Methods: Nude mice implanted with human UMSCC2 head and neck tumors were treated with ZD6474 alone (30 mg/kg/day), RT alone (2× 3 Gy per week for 2 weeks) or with combinations of ZD6474 with RT concomitantly, RT followed by ZD6474, or ZD6474 followed by RT. Tumor and plasma samples were also collected during ZD6474 therapy and drug levels measured.
Results: The effects of each regimen on tumor growth are outlined in Table 1. Plasma ZD6474 levels were 3.64±1.12 μM and tumor levels were 0.073±0.024 μmol/g as determined 6 hours after dosing for 5 consecutive days in a subset of animals treated with ZD6474 alone.

Table 1. UMSCC2 tumor growth delay

	Untreated (control)	RT alone (days 1–14)	ZD6474 alone (days 1–14)	ZD6474 plus RT (days 1–14)	RT (days 1–14) plus ZD6474 (days 15–28)	ZD6474 (days 1–14) plus RT (days 15–28)
Mean tumor doubling time (days, ± SD)	14.3±6.0	18.9±5.6	31.1±13.2†	35.7±6.8†‡	30.7±11.2†	22.8±7.1
Median tumor doubling time (days)	11.0	17.0	36.0	37.0	29.5	22.5
Fraction of animals with tumors that did not double in size by day 47	0/9	0/9	1/8	4/10	3/9	5/9

† Significantly different from untreated. ‡ Significantly different from RT alone. Level of significance is $P<0.05$ as determined by ANOVA analysis with Tukey's pairwise multiple comparison

Conclusions: In this model, concurrent RT/ZD6474 treatment afforded the greatest therapeutic benefit in terms of tumor growth delay. In all the combination groups, the number of animals *not* achieving a doubling of tumor start size was greater than in the control or single-agent treatment groups, suggesting a potential benefit for all schedules of combined RT and ZD6474 therapy examined. However, tumor doubling time was not significantly increased in the combination groups compared with ZD6474 alone. Pharmacokinetic data showed that plasma levels of ZD6474 obtained at 30 mg/kg/day were within the range of plasma drug levels seen in patients in Phase I studies. Studies are ongoing to elucidate the mechanism by which ZD6474 enhances RT, and to determine whether optimal combination schedules are tumor cell line-dependent.

143 POSTER

The interferon-inducible GTPase MxA is a metastasis suppressor

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To identify pathways controlling prostate cancer metastasis we performed differential display analysis of the human prostate carcinoma cell line PC-3 and its highly metastatic derivative PC-3-M. MxA, a 78-kDa interferon-inducible GTPase, was expressed in PC-3 but not in PC-3-M cells. Although MxA was silent in PC-3-M cells, the gene was present in Southern analysis and inducible by interferon alpha. Stable expression of MxA in PC-3-M cells markedly inhibited in vitro motility and invasion. These effects were reversed by an inactivating point mutation (T103A) of the MxA GTPase. Neither wild-type nor mutant MxA affected PC-3-M growth in vitro. GST pull-down and co-immunoprecipitation studies demonstrated that recombinant and endogenous MxA associate with tubulin, and this association was eliminated in the T103A MxA mutant. Stable expression of MxA in highly metastatic Lox melanoma cells also strongly inhibited motility and invasion in vitro, demonstrating MxA activity is not limited to one cell line or cell of origin. In an experimental metastasis model in which PC-3-M-Neo or PC-3-M-MxA cells were injected intrasplenically followed 60 seconds later by splenectomy, MxA expression markedly inhibited development of hepatic metastases. To identify small molecules with metastasis inhibitory activity, we established a high-throughput system and screened the NCI diversity set. Several hits were obtained that induced MxA protein and inhibited motility. Recently a number of studies have documented downregulation of interferon-activated genes, including MxA, in association with prostate cancer progression. The data presented here identify MxA as a novel, inducible metastasis suppressor and a new target for development of antimetastasis therapeutics.

144 POSTER

Develop novel cancer drug that controls angiogenesis factor expression post-transcriptionally

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Vascular endothelial growth factor (VEGF) is a key regulator for angiogenesis and is an important causative factor for the pathogenesis of cancers, diabetic retinopathy and exudative macular degeneration. Both the stability and translation efficiency of the VEGF transcript is controlled by sequences in the 5′- and 3′-untranslated regions (UTRs). The 5′-UTR contains an internal ribosomal entry site (IRES) and mediates cap-independent translation initiation while the 3′-UTR harbors multiple AU-rich (AUR) stability determinants that have been previously shown to regulate turnover of VEGF mRNA. Even though normal cap-dependent translation is dramatically impaired under hypoxic conditions, translation of the VEGF protein still occurs because of its IRES and AURs. Thus, this form of post-transcriptional regulation allows cells to produce large amounts of VEGF protein to support either further tumor growth or aberrant neovascularization in ocular diseases under hypoxic conditions. The unique regulatory sequences of VEGF UTRs have led us to initiate drug discovery and development efforts to identify novel anti-angiogenesis drugs for the treatment of cancer and ocular neovascular diseases. Using one of our proprietary platform technologies GEMS (Gene Expression Modulation by Small molecules), we have identified a series of molecular scaffolds that inhibit the expression of VEGF post-transcriptionally with EC_{50} values in the low nanomolar range. Selectivity studies demonstrated there is a subset of compounds that selectively inhibit VEGF production. Oral administration of these specific VEGF inhibitors has proven effective in reducing intratumor VEGF levels, inhibiting tumor angiogenesis and tumor growth in human tumor xenograft models. Pre-clinical studies designed to evaluate bioavailability, half-life and other pharmaceutical properties are in progress. This novel approach of targeting angiogenesis factors could yield inhibitors that have advantages over agents that either sequester VEGF itself or inhibit phosphorylation of its receptor. A drug that acts via a novel mechanism of action may have favorable synergistic activity with other drugs in clinical/development.

145 POSTER

A mitogenic-independent mechanism for ErbB receptor-induced tumour cell invasion

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Background: Aberrant expression of members of the ErbB/HER family of tyrosine kinase receptors has been associated with increased susceptibility for breast cancer dissemination to distant organs; the molecular mechanisms are not fully understood. We reported earlier that ErbB receptors greatly impact on tumor microenvironment, including deregulation of several markers of the extracellular matrix and angiogenesis (Cancer Res, 63:3764, 2003; Molecular Biology of the Cell, 13:4029, 2003). Here, we investigated the mechanisms by which overexpression of single or paired combinations of ErbB receptors regulates the turnover of focal adhesion complexes and cell migration in in-vitro 3-dimensional system and in animal models.
Methods: ErbB receptors were overexpressed using a retroviral bicistronic system. Cell invasion was examined in the 3-d system by the Boyden chamber assay, wound healing, and *in-vivo* in mice transplanted with tumor cells. Protein expression and phosphorylation were examined by western blot and immunoprecipitation assays. siRNA technology was used to interfere with the expression/function of specific protein of the focal adhesion complexes.
Results: We demonstrated that overexpression of ErbB-induces differential motile and invasive properties in *in-vitro* 3-D conditions that are dependent on the type of ErbB being overexpressed; e.g. cells overexpressing ErbB-2/3 were highly invasive. ErbB regulates the turnover of focal adhesion complexes and interacts with protein complexes containing the focal adhesion kinase (FAK). FAK is found to be required for ErbB-induced tumor progression and invasion. Both *in-vitro* and *in-vivo* the motile and invasive properties induced by ErbB in FAK deficient cells were significantly reduced but not abolished; this can be restored by re-expression of wild type FAK but not a mutant FAK that lacks the paxillin interaction site. Furthermore, inactivation of endogenous FAK or paxillin in invasive rodent and human cancer cells overexpressing ErbB receptors, by expression of siRNA or FRNK (a naturally occurring mutant of FAK), reduced cell invasion. No correlation between FAK phosphorylation status and ErbB-induced tumor invasion was observed. In contrast, confocal studies revealed that ErbB colocalizes with focal adhesion proteins on distinct protrusion structures of migratory cells. This colocalization is competed by ErbB peptides and was not observed in cells with low ErbB

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.

147 POSTER

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.

148 POSTER

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.

149 POSTER

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