liver dysfunction was reported by 1 pt each. Two (4.8%) pts, both with thyroid carcinoma, achieved a partial response, and 11 pts (26.2%) had stable disease, with a median duration time of 5.6 months (range: 2.7–9.0 months).
Conclusions: In this safety study, the addition of enzastaurin to standard pemetrexed infusion did not result in additional pemetrexed-related toxicities. Thus, it appears that enzastaurin can be safely combined with pemetrexed. In addition, the antitumor responses observed suggest that this combination should be further evaluated for antitumor activity.

65 POSTER

Safety of Volociximab as a monotherapy and in combination with chemotherapy the result of three phase II studies

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Background: Volociximab is a novel high-affinity chimeric (82% human/18% murine) IgG4 monoclonal antibody that specifically binds $\alpha5\beta1$ integrin. Volociximab is being developed as an anti-angiogenic agent targeting $\alpha5\beta1$ integrin for the treatment of solid tumors. The mechanism of action of volociximab is distinct from that of other anti-angiogenic agents because it acts downstream and is independent of the growth factors that stimulate angiogenesis, such as vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF).
Methods: A total of 100 patients (pts) have been treated with volociximab, 10 mg/kg IV every 2 weeks in 3 different multicenter, open label, single cohort phase II studies. Forty pts with refractory or relapsed metastatic renal cell carcinoma (RCC) received volociximab as a single agent until disease progression, forty pts with metastatic melanoma received volociximab with DTIC, 1 g/m^2 monthly, twenty pts with metastatic adenocarcinoma of the pancreas (MPC) received volociximab every 2 weeks with gemcitabine (Gem), 1 g/m^2 q3w. Pts were evaluated for safety and efficacy every 8 weeks or until disease progression using RECIST criteria. An independent data safety monitoring board was utilized to review safety data.
Results: A total of 100 pts were evaluated for safety using (NCI-CTC). Ninety-eight pts (98%) reported at least one AE; 26 pts (26%) had grade 1 and 39 pts (39%) had grade 2. Twenty-five pts (25%) had grade 3 AEs and eight pts (8%) had grade 4 AE's. The total number of pts who had grade 3 or 4 AEs considered possibly or probably related to volociximab were 11 pts (11%) and 3 pts (3%), respectively. Twenty-nine pts (29%) had an SAE which in 11 pts (11%) were considered to be possibly related to volociximab. The most common all-grade AEs for RCC were fatigue in 25 pts (62.5%) and nausea in 13 pts (32.5%) of which none were grade 3 or 4. In the melanoma study nausea was observed in 20 pts (50%) and fatigue in 17 pts (42.5%); none were grade 3 or 4. In MPC, nausea was reported in 13 pts (65%), vomiting in 12 pts (60%) and constipation in 10 pts (50%). All AEs were grade 1 and 2 except 1 pt (5%) had grade 3 vomiting.
Conclusion: Volociximab is well tolerated as a single agent and in combination with chemotherapy. Side effects seen in melanoma and MPC are similar to those expected from Gem and DTIC. Volociximab is currently being evaluated at 15 mg/kg qw in ongoing trials in RCC, MPC and Melanoma.

66 POSTER

Functional biomarkers to select dose and predict tumor response to anti-VEGF drugs

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For efficient drug development, biomarkers are needed to aid selection of optimal dose and schedule, to select patients and to predict tumor response to treatment. Biomarkers may be molecular or, alternatively, functional parameters that are altered as a consequence of the action of a drug on its molecular target. For the development of anti-angiogenic drugs, direct molecular markers have proven difficult because of the low expression of the target within the tumor mass and limitations for repeated tumor tissue sampling. Functional parameters that can be measured easily and repeatedly before and during drug treatment would overcome these difficulties.
We have evaluated several physiological and tumor-related functional parameters in an orthotopic breast cancer model (BN472) in rats using, PTK787/ZK222584*, a VEGF receptor tyrosine kinase inhibitor currently in phase III clinical trials for cancer. Blood pressure (BP), heart rate (HR), body temperature (BT) and interstitial tumor pressure (IFP) were measured in conscious freely moving rats by telemetry.
PTK787/ZK induced dose-dependent and significant decreases in HR, BT and IFP and increases in BP. The changes in all of these parameters occurred in the same dose range (12.5−100 mg/kg p.o.) but the duration of the responses varied. Responses in BT and HR were more transient than for BP. IFP remained changed over a longer period after a single dose, was further reduced after repeated dosing and after several doses, remained continuously lowered. Effects on BP, HR and BT probably reflect effects of VEGF inhibition on normal physiological mechanisms, whereas the effects on IFP reflect changes in the tumor vasculature. All parameters might be useful for selection of effective doses for complete inhibition of VEGF activated pathways, whereas IFP may be useful to select the dose needed to impact tumor growth. Since the acute response in IFP after acute dosing correlated with effects on tumor size after chronic dosing, IFP may also be a parameter that could predict tumor response to treatment.
In conclusion, our data demonstrate that functional biomarkers may be used to assess effects of VEGF inhibition on normal physiological mechanisms (on target side effects) or effects on tumor growth. Some of these parameters may also be useful to predict anti-angiogenic and anti-tumor response.
*PTK787/ZK222584 is jointly developed by Novartis and Schering AG.

67 POSTER

Membrane-type I matrix metalloproteinase is tyrosine phosphorylated on its cytoplasmic domain: role in in vitro angiogenesis

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Background: Membrane-type 1 matrix metalloproteinase (MT1-MMP) is a transmembrane matrix metalloproteinase (MMP) that plays an important role in both tumor cell migration and angiogenesis. In addition to its matrix-degrading activity, the cytoplasmic domain of MT1-MMP has been suggested to be important for both processes although the mechanisms involved remain poorly understood.
Methods: COS-7 cells were transfected with MT1-MMP cDNA constructs and phosphorylation studies were performed using immunoprecipitation techniques and two-dimensional gel electrophoresis. We have also produced a phosphospecific antibody against MT1-MMP. *In vitro* angiogenesis was performed in human umbilical vein endothelial cells (HUVEC) and bovine aortic endothelial cells (BAEC), by measuring morphogenic differentiation into capillary-like structures on Matrigel® and cell migration in Boyden® chambers.
Results: In this study, we show for the first time that MT1-MMP is tyrosine phosphorylated on its cytoplasmic domain, and that this phosphorylation requires the kinase Src. MT1-MMP tyrosine phosphorylation is induced by stimulation of endothelial cells with the proangiogenic factor sphingosine-1-phosphate (S1P), and is important for *in vitro* angiogenesis since a MT1-MMP mutant lacking the phosphorylated tyrosine residue failed to promote endothelial cell migration and their morphogenic differentiation into capillary-like structures.
Conclusion: Given that pharmacological inhibition of MMP catalytic activities has been shown to induce several undesirable side-effects, these findings suggest that the inhibition of MT1-MMP tyrosine phosphorylation may represent an unexpected alternative strategy for antiangiogenic drug development.

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Matrix metalloproteinase-2 (MMP-2) and -9 (MMP-9) and their tissue inhibitors (TIMP-1 and TIMP-2) in differential diagnosis between low malignant potential (LMP) and malignant ovarian tumors

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Background: Ovarian tumors of low malignant potential (LMP), also called borderline ovarian tumors, account for about 10% to 15% of all epithelial ovarian malignancies. The most important criterion for LMP ovarian tumor is the lack of invasion. The overall survival of patients with a LMP tumor is significantly better compared to those with a malignant ovarian tumor. Preoperative differential diagnosis between LMP and malignant ovarian tumors is often difficult. At least in advanced ovarian cancer,

the most significant factor that predicts survival is the presence of a gynecologic oncologist at the operation. Therefore, it would be useful to differentiate between LMP and malignant ovarian tumors before surgery in order to choose the hospital where the surgery is performed. Matrix metalloproteinases have been linked to aggressive behavior in ovarian malignancies. This study aimed to evaluate whether circulating matrix metalloproteinase's (MMP-2, MMP-9, MMP-2/TIMP-2 complex) or their tissue inhibitors (TIMP-2, TIMP-2) could be used as preoperative serum markers in differentiating between LMP and malignant ovarian tumors.
Materials and Methods: The study population consisted of 61 patients with ovarian neoplasm's (28 benign, 11 LMP and 22 malignant). Venous blood samples were collected before surgery and stored at −70ºC until assayed. All patient groups included both pre- and postmenopausal women. The LMP and malignant tumors were staged according to the International Federation of Gynecology and Obstetrics (FIGO). The immunoreactive proteins for MMP-2, MMP-9, MMP-2/TIMP-2 complex, TIMP-1 and TIMP-2 were assayd from the sera of the patients with benign, LMP and malignant ovarian tumors using enzyme-linked immunoasay (ELISA).
Results: Serum TIMP-1 values signifincantly increased from benign (median 250 ug/l, range 137–616 ug/l) to LMP (median 357 ug/l, range 63–587 ug/l) and further to malignant (median 443 ug/l, range 199–983 ug/l) ovarian neoplasms ($p < 0.001$). There was a significant difference in the rations of TIMP-1 to MMP-2 and TIMP-1 to MMP-2/TIMP-2 complex between the patients with a benign versus malignant and a LMP versus malignant tumor.
Conclusion: We conclude that the value of circulating TIMP-1 and the rations of TIMP-1 to MMP-2 and TIMP-1 to MMP-2/TIMP-2 complex may be valuable for differentiating between LMP and malignant ovarian tumors. Our data also suggest that LMP ovarian tumors are more similar to benign than malignant ovarian neoplasms.

69 POSTER

Therapeutic effect of everolimus (RAD001) in combination with antiangiogenic chemotherapy for gastric cancer in vivo

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Background: mTOR signalling is critical for cancer cell proliferation and expression of malignancy associated proteins, including HIF-1 alpha. Components of the mTOR pathway are frequently deregulated in gastric cancer. Inhibitors of mTOR like everolimus are currently being evaluated in clinical trials, but show only moderate activity when administered as single agents. We therefore evaluated the therapeutic effect of everolismus (RAD001) in combination with antiangiogenic chemotherapy for gastric cancer.
Materials and Methods: In vitro, effects of everolimus on mTOR signalling, HIF-1α expression and VEGF secretion were assessed by immunoblotting or ELISA. Gastric cancer cell proliferation and cell cycle distribution were evaluated by electronic cell counting and flow cytometry, respectively.
In vivo, the activity of everolimus in combination with cyclophosphamide at antiangiogenic schedule on NCI-N87 gastric cancer xenografts was studied. Ki-67 expression, activation of caspase 3, HIF-1α expression patterns and microvascular density (MVD) of tumors were investigated by immunohistochemistry. Levels of circulating endothelial progenitor cells (CEPs) were measured by flow cytometry. In a second experiment, the antitumor activity of everolimus in combination with cyclophosphamdie at metronomic schedule was studied.
Results: Everolimus decreases proliferation without inducing cell death and attenuates production of HIF-1α and VEGF in gastric cancer cells in vitro.
In vivo, everolimus treatment markedly inhibits tumor xenograft growth. Moreover, the combination of everolimus with cyclophosphamide at antiangiogenic schedule shows superior anti-tumor activity compared to either monotherapy ($p < 0.01$). Combination of everolimus with antiangiogenic cyclophosphamide results in significantly decreased MVD of tumors ($p < 0.01$). CEP levels tend to reflect microvascular density and antitumor activity. Furthermore, the combination of everolimus with metronomically administered cyclophosphamide is superior to everolimus monotherapy ($p < 0.01$).
Conclusion: mTOR inhibition by everolimus shows significant activity in a preclinical model of gastric cancer. Combination of everolimus with cyclophosphamide at antiangiogenic or metronomic schedule might be a promising approach for the treatment of gastric cancer patients.

70 POSTER

Autocrine character of VEGF signalling in astroglial tumors

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Angiogenesis is required for many physiological and pathological processes such as embryonic development, tissue regeneration, tumor growth and dissemination. The genetic background of the angiogenic switch during tumor progression is not fully understood. Hypervascularity, striking tumor angiogenesis, focal necrosis, and rapid cellular proliferation are key features of glioblastoma (GB). To explore the possibility that VEGF may act as a driving force in the progression of low grade to high grade glioma, more detailed study of VEGF signalling pathway is indispensable.
Material and Methods: VEGF, FLT-1 and KDR expression in astroglial cell lines A172, U87MG and T98G was examined by RT-PCR, Western blotting and indirect immunofluorescence. VEGF, FLT-1 and KDR expression in a group of low grade and high grade glioma samples was investigated by immunohistochemistry (IHC). The effect of VEGF on astroglial cells was determined by cell viability assay (MTT). Induction of G1/S transition was examined by bromodeoxyuridine (BrdU) incorporation 30, 60 minutes and 12 hours after VEGF treatment, respectively. Changes in total protein and phosphorylation levels of key MAPK and PI3K signalling pathways were detected using Western blotting. GW5074 (c-Raf inhibitor) was used to abrogate the effect of VEGF on MAPK phosphorylation.
Results: VEGF expression in astroglial cell lines A172, U87MG and T98G revealed cytoplasmic distribution; FLT-1 and KDR were immunodetected mainly on cell surfaces. IHC showed cytoplasmic expression of KDR and FLT-1. Results from MTT and BrdU incorporation implied mitogenic potential of VEGF on astroglial cell lines. Stimulation by VEGF significantly increased phosphorylation levels of ERK1/2$^{Thr202/Tyr204}$, Akt^{Thr308}, STAT3^{Tyr705} and p70S6K^{Thr389}. VEGF caused increased protein levels of cyclin D_1, p27^{Kip1}, and androgen receptor. Uses of c-Raf inhibitor GW5074 abrogated the effect of VEGF.
Conclusions: It is suggested that tumor angiogenesis in astroglial tumors is regulated by VEGF in a paracrine manner. It has been reported that the PI3K pathway, but not MAPK pathway, plays an important role in the VEGF signalling in endothelial cells. VEGF signalling in astroglial cell lines is coupled in major part to MAPK pathway. Thus, VEGF might fulfil a fundamental role as an autocrine/paracrine regulator in GB, thereby facilitating tumor proliferation and subsequent invasion. This work was supported by grants IGAMZCRNR/7828–3 and MSM6198959216.

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Antiangiogenic inhibitor axitinib (AG-013736) renders significant growth inhibition of bevacizumab-refractory xenograft tumors

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The VEGF pathway is essential to the processes of angiogenesis and tumor progression. Axitinib (AG-013736), an oral RTK inhibitor with picomolar potency against VEGF RTKs (receptor 1, 2 and 3) and nanomolar potency against PDGFRs and KIT, has shown encouraging single agent activity in multiple clinical tumor types, including mRCC. Bevacizumab, an anti-VEGF-A monoclonal antibody, is the first approved anti-angiogenic agent used in clinic in combination with 5-FU for the treatment of colorectal cancer. In combination with various chemotherapeutic agents, bevacizumab is currently under intensive Phase 2 and 3 clinical investigations for its potential broad therapeutic utilities. As a single agent, however, bevacizumab showed limited benefit in multiple clinical cancers. We tested the hypothesis whether axitinib could provide added benefit to bevacizumab in preclinical models. Anti-tumor efficacy was tested in MV522 human colon carcinoma and M24met human melanoma xenograft models. Both models do not express endogenous VEGFRs and PDGFRs and they secret appreciable levels of human VEGF-A, target of bevacizumab. In the MV522 model, bevacizumab treatment at the maximum dose (5 mg/kg 2qwk $\times$3) resulted in 32% tumor growth inhibition (TGI), whereas axitinib at ED_{80} (30 mg/kg, PO, BID) produced 71% TGI. Non-responders to bevacizumab treatment were randomized and switched to treatment with axitinib, which resulted in a 67% TGI compared to bevacizumab alone. Immunohistochemistry confirmed a greater anti-angiogenesis by axitinib than bevacizumab. In the M24met spontaneous metastasis model, bevacizumab was less active than axitinib in metastasis inhibition determined by lymph node tumor burden, lung metastasis and survival. Co-administration of axitinib with bevacizumab did not significantly improve anti-metastasis efficacy over axitinib alone. *Ex vivo* analysis is underway to explain the molecular contributors to the above observation. In addition, anti-tumor and anti-metastasis activity of axitinib $\pm$ bevacizumab $\pm$ docetaxel has also been investigated in various preclinical models. Results of these studies will be reported in detail.