provided growth delays of 10 and >28 days, respectively in the two above experiments. Plasma DMXAA concentrations were measured up to 6 h after DMXAA administration, with and without diclofenac, and demonstrated no significant change in DMXAA pharmacokinetics. Tumour tissue DMXAA concentrations were also unchanged for up to 4.5 h. Plasma 5-HIAA concentrations, measured after 4 h, were proportional to DMXAA dose. Administration of diclofenac alone caused a dose-dependent increase in 5-HIAA, and co-administration with DMXAA provided an additive effect on 5-HIAA concentration.
Conclusions: Administration of diclofenac at a pharmacological dose caused large increase in the antitumour activity of DMXAA in a murine tumour model. Similar increases in activity have been observed for salicylate and rofecoxib (data not shown). The ability of NSAIDs to prevent local protective effects of prostaglandins released in response to vascular injury may explain this effect. Co-administration of NSAIDs may have general utility in therapies targeting the tumour vasculature.
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139 POSTER

BAY 57-9352: an inhibitor of VEGFR-2 and PDGFR receptor tyrosine kinases that demonstrates anti-angiogenic activity in vitro and in vivo

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Tumor angiogenesis depends on proliferation, maturation and survival of endothelial cells along with key components of the supporting stroma such as smooth muscle cells. Endothelial cell proliferation and survival is stimulated via VEGFR-2 while PDGFR activation results in smooth muscle cell proliferation. Blockade of VEGFR-2 kinase activity has been shown to inhibit tumor growth in a variety of preclinical models. The present studies describe a novel, small molecule, BAY 57-9352, that inhibits both VEGFR-2 and PDGFR tyrosine kinases. In a biochemical assay, this compound inhibits VEGFR-2 and PDGFR with IC_{50}s of 6 nM and 15 nM, respectively. VEGF-dependent receptor autophosphorylation in mouse fibroblasts that express human VEGFR-2 is inhibited *in vitro* by BAY 57-9352 with an IC_{50} of 19 nM. Similar results are observed with VEGF-stimulated human endothelial cells (ECs) *in vitro*: BAY 57-9352 inhibits EC proliferation with an IC_{50} of 26 nM and Western blot analysis of treated cells confirmed the dose-dependent inhibition of VEGFR-2 autophosphorylation. *In vitro* treatment with BAY 57-9352 of human aortic smooth muscle cells (SMCs) that respond to PDGF inhibited SMC proliferation and inhibition of receptor autophosphorylation after treatment was confirmed by Western blotting. The proliferation of many epithelial-derived tumor cells is independent of VEGFR-2 or PDGFR *in vitro*, and consistent with this, BAY 57-9352 up to 20 μM exhibited no effect on the proliferation *in vitro* of a panel of human tumor cell lines. By contrast, *in vivo* administration of BAY 57-9352 results in inhibition of tumor growth in human tumor xenograft models. Based on the favorable *in vitro* and *in vivo* profile, BAY 57-9352 has advanced to Phase 1 clinical trials as an anti-angiogenic agent.

140 POSTER

A two-stage phase II study of the matrix metalloproteinase inhibitor (MMPI) Col-3 in patients with advanced soft tissue sarcoma (ASTS) – report of Stage I data

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Background: Col-3 (Metastat; Collagenex Pharmaceuticals, Newton, PA) is a tetracycline analog that specifically inhibits the production and activation of MMP-2 and MMP-9. A phase I study has established the tolerability of continuous uninterrupted dosing of Col-3, with photosensitivity and malaise as principal toxicities. Col-3 is exceptional amongst MMPIs, having demonstrated clinical benefit in pts with ASTS, as well as a 44% overall response in patients with AIDs-related KS. Phase II studies of active agents in ASTS, in patients previously treated with anthracyclines and ifosfamide, show that » 40% of pts have progression of disease (PD) at first evaluation. With this in mind, and the demonstrated potential for Col-3 to delay tumor progression, we designed a two-stage Phase II study to determine the proportion of ASTS pts with PD at 8 weeks following Col-3 therapy. Applying the two-stage design, with multinomial stopping rules, 15 pts are evaluated in stage I. If >4 of the first 15 pts develop PD on first evaluation, the likelihood that the true proportion of pts with early PD is <40% is <10% and the trial will be terminated. Otherwise if <13 of 30 pts develop PD, the drug will be considered of interest to pursue phase III evaluation.
Patients and Methods: Pts with ASTS meeting inclusion criteria and willing to minimize sun exposure are eligible. COL-3 is administered by continuous uninterrupted oral dosing at 50 mg/m2/d in 28 day cycles until PD.
Results: Twelve pts (5M/7F), median age 52 yrs (range 38–84) and PS0–2 with ASTS (leiomyosarcoma n=5, liposarcoma n=2, other n=5) have enrolled. With one exception, all pts have failed at least 2 prior chemotherapy regimens. Median treatment cycles administered is 3 (range 1–6). Grade 3 toxicities include photosensitivity (1), transaminitis (2) and reversible anemia (4) requiring transfusion in 2 pts. Most common < Grade 2 toxicities include fatigue, photosensitivity, anemia and transaminitis. Assessment of initial response at 8 weeks showed 8 of 11 (73%) evaluable pts with stable disease. Two pts had PD and 1 pt with clinically SD had discontinued therapy early by choice. Median duration of SD in evaluable pts is 14 weeks (range 11–24), median TTP has not been reached and six pts continue on therapy.
Conclusion: With stage I of this Phase II trial nearing completion, COL-3 appears to delay tumor progression with an encouraging 73% of ASTS pts maintaining SD beyond 8 weeks. Accrual to this study continues.

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The spectrum-selective kinase inhibitor EXEL-0999 inhibits mitogenic and angiogenic kinases, and causes rapid tumor vasculature destruction and regression in mouse xenograft models

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Receptor tyrosine kinases (RTKs) such as VEGFRs, FGFRs, PDGFRs, KIT and FLT3 play roles in tumor angiogenesis and/or tumor cell proliferation. EXEL-0999 is a potent, orally-available small molecule inhibitor of these RTKs, with low nanomolar potency in biochemical enzyme assays for VEGFRs 1–3, PDGFR-α and β, FGFRs 1 and 3, KIT, and FLT3. EXEL-0999 also inhibits RTK autophosphorylation in cell-based assays, with high potency against VEGFR2, VEGFR3, FGFR1, PDGFR-β, FLT3, and KIT. In functional angiogenesis assays in vitro, EXEL-0999 inhibits tubule formation and migration of endothelial cells in culture in response to VEGF or bFGF. EXEL-0999 also displays potent anti-proliferative activity against a variety of tumor cell lines in vitro.
In pharmacodynamic studies in nude mice, EXEL-0999 exhibits potent inhibition of VEGFR2, PDGFR-β, FGFR1, FLT3, and KIT, and shows sustained duration of action after a single oral dose. To determine the effect of EXEL-0999 in vivo on tumor cell proliferation and tumor angiogenesis, the compound was administered daily to nude mice bearing MDA-MB-231 human breast carcinoma xenografts. Tumors were harvested 4h to 96h after initiation of treatment, and analyzed histologically for vessel density, tumor cell proliferation, and cell death. EXEL-0999 caused a rapid destruction of the tumor vasculature, with tumor and endothelial cell death evident 2h to 4h after administration of the first dose. Longer exposure to the drug (24h to 96h) resulted in large decreases in vessel density and proliferating cells, and large increases in tumor necrosis. EXEL-0999 targets endothelial cells selectively in the tumor vasculature, as effects on endothelial cells were not observed in normal tissues such as liver, kidney, lung, intestines, and brain. These acute effects of EXEL-0999 translate to potent anti-tumor activity in efficacy studies, with once-daily oral administration causing substantial tumor growth inhibition of MDA-MB-231, PC-3, Calu6, HT-29, and A431 human tumor xenografts, as well as regression of larger, well established MDA-MB-231 xenografts. In a model of FLT3-driven leukemia, EXEL-0999 substantially increased the survival of nude mice injected intravenously with cells expressing human FLT3-ITD. Overall, these data indicate that targeting a spectrum of kinases including VEGFRs, FGFRs, PDGFRs, KIT and FLT3 with EXEL-0999 causes dramatic vascular destruction and shrinkage of solid tumors, and increased survival of leukemic mice, and provide a rational basis for clinical development of EXEL-0999 for treatment of solid tumors and FLT3-driven leukemia.

142 POSTER

Impact of scheduling on combined ZD6474 and radiotherapy in head and neck tumor xenografts

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Background: ZD6474 is a novel, orally available inhibitor of vascular endothelial growth factor receptor-2 tyrosine kinase activity with additional activity against epidermal growth factor receptor tyrosine kinase. ZD6474 has demonstrated enhanced efficacy in combination with radiation therapy (RT) in human tumor models and this study aimed to identify the optimal scheduling for this treatment regimen.

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