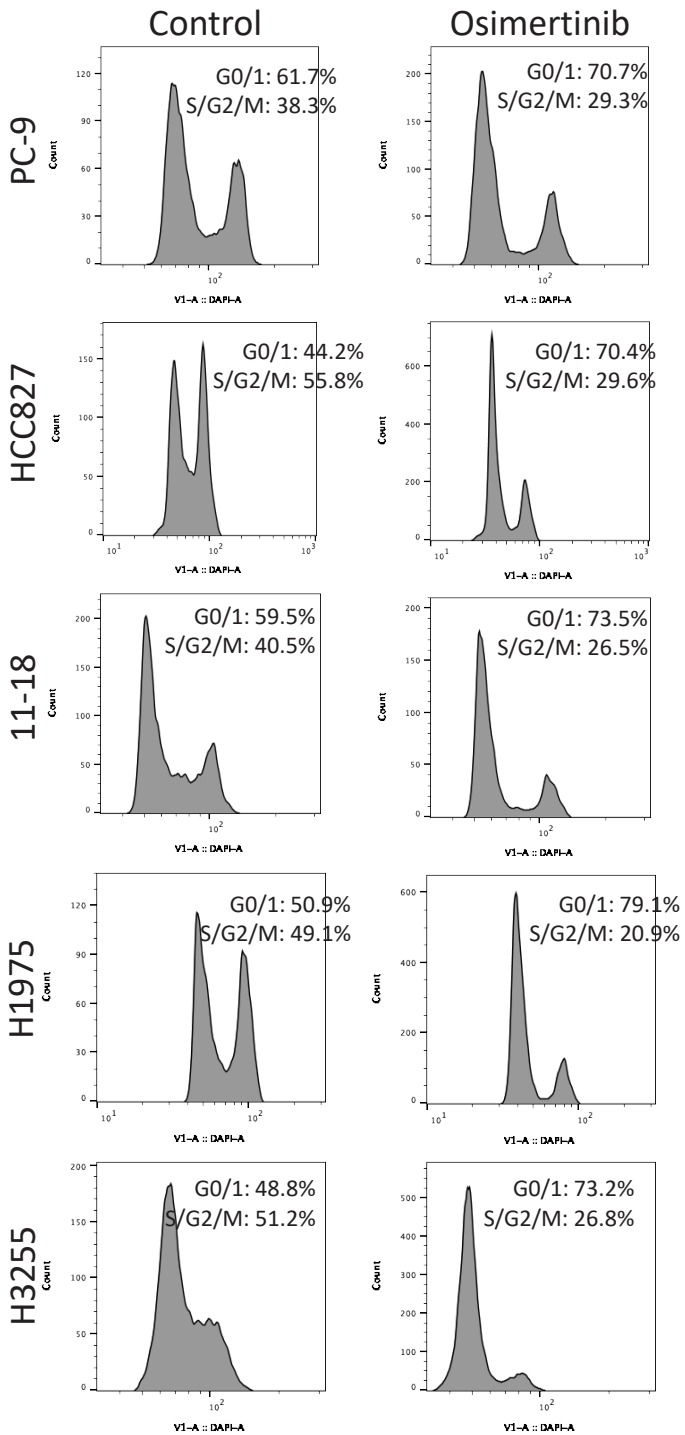


Figure S1 Representative cell cycle histogram in *Figure 1A*. Cell cycle analysis was performed using DAPI staining, followed by flow cytometry. The cells were treated for 24 h with dimethyl sulfoxide or 100 nM osimertinib. DAPI, 4',6-diamidino-2-phenylindole, dihydrochloride.

https://dx.doi.org/10.21037/tlcr-23-99

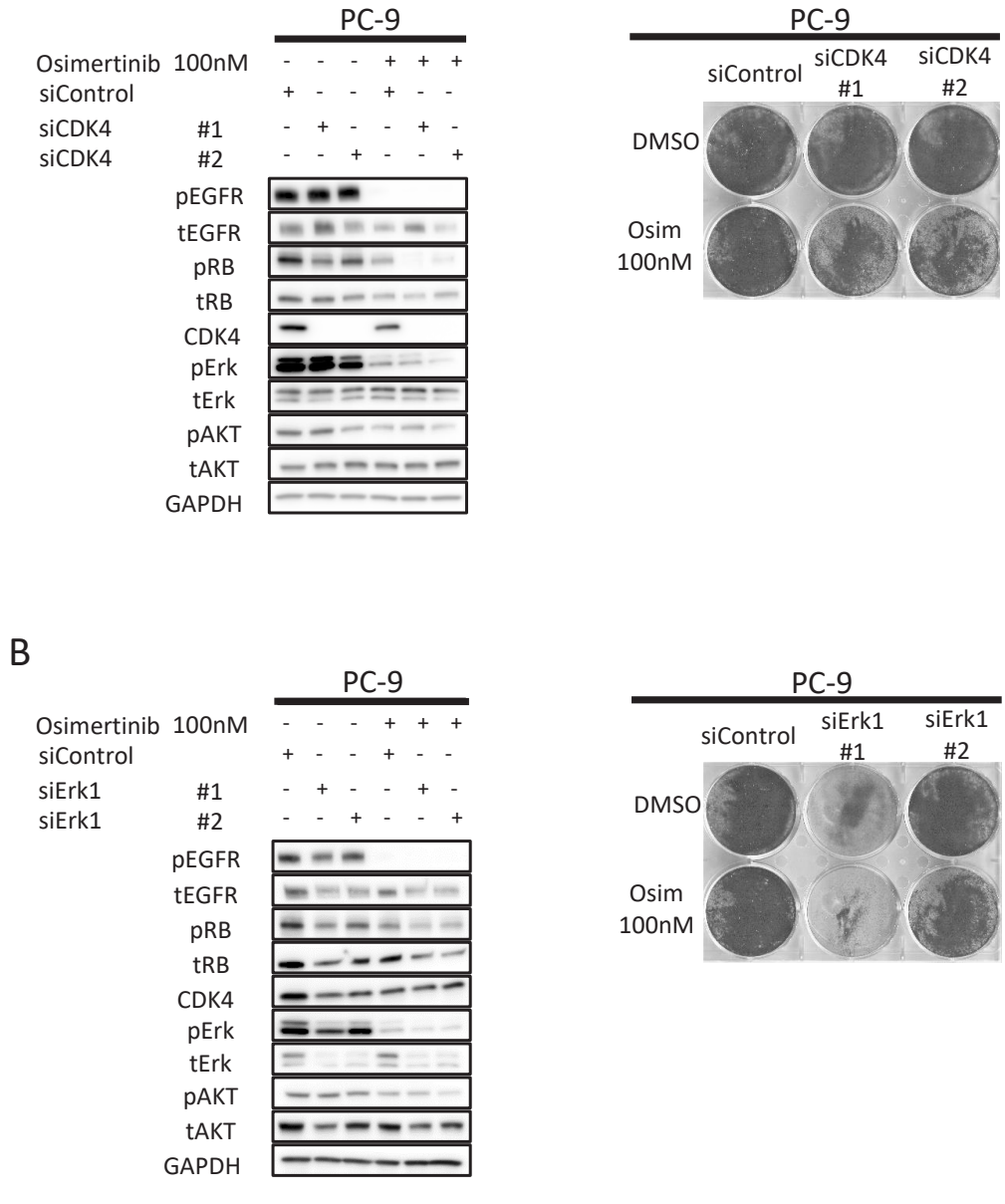


Figure S2 Effects of CDK4 and ERK1 silencing combined with Osim and CDK4/6 inhibitor on oncogenic signaling pathways and cell growth. (A) Two different CDK4-targeting siRNAs were used. Immunoblots of the indicated proteins in PC-9 cells treated with Osim (100 nmol/L) for 24 h. Crystal violet-stained cells after drug loading for 6 days. PC-9 cells were treated with 100 nmol/L Osim (magnification, 1×). (B) Two different ERK1-targeting siRNAs were used. Immunoblots of the indicated proteins in PC-9 cells treated with Osim (100 nmol/L) for 24 h. Crystal violet-stained cells after drug loading for 6 days (magnification, 1×). PC-9 cells were treated with 100 nmol/L Osim. Osim, osimertinib; CDK, cyclin-dependent kinase; p, phosphorylated; t, total; EGFR, epidermal growth factor receptor; RB, retinoblastoma protein; DMSO, dimethyl sulfoxide; siRNA, small interfering RNA.

https://dx.doi.org/10.21037/tlcr-23-99

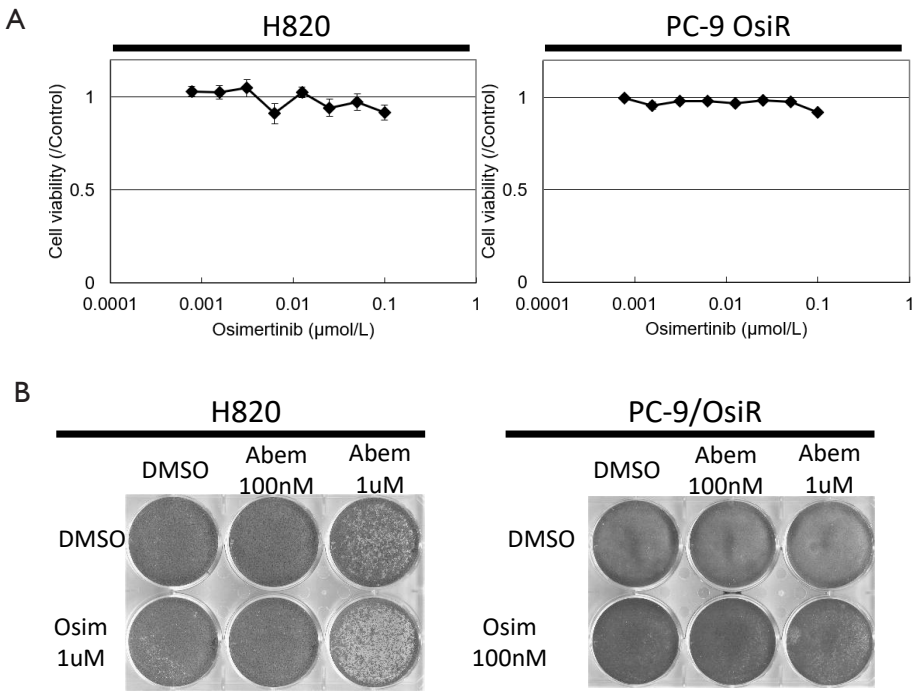
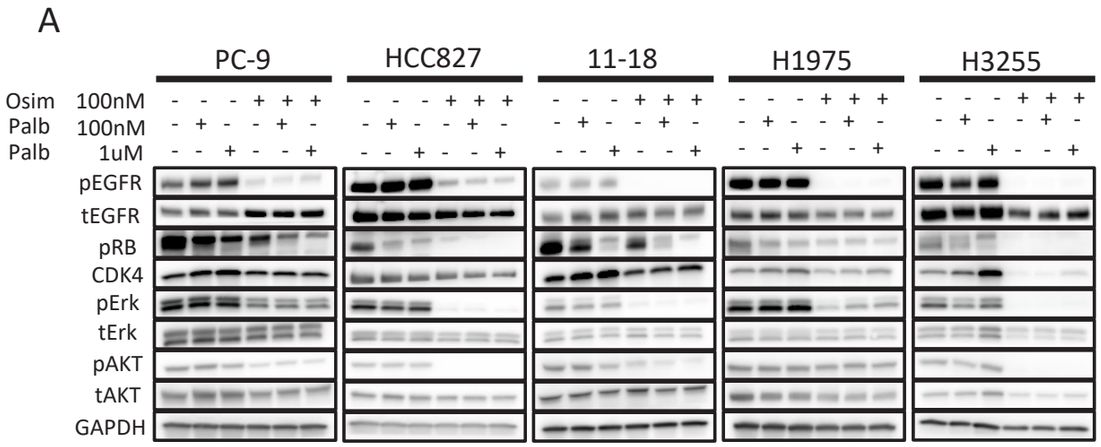
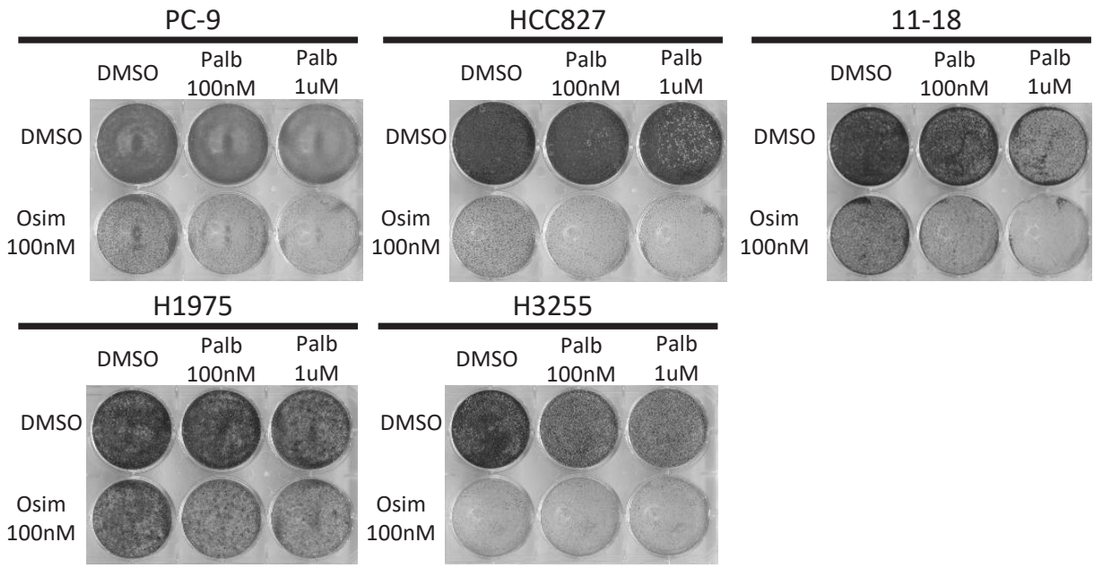
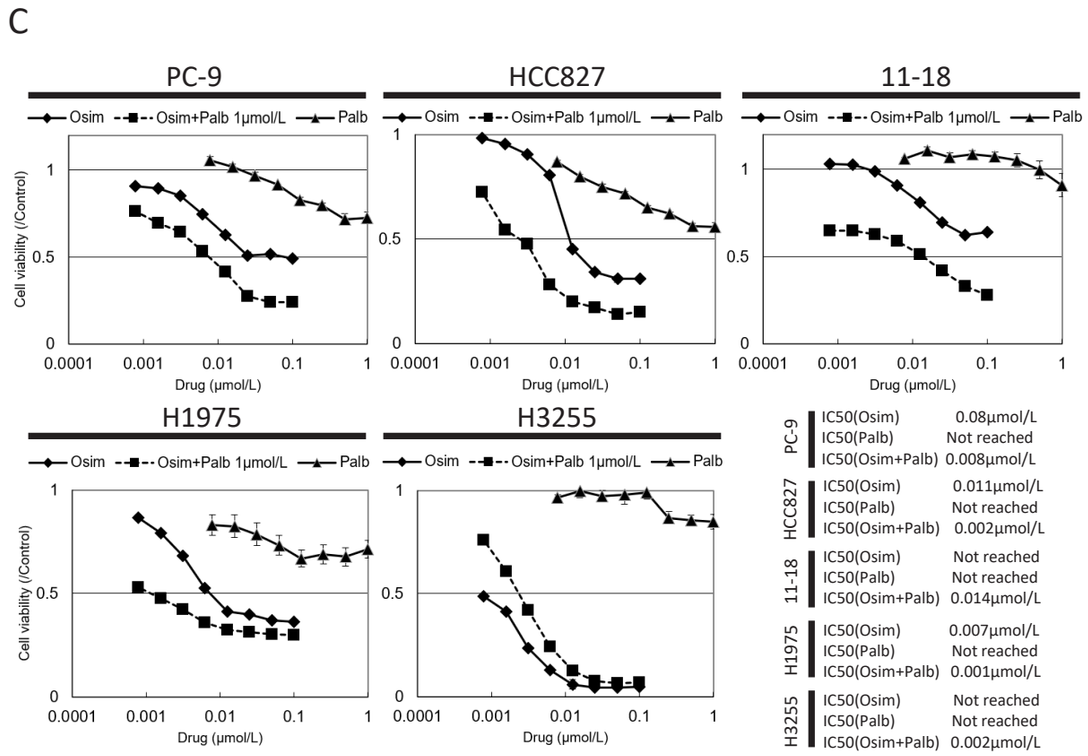


Figure S3 Effect of combination therapy with Abem on cell proliferation in Osim-resistant cell lines. (A) Viability curves for cell lines treated with Osim. Osim was loaded for 96 h. Error bars represent standard error. (B) Crystal violet-stained cells after drug loading for 6 days (magnification, 1×). Cancer cells were treated with 100 nmol/L or 1 μmol/L Abem combined with 100 nmol/L Osim for 6 days. DMSO, dimethyl sulfoxide; Osim, osimertinib; Abem, abemaciclib.

https://dx.doi.org/10.21037/tlcr-23-99







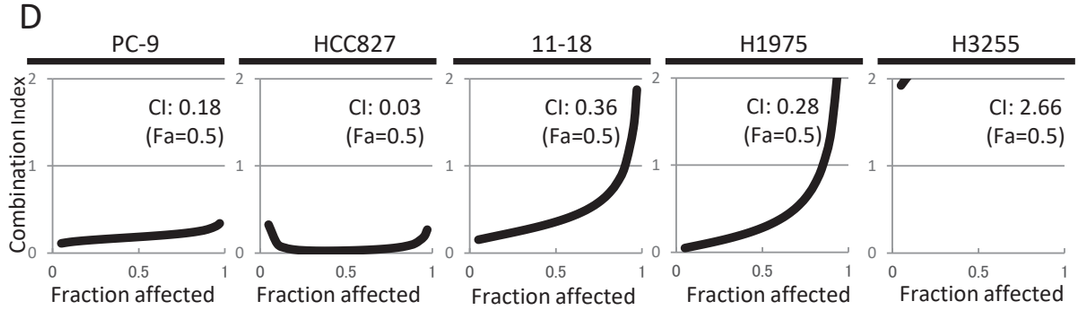


Figure S4 Combined effect of Osim and Palb on the cell cycle, related protein activity, and cell proliferation. (A) Immunoblots of the indicated proteins in PC-9, HCC827, 11-18, H1975, and H3255 cells treated with 100 nmol/L or 1 μmol/L Palb combined with 100 nmol/L Osim for 48 h. (B) Crystal violet-stained cells after drug loading for 6 days (magnification, 1×). Cancer cells were treated with 100 nmol/L or 1 μmol/L Palb combined with 100 nmol/L Osim for 6 days. DMSO was used at a calculated concentration of 0.1%. (C) Cell viability curves for cell lines treated with the Osim alone (rhombuses), Palb alone (triangles), or Osim and Palb (1 μmol/L; squares). All drugs were loaded for 96 h. Error bars represent the standard error. (D) The ratio of the Osim and Palb concentrations was 1:10. Osim, osimertinib; Palb, palbociclib; IC50, 50% inhibition concentration; CI, combination index; Fa, fraction affected; DMSO, dimethyl sulfoxide.

https://dx.doi.org/10.21037/tlcr-23-99