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Original Articles

Triple combination therapy comprising osimertinib, an AXL inhibitor, and an FGFR inhibitor improves the efficacy of EGFR-mutated non-small cell lung cancer

Ryota Nakamura [a], Tadaaki Yamada [a,*], Shinsaku Tokuda [a], Kenji Morimoto [a], Yuki Katayama [a], Yohei Matsui [a], Soichi Hirai [a], Masaki Ishida [a], Hayato Kawachi [a], Ryo Sawada [a], Yusuke Tachibana [a], Atsushi Osoegawa [b], Mano Horinaka [c], Toshiyuki Sakai [c], Tomoko Yasuhiro [d], Ryohei Kozaki [d], Seiji Yano [e,f,g], Koichi Takayama [a]

[a] Department of Pulmonary Medicine, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Kyoto, Japan
[b] Department of Thoracic and Breast Surgery, Oita University Faculty of Medicine, Oita, Japan
[c] Department of Drug Discovery Medicine, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Kyoto, Japan
[d] Research Center of Oncology, Discovery and Research, Ono Pharmaceutical Co., Ltd., Osaka, Japan
[e] Department of Respiratory Medicine, Kanazawa University Graduate School of Medical Sciences, Kanazawa, Japan
[f] Division of Medical Oncology, Cancer Research Institute, Kanazawa University, Kanazawa, Japan
[g] WPI-Nano Life Science Institute (WPI-Nano LSI), Kanazawa University, Kanazawa, Japan

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ABSTRACT

We previously reported that combined therapy with epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKI) osimertinib and AXL inhibitor ONO-7475 is effective in preventing the survival of drug-tolerant cells in high-AXL-expressing EGFR-mutated non-small cell lung cancer (NSCLC) cells. Nevertheless, certain residual cells are anticipated to eventually develop acquired resistance to this combination therapy. In this study, we attempted to establish a multidrug combination therapy from the first-line setting to overcome resistance to this combination therapy in high-AXL-expressing EGFR-mutated NSCLC. siRNA screening assay showed that fibroblast growth factor receptor 1 (FGFR1) knockdown induced pronounced inhibition of cell viability in the presence of the osimertinib–ONO-7475 combination, which activates FGFR1 by upregulating FGF2 via the c-Myc pathway. Cell-based assays showed that triple therapy with osimertinib, ONO-7475, and the FGFR inhibitor BGJ398 significantly increased apoptosis by increasing expression of proapoptotic factor Bim and reduced cell viability compared with that observed for the osimertinib–ONO-7475 therapy. Xenograft models showed that triple therapy considerably suppressed tumor regrowth. A novel therapeutic strategy of additional initial FGFR1 inhibition may be highly effective in suppressing the emergence of osimertinib- and ONO-7475-resistant cells.

1. Introduction

Lung cancer is the leading cause of cancer-related deaths worldwide [1]. Epidermal growth factor receptor (EGFR) mutations are major driver gene mutations in non-small cell lung cancer (NSCLC) and are associated with a high risk of distant metastasis and poor prognosis [2–4]. Monotherapy with the third-generation EGFR tyrosine kinase inhibitor (EGFR-TKI) osimertinib exhibited a high overall response rate, leading to overall survival (OS) benefit in patients with advanced EGFR-mutated NSCLC compared with those administered the first-generation EGFR-TKI monotherapy. However, approximately 20 % of patients with advanced EGFR-mutated NSCLC did not achieve clinical response to osimertinib. Furthermore, even among patients who achieved clinical response, >30 % experienced tumor recurrence within 1 year, implying that eventual tumor recurrence is inevitable [5]. Various underlying mechanisms, including hepatocyte growth factor overexpression and BCL2-like 11 deletion polymorphisms, may cause primary resistance to EGFR-TKIs [6,7].

* Corresponding author. Department of Pulmonary Medicine, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, 465, Kajii-cho, Kamigyo-ku, Kyoto, Japan.
E-mail address: tayamada@koto.kpu-m.ac.jp (T. Yamada).

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Drug-tolerant (DT) cells are a small subset of tumor cells that are in a reversible tolerant state acquired in response to initial drug exposure. The maintenance of DT cells is the foundation for the development of acquired resistance to targeted therapy [8,9]. We previously reported that AXL, which is a member of the TYRO3, AXL, and MERTK family of receptor tyrosine kinases, maintained a survival signal as adaptive resistance when EGFR-mutated NSCLC cells with high AXL expression were exposed to an EGFR-TKI. Additionally, EGFR-TKI and AXL inhibitor combination inhibited the proliferation of these cells predominantly by modulating AKT activity [10,11]. Moreover, patients with high-AXL-expressing EGFR-mutated NSCLC accounted for 26.1 % of the cases and had shorter progression-free survival after osimertinib therapy in comparison with those with low-AXL-expressing tumors [12]. Based on these studies, a clinical trial is ongoing to evaluate the safety and efficacy of combining osimertinib with the AXL inhibitor ONO-7475 for treating patients with untreated advanced EGFR-mutated NSCLC in Japan (jRCT2051210045). However, even with this treatment combination, acquired resistance was expected to develop eventually in clinical settings [11]. Thus, the development of therapeutic strategies for eradicating tumor cells or achieving long-term remission is needed for patients with high-AXL-expressing EGFR-mutated NSCLC.

A phase III trial in patients with BRAF-mutated colorectal cancer showed that an initial triple combination therapy with EGFR, BRAF, and MEK inhibitors significantly prolonged OS compared with that achieved with a standard therapy [13]. In recent years, combination therapy for EGFR-mutated NSCLC with three or more molecular targeted agents, including EGFR inhibitors, has been a potentially promising therapeutic strategy for preventing the development of acquired resistance [14,15].

In this study, we aimed to determine whether an initial combination therapy with multiple agents, including osimertinib and ONO-7475, would be a promising therapeutic approach for high-AXL-expressing EGFR-mutated NSCLC. We show that the activation of fibroblast growth factor receptor 1 (FGFR1) through the upregulation of FGF2 activates ERK signaling and maintains the survival of tumor cells exposed to osimertinib and ONO-7475 by suppressing the expression of the proapoptotic factor Bim. The triple combination therapy with osimertinib, ONO-7475, and the FGFR inhibitor BGJ398 remarkably reduces cell viability, inhibits the emergence of DT cells, and prevents tumor regrowth in cell-derived xenograft (CDX) models.

2. Materials and methods

2.1. Cell culture and reagents

In this study, 12 human EGFR-mutated NSCLC cell lines were used. H1650, HCC827, and HCC4006 cells were purchased from the American Type Culture Collection (Manassas, VA); PC-9 cells were purchased from RIKEN Cell Bank (Ibaraki, Japan). PC-9GXR cells containing deletions in EGFR exon 19 and T790M mutation were established at Kanazawa University from PC-9 cell xenograft tumors in nude mice that had acquired resistance to the first-generation EGFR-TKI gefitinib [10]. PC-9DR1 and PC-9DR2 cells containing deletions in EGFR exon 19 and T790M mutation were established at Kyoto Prefectural University of Medicine from PC-9 cell xenograft tumors in severe combined immunodeficient (SCID) mice that had acquired resistance to the second-generation EGFR-TKI dacomitinib [16]. H1975 cells containing EGFR-L858R/T790M double mutations were provided by Dr. Yoshitaka Sekido (Aichi Cancer Center Research Institute, Aichi, Japan) and Dr. John D. Minna (University of Texas Southwestern Medical Center, Dallas, TX). HCC4011 and H3255 cells were provided by Dr. David P. Carbone (Ohio State University Comprehensive Cancer Center, Columbus, OH). The patient-derived cell line KPP-03 with an EGFR-L858R mutation was established from tumor cells in pleural effusion [17]. The patient-derived cell line G719S-GR with an EGFR-G719S mutation was established from malignant pleural effusion of a patient whose tumor developed acquired resistance to gefitinib treatment [18]. The cells were maintained in RPMI 1640 medium with 10 % fetal bovine serum (FBS) and 1 % penicillin/streptomycin under a 5 % CO_2 atmosphere at 37 °C. All cells were passaged for <3 months and tested for mycoplasma using a MycoAlert Mycoplasma Detection Kit (Lonza, Basel, Switzerland). Osimertinib (third-generation EGFR-TKI), BGJ398 (pan-FGFR inhibitor), buparlisib (PI3K inhibitor), PD173074 (pan-FGFR inhibitor), pemigatinib (pan-FGFR inhibitor), and trametinib (MEK inhibitor) were obtained from Selleck Chemicals (Houston, TX). ONO-7475 was supplied by Ono Pharmaceutical Co., Ltd (Osaka, Japan). Recombinant FGF2 was obtained from R&D Systems (Minneapolis, MN).

Additional methods are included in Supplementary Methods.

3. Results

3.1. FGFR1 knockdown sensitizes EGFR-mutated NSCLC cells to osimertinib + ONO-7475

We tested 12 EGFR-mutated NSCLC cell lines and divided them based on high or low levels of AXL expression (Supplementary Fig. 1A). The IC50 of osimertinib was significantly higher in cells with higher AXL expression than in those with lower AXL expression (Supplementary Figs. 1B and 1C). To overcome insensitivity to osimertinib, we examined the potency of its combination with ONO-7475 on the viability of high-AXL-expressing EGFR-mutated NSCLC cell lines. H1650 cells exhibited the lowest sensitivity to the osimertinib + ONO-7475 combination in cell viability assay, which is consistent with the findings from both short- and long-term exposure in *in vitro* models (Fig. 1A, B, and 1C). To identify therapeutic targets that enhance osimertinib + ONO-7475 efficacy, we conducted a synthetic lethality screening using an siRNA library by targeting human receptor tyrosine kinases in H1650 cells. The INSR- and FGFR1-targeting siRNAs were associated with the highest and second-highest suppression of cell viability in H1650 cells, respectively, in the presence of osimertinib + ONO-7475 (Fig. 1D). The downstream effects of INSR signaling are vital for glucose homeostasis in many cell types. It was deemed unsuitable as a candidate molecule for combination therapy because of the potential for serious adverse events associated with its control and removed from the list of candidates. We focused on FGFR1 as a therapeutic target owing to its significant potential for clinical applicability. Among the FGFR family members, FGFR3 is important for tolerance to EGFR-TKIs in EGFR-mutated NSCLC cells [19]. Cell viability assay using specific siRNAs targeting each FGFR member to determine the FGFR family member essential for the survival of high-AXL-expressing EGFR-mutated NSCLC cells exposed to osimertinib + ONO-7475 showed that FGFR1 knockdown in both PC-9 and H1650 cells resulted in a more pronounced enhancement of the effect of osimertinib + ONO-7475 on cell viability compared with that observed after FGFR2 or FGFR3 knockdown. Thus, FGFR1 plays crucial roles in the tolerance of high-AXL-expressing EGFR-mutated NSCLC cells to osimertinib + ONO-7475 (Fig. 1E and Supplementary Fig. 2). To further validate the effects of FGFR1 knockdown, we transfected PC-9, PC-9GXR, and H1650 cells with FGFR1-specific siRNAs and cultured them with or without osimertinib + ONO-7475. Over 7 days, FGFR1 knockdown enhanced the inhibitory effects of osimertinib + ONO-7475 on cell viability (Fig. 1F and Supplementary Fig. 2). FGFR1-specific siRNAs in combination with osimertinib + ONO-7475 suppressed ERK phosphorylation compared with that observed in cells treated with a control siRNA and osimertinib + ONO-7475 combination (Fig. 1G). In contrast, FGFR1-specific siRNAs and osimertinib + ONO-7475 did not exert any additional effect on AKT phosphorylation compared with that observed for control siRNA and osimertinib + ONO-7475. To elucidate the signaling pathways involved in ensuring the survival of high-AXL-expressing EGFR-mutated NSCLC cells exposed to osimertinib + ONO-7475, we investigated the effect of downstream molecules on cell viability in PC-9 and H1650 cells. The MEK inhibitor trametinib exerted additional effects when administered osimertinib + ONO-7475

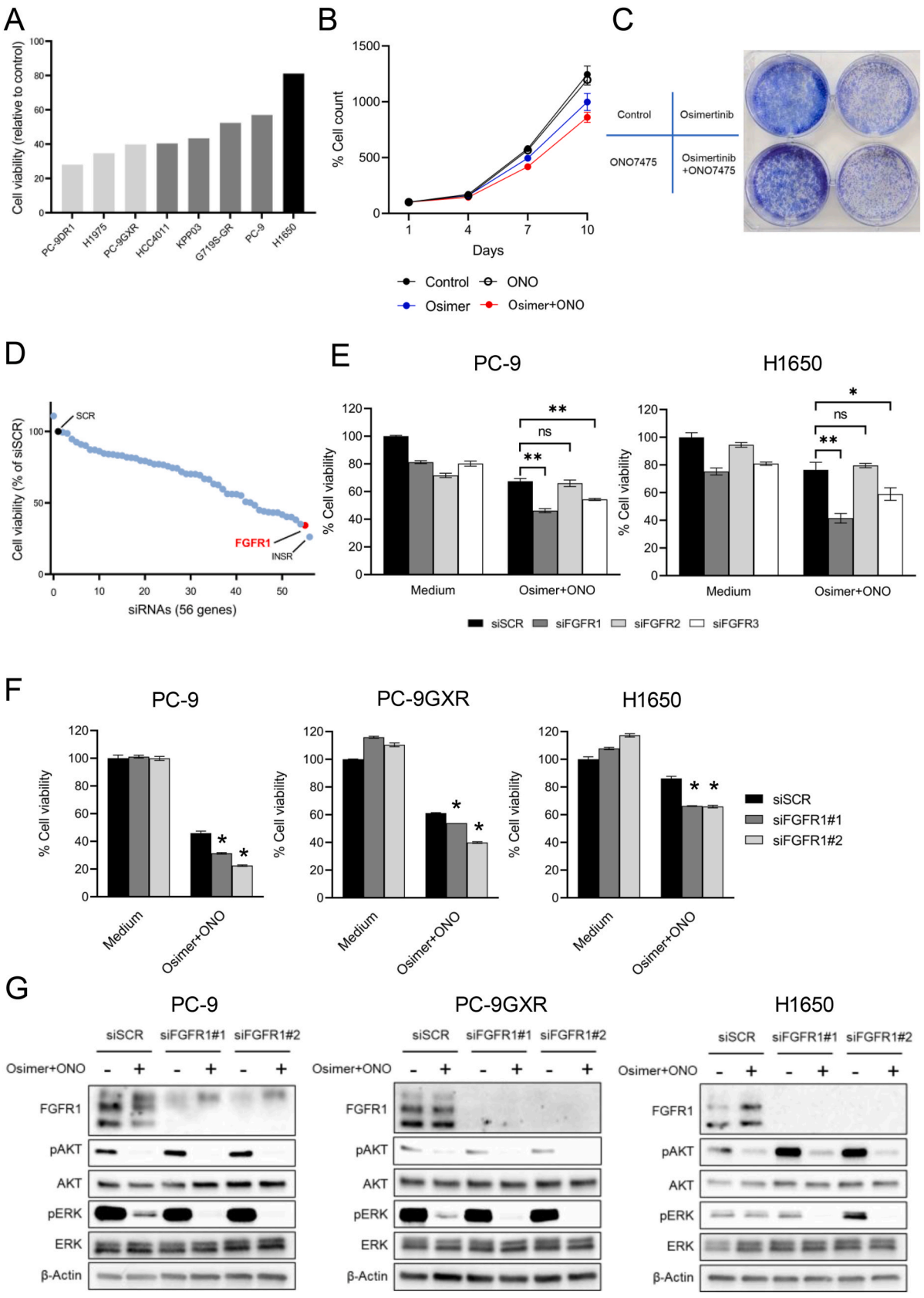

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Fig. 1. FGFR1 knockdown sensitizes EGFR-mutated NSCLC cells to osimertinib plus ONO-7475
A High-AXL-expressing EGFR-mutated NSCLC cell lines were treated with DMSO or a combination of osimertinib (100 nmol/L) and ONO-7475 (100 nmol/L) for 4 days. Cell viability was determined using the MTT assay. Cell viability percentage for each cell line against control when treated with the combination therapy is shown. Black, dark gray, and light gray bars indicate approximately 60 %, 40–60 %, and 40 % cell viability, respectively. **B** H1650 cells were treated with DMSO, osimertinib (100 nmol/L) alone, ONO-7475 (100 nmol/L) alone, or a combination of these drugs for the indicated time durations. The drugs were replenished every 72 h. The cell counts were assessed using the MTT assay. **C** H1650 cells were treated with DMSO, osimertinib (100 nmol/L) alone, ONO-7475 (100 nmol/L) alone, or a combination of these drugs for 9 days. The drugs were replenished every 72 h. The cells were stained with crystal violet. **D** H1650 cells were transfected with a scrambled control siRNA or 56 receptor tyrosine kinase (RTK)-specific siRNAs. After 24 h of transfection, the cells were incubated with osimertinib (100 nmol/L) plus ONO-7475 (100 nmol/L) for 72 h. The percentage cell viability for each RTK-specific siRNA against that of the scrambled control siRNA was assessed using the MTT assay. **E** PC-9 and H1650 cells were transfected with a scrambled control siRNA, FGFR1-specific siRNA (#2), FGFR2-specific siRNA, or FGFR3-specific siRNA. After 24 h of transfection, the cells were incubated with DMSO or a combination of osimertinib (100 nmol/L) and ONO-7475 (100 nmol/L) for 72 h. Cell viability was determined using the MTT assay. $*P < 0.05$, $**P < 0.005$. **F** PC-9, PC-9GXR, and H1650 cells were transfected with a scrambled control siRNA or FGFR1-specific siRNAs (#1 and #2). After 24 h of transfection, the cells were incubated with DMSO or a combination of osimertinib (100 nmol/L) and ONO-7475 (100 nmol/L) for 7 days. The drugs were replenished every 72 h. The cell viability was determined using the MTT assay. $*P < 0.05$. **G** PC-9, PC-9GXR, and H1650 cells were transfected with a scrambled control siRNA or FGFR1-specific siRNAs (#1 and #2). After 24 h of transfection, the cells were incubated with DMSO or a combination of osimertinib (100 nmol/L) and ONO-7475 (100 nmol/L) for 24 h. The cells were lysed, and the indicated proteins were detected using the western blotting assay.

than that of the PI3K inhibitor buparlisib, indicating the involvement of the MEK/ERK pathway but not that of the PI3K/AKT pathway in ensuring the survival of high-AXL-expressing EGFR-mutated NSCLC cells exposed to osimertinib + ONO-7475 (Supplementary Figs. 3A and 3B). We further evaluated whether osimertinib + ONO-7475 treatment induces feedback ERK activation. The osimertinib + ONO-7475 treatment-induced decrease in ERK phosphorylation in PC-9 and H1650 cells started to recover on day 3 and day 2, respectively (Supplementary Fig. 4). These results indicated that FGFR1 knockdown might result in the suppression of the ERK signaling, which sensitizes high-AXL-expressing EGFR-mutated NSCLC cells to osimertinib + ONO-7475.

3.2. FGFR inhibitors augment osimertinib + ONO-7475 combination treatment efficacy in EGFR-mutant NSCLC cells

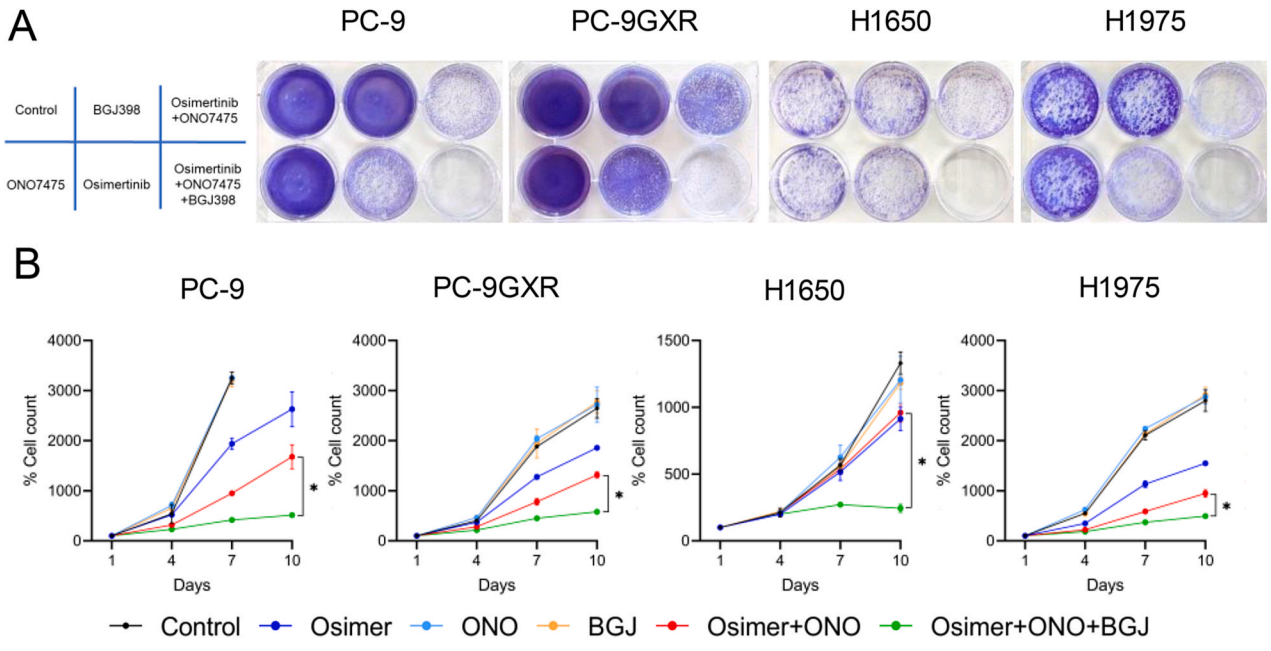
We evaluated whether FGFR inhibitors increased the sensitivity of high-AXL-expressing EGFR-mutated NSCLC cell lines to osimertinib + ONO-7475. Notably, pan-FGFR inhibitors (BGJ398, pemigatinib, and PD173074) with osimertinib + ONO-7475 suppressed the proliferation of the high-AXL-expressing residual PC-9, PC9GXR, H1650, and H1975 cells over an extended duration (Fig. 2A and B, and Supplementary Fig. 5). Western blotting after FGFR1 immunoprecipitation confirmed that BGJ398 inhibited FGFR1 phosphorylation (Supplementary Fig. 6). Osimertinib + ONO-7475 + BGJ398 triple combination therapy for 4 and 48 h produced notable inhibition of ERK phosphorylation than that observed after treatment with osimertinib + ONO-7475 (Fig. 2C and D). These results indicated that combined treatment with FGFR inhibitors may increase cell sensitivity to osimertinib + ONO-7475 primarily by modulating the ERK activity and reducing the viability of high-AXL-expressing EGFR-mutated NSCLC cells.

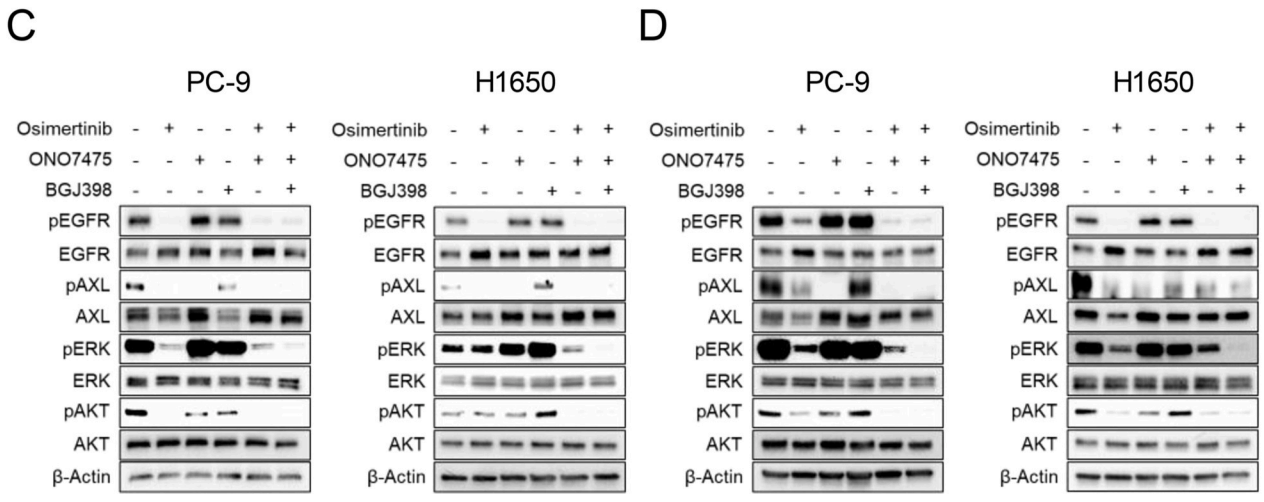
Migration assay in PC-9 and H1650 cells showed that the triple combination therapy significantly reduced the number of migrating cells compared with that observed after osimertinib + ONO-7475 treatment, indicating that osimertinib + ONO-7475 + BGJ398 therapy may suppress motility in high-AXL-expressing EGFR-mutated NSCLC cells (Fig. 2E and F).

3.3. FGF2 upregulation via c-myc induces resistance in EGFR-mutated NSCLC cells to osimertinib + ONO-7475

Real-time polymerase chain reaction (PCR) array analysis to evaluate the effect of osimertinib + ONO-7475 treatment on autocrine FGFR ligand production showed that FGF2 was the most upregulated among all detected FGFR ligands after osimertinib + ONO-7475 treatment (Fig. 3A). Real-time PCR and western blotting analyses showed that osimertinib + ONO-7475 treatment upregulated FGF2 expression in PC-9 cells at both mRNA and protein levels (Supplementary Figure 7 and Fig. 3B). Recombinant FGF2 stimulation promoted FGFR1 phosphorylation, as evidenced by western blotting analysis after FGFR1 immunoprecipitation (Supplementary Fig. 8). Investigating FGF2 involvement in cell tolerance to osimertinib + ONO-7475 showed that FGF2 knockdown enhanced the inhibitory effects of osimertinib + ONO-7475 on cell viability in PC-9 and H1650 cells (Fig. 3C). Western blotting analysis showed that treatment with FGF2-specific siRNA + osimertinib + ONO-7475 induced higher suppression of ERK phosphorylation than that observed with control siRNA + osimertinib + ONO-7475 (Fig. 3D). Examining the effect of recombinant FGF2 stimulation on PC-9 and H1650 cell sensitivity to osimertinib + ONO-7475 showed that recombinant FGF2 reduced the sensitivity to osimertinib + ONO-7475, whereas the pan-FGFR-inhibitor BGJ398 restored the sensitivity of these cells to osimertinib + ONO-7475 in the presence of recombinant FGF2 (Supplementary Fig. 9A). Western blotting analysis showed that recombinant FGF2 stimulation induced ERK phosphorylation in cells treated with osimertinib + ONO-7475, whereas BGJ398 inhibited the recombinant FGF2-induced phosphorylation compared with that observed after osimertinib + ONO-7475 treatment (Supplementary Fig. 9B). In addition to binding to FGFR1, FGF2 also binds to the other FGFR family members, which are the targets of pan-FGFR inhibitors [20]. To confirm that recombinant FGF2 stimulation reduced cell sensitivity to osimertinib + ONO-7475 through FGFR1 activation, we evaluated the effects of FGFR1, FGFR2, or FGFR3 knockdown on cell viability in recombinant FGF2-stimulated cells. In contrast to that observed after FGFR1 knockdown, FGFR2 or FGFR3 knockdown did not reduce recombinant FGF2-induced tolerance of PC-9 cells to osimertinib + ONO-7475 (Supplementary Fig. 10). Thus, FGF2 induces resistance to osimertinib + ONO-7475 in high-AXL-expressing EGFR-mutated NSCLC cells primarily through FGFR1 activation.

Next, we sought to clarify the mechanisms underlying osimertinib + ONO-7475 treatment upregulated FGF2 expression in PC-9 cells. FGF2 is a crucial factor required for maintaining the undifferentiated state in embryonic stem cells and induced pluripotent stem cells [21]. Osimertinib + ONO-7475 + BGJ398 triple combination therapy significantly suppressed colony formation on soft agar, which is an indicator of cancer stemness [22], compared with that observed after osimertinib + ONO-7475 treatment (Fig. 3E). Therefore, we hypothesized that the stemness regulators modulate the FGF2-FGFR1 activation signal. We screened for changes in stemness regulator expression levels and verified that only c-Myc expression significantly increased after osimertinib + ONO-7475 treatment (Fig. 3F). To investigate c-Myc involvement in FGF2 upregulation, we examined the effects of c-Myc knockdown using a specific siRNA in PC-9 cells. c-Myc knockdown suppressed FGF2 upregulation on mRNA and protein levels in osimertinib + ONO-7475-treated cells (Fig. 3G and Supplementary Fig. 11A). Additionally, it enhanced the inhibitory effects of osimertinib plus ONO-7475 on cell viability (Fig. 3H). Conversely, c-Myc overexpression in PC-9 cells induced FGF2 upregulation at mRNA and protein levels in the presence or absence of osimertinib + ONO-7475 (Fig. 3I and





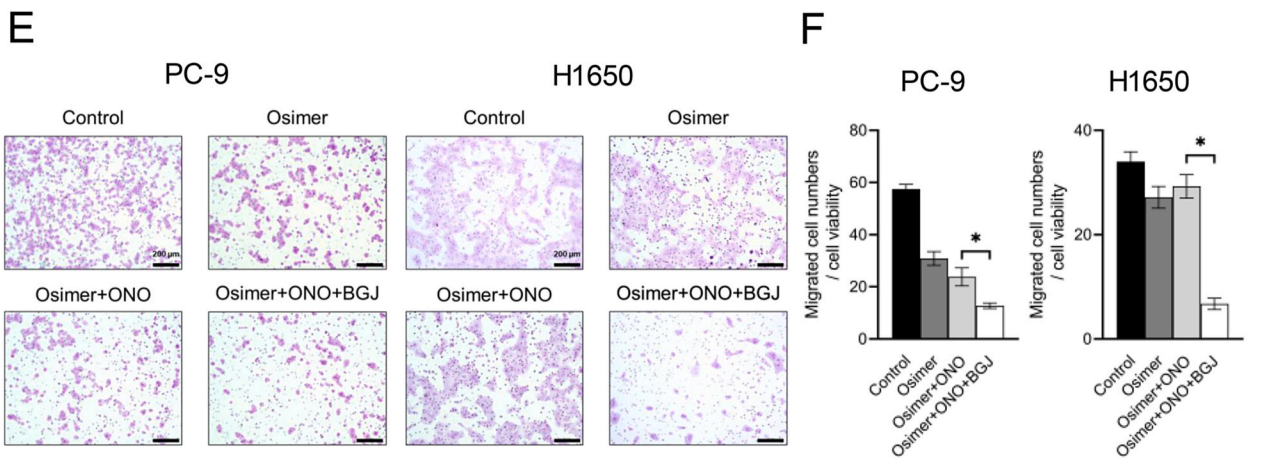


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Fig. 2. FGFR inhibitors augment the efficacy of osimertinib plus ONO-7475 combination treatment in EGFR-mutant NSCLC cells
A PC-9, PC-9GXR, H1650, and H1975 cells were treated with DMSO, osimertinib (100 nmol/L) alone, ONO-7475 (100 nmol/L) alone, BGJ398 (1.0 μmol/L) alone, osimertinib and ONO-7475, or all the three drugs for 9 days. The drugs were replenished every 72 h. The cells were stained with crystal violet. **B** PC-9, PC-9GXR, H1650, and H1975 cells were treated with DMSO, osimertinib (100 nmol/L) alone, ONO-7475 (100 nmol/L) alone, BGJ398 (1.0 μmol/L) alone, osimertinib and ONO-7475, or all three drugs for the indicated time durations. The drugs were replenished every 72 h. The cell counts were assessed using the MTT assay. $^*P < 0.05$. **C, D** PC-9 and H1650 cells were incubated with DMSO, osimertinib (100 nmol/L) alone, ONO-7475 (100 nmol/L) alone, BGJ398 (1.0 μmol/L) alone, osimertinib and ONO-7475, or all three drugs for 4 h (C) and 48 h (D). The indicated proteins were detected using the western blotting assay. **E, F** PC-9 and H1650 cells were treated with DMSO, osimertinib (100 nmol/L) alone, osimertinib and ONO-7475 (100 nmol/L), or osimertinib, ONO-7475, and BGJ398 (1.0 μmol/L) combination for 24 h. Representative Giemsa staining images (Scale bar, 200 μm). (E) Mean percentage of four evaluated areas divided by the cell viability as determined using the MTT assay (F). $^*P < 0.05$.

Supplementary Fig. 11B). Additionally, c-Myc overexpression reduced the inhibitory effects of osimertinib + ONO-7475 on cell viability, whereas BGJ398 restored the cell sensitivity to osimertinib + ONO-7475 (Fig. 3J). Thus, c-Myc accelerates the adaptive resistance of cells to osimertinib + ONO-7475 by at least partly promoting the FGF2-related pathway.

3.4. BGJ398 enhances apoptosis via Bim upregulation in EGFR-mutated NSCLC cells

We examined the mechanisms underlying the efficacy of osimertinib + ONO-7475 + BGJ398 triple combination therapy in inducing apoptosis in PC-9 and H1650 cells. Flow cytometry analysis showed that the apoptotic cell percentage was higher after osimertinib + ONO-7475 + BGJ398 treatment than after osimertinib alone and osimertinib + ONO-7475 treatments (Fig. 4A and Supplementary Fig. 12). Additionally, osimertinib + ONO-7475 + BGJ398 treatment enhanced Bim accumulation compared with that induced by osimertinib alone or osimertinib + ONO-7475 treatments (Fig. 4B). Bim was knocked down using a specific siRNA to investigate its significance in apoptosis induction by the triple combination therapy (Supplementary Fig. 13). Bim knockdown showed results consistent with those of flow cytometry analysis; osimertinib + ONO-7475 + BGJ398 significantly increased caspase-3/7 activity compared with that of osimertinib alone or osimertinib + ONO-7475 treatments, whereas Bim knockdown notably mitigated this increase (Fig. 4C). Thus, osimertinib + ONO-7475 + BGJ398 increased Bim expression and induced apoptosis (Fig. 4D).

3.5. BGJ398 suppresses the emergence and maintenance of cells tolerant to osimertinib + ONO-7475

We examined the role of FGFR signaling in the maintenance of DT cells exposed to osimertinib + ONO-7475. We isolated DT cells from PC-9 and H1650 cells after exposing them to high doses of osimertinib (3.0 μmol/L) plus ONO-7475 (1.0 μmol/L) for 9 days [11].

To confirm that the drug tolerance of DT cells is reversible, we cultured these cells under a drug-free condition for 15 days (DF cells) (Fig. 5A). Compared with that of the parental or DF cells, a large proportion of PC-9 and H1650 DT cells was in the G1 phase of the cell cycle (Fig. 5B). The growth rate of these cells was slower than that of the parental or DF cells (Supplementary Fig. 14) [8]. As expected, the cell viability assay showed that these DT cells were resistant to osimertinib alone or osimertinib + ONO-7475 treatments compared with that of their parental counterparts. In contrast, treatment with BGJ398 alone decreased DT cell viability but not that of the parental and DF cells (Fig. 5C). The DT cells exhibited increased FGFR1 levels compared with that in the parental cells. In contrast, FGF2 was only upregulated in PC-9DT cells but not in H1650DT cells compared with that observed in the respective parent counterparts (Fig. 5D). Although osimertinib alone and osimertinib + ONO-7475 treatments did not inhibit ERK phosphorylation, BGJ398 suppressed its phosphorylation in DT cells (Fig. 5E). Thus, activated FGFR1 played a critical role in the emergence and the maintenance of DT cells exposed to osimertinib + ONO-7475 and that FGFR inhibitors may suppress DT cell formation in combination with osimertinib + ONO-7475.

3.6. Osimertinib + ONO-7475 + BGJ398 triple combination therapy prevents cell line-derived xenograft tumor regrowth

We evaluated the effects of the osimertinib + ONO-7475 + BGJ398 in CDX models. Mice were continuously administered osimertinib alone, ONO-7475 alone, BGJ398 alone, osimertinib plus ONO-7475, or the triple combination. Treatment with osimertinib alone or osimertinib + ONO-7475 caused tumor regression within 1 week but tumor regrowth occurred within 2 weeks. In contrast, osimertinib + ONO-7475 + BGJ398 treatment caused tumor regression compared with that in the osimertinib alone or osimertinib + ONO-7475 treatment groups, and tumor size were maintained for 6 and 8 weeks in H1650 cells and PC-9 cells, respectively (Fig. 6A and B). The number of Ki67-positive proliferating tumor cells was significantly lower in both H1650 and PC-9 tumors treated with osimertinib + ONO-7475 + BGJ398 than in those treated with osimertinib alone or osimertinib + ONO-7475 (Fig. 6C, D, 6E, and 6F). Additionally, TUNEL-positive tumor cell proportion significantly increased in both H1650 and PC-9 tumors after osimertinib + ONO-7475 + BGJ398 therapy than in tumors treated with osimertinib alone or osimertinib + ONO-7475 (Fig. 6C, D, 6G, and 6H). These results were consistent with cell line-based analysis results. Osimertinib + ONO-7475 + BGJ398 treatment reduced phosphorylated ERK levels in H1650 cell-derived tumors and induced the expression of Bim and cleaved PARP compared with that observed after osimertinib alone or osimertinib + ONO-7475 treatments (Fig. 6I). No apparent adverse effects including weight loss were observed during these treatments in both H1650 and PC-9 tumors (Supplementary Fig. 15). Thus, osimertinib + ONO-7475 + BGJ398 therapy was safe and may potentially prevent tumor regrowth in high-AXL-expressing EGFR-mutated NSCLC tumors by inhibiting cell proliferation and promoting apoptosis.

4. Discussion

Clinical application of EGFR-TKIs remarkably improves patient prognosis in EGFR-mutated NSCLC. However, the response duration to EGFR-TKI treatment remains limited because of adaptive resistance mechanisms, leading to the development of acquired resistance. Therefore, better initial treatment strategies using EGFR-TKIs are desired to further improve patient prognosis in EGFR-mutated NSCLC. Currently, various initial combination treatments with EGFR-TKIs are being developed for preclinical studies in EGFR-mutated lung cancer [10,15,19,23–25]. We previously reported that a combination therapy targeting EGFR and AXL, which is currently under development for clinical application, is a promising therapeutic strategy for high-AXL-expressing EGFR-mutated NSCLC. However, it may be further enhanced by improving disease control [10,11]. In this study, we have elucidated the mechanism underlying adaptive resistance to (EGFR-TKI) osimertinib and (AXL inhibitor) ONO-7475 combination therapy and demonstrated the promising potential of the triple combination therapy targeting EGFR, AXL, and FGFR in high-AXL-expressing EGFR-mutated NSCLC. Both AXL and FGFR family members confer drug resistance in driver oncogene-positive cancer cells, including EGFR-mutated NSCLC [10,11,19,25–28]. This is the first study to suggest the potential clinical utility of this triple combination in cancer treatment.

PI3K/Akt/mTOR and Ras/Raf/MEK/ERK pathways are crucial for

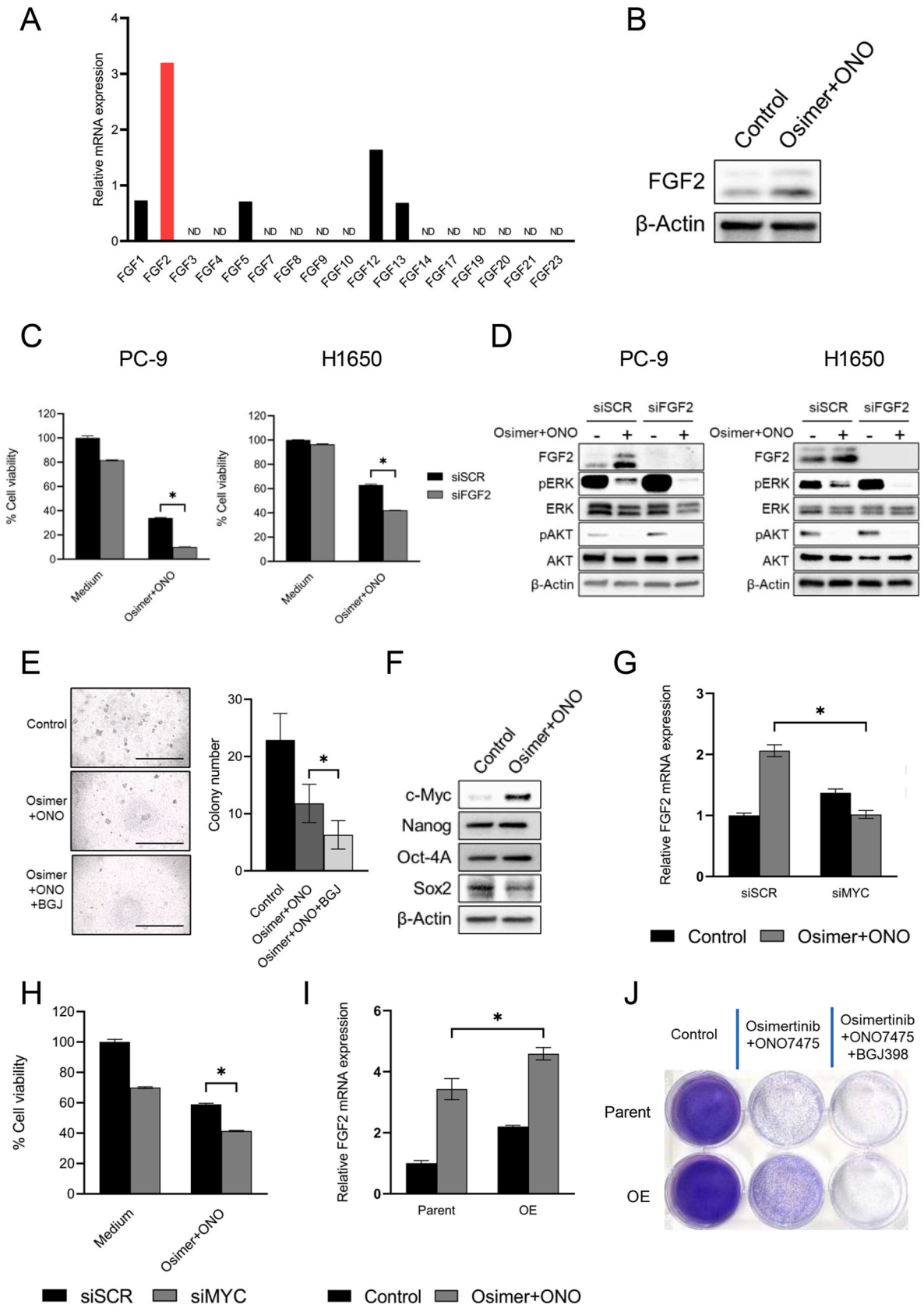

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Fig. 3. FGF2 upregulation via c-Myc induces EGFR-mutated NSCLC cell resistance to osimertinib plus ONO-7475
A PC-9 cells were incubated with osimertinib (100 nmol/L) and ONO-7475 (100 nmol/L) combination for 96 h, and the expression of FGFR ligands was analyzed using TaqMan™ arrays. ND, not detected. **B** PC-9 cells were incubated with DMSO or osimertinib (100 nmol/L) and ONO-7475 (100 nmol/L) combination for 96 h, and the indicated proteins were detected using the western blotting assay. **C** PC-9 and H1650 cells were transfected with a scrambled control siRNA or an FGF2-specific siRNA. After 24 h of transfection, the cells were incubated with DMSO or osimertinib (100 nmol/L) and ONO-7475 (100 nmol/L) combination for 96 h. Cell viability was determined using the MTT assay. $^{*}P < 0.05$. **D** PC-9 and H1650 cells were transfected with a scrambled control siRNA or FGF2-specific siRNA. After 24 h of transfection, the cells were incubated with DMSO or osimertinib (100 nmol/L) and ONO-7475 (100 nmol/L) combination for 96 h, and the indicated proteins were detected using the western blotting assay. **E** PC-9 cells were seeded on soft agar for colony formation and were treated with DMSO, osimertinib (100 nmol/L) and ONO-7475 (100 nmol/L) combination, or osimertinib, ONO-7475, and BGJ398 (1.0 μmol/L) combination for 14 days. Representative images (Scale bar, 50 μm) and mean colony number in 10 evaluated areas. $^{*}P < 0.05$. **F** PC-9 cells were incubated with DMSO or osimertinib (100 nmol/L) and ONO-7475 (100 nmol/L) combination for 96 h, and the indicated proteins were detected using the western blotting assay. **G** PC-9 cells were transfected with a scrambled control siRNA or MYC-specific siRNA. After 24 h of transfection, the cells were incubated with DMSO or osimertinib (100 nmol/L) and ONO-7475 (100 nmol/L) combination for 96 h, and the *FGF2* mRNA levels were measured using the qPCR assay. $^{*}P < 0.05$. **H** PC-9 cells were transfected with a scrambled control siRNA or MYC-specific siRNA. After 24 h of transfection, the cells were incubated with DMSO or osimertinib (100 nmol/L) and ONO-7475 (100 nmol/L) combination for 96 h. Cell viability was determined using the MTT assay. $^{*}P < 0.05$. **I** PC-9 cells and PC-9 cells overexpressing c-Myc were incubated with DMSO or osimertinib (100 nmol/L) and ONO-7475 (100 nmol/L) combination for 96 h, and the FGF2 mRNA levels were measured using the qPCR assay. $^{*}P < 0.05$. **J** PC-9 cells (1×10^4 cells) and PC-9 cells overexpressing c-Myc (1×10^4 cells) were treated with DMSO, osimertinib (100 nmol/L) and ONO-7475 (100 nmol/L), or osimertinib, ONO-7475, and BGJ398 (1.0 μmol/L) combination for 9 days. The drugs were replenished every 72 h. The cells were then stained with crystal violet.

cancer cell survival and proliferation [29]. The addition of AXL inhibition predominantly contributed to cell viability suppression by inhibiting AKT signaling in high-AXL-expressing EGFR-mutated NSCLC cells treated with EGFR-TKIs [10]. Importantly, prolonged exposure to this combination therapy resulted in the reinvigoration of ERK signaling due to the reactivation of FGFR. In contrast, the triple combination therapy targeting EGFR, AXL, and FGFR effectively suppressed both ERK and AKT signaling in high-AXL-expressing EGFR-mutated NSCLC, suggesting that co-inhibition of AXL and FGFR1 may potentially eliminate crucial cell survival signals associated with adaptive tolerance of high-AXL-expressing EGFR-mutated NSCLC cells to osimertinib.

Several initial combinations of multiple kinase inhibitors for specific tumor populations have garnered attention as therapeutic strategies aimed at eradicating lung cancer cells [14,15]. The efficacy and safety of the pan-FGFR inhibitor BGJ398 in patients with unresectable locally advanced or metastatic cholangiocarcinoma with an FGFR2 fusion or other rearrangement was verified in a phase II clinical trial. BGJ398 has been approved by the United States Food and Drug Administration [30]. In this study, experiments using CDX models indicated the potential efficacy and safety of the osimertinib + ONO-7475 + BGJ398 for patients with EGFR-mutated NSCLC. However, its clinical application for treating patients with lung cancer needs to be further evaluated.

We elucidated the mechanisms underlying adaptive resistance through the activation of the FGF2-FGFR1 pathway in EGFR-mutated NSCLC treated with a combination of osimertinib and ONO-7475. The FGF2-FGFR1 pathway is a promising therapeutic target for melanoma and gastrointestinal stromal tumors [31,32]. In EGFR-mutated NSCLC, activation of this pathway induces intrinsic and acquired resistance to EGFR-TKIs [25,33–35]. Moreover, FGFR1 overexpression decreases EGFR-mutated NSCLC cell sensitivity to EGFR-TKIs but increases that to FGFR inhibitors [36]. In DT cell experiments, FGFR1 activation played a crucial role in the emergence of EGFR-mutated NSCLC cells tolerance to the osimertinib + ONO-7475 combination, suggesting that therapeutic intervention led to the survival of the cell population characterized by relatively higher dependence on FGFR1 signaling as a mechanism for drug tolerance.

c-Myc forms heterodimers with its partner protein, MAX, and regulates the expression of various genes associated with cell behavior, including proliferation, differentiation, and apoptosis [37]. It plays a pivotal role in tumorigenesis and is amplified in various tumors [38]. c-Myc promotes FGF2 expression via miR-15-16 repression in tumor cells exposed to hypoxia [39]. In this study, c-Myc suppression significantly inhibited FGF2 upregulation induced by the osimertinib + ONO-7475, whereas its overexpression significantly upregulated FGF2 expression. Thus, c-Myc plays an important role in establishing adaptive resistance in EGFR-mutated NSCLC by activating FGF2-FGFR1-ERK signaling. However, the mechanism underlying c-Myc upregulation after treatment with osimertinib + ONO-7475 has not been elucidated, which is a limitation of this study.

Bim upregulation is pivotal in EGFR-TKI-induced apoptosis of EGFR-mutated NSCLC cells [40]. Bim expression is regulated predominantly by its ERK-dependent phosphorylation at Ser69 [41–43]. In this study, the addition of an FGFR inhibitor promoted apoptosis of EGFR-mutated NSCLC cells by promoting Bim accumulation by suppressing ERK phosphorylation.

In summary, adaptive resistance of high-AXL-expressing EGFR-mutated NSCLC to osimertinib + ONO-7475 relies on FGF2-FGFR1-ERK pathway activation. Importantly, osimertinib + ONO-7475 + BGJ398 triple combination therapy effectively suppressed the survival of high-AXL-expressing EGFR-mutated NSCLC cells by enhancing apoptosis via Bim upregulation and elicited antitumor effects by reducing cell migration. These results indicate that osimertinib + ONO-7475 + BGJ398 may potentially improve outcomes in EGFR-mutated NSCLC. Future studies are warranted in clinical settings to assess the safety and efficacy of this combination therapy for patients with EGFR-mutated NSCLC.

Ethical statement

The mouse experimental protocols were approved by the institutional review board of Kyoto Prefectural University of Medicine (Kyoto, Japan; approval no. M2022-128). According to institutional guidelines, surgeries were performed after the animals had been anesthetized with sodium pentobarbital and efforts were made to minimize animal suffering.

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Data availability

The data that support the findings of this study are available on request from the corresponding author.

CRediT authorship contribution statement

Ryota Nakamura: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Tadaaki Yamada:** Writing – review & editing, Writing – original draft, Resources, Project administration, Funding

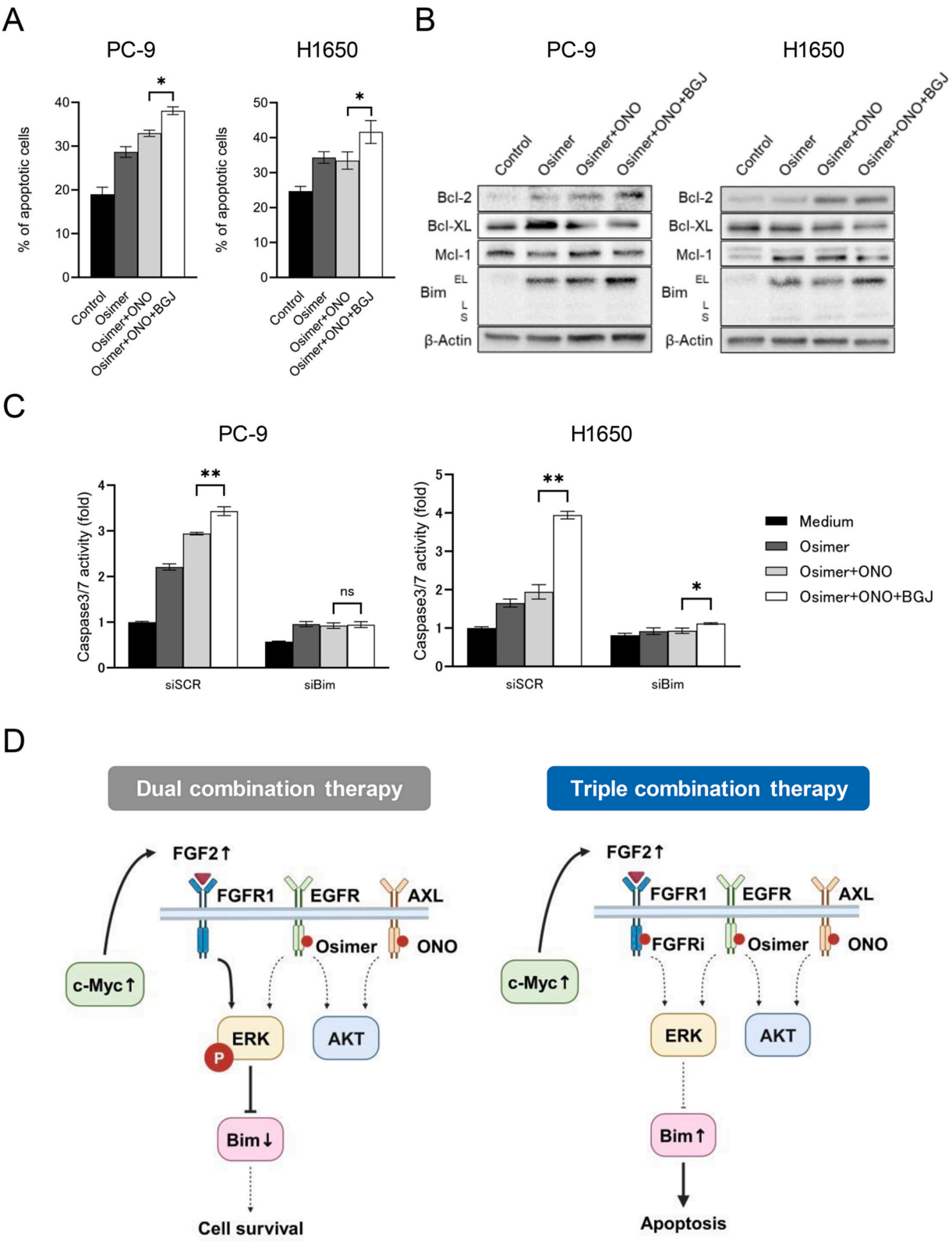

Fig. 4. FGFR inhibitor BGJ398 enhances apoptosis via Bim upregulation in EGFR-mutated NSCLC cells
A PC-9 and H1650 cells were incubated with DMSO, osimertinib (100 nmol/L) alone, osimertinib and ONO-7475 (100 nmol/L) combination, or osimertinib, ONO-7475, and BGJ398 (1.0 μmol/L) combination for 48 h. Cell apoptosis was analyzed using flow cytometry. The percentages of apoptotic cells are shown. *$P < 0.05$. **B** PC-9 and H1650 cells were incubated with DMSO, osimertinib (100 nmol/L) alone, osimertinib and ONO-7475 (100 nmol/L) combination, or osimertinib, ONO-7475, and BGJ398 (1.0 μmol/L) combination for 72 h. The indicated proteins were detected using the western blotting assay. **C** PC-9 and H1650 cells were transfected with a scrambled control siRNA or Bim-specific siRNA. After 24 h of transfection, the cells were incubated with DMSO, osimertinib (100 nmol/L) alone, osimertinib and ONO-7475 (100 nmol/L) combination, or osimertinib, ONO-7475, and BGJ398 (1.0 μmol/L) combination for 48 h. Caspase-3/7 activity was measured. *$P < 0.05$, **$P < 0.005$. **D** Schematic of the adaptive drug tolerance mechanisms, including activation of FGF2-FGFR1-ERK signaling via c-Myc and avoiding cell apoptosis through Bim degradation in EGFR-mutated NSCLC cells.

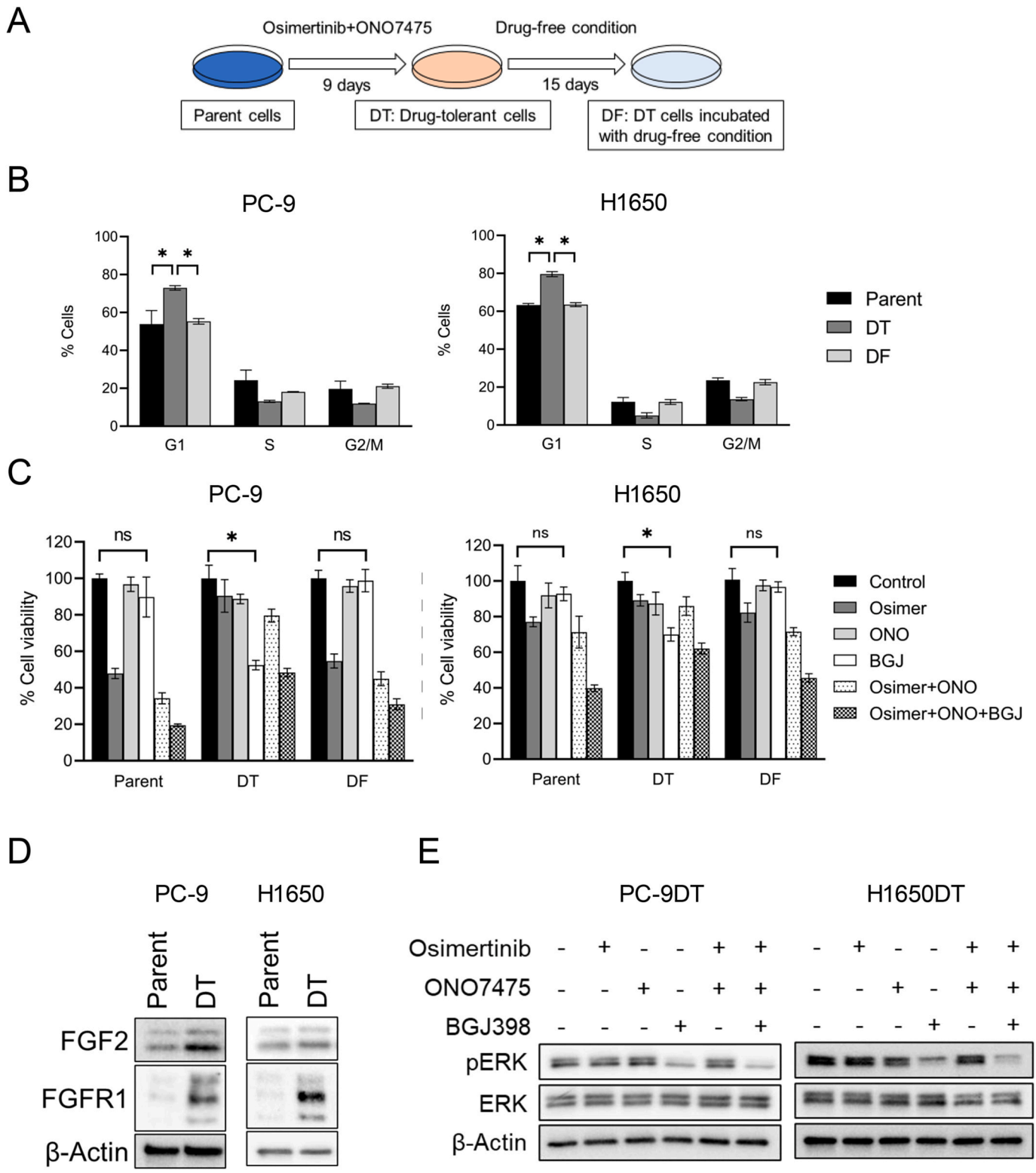

Fig. 5. FGFR inhibitor BGJ398 suppresses emergence and maintenance of cell tolerance to osimertinib plus ONO-7475
A PC-9 and H1650 cells were left untreated (left) or treated with osimertinib (3.0 μmol/L) plus ONO-7475 (1.0 μmol/L) for 9 days (Drug-tolerant (DT) cells; middle). DT cells were incubated with drug-free medium for 15 days (Drug-free (DF) cells; right). **B** The cell cycle of parent, DT, and DF cells was analyzed using flow cytometry. The percentages of cell populations in the various cell-cycle phases are shown. *$P < 0.05$. **C** Parent, DT, and DF cells were treated with DMSO, osimertinib (100 nmol/L) alone, ONO-7475 (100 nmol/L) alone, BGJ398 (1.0 μmol/L) alone, osimertinib and ONO-7475 combination, or all three drugs for 96 h. Cell viability was assessed using the MTT assay. *$P < 0.05$. **D** Indicated protein levels in parent and DT cells were determined using the western blotting assay. **E** DT cells were incubated with DMSO, osimertinib (100 nmol/L) alone, ONO-7475 (100 nmol/L) alone, BGJ398 (1.0 μmol/L) alone, osimertinib and ONO-7475 combination, or all three drugs for 24 h. Indicated protein levels were detected using the western blotting assay.

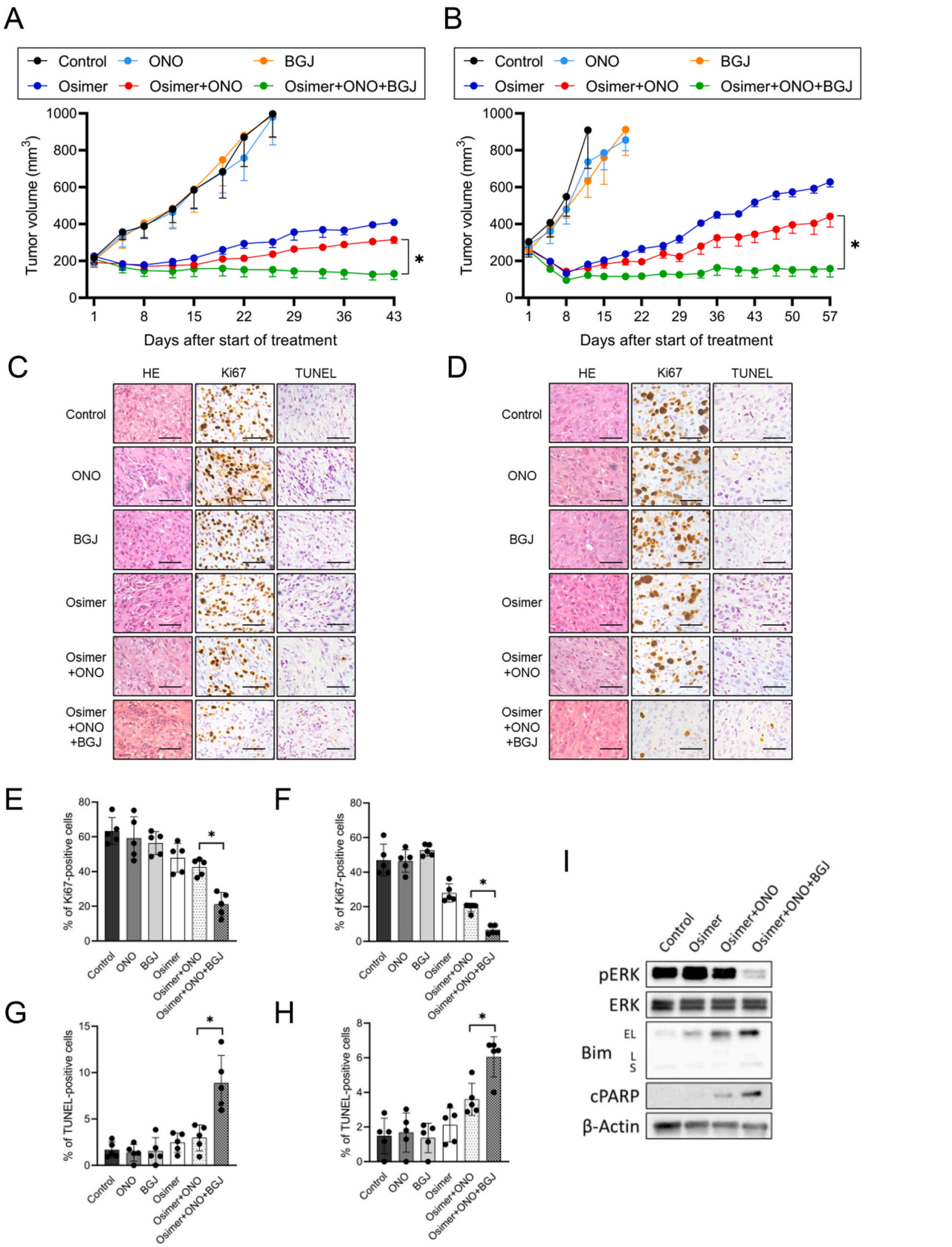

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Fig. 6. Triple combination therapy with osimertinib, ONO-7475, and BGJ398 prevents regrowth of cell line-derived xenograft tumors
A, B H1650 cell line-derived xenograft (CDX; $n = 6$) (A) and PC-9 CDX ($n = 5$) (B) tumors were treated with a vehicle (control), osimertinib (5.0 mg/kg), ONO-7475 (10 mg/kg), BGJ398 (15 mg/kg), osimertinib plus ONO-7475, or osimertinib plus ONO-7475 plus BGJ398 via oral gavage. Tumor volume was measured from the start of treatment and is shown as mean ± SEM. $^*P < 0.05$. **C-H** H1650 CDX tumors and PC-9 CDX tumors were treated with a vehicle (control), osimertinib (5.0 mg/kg), ONO-7475 (10 mg/kg), BGJ398 (15 mg/kg), osimertinib plus ONO-7475, or osimertinib plus ONO-7475 plus BGJ398 for 4 days via oral gavage. Representative images of H1650 (C) and PC-9 (D) tumors immunohistochemically stained with antibodies against human Ki-67 and subjected to TUNEL assay (Scale bar, 50 μm). Proliferating cells were quantified using the Ki-67-positive proliferation index (percentage of Ki-67-positive cells) in H1650 (E) and PC-9 (F) tumors. $^*P < 0.05$. Data are represented as mean ± S.D. Apoptotic cells were quantified using the percentage of TUNEL-positive (apoptotic) cells in H1650 (G) and PC-9 (H) tumors. $^*P < 0.05$. Data are represented as mean ± S.D. **I** H1650 CDX tumors treated with vehicle (control), osimertinib (5.0 mg/kg), osimertinib plus ONO-7475 (10 mg/kg), or osimertinib plus ONO-7475 plus BGJ398 (15 mg/kg) for 4 days and lysed. The indicated proteins were detected using the western blotting assay.

acquisition, Conceptualization. **Shinsaku Tokuda:** Resources, Methodology. **Kenji Morimoto:** Investigation. **Yuki Katayama:** Investigation. **Yohei Matsui:** Investigation. **Soichi Hirai:** Investigation. **Masaki Ishida:** Investigation. **Hayato Kawachi:** Investigation. **Ryo Sawada:** Investigation. **Yusuke Tachibana:** Investigation. **Atsushi Osoegawa:** Resources. **Mano Horinaka:** Supervision, Resources. **Toshiyuki Sakai:** Supervision, Resources. **Tomoko Yasuhiro:** Resources. **Ryohei Kozaki:** Resources. **Seiji Yano:** Resources. **Koichi Takayama:** Supervision.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

T. Yamada received commercial research grants from Ono Pharmaceutical, Janssen Pharmaceutical K.K., AstraZeneca, and Takeda Pharmaceutical Company Limited and speaking honoraria from Eli Lilly and Chugai-Roche. H. Kawachi received personal fees from Ono Pharmaceutical, Bristol-Myers Squibb, Chugai Pharmaceutical, AstraZeneca, Taiho Pharmaceutical, Eli Lilly Japan, and MSD outside the purview of the submitted work. A. Osoegawa received consultant honoraria from AstraZeneca and speaking honoraria from AstraZeneca, MSD Japan, Chugai Pharmaceutical, Bristol Myers Squibb, and Ono Pharmaceutical. T. Sakai received research grants from Otsuka Pharmaceutical, Taiho Pharmaceutical, and Oncolys BioPharma and a patent fee from JT Pharmaceutical. T. Yasuhiro and R. Kozaki are paid employees of Ono Pharmaceutical. S. Yano received research grants from Chugai-Roche and Boehringer-Ingelheim and speaking honoraria from Amgen, Chugai-Roche, Boehringer-Ingelheim, Novartis, and Pfizer. K. Takayama received research grants from Chugai-Roche and Ono Pharmaceutical and personal fees from AstraZeneca, Chugai-Roche, MSD-Merck, Eli Lilly, Boehringer-Ingelheim, and Daiichi-Sankyo. The other authors have no conflicts of interest to declare.

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Appendix A. Supplementary data

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References

[1] H. Sung, J. Ferlay, R.L. Siegel, M. Laversanne, I. Soerjomataram, A. Jemal, et al., Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, CA A Cancer J. Clin. 71 (2021) 209–249, https://doi.org/10.3322/caac.21660.
[2] D. Fujimoto, H. Ueda, R. Shimizu, R. Kato, T. Otoshi, T. Kawamura, et al., Features and prognostic impact of distant metastasis in patients with stage IV lung adenocarcinoma harboring EGFR mutations: importance of bone metastasis, Clin. Exp. Metastasis 31 (2014) 543–551, https://doi.org/10.1007/s10585-014-9648-3.
[3] G. Han, J. Bi, W. Tan, X. Wei, X. Wang, X. Ying, et al., A retrospective analysis in patients with EGFR-mutant lung adenocarcinoma: is EGFR mutation associated with a higher incidence of brain metastasis? Oncotarget 7 (2016) 56998–57010, https://doi.org/10.18632/oncotarget.10933.
[4] F. Hsu, A. De Caluwe, D. Anderson, A. Nichol, T. Toriumi, C. Ho, EGFR mutation status on brain metastases from non-small cell lung cancer, Lung Cancer 96 (2016) 101–107, https://doi.org/10.1016/j.lungcan.2016.04.004.
[5] S.S. Ramalingam, J. Vansteenkiste, D. Planchard, B.C. Cho, J.E. Gray, Y. Ohe, et al., Overall survival with osimertinib in untreated, EGFR-mutated advanced NSCLC, N. Engl. J. Med. 382 (2020) 41–50, https://doi.org/10.1056/NEJMoa1913662.
[6] S. Yano, T. Yamada, S. Takeuchi, K. Tachibana, Y. Minami, Y. Yatabe, 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. 6 (2011) 2011–2017, https://doi.org/10.1097/JTO.0b013e31823ab0dd.
[7] K.P. Ng, A.M. Hillmer, C.T.H. Chuah, W.C. Juan, T.K. Ko, A.S.M. Teo, et al., A common BIM deletion polymorphism mediates intrinsic resistance and inferior responses to tyrosine kinase inhibitors in cancer, Nat. Med. 18 (2012) 521–528, https://doi.org/10.1038/nm.2713.
[8] S.V. Sharma, D.Y. Lee, B. Li, M.P. Quinlan, F. Takahashi, S. Maheswaran, et al., A chromatin-mediated reversible drug-tolerant state in cancer cell subpopulations, Cell 141 (2010) 69–80, https://doi.org/10.1016/j.cell.2010.02.027.
[9] Y. Pu, L. Li, H. Peng, L. Liu, D. Heymann, C. Robert, et al., Drug-tolerant persister cells in cancer: the cutting edges and future directions, Nat. Rev. Clin. Oncol. 20 (2023) 799–813, https://doi.org/10.1038/s41571-023-00815-5.
[10] H. Taniguchi, T. Yamada, R. Wang, K. Tanimura, Y. Adachi, A. Nishiyama, et al., AXL confers intrinsic resistance to osimertinib and advances the emergence of tolerant cells, Nat. Commun. 10 (2019) 259, https://doi.org/10.1038/s41467-018-08074-0.
[11] N. Okura, N. Nishioka, T. Yamada, H. Taniguchi, K. Tanimura, Y. Katayama, 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. 26 (2020) 2244–2256, https://doi.org/10.1158/1078-0432.CCR-19-2321.
[12] A. Yoshimura, T. Yamada, M. Serizawa, H. Uehara, K. Tanimura, Y. Okuma, et al., High levels of AXL expression in untreated EGFR-mutated non-small cell lung cancer negatively impacts the use of osimertinib, Cancer Sci. 114 (2023) 606–618, https://doi.org/10.1111/cas.15608.
[13] S. Kopetz, A. Grothey, R. Yaeger, E. Van Cutsem, J. Desai, T. Yoshino, et al., Encorafenib, binimetinib, and cetuximab in BRAF V600E-mutated colorectal cancer, N. Engl. J. Med. 381 (2019) 1632–1643, https://doi.org/10.1056/NEJMoa1908075.
[14] J.M. Fernandes Neto, E. Nadal, E. Bosdriesz, S.N. Ooft, L. Farre, C. McLean, et al., Multiple low dose therapy as an effective strategy to treat EGFR inhibitor-resistant NSCLC tumours, Nat. Commun. 11 (2020) 3157, https://doi.org/10.1038/s41467-020-16952-9.
[15] K.J. Kurppa, Y. Liu, C. To, T. Zhang, M. Fan, A. Vajdi, et al., Treatment-induced tumor dormancy through YAP-mediated transcriptional reprogramming of the apoptotic pathway, Cancer Cell 37 (2020) 104–122.e12, https://doi.org/10.1016/j.ccell.2019.12.006.
[16] Y. Katayama, T. Yamada, S. Tokuda, N. Okura, N. Nishioka, K. Morimoto, et al., Heterogeneity among tumors with acquired resistance to EGFR tyrosine kinase inhibitors harboring EGFR-T790M mutation in non-small cell lung cancer cells, Cancer Med. 11 (2022) 944–955, https://doi.org/10.1002/cam4.4504.
[17] R. Nakamura, H. Fujii, T. Yamada, Y. Matsui, T. Yaoi, M. Honda, et al., Analysis of tumor heterogeneity through AXL activation in primary resistance to EGFR tyrosine kinase inhibitors, JTO Clin Res Rep 4 (2023) 100525, https://doi.org/10.1016/j.jtocrr.2023.100525.
[18] A. Osoegawa, T. Hashimoto, Y. Takumi, M. Abe, T. Yamada, R. Kobayashi, et al., Acquired resistance to an epidermal growth factor receptor-tyrosine kinase inhibitor (EGFR-TKI) in an uncommon G719S EGFR mutation, Invest. N. Drugs 36 (2018) 999–1005, https://doi.org/10.1007/s10637-018-0592-y.
[19] S. Raoof, I.J. Mulford, H. Frisco-Cabanos, V. Nangia, D. Timonina, E. Labrot, et al., Targeting FGFR overcomes EMT-mediated resistance in EGFR mutant non-small cell lung cancer, Oncogene 38 (2019) 6399–6413, https://doi.org/10.1038/s41388-019-0887-2.
[20] X. Zhang, O.A. Ibrahimi, S.K. Olsen, H. Umemori, M. Mohammadi, D.M. Ornitz, Receptor specificity of the fibroblast growth factor family. The complete mammalian FGF family, J. Biol. Chem. 281 (2006) 15694–15700, https://doi.org/10.1074/jbc.M601252200.
[21] M. Mossahebi-Mohammadi, M. Quan, J.S. Zhang, X. Li, FGF signaling pathway: a key regulator of stem cell pluripotency, Front. Cell Dev. Biol. 8 (2020) 79, https://doi.org/10.3389/fcell.2020.00079.
[22] V. Rajendran, M.V. Jain, In vitro tumorigenic assay: colony forming assay for cancer stem cells, Methods Mol. Biol. 1692 (2018) 89–95, https://doi.org/10.1007/978-1-4939-7401-6_8.

[23] K.N. Shah, R. Bhatt, J. Rotow, J. Rohrberg, V. Olivas, V.E. Wang, et al., Aurora kinase A drives the evolution of resistance to third-generation EGFR inhibitors in lung cancer, Nat. Med. 25 (2019) 111–118, https://doi.org/10.1038/s41591-018-0264-7.

[24] R. Wang, T. Yamada, K. Kita, H. Taniguchi, S. Arai, K. Fukuda, et al., Transient IGF-1R inhibition combined with osimertinib eradicates AXL-low expressing EGFR mutated lung cancer, Nat. Commun. 11 (2020) 4607, https://doi.org/10.1038/s41467-020-18442-4.

[25] K. Furugaki, T. Fujimura, H. Mizuta, T. Yoshimoto, T. Asakawa, Y. Yoshimura, et al., FGFR blockade inhibits targeted therapy-tolerant persister in basal FGFR1- and FGF2-high cancers with driver oncogenes, npj Precis. Oncol. 7 (2023) 107, https://doi.org/10.1038/s41698-023-00462-0.

[26] Z. Zhang, J.C. Lee, L. Lin, V. Olivas, V. Au, T. LaFramboise, et al., Activation of the AXL kinase causes resistance to EGFR-targeted therapy in lung cancer, Nat. Genet. 44 (2012) 852–860, https://doi.org/10.1038/ng.2330.

[27] H.S. Solanki, E.A. Welsh, B. Fang, V. Izumi, L. Darville, B. Stone, et al., Cell type-specific adaptive signaling responses to KRASG12C inhibition, Clin. Cancer Res. 27 (2021) 2533–2548, https://doi.org/10.1158/1078-0432.CCR-20-3872.

[28] H. Kitai, H. Ebi, S. Tomida, K.V. Floros, H. Kotani, Y. Adachi, et al., Epithelial-to-mesenchymal transition defines feedback activation of receptor tyrosine kinase signaling induced by MEK inhibition in KRAS-mutant lung cancer, Cancer Discov. 6 (2016) 754–769, https://doi.org/10.1158/2159-8290.CD-15-1377.

[29] V. Asati, D.K. Mahapatra, S.K. Bharti, PI3K/Akt/mTOR and Ras/Raf/MEK/ERK signaling pathways inhibitors as anticancer agents: structural and pharmacological perspectives, Eur. J. Med. Chem. 109 (2016) 314–341, https://doi.org/10.1016/j.ejmech.2016.01.012.

[30] M. Javle, S. Roychowdhury, R.K. Kelley, S. Sadeghi, T. Macarulla, K.H. Weiss, et al., Infigratinib (BGJ398) in previously treated patients with advanced or metastatic cholangiocarcinoma with FGFR2 fusions or rearrangements: mature results from a multicentre, open-label, single-arm, phase 2 study, Lancet Gastroenterol Hepatol 6 (2021) 803–815, https://doi.org/10.1016/S2468-1253(21)00196-5.

[31] Y. Wang, D. Becker, Antisense targeting of basic fibroblast growth factor and fibroblast growth factor receptor-1 in human melanomas blocks intratumoral angiogenesis and tumor growth, Nat. Med. 3 (1997) 887–893, https://doi.org/10.1038/nm0897-887.

[32] F. Li, H. Huynh, X. Li, D.A. Ruddy, Y. Wang, R. Ong, et al., FGFR-mediated reactivation of MAPK signaling attenuates antitumor effects of imatinib in gastrointestinal stromal tumors, Cancer Discov. 5 (2015) 438–451, https://doi.org/10.1158/2159-8290.CD-14-0763.

[33] H. Terai, K. Soejima, H. Yasuda, S. Nakayama, J. Hamamoto, D. Arai, et al., Activation of the FGF2-FGFR1 autocrine pathway: a novel mechanism of acquired resistance to gefitinib in NSCLC, Mol. Cancer Res. 11 (2013) 759–767, https://doi.org/10.1158/1541-7786.MCR-12-0652.

[34] K.E. Ware, T.K. Hinz, E. Kleczko, K.R. Singleton, L.A. Marek, B.A. Helfrich, et al., A mechanism of resistance to gefitinib mediated by cellular reprogramming and the acquisition of an FGF2-FGFR1 autocrine growth loop, Oncogenesis 2 (2013) e39, https://doi.org/10.1038/oncsis.2013.4.

[35] K. Azuma, A. Kawahara, K. Sonoda, K. Nakashima, K. Tashiro, K. Watari, et al., FGFR1 activation is an escape mechanism in human lung cancer cells resistant to afatinib, a pan-EGFR family kinase inhibitor, Oncotarget 5 (2014) 5908–5919, https://doi.org/10.18632/oncotarget.1866.

[36] A. Quintanal-Villalonga, S. Molina-Pinelo, C. Cirauqui, L. Ojeda-Márquez, Á. Marrugal, R. Suarez, et al., FGFR1 cooperates with EGFR in lung cancer oncogenesis, and their combined inhibition shows improved efficacy, J. Thorac. Oncol. 14 (2019) 641–655, https://doi.org/10.1016/j.jtho.2018.12.021.

[37] C.V. Dang, MYC on the path to cancer, Cell 149 (2012) 22–35, https://doi.org/10.1016/j.cell.2012.03.003.

[38] R. Beroukhim, C.H. Mermel, D. Porter, G. Wei, S. Raychaudhuri, J. Donovan, et al., The landscape of somatic copy-number alteration across human cancers, Nature 463 (2010) 899–905, https://doi.org/10.1038/nature08822.

[39] G. Xue, H.L. Yan, Y. Zhang, L.Q. Hao, X.T. Zhu, Q. Mei, et al., c-Myc-mediated repression of miR-15-16 in hypoxia is induced by increased HIF-2α and promotes tumor angiogenesis and metastasis by upregulating FGF2, Oncogene 34 (2015) 1393–1406, https://doi.org/10.1038/onc.2014.82.

[40] D.B. Costa, B. Halmos, A. Kumar, S.T. Schumer, M.S. Huberman, T.J. Boggon, et al., BIM mediates EGFR tyrosine kinase inhibitor-induced apoptosis in lung cancers with oncogenic EGFR mutations, PLoS Med. 4 (2007) 1669–1679, https://doi.org/10.1371/journal.pmed.0040315.

[41] M. Marani, D. Hancock, R. Lopes, T. Tenev, J. Downward, N.R. Lemoine, Role of Bim in the survival pathway induced by Raf in epithelial cells, Oncogene 23 (2004) 2431–2441, https://doi.org/10.1038/sj.onc.1207364.

[42] F. Luciano, A. Jacquel, P. Colosetti, M. Herrant, S. Cagnol, G. Pages, et al., Phosphorylation of Bim-EL by ERK1/2 on serine 69 promotes its degradation via the proteasome pathway and regulates its proapoptotic function, Oncogene 22 (2003) 6785–6793, https://doi.org/10.1038/sj.onc.1206792.

[43] R. Ley, K. Balmanno, K. Hadfield, C. Weston, S.J. Cook, Activation of the ERK1/2 signaling pathway promotes phosphorylation and proteasome-dependent degradation of the BH3-only protein, Bim, J. Biol. Chem. 278 (2003) 18811–18816, https://doi.org/10.1074/jbc.M301010200.