that mTOR only partially regulates VEGF under hypoxia. To determine whether VEGF production was controlled by Akt signaling, independent of mTOR, we exposed cells to the Akt-selective inhibitor A443654 for 24 hr and VEGF secreted into medium determined. A443654 completely inhibited signaling through Akt as judged by loss of detectable phospho-S6 protein, and hypophosphorylation of GSK-3b. Of note inhibition of Akt was more effective than rapamycin in blocking hypoxia-driven VEGF in 3/3 RMS and 2/4 NB cell lines. Combination of A443654 with rapamycin was additive or synergistic and completely blocked hypoxia-driven increases in VEGF. Because inhibition of Akt may result in unacceptable toxicity (hyperglycemia and hyperinsuliemia), we have explored the effect of blocking the IGF-1R using an antibody (CP-751871) as an alternative strategy. Administration of 0.25 mg to tumor bearing mice resulted in dramatic downregulation of IGF-1R in 4/5 sarcoma xenograft models, associated with a dramatic decrease in pAkt and pS6 levels.
Conclusions: These preliminary results suggest that direct inhibition of IGF-1R may be an interesting approach to modulating VEGF in pediatric sarcoma, and other solid tumors.

45 POSTER

Focal adhesion kinase is a key signalling intermediate in interleukin-8 promoted chemotaxis and adhesion of prostate cancer cells to bone marrow endothelium

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Purpose: To characterize the biochemical and functional relationship between interleukin-8 (IL-8) signaling and focal adhesion kinase (FAK) activation and to define their importance to prostate cancer metastasis.
Experimental design: Immunohistochemistry (IHC) conducted on human prostate biopsy tissue was used to determine the phosphorylation status of FAK in tumour cells relative to IL-8 expression. Experiments using metastatic prostate cancer PC3 cells established the biochemical, molecular and functional importance of FAK to IL-8 promoted cell motility and adhesion to bone marrow endothelial cells (BMECs).
Results: IHC demonstrated normal prostate epithelium to be devoid of FAK expression/activation but expression and autophosphorylation of FAK was detected in tumour cells of locally-invasive and hormone-independent prostate tissue. Statistical analysis confirmed that IL-8 expression correlated with increased autophosphorylation of FAK on Tyr^{397} in prostate cancer cells ($p < 0.001$). Stimulation of PC3 cells with IL-8 induced cell polarization and promoted the redistribution of FAK to sites of focal adhesion. Immunoblotting confirmed that IL-8 induced time-dependent phosphorylation of FAK on Tyr^{397}, Tyr^{576} and Tyr^{925}, that was mediated by a complex signaling cascade downstream of CXCR1 and CXCR2 receptors. Inhibition of FAK activity, using the dominant-negative FRNK construct or through RNAi-mediated depletion of FAK, attenuated IL-8-promoted activation of Rac-GTPase in pull-down assays, abrogated IL-8 promoted chemotaxis and attenuated IL-8 potentiated adhesion of PC-3 cells to BMECs, respectively.
Conclusions: IL-8 signaling regulates FAK activation in prostate cancer cells and is functionally important in mediating IL-8 promoted cell motility and adhesion, consistent with the metastasis promoting function of this chemokine. Our results describe a novel molecular basis to IL-8 promoted metastasis of prostate cancer and indicate the potential therapeutic significance of attenuating IL-8 expression in prostate cancer.

46 POSTER

Genetically engineered PAI-1 in anti-angiogenic and anti-metastatic therapy

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Abnormal proteolytic activity of urokinase (uPA) is one of the factors causing metastasis and angiogenesis. Thus eradication of uPA activity might result in inhibition of these processes. The small molecular inhibitors of uPA and plasminogen activator inhibitor (PAI-1) have been successfully used to reduce the angiogenesis and tumor growth. While uPA inhibitors were used we have observed reduction of sprout formation of human vascular endothelial cells, lowering blood vessel density in chick embryo chorioallantoic membrane (CAM) assay and reduction of tumor size of xenografts of human prostate LnCAP and DU145 cancer cells.
Plasminogen activator inhibitor (PAI-1) that inhibits the uPA could be used as an inhibitor of angiogenesis. However, wild PAI-1 is not stable and converts into the latent form in t1/2 $\approx$ 2 hours. This conversion is associated with partial insertion of the reactive loop (P4−P10′) into the PAI-1 molecule. In such conformation, P1−P1′ are not accessible for reaction with uPA. By 2 to 6 point mutations that could form disulfide bridges in PAI-1s in proximity of A3, A5 strands, we have extended half-life of this protein up to ~650 h. This PAI-1 is called VLHL PAI-1 and is fully functional as demonstrated by uPA inhibition. Additional, genetically engineered clone was produced by deleting part of this protein and lowering its size to create molecule that is more therapeutically desired. Mutant of Arg346 $\rightarrow$ Ala produced VLHLns PAI-1 that do not react with uPA and will be used as negative control.
Using baculovirus expression system PAI-1s were expressed in Sf9 insect cells and purified using affinity tag (6His). In single step purification we achieve +95% purity. The identity of PAI-1 was conformed by tandem liquid chromatography-mass spectroscopy. Disulfide bridge of VLHL PAI-1 could be reduced by DTT and reduced cysteine can't keep A3 and A5 strands together that is prerequisite of extending PAI-1 activity. Reduced form of VLHL PAI-1s convert into latent form as wPAI-1 does, and do not inhibit uPA. These conform our assumption of importance of the disulfide bridges in extending the half life of PAI-1.
Novel PAI-1 was fully functional against uPA and showed anti-angiogenic activity in the in vitro and in vivo models. Such prolonged serpin activity, which is therapeutically desired in cancer treatment could launch a new class of novel anti-cancer agents based on Cys mutated PAI-1s.

47 POSTER

A phase II study of Sorafenib (BAY 43–9006) in recurrent and/or metastatic squamous cell carcinoma of the head and neck (SCCHN) and nasopharyngeal cancer (NPC): final results

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Background: Sorafenib is an oral multi-kinase inhibitor targeting Raf kinase and VEGFR-2 among others. As the Ras-Raf-MAPK-ERK signaling pathway and angiogenesis are thought to play a significant role in the pathogenesis of head and neck cancers, we conducted a phase II study of sorafenib in recurrent and/or metastatic SCCHN and NPC to determine its efficacy and safety in this patient population.
Patients and Methods: This is a single arm, two-stage phase II trial. Sorafenib was administered orally at 400 mg BID continuously. Patients had $\leqslant$1 line of chemo for recurrent disease, performance status (PS) ECOG 0–2, and adequate organ function. Response was evaluated every 8 weeks according to RECIST criteria. At the end of stage one, efficacy criteria for further accrual were not met but the study was amended to enroll an additional 5 patients for pharmacodynamic evaluations. The biologic effects of sorafenib on tumors were assessed before and 4 weeks after treatment initiation.
Results: We enrolled 28 patients, of whom 27 and 26 were eligible for toxicity and efficacy evaluations. Median age was 53 years (range 37−77); 63% patients were male; 89% had PS 0 or 1; 74% SCCHN and 26% NPC; 70% of patients received prior chemotherapy, 48% had prior first-line chemotherapy for their recurrent and/or metastatic disease. In total, 72 cycles have been administered with a median of 2 cycles per patient (range 1–7). Most common adverse events (AE), at least possibly related to sorafenib, were fatigue in 79%, lymphopenia in 42%, mucositis in 42%, anemia in 35%, hand-foot skin reaction in 29% and hypertension in 28% of cycles. Most common grade 3 AEs were lymphopenia and fatigue in 17% and 7% of cycles. No grade 4 AEs were observed, 2 deaths on study were unlikely related to sorafenib. One patient with SCCHN (3.7%) had a confirmed partial response, 10 (37%) had stable disease ranging from 2 to 6 cycles and 15 patients (55.6%) had progressive disease. Median time to progression was 1.8 months (95% CI: 1.6–3.4) and median overall survival was 4.2 months (95% CI: 3.6–8.7). Results of the PD analysis will be presented at the meeting.
Conclusions: Sorafenib was well tolerated in this group of patients. Although the criteria for the second stage were not met, single-agent sorafenib has modest anti-tumor activity, comparable to single-agent erlotinib and gefitinib. Further evaluation of sorafenib in combination with other agents may be warranted in SCCHN and NPC.

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Distinct gene expression profiles and cell death pathways in clear-cell renal cell carcinoma (CCRCC) and colorectal carcinoma (CRC) cells:r elationship to hypoxia, von Hippel Lindau protein (pVHL) expression and anti-tumor activity of sorafenib

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Background: The interaction between pVHL and hypoxia is important in regulating target genes in CCRCC cells. Sorafenib, a multi-kinase inhibitor

is clinically effective for the treatment of metastatic CCRCC and we sought to further define mechanisms governing activity of this agent in cell lines that lacked pVHL (VHL^-) or expressed wild-type pVHL (VHL^+).
Material and Methods: The CCRCC cell lines evaluated were: CAKI-1 (VHL^+), CAKI-2 (VHL^-), an isogenic pair of 786-O-neo (vector control, VHL^-) or 786-O-VHL (VHL^+) cells. HCT-116/p53 +/+ (VHL^+) colorectal carcinoma (CRC) cells were also evaluated. Cells were treated in the absence (control) or presence of sorafenib (2.5−20 μM) for 24–96 hours at 37ºC in an atmosphere of either normoxia (21% O_2) or hypoxia (1% O_2), 5% CO_2 and the remainder N_2. Gene expression analysis of control and sorafenib treated cells was carried out using a custom cancer cDNA array and real-time RT-PCR. Fluorescence microscopy following staining with Hoechst 33342 plus propidium iodide was used to analyze cell death by apoptosis and/or necrosis.
Results: In VHL^+ CCRCC cells, exposure to 1% O_2 relative to 21% O_2, led to a gene expression profile that was distinct from CCRCC VHL^- cells, which included increased expression (2 to 5-fold) of angiogenesis (VEGF) and anti-apoptosis (TNFAIP3 & MCF2) genes and a decreased (>2-fold) expression of an apoptotic (TNFRSF25) gene. The changes in gene expression profile in CRC HCT-116/p53 +/+ (VHL^+) cells exposed to 1% O_2 relative to 21% O_2, while similar to CCRCC cells, differed in a >3-fold increase in expression of the apoptotic gene, TNFRSF25. Although exposure to 1% O_2 led to ~2-fold resistance to the anti-proliferative effects of sorafenib in CCRCC cells that were VHL^+ relative to VHL^- cells, sorafenib treatment in 1% O_2 led to a >2-fold decrease in expression of the angiogenesis and anti-apoptotic genes. Treatment with sorafenib (10–20 μM) for 48h followed by staining with Hoechst plus propidium iodide showed that while cell death was primarily (>80%) by necrosis in CCRCC cells, apoptotic cell death was the predominant (>95%) mechanism in HCT-116 cells. Apoptotic or necrotic cell death induced by sorafenib was unaffected by VHL status and normoxia or hypoxia.
Conclusions: In contrast to CCRCC VHL^+ cells, hypoxia led to upregulation of the apoptotic gene TNFRSF25 in the VHL^+ CRC cells. Anti-proliferative effects of sorafenib were primarily by necrosis in CCRCC cells and by apoptosis in CRC cells.

49 POSTER

Experimental tumor models with high LDH activity are efficiently targeted by treatment with PTK787/ZK222584, an oral multi-VEGF tyrosine kinase inhibitor

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Background: PTK 787/ZK 222584 (PTK/ZK) is a small molecule anti-angiogenic inhibitor that blocks all known VEGF receptor tyrosine kinases. Recent clinical data strongly suggested that colorectal cancer patients with a high serum LDH (lactate dehydrogenase) activity did preferentially benefit from treatment with PTK/ZK. Like VEGF LDH can be up-regulated by hypoxia which may be the link between these two processes. Here, we present early findings on the relationship between LDH activity and targeting the VEGF signaling pathway in vivo using a small molecule inhibitor.
Material and Methods: For in vivo analyses of PTK/ZK's effects on tumor growth a number of tumor cell lines of different origin were transplanted either onto nude or immune-competent mice. PTK/ZK was applied mostly with a dose of 100 mg/kg daily p.o. During the course of the experiments, tumor area/volume and mouse body weights were recorded, and following experimentation, the animals were sacrificed and tumor weight was determined. The blood was collected and the serum was used for the determination of the LDH enzyme activity.
Results: In all tumor models with a LDH activity higher than ~4,500 U/L treatment with PTK/ZK was efficacious, i.e. a tumor growth inhibition of >50%. In models with a lower LDH activity PTK/ZK was in general less efficacious. However, interestingly, some models were found where PTK/ZK was efficacious despite a lower LDH activity, e.g, DU145 a hormone-independent human prostate carcinoma model.
Conclusion: PTK/ZK is a multi-VEGF receptor tyrosine kinase inhibitor with potent anti-angiogenic activity in a variety of tumor models. Our recent data strongly suggest that preferentially, but not exclusively, tumor models with a high LDH activity, can be efficiently targeted by treatment with PTK/ZK. These pre-clinical findings are in strong accordance with recent findings from phase III clinical studies with PTK/ZK in which patients with a high LDH activity did preferentially benefit from treatment with this compound. Thus, if these clinical and pre-clinical findings can be confirmed in future clinical trials LDH may serve as a patient stratification marker and prognostic factor for PTK/ZK treatment.

50 POSTER

Inhibition of Src kinase with the dual Src/Abl kinase inhibitor AZD0530 reduces bladder tumour growth and the development of mixed osteolytic/osteosclerotic lesions in bone

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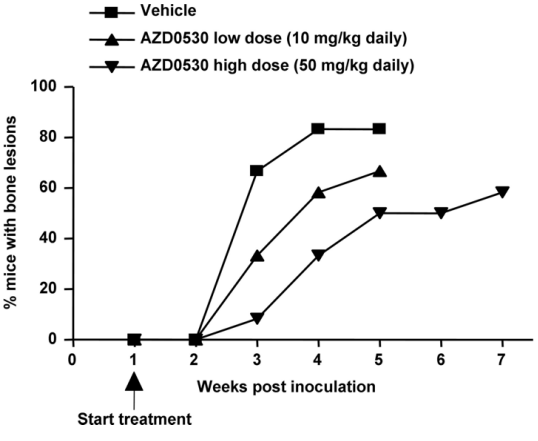
Src kinase plays a central role in growth factor and integrin signalling, regulating a diverse array of cellular functions including proliferation, migration and invasion. Recent studies have demonstrated that Src activity is frequently elevated in human tumours and correlates with disease stage. In normal mice, disruption of Src impairs osteoclast bone resorbing activity, resulting in osteopetrosis. In the osteoblast lineage, Src signalling is also important for osteoblast differentiation and for prevention of osteocyte apoptosis. In bladder cancer, c-Src kinase activity has been reported in tissue lysates at all stages of carcinogenesis, indicating that c-Src is expressed throughout tumour development. We have used the metastasis-selected B1 variant of the TSU-Pr1 human bladder carcinoma cell line, which induces mixed osteolytic/osteosclerotic lesions in bone, to examine the impact of inhibition of Src kinase using AZD0530 on the growth of bladder cancer tumours and the nature of associated bone lesions. AZD0530 is a dual specific Src/Abl kinase inhibitor, which has been shown to reduce biomarkers of bone resorption in healthy volunteers, and thus may have therapeutic benefit in treating osteoclast-driven metastatic bone disease.
Male SCID mice were inoculated with 10^4 TSU-Pr1-B1 cells intratibially (n = 12/group). After allowing one week for tumour establishment, mice were gavaged daily with either Src inhibitor AZD0530 (50 mg/kg or 10 mg/kg) or vehicle (1% polysorbate 80). The development of bone lesions was tracked using weekly high resolution x-rays (Faxitron). At the end of the experiment, which was 5 weeks post-tumour cell inoculation for vehicle and low-dose AZD0530, and 7 weeks post-inoculation for high dose AZD0530 (once bone lesions had reached a similar size to that observed in the vehicle group), all tibia were collected and examined histologically.
Treatment of tumour bearing mice with the novel Src inhibitor AZD0530 significantly inhibited both tumour growth and development of bone lesions (both number and size) in a dose-dependent manner. However, once bone lesions developed, the mixed osteolytic/osteosclerotic nature of lesions was not altered by AZD0530 treatment. These observations suggest that the use of AZD0530 may provide an effective treatment in inhibiting metastatic bone lesions, particularly those in which aberrant osteoclast activity plays an important role.



51 POSTER

Phase 1 single-dose safety, PK, and food-effect study of PTC299, a novel VEGF expression inhibitor for treatment of solid tumors

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Background: VEGF production is highly regulated posttranscriptionally through the 5′- and 3′-untranslated regions (UTRs) of VEGF mRNA. PTC299 is a novel, orally bioavailable, small molecule designed to act through the 5′-UTR to inhibit VEGF production. In multiple preclinical human tumor xenograft models, PTC299 reduces tumor and circulating