bevacizumab; 50% for LC06; 78% for the combination and 87% for TAvi6. TAvi6 was able to suppress growth of tumors refractory to 1st-line treatment with bevacizumab (Colo205 xenograft) and suppressed metastasis to the lung significantly (KPL-4 xenograft). In the s.c. Calu-3 xenograft we observed a strong inhibition of angiogenesis by in vivo and ex vivo imaging and an advantage of TAvi6 compared to the antibody combination. In the VEGF-induced cornea-pocket assay TAvi6 resulted in a complete shutdown of angiogenesis.
Conclusions: We have generated a novel tetravalent IgG-like bispecific antibody targeting VEGF-A and Ang-2 simultaneously. TAvi6 shows identical properties compared to the respective parental antibodies. In particular, TAvi6 blocks angiogenesis in vivo efficacously and mediates strong tumor growth inhibition in various xenograft models with a slight advantage of TAvi6 over the combination of the respective single agents bevacizumab and LC06 in several models.

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Therapy monitoring of Sorafenib effect on experimental prostate carcinomas using dynamic contrast-enhanced (DCE)-MRI with Gadobutrol and immunohistochemical analyses

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Background: To investigate and quantify the anti-angiogenic effect of the multikinase inhibitor Sorafenib on experimental prostate carcinomas in rats with Gadobutrol-enhanced dynamic contrast-enhanced (DCE-) MRI assays of tumor perfusion and tumor endothelial permeability. Non-invasive MRI results were correlated with immunohistochemistry.
Material and Methods: A total of 20 Copenhagen rats implanted with subcutaneous prostate carcinoma allografts (MLLB-2), randomized to either the treatment (n = 10) or the control group (n = 10), were imaged on day 0 and day 7 using DCE-MRI at 3T enhanced with Gadobutrol (Gadovist®, Bayer Schering Pharma, Berlin, Germany). The treatment group received daily applications of Sorafenib (Nexavar®, Bayer Healthcare, Leverkusen, Germany, 10 mg/kg bodyweight) via gavage; the control group was treated with the solvent of Sorafenib (Cremophor/Ethanol). SI-time curves were analyzed with PMI 0.4 software using a two-compartment kinetic model. Target parameters were tumor perfusion (PF, ml/100ml/min) and microvessel permeability (endothelial transfer constant K^{PS}). Tumors were excised on day 7 for immunohistochemical of tumor vascularity (RECA-1), cell proliferation (TUNEL) and cell apoptosis (Ki-67).
Results: Tumor perfusion in treated prostate carcinoma allografts, quantified by DCE-MRI, declined significantly from day 0 to day 7 (47.9±36.9 vs. 24.4±18.5 ml/100ml/min, $p < 0.05$). In the control group, tumor perfusion increased significantly from day 0 to day 7 (37.6±12.3 vs. 49.8±15.0 ml/100 ml/min, $p < 0.05$). No significant change in endothelial permeability was observed either in the therapy or in the control group ($p > 0.05$). Immunohistochemical measurements of tumor vascularity demonstrated a significantly lower area density of endothelial cells in the therapy than in the control group (RECA-1 5.1±1.9 vs. 23.1±7.7, $p < 0.05$). Tumor cell proliferation was significantly ($p < 0.05$) lower in the therapy than in the control group (Ki-67 847±307 vs. 1692±469). In the Sorafenib-treated therapy group the area density of apoptotic cells was significantly ($p < 0.05$) higher than in the control group (TUNEL 427±283 vs. 218±312).
Conclusions: Tumor perfusion measured by Gadobutrol-enhanced DCE-MRI can be applied to monitor the anti-angiogenic effects of Sorafenib on prostate carcinoma allografts. Correspondingly, immunohistochemical analyses revealed an anti-angiogenic, anti-proliferative and pro-apoptotic effect of Sorafenib on the investigated prostate carcinoma model.

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E-cadherin plasticity in tumor-initiating stem-like cells regulates prostate cancer cell invasion

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Background: The mechanisms contributing to prostate cancer cell dissemination and subsequent metastatic lesions remain poorly understood. We have previously isolated tumor-initiating E-Cadherin-positive prostate cancer cell subpopulations from the mixed EMT marker-expressing DU145 and PC-3 prostate cancer cell lines. We herein characterized the invasive properties of the highly purified E-Cadherin-positive subpopulations of the DU145 and PC-3 cell lines.
Materials and Methods: Prostate cancer DU145 and PC-3 cells were separated into E-Cadherin positive (E-Cad+) and negative (E-Cad−) subpopulations by flow cytometric sorting. Sorted cells were plated onto Matrigel-coated porous membranes and incubated for varying times. To examine spheroid formation, invaded cell populations were harvested and plated in serum-free medium on low-attachment plates for 3 days. Total mRNA was collected from the sorted top invasion chamber populations at varying time points, and real-time RT-PCR was performed; gene expression changes were calculated by the $\Delta\Delta C_t$ method using GAPDH as an internal control.
Results: The E-Cad+ subpopulation, following cell sorting and plating, was highly invasive compared to the E-Cad− subpopulation. Invaded E-Cad+ cells efficiently formed E-Cadherin- and CD44-expressing spheroids. E-Cad+ cells invaded through the membrane in a time-dependent manner, during which E-Cadherin was drastically reduced. E-Cad expression was restored approximately 5 h after E-Cad+ cell invasion. Examination of the E-Cad repressor genes revealed increased Slug levels concomitant with the loss of E-Cadherin expression. Targeted knockdown of E-Cad expression in the E-Cad+ cell population reduced Sox2 and OCT3/4 expression in parental PC-3 cells, which exhibited reduced cellular invasion. However, despite efficient E-Cad knockdown in parental DU145 cells, Sox2 and OCT3/4 were not reduced; cells did not display reduced invasion. Furthermore, targeted knockdown of Sox2 or OCT3/4 in either DU145 or PC-3 cells significantly reduced both E-Cad and cellular invasion.
Conclusions: Tumor-initiating prostate cancer stem-like cells require the expression of the early progenitor markers Sox2 and OCT3/4, as well as E-Cad modulation, which is a permissive factor for invasion *in vitro*. Therefore, we propose a model in which the post-EMT prostate cancer phenotype progresses to frank invasion, which requires E-Cadherin plasticity.

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Inhibiting signalling by erbB receptor tyrosine kinases with AZD8931, a potent reversible small molecule inhibitor, reduces intestinal adenoma formation in the ApcMin/+ mouse model

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Abstract: The erbB protein family plays major roles in tumour cell survival, proliferation and vascularisation in many cancers including colon. Pharmacological targeting of EGFR (erbB1) or erbB2 has led to modest clinical results in colorectal patients. ErbB3 has been shown to contribute to EGFR inhibitor resistance in some cancer types. AZD8931, a novel small-molecule inhibitor with equipotency against signalling by EGFR, erbB2 and erbB3 receptors (Hickinson *et al.* 2010), has demonstrated broader preclinical anti-tumour efficacy than agents with a narrower spectrum of erbB family activity (e.g. gefitinib, lapatinib). To determine the effect of AZD8931 on intestinal tumourigenesis, studies were conducted in the multiple intestinal neoplasia ($Apc^{Min/+}$) mouse model. Previous work has demonstrated erbB family activity in this model and its importance in adenoma formation (Lee *et al.* 2009).
Method: In situ hybridization (ISH) was used to determine the expression level and pattern of the erbB family in intestinal adenomas and normal tissue of $Apc^{Min/+}$ mice. In pharmacological studies, $Apc^{Min/+}$ mice received AZD8931 (50 mg/kg/dose BID) or vehicle (n = 15 per group) for 3 weeks. Animals were then humanely culled and small bowel (SB) and colons isolated and examined under a dissecting microscope for tumour burden, calculated by the product of adenoma number and volume.
Results: ISH analysis indicated strong expression of erbB2 and erbB3 throughout the adenomas. EGFR expression was mainly localised to the periphery of the adenomas, whilst erbB4 was virtually absent in both tumour and normal tissue. Compared with vehicle treated animals, AZD8931 significantly reduced adenoma number in the SB by 35% ($P > 0.01$). Adenoma diameter was also reduced in the SB by 32%, producing a 75% overall decrease of mean adenoma burden, $P = 0.01$. In the colon a noticeable adenoma reduction of 60% was recorded ($P = 0.17$). Splenomegaly (spleen enlargement), an associated marker of tumour load in this model, was also significantly reduced (52%, $P = 0.001$) by AZD8931 treatment.
Conclusion: Treatment of $Apc^{Min/+}$ mice with AZD8931 results in a significant decrease in adenoma number, diameter and overall tumour burden compared with control animals. Taken together these results provide a scientific rationale for studying broad spectrum equipotent erbB inhibitors in intestinal tumourigenesis. AZD8931 is currently undergoing evaluation in phase II clinical trials.