VALIDATION DATA SETS

Triggers:

1. Increase in EGFR Expression
2. Increase in PDGFRA Expression
3. Increased in IGFR Expression
4. An increase in p53 and PTEN mutation
5. Low availability of Oxygen with prevailing hypoxic conditions

STUDY 1: Increase in BCL2 with Disease Progression

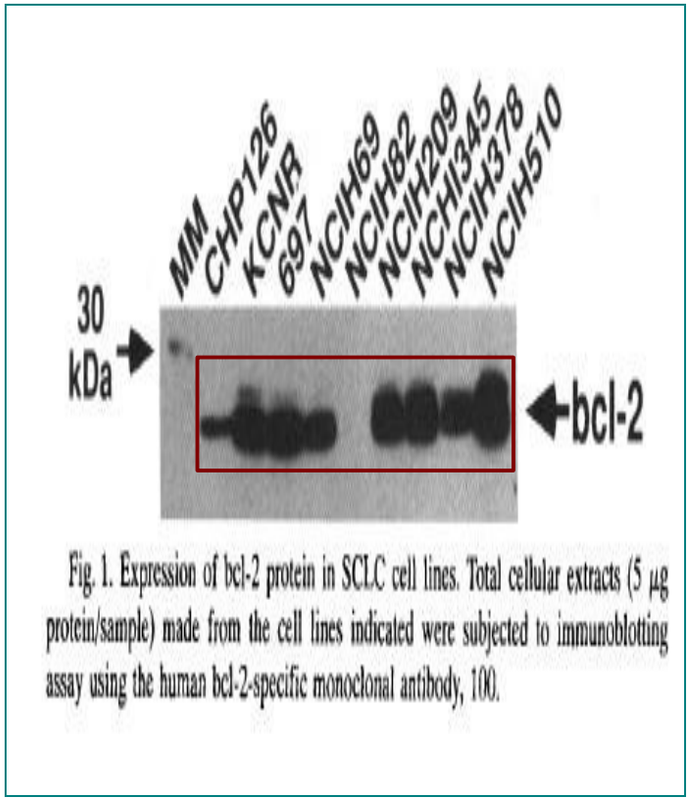
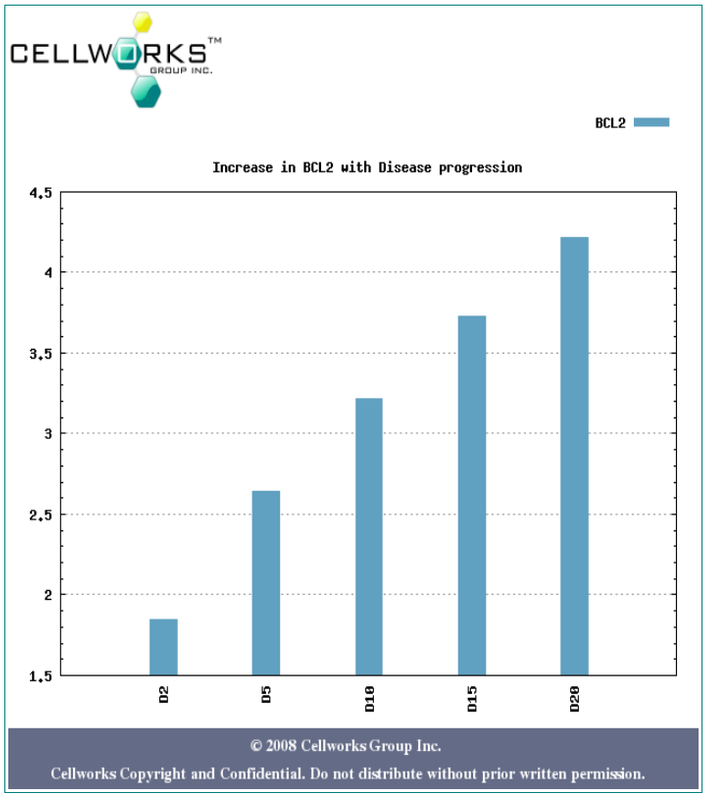


Fig. 1. Expression of bcl-2 protein in SCLC cell lines. Total cellular extracts (5 μg protein/sample) made from the cell lines indicated were subjected to immunoblotting assay using the human bcl-2-specific monoclonal antibody, 100.

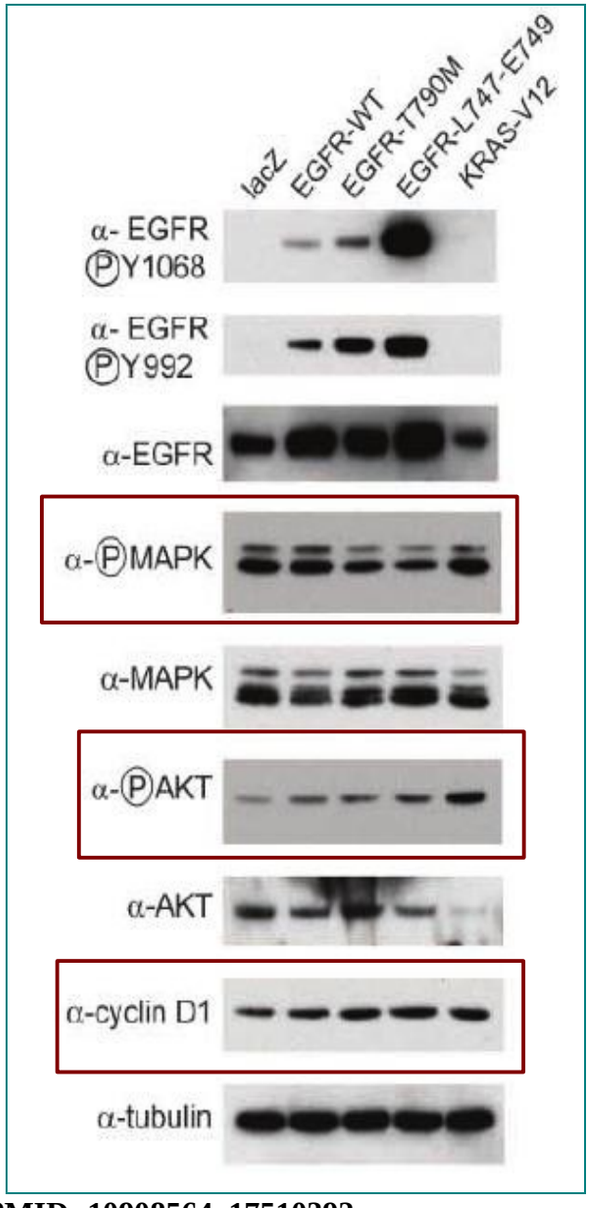


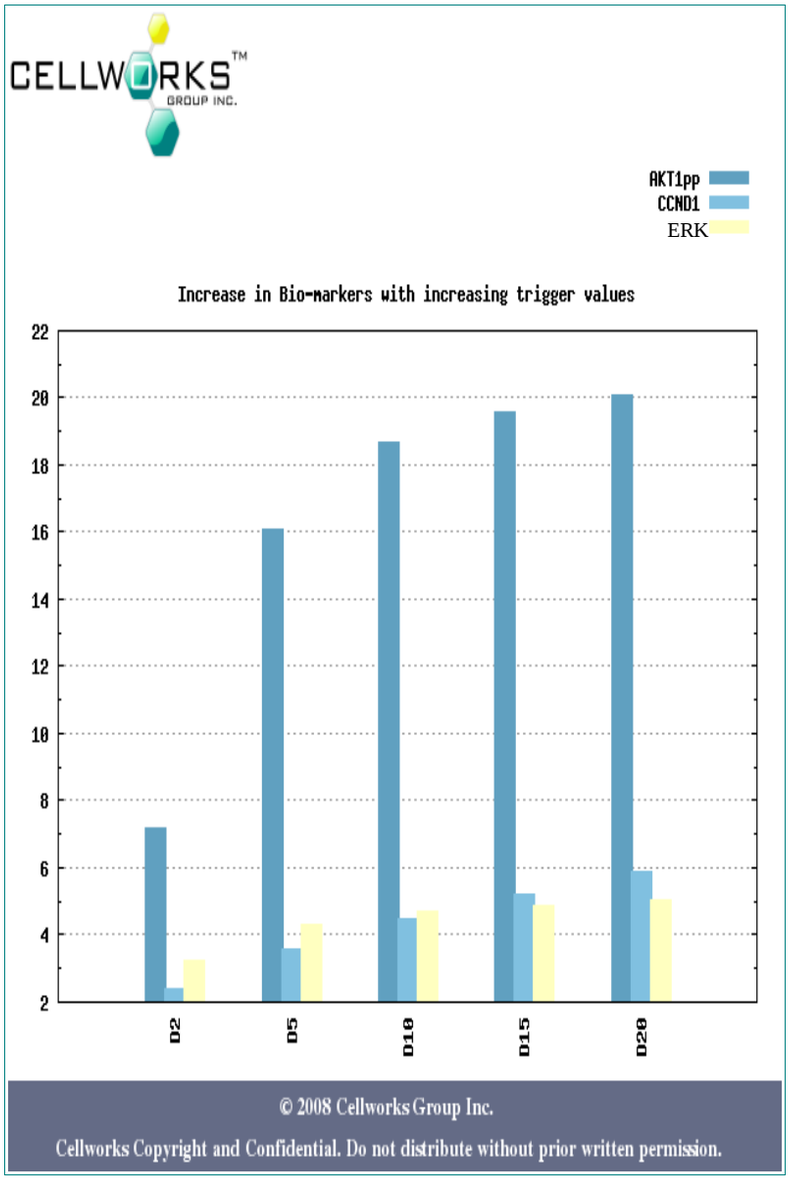
PMID 8261463

Experimental Information: The above plot shows an increase in BCL2 expression in Cancer cell lines.

Virtual Study Result: the above bar graph shows an increase in BCL2 levels with increasing trigger values

STUDY 2: Increase in AKT, CCND1 and ERK with Disease Progression



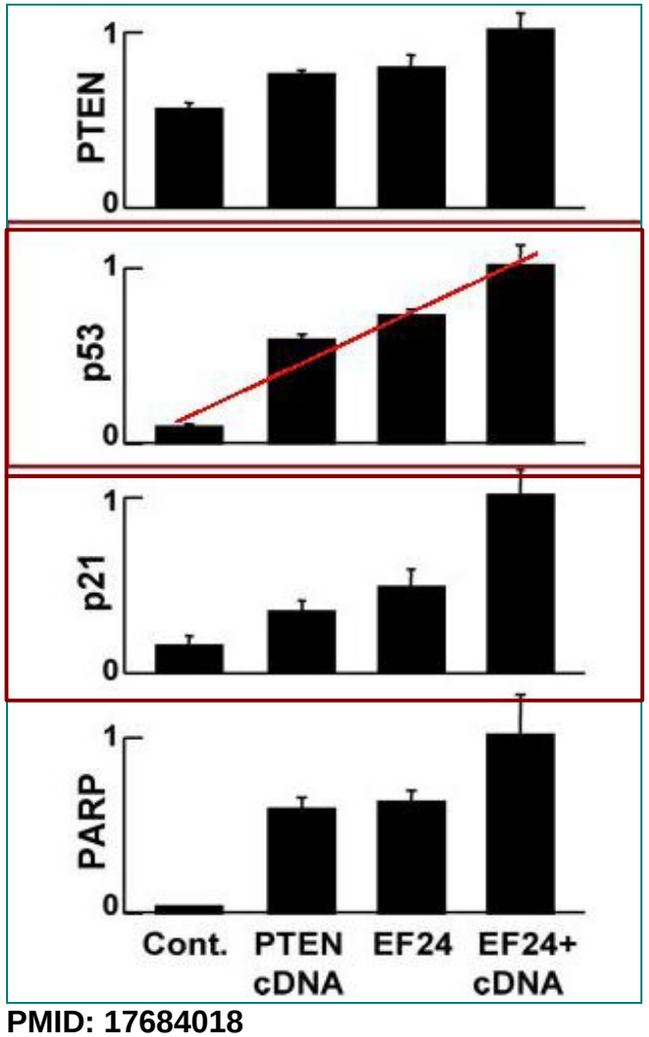


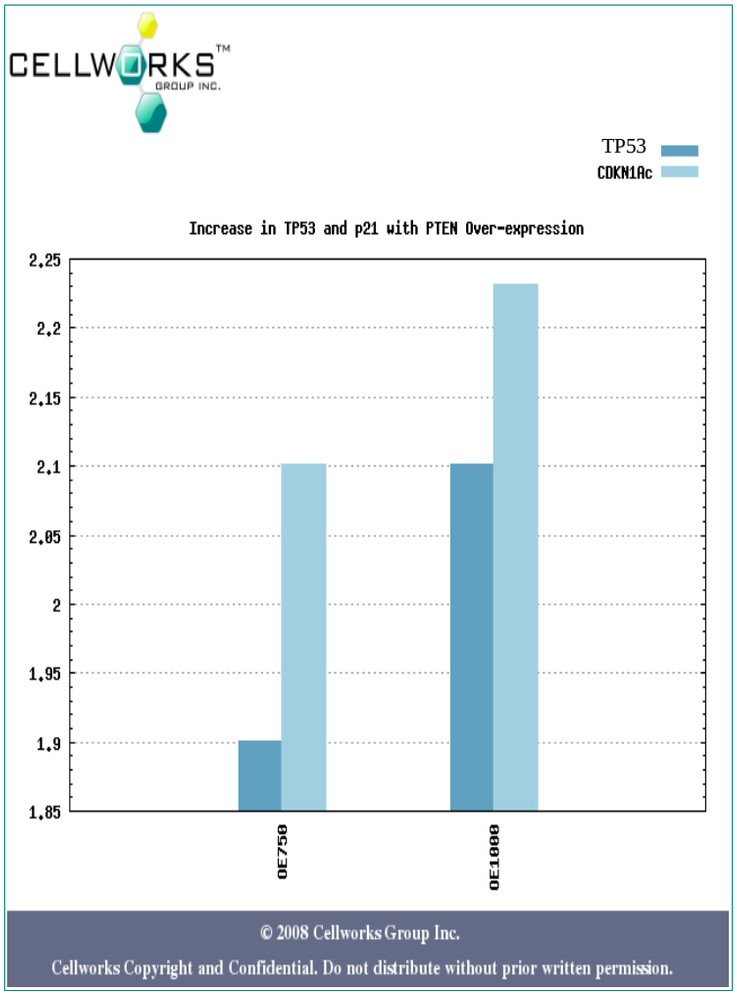
PMID: 10908564, 17510392

Experimental Information: Colony formation growth assay for EGFR-T790M. Viral transduction and selection of HBEC cells stably expressing EGFR were done. Cells (200) were plated in triplicate and grown in keratinocyte serum-free medium (with 50 Ag/mL bovine pituitary extract and without EGF). After 10 d, cells were stained with methylene blue. Western blots for EGFR,EGFR-Y1068, EGFR-Y992, MAPK, MAPK (T202/Y204), AKT, AKT (S473), cyclin D1, and a-tubulin in the HBEC stable cell lines were done as indicated.

Virtual Study Result: The above plot shows an increase in AKT, CCND1 and ERK with increasing trigger values

STUDY 3: Increase in TP53 and p21 with PTEN Over-expression



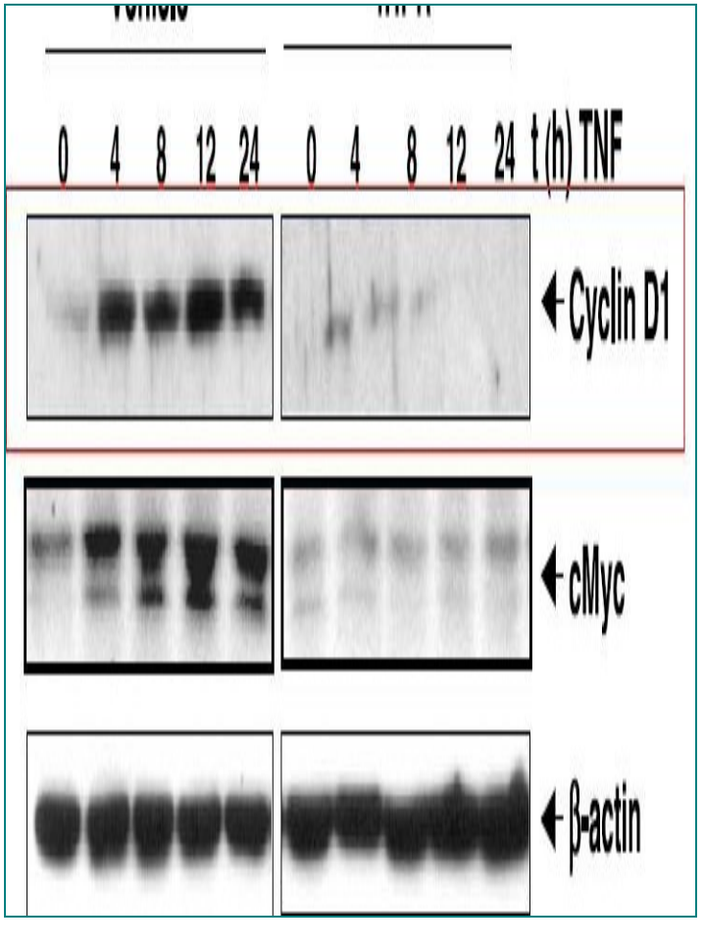


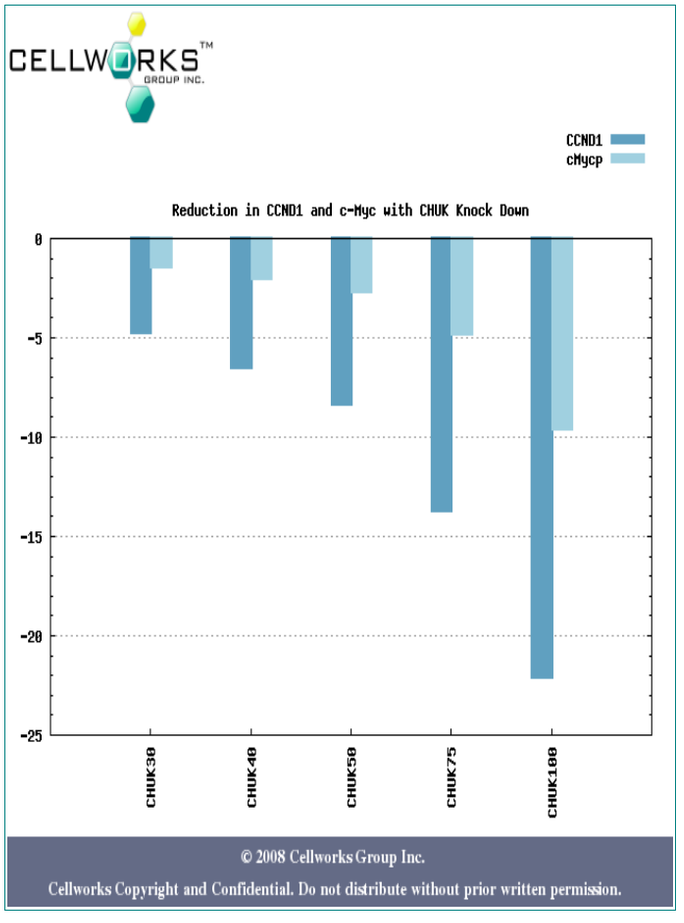
PMID: 17684018

Experimental Information: The PTEN/FLAG gene was transfected into cells and then treated with 2 μM EF24 for 24 h. The effect of PTEN overexpression and EF24 treatment on the apoptotic markers in CR cells. Expression of PTEN, p53, p21, pAkt, Akt, cleaved caspase-3, PARP, and FLAG expression in untransfected cells treated without (Control) and with EF24 treatment (EF24), and cDNA-transfected cells without (cDNA) and with EF24 treatment (EF24 + cDNA) were determined by Western blot.

Virtual Study Result: The above plot shows an increase in wild type p53 protein and the cell cycle inhibitor CDKN1A with increase in PTEN expression

STUDY 4: Reduction in CyclinD1 and c-MYC with CHUK Knock down



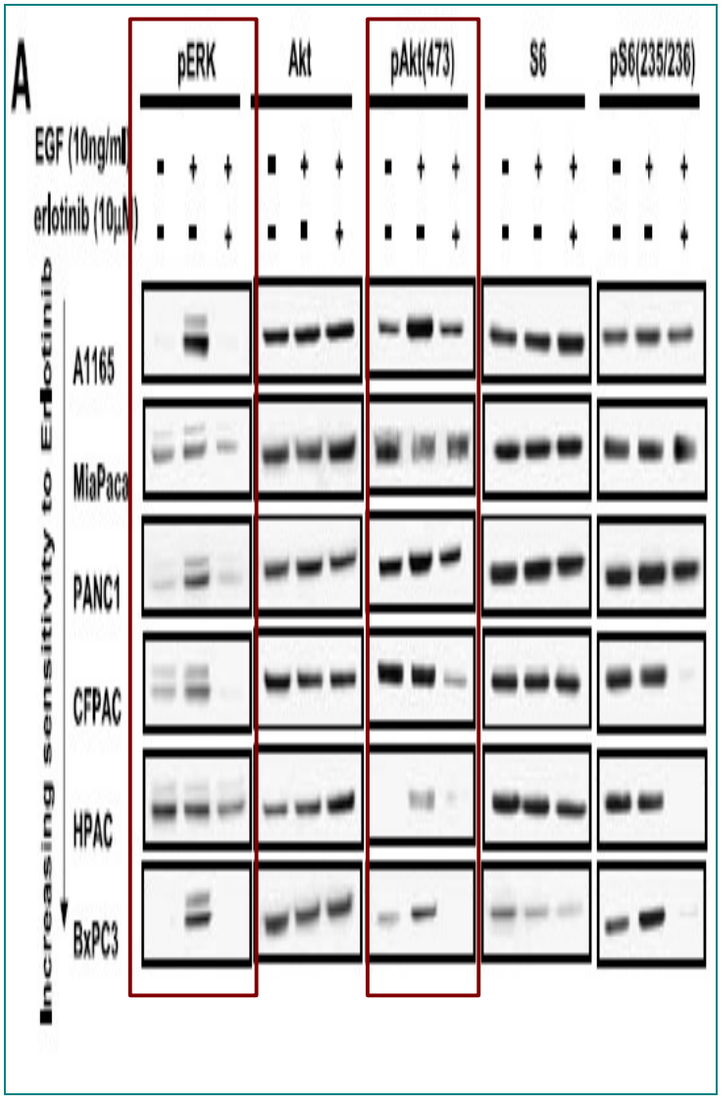


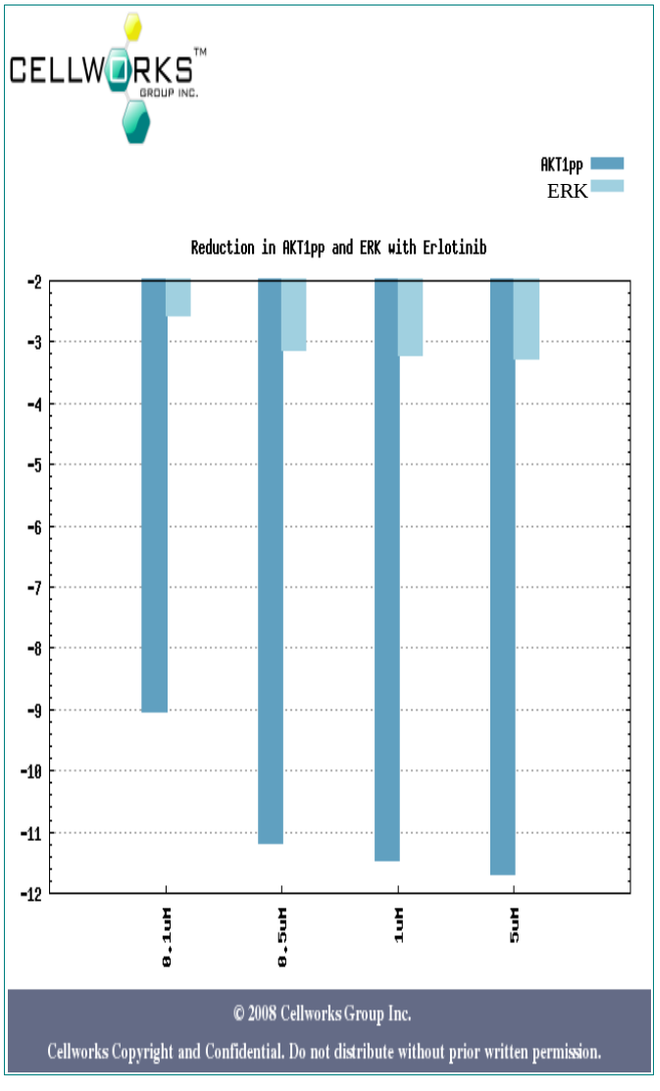
PMID: 16230421

Experimental Information: 4-HPR blocked the phosphorylation and degradation of IKK through the inhibition of activation of IKK and this led to suppression of the phosphorylation and nuclear translocation of p65. 4-HPR inhibits cyclin D1 and c-*myc* expression induced by TNF. H1299 cells (2 x 10^6/mL) were left untreated or incubated with 25 µmol/L 4-HPR for 24 hours and then treated with 0.1 nmol/L TNF for different times. Whole-cell extracts were prepared, and whole-cell lysate (80 µg) was analyzed by Western blotting using antibodies against cyclin D1 and c-*myc*

Virtual Study Result: The above plot shows reduction in CyclinD1 and cMYc levels with reduction in IKKa activity

STUDY 5: Reduction in ERK and AKT1pp with Erlotinib



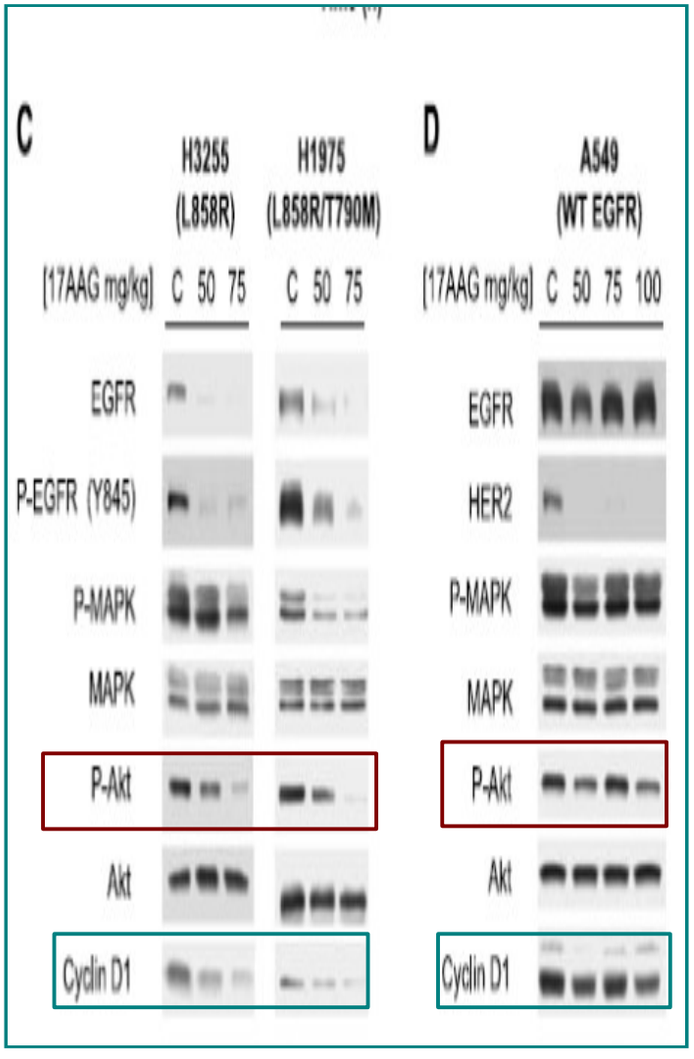


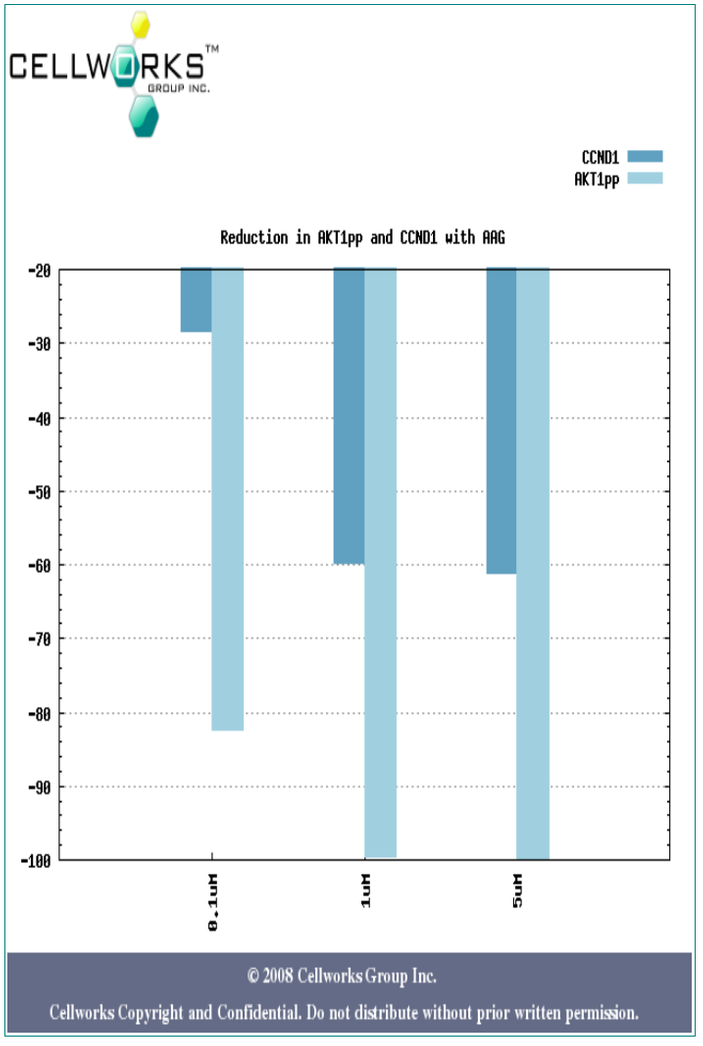
PMID 16928826

Experimental Information: Effect of EGF and erlotinib on the phosphorylation of ERK, Akt, and S6 in a panel of three erlotinib-insensitive (A1165, MiaPaca-2, and Panc1) and three erlotinib-sensitive (CFPAC, HPAC, BxPC3) cell lines

Virtual Study Result: The above plot shows reduction in phosphorylated AKT1 and ERK levels with EGFR inhibition through TKI erlotinib

STUDY 6: Reduction in CyclinD1 with AAG



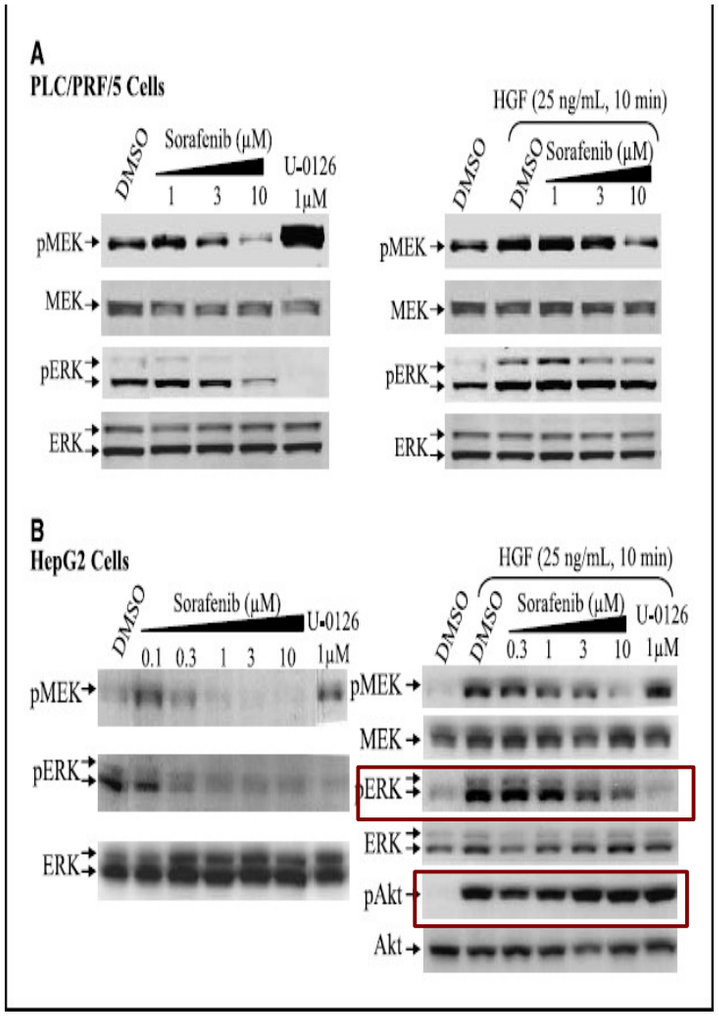


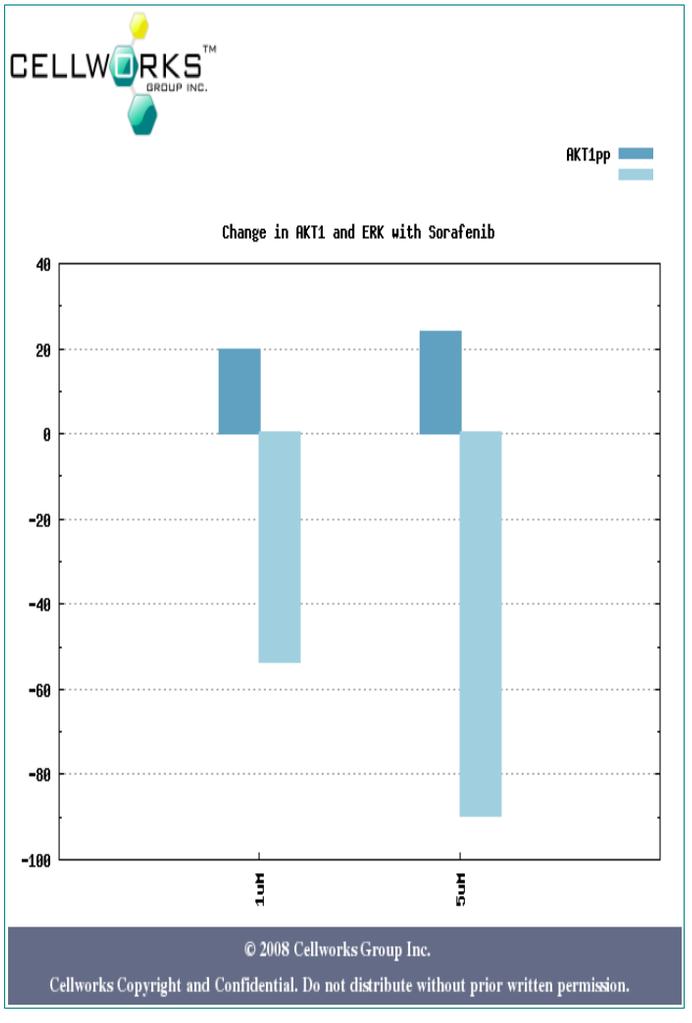
PMID : 18199556

Experimental Information: 17-AAG, at nontoxic doses, down-regulates mutant EGFR expression in xenograft tumors. For dose-response studies, mice were treated with either 50 or 75 mg/kg of 17-AAG and sacrificed 6 h later. In time course experiments mice were treated with a single dose of 75 mg/kg of 17-AAG and sacrificed at the time points indicated (0–48 h). A, immunoblots of EGFR, Raf-1, Akt, and cyclin D1 in H3255 and H1975 xenografts treated with 17-AAG.

Virtual Study Result: The above plot shows reduction in phosphorylated AKT1, ERK and CyclinD1 levels with Hsp90 inhibition

STUDY 7: Reduction in CyclinD1 with Sorafenib



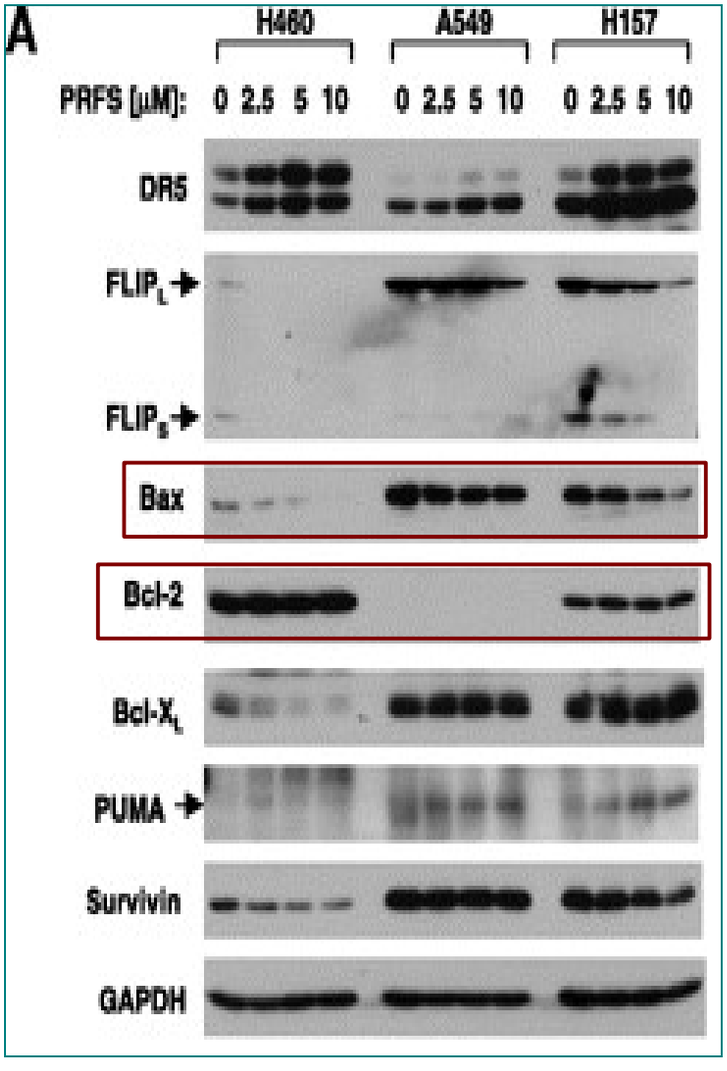


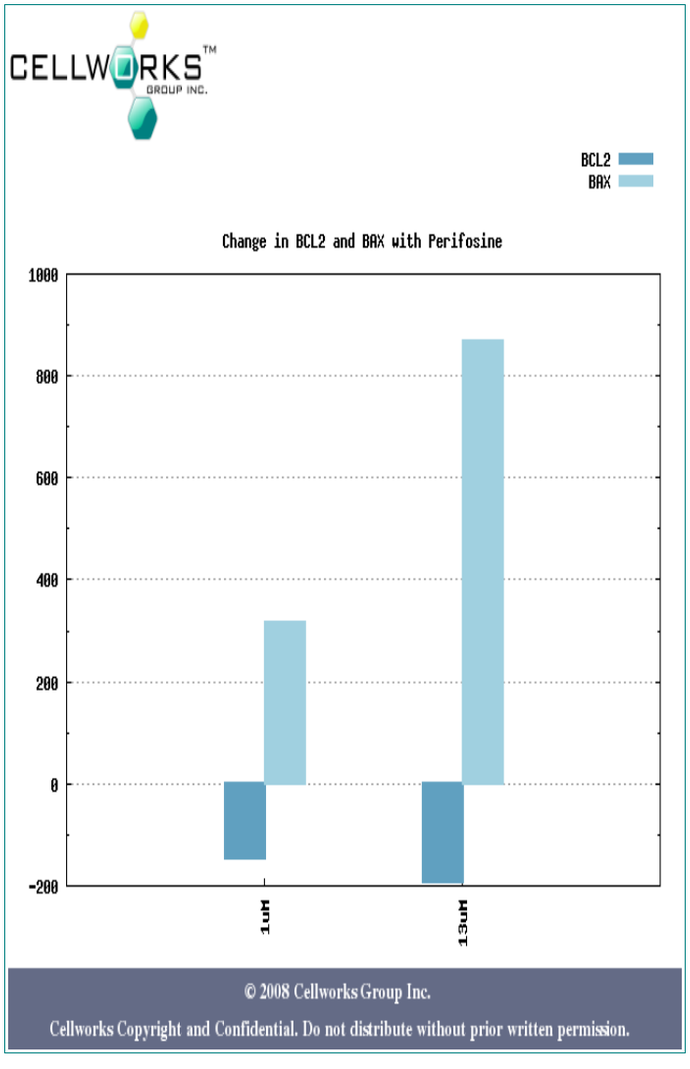
PMID: 17178882

Experimental Information: Sorafenib inhibited MEK and ERK phosphorylation at a concentration of between 3 and 10 umol/L in PLC/PRF/5 cells (p53 mutant) (Fig. A) and between 1 and 3 Amol/L in HepG2 p53 wild type) cells (Fig. B). Both of them HCC (Human Hepatocellular carcinoma) cell lines

Virtual Study Result: The above plot shows reduction in phosphorylated ERK with Nexavar and increase in phosphorylated ERK

STUDY 8: Reduction in BCL2 in Perifosine



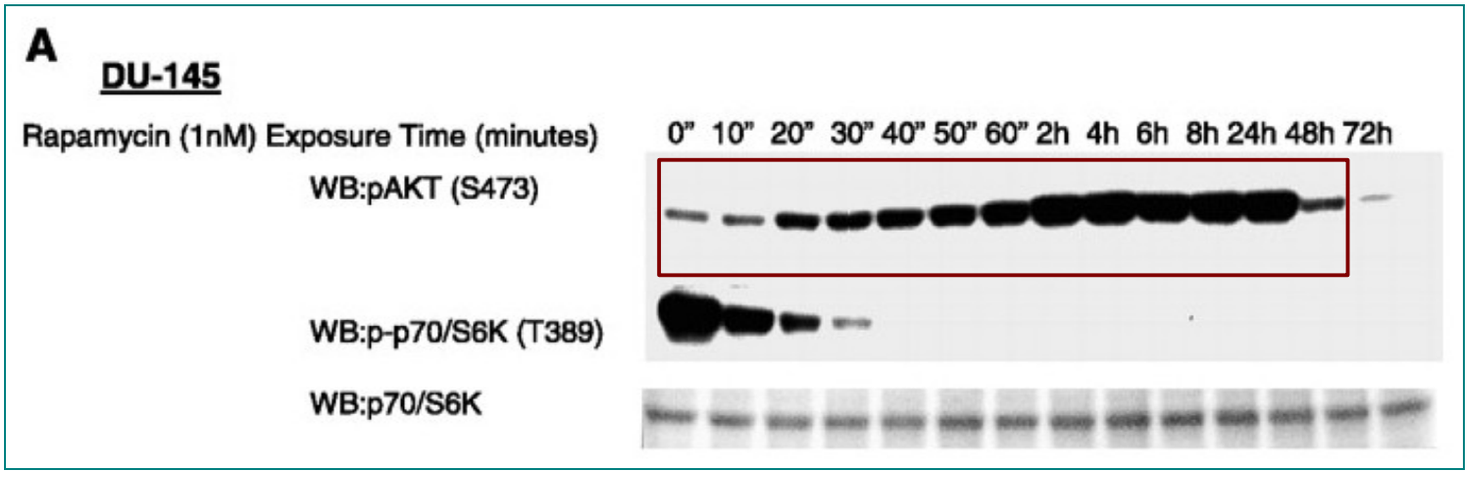


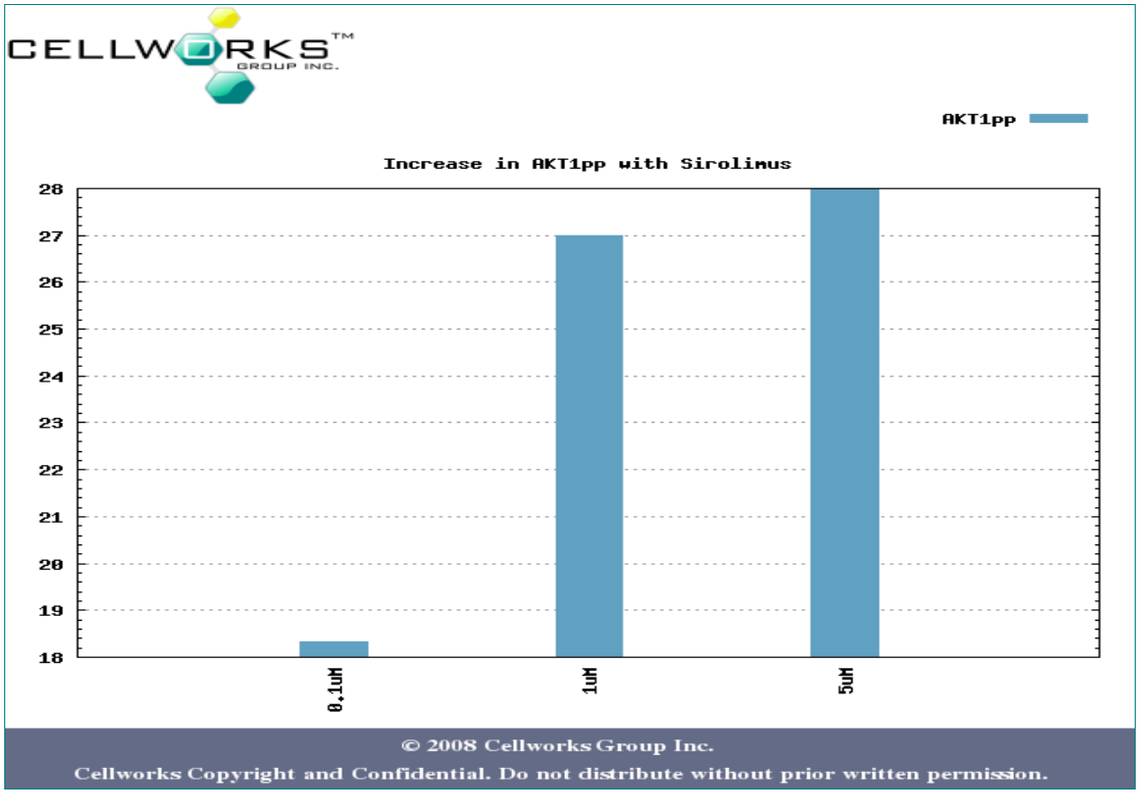
PMID: 17604333

Experimental Information: Human NSCLC(non–small cell lung cancer) cells were treated with given concentrations of Perifosine. Perifosine decreased Bcl-XL levels in H460 cells that were very sensitive to perifosine, but not in A549 and H157 cells that were less sensitive to perifosine

Virtual Study Results: The above plot shows reduction in BCL2 and BCL-xL protein with AKT inhibition

STUDY 9: Increase in AKT1pp with Sirolimus





PMID 16452206

Experimental Information: mTOR inhibition activates Akt in tumor cells. *A,* 1 nmol/L rapamycin treatment induced S473 Akt and S21/9 GSK3α /ß phosphorylation *in vitro* in a DU-145 prostate cancer cell line.

Virtual Study Results: The above plot shows increase in phosphorylated AKT with mTOR inhibition