

Analysis of Tumor Heterogeneity Through AXL Activation in Primary Resistance to EGFR Tyrosine Kinase Inhibitors



Ryota Nakamura, MD,^a Hiroyuki Fujii, MD,^a Tadaaki Yamada, MD, PhD,^{a,*} Yohei Matsui, MD,^a Takeshi Yaoi, MS,^b Mizuki Honda, MD,^c Noriyuki Tanaka, MD, PhD,^c Aya Miyagawa-Hayashino, MD, PhD,^c Akihiro Yoshimura, MD, PhD,^a Kenji Morimoto, MD, PhD,^a Masahiro Iwasaku, MD, PhD,^a Shinsaku Tokuda, MD, PhD,^a Young Hak Kim, MD, PhD,^a Eiichi Konishi, MD, PhD,^c Kyoko Itoh, MD, PhD,^b Koichi Takayama, MD, PhD^a

^aDepartment of Pulmonary Medicine, Kyoto Prefectural University of Medicine, Kyoto, Japan

^bDepartment of Pathology and Applied Neurobiology, Kyoto Prefectural University of Medicine, Kyoto, Japan

^cDepartment of Surgical Pathology, Kyoto Prefectural University of Medicine, Kyoto, Japan

Received 22 December 2022; revised 24 April 2023; accepted 8 May 2023

Available online - 12 May 2023

ABSTRACT

Introduction: EGFR tyrosine kinase inhibitors are standard therapeutic agents for patients with advanced NSCLC harboring EGFR mutations. Nevertheless, some patients exhibit primary resistance to EGFR tyrosine kinase inhibitors in the first-line treatment setting. AXL, a member of the TYRO3, AXL, and MERTK family of receptor tyrosine kinases, is involved in primary resistance to EGFR tyrosine kinase inhibitors in EGFR-mutated NSCLC.

Methods: We investigated spatial tumor heterogeneity using autopsy specimens and a patient-derived cell line from a patient with EGFR-mutated NSCLC having primary resistance to erlotinib plus ramucirumab.

Results: Quantitative polymerase chain reaction analysis revealed that AXL mRNA expression differed at each metastatic site. In addition, AXL expression levels were likely to be negatively correlated with the effectiveness of erlotinib plus ramucirumab therapy. Analysis of a patient-derived cell line established from the left pleural effusion before initiation of treatment revealed that the combination of EGFR tyrosine kinase inhibitors and an AXL inhibitor remarkably inhibited cell viability and increased cell apoptosis in comparison with EGFR tyrosine kinase inhibitor monotherapy or combination therapy of these inhibitors with ramucirumab.

Conclusions: Our observations suggest that AXL expression may play a critical role in the progression of spatial tumor heterogeneity and primary resistance to EGFR tyrosine kinase inhibitors in patients with EGFR-mutated NSCLC.

© 2023 The Authors. Published by Elsevier Inc. on behalf of the International Association for the Study of Lung Cancer. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Keywords: AXL; Drug resistance; EGFR tyrosine kinase inhibitor; Heterogeneity; Non-small cell lung cancer

Introduction

Lung cancer is the leading cause of cancer-related death worldwide.¹ EGFR mutations are major driver

*Corresponding author.

Disclosure: Dr. Yamada received commercial research grants from Pfizer, Ono Pharmaceutical, Janssen Pharmaceutical K.K., AstraZeneca, and Takeda Pharmaceutical Company Limited and speaking honoraria from Eli Lilly. Dr. Takayama received research grants from Chugai-Roche and Ono Pharmaceutical and personal fees from AstraZeneca, Chugai-Roche, Merck Sharp & Dohme-Merck, Eli Lilly, Boehringer Ingelheim, and Daiichi Sankyo. The remaining authors declare no conflict of interest.

Address for correspondence: Tadaaki Yamada, MD, PhD, Department of Pulmonary Medicine, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, 465, Kajii-cho, Kamigyo-ku, Kyoto, Japan. E-mail: tayamada@koto.kpu-m.ac.jp

Cite this article as: Nakamura R, Fujii H, Yamada T, et al. Analysis of tumor heterogeneity through AXL activation in primary resistance to EGFR tyrosine kinase inhibitors. *JTO Clin Res Rep.* 2023;4:100525.

© 2023 The Authors. Published by Elsevier Inc. on behalf of the International Association for the Study of Lung Cancer. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

ISSN: 2666-3643

<https://doi.org/10.1016/j.jtocr.2023.100525>

gene mutations in NSCLC. EGFR tyrosine kinase inhibitor (TKI) monotherapy is highly effective in patients with EGFR-mutated NSCLC. Nevertheless, approximately 20% of patients with EGFR-mutated NSCLC do not achieve clinical response to such first-line treatments.²⁻⁴ To overcome these issues, combined therapeutic strategies, including EGFR TKIs plus antiangiogenesis agents or chemotherapy, have been recently approved in several countries for the first-line treatment of patients with EGFR-mutated NSCLC.^{3,5}

Various underlying mechanisms, including hepatocyte growth factor overexpression and deletion polymorphisms of BCL2-like 11, have been reported to cause primary resistance to EGFR TKIs.^{6,7} Our previous studies reported that AXL, a member of the TYRO3, AXL, and MERTK family of receptor tyrosine kinases, maintained a survival signal as a bypass pathway when EGFR-mutated NSCLC cell lines with high AXL expression were exposed to EGFR TKIs, and that a combination of EGFR TKIs and AXL inhibitors was effective for these cell lines.^{8,9} Our recent prospective observational study also revealed that in patients with EGFR-mutated NSCLC, high tumor AXL levels were significantly associated with a shorter progression-free survival after third-generation EGFR TKI osimertinib therapy in comparison with low AXL levels.¹⁰ On the basis of these studies, a clinical trial is ongoing to evaluate the safety and efficacy of the combination of osimertinib and ONO-7475, an AXL inhibitor, in patients with untreated advanced EGFR-mutated NSCLC in Japan (jRCT2051210045).

Intratumor heterogeneity is associated with the effectiveness of targeted therapies for various types of cancer.¹¹⁻¹³ Therefore, understanding the involvement of spatial tumor heterogeneity as a primary resistance mechanism against molecular-targeted therapy in patients with NSCLC is important. In this study, to reveal the clinical impact of spatial tumor heterogeneity in patients with EGFR-mutated NSCLC, we evaluated the relationship between the clinical response and AXL expression levels in autopsy tumor samples from a patient with advanced EGFR-mutated NSCLC who had primary resistance to erlotinib plus ramucirumab. Furthermore, we performed cell line-based analysis to evaluate the efficacy of the combination of EGFR TKIs and an AXL inhibitor by using a patient-derived cell line established from left pleural effusion before initiation of the treatment.

Material and Methods

The Patient

The patient was a 67-year-old man who underwent left upper lobectomy for localized NSCLC (pT2aN0M0) in January 2021. During postoperative adjuvant chemotherapy

with S-1, he had recurrence in July 2021. Because the EGFR-L858R mutation was detected from a recurrent site, combination therapy with the EGFR TKI erlotinib and the anti-vascular epithelial growth factor receptor 2 antibody ramucirumab was initiated. The patient died owing to cancer progression 15 days after initiation of the treatment. Postmortem autopsy was performed, and samples were obtained from various metastatic sites by macroscopically cutting out part of the tumor.

Real-Time Polymerase Chain Reaction

Autopsy samples were homogenized using a homogenizer. Total RNA was purified using the NucleoSpin RNA Plus (Takara Bio). Complementary DNA was synthesized using PrimeScript RT Master Mix (Perfect Real Time) (Takara Bio). Real-time polymerase chain reaction (PCR) was performed using a QuantStudio 3 Real-Time PCR system (Applied Biosystems) and THUNDERBIRD Next SYBR quantitative PCR Mix (TOYOBO). Gene expression levels were calculated using the $2^{-\Delta\Delta CT}$ method.¹⁴ Glyceraldehyde 3-phosphate dehydrogenase was used as an internal control. The primer sequences were as follows: EGFR L858R (F: 5'-GCATTTGC-CAAGTCCTACAGA-3', R: 5'-CTATCAATGCAAGCCACGG-3'), AXL (F: 5'-GGTGGCTGTGAAGACGATGA-3', R: 5'-CTCAGATACTCCATGCCACT-3'), and glyceraldehyde 3-phosphate dehydrogenase (F: 5'-GCCAAATATGATGACATCAAGAAGG-3', R: 5'-GGTGTGCTGTTGAAGTCAGAG-3').

Patient-Derived Cell Line Establishment

Tumor cells were collected by centrifugation from the left pleural effusion of the patient before initiation of erlotinib plus ramucirumab therapy. We established the patient-derived cell line KPP-03 from tumor cells, as described previously.¹⁵ EGFR-L858R mutation was confirmed using the PNA-LNA PCR clamp method (LSI Medience Corporation).

Cell Culture and Reagents

HCC827 and PC-9 cells were purchased from the American Type Culture Collection and RIKEN Cell Bank, respectively. H1975 cells containing EGFR-L858R and T790M double mutation were provided by Dr. Yoshitaka Sekido (Aichi Cancer Center Research Institute) and Dr. John D. Minna. The cells were maintained in RPMI 1640 medium with 10% fetal bovine serum and 1% penicillin and streptomycin in 5% CO₂ at 37°C. All cells were passaged for less than 3 months and tested for mycoplasma using a MycoAlert Mycoplasma Detection Kit (Lonza). Erlotinib (first-generation EGFR TKI), osimertinib (third-generation EGFR TKI), ONO-7475 (AXL inhibitor), and ramucirumab (human anti-vascular

epithelial growth factor receptor 2 antibody) were obtained from Selleck Chemicals.

Western Blotting

Western blotting was performed as described previously⁸ using primary antibodies for p-AXL (Tyr702), t-AXL, p-EGFR (Tyr1086), β -actin (13E5), p-Akt (Ser473), t-Akt, p-Erk1/2 (Thr202/Tyr204), t-ERK1/2 (1:1000; Cell Signaling Technology), and t-EGFR (1:1000; R&D Systems).

Cell Viability Assay

The cells (3×10^3 cells/well) were cultured with the indicated drugs for 72 hours. Cell viability was determined using the MTT assay (Sigma-Aldrich). Absorbance was measured using a microplate reader. The cells were also treated for 9 days, with the drugs replenished every 72 hours. The cells were stained with crystal violet and examined visually.

Apoptotic Cell Analysis

KPP-03 cells (3×10^5) were treated with the indicated drugs for 48 hours. Then, the cells were collected and incubated with Annexin V-FITC and propidium iodide for 15 minutes, after which they were analyzed using a BD Accuri C6 Plus Flow Cytometer (Becton, Dickinson & Company). Data were analyzed using FlowJo software (FlowJo LLC), as reported previously.¹⁶

siRNA Transfection

Cells were transfected with a Silencer Select small interfering (si)RNA for AXL (s1845; Invitrogen) using Lipofectamine RNAi-MAX (Invitrogen) according to the manufacturer's instructions. The Silencer Select siRNA for negative control number 1 (Invitrogen) was the scrambled control. AXL knockdown was confirmed using Western blotting. Each sample was independently analyzed at least three times.

Statistical Analysis

Data from the MTT assay and flow cytometry were expressed as mean \pm SD. Significant differences were analyzed using unpaired *t* tests using Prism 9.0 (GraphPad Software), with two-sided *p* values of less than 0.05 considered significant.

Ethical Consideration

This study was approved by the institutional review board of Kyoto Prefectural University of Medicine (No. ERB-C-977). Written informed consent was obtained from the patient and his family for the establishment of the cell line, use of the tumor specimens, and publication of the medical records and research results.

Results

Case Report

The patient was a 67-year-old man who underwent left upper lobectomy for localized NSCLC (pT2aN0M0) in January 2021. Postoperative recurrence was diagnosed using computed tomography (CT)-guided lung biopsy for a lung nodule during postoperative adjuvant chemotherapy with S-1 (80 mg/m², d 0–13) in July 2021. The EGFR-L858R mutation was detected using the OncoPrint Dx Target Test (Thermo Fisher Scientific) with a CT-guided biopsy specimen. Although combination therapy with erlotinib (150 mg/d) and ramucirumab (10 mg/kg) was initiated, the patient died owing to cancer progression 15 days after initiation of the treatment (Fig. 1A). In a comparison of CT scan results taken 9 days after initiation with those taken before initiation of the treatment, tumors in the right and left lower lobes had no remarkable changes, whereas a tumor in the right upper lobe increased rapidly, and left pleural effusion increased (Fig. 1B). These different observations may have been influenced by the heterogeneity among the metastatic tumors.

AXL Expression Levels Are Negatively Correlated With the Effectiveness of Treatment Regimens Including EGFR TKIs

To investigate the spatial tumor heterogeneity in relation to AXL expression, autopsy specimens from multiple metastatic sites were obtained, and AXL mRNA expression levels were compared using quantitative PCR. AXL mRNA expression levels of tumors in the right upper lobe and left pleura, which revealed tumor progression, were higher than those of tumors in the right and left lower lobes, which revealed stable disease (Fig. 1C). In comparison with AXL mRNA expression, EGFR-L858R-mutated mRNA expression revealed no remarkable association with the clinical response to treatment (Fig. 1D). To quantify the fraction of the tumor cells, AXL mRNA expression levels were divided by EGFR-L858R-mutated mRNA expression levels. The number of refractory tumors having disease progression was higher than that of the other tumors (Fig. 1E). Taken together, these findings suggest that AXL expression in the tumors may be negatively related to the effectiveness of EGFR TKIs and may lead to spatial tumor heterogeneity.

Patient-Derived Cell Lines With High AXL Expression Were Insensitive to Standard Treatments With EGFR TKIs

We next established the patient-derived cell line KPP-03 from the left pleural effusion of this patient before initiation of erlotinib plus ramucirumab therapy. The

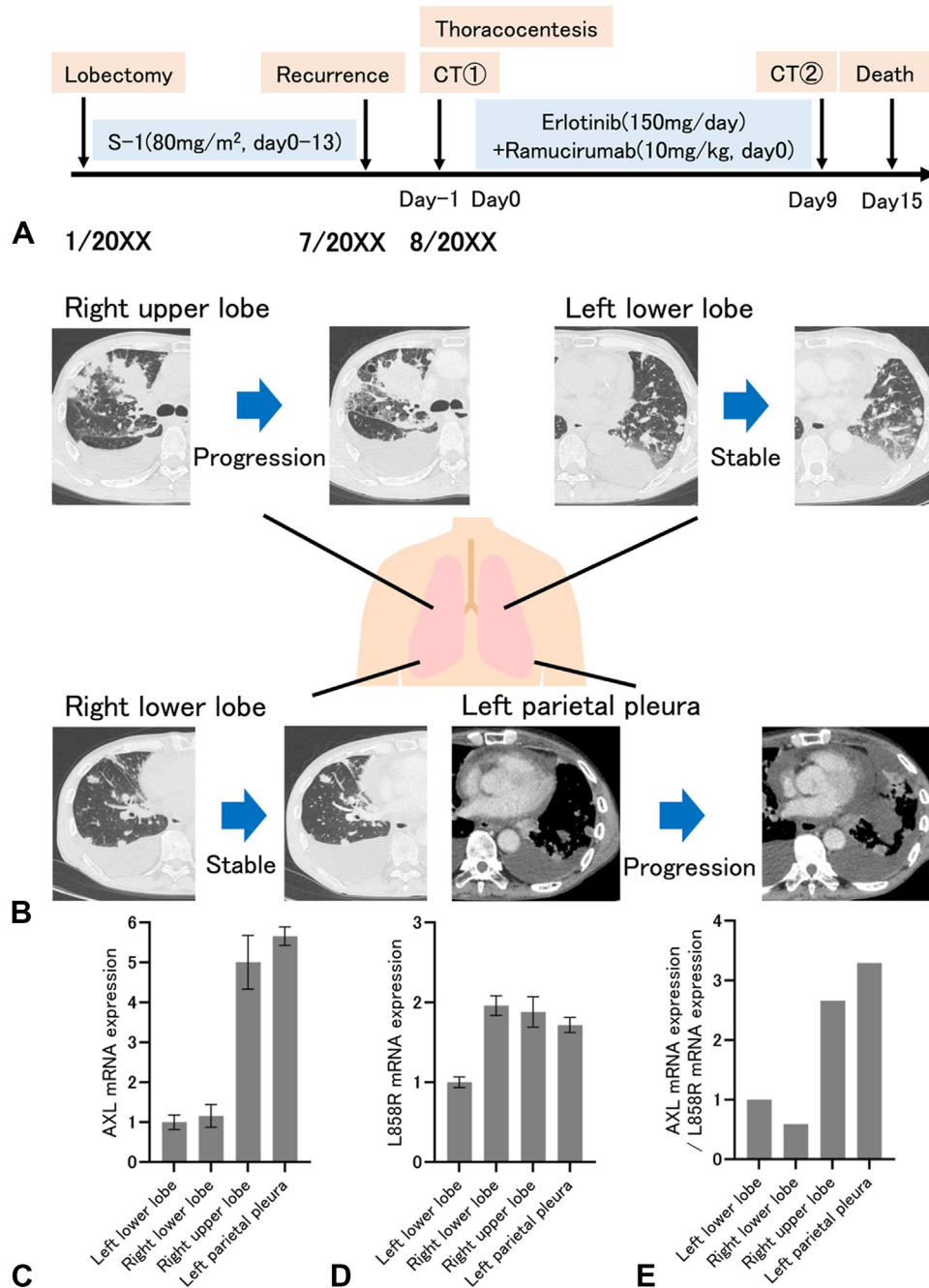


Figure 1. AXL expression levels are negatively correlated with the efficacy of EGFR TKI treatment: clinical case. (A) A 67-year-old man underwent left upper lobectomy for localized NSCLC (pT2aN0M0) in January 2021. Postoperative recurrence was diagnosed in July 2021 during postoperative adjuvant chemotherapy with S-1. A CT scan and left thoracocentesis were performed on the day before initiation of erlotinib plus ramucirumab. A CT scan was performed 9 days after initiation of treatment, and the treatment was discontinued. The patient died 15 days after initiation of the treatment. (B) CT scans taken before initiation of the treatment and those taken 9 days after initiation of the treatment. (C) The AXL mRNA expression levels of autopsy specimens measured by qPCR. (D) The EGFR-L858R-mutated mRNA expression levels of autopsy specimens measured by qPCR. (E) The AXL mRNA expression levels divided by the EGFR-L858R-mutated mRNA expression levels. CT, computed tomography; qPCR, quantitative polymerase chain reaction; TKI, tyrosine kinase inhibitor.

EGFR-L858R mutation was detected in the KPP-03 cells, which had higher AXL expression than HCC-827 cells with low AXL levels and PC-9 cells with high AXL levels^{8,9} (Fig. 2A and B). The growth inhibitory assay revealed that the KPP-03 and PC-9 cells had lower

sensitivity to erlotinib plus ramucirumab than the HCC-827 cells. Similar results were observed in osimertinib treatment (Fig. 2C). We evaluated the knockdown effect of AXL using a specific siRNA to observe the dependency of cell viability on AXL signaling during EGFR TKI

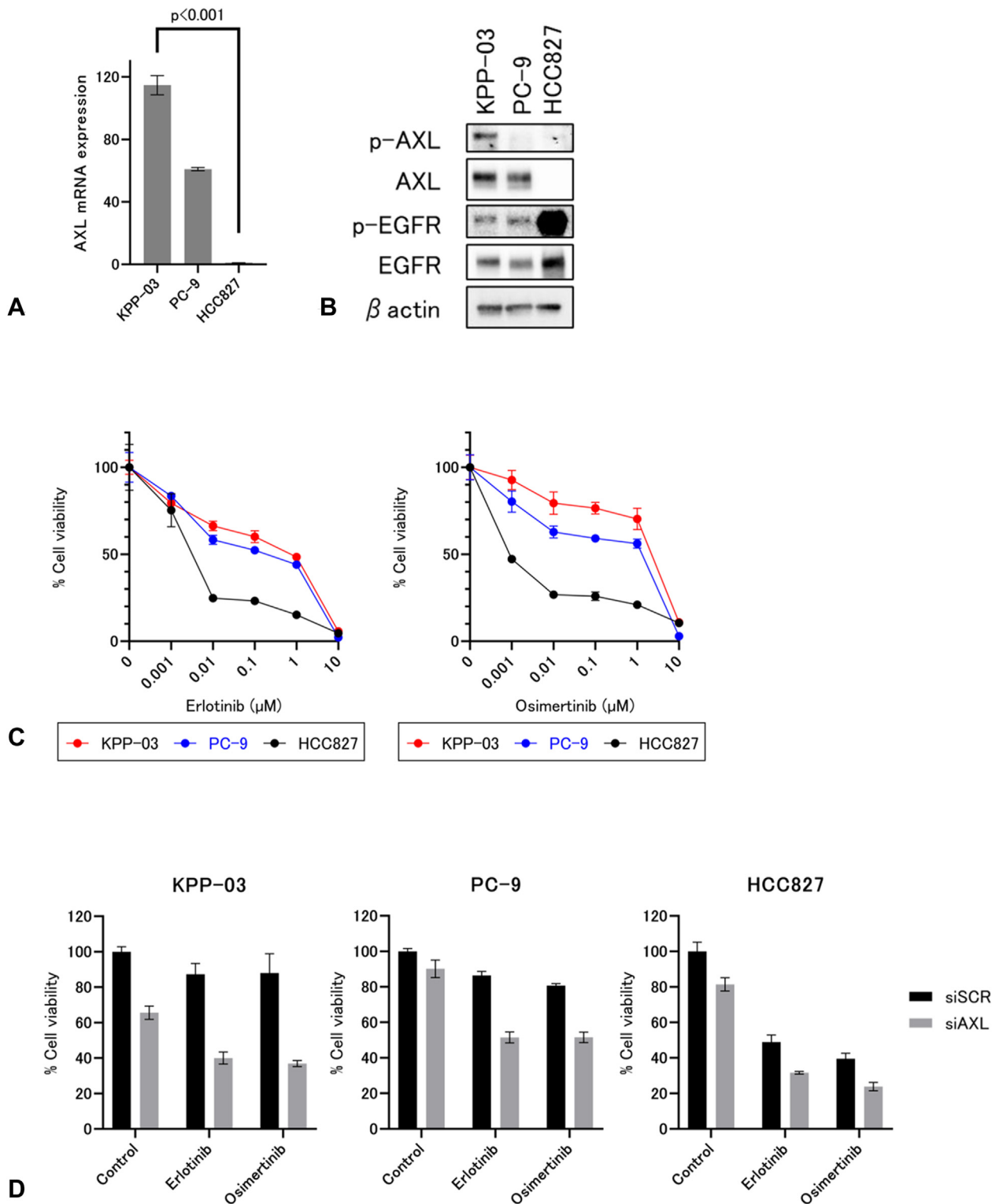


Figure 2. The patient-derived cell line KPP-03 with high AXL expression was insensitive to EGFR TKIs. (A) The AXL mRNA expression levels of KPP-03, PC-9, and HCC827 cells measured by the qPCR assay. (B) The indicated proteins of KPP-03, PC-9, and HCC827 cells detected by the Western blotting assay. (C) KPP-03, PC-9, and HCC827 cells were incubated with erlotinib plus ramucirumab (150 ng/ μ L) or osimertinib for 72 hours. Cell viability was determined using the MTT assay. (D) KPP-03, PC-9, and HCC827 cells treated with nonspecific control or AXL-specific siRNA were incubated with or without erlotinib (100 nmol/L) or osimertinib (100 nmol/L) for 72 hours, and cell viability was detected using MTT assays. p-, phosphorylated-; qPCR, quantitative polymerase chain reaction; si, small interfering.

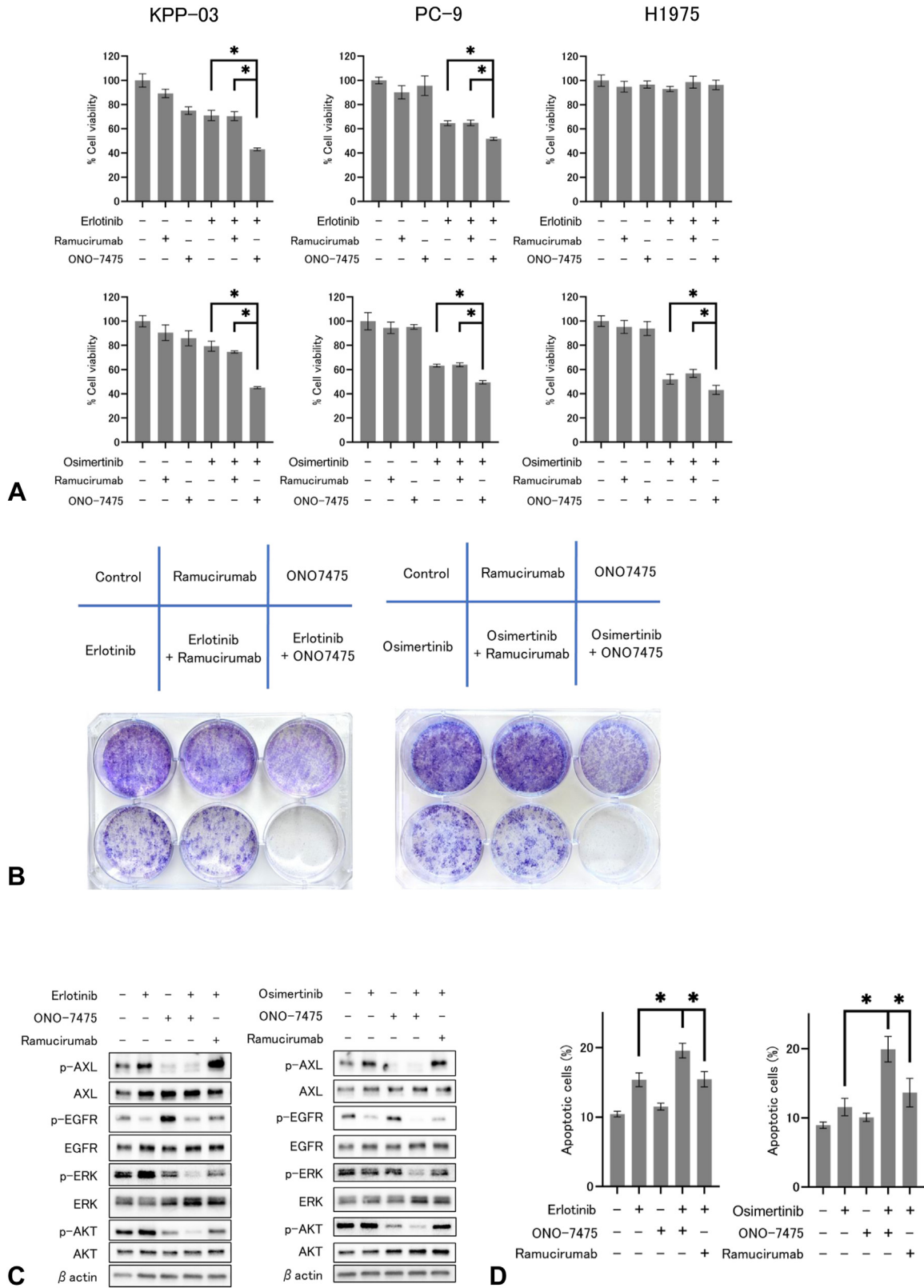


Figure 3. Combination of EGFR TKIs and an AXL inhibitor overcomes primary resistance caused by AXL signal activation. (A) KPP-03, PC-9, and H1975 cells were treated with EGFR TKIs alone (erlotinib 100 nmol/liter or osimertinib 100 nmol/liter), ONO-7475 (100 nmol/L) alone, ramucirumab (150 ng/μL) alone, EGFR TKIs with ONO-7475, or EGFR TKIs with ramucirumab for 72 hours. Cell viability was assessed using the MTT assay. (B) KPP-03 cells were treated with EGFR TKIs alone (erlotinib 100 nmol/L or osimertinib 100 nmol/L), ONO-7475 (100 nmol/L) alone, ramucirumab (150 ng/μL) alone, EGFR TKIs with

treatment. Treatment with AXL-specific siRNA enhanced the inhibitory effects of high AXL-expressed KPP-03 and PC-9 cells on EGFR TKIs than that of low AXL-expressed HCC827 cells, suggesting that AXL signaling might reduce drug tolerance to EGFR TKIs in high AXL-expressed cells (Fig. 2D and Supplementary Fig. 1).

The Combination of EGFR TKIs and an AXL Inhibitor Overcame Primary Resistance to EGFR TKIs in the Patient-Derived Cell Line

We performed *in vitro* experiments using KPP-03, PC-9, and H1975 cells to evaluate whether combining EGFR TKIs and an AXL inhibitor could inhibit the proliferation of cells expressing high AXL levels (Supplementary Fig. 2). Combining erlotinib or osimertinib with the AXL inhibitor—ONO-7475—considerably inhibited cell proliferation more than EGFR TKI monotherapy and the combination with ramucirumab in KPP-03, PC-9, and H1975 cells, except for erlotinib treatment for H1975 cells (Fig. 3A). In addition, the continuous co-treatment of KPP-03 cells with EGFR TKIs and ONO-7475 for 9 days reduced cell viability than EGFR TKI monotherapy or the combination with ramucirumab (Fig. 3B). Western blotting analysis indicated that the combination of EGFR TKIs and ONO-7475 suppressed the phosphorylation of ERK and AKT in comparison with EGFR TKI monotherapy and the combination with ramucirumab in KPP-03 cells (Fig. 3C). Apoptosis analysis revealed that the combination of EGFR TKIs and ONO-7475 considerably increased cell apoptosis in comparison with EGFR TKI monotherapy and the combination with ramucirumab in KPP-03 cells (Fig. 3D). These results indicated that AXL activation induced primary resistance to EGFR TKIs and that an initial combination of EGFR TKIs and an AXL inhibitor could overcome the insensitivity to EGFR TKI monotherapy and the combination with ramucirumab in AXL-overexpressing cells.

Discussion

Treatment with EGFR TKIs is the standard strategy for patients with untreated advanced EGFR-mutated NSCLC.^{17–19} Nevertheless, some patients have primary resistance to EGFR TKI treatment.^{2–4} High AXL protein levels in tumors are related to primary resistance and poor clinical response to osimertinib in patients with advanced EGFR-mutated NSCLC.¹⁰ On the basis of these

clinical issues, elucidation of the mechanism of primary resistance to EGFR TKIs and development of novel therapies to overcome such primary resistance in patients with EGFR-mutated NSCLC are essential. Intratumor heterogeneity has been reported to be an important factor in primary resistance to EGFR TKIs.²⁰ Nevertheless, intratumor heterogeneity has not yet been evaluated in relation to AXL expression in patients with EGFR-mutated NSCLC. In this study, we investigated the effect of AXL activation on spatial tumor heterogeneity by using autopsy specimens and a patient-derived cell line from a patient with EGFR-mutated NSCLC who had primary resistance to erlotinib plus ramucirumab.

Our observations revealed that AXL mRNA expression levels in autopsy samples were likely correlated with the clinical response to erlotinib plus ramucirumab. In addition, the patient-derived cell line KPP-03, which had high AXL expression, was less sensitive to erlotinib plus ramucirumab than HCC827 cells expressing lower levels of AXL, and the combination of EGFR TKIs and an AXL inhibitor overcame the resistance to current standard treatments. These findings suggest that AXL expression levels in tumors could predict the effective lesions for initial combined inhibition of AXL and EGFR in EGFR-mutated NSCLC tumors.

The tumor microenvironment (TME) has been reported to promote spatial tumor heterogeneity in lung cancer.²¹ The hypoxic environment in tumors is a critical factor in the TME. A preclinical study reported that hypoxia-inducible factors directly activated AXL expression in renal cell cancer.²² In this study, tumors with high AXL expression levels in the right upper lobe were larger than those with low AXL expression in the right and left lower lobes. These findings suggest that enrichment of AXL expression may be induced by the hypoxic environment in large tumors. Nevertheless, this is a limited analysis; therefore, further large-scale investigations are required to confirm our observations.

We revealed that the combination of EGFR TKIs and an AXL inhibitor ONO-7475 effectively suppressed phosphorylation of ERK and AKT in KPP-03 cells, indicating that KPP-03 cells depend on the AXL cascade for resistance to EGFR TKIs. The cancer immune environment is one of the major factors involved in the regulation of tumorigenicity. AXL has been reported to be involved in the immune environment and is currently being identified as a novel therapeutic target.^{23,24} Thus,

ONO-7475, or EGFR TKIs with ramucirumab for 9 days. Cells were stained with crystal violet. (C) KPP-03 cells were incubated with EGFR TKIs alone (erlotinib 100 nmol/L or osimertinib 100 nmol/L), ONO-7475 (100 nmol/L) alone, EGFR TKIs with ONO-7475, or EGFR TKIs with ramucirumab (150 ng/ μ L) for 4 hours, and the indicated proteins were then detected by Western blotting assay. (D) KPP-03 cells were treated with EGFR TKIs alone (erlotinib 100 nmol/L or osimertinib 100 nmol/L), ONO-7475 (100 nmol/L) alone, EGFR TKIs with ONO-7475, or EGFR TKIs with ramucirumab (150 ng/ μ L) for 48 hours, and apoptotic cells were then detected by flow cytometry. p-, phosphorylated-; TKI, tyrosine kinase inhibitor.

these observations suggest that in vivo models and clinical practice might increase the dependency of AXL signaling on resistance to EGFR TKIs through its effects on the TME.^{25,26}

This study revealed a fourfold difference in AXL expression among the metastatic lesions, suggesting that analysis by a single biopsy may underestimate the evaluation of AXL expression levels in whole tumors. Nevertheless, multiple biopsies from multiple metastatic sites are difficult to perform; therefore, evaluation by liquid biopsy when administering treatment might be useful.

This study had some limitations. First, we observed a single case. Therefore, we could not conclude the relationship between AXL expression in tumors and the effectiveness of EGFR TKIs. Nevertheless, a previous study of BRAF-mutant melanoma revealed that heterogeneity of AXL expression could affect the efficacy of MAPK pathway inhibitors, which suggested that AXL expression heterogeneity might affect the efficacy of molecular-targeted therapy, including EGFR TKIs.²⁷ Second, the patient died shortly after initiating the treatment. Therefore, we could not evaluate the treatment efficacy after a sufficient period, which might lead to underestimating the changes in small lesions. Third, we did not establish patient-derived cell lines from multiple lesions in the present study; therefore, we could not compare the results of drug sensitivity in multiple lesions in vitro. Additional studies on the clinical efficacy of concomitant AXL inhibitors on the basis of AXL expression levels are required. Fourth, other primary resistance mechanisms were not evaluated. Nevertheless, the combination of EGFR TKIs and an AXL inhibitor remarkably suppressed the viability of KPP-03 cells, suggesting that high AXL expression might be the major resistance mechanism of EGFR TKIs.

In conclusion, AXL expression in tumors may play a critical role in the progression of spatial tumor heterogeneity and primary resistance to EGFR TKIs in patients with EGFR-mutated NSCLC. In such cases, an initial combination of EGFR TKIs and AXL inhibitors may be a promising therapeutic option for patients with EGFR-mutated NSCLC.

CRediT Authorship Contribution Statement

Ryota Nakamura: Conceptualization, Data curation, Formal analysis, Methodology, Visualization, Writing—original draft preparation.

Hiroyuki Fujii: Conceptualization, Resources.

Tadaaki Yamada: Conceptualization, Funding acquisition, Project administration, Methodology, Writing—original draft preparation.

Yohei Matsui, Takeshi Yaoi, Mizuki Honda, Noriyuki Tanaka, Aya Miyagawa-Hayashino: Resources.

Akihiro Yoshimura, Kenji Morimoto, Masahiro Iwasaku, Shinsaku Tokuda, Young Hak Kim: Writing—review and editing.

Eiichi Konishi, Kyoko Itoh, Koichi Takayama: Supervision.

Acknowledgments

This work was supported by the Takeda Science Foundation and the Princess Takamatsu Cancer Research Fund. The authors thank Editage (www.editage.jp) for help with the English-language editing.

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.2023.100525>.

References

1. Sung H, Ferlay J, Siegel RL, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin.* 2021;71:209-249.
2. Soria JC, Ohe Y, Vansteenkiste J, et al. Osimertinib in untreated EGFR-mutated advanced non-small-cell lung cancer. *N Engl J Med.* 2018;378:113-125.
3. Nakagawa K, Garon EB, Seto T, et al. Ramucirumab plus erlotinib in patients with untreated, EGFR-mutated, advanced non-small-cell lung cancer (RELAY): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet Oncol.* 2019;20:1655-1669.
4. Wu YL, Cheng Y, Zhou X, et al. Dacomitinib versus gefitinib as first-line treatment for patients with EGFR-mutation-positive non-small-cell lung cancer (ARCHER 1050): a randomised, open-label, phase 3 trial. *Lancet Oncol.* 2017;18:1454-1466.
5. Hosomi Y, Morita S, Sugawara S, et al. Gefitinib alone versus gefitinib plus chemotherapy for non-small-cell lung cancer with mutated epidermal growth factor receptor: NEJ009 study. *J Clin Oncol.* 2020;38:115-123.
6. Yano S, Yamada T, Takeuchi S, et al. Hepatocyte growth factor expression in EGFR mutant lung cancer with intrinsic and acquired resistance to tyrosine kinase inhibitors in a Japanese cohort. *J Thorac Oncol.* 2011;6:2011-2017.
7. Ng KP, Hillmer AM, Chuah CT, et al. A common BIM deletion polymorphism mediates intrinsic resistance and inferior responses to tyrosine kinase inhibitors in cancer. *Nat Med.* 2012;18:521-528.
8. Taniguchi H, Yamada T, Wang R, et al. AXL confers intrinsic resistance to osimertinib and advances the emergence of tolerant cells. *Nat Commun.* 2019;10:259.
9. Okura N, Nishioka N, Yamada T, et al. ONO-7475, a novel AXL inhibitor, suppresses the adaptive resistance to initial EGFR-TKI treatment in EGFR-mutated non-small cell lung cancer. *Clin Cancer Res.* 2020;26:2244-2256.

10. Yoshimura A, Yamada T, Serizawa M, et al. High levels of AXL expression in untreated EGFR-mutated non-small cell lung cancer negatively impacts the use of osimertinib. *Cancer Sci.* 2023;114:606-618.
11. Russo M, Siravegna G, Blaszkowsky LS, et al. Tumor heterogeneity and lesion-specific response to targeted therapy in colorectal cancer. *Cancer Discov.* 2016;6:147-153.
12. Kwak EL, Ahronian LG, Siravegna G, et al. Molecular heterogeneity and receptor coamplification drive resistance to targeted therapy in MET-amplified esophagogastric cancer. *Cancer Discov.* 2015;5:1271-1281.
13. Juric D, Castel P, Griffith M, et al. Convergent loss of PTEN leads to clinical resistance to a PI(3)K α inhibitor. *Nature.* 2015;518:240-244.
14. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2⁻($\Delta\Delta C_T$) Method. *Methods.* 2001;25:402-408.
15. Tsuji T, Ozasa H, Aoki W, et al. YAP1 mediates survival of ALK-rearranged lung cancer cells treated with alectinib via pro-apoptotic protein regulation. *Nat Commun.* 2020;11:74.
16. Yang S, Meng J, Yang Y, et al. A HSP60-targeting peptide for cell apoptosis imaging. *Oncogenesis.* 2016;5:e201.
17. Ettinger DS, Wood DE, Aisner DL, et al. Non-small Cell Lung Cancer, Version 3.2022, NCCN Clinical Practice Guidelines in Oncology. *J Natl Compr Canc Netw.* 2022;20:497-530.
18. Planchard D, Popat S, Kerr K, et al. Metastatic non-small cell lung cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol.* 2018;29(suppl 4):iv192-iv237.
19. Ninomiya K, Teraoka S, Zenke Y, et al. Japanese lung cancer society guidelines for stage IV NSCLC with EGFR mutations. *JTO Clin Res Rep.* 2021;2:100107.
20. Taniguchi K, Okami J, Kodama K, Higashiyama M, Kato K. Intratumor heterogeneity of epidermal growth factor receptor mutations in lung cancer and its correlation to the response to gefitinib. *Cancer Sci.* 2008;99:929-935.
21. Junttila MR, de Sauvage FJ. Influence of tumour micro-environment heterogeneity on therapeutic response. *Nature.* 2013;501:346-354.
22. Rankin EB, Fuh KC, Castellini L, et al. Direct regulation of GAS6/AXL signaling by HIF promotes renal metastasis through SRC and MET. *Proc Natl Acad Sci U S A.* 2014;111:13373-13378.
23. Skinner HD, Giri U, Yang LP, et al. Integrative analysis identifies a novel AXL-PI3 kinase-PD-L1 signaling axis associated with radiation resistance in head and neck cancer. *Clin Cancer Res.* 2017;23:2713-2722.
24. Tsukita Y, Fujino N, Miyauchi E, et al. Axl kinase drives immune checkpoint and chemokine signalling pathways in lung adenocarcinomas. *Mol Cancer.* 2019;18:24.
25. Bárcena C, Stefanovic M, Tutusaus A, et al. Gas6/Axl pathway is activated in chronic liver disease and its targeting reduces fibrosis via hepatic stellate cell inactivation. *J Hepatol.* 2015;63:670-678.
26. Xiao Y, Zhao H, Tian L, et al. S100A10 is a critical mediator of GAS6/AXL-induced angiogenesis in renal cell carcinoma. *Cancer Res.* 2019;79:5758-5768.
27. Boshuizen J, Koopman LA, Krijgsman O, et al. Cooperative targeting of melanoma heterogeneity with an AXL antibody-drug conjugate and BRAF/MEK inhibitors. *Nat Med.* 2018;24:203-212.