Supplementary Figure S1. Effects of infigratinib and afatinib on osimertinib resistance.

(A) MTT assays were performed to assess the growth-inhibitory effects of combined treatment for 72 h with infigratinib (1 µM) and increasing osimertinib concentrations in PC9-OR#1 cells (n = 3). The results are expressed as the mean ± SEM. (B) MTT assays were performed to assess the growth-inhibitory effects of combined treatment for 72 h with afatinib (0.03 µM) and increasing osimertinib concentrations in PC9-OR#1, PC9-OR#2 and PC9-OR#3 cells (n = 3). The results are expressed as the mean ± SEM.
Supplementary Figure S2. Analysis of PC9-OR#2 cell lines.

(A) Ras-GTP activity in PC9-OR#2 cells was measured using the Ras Activation Assay Kit. (B) Control and KRAS siRNAs were transfected into parental PC9-ffluc cells and PC9-OR#2 cells. At 24 h post-transfection, the cells were treated with 1 μM osimertinib, and cell viabilities were measured after 72 h via MTT assays (n = 3) (C); after 1 week, the cells were stained with crystal violet and examined visually. (D) Control and KRAS siRNAs were transfected into the resistant clonal cell lines. At 24 h post-transfection, the cells were treated with 1 μM osimertinib for 72 h, after which cell lysates were obtained and analyzed by immunoblotting with the indicated antibodies.
Supplementary Figure S3. Effects of infigratinib and afatinib on osimertinib resistance in PC9-OR#2 clone cells.

(A) The expression levels of pHER2 and HER2 were evaluated by western blot analysis. (B) MTT assays were performed to assess the growth-inhibitory effects of combined treatment for 72 h with infigratinib (1 µM) or afatinib (0.03 µM) and increasing osimertinib concentrations in PC9-OR#2 clone cells (n = 3).
Supplementary Figure S4. Effects of trametinib on osimertinib resistance.

(A) MTT assays were performed to assess the growth inhibition of H1975-KRAS-G12V cells treated with increasing concentrations of osimertinib in combination with 0.03 μM trametinib for 72 h (n = 3). (B) H1975-KRAS-G12V cells were treated with 1 μM osimertinib and/or 0.03 μM trametinib for 72 h and 2 h, respectively. Changes in the expression levels of the indicated proteins were evaluated by western blot analysis.
Supplementary Figure S5. Effects of trametinib on PC9-OR#2 cells.

(A) MTT assays were performed to assess the growth-inhibitory effects of combined treatment for 72 h with trametinib (0.03 μM) and increasing osimertinib concentrations in PC9-OR#2 cells (n = 3). The calculated IC50 values are shown. The results are expressed as the mean ± SEM. (B) PC9-OR#2 cells were treated with 1 μM osimertinib and/or 0.03 μM trametinib for 72 h and 2 h, respectively. Changes in the expression levels of the indicated proteins were assessed by western blot analysis.
Supplementary Figure S6. Effects of trametinib on osimertinib resistance in a mouse model of leptomeningeal carcinomatosis.

(A) PC9-OR#2 cells (4 × 10^5 cells/0.1 mL) were inoculated in the leptomeningeal space of SHO-SCID mice (n = 15). At the beginning of day 8, the mice were orally administered with osimertinib (25 mg/kg) and trametinib (0.6 mg/kg) each day, randomized into four groups (control group, n = 4; trametinib group, n = 4; osimertinib group, n = 4; combination-treatment group; n = 3), and treated daily with trametinib (0.6 mg/kg) and/or osimertinib (25 mg/kg). Luminescence was measured twice per week until the experiment was terminated. The error bars represent the SEM. A two-sided Student’s t-test was used for comparisons between two groups. The threshold for statistical significance was designated as P < 0.05, when compared with the combination-treatment group. (B) Representative luminescence images in the mice and fluorescence in the brain lesions are shown.
Supplementary Figure S7. Immunohistochemistry of brain samples.

Four days following the start of treatment, we stained the cancer cells with hematoxylin and eosin, and performed luciferase staining.