Resminostat, a histone deacetylase inhibitor, circumvents tolerance to EGFR inhibitors in EGFR-mutated lung cancer cells with BIM deletion polymorphism

Sachiko Arai1, Shinji Takeuchi1,2, Koji Fukuda1,2, Azusa Tanimoto1, Akihiro Nishiyama1, Hiroaki Konishi2, Akimitsu Takagi3, Hiroyuki Takahashi4, S. Tiong Ong5,6,7,8, and Seiji Yano1,2.

1Division of Medical Oncology, Cancer Research Institute, Kanazawa University, Kanazawa, Japan, 2Nano Life Science Institute, Kanazawa University, Kanazawa, Japan, 3Yakult Central Institute, Yakult Honsha Co., Ltd., Kunitachi, Tokyo, Japan, 4Pharmaceutical business division, Yakult Honsha Co., Ltd., Tokyo, Japan, 5Cancer and Stem Cell Biology Signature Research Program, Duke-NUS Medical School, Singapore, 6Department of Haematology, Singapore General Hospital, Singapore, 7Department of Medical Oncology, National Cancer Centre Singapore, Singapore, 8Department of Medicine, Duke University Medical Center, Durham, NC, United States of America

Abstract: Drug-tolerant cells are mediators of acquired resistance. BIM-intron2 deletion polymorphism (BIM-del) is one of the mechanisms underlying the resistance to epidermal growth factor tyrosine kinase inhibitor (EGFR-TKI)-mediated apoptosis that induces drug tolerance. Here, we investigated whether resminostat, a histone deacetylase inhibitor, circumvents BIM-del-associated apoptosis resistance. The human EGFR-mutated non-small cell lung cancer (NSCLC) cell line PC-9 and its homozygous BIM-del-positive variant (PC-9 i2- / -), established by editing with zinc finger nucleases, were used. In comparison with PC-9 cells, PC-9 BIM i2- / - cells were less sensitive to apoptosis mediated by EGFR-TKIs such as gefitinib and osimertinib. The combined use of resminostat and an EGFR-TKI preferentially induced the expression of the pro-apoptotic BIM transcript containing exon 4 rather than that containing exon 3, increased the level of pro-apoptotic BIM protein (BIMEL), and stimulated apoptosis in vitro. In a subcutaneous tumor model derived from PC-9 BIM i2- / - cells, gefitinib monotherapy decreased tumor size but retained residual lesions, indicative of the presence of tolerant cells in tumors. The combined use of resminostat and gefitinib increased BIMEL protein level and induced apoptosis, subsequently leading to the remarkable shrinkage of tumor. These findings suggest the potential of resminostat to circumvent tolerance to EGFR-TKIs associated with BIM deletion polymorphism. J. Med. Invest. 67: 343–350, August, 2020

Keywords: drug tolerance, BIM polymorphism, EGFR tyrosine kinase inhibitor, lung cancer

INTRODUCTION

Non-small cell lung cancer (NSCLC) with classical epidermal growth factor receptor (EGFR) mutations such as exon 19 deletion and L858R point mutation is highly sensitive to EGFR tyrosine kinase inhibitors (EGFR-TKIs), including the first-generation inhibitor gefitinib and the third-generation inhibitor osimertinib (1, 2). However, almost all patients experience disease recurrence, owing to the acquired resistance to EGFR-TKIs. Recent studies have revealed the presence of a small population of cells that adapts to the initial treatment with EGFR-TKIs as persisters, which form the basis for acquired resistant lesions (3). Elevation of the adaptation mechanism following initial treatment with EGFR-TKIs may allow development of novel initiation therapies to eradicate tumor cells and improve the therapeutic outcome in advanced EGFR-mutated NSCLC by preventing the development of acquired resistance.

The decrease in the activity of BIM, also known as Bcl-2-like protein 11, a pro-apoptotic molecule that belongs to the Bcl-2 family, has been recognized as one of mechanisms underlying the intrinsic resistance or tolerance to EGFR-TKIs. BIM expression upregulation is essential for the induction of apoptosis in NSCLC cells carrying EGFR mutations following treatment with first- to third-generation EGFR-TKIs. On the contrary, low BIM protein level is associated with resistance or tolerance to EGFR-TKIs (4, 5). A functional BIM deletion polymorphism, specifically a 2,903 bp deletion in intron 2, was reported in East Asian individuals (13%–18%) (6) and South American patients with NSCLC (15.7%) (7) and was associated with poor response to EGFR-TKIs (6, 8). Mechanistically, BIM deletion results in the mutually exclusive splicing of exon 3 over the BH3-encoding (pro-apoptotic) exon 4 of the BIM pre-mRNA, leading to the production of an inactive BIM protein isoform (BIMγ). This protein lacks the BH3 domain, and its production results in the reduction in the expression of the pro-apoptotic BIM protein isoform (BIMβ) in EGFR-mutated lung cancer cell lines upon TKI exposure, thereby inducing TKI resistance (6). Several meta-analyses have reported an association between BIM deletion polymorphism and shorter progression-free survival (PFS) in patients with NSCLC carrying EGFR mutations who received either gefitinib or erlotinib (9-14). Therefore, the restoration of BIM activity may be an important strategy to overcome the intrinsic resistance or tolerance to EGFR-TKI in patients with EGFR-mutated NSCLC with BIM deletion.

Histone deacetylase (HDAC) is one of the targets for cancer treatment. For instance, vorinostat (suberoylanilide hydroxamic acid [SAHA]) is a small molecule inhibitor of pan HDACs that targets both class I HDACs (HDAC-1, 2, 3, and 8) and class IIb HDACs (HDAC-6 and 10), causes acetylation of histone proteins, and induces cell differentiation, cell cycle arrest, and apoptosis in several types of tumor cells (15). Vorinostat monotherapy has been approved for cutaneous T-cell lymphoma. We have previously reported that the combination of vorinostat and gefitinib...
could preferentially induce the transcription of the pro-apoptotic exon 4-containing BIM isoform over that of the inactive exon 3-containing isoform, thereby resensitizing the BIM deletion-containing EGFR-mutated NSCLC cell lines to TKIs in vitro and in vivo (19). Based on our preclinical findings, we performed a phase I study, VICTORY-J, in patients with EGFR-mutated NSCLC harboring BIM deletion polymorphism to evaluate the safety of the combined therapy with vorinostat and gefitinib. Vorinostat at 400 mg/day b.i.w.e. combined with gefitinib 250 mg/day was the recommended dose in phase II studies (17). As panobinostat, another pan-HDAC inhibitor, has been recently approved for multiple myeloma (18), newer generations of HDAC inhibitors are currently under development.

Resminostat is a potent inhibitor of class I, IIb, and IV HDACs. It induces hyperacetylation of histone H4 in multiple myeloma cells (19). Safety and efficacy of resminostat were evaluated in various clinical studies for hematopoietic carcinoma, non-small cell lung cancer, biliary tract cancer and Hodgkin’s lymphoma (20-24). Its activity is being evaluated in a clinical trial (25).

In the present study, we examined the effect of resminostat on the susceptibility of EGFR-mutated NSCLC cell lines with BIM deletion polymorphism to an EGFR-TKI in vitro and in vivo.

MATERIALS AND METHODS

Cell lines and reagents

PC-3 cells, derived from a Japanese female patient with NSCLC carrying an exon 19 deletion in EGFR and differing from the prostate cancer cell line PC-3 (ATCC CRL1435), were purchased from the Human Science Research Resource Bank. The NSCLC cell line, PC-9 carrying EGFR mutations, was obtained from the RIKEN Cell Bank (Ibaraki, Japan). PC-9 cells with a homozygous BIM-intron 2 deletion polymorphism (PC-9 BIMdel) were established by editing with zinc finger nuclease, as previously reported (26). PC-9 GXR cells carrying deletions in the EGFR exon 19 and T790M mutation were established at Kanazawa University (Kanazawa, Japan) from PC-9 cell xenograft tumors in nude mice that had acquired resistance to gefitinib (27). PC-3 and the other three cell lines were maintained in Dulbecco’s modified Eagle’s medium (DMEM) and Roswell Park Memorial Institute (RPMI)-1640 medium, respectively, both supplemented with 10% fetal bovine serum (FBS) and antibiotics. Resminostat was produced by Yakult Honsha Co., Ltd (Tokyo, Japan). Gefitinib, osimertinib, and vorinostat were obtained from the RIKEN Cell Bank (Ibaraki, Japan). Gefitinib, osimertinib, and vorinostat were both supplemented with 10% fetal bovine serum (FBS) and Park Memorial Institute (RPMI)-1640 medium, respectively, in Dulbecco’s modified Eagle’s medium (DMEM) and Roswell Park Memorial Institute (RPMI)-1640 medium, respectively. Safety and efficacy of resminostat were evaluated in various clinical studies for hematopoietic carcinoma, non-small cell lung cancer, biliary tract cancer and Hodgkin’s lymphoma (20-24). Its activity is being evaluated in a clinical trial (25). In the present study, we examined the effect of resminostat on the susceptibility of EGFR-mutated NSCLC cell lines with BIM deletion polymorphism to an EGFR-TKI in vitro and in vivo.

Genotype and expression analysis of BIM

Genomic DNA was amplified using a Veriti Thermal Cycler (Applied Biosystems) with REDAccuTag LA DNA Polymerase (Sigma). The PCR amplitcons for the wild-type (4226 bp) and deletion (1323 bp) alleles were separated by agarose gel electrophoresis.

RNA interference

The cells (2 × 10⁵ cells/well) cultured in a medium containing 10% FBS (antibiotic-free) for 24 h were treated with a Stealth RNAi small-interfering RNA (siRNA) against BIM and Stealth RNAi siRNA Negative Control Lo GC (Invitrogen) using Lipofectamine RNAiMAX (Invitrogen) for 48 h.

Western blot analysis

Western blotting was conducted using antibodies against phospho-EGFR (Tyr1068), protein kinase B (Akt) (Ser473), cleaved poly (ADP-ribose) polymerase (PARP), cleaved caspase-3, acetylated histone H3 (Lys27), BIM, and β-actin (Cell Signaling Technology); phospho-extracellular signal-regulated kinase 1/2 (ERK1/2; Thr202/Tyr204), ERK1/2, and EGFR (R&D Systems). Blots were subsequently incubated with horse-radish peroxidase-conjugated secondary antibodies specific to goat or rabbit immunoglobulin G, and the signals were detected by enhanced chemiluminescence using SuperSignal West Dura Extended Duration Substrate (Thermo Fisher Scientific).

Real-time reverse-transcriptase quantitative PCR (RT-qPCR)

Total cellular RNA was extracted from cells using ISOGEN (NIPPON GENE) in accordance with the manufacturer’s instructions. Reverse transcription was performed using SuperScript VILO cDNA synthesis Kit and Master Mix (Invitrogen). Expression of BIM mRNA was quantitatively measured by Viia 7 Real-Time PCR System (Applied Biosystems) using the following primers: BIM exon 2A (forward 5′-ATGGCAAAGCAACCTTCTGATG-3′ and reverse 5′-GGCTCTGTCGTGAGGGGTG-3′) and exon 3 (forward 5′-CAATGGTAGTCATCCTAGAGG-3′ and reverse 5′-GACAAATGTCCTCAGGAAGGG-3′); BIM exon 4 (forward 5′-TTCTATGAGGGCAGCTGAAAC-3′ and reverse 5′-CCTCCTTGCATAGTAAAGCTGTT-3′); and b-actin (forward 5′-GGACTTGGACGAAAGATGAGG-3′ and reverse 5′-AGCAGTGTTGGCGGTACG-3′).

Staining for live cells and dead cells

Cell death induced by the drugs was determined through the use of Fluoroskan Ascent FL (Thermo Fisher Scientific) using the Live or Dead cell viability Assay Kit (AAT Bioquest Inc, Sunnyvale, CA), which detected and quantified living cells with Cellbrite™ Orange at 630 nm and apoptotic cells with Blue™ DCS1 at 420 nm using the microplate reader.

Subcutaneous xenograft models

Male BALB/cAcl-nu/nu mice, aged 5 to 6 weeks, were obtained from CLEA Japan Inc., and subcutaneously injected with cultured tumor cells (5 × 10⁶ cells/0.1 mL) into their flanks. Once the tumor volume reached 100 to 200 mm³, the mice were randomized and treated once daily with gefitinib and/or resminostat. Each tumor was two-dimensionally measured, and the volume was calculated using the following formula: tumor volume (mm³) = 1/2 × length (mm) × width (mm)². The study protocol was approved by the Ethics Committee on the Use of Laboratory Animals and the Advanced Science Research Center, Kanazawa University, Kanazawa, Japan.

RESULTS

Resminostat upregulates BIM expression in EGFR-mutant NSCLC cell lines harboring BIM deletion polymorphism

We first examined the BIM-intron2 deletion polymorphism in EGFR-mutant NSCLC cell lines by PCR. PC-9 cells had wild-type alleles with a PCR product of 4.2 kb. Consistent with a previous report (16, 26), PC-3 cells were heterozygous for BIM deletion polymorphism, as evident from the PCR products of 4.2 kb and 1.3 kb. Western blot analysis revealed the markedly lower
expression of the major proapoptotic BIM protein (BIM<sub>EL</sub>) in PC-3 and PC-9BIM<sup>−/−</sup> cells than in PC-9 cells (Fig. 1A). We have previously reported that the HDAC inhibitor vorinostat upregulates the level of acetylated histone H3 and BIM with the BH3 domain in EGFR-mutated NSCLC cells heterozygous for BIM deletion (16). In line with this observation, we found that vorinostat dose-dependently increased the expression of acetylated histone H3 and BIM with the BH3 domain in PC-9BIM<sup>−/−</sup> cells (Fig. 1B) homozygous for BIM deletion polymorphism as well as in PC-3 cells (Fig. 1C). Under same experimental conditions, resminostat increased the expression of acetylated histone H3 and BIM with the BH3 domain (BIM<sub>EL</sub>, BIM<sub>L</sub>, and BIM<sub>S</sub>) in PC-9BIM<sup>−/−</sup> (Fig. 1D) and PC-3 (Fig. 1E) cells in a dose-dependent manner. These results indicate that resminostat could upregulate the expression of the pro-apoptotic BIM protein in EGFR-mutated NSCLC cells that are either heterozygous or homozygous for BIM deletion polymorphism.

Resminostat upregulates BIM expression and efficiently induces apoptotic proteins in combination with EGFR-TKIs

We investigated whether the addition of resminostat to EGFR-TKIs results in the induction of apoptosis of EGFR-mutant NSCLC cells homozygous for BIM deletion polymorphism. In parental PC-9 cells, gefitinib inhibited the phosphorylation of EGFR and the downstream molecules (AKT and ERK), increased the expression of the major pro-apoptotic BIM isoform (BIM<sub>EL</sub>), and induced the expression of apoptotic markers (cleaved caspase-3 and cleaved PARP) (Fig. 2A). On the contrary, while gefitinib inhibited the phosphorylation of EGFR and downstream molecules (AKT and ERK), it had minimal effects on the expression of BIM<sub>EL</sub> and apoptotic markers in PC-9BIM<sup>−/−</sup> cells (Fig. 2A). However, the combination of resminostat and gefitinib markedly increased the expression of BIM<sub>EL</sub> as well as the apoptosis markers cleaved caspase-3 and cleaved PARP (Fig. 2A).

Figure 1. Resminostat upregulates BIM expression in EGFR-mutant NSCLC cell lines harboring BIM deletion polymorphism

A. top, PCR products from three EGFR-mutated NSCLC cell lines generated by primers flanking the deletion regions. The PCR products of 4.2 and 1.3 kb correspond to the alleles without and with deletion, respectively, in the presence of both products, indicating the heterozygosity of the deletion polymorphism. Bottom, the level of the expression of the products BIM<sub>EL</sub>, BIM<sub>L</sub>, and BIM<sub>S</sub> in each line B-E, PC-9BIM<sup>−/−</sup> cells and PC-3 cells were incubated with serial dilutions of vorinostat (B, C) or resminostat (D, E) for 24 h. The cell lysates were harvested and the indicated proteins were analyzed by western blotting.

Figure 2. Resminostat upregulates BIM expression and efficiently induces pro-apoptotic markers in combination with EGFR-TKIs

A. PC-9 and PC-9BIM<sup>−/−</sup> cells were incubated with gefitinib (1 µM) and/or resminostat (5 µM) for 12 h. The cell lysates were harvested and the indicated proteins were analyzed by western blotting. B. PC-9 GXR and PC-9BIM<sup>−/−</sup>GR cells were incubated with osimertinib (1 µM) and/or resminostat (5 µM) for 12 h. The cell lysates were harvested and the indicated proteins were determined by western blotting.
Similar results were obtained when resminostat was combined with the third-generation EGFR-TKI osimertinib (Fig. 2B).

In PC-9 GXR cells with EGFR-T790M mutation, osimertinib inhibited the phosphorylation of EGFR and the downstream molecules AKT and ERK, increased the level of BIMm, and induced the expression of cleaved caspase-3 and cleaved PARP. PC-9BIMisoGR cells were positive for EGFR-T790M and resistant to gefitinib, and were derived following continuous exposure to gefitinib. In PC-9BIMisoGR cells, osimertinib inhibited the phosphorylation of EGFR and the downstream molecules but minimally increased the expression of BIMm and apoptotic markers (Fig. 2C). However, the combination of resminostat and osimertinib increased the expression of BIMm as well as the apoptosis markers cleaved caspase-3 and cleaved PARP (Fig. 2C).

We further evaluated whether resminostat combined with EGFR-TKI induced apoptosis by cytochemical staining for live cells (Cellbrite™ Orange-positive) and apoptotic cells (BlueTM DCS1-positive). Gefitinib remarkably increased the number of apoptotic cells in PC-9 cells but not PC-9BIMiso- cells. Under these experimental conditions, gefitinib combined with resminostat discernibly increased the number of apoptotic cells in PC-9BIMiso- cells (Fig. 3). These results indicate that resminostat upregulates BIM expression and efficiently induces apoptotic in combination with EGFR-TKIs.

**Resminostat induces apoptosis of PC-9BIMiso- cells through the upregulation of BIM expression**

To investigate whether the induction of apoptosis with the combined use of resminostat and EGFR-TKIs relies on the expression of BIM protein, PC-9BIMiso- cells were transfected with BIM-specific siRNA and then treated with resminostat and gefitinib for 48 h. We found that the knockdown of BIM protein expression mediated by BIM-specific siRNA resulted in the abrogation of apoptosis, as evident from the absence of cleaved PARP in resminostat- and gefitinib-treated PC-9BIMiso- cells (Fig. 4A). Analysis of BIM transcripts revealed that resminostat alone induced BIM mRNA expression, and this effect was enhanced in the presence of gefitinib. Moreover, the combination of resminostat and gefitinib preferentially induced the expression of the transcripts containing exon 4 over those containing exon 3 (Fig. 4B, C). These findings suggest that the combined use of resminostat and EGFR-TKIs results in the upregulation of the expression of the pro-apoptotic BIM protein and induction of apoptosis of EGFR-mutated NSCLC cells homozygous for BIM deletion polymorphism in vitro.

**Resminostat combined with gefitinib regresses the growth of the tumors derived from EGFR-mutated NSCLC cells with homozygous BIM deletion polymorphism in vivo**

We next examined the effect of the combination of gefitinib and resminostat on EGFR-mutated NSCLC cells homozygous for BIM deletion polymorphism in vivo. PC-9 and PC-9BIMiso- cells were subcutaneously implanted in mice to produce tumors. The mice were then treated with gefitinib, resminostat, or the combination of both. Gefitinib alone markedly reduced the volume of the xenograft tumor induced by PC-9 cells (Fig. 5A). Although gefitinib monotherapy prevented the enlargement of the tumor produced by PC-9BIMiso- cells harboring homozygous BIM-intron2 deletion...
Figure 4. Resminostat induces apoptosis of PC-9BIM<sup>+</sup> cells through the upregulation of BIM expression
A, PC-9BIM<sup>+</sup> cells were transfected with BIM or control siRNA for 24 h before gefitinib (1 µM) and resminostat (3 µM) treatment for 48 h. B, PC-9BIM<sup>+</sup> cells were treated with gefitinib (1 µM) and/or resminostat (3 µM) for 12 h. The levels of various transcripts containing exon 2A, 3, or 4 are expressed after normalization to actin level. C, Ratio of exon 3-containing transcripts to exon 4-containing transcripts in PC-9BIM<sup>+</sup> cells after treatment with each compound.

Figure 5. Resminostat combined with gefitinib regresses the growth of the tumors derived from EGFR-mutated NSCLC cells with homozygous BIM deletion polymorphism in vivo
The antitumor activity of gefitinib and/or resminostat in mouse xenograft models of PC-9 and PC-9BIM<sup>+</sup> tumors. Nude mice bearing established tumors with PC-9 (A) or PC-9BIM<sup>+</sup> (B) cells were treated with 25 mg/kg gefitinib and/or 60 mg/kg resminostat once daily for 15 days. Tumor volume was measured using calipers on the indicated days. Mean ± SE of tumor volumes are shown for groups of 6 to 7 mice. C, The body weight of mice examined in A and B was evaluated. D, Tumors were harvested 4 h after four consecutive treatments with each compound, and the levels of protein in tumor lysates were detected by western blotting.
polymorphism, we failed to observe complete tumor regression. This observation indicates the tolerance of PC-9BIMi2/+ cells to gefitinib in vivo. Resminostat monotherapy, on the contrary, slightly inhibited tumor growth but markedly reduced the tumor volume in combination with gefitinib (Fig. 5D). None of the mice treated with these agents showed any macroscopic adverse effects, including loss of body weight (Fig. 5C). To clarify the mechanisms underlying the effect of resminostat and gefitinib in vivo, we performed western blot analysis on tumor lysates. As a result, we found that gefitinib induced the cleavage of caspase-3 in PC-9 tumors. In PC-9BIMi2/+ tumors, treatment with gefitinib or resminostat had no effect on caspase-3 cleavage, which was increased along with BIM expression following the combination treatment with both agents (Fig. 5D). These findings indicate that the combination of resminostat and gefitinib increases BIM protein expression and induces tumor cell apoptosis, resulting in the shrinkage of the tumors produced by EGF-R-mutated NSCLC cells carrying homozygous BIM deletion polymorphism.

DISCUSSION

Drug tolerant cells, also called as drug persisters or minimal residual lesions at dormant state, serve as a reservoir for the development of acquired resistance (3). Tolerance to EGFR-TKIs is mediated by several mechanisms, including activation of insulin-like growth factor receptor 1 (IGF-1R) (3), AXL (27), and is mediated by several mechanisms, including activation of in-histone and non-histone protein, and their profile of interacting proteins tends to be different for different HDAC inhibitors, and it was not depended on a class of HDAC inhibitor (31). It was also reported that mechanisms of antitumor effect of HDAC inhibitors may be different and depend on HDAC inhibitors (32). We speculated that the difference of the profile of interacting proteins may contribute to a difference of safety and efficacy profile of HDAC inhibitors. Therefore, we examined resminostat efficacy as a new HDAC inhibitor other than vorinostat that we previously reported the efficacy.

We have previously conducted investigator initiated trials for BIM deletion/EGFR mutation double-positive NSCLC patients to determine the safety of vorinostat-gefitinib combination and evaluate the pharmacodynamic biomarkers of vorinostat activity (17). We failed to notice any dose-limiting toxicity, and proposed 400 mg vorinostat as the recommended phase II dose. Although this was a phase I study with limited number of patients, the median PFS was 5.2 months (95% confidence interval 1.4–15.7); the disease control rate at 6 weeks was 83.3% (10/12) in a previous heavily-treated patient population (17). Moreover, the analysis of peripheral blood mononuclear cells revealed that vorinostat preferentially induced the expression of BIM mRNA containing exon 4 over that containing exon 3, acetylated histone H3 protein, and pro-apoptotic BIMi2 protein in 11/11, 10/11, and 5/11 patients, respectively (17). These data indicate that the BIM mRNA exon3/exon4 ratio in PBMCs may be a useful pharmacodynamic marker for treatment (17). As osimertinib is recognized as one of standard first-line treatment for EGFR-muta-
tated NSCLC (2), the use of the combination of osimertinib and new-generation HDAC inhibitors, including resminostat, along with the monitoring of this pharmacodynamic marker may be worth testing in BIM deletion polymorphism-positive EGFR-mu-
tated NSCLC.

In conclusion, resminostat with HDAC3 inhibitory activity could preferentially induce the expression of the pro-apoptotic BIM transcript containing exon 4 rather than the BIM transcript containing exon 3; it also increased pro-apoptotic BIM protein (BIMi2) level and stimulated apoptosis in vitro. In addition, the combined use of resminostat and gefitinib increased BIMi2 protein level and induced apoptosis, thereby leading to a remarkable shrinkage of tumors produced by EGF-R-mutated NSCLC with BIM deletion polymorphism. These findings suggest the potential application of resminostat to circumvent tolerance to EGFR-TKIs associated with BIM deletion polymorphism.

DETAILED CONFLICT OF INTEREST STATEMENTS

S. Yano obtained commercial research grants from Yakult Honsha, Chugai Pharmaceutical, Boehringer-Ingelheim, Novartis, and has received speaking honoraria from AstraZeneca, Chugai Pharmaceutical, Boehringer-Ingelheim, Novartis, and Pfizer. H. Konishi, A. Takagi, and H. Takahashi is an employee of Yakuruto Honsha, Co., Ltd. No potential conflicts of interest were disclosed by the other authors.

ACKNOWLEDGEMENTS

This work was supported by JSPS KAKENHI Grant Number 19H03665 (to S. Yano), 19K16738 (to S. Arai), and 17K09649 (to S. Takeuchi).

REFERENCES

1. Maemondo M, Inoue A, Kobayashi K, Sugawara S, Oizumi

S. Arai, et al. Resminostat in EGFR-mutated lung cancer
S, Isobe H, Gemma A, Harada M, Yoshizawa H, Kinoshita I, Fujita Y, Okinaga S, Hirano H, Yoshimori K, Harada T, Ogura T, Ando M, Miyazawa H, Tanaka T, Saito J, Hagimura K, Morita S, Nukiwi T; North-East Japan Study Group: Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. N Engl J Med 362: 2390-2398, 2010

2. Soria JC, Ohe Y, Vansteenkiste J, Reungwetwattana T, Chewaskulyong B, Lee KH, Dechakunpluk A, Imamura F, Nomami N, Kurata T, Okamoto I, Zhou C, Cho BC, Cheng Y, Cho EK, Voon PJ, Planchard D, Su WC, Gray JE, Lee SM, Hodge R, Marotti M, Rukazenkov Y, Ramalingam SS; FLAURA Investigators: Osimertinib in untreated EGFR-mutated advanced non-small-cell lung cancer. N Engl J Med 378: 113-125, 2018

3. Sharma SV, Lee DY, Li B, Quinlan MP, Takahashi F, Maheswaran S, McDermott U, Azizian N, Zou L, Fischbach MA, Wong KK, Brandstetter K, Wittner B, Ramaswamy S, Classon M, Settlur J: A chromatin-mediated reversible drug-tolerant state in cancer cell subpopulations. Cell 141: 69-80, 2010

4. Faber AC, Corcoran RB, Ebi H, Sequist LV, Waltsman BA, Chung E, Inacio J, Dimangurthy SR, Pollack SF, Song Y, Muzikansky A, Lifshits E, Roberge S, Coffman EJ, Benes CH, Gómez HL, Basgel J, Arteaga CL, Rivera MN, Dias-Santagata D, Jain RK, Engelmann JA: BIM expression in treatment-naïve cancers predicts responsiveness to kinase inhibitors. Cancer Discov 1: 352-365, 2011

5. Costa C, Molina MA, Drozdowskyj A, Giménez-Capitán A, Bertran-Alamillo J, Karachaliou N, Gervais R, Massuti B, Wei J, Moran T, Majem F, Felip E, Carcereny E, García-Campello R, Viteri S, Taron M, Ono M, Giannikopoulos P, Bivona T, Rosell R: The impact of EGFR T790M mutations and BIM mRNA expression on outcome in patients with EGFR-mutant NSCLC treated with erlotinib or chemotherapy in the randomized phase III EURTAC trial. Clin Cancer Res 20: 2001-2010, 2014

6. Ng KP, Hillmer AM, Chunh CT, Juan WC, Ko TK, Tsao AS, Arriaratne PN, Takahashi N, Sawada K, Foi Y, Soh S, Lee WH, Huang JW, Allen JC Jr, Woo XY, Nagarajan N, Kumar V, Thalamuthu A, Poh WT, Ang AL, Mya HT, How GY, Yang LY, Koh LP, Chowby B, Chang CT, Nadarajan VS, Chng WJ, Than H, Lim LC, Goh YT, Zhang S, Poh D, Tan P, Seet JE, Ang MK, Chau NM, Ng QS, Tan DS, Soda M, Isobe K, Nöthen MM, Wong TY, Shahab A, Ruan X, Cacheux-Rataboul V, Sung WK, Tan EH, Yatabe Y, Mano H, Sook RA, Chin TM, Lim WT, Ruan Y, Ong ST: A common BIM deletion polymorphism mediates intrinsic resistance and inferior responses to tyrosine kinase inhibitors in cancer. Nat Med 18: 528-534, 2012

7. Cardona AF, Rojas L, Wills B, Arrieta O, Carranza H, Vargas VS, Chng WJ, Than H, Lim LC, Goh YT, Zhang S, Ruan X, Cacheux-Rataboul V, Sung WK, Tan EH, Yatabe Y, Mano H, Sook RA, Chin TM, Lim WT, Ruan Y, Ong ST: A common BIM deletion polymorphism mediates intrinsic resistance and inferior responses to tyrosine kinase inhibitors in cancer. Nat Med 18: 528-534, 2012

8. Isobe K, Hata Y, Tochigi N, Kaburaki K, Kobayashi H, Makino T, Otsuka H, Sato F, Ishida F, Kikuchi N, Hirota N, Sato K, Sano G, Sugino K, Sakamoto S, Takai Y, Shibuya K, Iyoda A, Homma S: Clinical significance of BIM deletion polymorphism in non-small-cell lung cancer with epidermal growth factor receptor mutation. J Thorac Oncol 9: 483-487, 2014

9. Ying HQ, Chen J, He BS, Pan YQ, Wang F, Deng QW, Sun HL, Liu X, Wang SK: The effect of BIM deletion polymorphism on intrinsic resistance and clinical outcome of cancer patient with kinase inhibitor therapy. Sci Rep 5: 11348, 2015

10. Ma JY, Yan HJ, Gu W: Association between BIM deletion polymorphism and clinical outcome of EGFR-mutated NSCLC patient with EGFR-TKI therapy: A meta-analysis. J Cancer Res Ther 11: 397-402, 2015

11. Huang WF, Liu AH, Zhao HJ, Dong HM, Liu LY, Cai SX: BIM gene polymorphism lowers the efficacy of EGFR-TKIs in advanced nonsmall cell lung cancer with sensitive EGFR mutations: a systematic review and meta-analysis. Medicine (Baltimore) 94: e1263, 2015

12. Nie W, Tao X, Wei H, Chen WS, Li B: The BIM deletion polymorphism is a prognostic biomarker of EGFR-TKIs response in NSCLC: a systematic review and meta-analysis. Oncotarget 6: 25696-25700, 2015

13. Zou Q, Zhan P, Lv T, Song Y: The relationship between BIM deletion polymorphism and clinical significance of epidermal growth factor receptor-mutated non-small cell lung cancer patients with epidermal growth factor receptor-tyrosine kinase inhibitor therapy: a metaanalysis. Transl Lung Cancer Res 4: 792-796, 2015

14. Su W, Zhang X, Cai X, Peng M, Wang F, Wang Y: BIM deletion polymorphism predicts poor response to EGFR-TKIs in nonsmall cell lung cancer: An updated meta-analysis. Medicine (Baltimore) 98: e14568, 2019

15. Sato A: Vorinostat approved in Japan for treatment of cutaneous T-cell lymphoma: status and prospects. Onco Targets Ther 5: 67-76, 2012

16. Nakagawa T, Takeuchi S, Yamada T, Ebi H, Sano T, Nanjo S, Ishikawa D, Sato M, Hasegawa Y, Sekido Y, Yano S: EGFR-TKI resistance due to BIM polymorphism can be circumvented in combination with HDAC inhibition. Cancer Res 73: 2428-2434, 2013

17. Takeuchi S, Hase T, Shimizu S, Ando M, Hata A, Murakami H, Kawakami T, Nagase K, Yoshimura K, Fujiwara T, Tanimoto A, Nishiyama A, Arai S, Fukuda K, Katakami N, Takahashi T, Hasegawa Y, Ko TK, Ong ST, Yano S: Phase I study of vorinostat with gefitinib in BIM deletion polymorphism/EGFR mutation double-positive lung cancer. Cancer Sci 111: 561-570, 2020

18. Sivaraj D, Green MM, Gasparetto C: Panobinostat for the management of multiple myeloma. Future Oncol. 13: 477-488, 2017

19. Mandl-Weber S, Meinel FG, Jankowsky R, Onducu F, Schmidmaier R, Baumann P: The novel inhibitor of histone deacetylase resminostat (RAS2410) inhibits proliferation and induces apoptosis in multiple myeloma (MM) cells. Br J Haematol 149: 628-639, 2010

20. Bitzer M, Horger M, Giannini EG, Ganten TM, Wörns MA, Siewe JT, Dollinger MM, Gerken G, Schuelen ME, Wege H, Zagonel V, Cillo U, Trevisani F, Santoro A, Montesarchio V, Malek NP, Holzapfel J, Herz T, Amendola AS, Poggero SR, Hauns B, Mais A, Lauer UM, Henning SW, Hentschel B: Resminostat plus sorafenib as second-line therapy of advanced hepatocellular carcinoma - The SHELTER study. J Hepatol 58: 280-288, 2016
22. Tambo Y, Hosomi Y, Sakai H, Nogami N, Atagi S, Sasaki Y, Kato T, Takahashi T, Seto T, Maemondo M, Nokihara H, Koyama R, Nakagawa K, Kawaguchi T, Okamura Y, Nakamura O, Nishio M, Tamura T: Phase I/II study of docetaxel combined with resminostat, an oral hydroxamic acid HDAC inhibitor, for advanced non-small cell lung cancer in patients previously treated with platinum-based chemotherapy. Invest New Drugs 35: 217-226, 2017.

23. Ikeda M, Ohno I, Ueno H, Mitsunaga S, Hashimoto Y, Okusaka T, Kondo S, Sasaki M, Sakamoto Y, Takahashi H, Hara R, Kobayashi S, Nakamura O, Morizane C: Phase I study of resminostat, an HDAC inhibitor, combined with S-1 in patients with pre-treated biliary tract or pancreatic cancer. Invest New Drugs 37: 109-117, 2019.

24. Walewski J, Paszkiewicz-Kozik E, Borsaru G, Hellmann A, Janikova A, Warszewska A, Mais A, Ammendola A, Herz T, Krauss B, Henning SW: Resminostat in patients with relapsed or refractory Hodgkin lymphoma: results of the phase II SAPHIRE study. Leuk Lymphoma 60: 675-684, 2019.

25. Oka T, Miyagaki T: Novel and future therapeutic drugs for advanced mycosis fungoides and Sézary Syndrome. Front Med (Lausanne) 6: 116, 2019.

26. Tanimoto A, Takeuchi S, Arai S, Fukuda K, Yamada T, Roca X, Ong ST, Yano S: Histone deacetylase 3 inhibition overcomes BIM deletion polymorphism-mediated osimertinib resistance in EGFR-mutant lung cancer. Clin Cancer Res 23: 3139-3149, 2017.

27. Taniguchi H, Yamada T, Wang R, Tanimura K, Adachi Y, Nishiyama A, Tanimoto A, Takeuchi S, Araujo LH, Beroni M, Yoshimura A, Shirtsu S, Matsumoto I, Watanabe S, Kikutchi T, Miura S, Tanaka H, Kitazaki T, Yamaguchi H, Muke H, Uchino J, Uehara H, Takayama K, Yano S: AXL confers intrinsic resistance to osimertinib and advances the emergence of tolerant cells. Nat Commun 10(1): 259, 2019.

28. Kurppa KJ, Liu Y, To C, Zhang T, Fan M, Vajdi A, Knelson EH, Xie Y, Lim K, Cejas P, Portell A, Lizotte PH, Ficarro SB, Li S, Chen T, Haikala HM, Wang H, Bahcall M, Gao Y, Shallout S, Boettcher S, Shin BH, Thai T, Wilkins MK, Tillgren ML, Mushiajian M, Xu M, Choi J, Bertram AM, Ebert BL, Beroukhim R, Bandopadhayay P, Awad MM, Gokhale PC, Kirschmeier PT, Marto JA, Camargo FD, Haq R, Pawelez CP, Wong KK, Barbie DA, Long HW, Gray NS, Jannes PA: Treatment-induced tumor dormancy through YAP-mediated transcriptional reprogramming of the apoptotic pathway. Cancer Cell 37: 104-122, 2020.

29. Kitazono S, Fujinami Y, Nakamichi S, Mizugaki H, Nokihara H, Yamamoto N, Yamada Y, Inukai E, Nakamura O, Tamura T: A phase I study of resminostat in Japanese patients with advanced solid tumors. Cancer Chemother Pharmacol 75: 1155-1161, 2015.

30. Mandl-Weber S, Meinel FG, Jankowsky R, Oduncu F, Schmidmaier R, Baumann PM: The novel inhibitor of histone deacetylase resminostat (RAS2410) inhibits proliferation and induces apoptosis in multiple myeloma (MM) cells. Br J Haematol 149: 518-528, 2010.

31. Li W, Zheng Sun Z: Mechanism of action for HDAC inhibitors: insights from omics approaches. Int J Mol Sci 20(7): 1616, 2019.

32. Eckschlager T, Plch J, Stiborova M, Hrabeta J: Histone deacetylase inhibitors as anticancer drugs. Int J Mol Sci 18(7): 1414, 2017.