cohorts to date in the US study and 4 patients have been enrolled in the UK study. GDC-0941 has been well tolerated clinically to date. At least one patient has been on study for at least 92 days. Preliminary PK data demonstrate dose proportional increases in Cmax and AUC in fasting conditions. PD assays, including assessment of pAKT levels in platelet rich plasma coinciding with PK evaluation, FDG-PET scans, and evaluation of tumor biopsies for marker modulation, are underway. Preliminary data show decreases in pAKT levels in platelet rich plasma in patients in cohort 2 that are reflective of drug levels in plasma.
Conclusions: GDC-0941 is a potent pan-PI3K inhibitor with promising preclinical activity. Ongoing Phase I studies have indicated favorable preliminary PK and safety profiles in the first few cohorts. Updated data including clinical PK and PD will be presented.

224 POSTER

Assessment of the antitumor activity of NVP-BEZ235 in experimental renal cell carcinoma models

M. Michel[1], J. Vaxelaire[1], M. Hattenberger[1], M. Vogelsanger[1], F. Stauffer[1], A. Theuer[1], C. Stephan[1], M. Ito[1], C. Garcia-Eccheverria[1]. [1]*Novartis Institutes for BioMedical Research, Oncology, Basel, Switzerland*

Background: Renal cell carcinoma (RCC) is among the ten leading causes of cancer-related deaths worldwide. Over the past few years, the understanding of cellular and molecular processes underlying the tumor biology of RCC has made substantial progress. A genetic change involved in RCC tumorigenesis (75% of cases) is inactivation of the von Hippel-Lindau (VHL) gene leading to constitutive induction of hypoxya-inducible factor (HIF). This and other genetic alterations often found in RCC (PTEN deletions or silencing) result in activation of PI3K and downstream effectors.
Materials and Methods: To study the potential use of the dual PI3K/mTor inhibitor NVP-BEZ235, which is currently in Phase I clinical trials, in the treatment of RCC, the compound was tested *in vitro* and *in vivo* in several genetically characterized human RCC models. As part of its *in vivo* evaluation, head-to-head studies were performed with RAD001, an allosteric mTORC1 kinase inhibitor that has been shown promise in the treatment of RCC.
Results: NVP-BEZ235 significantly inhibited the proliferation (GI_{50} <50 nM) of a panel of human RCC cell lines −A498, RENCA; SK-RC-01, 786−0, ACHN, Caki1, Caki2, and Sk-RC-02- by specifically blocking the phosphorylation and activation of Akt. No correlation was found between specific genetic alternations and tumor cell line sensitivity. A similar experimental observation was obtained when the compound was tested under anchorage independent conditions. To confirm these cellular findings, several *in vivo* efficacy studies were conducted using human tumor RCC models with different genetic alterations. When administered orally at 30 mg/kg/day, a statistically significant antitumor activity (R 25%) was obtained in the 786−0 tumor model (PTEN deletion, VHL mutant). Under the same experimental conditions, RAD001, which was administered orally at 10 mg/kg/day, displayed a similar antitumor effect. Different results were obtained when NVP-BEZ235 was tested in the Caki-1 RCC model (VHL wild-type). Altough efficient blockade of pAkt and pS6 was confirmed upon *ex-vivo* analysis of tumor tissues at the end of the efficacy study, NVP-BEZ235 did not show a significant antitumor effect in this model.
Conclusions: Our results suggest that concomitant blockade of PI3K and mTOR by low molecular mass kinases inhibitors like NVP-BEZ235 may represent an effective therapeutic strategy for patients with RCC, in particular tumors that harbor VHL mutations.

225 POSTER

Evaluation of antitumor activity of a novel PI3K inhibitor ZSTK474 by various human cancer xenograft models

T. Yamori[1], M. Okamura[1]. [1]*Japanese Foundation for Cancer Research, Division of Molecular Pharmacology Cancer Chemotherapy Center, Tokyo, Japan*

Phosphatidylinositol 3-kinase (PI3K) is a key enzyme playing important roles in various cellular functions such as cell growth, survival and metabolism. The dysregulation of PI3K is known to associate with carcinogenesis and cancer progression. Therefore, PI3K is a potential molecular target. We recently developed a novel PI3K inhibitor ZSTK474, 2-(2-difluoromethylbenzimidazol-l-yl)-4, 6-dimorpholino-1,3,5-triazine, and demonstrated its therapeutic efficacy in some in vivo models (Yaguchi S. et al. J Natl Cancer Inst 2006; 98: 545−56). ZSTK474 is a pan-PI3K inhibitor and inhibits PI3K in a ATP-competitive manner (Kong D. et al. Cancer Sci 2007; 98: 1638−42). The purpose of this study was to further evaluate its efficacy in various types of human cancer xenograft. We subcutaneously transplanted 24 human cancer xenografts derived from 9 different organs into nude mice. When the tumor volume reached approximately 100 mg (Day 0), oral administration of ZSTK474 (200 or 400 mg/kg/day) was started and continued for 14 days. The tumor size was measured, and the tumor growth inhibition (T/C %) was examined. A wide antitumor spectrum across the 24 xenografts was observed without severe toxicity, which demonstrated again the in vivo efficacy of ZSTK474. However, the values of T/C varied from 10% to 70% depending on the xenograft type. These results suggested that the sensitivities of human cancers to ZSTK474 are diverse. Therefore, identification of biomarkers predicting its efficacy would be necessary.

226 POSTER

Anti-angiogenic activity of a novel PI3K inhibitor, ZSTK474

D. Kong[1], H. Yoshimi[1], T. Yamori[1]. [1]*Japanese Foundation for Cancer Research, Department of Molecular Pharmacology Cancer Chemotherapy Center, Tokyo, Japan*

PI3 Kinase (PI3K) dysregulations are known to be involved in tumor initiation and progression. It was recently reported that PI3K promotes angiogenesis via inducing expressions of HIF-1a and vascular endothelial growth factor (VEGF), which are well known to play important roles in angiogenesis. ZSTK474, 2-(2-difluoromethylbenzimidazol-l-yl)-4, 6-dimorpholino-1,3,5-triazine, is a novel ATP-competitive pan-PI3K inhibitor that we developed as an anticancer drug in recent years. (Kong D., et al. Cancer Sci 2007, 98: 1638−42) ZSTK474 showed favorable in vivo antitumor effect on human cancer xenografts without obvious toxicity observed. (Yaguchi S., et al. J Natl Cancer Inst 2006; 98: 545–556) The purpose of the present study is to examine the anti-angiogenic effect of ZSTK474. WST-8 assay was utilized to evaluate the growth inhibition activity of ZSTK474 against human umbilical vein endothelial cells (HUVEC). ZSTK474 dose-dependently inhibited HUVEC growth at submicromolar concentrations. Furthermore, Matrigel capillary-like tube formation assay indicated that ZSTK474 blocked in vitro tube formation by HUVEC; and Boyden chamber assay showed that ZSTK474 inhibited VEGF-induced migration of HUVEC with a comparable activity with SU5416, a well-known VEGF-R inhibitor. On the other hand, ZSTK474 treatment inhibited expressions of p-Akt, HIF-1a and VEGF in RXF-631L renal cancer cells. Finally, immunohistochemical staining with anti-CD31 antibody of tumor tissues from RXF-631L mouse xenograft models showed the significant reduction of the microvessels in ZSTK474-treated mice compared to vehicle group. These results indicated that ZSTK474 had anti-angiogenic activity which might be attributed to inhibition of the expression of HIF-1a and VEGF via PI3K-Akt pathway.

227 POSTER

Anti-angiogenic effects of PI3K/Akt/mTOR pathway inhibitors

L. Friedman[1], M. Belvin[1], J. Le Couter[1], K. Lin[1], L. Robillard[1], D. Sampath[1], H. Stern[1], U. Vijapurkar[1], W. Ye[1], G. Plowman[1]. [1]*Genentech Inc., Research, South San Francisco, CA, USA*

Background: The PI3K signaling pathway is important for VEGF mediated angiogenesis. We utilized selective small molecule inhibitors of PI3K alpha/delta, pan-PI3K (GDC-0941), Akt, TORC1, and mTOR/PI3K to investigate the signaling and mechanistic effects of inhibition at various nodes in the pathway.
Methods: HUVEC sprouting and migration assays were carried out in vitro. In vivo experiments for compound effects on neovascularization were performed in mice. Endothelial specific proliferation in xenograft tumors was assessed utilizing FFPE sections immunostained with MECA32 and Ki67. Combination efficacy studies of various pathway inhibitors with the anti-VEGF antibody B20 were carried out in xenograft tumor models.
Results: Inhibition of PI3K, Akt, or PI3K/mTOR using small molecule inhibitors all caused a decrease in VEGF expression in cultured tumor cells. While PI3K and PI3K/mTOR inhibitors robustly diminished HUVEC sprouting and migration in a dose-dependent manner, Akt inhibition was less effective. In HUVEC migration the TORC1 inhibitor had a broad effective range, although the maximum effect was 50% in VEGF-induced response. Dual mTOR/PI3K inhibitors produced a significant decrease in the microvascular density in the pancreas, smooth muscle, and trachea of perinatal mice. Xenograft tumor studies indicate inhibitors of the PI3K pathway have roles in targeting the tumor cells directly as well as tumor vasculature. Dual mTOR/PI3K inhibitors produced a greater decrease, compared with selective PI3K inhibitors, in the number of proliferating endothelial cells in xenografts. Combination with anti-VEGF antibody resulted in enhanced efficacy for the Akt inhibitor and PI3K inhibitors.
Conclusions: Dual PI3K/mTOR inhibition had significant effects on endothelial cell sprouting and migration in culture, and in vivo treatment resulted in decreased microvascular density, decreased tumor angiogenesis, and decreased endothelial cell survival. Effects of other inhibitors of the pathway were less robust.