to be important in cell survival including EGFR, Akt, Stat3, c-Src, PIM-1, and Bcl-2 proteins.
Results: Cells dependent on EGFR for survival demonstrated increased sensitivity to LBH589 and underwent apoptosis following exposure to these agents. LBH589 inhibits the binding of Hsp90 to EGFR. LBH589 selectively depleted proteins important in signaling cascades in cell lines harboring EGFR kinase mutations, such as EGFR, Stat3, and Akt. In addition, we found depletion of Stat3-dependent survival proteins including Bcl-xL, Mcl-1, and Bcl-2. Conversely, LBH589 had no effect on apoptosis in cells not dependent on EGFR for survival and no changes were identified in EGFR, Stat3, Akt, or Stat3-dependent survival proteins.
Conclusions: Based on these results, LBH589 can trigger apoptosis in EGFR-dependent lung cancer cells and depletes levels of key signaling cascades important in tumor survival.

605 POSTER

Modulation of the HSP90 co-chaperone AHA1 affects client protein activity and increases cellular sensitivity to the HSP90 inhibitor 17-allylamino-17-demethoxygeldanamycin (17-AAG)

J. Holmes, S. Sharp, P. Workman. *Institute of Cancer Research, Sutton, United Kingdom*

AHA1 (Activator of HSP90 ATPase) is a co-chaperone of the ATP-dependent molecular chaperone HSP90, which is involved in the maturation and function of several oncogenic client proteins (Maloney A, Workman P. Expt Opin Biol Ther 2:3−24, 2002). HSP90, in association with its co-chaperones, operates as part of a multimeric complex driven by the binding and hydrolysis of ATP. The intrinsic ATPase activity of the human HSP90 has been shown to be significantly increased by AHA1 *in vitro*. Inhibition of HSP90 by the first-in-class HSP90 ATPase-inhibitor 17-AAG results in cessation of cell growth and the degradation of client proteins such as C-RAF and CDK4 via the ubiquitin proteasome pathway. Co-chaperones such as AHA1 and HSP72 have also been shown to be upregulated with 17-AAG treatment as a result of stress-induced transcription. As AHA1 is known to increase the ATPase activity of HSP90, we hypothesised that modulation of AHA1 expression could influence HSP90 activity and the cellular response to treatment with 17-AAG. We have previously shown that when AHA1 is knocked down using RNA interference, there is a significant ($P < 0.05$) increase in sensitivity to 17-AAG, as demonstrated by a 2–3 fold increase in detached cells (Holmes, J *et al*, Clinical Cancer Research 11(24 Suppl): 9157s, 2005). Further investigation into the effects of AHA1 modulation on cellular sensitivity to 17-AAG has shown that overexpression of AHA1 (3.5−10 fold) had no effect on sensitivity to 17-AAG. Using RNA interference and our AHA1 overexpression model, the role of AHA1 on HSP90 client protein activity has been investigated. When AHA1 protein expression was knocked down (~80%) by siRNA oligonucleotides there was no effect on HSP90 client proteins C-RAF, ERBB2 or CDK4. Similar results were obtained when AHA1 was overexpressed. Interestingly, however, MEK1/2 and ERK1/2 phosphorylation were decreased when AHA1 was knocked down with no change in the total protein levels. Moreover, overexpression of AHA1 resulted in an increase in phosphorylation of MEK1/2 and ERK1/2. These results would suggest that AHA1 may have a role in client protein activation, and modulation of AHA1 could be a therapeutic strategy to increase sensitivity to HSP90 inhibitors.

606 POSTER

Augmented growth inhibition of human NSCLC cells resistant to EGFR-tyrosine kinase inhibitor (TKI) by a combination of dual TKI of EGFR/VEGFR2 (AEE788) and mTOR inhibitor (RAD001)

K. Soejima, I. Nakachi, K. Naoki, H. Watanabe, I. Kawada, H. Yasuda, A. Ishizaka. *Keio University, Pulmonary Medicine, Tokyo, Japan*

Background: EGFR-TKI such as gefitinib and erlotinib show anti-tumor activity in a subset of non-small cell lung cancer (NSCLC) patients having mutations of EGFR gene. However, clinical resistance to EGFR-TKI is observed in spite of the initial response. Recent work shows such resistance can be caused by a secondary mutation, T790M in the EGFR-TK domain. Several studies suggested the importance of the EGFR downstream kinases as potential drug targets. The aim of this study is to evaluate the efficacy of alternative small molecules which inhibit other targets than EGFR-TK, such as AEE788 and RAD001, for NSCLC cell lines. AEE788 is a dual TKI for EGFR and vascular endothelial growth factor receptor 2 (VEGFR2), while RAD001 is an inhibitor of the mammalian target of rapamycin (mTOR).
Methods: We used 3 human NSCLC cell lines, namely, A549, H1650 and H1975. A549 has wild type EGFR, H1650 harbors a deletion mutation in exon 19, while H1975 possesses double mutations at L858R and T790M, which account for sensitiveness and resistance to EGFR-TKI, respectively. We first treated these cells with AEE788 or RAD001 as a single agent, then tried combination of two agents and evaluated the effect on cell growth as well as the induction of apoptosis.
Results: AEE788, as a single agent, significantly reduced the proliferation of all cell lines dose-dependently. The degree of reduction, however, was much less in H1975 compared to other cell lines. The reduction was independent of inhibition of EGFR-TK activity as the status of p-EGFR was unchanged in H1975 after AEE788 treatment, suggesting the inhibition of other pathways, such as VEGFR by AEE788. RAD001 single-treatment also showed the growth inhibition of all cells with less effect in H1970 than in A549. The combined treatment with AEE788 and RAD001 showed no additional effect compared to AEE788 alone on growth inhibition in A549 and H1650. On the other hands, this combination resulted in effective and additional growth inhibition against H1975 and was related to induction of apoptosis.
Conclusions: AEE788 and RAD001 will be possible novel candidates for the treatment of NSCLC patients and will be especially useful to overcome the acquired resistance to EGFR-TKI when used in combination.

607 POSTER

Impressive anti-tumor activity of combined erbB1 and erbB2 blockade: a phase I and pharmacokinetics (PK) study of OSI-774 (Erlotinib; E) and Trastuzumab (T) in combination with weekly Paclitaxel (P) in patients (pts) with advanced solid tumors

M. Beeram[1], J.S. De Bono[2], E.K. Rowinsky[2], P. O'Rourke[2], A. Ricart[2], L.A. Hammond[2], A. Patnaik[2], M. Mita[2], A. Mita[2], A.W. Tolcher[2]. *[1]University Of Texas Health Science Center At San Antonio, Medicine/Medical Oncology, San Antonio, TX, USA; [2]Institute For Drug Development, Cancer Therapy And Research Center, San Antonio, TX, USA*

Background: Co-expression of erbB1 and erbB2 receptors confers a growth advantage in erbB2 overexpressing (+) cancers. Specifically, co-expression alters the normal rapid internalization and inactivation of erbB1, slows dissociation of the erbB1 ligand-receptor complex and degradation of the active receptor. Co-targeting both receptors together may offer a therapeutic advantage over targeting erbB2 alone, especially in T refractory solid tumors. A phase I and PK study was launched to determine the toxicity and recommended dose of continuous daily oral E in patients with erbB2 + cancers along with weekly P and T, especially since T improves survival in combination with chemotherapy.
Methods: Eligible pts were treated with weekly T IV (2 mg/kg/wk) along with weekly P (starting at 80 mg/m^2) and escalating doses of E po daily for 28-days. MUGA scans were performed at baseline and every 2 cycles (8 weeks). Two schedules – 3 out of 4 weeks and continuous weekly P and T were explored.
Results: 24 patients [breast (22), Colon (1), ovary (1)] have received 97 courses [median 2, range 1–13] in 5 cohorts. Doses of different drugs were E 50–150 mg (50 mg, 3 pts; 100 mg, 15 pts; 150 mg, 6 pts), P 80−90 mg/m^2 weekly for 3 out of 4 weeks and weekly T. Patients were women with median age 54 years [range 37−75] and PS 0 (5), PS 1 (17) or PS 2 (2). The proportion of patients positive for hormone receptors was 10/18. Thirteen patients received prior T and 8 patients had received T, including 6 patients who had previously received the combination TH. Also 13 patients had received Docetaxel (4 in combination with P). Dose limiting grade (gr) 3 diarrhea and gr 3 dermatitis was seen in 1 pt at 100 mg of E and 80 mg/m^2 of P. Other toxicities included gr 2 diarrhea, skin rash, fatigue, neutropenia and alopecia. Significant asymptomatic drop in LVEF was noted in 4 pt. One complete and four partial responses have been seen in pts with breast cancers, 2 of them have previously failed to T therapy and 3 had failed to taxanes. Three breast cancer pts experienced stable disease lasting 13, 11 and 6 courses, respectively. Preliminary PK data does not suggest a clinically relevant interaction between the 3 agents.
Conclusions: E combined with T and P provides a well-tolerated, targeted therapy with impressive anti-tumor activity in T-refractory breast carcinoma. Dose escalation was discontinued on the continuous dosing schedule as two patients experienced DLT. Expanded accrual is ongoing for the interrupted dosing schedule of 3 out of 4 weeks therapy at full doses of all three agents (MTD) to further characterize the toxicities.

608 POSTER

Targeting aberrant PI-3 kinase pathway signaling by dual inhibition of Akt and p70S6K

P. Lamb. *Exelixis Inc., South San Francisco, USA*

The PI-3 kinase pathway is frequently dysregulated in cancer cells, and is implicated in multiple aspects of tumor growth and survival. In addition, resistance to many anticancer agents (including receptor tyrosine kinase inhibitors and genotoxic agents) has been attributed to failure to downregulate PI-3 kinase pathway signaling. Current inhibitors of this

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 ± 0.27, 0.61 ± 0.16, $4.56\pm1.32\,\mu$M, respectively. A dose dependent inhibition of cell growth was also observed after gemcitabine treatment with IC50s of 0.08 ± 0.01 (MIA PaCa-2), 0.10 ± 0.02 (Capan-1), 0.178 ± 0.039 (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