**543** POSTER

**Inhibition of Hsp90 function downregulates EGFR and sensitizes EGFR-mutant xenografts to paclitaxel**

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**Background:** Somatic mutations in the kinase domain of the epidermal growth factor receptor (EGFR) receptor tyrosine kinase (RTK) are found in a subset of patients with lung cancer. These mutations correlate with response to the EGFR tyrosine kinase inhibitors (TKIs) gefitinib and erlotinib. Resistance to these agents invariably develops and current treatment strategies have limited efficacy in this setting. Hsp90 is a molecular chaperone required for protein refolding and the conformational maturation of a variety of signaling proteins. Hsp90 inhibitors such as 17AAG (17-allylamino-17-demethoxy geldanamycin) induce the degradation of "client" proteins which require Hsp90 for maturation, activation or stability.
**Methods:** We studied the effects of 17AAG alone and in combination with paclitaxel on EGFR expression and signaling, cell proliferation and xenograft tumor growth, using a panel of isogenic and human lung cancer cell lines expressing wt EGFR and EGFR kinase domain mutants.
**Results:** The EGFR point and deletion mutants found commonly in the tumors of patients who respond to EGFR TKIs are potently degraded by 17AAG. Although wild-type EGFR was also degraded by 17AAG, its degradation required higher concentrations of drug and a long-duration of exposure to 17AAG than the EGFR mutants. Degradation of mutant EGFR was accompanied by downregulation of Akt and MAPK activity, loss of cyclin D1 expression, growth arrest and induction of apoptosis. A single dose of 17AAG was sufficient to induce the dose-dependent degradation of mutant EGFR and downregulate Akt and MAPK activity in tumor xenografts. 17AAG at non-toxic doses (75–100 mg/kg 3×/wk) was active as a single agent in all three EGFR mutant xenograft models studied. These included tumors with the L858R point mutation (H3255), an exon 19 deletion (H1650) and the L858R-T790M double mutant (H1975). These later two cell lines are resistance to EGFR TKIs. Full doses of 17AAG and paclitaxel could be co-administered without evidence of enhanced toxicity to mice bearing H1650 and H1975 xenografts, and the combination of 17-AAG and paclitaxel was significantly more effective than either drug alone.
**Conclusions:** 17AAG induces the degradation of mutant EGFR and has anti-tumor activity alone and in combination with paclitaxel in tumor xenografts with EGFR kinase domain mutations. These data suggest that Hsp90 inhibition may represent an effective treatment strategy for patients with EGFR TKI-resistant lung cancer.

**544** POSTER

**SNX5542, an oral Hsp90 inhibitor, causes Her2 degradation and inhibition of tumor growth in models of Her2 amplified breast cancer.**

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**Background**: Hsp90 is a molecular chaperone required for the conformational maturation and stability of a variety of signaling proteins. Natural products that bind to the Hsp90 ATP pocket (e.g. geldanamycin) cause the proteosomal degradation of Hsp90 "client" proteins. We find the HER2 oncogene to be the client that is most sensitive to Hsp90 inhibition. Moreover, 17-AAG (a geldanamycin derivative) has shown promising clinical activity in patients with HER2 amplified breast cancer. However, the limitations of the natural product inhibitors have prompted us to develop selective, small-molecule inhibitors of Hsp90.
**Methods**: We compared the pharmacokinetics, pharmacodynamics and anti-tumor effects of 17-AAG with SNX-2112 and its pro-drug SNX5542, a water-soluble, orally bioavailable Hsp90 inhibitor. Drug accumulation and changes in Hsp90 client expression were assayed in both normal and tumor tissues.
**Results**: SNX2112 has similar potency to 17-AAG across a panel of human cancer cell lines. For example, SNX2112 inhibits the growth of HER2 amplified breast cancer cells at IC50s below 5 nM. SNX2112 causes degradation of HER2 and inactivation of AKT and ERK signaling. This results in downregulation of cyclinD1 expression and induction of a pro-apoptotic response. We evaluated the pharmacokinetics and tissue localization of SNX5542 in BT-474 tumor bearing mice. SNX5542 accumulated in tumors to concentrations over ten-fold higher than those in normal tissues (e.g. lung, liver, and brain). A single, non-toxic, oral dose of SNX5542 (75–150 mg/kg) was effective in potently downregulating the expression of HER2 in BT-474 tumor xenografts for over 24 hours. The degradation of HER2 was associated with inhibition of AKT and ERK activity, loss of cyclinD1 expression, and induction of apoptosis in the tumors of SNX5542-treated mice. Degradation of HER2 was accompanied by tumor regression in mice treated 3–5×/week with SNX-5542. For example, treatment of BT474 xenografts with SNX5542 50 mg/kg 5×/week was non-toxic and resulted in a greater than 50% tumor regression (vs. 300% increase in vehicle-treated control tumors). These effects were durable and preserved for 3 weeks beyond the cessation of treatment.
**Conclusions**: Oral SNX5542 potently degrades HER2, induces apoptosis and tumor regression in HER2-dependent breast cancer models. The results suggest that SNX5542 may represent a promising strategy for the treatment of advanced breast cancers.

**545** POSTER

**SNX-2112: a novel, selective, potent small molecule inhibitor of Hsp90 with unique pharmacodynamic properties**

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**Background:** The preclinical and clinical data for HSP90 inhibitors such as 17-AAG and 17-DMAG, both derivatives of geldanamycin (GDN), support this molecular chaperone as a promising target for cancer therapy. However, the utility of these GDN derivatives has been limited due to potential off-target toxicity and formulation challenges. For this reason, there continues to be a growing interest in orally-active, small molecule HSP90 inhibitors. Serenex has discovered and developed novel small molecules, represented by SNX-2112, that display the desired pharmacodynamic profile. Here we describe the preclinical evaluation of this compound.
**Methods:** SNX-2112 was identified and characterized using Serenex's proprietary purine proteome mining technology, which enables evaluation of binding to the ATP site of HSP90 simultaneously with hundreds of purine binding proteins. This allowed simultaneous assessment of Hsp90 affinity and target selectivity. In parallel, compounds were evaluated in tumor cell proliferation assays as well as high-content screens for HSP90 client effects. For *in vivo* evaluation, SNX-2112 was delivered using the prodrugs SNX-5422 and SNX-5542 to facilitate formulation. Xenografts were performed with oral administration in D5W using a 3 times weekly regimen, compared to 17-DMAG on the previously reported MTD dose and regimen. Tumor progression and body weight was monitored while plasma, tumor and other tissues were harvested for evaluation of HSP90 client proteins.
**Results:** SNX-2112 exhibits a higher affinity for the ATP site of HSP90 than 17-AAG and 17-DMAG. Against a panel of more than 12 tumor cell lines, SNX-2112 blocked cell proliferation with a potency of 1–140 nM. One significant difference between SNX-2112 and the GDN derivatives was its effects on HSP90 clients and their downstream signaling markers. Whereas 17-AAG affected the Akt pathway in A375 cells, as measured by phosphorylated S6 kinase levels, with a potency of 308 nM, SNX-2112 was nearly 100-fold more active with an IC50 value of 4 nM. As expected, SNX-2112 exhibits potent pro-apoptotic effects in cellular assays, as measured by caspase induction. In animal studies, SNX-2112, delivered as a prodrug, consistently delayed tumor growth more profoundly than GDN derivatives. 17-DMAG at the reported MTD caused a tumor growth delay in HT29 xenograft of 2%, while SNX-5422 at 100 mg/kg PO 3X weekly had a delay of 52%.
**Conclusions:** SNX-2112 exhibits an *in vitro* HSP90 inhibitory profile consistent with its high potency and selectivity. This compound uniformly targets both the pro-proliferation pathways driven by Her2 and ERK as well as the anti-apoptotic Akt pathway and exhibits potent *in vivo* anti-tumor activity that extends significantly beyond the effects observed with GDN analogs.

**546** POSTER

**Efficacy of a selective small molecule IGF-IR kinase inhibitor in mouse xenografts models as a single agent and in combination with erlotinib**

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Insulin-like growth factor-I receptor (IGF-IR) and its ligands, IGF-I and IGF-II, are upregulated in a variety of human cancers. In tumors, such as colorectal and NSCLC cancers, which may drive their own growth and survival through autocrine expression of IGF-II, the role of IGF-IR is especially critical. In addition, activation of the IGF-IR protects tumor cells from apoptosis induced by a variety of anti-cancer agents. Thus IGF-IR represents as an important therapeutic target for the treatment of human cancer. Here, we present a novel small molecule IGF-IR kinase inhibitor, *cis*-3-[3-(4-methyl-piperazin-1-yl)-cyclobutyl]-1-(2-phenyl-quinolin-7-yl)-imidazo[1,5-a]pyrazin-8-ylamine (Compound-1) that was identified through a structure-based drug design approach. This compound displayed a cellular $IC_{50}$ of 19 nM for inhibition of ligand-dependent autophosphorylation of IGF-IR with 14-fold cellular selectivity over the human

insulin receptor. It had minimal activity against a panel of 30 other protein kinases. Compound-1 also abolished IGF-induced anchorage-independent growth and activation of downstream pathways pAkt, pErk1/2 and p-p70S6K in both IGF-IR transfectant cells and a GEO human colorectal cancer cell line. Analysis of GEO cells revealed an active IGF-II/IGF-IR autocrine loop. Robust anti-tumor efficacy was achieved in a GEO xenograft model with Compound-1 administrated orally once daily, which correlated with the degree and duration of inhibition of tumor IGF-IR phosphorylation by Compound-1. No substantial blood glucose changes were observed in mice at efficacious dose levels. Moreover, when Compound-1 was tested in EGFR inhibitor erlotinib (Tarceva®)-sensitive (H292), moderately sensitive (H441) and insensitive (H460) NSCLC lines alone or in combination with erlotinib *in vitro* and *in vivo,* synergistic effects were achieved in both H292 and H441 cell lines, whereas minimal potentiation was observed in H460 cells. Analysis of downstream pathways to EGFR and IGF-IR indicated that Compound-1 together with erlotinib effectively blocked pAkt and pErk1/2 in H292 and H441 cells, which in turn led to significant apoptosis, whereas in H460 cells there were minimal effects of the combination on the downstream pathways. Accordingly, tumor growth of H292 exhibited a durable cure in 3/8 mice when erlotinib was co-administrated with this IGF-IR inhibitor orally once daily. Significant tumor regression was also observed in H441 tumors in response to the combination treatment. Thus potent and selective IGF-IR kinase inhibitors like Compound-1 may have broad clinical utility for treatment of cancers as a single agent and in combination with EGFR inhibitors such as erlotinib.

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**Novel small-molecule inhibitors of STAT3 that selectively induce antitumor cell activity**

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The Signal Transducer and Activator of Transcription (STAT) family of proteins were originally discovered as mediators of cytokine and growth factor responses. Evidence has accumulated demonstrating that persistently-active STAT3, one of the family members, is a critical mediator of events that contribute to carcinogenesis and tumor progression. Aberrant STAT3 activity mediates dysregulated growth and survival, promotes angiogenesis and modulates immune responses. Of clinical significance, constitutive STAT3 activation is detected in breast, prostate, and colon cancers, non-small cell lung cancer, squamous cell carcinoma of the head and neck, as well as many other solid and hematological tumors. Inhibition of aberrant STAT3 induces antitumor cell activity and tumor regression in mouse model studies, thus providing therapeutic relevance to STAT3 inhibition and a validation of STAT3 as a cancer drug target. We have identified a diverse group of small-molecule STAT3 inhibitors by structure-based design with computational modeling that exploited the key structural requirements for binding of the native STAT3 phosphotyrosine (pTyr) peptide to the SH2 domain of STAT3. In addition, we performed random screening of compound libraries for binders of the STAT3 SH2 domain or inhibitors of STAT3 DNA-binding activity. Current inhibitors, such as non-peptide analogs of the STAT3 pTyr peptide (PpYLKTK) as disruptors of STAT3 dimerization, structurally-diverse compounds that bind the STAT3 SH2 domain, as well as novel platinum (IV) complexes that interact with the STAT3 DNA-binding domain, potently inhibit constitutive STAT3 activation and signaling in malignant cells. Moreover, these small-molecule STAT3 inhibitors selectively block tumor cell growth and induce apoptosis of malignant cells harboring aberrant STAT3 activity. Consistent with these findings, molecular analysis reveals down-regulation by STAT3 inhibitors of known STAT3-regulated genes, including Cyclin D1, Bcl-xL, Bcl-2 and Survivin, in malignant cells. Together, our studies demonstrate the feasibility of current efforts to develop small-molecule STAT3 inhibitors as anticancer drugs. Our findings also provide proof-of-concept for the antitumor cell effects of novel small-molecules that inhibit aberrant STAT3.

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**Active mutant epidermal growth factor receptor undergoes less protein degradation due to diminished binding to c-Cbl ubiquitin ligase**

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**Background:** Recently, new research has revealed that gefitinib (Iressa) sensitivity in non-small-cell lung cancer (NSCLC) is associated with activating mutations in the epidermal growth factor receptor (EGFR). Previously, we identified a 15-bp deletion mutation (2411 to 2425) in a NSCLC cell line, PC-9 (AACR 2004, abs 1221). This cell line is 1000 times more sensitive to gefitinib as compared with another NSCLC cell line, PC-14, which expresses wild-type EGFR. It has been reported that autophosphorylation of mutant EGFR is significantly prolonged as compared with the wild type EGFR (Lynch et al NEJM 2004). The same observation is seen in PC-9. EGFR is known to be degraded by lysosomal proteases and 26S proteasome after autophosphorylation, and in the latter case, the degradation occurs through ubiquitinylation of EGFR by c-Cbl ubiquitin ligase. To explore the mechanism of sustained autophosphorylation of the mutant EGFR, we examined the difference in EGFR degradation activities between the wild type EGFR and the mutant EGFR.
**Material and Methods:** To clarify the mechanism, we used expression vectors of wild type EGFR and mutant EGFR with the 15-bp deletion found in PC-9, and established stable transfected cell lines, named 293_pEGFR and 293_pΔ15, respectively. EGFR internalization and degradation activities were measured by using $^{125}$I-EGF. EGFR-bound c-Cbl was immunoprecipitated by EGFR-specific antibody and the binding activity was detected by immunobloting. Site specific c-Cbl phosphorylations were detected by immunobloting using respective anti phospho-c-Cbl antibodies.
**Results:** After TGF-α exposure, degradation rate of EGFR was about 10-fold higher in PC-14 as compared with that in PC-9. In 293_pΔ15, EGFR degradation activities and c-Cbl-binding to this receptor after TGF-α stimulation were significantly decreased as compared with 293_pEGFR. Although Tyr-700, Tyr-731, and Tyr-774 residues of c-Cbl were significantly phosphorylated by TGF-α exposure in 293_pEGFR, these residues, excluding Tyr-774, were not phosphorylated in 293_p15.
**Conclusion:** Based on these results, we concluded that mutant EGFR with a 15-bp deletion undergoes less protein degradation than wild type EGFR due to diminished binding to c-Cbl ubiquitin ligase. There is a possibility that the mutant EGFR alters substrate specificity and the alteration of phosphorylation status in c-Cbl possibly decreases its binding to EGFR.

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**The RANKL inhibitor osteoprotegerin (OPG) inhibits tumor growth, prevents tumor-induced osteolysis, and significantly improves survival in a mouse model of breast cancer bone metastasis**

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**Background:** Bone metastases from breast cancer cause severe skeletal morbidity including fractures and hypercalcemia. Tumor cells in bone can induce activation of osteoclasts, which mediate bone resorption and release of growth factors from the bone matrix, resulting in a cycle of bone breakdown and tumor proliferation. RANKL acts as an essential mediator of osteoclast function and survival, which is opposed by a soluble decoy receptor, OPG. RANKL inhibition by OPG-Fc can prevent tumor-induced osteolysis and decrease skeletal tumor burden. Using bioluminescence imaging (BLI) in a mouse model, we monitored the anti-tumor efficacy of RANKL inhibition on MDA-MB-231 human breast cancer cells longitudinally and tested the role of RANKL inhibition in overall survival.
**Methods:** After injection of luciferase-labeled MDA-231 cells into the left cardiac ventricle, tumor burden was tracked non-invasively by BLI. BLI measurements of the hind limbs were correlated to histological analysis of tumor growth, and osteolysis was analyzed by x-ray histomorphometry. Osteoclasts were measured by TRAP staining in sections of femurs and tibias. OPG-Fc (0.3 and 3.0 mg/kg, 2×/week) was administered in both prevention (begin day 0) and therapeutic (begin day 7) settings. In the therapeutic setting, mice with established bone metastases continued treatment until they became moribund and/or experienced hind limb paralysis, at which point they were euthanized (blinded analysis). Based on these criteria, a Kaplan Meier analysis of survival was performed.
**Results:** In the prevention setting, OPG-Fc (3.0 mg/kg) resulted in a 78% decrease ($p = 0.0002$) in BLI tumor burden vs. vehicle at day 25. Tumor burden as measured by histology also decreased by 78%