panobinostat in NSCLC and small-cell lung cancer (SCLC) cell lines and in xenograft models.
Materials and Methods: Effects of panobinostat on proliferation (IC50) and viability (LD_{50}) of a panel of SCLC and NSCLC cell lines were measured by MTS assay. Histone acetylation levels were assessed by immunoblotting. Single-agent activity or effect in combination with standard chemotherapeutic agents was assessed in human cell lines or primary tumor xenograft models of NSCLC and SCLC. In SCLC models, panobinostat was combined with cisplatin and etoposide.
Results: Panobinostat significantly inhibited growth of all treated lung cancer cell lines at low nanomolar concentrations (IC_{50} 5−85 nM). Interestingly, whereas low nanomolar concentrations of panobinostat induced death of all SCLC cell lines treated (LD_{50} 2.5−84 nM), only a subset of NSCLC cell lines were killed (LD_{50} 59−>1000 nM). In addition, panobinostat treatment led to increased histone acetylation, upregulation of *p21* expression, and caspase activation in SCLC cell lines, consistent with its potent effect on inhibiting cell viability. *In vivo*, single-agent panobinostat induced profound tumor regression in SCLC models and tumor growth inhibition in NSCLC models. In human cell line and patient-derived primary xenografts of SCLC tumors in mice, combination of panobinostat with standard chemotherapeutic agents resulted in enhanced anti-tumor activity compared with the effect of the single agents.
Conclusions: Panobinostat exhibits significant anticancer effects in SCLC and NSCLC in both *in vitro* and *in vivo* models at clinically attainable concentrations. These studies support the ongoing clinical evaluation of panobinostat as a promising novel therapy in the treatment of lung cancer.

152 POSTER

Prevention and treatment of bortezomib-induced peripheral neuropathy by the Hsp90 inhibitor tanespimycin (KOS-953) in the rat

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Background: Chemotherapy-induced peripheral neuropathy (CIPN) is a severe adverse effect for many chemotherapeutics, including taxanes, vinca alkaloids, platinum compounds, as well as the proteasome inhibitor, bortezomib. CIPN can be a dose limiting toxicity requiring a reduction of the therapeutic dose in order for patients to achieve tolerability, thus diminishing their potential for a successful therapeutic outcome. Prevention or reversal of CIPN can enable patients to tolerate the recommended therapeutic dose, prolong the time on drug and thus enhance the potential for a successful chemotherapeutic treatment outcome. Tanespimycin is a potent Hsp90 inhibitor that has shown anticancer activity when combined with standard of care in multiple myeloma and in HER2-positive metastatic breast cancer. The incidence and severity of CIPN in multiple myeloma patients treated with tanespimycin combined with bortezomib was reduced compared to the incidence commonly observed in patients treated with bortezomib alone. Based on this clinical observation, we conducted studies in a rat model of bortezomib-induced peripheral neuropathy to confirm the neuroprotective effect of tanespimycin.
Materials and Methods: Male Sprague-Dawley rats were treated intravenously with either bortezomib (0.2 mg/kg, twice weekly) or bortezomib combined with tanespimycin (0.2 mg/kg for bortezomib and 20 mg/kg for tanespimycin, twice weekly). Neuropathy symptoms were measured by paw withdraw pressure threshold using a von Frey Hair asthesiometer.
Results: Rats developed CIPN after four doses of bortezomib at 0.2 mg/kg, administered intravenously twice weekly. In contrast, when rats were given a combination treatment of bortezomib (0.2 mg/kg, twice weekly) and tanespimycin (20 mg/kg, twice weekly), they were completely devoid of CIPN, as measured by the paw withdraw threshold tests using a von Frey Hair asthesiometer. Furthermore, after rats had developed CIPN with 6 doses of bortezomib, their symptoms of neuropathy had disappeared after two doses of tanespimycin, while maintaining bortezomib treatment at the same time.
Conclusion: The Hsp90 inhibitor tanespimycin prevents and alleviates CIPN induced by bortezomib in rats, thus confirming and further substantiating its neuroprotective effect in humans.

153 POSTER

IPI-493, a potent, orally bioavailable Hsp90 inhibitor of the ansamycin class

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Background: The cellular chaperone heat shock protein 90 (Hsp90) has emerged as an important target in cancer due to its essential role in several key oncogenic signaling pathways. In several types of cancer (melanoma, NSCLC, breast cancer) high expression of Hsp90 has been associated with either disease progression or decreased survival. Several classes of Hsp90 inhibitors have recently advanced into clinical trials including ansamycin derivatives that are semi-synthetic derivatives of the natural product geldanamycin (e.g. 17-AAG, IPI-504, 17-DMAG) or small molecule synthetic derivatives designed from structure-based drug design (e.g. purine derivatives, isoxazoles, pyrazoles). Geldanamycin derivatives incorporate the advantages of natural products (high affinity and selectivity) but certain derivatives have suffered from either unacceptable toxicity (Geldanamycin, DMAG) or low solubility/oral bioavailability (17-AAG). We have developed an oral formulation for 17-AG (IPI-493), the primary long-lived metabolite of IPI-504 and 17-AAG and report herein its in vitro and in vivo properties.
Results: Multiple formulations of IPI-493 were designed and tested for oral bioavailability. Formulations were identified that led to significantly improved systemic exposure in beagle dogs after oral administration. Similar formulations also led to high IPI-493 exposure in mice following oral dosing. In a mouse xenograft model of TKI resistant NSCLC known to be sensitive to Hsp90 inhibitors (NCI-H1975), this optimal formulation of IPI-493 inhibited tumor growth by 87% at an oral dose of 100 mg/kg, QOD. We have also characterized the biochemical and cellular activity of IPI-493. The affinity of IPI-493 to purified Hsp90 is high and not significantly influenced by reduction to the hydroquinone (Ki 17-AG quinone = 21 nM, Ki 17-AG hydroquinone = 3 nM). This is in marked contrast to other ansamycin derivatives (e.g. 17-AAG) where the hydroquinone (IPI-504) is approximately 30 times more potent than the quinone derivative. When tested against a panel of normal and cancer cell lines, IPI-493 selectively inhibits the growth of cancer cells over normal cells. Unexpectedly, in a subset of cancer cell lines we find IPI-493 to be significantly more potent than 17-AAG.
Conclusion: We have developed an oral formulation for 17-AG (IPI-493), the major metabolite of IPI-504 and 17-AAG. This compound binds tightly to purified Hsp90 and the binding is not significantly dependent on the redox environment. Furthermore, IPI-493 is more potent than 17-AAG and has a longer half-life in vivo. To our knowledge, this is the first successful development of 17-AG as a potential therapeutic, as demonstrated by in vivo efficacy data. IPI-493 is expected to enter Phase 1 clinical development in 2008.

154 POSTER

Hsp90 is expressed and represents a novel target in human oesophageal cancer using the inhibitor 17-allylamino-17-demethoxy-geldanamycin

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Background: Esophageal cancer is an aggressive disease. At diagnosis, over 50% of patients present with distant metastasis and standard treatments are in most cases not effective enough. In cancer cells, Hsp90 functions to protect and stabilize overexpressed or mutated signal transduction proteins, thus indirectly promoting cell growth and survival. The aim of this study was to investigate the role of Hsp90 in esophageal cancer, which may consequently serve as a therapeutic target for treatment of oesophageal cancer.
Methods: Hsp90 expression of 81 oesophageal cancer patients was investigated by immunohistochemical staining. Hsp90 expression in cell lines and EGF receptor signalling pathway was analysed by western blot. For proliferation analysis, cells were treated with 17-allylamino-17-demethoxygeldanamycin (17-AAG), an inhibitor of Hsp90, at increasing concentrations and counted in a cell counter (Beckman). For irradiation and clonogenic survival assay, cells were exposed to irradiation of 2−8 Gy and colonies allowed to form for 10 days. After haematoxylin staining the number of colonies were counted.
Results: In squamous cell carcinoma, a marked upregulation of Hsp90 could be noted in dysplastic epithelium and invasive cancer compared to normal epithelium. Regarding adenocarcinoma, Hsp90 was expressed in neoplastic epithelium but to a certain extent also in normal non-neoplastic glands. Hsp90 was abundantly expressed in nine esophageal carcinoma cell lines analyzed. An interaction between EGF receptor and Hsp90 was seen, suggesting that the EGF receptor is an Hsp90 client protein. Cells were treated with increasing concentrations of 17-AAG, and a significant downregulation of EGF receptor in a dose- and time-dependent manner was observed. In addition, EGF-induced activation of the downstream signaling proteins Erk and Akt was inhibited by 17-AAG. Evaluating Hsp90 as a therapeutic target in esophageal cancer, 17-AAG was used in cell proliferation experiments, where a dose- and time-dependent reduction in

cell proliferation was seen. Exploring the possibility of radiosensitization, results showed that treatment with 17-AAG indeed sensitized esophageal cancer cells towards gamma photon radiation in an at least addidative manner.
Conclusions: Hsp90 was specifically expressed in oesophageal cancer. Our data support the notion that Hsp90 inhibition may be a way to sensitize esophageal tumor cells toward radiotherapy, conceivably by downregulation of EGF receptor expression and inhibition of downstream proliferative and anti-apoptotic signaling pathways.

155 POSTER
Pharmakokinetic/phamacodynamic relationship in human xenograft models and PBMC's treated with the Hsp90 inhibitor NVP-AUY922

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NVP-AUY922 is a synthetic inhibitor of Heat Shock Protein 90 (Hsp90) ATPase activity. Hsp90 is a ubiquitously expressed molecular chaperone which plays an important role in the conformational maturation and activation of a large number of "client" proteins that have been implicated in oncogenesis. Hsp90 has attracted considerable interest as a therapeutic target for cancer treatment since Hsp90 ATPase inhibition induces the simultaneous degradation of multiple oncogenic proteins. Antitumor activity and tolerability of NVP-AUY922 was determined in preclinical cancer animal models. The pharmacokinetic profile of NVP-AUY922 in plasma and tumor tissues was evaluated at well tolerated, efficacious dose levels. We observed tumor specific compound retention and rapid tissue and plasma clearance. *Ex vivo* PK/PD analyses of tumor tissues upon acute dose or after termination of *in vivo* efficacy studies showed a time-dependent correlation between compound concentration and down-regulation of target proteins. In addition to the effect on targeted oncogenic proteins, other markers of activity were investigated.
Disruption of the Hsp90 chaperone hetero-complex, resulting in a loss of Heat Shock Factor-1 (HSF-1) repression and induction of Heat shock protein 70 (Hsp70) is considered to be one of the most robust biomarkers to detect inhibition of Hsp90 ATPase activity in clinical trials. In the current dose escalation study in patients with solid tumors, induction of Hsp70 is being evaluated for pharmacodynamic effect of AUY922 treatment in a surrogate tissue, peripheral blood monocytes (PBMC), at multiple time-points. In concordance with pre-clinical studies, there is a good correlation between NVP-AUY922 blood concentration and the effect on Hsp70 levels in PBMCs.

156 POSTER
BIIB021, a fully synthetic oral small molecule inhibitor of Hsp90, shows potent anti-tumor activity as a single agent and in combination with standard of care therapies in preclinical tumor models

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The heat shock protein 90 (Hsp90) is a molecular chaperone that functions in the maturation and stabilization of its so called client proteins. Hsp90, in complex with other co-chaperone proteins, catalyzes the conformational changes required for client protein function via its ATPase activity. Mutant and over-expressed oncoproteins that drive malignant progression are particularly dependent on Hsp90 chaperone activity. In tumor cells, inhibition of Hsp90 results in degradation of these proteins followed by cell death making Hsp90 a target of substantial interest for cancer therapy. BIIB021, formerly known as CNF2024, is a novel, fully synthetic oral inhibitor of Hsp90. BIIB021 binds competitively with geldanamycin in the ATP binding pocket of Hsp90. In cell-based assays with a variety of human cell lines, BIIB021 induced the degradation of HER-2 and other key client proteins including AKT, ERK, Raf-1, EGFR. BIIB021 upregulated the expression of the heat shock proteins including Hsp70 and Hsp27, in a similar manner to other Hsp90 inhibitors. Oral administration of BIIB021 led to the degradation of HER-2, the induction of apoptosis and the inhibition of tumor growth in several human tumor xenograft models. BIIB021 showed antitumor activity when administered on both daily and intermittent dosing schedules. Furthermore, the administration of BIIB021 in combination with a range of standard of care chemotherapies and molecularly targeted therapies enhanced the activity of either monotherapy alone. BIIB021 is a promising new Hsp90 inhibitor that is fully synthetic and designed to be given orally, thereby supporting flexible therapeutic dosing schedules. BIIB021 is currently undergoing Phase 1 and Phase 2 clinical trials in hematological and solid tumors.

Hormonal agents

157 POSTER
BMS-641988: A highly potent and rationally designed inhibitor of the androgen receptor (AR), with efficacy in castration resistant human prostate cancer xenograft models

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Background: 1st-line androgen ablation therapy (AAT) is a highly effective treatment for metastatic prostate cancer (PC), however, most patients will progress to a castration resistant form of the disease. Research suggests that reactivation of the AR pathways is a major factor leading to castration resistant PC, suggesting that new agents that act at the level of the AR should be effective in treating this disease.
Methods: Through AR co-crystal structures, we applied structure based drug design to create a new, highly potent inhibitor of the AR, BMS-641988 (988). The activity of 988 was examined in a series of *in vitro* reporter assays and *in vivo* PD and human PC xenograft models.
Results: 988 demonstrates increased potency compared to the clinically used anti-androgen bicalutamide (BC), in both binding affinity to AR and inhibition of AR mediated transactivation in cell based reporter assays.

Table 1

	BMS-641988	bicalutamide
Radioligand competitive binding assay in MDA-MB453 cells		
Ki (nM)	1.8±02	37±3
PSA driven reporter transactivation		
MDA-MB-453 (wtAR) IC50 (nM)	11±3	166±38
LNCaP (mutant AR) IC50 (nM)	110±62	680±376
CWR22rv1 (mutant AR) IC50 (nM)	322±130	3432±962

988 potently inhibits the AR dependent growth of the prostate and seminal vesicle in a mature and immature rat prostate weight model. In these models, 988, compared to BC, created a proteomics and prostate histological profile that better recapitulated that of surgical castration. 988 exhibited a greater average %-tumor growth inhibition compared to BC (D%TGI of >90 vs. <50) in the human PC xenograft model CWR-22LD1 (LD1), an androgen insensitive variant of the CWR22 line. 988 is efficacious in a LD1 model made refractory to treatment with BC. 988 is highly efficacious in the LuCaP 23.1 human PC xenograft model, a model derived a patient who failed AAT, inducing stasis at 3 mg/kg (po). Using the LD1 line, in a genome wide AR-positioning analysis of cells treated with either 988 or BC, and in a transcriptomic analysis of tumors from mice treated with 988, BC, or surgical castration, 988 promotes a gene expression profile distinct from BC and more similar to castration.
Conclusion: BMS-641988 shows potent activity in a variety of PC tumor lines and xenograft models derived from patients who failed AAT. Compared to BC, 988 generates a profile more indicative of full castration in rodents. Based on the promising preclinical activity of BMS-641988, it has been advanced into clinical development for the treatment of PC.