bounded by endothelial cells while others were bounded by tumour cells. Administration of DMXAA in a previously determined optimal schedule (20 mg/kg followed by two 5 mg/kg doses at 4 and 8 hours; repeated after 11 days) to mice with NZM7 xenografts induced extensive tumour necrosis with a tumour growth delay of 19 days (2/6 cures).
Conclusions: DMXAA has a significant effect on the function of tumour cells exhibiting features of vasculogenic mimicry. This may be of importance to its action in clinical trials.
Support: This work was supported by an Antisoma Post-doctoral Fellowship.

150 POSTER

Molecular tumor characteristics and response to bevacizumab plus irinotecan/5-fluorouracil/leucovorin in metastatic colorectal cancer

H. Koeppen[1], W. Ince[1], E. Holmgren[2], M. Zhang[2], P. Tobin[1], R. Zhang[3], W. Novotny[2], K. Hillan[1]. [1]*Genentech, Inc., Pathology, South San Francisco, USA;* [2]*Genentech, Inc., BioOncology, South San Francisco, USA;* [3]*Genentech, Inc., Molecular Biology, South San Francisco, USA*

Background: Bevacizumab (BV) is a recombinant, humanized monoclonal antibody directed against vascular endothelial growth factor (VEGF) that has demonstrated survival benefit in first-line treatment of patients with metastatic carcinoma of the colon or rectum. In a phase III study, the addition of BV to irinotecan/5-fluorouracil/leucovorin (IFL) first-line therapy resulted in a 34% reduction in the daily hazard of death compared to IFL alone (HR = 0.66; p=0.00004) (ASCO 2003). Submission of tumor specimens was optional in this study. We sought to explore the effects of baseline molecular tumor characteristics on survival, PFS, and objective response rate.
Methods: Tumor material was analyzed from 232 of the 923 patients in the study; consisting of either tumor cores isolated from paraffin blocks and placed into tissue microarrays or unstained tissue sections. The analysis included *in situ* hybridization (ISH) for VEGF RNA and immunohistochemistry (IHC) for p53 protein (DO-7 antibody, DAKO). Mutational analysis for *KRAS* (exon 1), *BRAF* (exon 15) and *TP53* (exons 5–8) was performed by DNA sequencing of tumor cells isolated by laser capture microdissection (PixCell II, Arcturus). Descriptive summaries of duration of survival, PFS and objective response were produced for each of the categorical variables listed above for each treatment arm. These descriptive summaries consisted of the hazard ratio from unstratified Cox regression and Kaplan-Meier estimates of median time to the event.
Results: 232 patients randomized to receive IFL alone (100) or IFL with BV (132) contributed to the calculation of the hazard ratios. The demographic and background characteristics were generally similar between the study as a whole and the subset in this analysis; this subset did have a higher percentage of subjects with ECOG PFS 0 (study: 57% versus subset: 64%). Mutations in the *KRAS*, *BRAF* and *p53* genes were observed in 35, 6 and 67% of patients, respectively. The type and frequency of p53 mutations were consistent with previously published data for colorectal adenocarcinomas. The IHC assay for p53 protein was positive in 72% of patients; the concordance rate between the two p53 assays was 66%. VEGF ISH on standard paraffin sections is in progress and results will be presented.
Conclusions: Patients benefited from the addition of BV to the chemotherapy regimen, as measured by duration of survival, independent of KRAS, BRAF or TP53 status.

		N	Median survival (mo)		Hazard Ratio
			IFL	IFL/BV	
All		232	17.5	26.5	0.54 (0.35–0.82)
KRAS	Mutant	76	14.9	19.9	0.75 (0.39–1.44)
	Wildtype	140	21.7	27.7	0.57 (0.32–1.01)
BRAF	Mutant	13	8.0	15.9	0.13 (0.02–0.70)
	Wildtype	196	18.7	26.4	0.52 (0.32–0.84)
TP53	Mutant	118	21.7	27.7	0.42 (0.23–0.78)
	Wildtype	58	17.5	not estimable	0.71 (0.31–1.61)
p53	Positive	163	17.6	26.4	0.70 (0.43–1.14)
	Negative	64	13.6	25.1	0.26 (0.11–0.64)

151 POSTER

AMG 706 first in human, open-label, dose-finding study evaluating the safety and pharmacokinetics (PK) in subjects with advanced solid tumors

R. Herbst[1], R. Kurzrock[1], M. Parson[2], R. Benjamin[1], L. Chen[1], C. Ng[1], M. Ingram[3], S. Wong[3], D. Chang[3], L. Rosen[2]. [1]*M.D. Anderson Cancer Center, Thoracic/Head & Neck Oncology, Houston, TX, USA;* [2]*CIMG, Santa Monica, CA, USA;* [3]*Amgen, Thousand Oaks, CA, USA*

Introduction: AMG 706 is a potent and selective small molecule inhibitor of multiple kinases, including vascular endothelial growth factor receptor, platelet derived growth factor receptor, and c-kit. To assess the safety, establish the maximum tolerated dose, and generate PK profiles of oral AMG 706, a clinical study in adult subjects was initiated.
Methods: individuals with advanced solid tumors, refractory to standard therapy or with no standard therapy available, were enrolled in this ongoing, open-label, dose-escalation study. Cohorts of 3 to 9 subjects were orally administered 50, 100, 125, or 175 mg once daily (QD) in an intermittent dose pattern of 21 days of dosing in a 28-day cycle. Subjects remained on study until tumor progression or unacceptable toxicities occurred.
Results: AMG 706 was generally well-tolerated up to 125 mg QD using the intermittent dose schedule. Most adverse events were mild to moderate in severity and reversible. Eight of the 9 subjects in the 125 mg QD cohort remained on study until day 50 including 1 subject with a grade (gr.) 3 hypertension and another with a gr. 3 creatinine and gr. 4 hyponatremia. Twenty-six of the 31 treated subjects reached the day 50 tumor assessment, revealing 1 (leiomyosarcoma) partial response, 3 (gastrointestinal stromal, thyroid, and carcinoid tumors) minor responses (−8% to −23% in the sum of the longest diameter of target lesions), and an additional 9 stable diseases (SD). Six subjects maintained SD for at least 134 days and 3 of these 6 subjects had SD for more than 218 days on study. AMG 706 demonstrated favorable bioavailability and half-life (about 7 hrs) at all dose levels. A single- and multiple-dose PK result comparison suggests that no significant accumulation of drug occurred during the first 3 weeks of AMG 706 administration.
Conclusions: AMG 706 appears to be safe and tolerable at daily doses up to 125 mg. Once daily dosing generated sustained exposure sufficient to elicit partial, minor and SD responses across multiple cancer types, suggesting that AMG 706 has broad anti-tumor activity.

152 POSTER

A synthetic Resveratrol analog inhibits the proangiogenic response of liver sinusoidal cells to tumor-derived factors during hepatic melanoma metastasis formation

O. Elvira[1], S. Clarisa[2], G. Natalia[2], L. Aritz[1], P.C. Fernando[3], V. Fernando[1]. [1]*University of the Basque Country, Cellular Biology and Histology, Leioa, Spain;* [2]*Dominion Pharmakine S.A., Derio, Spain;* [3]*University of the Basque Country, Organic Chemistry, San Sebastian, Spain*

Background: Resveratrol (3,5,4′-trihydroxystilbene) is a naturally occurring phytoalexin with cancer chemopreventive properties. Widespread interest in this molecule and synthetic stilbene analogues have arisen in recent years due to the discovery of its antioxidant, antiinflammatory, antiangiogenic and anti-carcinogenic activities.
Materials and Methods: Using resveratrol as prototype, we synthesized compound 5-((E)-(4-hydroxyphenylimino)methyl)benzene-1,3-diol, an unnatural resveratrol analog (FAS21) obtained in high yield by condensation between readily available reagents 4-aminophenol and 3,5-dihydroxybenzaldehyde. Then, the effects of trans-resveratrol were compared with those of FAS21 through an *in vivo* model of hepatic metastasis by intrasplenically injected B16 melanoma cells. Because tumor-activated hepatic sinusoidal cells contribute to tumor growth via NfkappaB and COX-2-dependent angiogenesis stimulation (Olaso et al, Hepatology 2003;37:674–85), we also investigated the antiangiogenic effect of resveratrol and FAS21 through tumor-hepatic sinusoidal cell interaction assays *in vitro*.
Results: Trans-resveratrol and FAS21, given orally as one single daily dose (1 mg/kg) since day 5 after B16M cell injection, reduced metastasis volume by 62% and metastasis number by 50%. Antitumor effect was selective on hepatic metastases having a sinusoidal-type angiogenesis, where microvessel density decreased while necrotic area increased. Consistent with *in vivo* data, both trans-resveratrol and FAS21 dose-dependently (5–25 μM) inhibited proliferative and migratory responses of human and murine hepatic myofibroblasts to human A375 melanoma and murine B16M-derived soluble factors. Trans-resveratrol also decreased by 70% human and murine hepatic sinusoidal endothelial cell migration towards tumor-conditioned media. The migration of human hepatic myofibroblasts in response to cytokines present in cultured melanoma cell supernatants

(i.e., VEGF, CM-GSF, bFGF, TGFbeta) was abrogated in the presence of trans-resveratrol and FAS21. Neither migration nor proliferation was altered by trans-resveratrol and FAS21 in basal medium-cultured hepatic cells. Consistent with in vitro inhibitory effects on liver sinusoidal cell-induced tumor cell growth, Ki67 staining of B16M cells decreased in metastasized livers from mice treated with trans-resveratrol and FAS21 as compared to controls. However, neither apoptosis nor toxicity was observed in tumor and host cells receiving above treatments under basal culture conditions.
Conclusions: These results demonstrate the potent antimetastatic effect of trans-resveratrol during hepatic melanoma metastasis formation, and suggest the potential therapeutic interest of the studied synthetic compound to target proangiogenic action of tumor-activated hepatic sinusoidal myofibroblasts.

153 POSTER

Inhibition of cytosolic superoxide dismutase (SOD1) in endothelial cells: a possible mechanism for the antiangiogenic properties of the copper depleting drug ATN-224

O. Betancourt, F. Donate, X. Guan, D. Shaw, A. Mazar, S. Pirie-Shepherd. *Attenuon, LLC, Biology, San Diego, USA*

Background: Copper has been demonstrated to be elevated in tumor tissue and plasma from patients with various malignancies. *In vitro* and *in vivo* animal studies have implicated copper in the process of angiogenesis and tumor progression, and the depletion of systemic copper using a copper binding agent such as tetrathiomolybdate has been demonstrated to inhibit tumor growth in animal models. However, the role of copper in mediating angiogenesis is poorly understood at the molecular level. Using a second generation tetrathiomolybdate analog (ATN-224, currently in Phase I clinical trials), we demonstrate the ability of this analog to inhibit SOD1 activity in endothelial cells by depleting copper from this enzyme. Further, we demonstrate that the inhibition of SOD1 activity by ATN-224 is sufficient to completely inhibit endothelial cell proliferation *in vitro* and angiogenesis *in vivo*.
Materials and Methods: Using a combination of *in vitro* cell proliferation assays and enzymatic assays we demonstrate that one of the anti-angiogenic targets for ATN-224 is intracellular CuZn-superoxide dismutase (SOD1). *In vivo* experiments extend these findings and show that SOD1 is a target for ATN-224 in animal models.
Results: Inhibition of angiogenesis by ATN-224 in the Matrigel plug takes place without changing the levels of copper in plasma or in tissue suggesting that depletion of systemic copper may not be required for the anti-angiogenic activity of the drug. ATN-224 is able to accumulate in proliferating human umbilical vein endothelial cells (HUVECs) and inhibits HUVEC SOD1 activity by removing the bound copper with an IC_{50} similar to that observed for the inhibition of HUVEC proliferation. The inhibition of HUVEC proliferation by ATN-224 *in vitro* can be substantially reversed using a synthetic porphyrin SOD mimetic (MnTBAP) that is not copper dependent. Similar results are observed *in vivo*, where the inhibition of angiogenesis by ATN-224 in a Matrigel plug model of angiogenesis is also reversed by MnTBAP. SOD1 inhibition by ATN-224 results in a concomitant increase in intracellular reactive oxygen species (ROS) in HUVECs. In pharmacodynamic studies, one dose of orally administered ATN-224 provides ~60% inhibition of SOD1 activity in red blood cells within 1 hour which is sustained for at least 6 hours. Inhibition of SOD1 activity in red blood cells was maintained at 30% even after 24 hours after a single dose of ATN-224.
Conclusions: These data suggest a molecular target for copper depletion therapy, demonstrate that SOD1 inhibition is achievable *in vivo* in cellular compartments after oral administration of drug and suggest that SOD1 may be a promising target for the inhibition of angiogenesis.

154 POSTER

The VEGFR-2 tyrosine kinase inhibitor, ZD6474, enhances the antitumor effect of radiation

D. Siemann, W. Shi. *Shands Cancer Center, University of Florida, Department of Radiation Oncology, Gainsville, USA*

Background: Angiogenesis is essential for the growth and metastatic spread of solid tumors. Vascular endothelial growth factor and its receptors are crucial to this process and therefore represent key targets for antiangiogenic cancer therapy. ZD6474 is an orally available inhibitor of vascular endothelial growth factor receptor-2 (VEGFR-2) tyrosine kinase activity with additional activity against epidermal growth factor receptor tyrosine kinase. The antitumor effects of this novel agent have been evaluated in combination with fractionated radiation therapy (RT) in a human colorectal cancer xenograft model (HT29).
Methods: The effects of increasing doses of ZD6474, either alone or in combination with radiation, on endothelial cell (HMVEC-L) and HT29 cell proliferation and survival were initially evaluated *in vitro*. *In vivo* studies were performed in mice bearing HT29 tumors grown intramuscularly in one hind limb. When tumors reached approximately 200 mm^3 animals were randomly assigned to receive 2 weeks' treatment with RT (2 Gy per fraction, Monday–Friday), ZD6474 alone (25 mg/kg/day, p.o. Monday–Friday), a combination of RT plus ZD6474 according to three schedules, or no treatment. In the combination groups, 2 weeks' treatment with ZD6474 preceded, followed, or was given concurrently with 2 weeks' RT. In the concurrent group ZD6474 was given 1 hour after each radiation dose. Tumor response was assessed using a growth delay assay.
Results: *In vitro*, ZD6474 (50 nM) significantly inhibited the proliferation of stimulated endothelial cells, but no inhibitory effects on HT29 tumor cells were observed at doses up to 5 μM. ZD6474 did not affect cell survival and did not enhance the extent of radiation-induced cell killing. *In vivo*, ZD6474 or RT alone led to tumor growth delays of approximately 13 and 18 days, respectively. Tumor responses were significantly greater when RT and ZD6474 were combined. Tumor growth delays of 36.5, 36 and 32 days were observed when ZD6474 was administered before, after, or concurrently with RT. No statistically significant differences were seen between these regimens. ZD6474 treatment was well tolerated with no obvious toxicities.
Conclusions: These results suggest that inhibition of tyrosine kinase signaling pathways with ZD6474 may provide an effective means by which to enhance the efficacy of RT in the treatment of solid tumors. ZD6474 is currently in Phase II clinical development.

155 POSTER

PTK787/ZK 222584 (PTK/ZK), a potent orally active and highly selective inhibitor of VEGFR kinases, is highly efficacious in various experimental tumor models either as mono- or combination therapy

H. Hess-Stumpp[1], M. Haberey[1], A. Reichel[2], K.H. Thierauch[1]. [1]*Schering AG, Experimental Oncology, Berlin, Germany;* [2]*Schering AG, Research Pharmacokinetics, Berlin, Germany*

Background: The recent approval of Avastin by the FDA was the starting point to introduce the concept of anti-angiogenesis therapy into clinical practice within the next years. PTK/ZK a small molecule which is currently in phase III clinical trials blocks in contrast to Avastin all known VEGFRs*. Here we summarize recent findings documenting its efficacy and good tolerability.
Material and Methods: PTK/ZK inhibitory activity was assayed *in vitro* with GST-fusion constructs of the various VEGFR-kinases or in the case of VEGFR 3 as inhibition of cellular receptor autophosphorylation in MVEC. For *in vivo* analyses various tumor cell lines of different tumor origin were xenografted onto nude mice. PTK/ZK was applied mostly with a dose of 50 mg/kg daily p.o. Cytostatic compounds were applied based on literature data or in-house experience. During the course of the experiments tumor areas and body weights were recorded, and following experimentation the animals were sacrificed and tumors weights were determined. Calculation of tumor concentrations of cytostatic agents after PTK/ZK treatment was done either by HPLC (oxaliplatin) or by radioisotope analysis (5-FU).
Results: To test the specificity of PTK/ZK as a tyrosine kinase inhibitor, almost 100 different kinases were analyzed (10 μM). In accordance with earlier results the VEGFR kinases 1 and 2 were found to be inhibited with an IC_{50} of 20–50 nM and VEGFR 3 in the cellular auophosphorylation assay with an IC_{50} of 20–30 nM. Other kinases inhibited were c-fms (IC_{50}-40 nM), c-kit (IC_{50}-364 nM), PDGFRß (IC_{50}-567 nM), lyn (IC_{50} – 1 μM) and c-raf (IC_{50} – 5 μM). All other kinases tested exhibited no inhibition (^{IC50} >10 μM). *In vivo* studies of PTK/ZK as a monotherapy revealed in most cases a tumor growth inhibition of ~50%. When PTK/ZK was used in combination studies generally additive effects were observed. In all cases the mice showed no acute signs of toxicity due to PTK/ZK treatment. In combination studies only body weight decreases due to the administration of cytostatic compounds were observed. The results of the cytostatic tumor concentrations analyses revealed no significant differences of the concentrations either with or without additional PTK/ZK treatment.
Conclusion: Since PTK/ZK is inhibiting only VEGFRs and a few other kinases the observed efficacy *in vivo* is mainly due to an inhibition of tumor angiogenesis. Thus, a concept is provided that efficaeous treatment of cancer may be possible without harmful side effects. However, since the majority of cancers is treated by a combination of various cytostatic compounds, our data show that the additional use of PTK/ZK in combination therapy may also yield additional benefit.
*PTK/ZK is co-developed by Schering AG, Berlin and Novartis.