectinib treatment in patients with ALK-positive NSCLC.

Materials and Methods

Patient Inclusion Criteria

For this retrospective study, we analyzed the clinical and molecular data of a total of 14,320 patients who were diagnosed with lung cancer and submitted samples for ALK molecular testing between January 2018 and June 2022 at Hunan Cancer Hospital, a tertiary cancer hospital in Hunan Province, People's Republic of China. The main study inclusion criteria are as follows: (1) histopathology confirmed NSCLC; (2) positive detection of ALK fusion either by Ventana ALK immunohistochemistry (IHC) or by DNA-based next-generation sequencing (NGS); (3) disease progression from first-line or second-line alectinib therapy; and (4) availability of imaging data (i.e., computed tomography or magnetic resonance imaging) at baseline and disease progression. The study protocol was approved by the Hunan Cancer Hospital Institutional Review Board (approval number: 2019-SSB-IIT-115). Written informed consent was waived, given the retrospective nature of the study.

Programmed Death Ligand-1 Expression

Programmed death ligand-1 (PD-L1) IHC of formalin-fixed paraffin-embedded tumor samples was performed using either clone 22C3 (pharmDx, Agilent Dako Omnis, Santa Clara, CA) or SP142 (Ventana, Roche Diagnostics, Indianapolis, IN) following standard protocol from the manufacturer. Each sample was assessed and scored by two qualified pathologists. The tumor proportion score (TPS) was calculated as a percentage of at least 100 viable tumor cells with membrane staining.

Next-Generation Sequencing

Tissue biopsy or blood samples from patients were submitted for DNA-based NGS to a centralized laboratory. DNA isolated from tissue biopsy or blood samples was subjected to library preparation using a commercially available panel that specifically targets 56 or 168 genes (Burning Rock Biotech, Guangzhou, People's Republic of China). All the panels cover relevant genomic regions for the detection of variants in genes that are relevant for NSCLC, including *ALK*, *BRAF*, *EGFR*, *ERBB2*, *KRAS*, *MET*, *RET*, *ROS1*, *TP53*, and *RB1*. The capture probes for detecting the *ALK* fusions in all gene panels were designed to interrogate the intronic regions of *ALK*, whereas no capture probe was designed to target the intronic regions of the fusion partner, including *EML4*. Subsequently, the prepared libraries underwent paired-end

sequencing on a Nextseq 500 instrument (Illumina, CA). The target sequencing depth was 1000× and 10,000× for tissue and plasma samples, respectively. Analysis was performed using an optimized bioinformatics pipeline for identifying somatic variants.

Efficacy and Safety Evaluation

Systemic response was assessed by the physician-in-charge in accordance with the Response Evaluation Criteria in Solid Tumors (version 1.1). Tumor assessments, including CNS metastases, were conducted using computed tomography or magnetic resonance imaging at baseline before initiating treatment (day 0) and after every two cycles (approximately 8 wks) until confirmation of disease progression. Each radiological image was independently evaluated by two radiologists. Progression-free survival (PFS) was calculated as the time interval from initiating treatment until confirmation of tumor progression, death from any cause, or the last date of follow-up.

Statistical Analysis

Continuous variables were summarized as means and standard deviations or medians with range and compared using unpaired *t* test or Wilcoxon signed rank test. Categorical variables were summarized by presenting the frequencies with their corresponding percentages and compared using chi-square or Fisher's exact test, as appropriate. Kaplan-Meier analysis was used to estimate the survival functions and log-rank test to determine the difference in survival outcomes between groups. The risk of intracranial progression was analyzed using multivariate logistic regression with stepwise regression to mitigate the potential impact of multicollinearity. All the tests conducted in this study were performed using a two-sided approach, and a p value of less than 0.05 was considered statistically significant. All statistical analyses were performed using R (version 3.3.3, the R Foundation for Statistical Computing, Vienna, Austria) and R Studio (version 1.1.383).

Result

Patient Characteristics

A flowchart of patient screening is summarized in Figure 1. The baseline characteristics of patients with ALK-positive NSCLC who received first-line or second-line

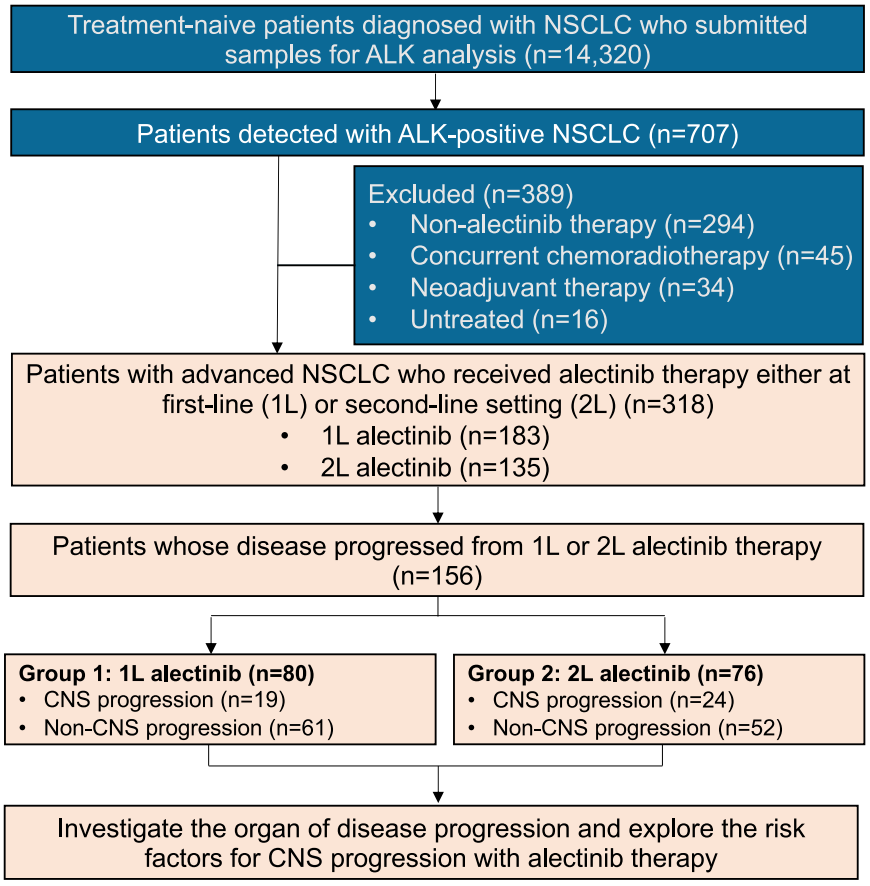

Figure 1. Flow diagram illustrating the patient selection and subgroup disposition. 1L, first-line; 2L, second-line; CNS, central nervous system.

alectinib therapy are listed in Table 1. The median age of the whole cohort was 50.8 years (range: 21–78 y). Among 318 patients diagnosed with ALK-positive NSCLC treated with alectinib, 44.3% were younger than 51 years old, female patients account for 54.7%, 66.7% were nonsmokers, 96.9% had Eastern Cooperative Oncology Group performance score of zero to one. Most patients (95.9%) had lung adenocarcinoma, and 33.9% had brain metastasis at baseline. The detection methods used to identify ALK positivity among the 318 patients included NGS (77.7%, n = 247), Ventana IHC (21.1%), and amplification refractory mutation system (1.2%). Among 251 patients identified as *ALK*-positive by NGS and ARMS, 14.7% were detected with a fusion gene containing the 3′-*ALK* and an additional 5′-*ALK* fusion, and 85.3% were detected with only the 3′-*ALK* fusion. Among these 251 patients who had DNA-based NGS data, 219 patients were detected with known *ALK* breakpoint variants, of which 37.0% (81 of 219) had variant 3a/b and 63.0% (138 of 219) non-3a/b variant. Concomitant mutations in tumor suppressor genes, including *TP53* (66 of 318), *PTEN*, and *RB1*, were detected in 23.0% (73 of 318) of patients, with 54.4% of patients not detected with mutations in these genes and gene mutation status in 22.6% of patients not evaluable for concomitant mutations due to the use of either a smaller panel for NGS or use of non-NGS molecular assay (i.e., amplification refractory mutation system or IHC). Among 318 ALK-positive patients, only 133 patients had data for PD-L1 expression by 22C3 or SP142 antibody. Of them, 33.1% (44 of 133) had PD-L1 TPS of $\geq$50%, 35.3% of patients (47 of 133) had PD-L1 TPS of 1% to 49%, and 31.6% (42 of 133) had PD-L1 TPS of less than 1%.

Treatment Outcomes With Alectinib

We analyzed the treatment outcomes of first-line and second-line alectinib in our cohort. Patients who received first-line alectinib had a median PFS of 36.0 months (95% confidence interval [CI]: 25.3–46.7); with a 1-year PFS rate of 73.0%, 2-year PFS rate of 61.3%, and 3-year PFS rate of 51.0% (Fig. 2*A*). Patients who received alectinib as second-line therapy had a median PFS of 12.0 months (95% CI: 8.2–15.8), with a 1-year PFS rate of 49.9%, 2-year PFS rate of 36.3%, and 3-year PFS rate of 23.6% (Fig. 2*B*).

We also performed subgroup analysis to understand the PFS with first-line alectinib and second-line alectinib for various molecular subgroups. In terms of *EML4-ALK* variants, we found that patients whose NSCLCs harbored variant 3a/b *EML4-ALK* had significantly shorter PFS with first-line alectinib than patients with non-3a/b variant (14.0 months versus 41.0 mo; hazard ratio [HR] = 2.32, 95% CI: 1.25–4.32, $p = 0.001$; Supplementary Fig. 1). Although we observed the PFS difference for first-line alectinib, patients with variant 3a/b *EML4-ALK* had statistically comparable PFS with second-line alectinib as patients with non-3a/b variant (21.2 months versus 11.0 mo; HR = 0.75, 95% CI: 0.44–1.28, $p = 0.29$; Supplementary Fig. 2).

In terms of concurrent *TP53* mutation status, we found that patients detected with concurrent *TP53* mutations and those who were not detected with concurrent *TP53* mutations had statistically comparable PFS with first-line alectinib (28.0 months versus 35.0 mo; HR = 1.27, 95% CI: 0.74–2.18, $p = 0.33$; Supplementary Fig. 3). Contrastingly, patients detected with concurrent *TP53* mutations had significantly shorter PFS with second-line alectinib as compared with patients who were not detected with concurrent *TP53* mutations (7.0 months versus 13.0 mo HR = 2.14, 95% CI: 1.09–4.18, $p = 0.026$; Supplementary Fig. 4).

We also analyzed the impact of the concurrent detection of 5′-*ALK* with *EML4-ALK*. Compared with patients detected with only the classic *EML4-ALK*, patients whose NSCLCs retained the 5′-*ALK* with *EML4-ALK* had significantly shorter PFS with first-line alectinib (12.0 months versus 37.3 mo; HR = 2.13, 95% CI: 1.04–4.37, $p = 0.006$; Supplementary Fig. 5) and second-line alectinib (5.0 mo versus 15.0 mo; HR = 3.58, 95% CI: 1.23–10.44, $p < 0.001$; Supplementary Fig. 6).

Pattern of Progression With Alectinib Treatment

Among the 318 patients with ALK-positive NSCLC treated with alectinib, a total of 156 patients experienced disease progression during the study period. Among them, 80 patients experienced disease progression on first-line alectinib and 76 patients experienced disease progression on second-line alectinib. We further analyzed the site of disease progression and stratified these patients into two subgroups on the basis of the extent of spread of the disease/metastatic progression as oligoprogression and nonoligoprogression. The criteria for defining oligoprogression were the detection of disease progression in three organs or less, three lesions or less originating from a single organ wherein the size of each lesion should be 3 cm or less. As depicted in Figure 3*A*, 55.0% (44 of 80) of patients who progressed after first-line alectinib had oligoprogression and 45.0% (36 of 80) had nonoligoprogression. Among the 44 patients with oligoprogression, disease progression was observed mostly in the brain (18.2%, eight of 44) and lung (47.8%, 21 of 44). Organs involved in disease progression of the 36 patients with nonoligoprogression were lung (75.0%, 27 of 36), brain (30.5%, 11 of 36), lymph nodes (25.0%, nine of 36), and pleural effusion (25.0%, nine of 36). As seen in Figure 3*B*, 63.2% (48 of 76) of patients whose disease progressed on second-line

Table 1. Baseline Clinical and Molecular Characteristics of the Study Cohort

Characteristics	Total (N = 318)	Alectinib Treatment Line		p^a
		First-Line Alectinib (n = 183)	Second-Line Alectinib (n = 135)	
Age (y), median (range)	50.8 (21-78)	50.8 (21-8)	50.7 (26-73)	0.86
Age (y)				
<51	141 (44.3)	83 (45.4)	58 (42.9)	0.76
≥51	177 (55.7)	100 (54.6)	77 (57.1)	
Sex				
Female	174 (54.7)	83 (45.4)	91 (67.4)	<0.001
Male	144 (45.3)	100 (54.6)	44 (32.6)	
Smoking history				
Never smoker	212 (66.7)	109 (59.6)	103 (76.3)	0.002
Smoker	106 (33.3)	74 (40.4)	32 (23.7)	
ECOG PS				
High (2-3)	10 (3.1)	2 (1.1)	8 (5.9)	0.014
Low (0-1)	308 (96.9)	181 (98.9)	127 (94.1)	
Clinical stage				
IIIb/IIIc	40 (12.6)	19 (10.4)	21 (15.6)	0.23
IV	278 (87.4)	164 (89.6)	114 (84.4)	
Brain metastases status				
With	108 (33.9)	53 (28.9)	55 (40.7)	0.028
Without	210 (66.1)	130 (71.1)	80 (59.3)	
Methods used for evaluating brain metastasis (n = 108)				
CT	38 (35.2)	16 (30.2)	22 (40.0)	0.28
MRI	70 (64.8)	37 (69.8)	33 (60.0)	
Local therapy received for management of brain metastasis (n = 39)				
WBRT	21 (53.8)	12 (50.0)	9 (60.0)	0.54
SBRT	18 (46.2)	12 (50.0)	6 (40.0)	
Histopathology				
Adenocarcinoma	305 (95.9)	176 (96.2)	129 (95.6)	0.78
Other[b]	13 (4.1)	7 (3.8)	6 (4.4)	
ALK detection method				
ARMS	4 (1.2)	4 (2.2)	0	0.08
NGS	247 (77.7)	146 (79.8)	101 (74.8)	
Ventana IHC	67 (21.1)	33 (18.0)	34 (25.2)	
ALK fusion type				
Non-*EML4-ALK*	13 (4.1)	5 (2.7)	8 (5.9)	0.13
3′-*ALK* Retain 5′-*ALK*	37 (11.6)	25 (13.7)	12 (8.9)	
ALK (IHC)	67 (21.1)	33 (18.0)	34 (25.2)	
EML4-ALK	201 (63.2)	120 (65.6)	81 (60.0)	
ALK fusion variant				
Variant 3a/b	81 (25.5)	41 (22.4)	40 (29.6)	0.17
Non-3a/b variant	138 (43.4)	87 (47.5)	51 (37.8)	
Unidentified	99 (31)	55 (30.1)	44 (32.6)	
TP53 concomitant mutation				
None	252 (79.2)	144 (78.7)	108 (80.0)	0.88
With	66 (20.8)	39 (21.3)	27 (20.0)	
PD-L1 TPS				
<1%	42 (13.2)	23 (12.6)	19 (14.1)	0.63
1%-49%	47 (14.8)	28 (15.3)	19 (14.1)	
≥50%	44 (13.8)	29 (15.8)	15 (11.1)	
Unknown	185 (58.2)	103 (56.3)	82 (60.7)	

Note: All values are n (%) unless otherwise specified.

[a]Statistical comparison performed using chi-square test except for age.

[b]Among 13 patients, six patients had adenosquamous carcinoma, four patients had mucoepidermoid carcinoma, and three patients had squamous cell carcinoma.

ARMS, amplification refractory mutation system; CT, computed tomography; ECOG PS, Eastern Cooperative Oncology Group performance status; IHC, immunohistochemistry; MRI, magnetic resonance imaging; NGS, next-generation sequencing; PD-L1, programmed death-ligand 1; SBRT, stereotactic body radiotherapy; TPS, tumor proportion score; WBRT, whole brain radiotherapy.

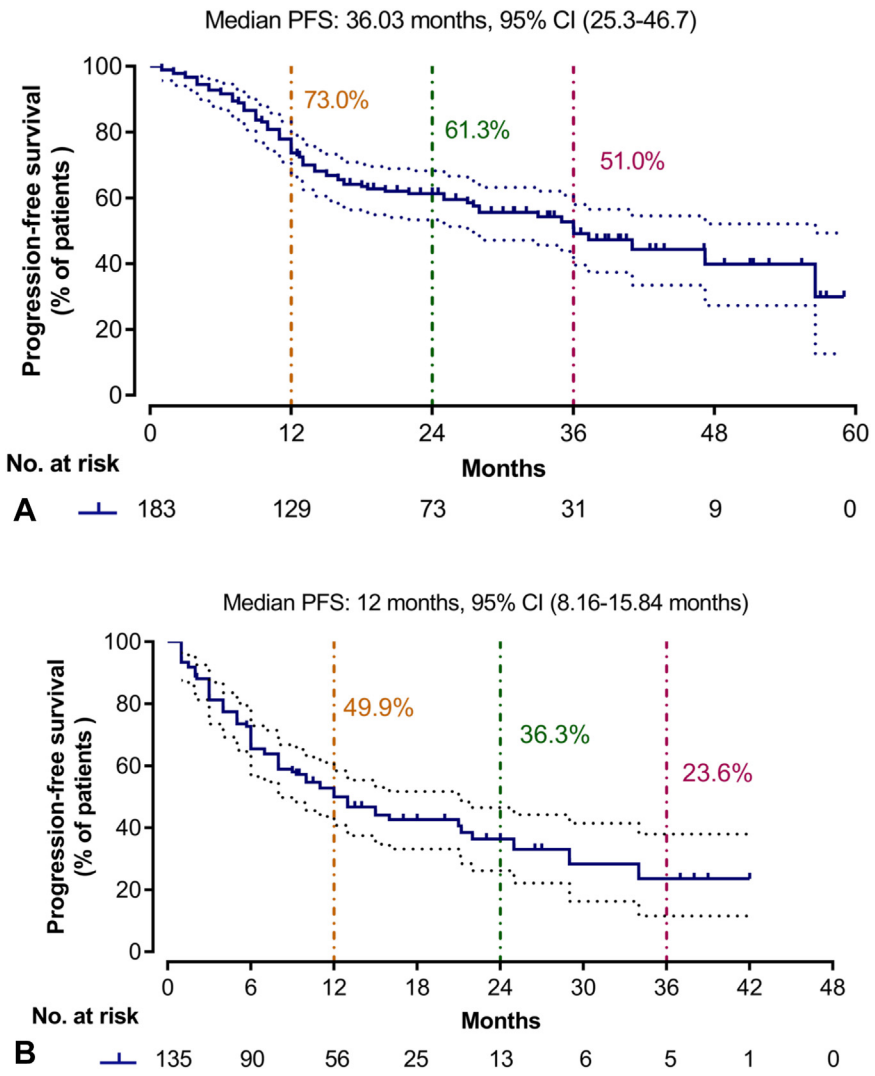

Figure 2. Kaplan-Meier survival curves illustrating the PFS of patients who received first-line alectinib (n = 183) *(A)* and second-line alectinib (n = 135) *(B)*. Tick marks indicate the censored data. Blue dotted lines above and below the curve correspond to the lower-bound and upper-bound 95% CI. Vertical dashed lines denote the PFS rates for 1 year (orange), 2 years (green), and 3 years (red). The risk table below indicates the number of events analyzed for the particular time point. CI, confidence interval; PFS, progression-free survival.

alectinib had oligoprogression and 36.8% (28 of 76) had nonoligoprogression. Among the 48 patients with oligoprogression, disease progression was most often observed in the brain (31.3%, 15 of 48). On the other hand, disease progression was found in the lung (67.8%, 19 of 28), brain (32.1%, nine of 28), bone (32.1%, nine of 28), and liver (28.5%, eight of 28) in 28 patients with nonoligoprogression.

Next, we compared the proportion of CNS progression after first-line and second-line alectinib treatment. The frequency of CNS progression was similar between patients who received first-line alectinib and second-line alectinib ($p > 0.05$), regardless of whether it was in the overall population (23.7% versus 31.5%; $p = 0.27$), the subset of patients with oligoprogression (18.1% versus 31.2%; $p = 0.15$) or patients who had nonoligoprogression (30.5% versus 32.1%; $p = 0.89$; Supplementary Fig. 7). The incidence rates of CNS progression were 23.7% after first-line alectinib therapy and 31.6% after second-line alectinib therapy.

Among the patients who received first-line alectinib treatment, brain metastases were detected at diagnosis in 35.6% (31 of 87) of patients with non-3a/b variant, and 21.9% (nine of 41) of patients with variant 3a/b. Radiotherapy was administered to 48.4% (15 of 31) of patients with non-3a/b with brain metastases at baseline (stereotactic body radiotherapy [SBRT], n = 7; whole brain radiotherapy [WBRT], n = 8), and 66.7% (six of nine) of patients with variant 3a/b with brain metastases at baseline (SBRT, n = 3; WBRT, n = 3). Among the

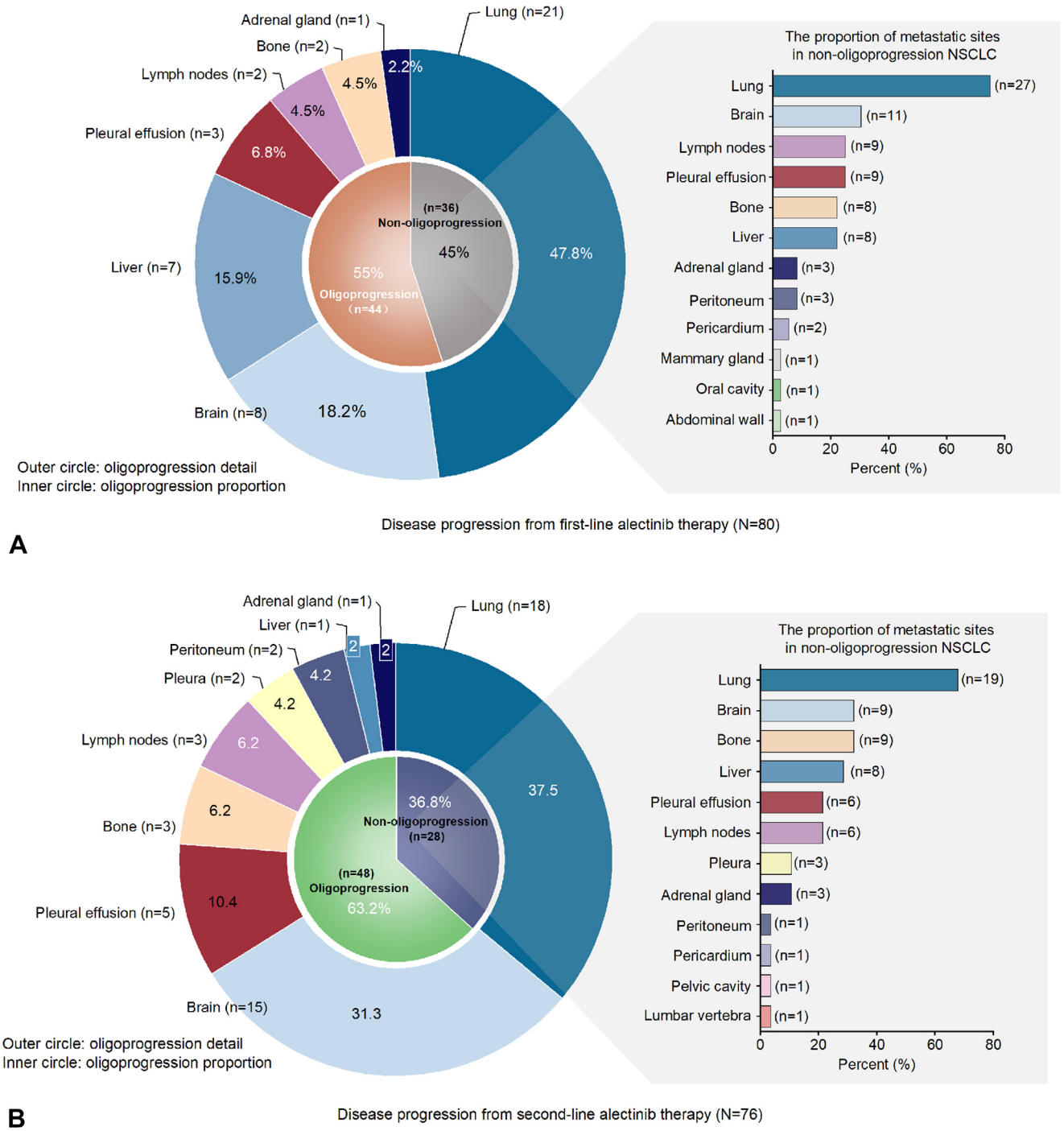

Figure 3. Organ distribution after progression from first-line or second-line alectinib therapy *(A, B)*. Donut charts illustrating the distribution of organ involvement at disease progression of patients who received first-line alectinib therapy (n = 80) *(A)* and those who received second-line alectinib therapy (n = 76) *(B)*. The inner circle depicts the distribution of disease progression into oligoprogression or nonoligoprogression. The outer circle illustrates the distribution of organs where disease progression was detected in the subgroup with oligoprogression. The histogram on the right specifies the distribution of organs where disease progression was detected in the subgroup with nonoligoprogression.

patients who received second-line alectinib treatment, brain metastases were detected before treatment of 47.1% (24 of 51) of patients with non-3a/b variant, and 27.5% (11 of 40) of patients with variant 3a/b. Radiotherapy was administered to 25.0% (six of 24) of patients with a non-3a/b variant with brain metastases at baseline (SBRT, n = 2; WBRT, n = 4) and 45.5% (five of 11) of patients with variant 3a/b with brain

metastasis (SBRT, n = 2; WBRT, n = 3). The difference in basic clinical characteristics between the cohorts contributes to the higher incidence of brain progression in patients with non-3a/b variant than those with variant 3a/b. Patients with non-3a/b variant had a higher proportion of brain metastases at baseline and received less radiotherapy, regardless of whether they received alectinib treatment in the first or second line. Patients who received second-line alectinib were treated with first-line crizotinib.

Univariate and Multivariate Analyses

We used univariate and multivariate analysis to identify baseline clinical and molecular factors that could be associated with an increased risk for CNS progression during alectinib therapy among patients with ALK-positive NSCLC. Patients without brain metastasis at baseline had a lower risk of CNS progression during alectinib treatment than patients with baseline brain metastasis (univariate OR = 0.35, $p = 0.002$; multivariate OR = 0.36, $p = 0.011$; Table 2). Compared with

Table 2. Univariate and Multivariate Logistic Regression in Predicting Intracranial Progression With Alectinib Treatment in 318 Patients With ALK-Positive NSCLC

Characteristics	Nonintracranial Progression (n = 275), n (%)	Intracranial Progression (n = 43), n (%)	OR (95% CI, *p* Value) (Univariate)	OR (95% CI, *p* Value) (Multivariate)
Age				
<51	122 (44.4)	19 (44.2)		
≥51	153 (55.6)	24 (55.8)	1.01 (0.53-1.92, $p = 0.983$)	1.46 (0.67-3.17, $p = 0.345$)
Sex				
Female	148 (53.8)	26 (60.5)		
Male	127 (46.2)	17 (39.5)	0.76 (0.40-1.47, $p = 0.416$)	0.50 (0.17-1.46, $p = 0.203$)
Smoking history				
Never smoker	183 (66.5)	29 (67.4)		
Smoker	92 (33.5)	14 (32.6)	0.96 (0.48-1.91, $p = 0.908$)	1.43 (0.46-4.44, $p = 0.538$)
ECOG PS score				
High (2-3)	7 (2.5)	3 (7)		
Low (0-1)	268 (97.5)	40 (93)	0.35 (0.09-1.40, $p = 0.138$)	0.38 (0.07-2.04, $p = 0.258$)
Stage				
IIIB/IIIC	36 (13.1)	4 (9.3)		
IV	239 (86.9)	39 (90.7)	1.47 (0.50-4.35, $p = 0.488$)	1.96 (0.54-7.07, $p = 0.303$)
Baseline brain metastasis status				
With	84 (30.5)	24 (55.8)		
Without	191 (69.5)	19 (44.2)	0.35 (0.18-0.67, $p = 0.002$)	0.36 (0.16-0.79, $p = 0.011$)
ALK fusion				
Non-*EML4-ALK*	11 (4)	2 (4.7)		
ALK (IHC)	64 (23.3)	3 (7)	0.26 (0.04-1.72, $p = 0.162$)	1.68 (0.07-39.83, $p = 0.748$)
EML4-ALK	170 (61.8)	31 (72.1)	1.00 (0.21-4.75, $p = 0.997$)	4.18 (0.41-42.90, $p = 0.228$)
3′-*ALK* Retain 5′-*ALK*	30 (10.9)	7 (16.3)	1.28 (0.23-7.14, $p = 0.776$)	3.25 (0.46-23.23, $p = 0.239$)
Fusion variant				
Non-V3a/b	110 (40)	28 (65.1)		
V3a/b	75 (27.3)	6 (14)	0.31 (0.12-0.80, $p = 0.015$)	0.35 (0.13-0.98, $p = 0.046$)
Unidentified	90 (32.7)	9 (20.9)	0.39 (0.18-0.88, $p = 0.022$)	2.20 (0.33-14.45, $p = 0.413$)
TP53 concomitant mutation				
None	218 (79.3)	34 (79.1)		
With	57 (20.7)	9 (20.9)	1.01 (0.46-2.23, $p = 0.976$)	0.93 (0.37-2.33, $p = 0.882$)
PD-L1 TPS				
<1%	39 (14.2)	3 (7)		
1%-49%	37 (13.5)	10 (23.3)	3.51 (0.90-13.78, $p = 0.071$)	4.28 (0.96-19.16, $p = 0.057$)
≥50%	29 (10.5)	15 (34.9)	6.72 (1.78-25.41, $p = 0.005$)	9.49 (2.16-41.65, $p = 0.003$)
Unknown	170 (61.8)	15 (34.9)	1.15 (0.32-4.16, $p = 0.835$)	1.42 (0.35-5.72, $p = 0.618$)
Treatment line				
Second-line	111 (40.4)	24 (55.8)		
First-line	164 (59.6)	19 (44.2)	0.54 (0.28-1.02, $p = 0.059$)	0.34 (0.15-0.76, $p = 0.008$)

CI, confidence interval; ECOG PS, Eastern Cooperative Oncology Group Performance Status; IHC, immunohistochemistry; PD-L1, programmed death ligand 1; TPS, tumor proportion score; V3a/b, variant 3a/b.

patients detected with the non-3a/b variant, patients with *EML4-ALK* variant 3a/b had statistically lower risk of CNS progression (univariate OR = 0.31, $p = 0.015$; multivariate OR = 0.35, $p = 0.046$; Table 2). Consistently, the incidence of CNS progression 1 year after treatment was numerically lower among patients with *EML4-ALK* variant 3a/b than those with the non-3a/b variant (5.9% versus 12.3%; Supplementary Fig. 8). Moreover, patients with PD-L1 TPS of 50% or higher were statistically more likely to develop CNS progression with alectinib therapy regardless of treatment line than those with PD-L1 TPS of less than 50% (univariate OR = 6.72, $p = 0.005$; multivariate OR = 9.49, $p = 0.003$; Table 2). Consistently, the incidence of CNS progression 1 year after treatment was numerically lower among patients with PD-L1 TPS of less than 1% than those with PD-L1 TPS of 50% or higher (8.5% versus 17.3%; Supplementary Fig. 9). Furthermore, patients who received first-line alectinib are less likely to experience CNS progression than those who received second-line alectinib (multivariate OR = 0.34, $p = 0.008$; Table 2). Stepwise regression for final variable screening identified four variables, namely *EML4-ALK* breakpoint variants, PD-L1 TPS, baseline brain metastasis status, and alectinib treatment line as variables associated with risk of CNS progression in patients with ALK-positive NSCLC treated with alectinib.

Furthermore, we have constructed a nomogram that incorporates the four clinical variables identified to be associated with CNS progression on the basis of multivariate regression analyses (Fig. 4*A*). Figure 4*B* plots the receiver operating characteristic curve, which shows the area under the curve of 0.793, with a specificity of 62.9% and sensitivity of 81.4% for the performance of the nomogram model in predicting the risk of CNS progression among patients with ALK-positive NSCLC after alectinib progression. The decision curve analysis complements the receiver operating characteristic curve and shows a high clinical value of the nomogram model (Fig. 4*C*).

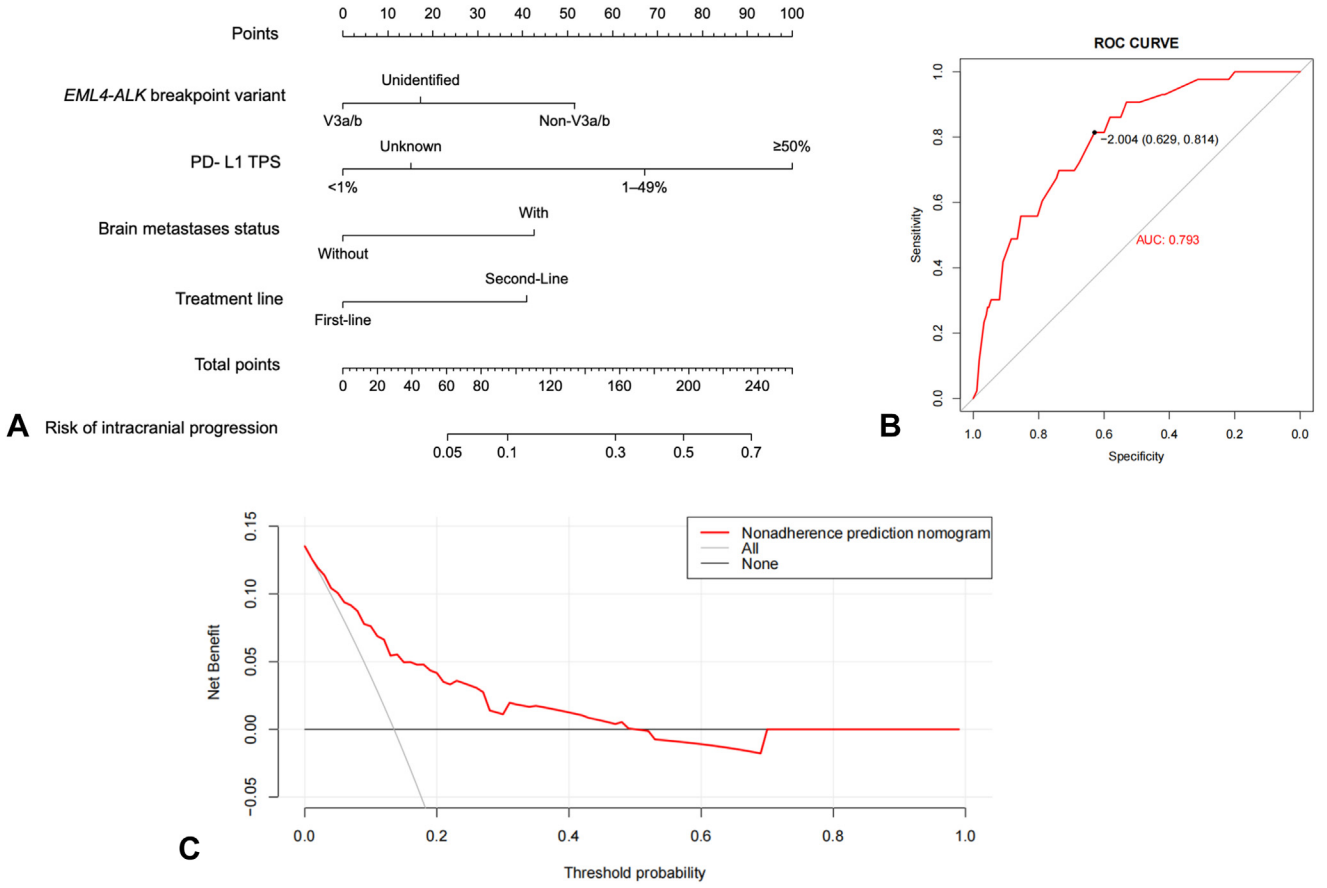

Figure 4. Nomogram-based model for predicting risk of CNS progression among patients with ALK-positive NSCLC. *(A)* Nomogram constructed on the basis of the four clinical variables found to be associated with the risk of CNS progression. *(B)* ROC curve plotting the performance of the nomogram in predicting the risk of CNS progression in patients with ALK-positive NSCLC after alectinib resistance. *(C)* DCA. ALK, anaplastic lymphoma kinase; CNS, central nervous system; DCA, decision curve analysis; ROC, receiver operating characteristic.

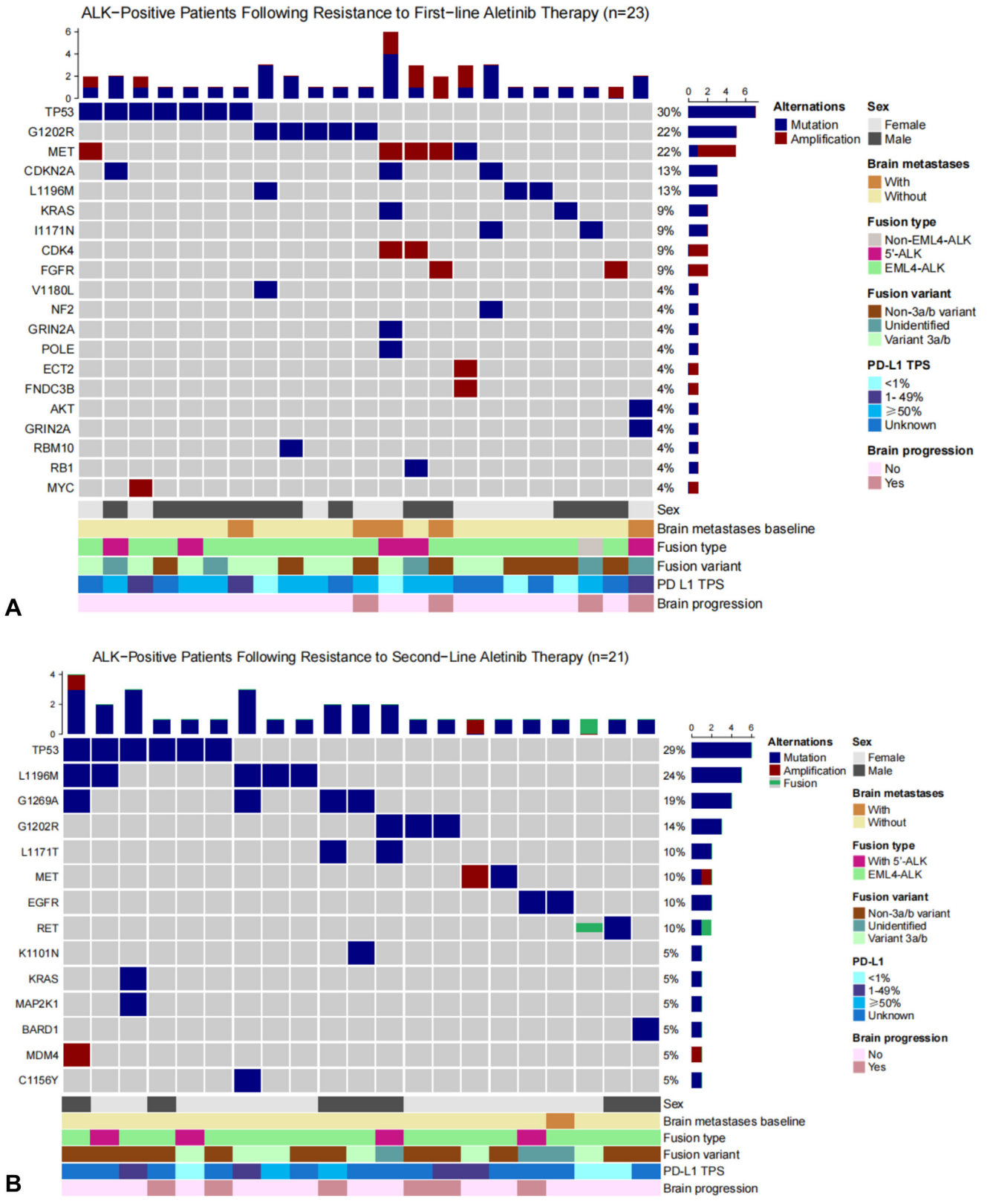

Figure 5. Oncoplot showing the molecular profile detected at disease progression of evaluable patients who received first-line alectinib therapy (n = 22) *(A)* and those who received second-line alectinib therapy (n = 21) *(B)*. Values on the left reveal the mutation rates of genes indicated on the right. Different colors denote different mutation types. The histogram on the right shows the proportion of mutation types detected per gene.

First-Line or Second-Line Molecular Resistance Profile of Alectinib

Lastly, we investigated the molecular resistance profile of patients treated with first-line or second-line alectinib therapy. The baseline somatic mutation landscape was comparable between patients who received first-line or second-line alectinib (Supplementary Figs. 10 and 11). *TP53* was the most common concomitant gene mutation detected in the two groups, detected in 21.3% (39 of 183) of patients who received first-line alectinib and 20.0% (27 of 135) of patients who received second-line alectinib. Among the 80 patients whose disease progressed with first-line alectinib, 23 patients submitted samples for molecular testing at disease progression (Fig. 5*A*). On the other hand, 21 of 76 patients treated with second-line alectinib underwent rebiopsy and submitted samples for molecular testing (Fig. 5*B*). As shown in Figure 5, the acquisition of secondary *ALK* mutations is the most common resistance mechanism, with *ALK* L1196M, G1202R, and G1202R as the most often detected mutations in patients who received first-line alectinib. Secondary *ALK* mutations were detected in 39.1% (nine of 23) of patients who received first-line alectinib and 47.6% (10 of 21) of patients who received second-line alectinib.

Discussion

Brain metastases impose a considerable humanistic burden, giving rise to notable disruptions in the quality of life as a result of the neurologic manifestations they entail.[17] Furthermore, brain metastases exert a detrimental impact on the survival prognosis of patients with lung cancer.[17] Despite the availability of an extensive number of literature dedicated to the treatment management of patients with ALK-positive NSCLC who developed brain metastases after ALK TKI treatment,[17,18] little is known about the prognostic factors, especially baseline factors associated with a higher risk of developing CNS progression. In this study, we investigated the pattern of disease progression by analyzing the sites of disease progression. We also investigated potential clinical factors that may increase the risk of CNS progression among patients with ALK-positive NSCLC who received alectinib treatment. These findings could impart valuable insights into optimal individualized management and improve the quality of life of patients with a higher risk of CNS progression.

TKIs offer the best initial response in treating tumors with driver mutations; nevertheless, disease progression eventually occurs, with half of the cases experiencing disease progression limited to a few sites.[19] This pattern of progression can often be explained by two mechanisms: (1) pharmacokinetic failure of the agent in the particular organ or (2) inherent heterogeneity within and between tumors.[20] In our study, we found that over half of patients who received either first-line or second-line alectinib treatment had oligoprogression, with a higher incidence of CNS progression in patients who received second-line alectinib.

Updated data from the ALEX study analyzed the impact of *EML4-ALK* fusion breakpoint subtypes on the efficacy of alectinib treatment and reported that patients harboring *EML4-ALK* breakpoint variants 1, 2, and 3a/b had comparable clinical outcomes as shown by the nonstatistically significant difference in PFS, objective response rate, or duration of response.[21] Our findings suggest that harboring the *EML4-ALK* non-3a/b breakpoint variant was associated with a higher risk of CNS progression during alectinib treatment than harboring the *EML4-ALK* variant 3a/b. In contrast, real-world evidence reported by El Shafie et al. found that patients with variant 3a/b *EML4-ALK* (n = 13) had an earlier intracranial progression, albeit a small cohort size.[22] The association between brain progression and *EML4-ALK* breakpoint variants that we observed in our study may only be secondary to the fundamental differences in baseline features and other confounders that exist between patients with *EML4-ALK* 3a/b and non-3a/b breakpoint variants. The relationship between brain progression and *EML4-ALK* breakpoint variants warrants further investigation.

Our study had several limitations. First, the retrospective nature of our study limits our analysis and conclusion as some of the patients included in the study have incomplete data on *ALK* fusion partner and breakpoint variants, and data on PD-L1 expression were only available for about one-third of the study cohort. Second, only a small number of patients submitted samples for genetic testing after developing CNS progression; hence, we were unable to perform further analyses such as comparing the risk of CNS progression according to the presence/lack of secondary *ALK* point mutations. Our study is hypothesis-generating and provides preliminary evidence for our findings. It would be meaningful to analyze the clinical data from a larger cohort of patients who received first-line alectinib treatment and who had no detectable brain metastasis at baseline to fully understand the clinical features and risks for CNS progression of this patient population.

Overall, our study provided real-world evidence on baseline molecular factors that could serve as prognostic markers for identifying patients with ALK-positive NSCLC who are at a higher risk of CNS progression despite being treated with first-line or second-line alectinib therapy. In particular, certain baseline features, including PD-L1 TPS of 50% or higher, are associated with a heightened risk of developing CNS progression

during alectinib therapy. These findings suggest that close monitoring of this patient subset could enable the implementation of prompt therapeutic intervention to mitigate and manage their risk and help prolong their quality of life.

CRediT Authorship Contribution Statement

Lianxi Song: Data curation, Methodology, Formal analysis, Visualization, Writing - original draft, Writing - review & editing.

Huan Yan: Software operation, Data validation, Writing - review & editing.

Qinqin Xu: Software operation, Data validation, Writing - review & editing, Formal analysis, Visualization.

Chunhua Zhou: Software operation, Data validation, Writing - review & editing. Juan Liang: Software operation, Data validation, Writing - review & editing.

Shaoding Lin: Critical comments and suggestions, Writing - review & editing.

Ruiguang Zhang: Formal analysis, Visualization, Software operation, Data validation, Writing - review & editing.

Juan Yu: Formal analysis, Visualization, Writing - review & editing.

Yang Xia: Formal analysis, Visualization, Writing - review & editing.

Nong Yang: Formal analysis, Critical comments and suggestions, Writing - review & editing.

Liang Zeng: Conceptualization, Organization, Data collection, Data curation, Methodology, Formal analysis, Auditing, Supervision, Project management, Funding acquisition, Writing - original draft, Writing - review &editing.

Yongchang Zhang: Conceptualization, Organization, Data collection, Auditing, Supervision, Project management, funding acquisition, Writing - review & editing.

Disclosure

The authors declare no conflict of interest.

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Supplementary Data

Note: To access the supplementary material accompanying this article, visit the online version of the *JTO Clinical and Research Reports* at www.jtocrr.org and at https://doi.org/10.1016/j.jtocrr.2024.100729.

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