

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 μ M) for 24–96 hours at 37°C in an atmosphere of either normoxia (21% O₂) or hypoxia (1% O₂), 5% CO₂ and the remainder N₂. 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% O₂ relative to 21% O₂, 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% O₂ relative to 21% O₂, while similar to CCRCC cells, differed in a >3-fold increase in expression of the apoptotic gene, TNFRSF25. Although exposure to 1% O₂ 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% O₂ led to a >2-fold decrease in expression of the angiogenesis and anti-apoptotic genes. Treatment with sorafenib (10–20 μ M) 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.

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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.

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POSTER

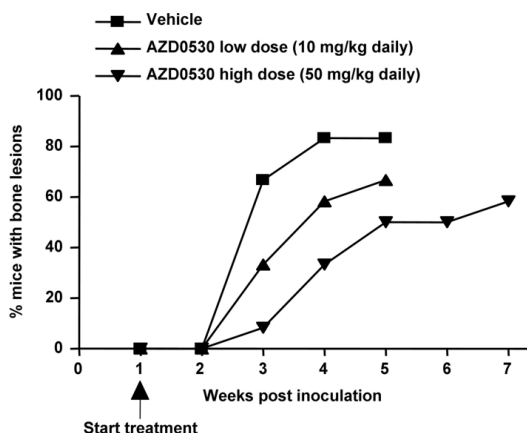
Inhibition of Src kinase with the dual Src/Abl kinase inhibitor AZD0530 reduces bladder tumour growth and the development of mixed osteolytic/osteosclerotic lesions in bone

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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 10⁴ 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 tibiae 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.



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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

plasma VEGF concentrations, decreases tumor microvessel density, and substantially slows tumor growth. When given together with cytotoxic agents or with bevacizumab, PTC299 enhances tumor growth delay.

Methods and Materials: This Phase 1 randomized, double-blind, placebo-controlled, escalating single-dose study in healthy adult volunteers includes 2 stages with dose finding performed in Stage 1 and food effects evaluated in Stage 2. To determine PTC299 safety, PK, and effects on plasma VEGF levels, subjects are followed with clinical observations, safety laboratory testing, and frequent plasma sampling.

Results: 8 subjects (4 males, 4 females; 6 PTC299, 2 placebo) have been enrolled at each of 4 progressively higher dose levels, for a total of 32 subjects with median age [range] of 45 [23–55] years. PTC299 has been well tolerated with no serious, dose-limiting, or definitively drug-related adverse events. PK data indicate dose-proportional increases in plasma exposures. Target trough plasma concentrations ($\geq 0.1 \mu\text{g/mL}$) active in xenograft models have been exceeded for ≥ 12 hours at doses $\geq 0.3 \text{ mg/kg}$, supporting the potential for dosing once or twice per day. The PK data are well-described by a 2-compartment model. Further accrual is ongoing.

Conclusions: PTC299 is the first drug specifically designed to modulate post-transcriptional control mechanisms to treat human disease. In this initial clinical study, PTC299 shows safety at plasma exposures associated with preclinical activity. Final data on dose ranging, safety, PK, food effects, and plasma VEGF levels will be presented at the meeting. PK modeling will be used to project an appropriate dosing regimen for subsequent multiple-dose studies in cancer patients.

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Preclinical development of PTC299: an orally bioavailable small molecule drug that selectively inhibits the production of VEGF protein, tumor growth, and microvessel density

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Background: We developed a phenotypic high-throughput screening platform, termed GEMS (Gene Expression Modulation by Small molecules), to identify small molecules that modulate gene expression by targeting processes controlled by the untranslated regions (UTRs) of the mRNA (e.g. message stability and translation initiation). This platform was utilized to identify molecules that inhibit the production of vascular endothelial growth factor A (VEGF). Following an extensive analoging campaign PTC299 was synthesized, evaluated biologically and pharmaceutically, and developed as an antiangiogenic agent for use in cancer and other pathologies with aberrant angiogenesis.

Materials and Methods: A high throughput screen was conducted using cells stably expressing a reporter gene under the control of UTRs from the VEGF mRNA. Hits that reduced the expression of the reporter were confirmed and SAR was driven by measuring inhibition of hypoxia-induced VEGF production in cells. VEGF protein was measured by ELISA.

Results: PTC299 inhibits the expression of all major isoforms of VEGF-A in cell culture across a wide variety of tumor types, with EC50 values in the low nanomolar range, and is highly selective for inhibition of VEGF expression when compared to a number of other growth factors, cytokines, and intracellular proteins. Results from mechanism of action studies suggest that PTC299 acts by inhibiting the 5' UTR-dependent translation of the VEGF protein. In xenograft-bearing animals treated with PTC299 there was a marked reduction of intratumor and circulating hVEGF (up to 90%) and a normalization of tumor vasculature within a matter of days. With continued dosing of PTC299, the reduction in VEGF correlated with a significant reduction in tumor growth in a number of subcutaneous and orthotopic xenograft models, including colorectal, NSCLC, breast, fibrosarcoma and neuroblastoma. PTC299 also demonstrated additive or synergistic activity in combination with a number of standard-of-care cytotoxics, including taxol, doxorubicin and CPT-11. PTC299, with a therapeutic window of >30 , was well-tolerated in IND-enabling toxicology studies that included multiday dosing in rats and dogs.

Conclusions: PTC299 is effective in reducing the production VEGF and controlling tumor growth in a number of model systems. IND-enabling toxicology studies have demonstrated a sufficient safety window and a dose-ranging Phase I clinical trial in healthy volunteers was initiated in April, 2006.

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POSTER

A phase II, multicenter, randomized clinical trial to evaluate the efficacy and safety of bevacizumab (Avastin®) in combination with either chemotherapy (docetaxel or pemetrexed) or erlotinib hydrochloride (Tarceva®) compared with chemotherapy alone for treatment of recurrent or refractory non-small cell lung cancer

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Background: Bevacizumab (B) is a recombinant, humanized anti-VEGF MAb. Erlotinib (E) is a potent, reversible, highly selective and orally available EGFR tyrosine-kinase inhibitor. Both compounds have demonstrated a survival benefit in the treatment of non-small cell lung cancer (NSCLC): bevacizumab when added to chemotherapy in the first line setting, and erlotinib when given alone in the 2nd/3rd line. In addition, a single arm phase I/II study of the combination of bevacizumab and erlotinib has shown encouraging survival and response rate data, with a favorable safety profile (Herbst et al, JCO 2005).

Methods: A multicenter, randomized phase II trial was conducted to evaluate the safety of combining bevacizumab with chemotherapy (docetaxel or pemetrexed), or with erlotinib; and to make a preliminary assessment of the efficacy of combining bevacizumab with chemotherapy or erlotinib relative to chemotherapy alone, as measured by progression-free survival. All patients had histologically confirmed non-squamous NSCLC and had experienced disease progression (clinical or radiographic) during or following one platinum-based regimen for advanced stage disease. Randomization was on a 1:1:1 basis to docetaxel or pemetrexed plus placebo v docetaxel or pemetrexed plus bevacizumab v bevacizumab plus erlotinib. Patients remained in the treatment phase of the study until documented radiographic or clinical disease progression or through 52 weeks of study treatment.

Results: Between August 2004 and November 2005, 120 patients were randomized and treated.

	Chemotherapy (n = 41)	Chemotherapy +B (n = 40)	B+E (n = 39)
Median PFS, mo	3.0	4.8	4.4
Adjusted Hazard Ratio (95% CI)	NA	0.66 (0.38, 1.16)	0.72 (0.42, 1.23)
Overall Survival, 6-month rate (%)	62.4	72.1	78.3
Response rate, n (%), CR/PR	5 (12.2)	5 (12.5)	7 (17.9)
Drug discontinuation due to AE, n (%)	10 (24.4)	10 (25.0)	4 (10.3)
SAEs, n (%)	22 (53.7)	16 (40.0)	13 (33.3)
Grade 5 drug-related AEs, n (%)	2 (4.9)	3 (7.5)	1 (2.6)
Grade 3–5 pulmonary hemorrhage	0	2(5)	1 (2.6)

Conclusions: The observed data favor the addition of bevacizumab to either chemotherapy or erlotinib over chemotherapy alone. No new or unexpected safety signals were noted. The toxicity profile of the bevacizumab-erlotinib combination is favorable when compared to either chemotherapy-containing group. The bevacizumab-erlotinib combination may represent an alternative to chemotherapy-based treatment in this setting. Updated OS data will be presented.

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POSTER

Changes in computed tomography perfusion scan parameters and circulating endothelial cells following bevacizumab administration in patients with advanced hepatocellular carcinoma

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Purpose: Hepatocellular carcinoma (HCC) is a highly vascular tumor with a poor prognosis. In a phase II study using bevacizumab (B) combined with gemcitabine and oxaliplatin (GEMOX) in advanced HCC, we examined changes in computed tomography (CT) perfusion scan parameters and circulating endothelial cells (CECs) as surrogate angiogenesis markers following B administration.