Table 1

Dose level	>50% Decline	>90% Decline
60 mg/day	41% (9/22)	9% (2/22)
150 mg/day	57% (13/23)	13% (3/23)
240 mg/day	50% (14/28)	29% (8/28)

Conclusions: MDV3100 is a novel AR antagonist in clinical investigation. The observed PSA responses are consistent with the inhibition of AR signaling. MDV3100 has been well-tolerated to date and appears to be a promising candidate for the treatment of CRPC. Pt recruitment and follow-up are continuing. The analyses of the associations among PSA, CTC, radiographic, and PET outcomes are ongoing and will be presented.

161 POSTER

Potent anticancer activity of panobinostat (LBH589) in models of hormone-refractory prostate cancer (HRPC): targeting the androgen receptor

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Background: Panobinostat (LBH589) is a highly potent pan-deacetylase (pan-DAC) inhibitor which has demonstrated preliminary clinical efficacy in hematologic and solid malignancies, including prostate cancer. Panobinostat inhibits the molecular chaperone heat shock protein 90, promoting degradation of client proteins such as the androgen receptor (AR) and human epidermal growth factor receptor type 2 (HER-2), which play critical roles in the growth and survival of prostate cancer. The anticancer effects of panobinostat were investigated in both *in vitro* and *in vivo* prostate cancer models, including effects on AR and HER-2 protein levels.
Materials and Methods: Cell lines of known AR status and different degrees of androgen dependence were treated with panobinostat. Effects on cell proliferation (IC_{50}) and viability (LD_{50}) were measured by MTS assay. Levels of target proteins were determined by immunoblotting. Mice bearing AR+, androgen-independent CWR22Rv1 prostate tumor xenografts were treated with panobinostat alone or in combination with docetaxel. Tumor growth inhibition and delay, and AR protein levels, were determined.
Results: Panobinostat inhibited growth of 10 prostate cancer cell lines (IC_{50} 0.9–22.4 nM) and induced potent cytotoxicity in AR+ prostate cancer cells (LD_{50} 20−81.9 nM). Interestingly, AR− cells were sensitive to the antiproliferative effect of panobinostat, but not to panobinostat-induced cell death (LD_{50} >1000 nM). Panobinostat treatment depleted AR and HER-2 in both androgen-dependent and -independent prostate cancer cells. In the hormone-refractory CWR22Rv1 tumor model, single-agent panobinostat induced prolonged tumor stasis, with concomitant depletion of AR from tumor tissues. The combination of panobinostat and standard of care agent docetaxel delayed tumor growth after cessation of treatment, and increased the time to study endpoint of 90 days or 2000 mm^3 tumor volume.
Conclusions: Panobinostat is a potent anticancer agent in both *in vivo* and *in vitro* models of prostate cancer. Panobinostat depletes AR and HER-2 in both AR+ androgen-dependent and -independent prostate cancer cells, and AR in an AR+ hormone-refractory prostate cancer xenograft model at clinically attainable levels. The combination of panobinostat with docetaxel *in vivo* results in enhanced anti-tumor effects and delay of tumor progression. These studies support the continued clinical investigation of panobinostat in HRPC.

162 POSTER

Effects of SOD2 silencing on androgen receptor function and gene regulation: implications for castration-resistant prostate cancer

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Background: Advanced prostate cancer is generally first treated with androgen deprivation therapy. However, tumors become resistant to and grow despite castrate levels of testosterone. Growth and proliferation of castration-resistant prostate cancer (CRPC) is mediated by gain-of-function changes in the androgen receptor (AR) and AR reactivation. Expression of manganese superoxide dismutase (SOD2), which regulates cellular reactive oxygen species, is markedly down-regulated in CRPC when compared to hormone responsive tumors.
Materials and Methods: Here, we knocked down SOD2 expression in AR-expressing LNCaP prostate cancer cells. We performed transcription factor DNA binding assays to determine changes in AR binding that occur with SOD2 knockdown. Furthermore, we performed DNA microarray analysis to identify gene expression changes induced in prostate cancer with SOD2 knockdown.
Results: Gene expression changes induced by SOD2 knockdown results in the up-regulation of genes which are also androgen responsive and 46% of genes up-regulated two-fold by the androgen ligand R1881 are also up-regulated to the same extent with SOD2 knockdown. The induction of many of these genes with SOD2 knockdown, such as VEGFA, is reversible with the antioxidant N-acetylcysteine (NAC), suggesting that this mechanism is directly linked to reactive oxygen species. Furthermore, an array for transcription factor DNA binding activity shows that SOD2 knockdown induces DNA binding by several transcription factors, including AR. SOD2 knockdown-induced AR activation was confirmed by electrophoretic mobility shift assay (EMSA) and was readily reversible with NAC.
Conclusions: These findings show that dysregulation of SOD2 induces AR activity in a reactive oxygen species-dependent manner, and suggests that there may be a role for antioxidant therapy in CRPC.

163 POSTER

The steroid sulfatase inhibitor BN83495 inhibits E1S-stimulated growth of DMBA-induced mammary tumour in rat

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Steroid sulfatase (STS) is a new target for the treatment of steroid hormone dependent diseases such as breast, prostate or endometrial cancer. In breast cancer, estrogens play a major role in the establishment of the disease and between one to two-thirds of tumours are estrogen receptor (ER) positive. Despite current hormonal treatments, improvement is still necessary to achieve better disease control and improve disease outcome. BN83495 is a non-steroidal, non estrogenic, potent, irreversible STS inhibitor that blocks both the formation of E1 from estrone sulfate and androstenediol from DHEAS. The ability of BN83495 to inhibit E1S-stimulated tumor growth in the rat was examined in a DMBA-induced mammary tumor model. Based on median tumor volume and the interquartile range at the end of the treatment period, BN83495 displayed the greatest antitumor activity compared to Tamoxifen or Fulvestrant. Addition of Fulvestrant or Tamoxifen to BN83495 did not improve the potent antitumor activity observed with BN83495 alone. Pharmacokinetic data of BN83945 and effects on estradiol levels are discussed. Altogether, these preclinical results have supported the entry of BN83945 into further clinical trials for estrogen receptor-positive breast cancer patients.

Metastasis and invasion

164 POSTER

Inhibition of CXCR-4 reduces breast cancer xenograft metastasis to multiple organs

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Regulation of metastasis occurs in part through chemokine secretion by distant organs/tissues. The chemokine CXCL12 is constitutively expressed in tissues where metastases develop, specifically lung, liver and bone. The primary receptor for CXCL12 is CXCR4. Expression of CXCR4 on breast cancer cells causes increased bone metastasis and poor overall survival in vivo. In addition, the CXCL12/CXCR4 pathway has been associated with increased migration and invasion of tumor cells in response to hypoxia and tumor angiogenesis through recruitment of bone marrow derived cells. It was hypothesized that treatment of mice with a CXCR4 antagonist would decrease the incidence of metastasis to bone and other organs in an in vivo model. The purpose of this study was to test the efficacy of CTCE-9908, a CXCR4 antagonist, as an antimetastatic agent for breast cancer. GFP-expressing MDA-MB-231 metastatic breast cancer cells were injected into the left cardiac ventricle or the tail vein of athymic mice. Mice were treated with 25 mg/kg CTCE-9908 daily beginning either the day previous to tumor cell injection or the day of tumor cell injection. After 6 or 8 weeks (intracardiac and tail vein injections, respectively),

the presence of foci in multiple organs was assessed using fluorescence microscopy. Incidence of metastasis decreased in the ovary and uterine horn, while incidence in all other organs was unaffected by CTCE-9908 treatment, regardless of injection site or treatment. Lung metastases from tail vein injections decreased only marginally by about 30% with CTCE-9908 treatment. After intracardiac injection, the number and size of the foci decreased in most organs with treatment. The number of foci per femur increased upon treatment with the CXCR4 inhibitor, but the size of foci was greatly decreased. The large metastases in the untreated animals likely obscured the small foci observed in the CTCE-9908 treated animals. Foci in the lung and heart were significantly decreased in number and size after CTCE-9908 treatment. Decreases in the number of foci, although not significant, were also noted in the liver, ribs, kidneys, pancreas and spleen. While treatment with CTCE-9908 did not decrease the incidence of metastasis as hypothesized, it decreased the metastatic burden in all organs examined. Animal survival was not measured but an increase in survival could be predicted as a result of the overall decrease in disease burden. The possible mechanisms of this decrease include changes in apoptosis, proliferation and angiogenesis as well as 'homing' of cells to the secondary sites. All of these will require further examination to understand fully the effect of CTCE-9908 on breast cancer metastasis. Preliminary results from a Phase I/II clinical trial with CTCE-9908 was presented in 2007. Final results are expected to be presented this year.

165 POSTER

Novel therapeutic efficacy of E7080 for controlling experimental metastases of human lung cancer cells in natural killer cell-depleted severe combined immunodeficient mice

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Background: Lung cancer is often characterized by rapid growth and metastatic spread. Because tumor growth and metastases are angiogenesis dependent, there is great interest in therapeutic strategies that aim to inhibit tumor angiogenesis.
Materials and Methods: The therapeutic efficacy of E7080, an orally available multiple tyrosine kinase inhibitor which inhibits VEGFR1−3, FGFR1−4, PDGFRs, RET etc., was examined in experimental multiple-organ metastasis models with human lung cancer cell lines (SBC-5, H1048 and PC14PE6) in natural killer cell-depleted severe combined immunodeficient mice.
Results: E7080 did not inhibit the proliferation of three human lung cancer cell lines (IC50 >1 microM), whereas it inhibited that of human microvascular endothelial cells induced by VEGF (IC50 0.3 nM) and bFGF (IC50 100 nM) in vitro. The large, medium and few amounts of VEGF were detected in the culture supernatant of PC14PE6, SBC-5 and H1048 cells, respectively. Intravenously inoculated human small cell lung cancer SBC-5 cells produced experimental metastases in the liver, lung, and bone on day 28, whereas H1048 cells produced the metastases in the liver, systemic lymph nodes, kidneys and bone on day 56. Human adenocarcinoma PC14PE6 cells yielded massive pleural effusion and lung metastases 28-days after intravenous inoculation. Daily treatment with E7080 (1, 3 and 10 mg/kg), started on day 14 (after the establishment of micrometastases), significantly reduced the amount of pleural effusion and the number of large (>2 mm) metastatic colonies (in the liver, lymph nodes and the lungs) and osteolytic bone lesions. E7080 treatment did not significantly reduce the number of small (<2 mm) metastatic lesions found in the lungs (SBC-5) or kidneys (H1048), consistent with an antiangiogenic mechanism of action. No significant adverse events of E7080 treatment, such as body weight loss were observed in these in vivo experiments. Histochemical analysis of metastatic deposits in the liver showed conspicuous necrosis, indicating that E7080 treatment inhibited angiogenesis in vivo.
Conclusions: These results suggest that E7080 may be of potential therapeutic value in inhibiting the growth of metastatic lung cancer in humans.

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Inhibitors of mitochondrial ATP synthesis show preferential cytotoxicity to pancreatic cancer cells under glucose-deprived conditions

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Background: The tumor microenvironment exerts an important influence on cancer progression. Because of the disorganized vascular systems in tumors, large areas of tissues are exposed to nutrient starvation and hypoxic conditions. Even under these severe growth conditions, certain cancers, such as a pancreatic cancer, which is characterized as hypovascular tumors, show an inherent ability to tolerate such severe conditions. Since chronic deprivation of nutrients seldom occurs in normal tissue, targeting nutrient-deprived cancer cells might be a promising strategy for the development of anticancer agents. The purpose of our study is to identify cytotoxic agents that function preferentially under nutrient-deprived conditions.
Materials and Methods: Human pancreatic cancer PANC-1 cells were cultured in nutrient-rich and nutrient-limited media. The cell survival was determined by the MTT method.
Results: Through screening cultured media of microorganisms and chemical compounds, we found that the NADH-ubiquinone reductase (complex I) inhibitor rotenone, the succinate-ubiquinone reductase (complex II) inhibitor atpenin A5, the ubiquinone-cytochrome c (complex III) inhibitor antimycin A3 and the F1F0-ATPase inhibitor (complex V) oligomycin exhibited preferential cytotoxicity to PANC-1 cells under nutrient-deprived conditions, but exhibited minimal cytotoxicity under nutrient-rich conditions. These compounds preferentially caused cell death under glucose-limiting condition, irrespective of the presence or absence of amino acids and/or serum. Although PANC-1 cells survived nutrient starvation even after 24 h, the intracellular ATP concentrations were markedly decreased. Therefore, inhibitors of mitochondrial ATP synthesis could exert preferential cytotoxicity under nutrient-deprived conditions.
Conclusions: These data indicate that inhibitors of mitochondrial ATP synthesis show preferential cytotoxicity to human pancreatic cancer PANC-1 cells under nutrient-deprived conditions. Therefore, these inhibitors may be useful for anticancer therapy and microenvironment-oriented therapeutic approaches could be a promising strategy for anticancer therapy.

167 POSTER

The EGFR-GEP100-Arf6 pathway in breast cancer invasion and metastasis

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Background: Expression of epidermal growth factor receptor (EGFR) is highly implicated in tumor malignancy. However, it awaits to be clarified whether there exist signaling pathways downstream of EGFR, that are specifically used for tumor invasion and metastasis though not generally used in normal cells. We have shown previously that a small GTPase Arf6 and its downstream effector AMAP1 are both highly overexpressed in invasive breast cancer cells and plays essential roles for their invasion and metastasis. Here, we identify a mechanism by which Arf6 is activated to induce tumor invasion and metastasis.
Material and Methods: We conducted siRNA-mediated knockdown of ArfGEFs expressed in highly invasive breast cancer MDA-MB-231 cells and examined their effects on their Matrigel chemoinvasion activities, in order to identify candidate GEFs responsible for invasion. Lung metastasis were assessed by use of mouse breast cancer 4T1/luc cells, by injecting them into fadpad of Balb/c mice.
Results: There are 16 genes encoded by human genome, bearing the Sec7 (ArfGEF) domain. We found that MDA-MB-231 cells express 10 different types of ArfGEFs and knockdown of GEP100, but not other ArfGEFs, blocked the Matrigel invasion activity. shRNA-mediated suppression of GEP100 also very effectively blocked invasion and metastasis of 4T1/luc cells in vivo. GEP100, via its PH domain, bound directly to phosphorylated Tyr1068 and Tyr1086 sites of EGFR to activate Arf6. Overexpression of GEP100, together with Arf6, caused non-invasive MCF7 cells to become invasive, which was dependent on EGF stimulation.