signaling pathway include rapamycin and related molecules, which inhibit the mTOR kinase. However, inhibition of mTOR and its downstream effector p70S6K can lead to upregulation of PI-3 kinase signaling, including activation of Akt and downstream survival pathways.
EXEL-9418 (XL418) is a potent inhibitor of Akt and p70S6K, two important kinases that mediate PI-3 kinase pathway signaling. In biochemical assays, EXEL-9418 inhibits Akt and p70S6K with $IC_{50}$ values in the low nanomolar range. In cellular assays, EXEL-9418 inhibits phosphorylation of ribosomal S6 protein (a substrate of p70S6K) and the Akt substrates GSK3β and PRAS40, and induces translocation of the FKHR transcription factor in tumor cells. Oral administration of EXEL-9418 in the A549 lung adenocarcinoma xenograft model inhibited p70S6K and Akt signaling, and these effects were correlated with inhibition of tumor cell proliferation and induction of apoptosis, respectively. In contrast, rapamycin inhibited proliferation but caused little or no apoptosis in this model. EXEL-9418 causes significant tumor growth inhibition in nude mouse xenograft models, with little effect on hematology and clinical chemistry parameters or body weight at efficacious doses. Moreover, combining EXEL-9418 with epidermal growth factor receptor (EGFR) inhibitors in an EGFR inhibitor-resistant cell line (MDA-MB-468) leads to downregulation of Akt and p70S6K signaling and a substantial increase in apoptosis compared to either agent alone, both in vitro and in vivo.
These data suggest that a dual inhibitor strategy, targeting both cell growth and cell survival pathways, may offer significant advantages over targeting growth signaling alone. Furthermore, such an inhibitor may have broad utility in potentiating the effects of EGFR inhibitors.

**609** POSTER

**Selective inhibition of Raf results in down regulation of the Ras/Raf/MEK/ERK pathway and inhibition of tumor growth in vivo**

S. Malek. *Exelixis, Drug Discovery, South San Francisco, USA*

The Ras/Raf/MEK/ERK signaling pathway is upregulated in approximately 30% of all human cancers, with activating Ras mutations evident in 15−30% of these cancers. Activating mutations of B-Raf, primarily B-RafV600E, have been identified in approximately 7% of human cancers, including 70% of malignant melanomas.
We have identified a highly potent and selective Raf kinase inhibitor, EXEL-2819 (XL281), which modulates MEK/ERK phosphorylation and tumor cell proliferation in vitro and in vivo. EXEL-2819 exhibits potent activity against c-Raf, B-Raf, and the activated mutant B-RafV600E in enzyme assays, with IC50 values in the low nanomolar range. EXEL-2819 is a highly selective inhibitor of Raf, with potency at least 250-fold greater for Raf compared to 100 other kinases. EXEL-2819 modulates the Raf/MEK/ERK pathway in a number of tumor cell lines harboring activating Ras and B-Raf mutations and potently inhibits the phosphorylation of MEK and ERK in these cells.
In pharmacokinetic studies, EXEL-2819 displays high oral bioavailability in mice, rats, dogs, and monkeys. In pharmacodynamic studies, administration of a single oral dose of EXEL-2819 results in decreased phosphorylation of MEK ($\leqslant$98% inhibition) and ERK ($\leqslant$78% inhibition) in xenograft tumors. In repeat-dose efficacy studies, EXEL-2819 inhibits tumor growth in a range of xenograft models including A375, MDA-MB-231, HCT116, and A431. Immunohistochemical analyses of tumors collected at the end of these studies reveal significant inhibition of phosphorylation of MEK and ERK, decreased cell proliferation (Ki67), and decreased tumor vascularization (CD31).
In summary, these data indicate that selectively targeting Raf kinases with EXEL-2819 results in substantial inhibition of the Raf/MEK/ERK pathway and of tumor growth in preclinical xenograft models, and provide a rational basis for the clinical development of this inhibitor for the treatment of solid tumors that rely on the Ras/Raf/MEK/ERK pathway.

**610** POSTER

**Synergistic cytotoxicity, inhibition of Akt and c-Kit phosphorylation and modulation of gene expression by sorafenib and gemcitabine in human pancreatic cancer cells**

R. Danesi, E. Giovannetti, S. Nannizzi, V. Mey, S. Ricciardi, C. Giazotto, M. Del Tacca. *Division of Pharmacology, Department of Internal Medicine, Pisa, Italy*

**Background:** pancreatic cancer is one of the most lethal tumours and, although gemcitabine produces a clinical meaningful response, there has been little improvement in prognosis. Therefore, research effort has focused on target-specific agents, such as sorafenib, which blocks both the RAF/MEK/ERK signaling pathway and receptors involved in neovascularization and tumour progression, including VEGFR-2 and c-Kit. We investigate whether sorafenib would be synergistic with gemcitabine against pancreatic cancer cell lines.
**Material and Methods:** cells were treated with sorafenib and gemcitabine, alone or in combination. Pharmacologic interaction was studied using the combination index (CI) method, while cell cycle was investigated with flow cytometry. Moreover, the effects of drugs on Akt and c-Kit phosphorylation, and on apoptosis induction were studied with ELISA and fluorescence microscopy, respectively. Finally, quantitative PCR analysis was performed to assess whether sorafenib modulated the expression of the gemcitabine activating enzyme deoxycytidine kinase (dCK) and the drug target ribonucleotide reductase (RR).
**Results:** sorafenib was cytotoxic against MIA PaCa-2, Capan-1, PANC-1 cells with IC50s of $3.48\pm0.27$, $0.61\pm0.16$, $4.56\pm1.32\,\mu$M, respectively. A dose dependent inhibition of cell growth was also observed after gemcitabine treatment with IC50s of $0.08\pm0.01$ (MIA PaCa-2), $0.10\pm0.02$ (Capan-1), $0.178\pm0.039$ (PANC-1) μM. The CI analysis showed synergism for both sequences. Flow cytometry demonstrated that gemcitabine enhanced cellular population in the S phase. Cell exposure to gemcitabine resulted in a significant Akt phosphorylation inhibition, whereas sorafenib exposure reduced c-Kit phosphorylation. Fluorescence microscopy demonstrated that cells treated with drugs and their combinations presented typical apoptotic morphology; in particular, drug combinations significantly increased apoptotic index with respect to single agents in Capan-1 and MIA PaCa-2 cells. PCR showed that sorafenib reduced the expression of RRM1 and RRM2 in MIA PaCa-2 and Capan-1 cells, enhancing the dCK/(RRM1$\times$RRM2) ratio ($p < 0.05$).
**Conclusions:** these data demonstrate that sorafenib and gemcitabine synergistically interact against pancreatic tumour cells, through suppression of Akt and c-Kit phosphorylation, induction of apoptosis and reduction of RRM1 and RRM2 gene expression, thus providing the experimental basis for developing this combination for the treatment of pancreas cancer.

**611** POSTER

**Sensitization of human prostate cancer cells to TRAIL/Apo2L by curcumin through inhibition of pro-survival Akt/NF-kB signaling pathways**

S. Gautam[1], D. Deeb[1], H. Jiang[2], X. Gao[1], S. Dulchavsky[1]. *[1]Henry Ford Health System, Surgery, Detroit, USA; [2]Henry Ford Health System, Neurology, Detroit, USA*

**Materials and Methods:** The expression levels of constitutively active Akt, NF-κB and NF-κB-dependent antiapoptotic proteins in LNCaP, PC3, and DU145 prostate cancer cells were determined by Western blotting. Curcumin, Akt inhibitor SH-6, and siRNA-Akt were used to sensitize cancer cells to TRAIL and to understand cross-talk between Akt and NF-κB signaling pathways and their role in resistance of prostate cancer cells to TRAIL-induced apoptosis.
**Results:** Each cancer cell line studied expressed transcriptionally active NF-κB which was inhibited by curcumin at concentration range of 10 to 30 μM. LNCaP and PC3 cells but not DU145 cells expressed p-Akt kinase which was also inhibited by curcumin. Inhibition of the NF-κB and p-Akt by curcumin sensitized cancer cells to TRAIL-induced cytotoxicity. Since NF-κB is a downstream target of p-Akt, we investigated whether inhibition of NF-κB by curcumin is mediated through suppression of Akt activation. Treatment of PC3 cells with SH-6, a specific inhibitor of Akt, or transfection with siRNA-Akt, not only inhibited p-Akt but also abrogated the nuclear expression of NF-κB. Furthermore, treatment with SH-6 or selective inhibition of Akt through siRNA-Akt inhibited NF-κB and sensitized cells to TRAIL. In contrast, SH-6 failed to inhibit NF-κB or sensitize DU145 prostate cancer cells to TRAIL as these cells do not express p-Akt. Because expression of antiapoptotic Bcl-2, Bcl-xL and XIAP is regulated by NF-κB, both curcumin and SH-6 decreased the levels of these proteins in PC3 cells through inhibition of NF-κB. Further, gene silencing of Bcl-2 with siRNA-Bcl-2 sensitized PC3 cells to TRAIL.
**Conclusions:** These data define a molecular pathway wherein curcumin sensitizes prostate cancer cells to TRAIL by inhibiting Akt-regulated NF-κB and NF-κB-dependent antiapoptotic Bcl-2, Bcl-xL and XIAP.

**612** POSTER

**Acquired resistance to drugs that yield PKCδ activation and PKCα inhibition modify adhesion and invasion in human cancer cells**

A. Ghoul[1], M. Serova[2], Y. Bieche[3], E. Phillips[1], E. Cvitkovic[4], S. Faivre[5], F. Lokiec[1], F. Calvo[2], E. Raymond[5]. *[1]Rene Huguenin Cancer Center, Pharmacology, Saint Cloud, France; [2]Saint-Louis Hospital, Pharmacology, Paris, France; [3]UPRES EA 3618, University René Descartes Paris 5, University René Descartes, Paris, France; [4]CAC Oncology, CAC Oncology, Le Kremlin-Bicêtre, France; [5]Department of Medical Oncology, Beaujon, University Hospital, Clichy, France*

**Background**: PKCs are serine/threonine kinases modulating proliferation, apoptosis and invasion in cancer cells. Among PKC modulators used in clinical trials the new agent PEP005 was shown to induce apoptosis by