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)

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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)

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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

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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

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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