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.

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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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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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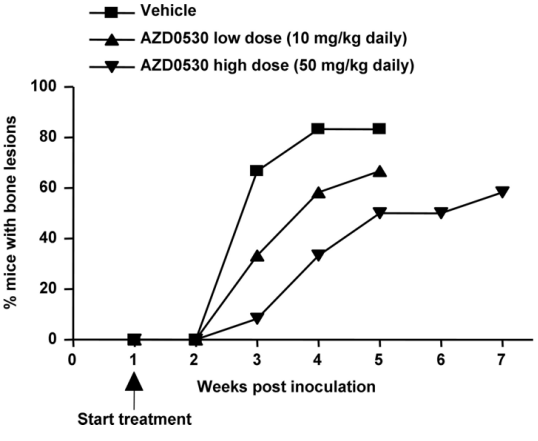
Src kinase plays a central role in growth factor and integrin signalling, regulating a diverse array of cellular functions including proliferation, migration and invasion. Recent studies have demonstrated that Src activity is frequently elevated in human tumours and correlates with disease stage. In 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.



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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