THERAPEUTIC ADVANCES in
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Study Protocol

Prospective observational study to explore genes and proteins predicting efficacy and safety of brigatinib for ALK-gene rearranged non-small-cell lung cancer: study protocol for ABRAID study (WJOG11919L)

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Abstract

Background: ALK-tyrosine kinase inhibitors (ALK-TKIs) are effective for treating non-small-cell lung cancer with *ALK* gene rearrangement; however, resistance is inevitable. Brigatinib is a unique ALK-TKI that is effective against many resistance mutations. However, data on factors associated with its efficacy and resistance mechanisms are limited.

Objectives: This study will evaluate the efficacy and safety of brigatinib in the real world and explore factors related to its efficacy, safety, and resistance mechanisms.

Design: Prospective observational study.

Ethics: This study is approved by the Ethics Committee of Wakayama Medical University. Written informed consent will be obtained from all patients before study-related procedures.

Methods and analysis: This study comprises three cohorts. Cohorts A, B, and 0 will enroll patients receiving alectinib as the first ALK-TKI, receiving alectinib as the first ALK-TKI and subsequently cytotoxic agents and/or lorlatinib after alectinib, and without a history of ALK-TKI, respectively. Overall, 100, 30, and 50 patients will be enrolled in Cohorts A, B, and 0, respectively. Circulating tumor DNA before starting brigatinib and at disease progression will be analyzed in all cohorts using a hypersensitive next-generation sequencing (NGS) PGDx Elio plasma resolve panel. Serum protein levels will be analyzed using the Milliplex xMAP assay system with a Luminex 200 (Luminex, Austin, USA). The enrollment period is 31 months and the patients will be observed for 2 years after enrollment. Archived tissues will be collected for NGS analysis, gene expression analysis, and immunohistochemistry staining 1 year after completion of registration. Quality of life and safety evaluation using electronic patient-reported outcomes will be investigated.

Discussion: This study will elucidate predictors of ALK-TKI efficacy and resistance mechanisms and evaluate the efficacy and safety of brigatinib in a real-world setting. The results will provide crucial information for establishing treatment strategies, discovering novel biomarkers, and developing new therapeutic agents.

Trial registration: UMIN000042439.

Keywords: alectinib, ALK inhibitors, brigatinib, non-small-cell lung cancer, observational study

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Introduction

Lung cancer was the leading cause of all cancer-related deaths worldwide in 2018, accounting for 18.4% (1.8 million) deaths.[1] Rearrangement of the ALK gene accounts for approximately 3–5% of all non-small-cell lung cancer (NSCLC) cases and tends to be more prevalent in young people.[2] In 2011, the Food and Drug Administration approved crizotinib, the first ALK tyrosine kinase inhibitor (TKI), for the treatment of advanced ALK-positive NSCLC. In 2014, a phase III trial in patients with untreated ALK gene metastases in advanced stages showed progression-free survival (PFS) superiority over standard chemotherapy,[3] cementing ALK-TKI as an essential drug. Subsequently, alectinib, lorlatinib, ensartinib, and brigatinib showed superiority over crizotinib in PFS in further phase III trials[4–7] and, currently, these three ALK-TKIs are widely used as first-line therapy. However, because most cases eventually lead to tumor progression, the development of sequencing strategies for ALK-TKI is an urgent issue.

Various reports exist on factors associated with the efficacy of ALK-TKI; for example, in 2018, Lin *et al.*[8] reported that the efficacy of lorlatinib differed between Variants 1 and 3 of the ALK gene and showed that Variant 3 led to more frequent G1202R resistance mutations than Variant 1 after progression on a second-generation ALK inhibitor. Other reports also suggest that lorlatinib and brigatinib had better PFS in variant 1 than in variant 3 [9,10]; however, reports also exist showing no such difference, including an analysis using data from the CROWN trial.[11,12] Thus, the significance of this finding is unclear. Moreover, mutations in genes other than ALK also affect ALK-TKI efficacy. The presence of TP53 mutations has been shown to reduce the efficacy of ALK-TKI, and an analysis of the ALTA-1L trial showed a trend toward shorter PFS in patients with TP53 mutations who received either brigatinib or crizotinib.[10] An analysis of the CROWN trial also showed a similar trend for lorlatinib[11,12]; however, no statistically significant difference was shown in this analysis and the significance of such compound mutations in ALK-TKI therapy is not fully understood.

Mutations in the tyrosine kinase domain of the ALK gene have been known to be one of the main resistance mechanisms in ALK-TKIs. For alectinib, the most widely used second-generation ALK-TKIs, approximately 50–60% of cases, had mutations in the ALK gene as a resistance mechanism, with I1171T/NS, V1180L, and G1202R being relatively common.[13] Because the meaning of the mutation varies depending on the drug, I1171T/N/S and V1180L are sensitive to ceritinib while reported to be resistant to crizotinib.[14] Brigatinib also has been reported to show sensitivity to I1171T/N/S and V1180L[13,15] and, among five patients with these mutations, one showed response and three achieved stable diseases.[16] Furthermore, brigatinib has limited sensitivity for G1202R.[14] However, the concentration of brigatinib that inhibited 50% of BA/F3 cells with G1202R mutation in Variant 3 was lower than that for cells with G1202R mutation in Variant 1,[15] and notably, G1202R is mostly found in Variant 3.[8] Considering these, such mutations may be predictive of brigatinib efficacy. Furthermore, it has been demonstrated that double and triple mutations occur, particularly when multiple ALK-TKIs are used[17,18] and that the appropriate agent for each mutation is also effective in such cases.[19] The accumulation of such information from clinical samples would therefore be crucial in developing appropriate treatment strategies for ALK-gene translocated NSCLC.[19]

Although varying by ALK-TKI, a significant proportion of cases with ALK-TKI resistance involve mechanisms other than ALK gene alterations, which are referred to as 'off-target' resistance. Various off-target resistance mechanisms exist,[14] including Met gene amplification,[20] Met signaling activation associated with hepatocyte growth factor (HGF)upregulation,[21] TP53 mutation,[22] the Src pathway,[23] and epidermal growth factor receptor (EGFR) pathway activation.[24] Such off-target resistance can occur in conjunction with on-target resistance, and this coexistence was reported in 5 of the 17 (29%) patients with multiple ALK-TKIs, which was higher than that in 2 of the 35 (6%) individuals with crizotinib alone.[25] In addition, the tumor microenvironment is associated with the efficacy of TKIs.[26] Hypoxia is reportedly involved in ALK-TKI resistance through the epithelial–mesenchymal transition.[27] In EGFR-TKIs, it has been recently reported that serum protein levels, including interleukin-6 (IL-6) and vascular endothelial growth factor (VEGF)[28,29] and tumor protein expression, such as EGFR,[30,31] programmed death-ligand 1 (PD-L1),[32,33] and CD47[34] are associated with efficacy and resistance. Similar resistance mechanisms may be observed for ALK-TKIs.

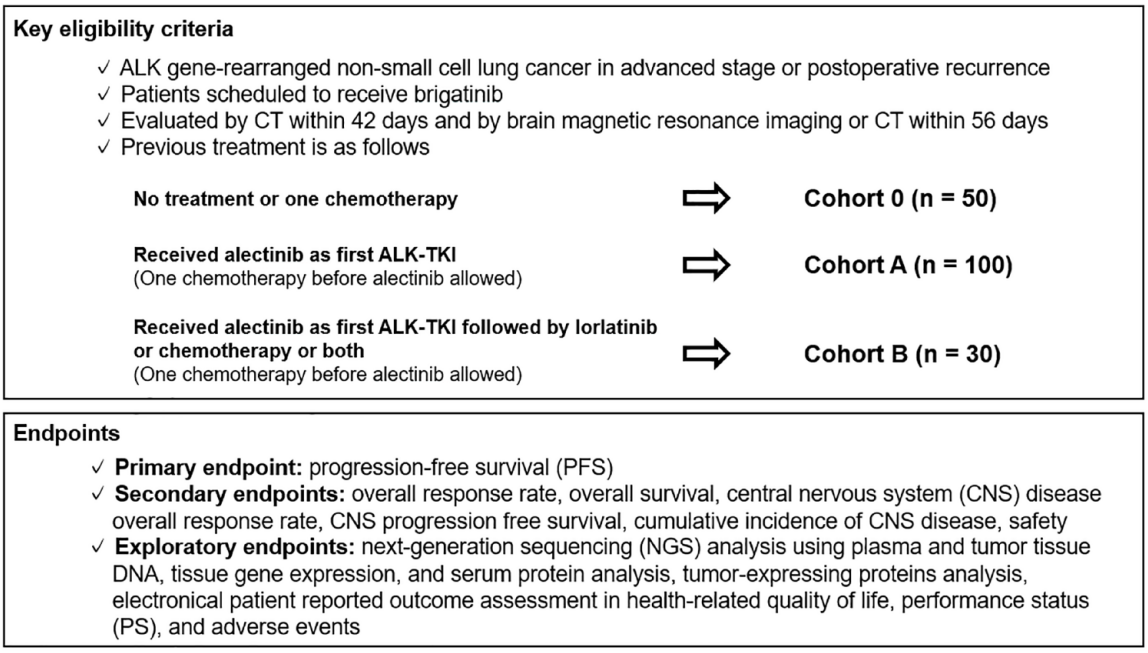

Figure 1. Protocol of this study.
ALK-TKI, ALK-tyrosine kinase inhibitor.

There is no clear indication of whether alectinib, lorlatinib, or brigatinib has the greatest efficacy for first-line therapy. Hence, elucidating the predictors of efficacy and resistance mechanisms for each of these drugs is essential to establishing the best sequencing strategy. In addition, data on brigatinib are relatively high in demand. Given these circumstances, evaluating its efficacy and safety in clinical practice, as well as elucidating the factors associated with its efficacy and resistance mechanisms, will be a major step toward establishing therapeutic strategies to utilize multiple second- and third-generation ALK-TKIs. Therefore, we have planned a multicenter observational study of brigatinib in three cohorts of patients with advanced ALK-rearranged NSCLC.

Study protocol

This study is prospectively enrolling patients with advanced ALK gene-rearranged NSCLC who receive brigatinib. Through this study, we aim to evaluate the efficacy and safety of brigatinib in the real world and explore factors related to its efficacy, safety, and resistance mechanisms.

This study includes the following three cohorts: A, B, and 0. Cohort A will enroll patients who receive alectinib as the first ALK-TKI, followed by brigatinib as an immediate subsequent therapy. This is the main cohort, and 100 patients will be enrolled. Cohort B will include 30 patients who receive alectinib as the first ALK-TKI, followed by chemotherapy and/or lorlatinib, and then brigatinib. Cohort 0 will comprise 50 patients who receive brigatinib as their first ALK-TKI. One chemotherapy regimen is allowed in all cohorts before the first ALK-TKI treatment (Figure 1). The eligibility criteria are as follows: (1) patients with advanced stages (IIIB, IIIC, IVA, and IVB) or those experiencing postoperative recurrence and ALK gene-rearranged NSCLC for which curative treatment is not indicated; (2) patients scheduled to receive brigatinib; and (3) patients who have received previous treatment: In Cohort A (brigatinib as second- or third-line therapy), alectinib was administered as the first ALK-TKI and was the most recent prior therapy. In Cohort B (brigatinib as 3rd–5th line therapy), alectinib was administered as the first ALK-TKI, and a cytotoxic agent (up to one regimen) and/or lorlatinib was administered after alectinib. Cytotoxic agents and lorlatinib are the most recent therapies. Cohort 0 (brigatinib as first- or second-line therapy): no prior use of ALK-TKIs. Up to one regimen of cytotoxic agents before the first ALK-TKI is allowed in all cohorts; (4) patients evaluated using computed tomography (CT), including the chest, within 42 days and brain magnetic resonance imaging or

CT within 56 days before the start of brigatinib; and (5) patients aged 20 years or older at the time consent was obtained.

The primary endpoint is PFS. The secondary endpoints are overall response rate, overall survival, time to treatment failure, duration of response, disease control rate, central nervous system (CNS) disease overall response rate, CNS disease control rate, CNS PFS, cumulative incidence of CNS disease, cumulative incidence of CNS disease progression, and incidence and severity of adverse events. Exploratory endpoints include next-generation sequencing (NGS) analysis using plasma and tumor tissue DNA, tissue gene expression, and serum and tumor-expressing proteins to explore factors associated with efficacy, safety, and resistance mechanisms to brigatinib. Plasma DNA will be collected before initiation of brigatinib and disease progression, and serum will be collected before initiation of brigatinib. Tumor tissues will be evaluated to the extent that is possible from incidental biopsies in clinical practice. In addition, patient-reported outcomes (PRO) will be assessed electronically in an exploratory manner in cases where additional consent is obtained. Specifically, health-related quality of life, performance status (PS), and adverse events will be assessed periodically. The impact of these assessments on clinical outcomes and the factors that influence participation in electronic PRO (ePRO) will be evaluated.

Treatment

All patients will receive brigatinib orally once daily for 7 days. Subsequently, 180 mg will be orally administered once daily. The dosage may be reduced according to the patient's condition and the investigators' clinical judgment.

Regarding circulating tumor DNA (ctDNA) analysis, we will collect plasma samples before treatment with brigatinib and after progressive disease, and ctDNA will be analyzed using hypersensitive NGS. For serum protein analysis, serum samples will be collected before brigatinib treatment. Furthermore, we will collect tumor tissue before the first ALK-TKI, after alectinib – before brigatinib, and after brigatinib – before the next treatment; we will also collect, when possible, and examine the tumor tissue DNA using NGS, tumor-expressing proteins using immunohistochemistry, and the gene expression profile of the tumor.

Participants' registration

After confirmation of eligibility and providing informed consent, eligible patients will be enrolled, and the investigator will initiate treatment with brigatinib. Recruitment will begin in May 2021 and continue until December 2023. Overall, 83 facilities, including community hospitals, academic hospitals, and cancer centers, in Japan will participate in this study.

DNA extraction from peripheral blood samples and tumor tissue

Regarding ctDNA analysis, blood samples will be collected using Streck Cell-Free DNA BCT blood collection tubes. Samples will be collected by SRL Inc. (Tokyo, Japan), and DNA will be extracted within 72 h of blood collection, according to the manufacturer's instructions. Briefly, blood will be centrifuged at 300*g* for 20 min and 5000*g* for 10 min at room temperature. Plasma DNA will be isolated using a QIAamp MinElute ccfDNA Midi Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Moreover, DNA concentration will be measured using a Qubit 3.0 Fluorometer (Life Technologies, Carlsbad, CA, USA) or NanoDrop 2000 (Fisher Scientific, Pittsburgh, USA). DNA and RNA will be extracted from formalin-fixed paraffin-embedded samples using SRL. Inc.

Next-generation sequencing

NGS will be performed using ctDNA extracted from the plasma. The PGDx Elio plasma resolve panel will be used to detect mutations and amplifications in 33 genes (Table 1). For tissue samples, the PGDx Elio tissue complete panel will be used to extract the mutations and amplifications of 507 genes when more than 50 ng of DNA is available (Supplemental Table S1).

Serum and tumor protein and gene expression analyses

Serum will be used to analyze the concentrations of 44 proteins (Table 2). The analysis will be performed using a Milliplex xMAP assay system with a Luminex 200. Gene expression in tissues will be analyzed using RNA extracted from tissues using the nCounter Analysis System (NanoString Technologies, Inc., Seattle, USA) Pancer IO360 panel. In the residual tissues, the expression of EGFR, Amphiregulin, CD47, PD-L1, CD8, and CD24 will be evaluated using immunostaining (Supplemental Table S2).

Table 1. Genetic analysis using circulating tumor DNA.

Sequence mutation analyses (33 genes)
AKT1, *BRCA1*, *CSF1R*, *HRAS*, *NTRK1*, *RET*, *ALK*, *BRCA2*, *EGFR*, *KIT*, *PDGFRA*, *ROS1*, *APC*, *BRIP1*, *ERBB2*, *KRAS*, *PIK3CA*, *TP53*, *ARID1A*, *CCND1*, *EZH2*, *MET*, *POLD1*, *ATM*, *CD274*, *FGFR1*, *MYC*, *POLE*, *BRAF*, *CDH1*, *FGFR2*, *NRAS*, and *RAF1*
Copy number variation (eight genes)
CCND1, *CD274*, *EGFR*, *ERBB2*, *KIT*, *MET*, *MYC*, and *FGFR2*
Translocation analyses (five genes)
ALK, *FGFR2*, *NTRK1*, *RET*, and *ROI*

Table 2. Cytokine analysis in the peripheral blood.

IL-1α/β	IL-1Ra	IL-2	IL-4	IL-5	IL-6	IL-7
IL-8	IL-10	IL-12	IL-13	IL-15	IL-17	EGF
CCL11	G-CSF	GM-CSF	GRO	IFN-α2	IFN-γ	CXCL10
CCL2	CCL7	CCL22	MIP-1α/β	TGF-α	MIP-1α/β	TNF-α/β
VEGF-A	Angiopoietin-2	BMP-9	EGF	FGF-2	Follistatin	HB-EGF
HGF	Leptin	PLGF	VEGF-C/D	TGF-β1/2/3		

Electronic patient-reported outcome

In patients who provide additional consent, adverse events, quality of life, and PS evaluations will be periodically conducted using Welby MyKarte ONC® (Welby, Tokyo, Japan). Adverse events will be assessed weekly using up to 21 questions on 11 items (anorexia, nausea, vomiting, diarrhea, shortness of breath, cough, swelling, rash, pruritus, fatigue, and anxiety) selected from the PRO version of the Common Terminology Criteria for Adverse Events 5.0. PS will be assessed every 4 weeks according to the Eastern Cooperative Oncology Group PS, and quality of life will be evaluated every 4 weeks using the European Organization for Research and Treatment QLQ-C30 and QLQ-LC13.

Statistical considerations

This clinical study aims to evaluate the efficacy and safety of brigatinib in advanced or recurrent ALK gene rearranged NSCLC after treatment with alectinib as the first ALK-TKI in the real world. The primary endpoint of this prospective observational study is PFS, and the number of patients in Cohort A is calculated according to the following hypothesis: a phase II study evaluating the efficacy of brigatinib in patients with resistance to second-generation ALK-TKIs, including alectinib, reported a median PFS of 6.4 months.[35] By contrast, a combination of platinum and pemetrexed in patients with second-generation ALK-TKI resistant, including alectinib, resulted in a median PFS of 4.3 months.[36] Assuming a PFS of 6.0 months for brigatinib, which is significantly higher than the 4.0 months that is expected for the platinum and pemetrexed combination, 90 patients would be the required number of individuals under the following conditions: 5% one-sided significance level, 80% power, 26 months of enrollment period, and 1 year of follow-up. Thus, the number of patients was set to 100 to allow for fewer dropouts.

Cohorts B and 0 are created to collect information in a clinical practice setting, and the necessary statistical number of cases is not calculated. As a realistic and feasible number of cases for exploratory analysis, we will enroll 30 and 50 patients in Cohorts B and 0, respectively.

The primary endpoint, PFS, is defined as the period beginning on the date of brigatinib

initiation and ending on the date of death from any cause, on the date of images confirming disease progression, or on the date of clinical diagnosis of disease progression, whichever occurs first. Disease progression is defined as an apparent worsening of imaging data, patient symptoms, or physical examination findings. The principal investigator or study investigator will determine disease progression with reference to Response Evaluation Criteria in Solid Tumors v1.1. The analysis will be performed on the entire cohort 1 year after the start of the follow-up period. At the end of the follow-up period, all endpoints will be analyzed after the data are confirmed based on a final survey.

Ethical consideration

This study will be conducted in accordance with the provisions of the Declaration of Helsinki and approved by the Ethics Committee of Wakayama Medical University (No. 3073). Written informed consent will be obtained from all patients before study-related procedures are performed. The trial registration number for this study is UMIN000042439.

Discussion

This prospective observational study has three main objectives. First, it aims to evaluate the efficacy and safety of brigatinib in a real-world setting. Second, it aims to explore predictive factors associated with the efficacy of brigatinib. Third, it aims to explore resistance mechanisms to brigatinib in each situation. We believe that these findings will contribute to developing appropriate treatment strategies for ALK-gene rearranged, advanced NSCLC.

Regarding the safety and efficacy of brigatinib, available data on brigatinib treatment after alectinib as the first ALK-TKI, which is the focus of cohort A in the ABRAID trial, are limited. Ou *et al.* reported a response rate of 26% with a median PFS of 3.8 months in 103 patients enrolled in a phase II study of brigatinib in individuals previously treated with ceritinib or alectinib, in the ALTA-2 trial. However, only 42 patients received alectinib as the first ALK-TKI.[37] J-ALTA study, a phase II study on brigatinib that included 47 patients previously treated with alectinib, also showed that brigatinib resulted in a response rate and median PFS of 34% and 7.3 months, respectively. However, 12 of the patients also received prior crizotinib, and only 35 received brigatinib after alectinib, which was administered as the first ALK-TKI.[38] Thus, Cohort A in this ABRAID trial is the largest prospective study to evaluate the efficacy and safety of brigatinib as a second ALK-TKI administered immediately after alectinib. In addition, to the best of our knowledge, no prospective clinical trials exist focusing on the efficacy and safety of brigatinib after third-line treatment or after treatment with two second-generation ALK-TKIs; therefore, the results of Cohort B, which enrolls this population, should be valuable. Furthermore, the J-ALTA study, which was conducted in Japan and included mostly Japanese participants, on brigatinib in patients with no prior ALK-TKI treatment resulted in response and 1-year PFS rates of 96.9% and 93% in 32 patients, respectively.[39] Cohort 0, which enrolls 50 ALK-TKI-naïve patients, is expected to confirm the results in a real-world setting. An analysis of 148 patients with ALK-positive lung cancers diagnosed between 2009 and 2021 showed that 54% received crizotinib and 44% alectinib as the first ALK-TKI with ceritinib, brigatinib, and lorlatinib as the second and subsequent ALK-TKI in 23%, 17%, and 29% of participants, respectively, in a real-world setting.[40] This indicates a lack of real-world data on brigatinib as the first ALK-TKI, as well as the need for a marker to select the second and subsequent ALK-TKI.

To explore predictive factors of efficacy, 180 ctDNA samples before brigatinib administration will be collected from the three cohorts. Samples from Cohort A will enable us to evaluate the association between resistance genetic alterations against alectinib and the efficacy of brigatinib. Although new mutations in the ALK gene including I1171T/NS, V1180L, and G1202R,[13] P53 mutations,[22] MET gene amplifications,[20] PIK3CA mutations,[41] and KRAS G12C mutations[42] are expected to occur after treatment with alectinib, there are few data about their impact on the efficacy of brigatinib or other ALK-TKI. Cohort B samples can be used to find genetic changes after alectinib followed by lorlatinib and to explore their association with the efficacy of brigatinib. After treatment with two second- or third-generation ALK-TKI administrations, multiple resistance mechanisms would be more likely to exist,[25] and evaluation of gene alterations alone may not be sufficient. Comparisons with cohorts 0 and A may provide an edge in clarifying this. Analysis of the association of ALK variants[13] and compound mutations like TP53 mutations[22] with the efficacy

of brigatinib using cohort 0 samples is important to confirm the results of similar analysis using samples from the ALTA-1L trial in the real-world setting.[10] It will also be of interest to explore whether an impact of ALK variants or compound mutations on ALK-TKI efficacy is similar when the ALK-TKI is administered as the second or third ALK-TKI. If it is, a treatment strategy that does not rely too much on ALK-TKI sequencing may be a preferred choice in such cases.

Moreover, this study will also evaluate protein levels in the pre-treatment serum and tumor tissue. Serum concentration of IL-6[27] and VEGF,[26] as well as expression of proteins such as EGFR,[30,31] PD-L1,[32,33] and CD47,[34] is associated with the efficacy of EGFR-TKI. Because these tumor microenvironment-related resistance mechanisms are not necessarily EGFR mutation-specific and have rarely been evaluated in ALK-TKI, such analysis may lead to the elucidation of new resistance mechanisms for ALK-TKI. The association between genetic abnormalities and the efficacy of ALK-TKI often differs between reports, suggesting the existence of important resistance mechanisms other than genetic abnormalities. Combined analysis of genetic abnormalities with serum proteins and/or tumor-expressing proteins may better predict the efficacy of ALK-TKI.

Data on the resistance mechanisms of brigatinib are similar to or less complete than those on other ALK-TKIs. This study explores resistance mechanisms when brigatinib is used as the first ALK-TKI, as the second ALK-TKI after alectinib, and as the third ALK-TKI after alectinib and lorlatinib, using ctDNA samples at progression or treatment discontinuation from each cohort. Notably, all 180 study participants have only these three patterns of pre-treatment history with ALK-TKIs. Therefore, this study will provide crucial information on how the pre-treatment history with ALK-TKIs affects the resistance mechanisms. It has been reported that the coexistence of ALK-resistant and TP53 mutations occurs more frequently when multiple ALK-TKIs are used[25]; however, it is not well understood whether similar events occur with other off-target mechanisms. Furthermore, alterations in resistance genes to brigatinib may depend on genetic alterations that existed prior to the brigatinib administration. Accumulating such data would enable us to choose the best ALK-TKI for each case considering not only the efficacy of the first ALK-TKI but also the efficacy of the second and third ALK-TKIs. Such exploration will also contribute to the development of new combination therapies and new ALK-TKI in the future.

The patient's assessment of the quality of life and adverse events using electronic devices is crucial, owing to their high sensitivity and the possibility of assessment over time.[43] However, such ePRO is technically difficult for older adults, and some patients may be reluctant to use such devices. In this observational study, we will use ePRO to evaluate the quality of life and adverse events in a distinctive population with ALK-positive lung cancer. Another important aspect of ePRO evaluation is the feasibility assessment. Little has been reported on what clinical context influences patient acceptance and continuation of ePRO. The results of this study will be useful for promoting the efficient use and dissemination of ePRO in clinical practice.

Conclusion

We evaluate the real-world clinical efficacy and safety of brigatinib in various treatment lines, including patients who received alectinib as their first ALK-TKI. Furthermore, we attempt to elucidate the efficacy-related and resistance mechanisms from multiple angles using genes and proteins in blood and tumor tissue. The results of this study are expected to be a major step forward in establishing therapeutic strategies for using multiple second- and third-generation ALK-TKIs, which is currently a major challenge in the treatment of ALK-positive lung cancers and may further lead to the development of novel biomarkers and therapeutic agents.

Declarations

Ethics approval and consent to participate

The study was approved by the Ethics Committee of the Wakayama Medical University (No. 3073) and all the participating facilities. All patients signed an informed consent form before enrollment.

Consent for publication

Not applicable.

Author contributions

Yuichi Ozawa: Project administration; Conceptualization; Methodology; Writing – original draft.

Yasuhiro Koh: Investigation; Conceptualization; Methodology; Resources; Writing – review & editing.

Tetsunari Hase: Resources; Writing – review & editing.

Kenji Chibana: Resources; Writing – review & editing.

Kyoichi Kaira: Resources; Writing – review & editing.

Kyoichi Okishio: Resources; Writing – review & editing.

Eiki Ichihara: Resources; Writing – review & editing.

Shuji Murakami: Resources; Writing – review & editing.

Mototsugu Shimokawa: Formal analysis; Methodology; Writing – review & editing.

Nobuyuki Yamamoto: Supervision; Funding acquisition; Comceptualization; Methodology; Writing – review & editing.

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Competing interests
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Availability of data and materials
All data generated or analyzed during this study are included in this published article.

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Supplemental material
Supplemental material for this article is available online.

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