Methods: The primary objective of this phase 1 study is to evaluate the safety and tolerability of ACE-041. Secondary objectives include identifying MTD, PK, preliminary activity on PD markers and antitumor activity by RECIST, PET-CT and DCE-MRI. Cohorts of 3−6 patients are being enrolled at escalating dose levels. ACE-041 is administered SC every 3 weeks for a total of 4 doses or until disease progression. Patients with confirmed stable or responding disease may continue treatment for up to 12 months.
Results: 19 patients (11M, 8F) have been enrolled. Five dose levels (0.1 to 1.6 mg/kg) have been completed; the sixth cohort (3.2 mg/kg) is ongoing. The t1/2 is approximately 10−15 days and the Tmax is 4−7 days. ACE-041 is well tolerated with no DLTs reported thus far. Common to this population, preliminary AEs included nausea, fatigue, anorexia, headache, fever and vomiting, which were generally of low grade toxicity. Stable disease was observed in 3 patients having previously progressed on chemo- and/or anti-VEGF therapy lasting at least 6 cycles; one aggressive carcinoid patient (6 cycles before progressing) and 2 patients still on treatment (NSCLC and head and neck) after 7 cycles. Additionally, in a heavily pre-treated NSCLC patient with an adrenal metastasis enrolled at the 1.6 mg/kg dose level, a positive major response on 18-FDG-PET was observed with a significant decrease in metabolic activity 2 weeks following the first dose.
Conclusions: ACE-041 is a first-in-class inhibitor of angiogenesis targeting ALK-1. Treatment thus far has been well tolerated and preliminary evidence of antitumor activity has now been observed in this first-in-human study. The study is ongoing and final results will be presented at the meeting.

466 POSTER DISCUSSION

PI3K inhibition is necessary and sufficient to induce an anti-angiogenic response in vivo based on suppression of tumor vascular structure

D. Sampath[1], T. Cao[2], J. Oeh[1], K.H. Barck[2], H.B. Reslan[2], S. Wyatt[2], S. Ungersma[2], H. Koeppen[3], L.S. Friedman[1], R.A.D. Carano[2].
[1]Genentech, Cancer Signaling and Translational Oncology, South San Francisco, USA; [2]Genentech, Biomedical Imaging, South San Francisco, USA; [3]Genentech, Pathology, South San Francisco, USA

Background: Vascular endothelial growth factor (VEGF) is a validated target for tumor angiogenesis and the PI3K pathway acts as a central mediator of VEGF driven endothelial cell survival and vascular permeability. While it has been demonstrated that a dual PI3K/mTOR inhibitor can suppress eNOS-induced vascular permeability and vasodilatation resulting in a reduction in the dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) parameter K^{trans}, the effects on vascular structure has not been elucidated [1]. Therefore, our aims were to further ascertain the role of PI3K pathway signaling on vascular structure and physiology by utilizing small molecule inhibitors that target PI3K (GDC-0941 and GNE-490), mTOR (rapamycin) or both (GDC-0980).
Methods: An array of in vivo imaging techniques were employed to evaluate the vascular response in human HM7 colorectal cancer xenografts: ex-vivo micro-computed tomography angiography (μCT-angio), DCE-MRI, vessel size imaging (VSI) by MRI and dynamic contrast-enhanced ultrasound (DCE-U/S) perfusion imaging.
Results: GDC-0980 strongly suppressed both tumor physiological and structural vascular parameters. The DCE-MRI parameter, K^{trans}, was reduced by 24% relative to the control group. DCE-U/S imaging showed that GDC-0980 reduced blood flow within the enhancement region by 8% and reduced the enhancement fraction (Ef) by 55%. GDC-0980 reduced μCT-angio vascular density (VD) by 57% relative to control. In-vivo VSI demonstrated a significant reduction in blood volume, the vessel density related parameter Q and increased vessel size; all changes consistent with a loss of small vessels. DCE-MRI and DCE-US demonstrate that GDC-0980 can suppress permeability and perfusion while uCT-angio, VSI and DCE-U/S Ef data indicates a strong effect on vascular structure. In addition, GDC-0941 also caused a significant decrease in VD while rapamycin did not. Interestingly, GNE-490, a pan-PI3K inhibitor that has similar pharmacokinetic parameters to GDC-0980, produced similar uCT-angio VD results as GDC-0980, suggesting that mTOR inhibition is not required for maintenance of vascular structural effects.
Conclusion: Inhibition of PI3K alone is necessary and sufficient to generate the dramatic physiological and structural changes in tumor vasculature that is characteristic of an anti-angiogenic response in vivo.

References

[1] Schnell et al., Cancer Res. 2008, p. 6598–6607.

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Updated efficacy and safety results for a randomized phase 2 trial of a tumor vascular disrupting agent fosbretabulin tromethamine (CA4P) with carboplatin (C), paclitaxel (P) and bevacizumab (B) in stage IIIB/IV non-squamous non-small cell lung cancer (NSCLC): The FALCON trial

E.B. Garon[1], J. Neidhart[2], J.D. Neidhart[2], F. Kabbinavar[1], N. Gabrail[3], M. Ribeiro de Oliveira[4], S.P. Lu[5], J. Balkissoon[5]. *[1]University of California Los Angeles, Medicine Division of Hematology/Oncology, Los Angeles CA, USA; [2]San Juan Oncology Associates, Hematology/Oncology, Farmington New Mexico, USA; [3]Gabrail Cancer Center, Hematology/Oncology, Canton Ohio, USA; [4]Northwest Medical Specialties, Hematology/Oncology, Tacoma Washington, USA; [5]OXiGENE, Oncology, South San Francisco California, USA*

Background: CA4P is a reversible tubulin-binding tumor vascular disrupting agent (VDA) that has previously shown clinical activity in combination with chemotherapy and antiangiogenic therapy. Enrollment of a phase 2 study evaluating CA4P in combination with C (Carboplatin) + P (Paclitaxel) + B (Bevacizumab) in advanced non-squamous NSCLC was recently completed.
Methods: In an open-label, randomized controlled study for patients with untreated, histologically-confirmed, stage IIIb or IV, non-squamous, NSCLC 60 patients were randomized to receive up to 6 cycles of C + P + B with (CA4P arm) or without CA4P (control arm). After 6 cycles of therapy, patients without progression continued to receive their randomized treatment B or B + CA4P until progression. The primary endpoint is progression-free survival (PFS). Secondary endpoints include response rate and overall survival.
Results: As of June, 2010, the target enrollment of 60 patients was completed. Of these, 53 patients (safety population) received treatment (26 in CA4P arm and 27 in control arm) by the most recent data analysis (May 6, 2010). 30 patients enrolled at least 12 months prior to the data analysis, and these patients composed the efficacy population. For this group, PFS was 6.9 months in the CA4P arm vs. 6.2 months in the control arm with a HR and 95%CI of 0.70 (0.27, 1.82). Partial responses were seen in 60% of patients for the CA4P arm vs. 40% for the control arm. Safety profiles in both treatment arms were comparable. Hypertension, mostly grade 1 and 2, and neutropenia were more frequent in the CA4P arm. Toxicities were manageable and did not result in differences in dose intensity between the two treatment arms. There were three reversible cardiac ischemia events in the CA4P arm, none of which required hospitalization. Updated safety and efficacy data will be presented.
Conclusions: The addition of CA4P to standard doses of C + P + B continues to be well tolerated with trends towards improved outcomes in the CA4P arm.

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Anti-tumoral and anti-metastatic activity of a tetravalent bispecific antibody (TAvi6) targeting VEGF and Angiopoietin-2

W. Scheuer[1], M. Thomas[1], M. Baehner[1], S. Seeber[1], H. Kettenberger[1], J. Schanzer[1], U. Brinkmann[1], M. Weidner[1], J. Regula[1], C. Klein[1]. *[1]Roche Diagnostics GmbH, Pharma Research, Penzberg, Germany*

Background: VEGF blockade has been validated clinically as a treatment for human cancers. Angiopoietin-2 (Ang-2) expression has been shown to function as a key regulator of blood vessel remodeling and tumor angiogenesis. In tumors Ang-2 is up-regulated and a bad prognostic factor. Recent data demonstrated that Ang-2 inhibition mediates antitumoral effects. We have generated TAvi6, a novel bispecific antibody targeting VEGF-A and Ang-2 and tested its anti-tumor efficacy. TAvi6 is a tetravalent IgG-like bispecific antibody based on bevacizumab and targets Ang-2 with 2 disulfide-stabilized scFvs (LC06) fused to the C-terminus of the heavy chain.
Material and Methods: TAvi 6 was profiled in biochemical and cellular (angiogenesis) assays. Antitumoral efficacy was assessed in established s.c. Colo205, s.c. Calu-3 and orthotopic i.m.f.p. KPL-4 xenografts in SCID beige mice. Mice were treated with bevacizumab or <Ang-2> antibody LC06 (10 mg/kg), the respective combination (each 10 mg/kg) and TAvi6 (13.3 mg/kg). In addition, TAvi6 was evaluated in tumors progressing after 1st-line treatment with Avastin, for inhibition of metastasis to the lung quantified by Alu-PCR and for inhibition of angiogenesis in the cornea micropocket assay. Tumors were explanted for histological analysis.
Results: In biochemical assays (affinity, Tie2-Ang-2 interaction) and cellular assays (Tie2 phosphorylation, HUVEC proliferation, tube formation) TAvi6 shows properties identical to the parental antibodies bevacizumab and LC06. In the orthotopic KPL-4–003 xenograft tumor growth inhibition was 79% for bevacizumab; 39% for LC06; 90% for the combination and 91% for TAvi6. In the s.c. Colo205–009 xenograft TGI was 66% for

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

C.C. Cyran[1], P.M. Paprottka[1], B. Schwarz[2], C.J. Bruns[2], O. Dietrich[1], R. Hinkel[1], J. Von Einem[1], M.F. Reiser[1], K. Nikolaou[1], B.J. Wintersperger[1]. *[1]University of Munich − Klinikum Grosshadern, Department of Clinical Radiology, Munich, Germany; [2]University of Munich − Klinikum Grosshadern, Department of Surgery, Munich, Germany*

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

K. Bae[1], N. Parker[2], J. Vieweg[1], D.W. Siemann[2]. *[1]University of Florida, Urology, Gainesville Florida, USA; [2]University of Florida, Radiation Oncology, Gainesville Florida, USA*

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

D. Alferez[1], A. Logié[2], P. Seedhar[3], R. Poulsom[3], T. Klinowska[2], R.W. Wilkinson[2]. *[1]University of Manchester, Experimental Oncology Group, Manchester, United Kingdom; [2]AstraZeneca Pharmaceuticals, Cancer Bioscience Alderley Park, Macclesfield Cheshire, United Kingdom; [3]London Research Institute Cancer Research UK, Histopathology Unit, London, United Kingdom*

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.