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.

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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.

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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.

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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