Supplementary Methods

Cell line derivation. MDA-MB-231 (NCI, 01/06), MDA-MB-435 (NCI, 01/06), PC-3 (NCI, 01/06), OVCAR-3 (ATCC, 06/87), A549 (ATCC, 12/02), DU 145 (04/89), NCI-H2122 (ATCC, 10/04), U-87 MG (ATCC, 07/93), ACHN (ATCC, 04/06), MDA-MB-468 (ATCC, 01/88), MCF-7 (ATCC, 03/86), BT-474 (ATCC, 12/87), IGR-OV1 (NCI, 01/06), RL95-2 (ATCC, 08/06), RT4 (ATCC, 08/06), C-33A (ATCC, 10/97), HCC70 (09/06), HCC1954 (ATCC, 09/06), BT-20 (ATCC, 06/90), A2780 (ECACC, 07/04), SCC-4 (ATCC, 10/06), OVCAR5 (NCI, 01/06), SK-OV-3 (ATCC, 09/91), HCT 116 (PFIZER, 01/92), NCI-H292 (ATCC, 11/02), NCI-H460 (ATCC, 04/01), RL (ATCC, 06/08), FaDu (ATCC, 06/05), COLO 205 (ATCC, 05/85), GEO (RPCI, 04/04), 786-0 (ATCC, 11/06), PANC-1 (ATCC, 01/00), HT-1080 (ATCC, 11/96), SW620 (ATCC, 04/90) and NCI-H23 (ATCC, 03/04). Rh1, Rh1mTORrr, Rh30, Rh30/mTORrr and Rh30/Rapa10K cells (Dr Peter Houghton, SJCRH)

Sandwich ELISA assay to measure p-4E-BP1 (T37/46). BT-474 cells were treated with OSI-027 in a concentration-dependent fashion for 2h in a 96-well plate and whole cell lysates were transferred to another plate coated with total 4E-BP1 goat antibody (Santa Cruz Biotechnology, sc-6024) and incubated overnight at 4^{o}C. Phospho-4E-BP1 bound to total 4E-BP1 was detected using p4E-BP1(T37/46) rabbit antibody and anti-rabbit HRP conjugated secondary antibody by chemiluminescence method. Luminescence signals of positive control, negative control and treated samples were used to calculate % inhibition and IC_{50} value.

Sandwich ELISA assay to measure pAKT (S473). BT-474 cells were treated with OSI-027 in a concentration-dependent fashion for 2h in a 96-well plate and whole cell lysates were analyzed for pAKT (S473) using Pathscan Phospho-AKT1 (S473) chemiluminescent ELISA kit from Cell Signaling Technology (cat# 7134). Luminescence signals of positive control, negative control and treated samples were used to calculate % inhibition and IC_{50} value.

Measurement of PK. Female CD-1 mice (5-8 weeks) were obtained from Charles River, and housed in an AALAC accredited animal facility. Animals were dosed with OSI-027 by oral gavage. The dose solutions were prepared by dissolving the sodium salt of OSI-027 in water by sonication, and were administered at 10 mL/kg. Plasma samples at multiple time points were obtained by cardiac puncture following euthanasia, and were analyzed by HPLC-MS/MS (Applied Biosystems 3000 mass spectrometer with Agilent 1100 HPLC) to determine the concentrations of OSI-027. Pharmacokinetic parameters were determined by noncompartmental modeling of the median (n=3) concentration data at each time point.

Supplementary Figure Legends

Supplementary Figure S1. Biochemical selectivity profile of OSI-027 against PI3K related kinases and other kinases. mTOR (native complex) assay was done at 1 mM ATP and all other kinases were tested at 100 μM ATP. mTOR (native complex), mTORC1 (native complex), mTORC2 (native complex) and PI3Kβ assays were done in-house and all other kinases were tested at Invitrogen using their Selectscreen kinase profiling service.

Supplementary Figure S2. Inhibition of pAKT (S473) and pAKT (T308) by OSI-027 in MDSA-MB-231 stimulated with insulin. MDA-MB-231 cells were serum starved overnight and OSI-027 was added to cells at varying concentrations. After 2h incubation cells were growth factor stimulated with 10 ng/mL insulin for 3 to 5 minutes, then rinsed with cold PBS and lysed for western blot analysis

Supplementary Figure S3. Mechanistic cell potency of OSI-027 in Sandwich ELISA assays. IC_{50} value of OSI-027 in BT474 breast cancer cell line for p-4E-BP1 (T37/46) and pAKT (S473) inhibition was determined as described under 'supplementary methods'

Supplementary Figure S4. Inhibition of IGF-1 stimulated pAKT (S473) by OSI-027 and not rapamycin in NCI-H460 NSCLC cells. Serum starved NCI-H460 cells were treated with OSI-027 (20 μM) and rapamycin (20 μM) for 24h and in the presence and absence of IGF-1 (10 ng/mL). Whole cell lysates was analyzed by WB for pAKT (S473).

Supplementary Figure S5. Anti-proliferative effects of rapamycin. Rapamycin-sensitive cell lines (IC_{50} <0.07 μM) were treated with rapamycin for 72h in a dose-dependent manner. Relative cell viabilities are measured using Cell TiterGlo and plotted as % DMSO control using Prism.

Supplementary Figure S6. Mouse PK of OSI-027 at 20 mpk, 100 mpk and 200 mpk. Pharmacokinetic parameters (Cmax and AUC) were determined by noncompartmental modeling of the median (n=3) concentration data at each time point.

Supplementary Figure S7. Western blot analysis of PD markers (p4E-BP1 and pAKT) in tumor lysates from MDA-MB-231 PD study (OSI-027, 60 mpk single dose).

Supplementary Figure S8. (A) PK-PD Correlation of OSI-027 in MDA-MB-231 PD model (OSI-027, 25 mg/kg, single dose) (B) Western blot analysis of PD markers (p4E-BP1 and pAKT) in tumor lysates from MDA-MB-231 PD study (OSI-027, 25 mg/kg, single dose)

Supplementary Figure S9. Western blot analysis of COLO 205 tumor lysates harvested at 8 & 24h after 14 day dosing with OSI-027 (65 mg/kg) or rapamycin (20 mg/kg).