have shown that 4-hydroxycoumarin (4-HC) reduces the motility and the adhesiveness to extracellular matrix proteins of B16-F10 melanoma cells. In this study, we have evaluated the effect of 4-HC on paxillin expression and signaling. Using Western Blot we found that 4-HC (500 μM) reduced the levels of paxillin; the alfa isoform decreased by 50% and the beta isoform diminished by 70%. RT-PCR assays showed that changes in both isoforms correlate with reductions in mRNA levels. Since tyrosine phosphorylation of paxillin is required for integrin-cytoskeleton crosstalk and can regulate its cellular localization, we analyzed the effect of 4-HC on phospho-paxillin content and on paxillin distribution. 4-HC treatment reduced the amount of tyrosine-phosphorylated paxillin and changed its distribution from a punctuate pattern to a perinuclear localization. In contrast, in the non-malignant cell line L929, 4-HC showed no effect on paxillin expression, phosphorylation or localization. Paxillin can also regulate the activation of Rac1 and RhoA; consequently, we performed pull-down assays in B16-F10 cells to evaluate the effect of 4-HC in the activation of these GTPases. 4-HC impaired the activation of both molecules; the active/total ratios were diminished by 65 and 75 % for Rac1 and RhoA respectively. Finally, in order to evaluate the importance of reduced paxillin expression and signaling in the formation of metastases, we injected *in vitro* treated B16-F10 cells into the tail vein of C57BL/6 mice. 4-HC inhibited by 85% the formation of experimental lung metastases. These results address the importance of paxillin in the formation of metastasis by melanoma cells, and suggest that 4-HC might be useful as an adjuvant in the therapy of melanoma.

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hTEM1 BAC Tg mice as a potential in vivo model system for evaluation of therapeutic antibodies against human TEM1

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Background: Tumor Endothelial Marker 1 (TEM1), also known as endosialin, is a transmembrane glycoprotein originally found to be selectively expressed by tumor endothelial cells. Later, TEM1 was described as being predominantly expressed by stromal fibroblasts and a subset of pericytes associated with tumor vessels. More recently, a study using mouse Tem1 KO mice demonstrated that Tem1 plays an important role in experimental tumor progression. Therefore, TEM1 may represent a potential target for cancer treatment.
Materials and Methods: As drug candidates, we raised fully human monoclonal antibodies (mAbs) against human TEM1 (hTEM1) utilizing the KM mice™. However, most of the mAbs were not cross-reactive to mouse Tem1 (mTem1). In order to evaluate efficacy of the mAbs *in vivo*, we generated hTEM1 transgenic (Tg) mice on a C57BL/6 background by using bacterial artificial chromosome (BAC) clones that contain h*TEM1* gene, expecting that those mice show natural expression pattern of hTEM1.
Results: One mouse line estimated to have a single copy number of the transgene was used for further analyses. As expected, hTEM1 was shown to be expressed in an organ-specific manner, suggesting that the Tg mice reproduced natural expression pattern of TEM1. Among major organs, expression level of hTEM1 mRNA was relatively high in heart and ovary compared with liver and spleen. Consistent with reported data on mouse Tem1 expression in normal mouse, semi-quantative RT-PCR indicated that expression level of hTEM1 mRNA in tumor tissues was significantly higher than those in normal tissues. In addition, in tumor tissues, hTEM1 was detected predominantly on stromal fibroblasts and pericytes by immunohistochemical analysis. Interestingly, spatial patterns and levels of hTEM1 expression varied dramatically by tumor cells implanted. For instance, B16 melanoma tissue expresses hTEM1 in the vasculature, whereas MCA207 sarcoma tissue expresses it independently of the vasculature.
Conclusions: These results suggest that studies using hTEM1 BAC Tg mice may provide useful information for development of new mAb drugs targeted to hTEM1. For further convenience, the hTEM1 Tg mice are being crossbred with mTem1 KO mice and SCID mice.

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Improved antitumor activity by combining ZD6474 (ZACTIMA) with radiotherapy and irinotecan in the LoVo human colorectal cancer xenograft model

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Introduction: ZD6474 is a once-daily oral inhibitor of VEGF-dependent tumor angiogenesis and EGFR-dependent tumor cell growth. The objective of the present study was to determine the tumor growth kinetics of the human LoVo colorectal xenograft model in response to radiotherapy (RT) or irinotecan (CPT-11) or both in the presence of ZD6474.
Methods: LoVo cells were injected subcutaneously into the right hind limb (5×10^6 cells in 100 μl PBS) of athymic NCR NUM mice and tumors were grown to a volume of 200–300 mm^3 before treatment. ZD6474 was administered at 50 mg/kg daily p.o. for 14 days starting on day 1. RT was given as three fractions (3×3 Gy) on days 1, 2 and 3. CPT-11 was given at 15 mg/kg i.p. on days 1 and 3. Tumor volumes were measured on a daily basis and calculated by measuring tumor diameters with digital calipers in two orthogonal dimensions.
Results: The kinetics of daily average increase in tumor volume changed with combination therapy after completion of ZD6474 (day 14). Therefore, two analyses were performed to determine tumor growth rates with combination therapy: (1) determination of tumor volume at completion of ZD6474 treatment (day 14); (2) time in days for tumors to reach 1000 mm^3. When tumor volumes were compared on day 14, there was a significant statistical difference between ZD6474 (465 mm^3) vs. combined modality treatment with ZD6474 + RT (291 mm^3) ($p = 0.037$) vs. ZD6474 + RT + CPT-11 (187 mm^3) ($p < 0.001$). Combined treatment with all three modalities was therefore better than ZD6474 alone and also significantly better than RT alone ($p < 0.001$) and CPT-11 alone ($p < 0.001$). However, when tumor growth delay was determined using time in days for tumors to reach 1000 mm^3 (days included time without ZD6474), the combinations of ZD6474 + RT or ZD6474 + CPT-11 + RT were not statistically significantly better than ZD6474 alone.
Conclusions: The response of LoVo colorectal tumors to RT and CPT-11 is improved with the addition of ZD6474. Furthermore, this study suggests that the improvement in response is dependent upon concurrent and post-sequencing of ZD6474 with cytotoxic therapy.

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Phase I study of pemetrexed followed by daily enzastaurin in patients with advanced or metastatic cancer

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Background: Enzastaurin, an oral serine/threonine kinase inhibitor, targets PKC and PI3K/AKT pathways to inhibit angiogenesis and tumor cell proliferation and to induce tumor cell death. Preclinical data suggest that the combination of enzastaurin and pemetrexed (Alimta®) produced additive or synergistic antitumor activity in tumor specimens. Objectives of this phase 1b study included evaluation of the safety, and antitumor activity of enzastaurin when combined with pemetrexed.
Materials and Methods: Patients (pts) with advanced or metastatic cancer who had at least 1 prior therapy received an intravenous dose of 500 mg/m^2 pemetrexed on day 1. On day 4 of cycle 1, a loading dose of 1200 mg enzastaurin (400 mg/3×/day) was given to achieve near steady-state concentrations. Starting on day 5 of cycle 1, 500 mg enzastaurin was administered orally, once daily (after breakfast) for the duration of treatment. This combination of oral enzastaurin and standard pemetrexed infusion was given in 21-day cycles for up to 6 cycles. Additional cycles were allowed for pts who benefited from the combination. Pts were also given oral folic acid daily and vitamin B_{12} every 9 weeks during pemetrexed therapy, and 5–7 days before cycle 1.
Results: Forty-two pts (16 male, 26 female; ECOG 0–2), with a median age of 59 years (range: 34−76 years), were treated with enzastaurin plus pemetrexed. Most patients (37/42) had received at least 1 prior chemotherapy. Thirty-six pts received ⩾2 cycles of treatment, of which 8 pts continued treatment for ⩾6 cycles. Colorectal cancer was the most frequent malignancy (26.2%). Drug-related hematological toxicities ⩾ grade 3 were anemia (n = 2), leukopenia (n = 1), thrombocytopenia (n = 4) and neutropenia (n = 3). Grade 3 ulcer and gastro-intestinal and

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.

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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.

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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,