expression. Unlike mitogenic signals, the regulation of focal adhesions and induction of cell migration by ErbB was not affected by herceptin, an anti-ErbB2 used in treatment regimens for metastatic breast cancer.
Conclusion: Our results provide a mechanistic model for ErbB-induced invasion that is distinct from ErbB-induced mitogenesis. The therapeutic implications of these results will be discussed. *Supported by the Canadian Breast Cancer Alliance (CBCRA) of the National Cancer Institute of Canada and the Cancer Research Society.*

146 POSTER

E7080, a novel multi-targeted tyrosine kinase inhibitor, exhibits anti-angiogenic activity via inhibition of KIT signalling in a small cell lung cancer xenograft model

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Stem cell factor (SCF) is an important growth factor that signals through a receptor tyrosine kinase KIT for amplification/mobilization of hematopoietic progenitor cells, which differentiate into blood and/or vascular endothelial cells. Recently, it was confirmed that KIT/SCF signaling played an important role in tumor angiogenesis by mobilizing endothelial progenitor cells (ECPs) and initiating branching from pre-existing vessels. SCF expression has been reported in several tumor types such as SCLC, NSCLC, colon, breast, and renal cancer. Among them, SCF and/or KIT are expressed in up to 70% of small cell lung cancer (SCLC), in which 50% are SCF-positive alone. Because the growth of KIT-positive SCLC is stimulated by SCF, which also acts to increase angiogenesis, inhibition of this signaling pathway is a promising therapeutic approach. In this study we evaluated the efficiency of E7080 in inhibiting SCF-driven angiogenesis in a SCLC xenograft.
E7080 is an oral multi-targeted tyrosine kinase inhibitor of VEGFRs (VEGFR1–3), FGFR1 and PDGFR-beta with IC50 values of 5–50 nM in cell free kinase assay. E7080 also inhibits KIT with IC50 value of 270 nM. In tube formation assay using human umbilical vein endothelial cells, E7080 inhibited angiogenesis driven by SCF in a dose dependent manner with an IC50 value of 5.2 nM. In this model, concomitant inhibition of KIT phosphorylation was seen. E7080 also inhibited angiogenesis driven by VEGF, with an IC50 value of 5.1nM. In order to assess the efficacy of E7080 in a SCLC xenograft model, H146, a KIT-negative and SCF-positive SCLC cell line was transplanted into mice. Oral administration of E7080 inhibited tumor growth at doses from 30 to 100 mg/kg (BID, QDx21) in a dose dependent manner and produced tumor regression at 100 mg/kg. Imatinib, a KIT kinase inhibitor, also inhibited tumor growth (160 mg/kg BID, QDx21), but it did not produce tumor regression. Treatment with anti-VEGF produced a similar pattern of growth inhibition to Imatinib. Our results indicate that E7080 achieved regression as a result of anti-angiogenic activity via inhibition of both KIT and VEGFR signaling indicating that E7080 has therapeutic potential in SCLC.

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Phase I pharmacokinetic (PK) and safety study of the antiangiogenic peptide ATN-161 (Ac-PHSCN-NH2) in patients with solid tumors

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Background: ATN-161 is a five-amino acid non-competitive inhibitor of the fibronectin synergy region, which plays a critical role in mediating tumor growth, survival and metastasis through interactions with integrins. ATN-161 binds to activated integrins $\alpha5\beta1$, $\alpha v\ \beta3$, and $\alpha v\ \beta5$ on tumor cells, and newly formed blood vessels and has potent anti-tumor activity in a variety of preclinical xenograft models including prostate, breast and colon cancers, either as monotherapy or in combination with chemotherapy. The safety and PK of ATN-161 were investigated in this first in human study.
Methods: Patients with advanced solid tumors refractory to standard therapy were enrolled in sequential dose cohorts to receive 0.1, 0.25, 0.5, 1.0, 2.0, 4.0, or 8.0 mg/kg ATN-161 administered as an IV bolus injection on a thrice-weekly schedule. PK sampling was performed on Day 1 over a 7-hour period after dosing.
Results: Twenty-three patients (10 women, 13 men; median age 64 years; ECOG 0–2) were enrolled to 7 dose levels, with a median treatment duration of two months (range 0.5–10). PK data at doses up to 0.5 mg/kg showed considerable interpatient variability, in part due to undetectable plasma concentrations at late time points. At the 1.0, 2.0 and 4.0 mg/kg dose levels pharmacokinetic parameters appeared dose-independent, with mean total clearance values that ranged from 10.5 to 14.5 ml/min/kg, and terminal elimination half-lives that ranged from 210 to 268 min. At the 8 mg/kg dose level, total clearance was reduced to about 7 ml/min/kg suggestive of saturable elimination.. There were no dose-limiting toxicities or treatment-related serious adverse events. Nineteen patients were evaluable for response. There have been no objective responses. One patient with ovarian cancer had stable disease for 10 months. Two other patients, one with renal cell cancer and one with adenoid cystic cancer of the hard palate, remain on study with stable disease in their 8th and 9th cycles, respectively.
Conclusions: ATN-161 can be safely administered as a thrice-weekly infusion of at least 4.0 mg/kg and higher doses are being explored in this dose-escalating Phase I clinical trial. A recommended Phase II dose has not yet been defined.

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BAY 57-9352: an inhibitor of VEGFR-2 and PDGFR receptor tyrosine kinases that demonstrates broad anti-tumor activity as a single agent in preclinical models

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BAY 57-9352 is an orally active, small molecule inhibitor of VEGFR-2 and PDGFR tyrosine kinases in clinical development that selectively blocks key regulators of tumor angiogenesis. To explore the spectrum of *in vivo* activity of BAY 57-9352, it was tested as a single agent in a panel of human tumor xenograft models representative of breast, colon, prostate and lung cancer. Human carcinoma cells from MDA-MB-231 breast carcinoma, Colo-205 colorectal carcinoma, DU-145 prostate carcinoma and H460 non-small cell lung carcinoma cell lines were implanted subcutaneously in NCr *nu/nu* mice. Studies were run as staged models and drug was administered by oral gavage beginning at the time of staging. BAY 57-9352 inhibited the growth of each tumor type in a dose-dependent manner during the period of drug administration. Immunohistochemical analysis was used to assess the effect of BAY 57-9352 treatment in MDA-MB-231 and Colo-205 tumor models on microvascular density. Following a single administration of BAY 57-9352, the endothelial cell (EC) content of tumor xenografts, as assessed by staining for CD31 and CD34 EC markers, was reduced by 50–70% within 24 hours of the first administration of BAY 57-9352. This finding is consistent with the role of VEGF as a survival factor for EC cell survival and is furthermore consistent with the rapid onset of tumor growth suppression *in vivo* observed following drug administration. These results demonstrate the anti-angiogenic and concomitant anti-tumor activity of BAY 57-9352 in models of human breast, colon, prostate and lung cancer.

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Inhibition of vasculogenic mimicry in melanoma by the antivascular drug 5,6-dimethylxanthenone-4-acetic acid (DMXAA)

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Background: The term "vasculogenic mimicry" is used to describe the ability of some malignant tumour cells to form blood conducting vessels *de novo* without the participation of endothelial cells. Tumour cells in such structures express endothelial-like markers, suggesting a genetic reversion to an embryonic-like genotype. In human cancers, vasculogenic mimicry occurs in breast, prostate and ovarian cancer as well as melanoma, and is associated with high tumour grade, development of distant metastasis and poor overall survival. DMXAA is a low molecular weight antivascular agent that is currently in clinical trial. It acts on the tumour vascular endothelial cells in both mice and humans to induce apoptosis and other effects. We wished to determine whether DMXAA has an effect on tumour cells exhibiting vasculogenic mimicry.
Methods: An early passage human melanoma line (NZM7) was grown both *in vitro* and as a xenograft *in vivo*. Observations were made with phase contrast, confocal laser scanning and transmission electron microscopy. A human angiogenesis gene array kit was used to analyse changes of *in vitro* gene expression.
Results: NZM7 cells lines formed tubular networks when cultured on Matrigel. Addition of DMXAA prevented network formation at a concentration (30 μg/ml) that did not inhibit growth when NZM7 were cultured as monolayers on tissue culture flasks. Microarray analysis of NZM7 cells growing on Matrigel showed that DMXAA (30 μg/ml) significantly inhibited expression of 14 endothelial – and vascular-associated genes, included VE-cadherin, Ephrin B4 and MMP-2. Electron microscopic analysis of NZM7 xenografts showed that some erythrocyte-containing vessels were

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.

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

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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

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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

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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