Results: *In vitro*, the presence of VEGF or EGF decreased paclitaxel-induced apoptosis in NCI-H441 and MLECs. ZD6474 treatment prevented this effect and decreased the IC_{50} of paclitaxel two-fold. *In vivo*, the most significant antitumor effects were seen in animals receiving combined ZD6474 and paclitaxel therapy. The lung weights in control, ZD6474, paclitaxel, and combined treatment groups were 0.46±0.07 g, 0.18±0.04 g, 0.29±0.06 g, and 0.02±0.001 g, respectively. Similar results were seen for pleural effusion, with 287±77 μl, 12±12 μl, 141±107 μl, and 0±0 μl in these groups, respectively. Pleural invasion was also most significantly reduced in the combination group. Immunohistochemical staining demonstrated that combined ZD6474/paclitaxel therapy induced more extensive tumor and endothelial cell apoptosis than either treatment alone.
Conclusions: These data suggest that the combination of ZD6474 and paclitaxel results in significant enhancement of antitumor and antivascular effects, which translate into significant therapeutic benefits *in vivo*, providing a basis for the design of clinical trials in human lung cancer patients.

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Antitumor therapy with VEGF receptor tyrosine kinase inhibitor ZD6474 in a mouse model of intestinal cancer

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Background: ZD6474 is a novel, orally active, small molecule inhibitor of vascular endothelial growth factor (VEGF) receptor tyrosine kinase, with additional activity against epidermal growth factor receptor (EGFR) tyrosine kinase. The aim of these studies was to evaluate the antitumor activity of ZD6474 in a spontaneous disease model of early intestinal cancer. The *Apc* $^{Min/+}$ mouse model is considered clinically relevant and well-characterized. Mice develop in excess of 30 adenomas throughout the intestinal tract, which arise stochastically because of a mutation in the adenomatous polyposis coli (APC) gene: by 6 weeks of age and onwards, mice have macroscopically detectable adenomas.
Methods: Two treatment periods were examined: (a) early intervention, where 6-week old C57BL/6J-*Apc* $^{Min/+}$ mice (n=12 per group, mixed male/female) were dosed (p.o., once-daily) until week 10 with either ZD6474 (50 mg/kg/day) or vehicle; and (b) late intervention, where 10-week old *Apc* $^{Min/+}$ mice (n=12 per group, mixed male/female) were given ZD6474 (50 mg/kg/day) or vehicle daily until week 14. Immediately following treatment, mice were humanely sacrificed and the number and size of polyps in the small and large intestines scored.
Results: In the early intervention study, administration of ZD6474 (50 mg/kg/day) was associated with a 46% and 76% reduction in polyp number in the small bowel and colon respectively (P=0.03). Polyp diameter was also significantly reduced in the small bowel, reducing mean polyp burden by 75%. In addition, micropolyp count and size were reduced. Small bowel polyp number and diameter were also decreased by ZD6474 in the late intervention study, with total polyp burden being reduced by 72% (P<0.01).
Conclusions: ZD6474 significantly reduced the number and size of polyps when administered at either an early or a late stage of polyp development. These results suggest that the angiogenic switch may occur at an early, premalignant stage of tumor development and that VEGF/VEGFR-2 signaling plays a key role in this process. The marked efficacy of ZD6474 at later stages of polyp development could potentially be attributable to effects on VEGF and EGF signaling.

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Anticancer effects of ZD6474 in gefitinib (Iressa™)-resistant lung cancer cell lines in vitro

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Background: Molecularly targeted therapies may hold the key to increasing survival rates in NSCLC patients. The epidermal growth factor receptor (EGFR) is overexpressed in 50–80% of NSCLC patients and is a primary target for therapeutic intervention.
Methods: The efficacy of ZD6474, an orally available inhibitor of vascular endothelial growth factor receptor-2 (VEGFR-2) tyrosine kinase activity with additional activity against EGFR tyrosine kinase, was evaluated in a panel of NSCLC lines well characterized in our lab for gefitinib sensitivity or resistance. *In vitro* analyses included MTT assay, FACS analysis, Western blotting and flow cytometry. Potential synergy between ZD6474, or gefitinib, with chemotherapy, radiation therapy, and other biologically targeted agents was examined.
Results: Gefitinib-sensitive NSCLC lines (IC_{50} <1 μM) were equally sensitive to ZD6474. Similarly, gefitinib-resistant cell lines (IC_{50} >10 μM), and those with intermediate sensitivity to gefitinib (IC_{50} 1–10 μM), showed comparable levels of resistance to ZD6474. As with gefitinib, EGFR expression did not predict sensitivity to ZD6474. We were unable to demonstrate VEGFR expression in NSCLC lines by FACS or Western blot analysis. By flow cytometry, ZD6474 induced a greater G_1 arrest than gefitinib in gefitinib-sensitive and -resistant lines. Western blot of the gefitinib-sensitive line H322 showed that Tyr-phosphorylation at EGFR residues 845, 992, and 1068 was reduced by both compounds but to a greater degree by gefitinib. Both agents reduced pERK1/2 in H322 cells, but did not affect pERK1/2 in the gefitinib-resistant line H1264. PTyr1248 on the HER2 receptor in gefitinib-sensitive Calu-3 cells, which overexpress this receptor, was also significantly reduced by both agents. We then evaluated potential *in vitro* synergy between ZD6474, or gefitinib, with chemotherapy, radiation, and other biologically targeted agents, using MTT assays. Additive to synergistic interactions were seen with gefitinib and ZD6474 in sensitive lines with radiation and LY294002, a PI3 kinase inhibitor. This was also seen in resistant lines but the concentrations required were >1 μM.
Conclusions: These data indicate the potential anticancer effects of ZD6474 in gefitinib-resistant tumors. This activity will be investigated further in ongoing *in vivo* studies where the primary effects of ZD6474 on tumor endothelium will be demonstrated.

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In vivo effects of a monoclonal antibody to the murine VEGFR-3 that antagonizes the binding of VEGF-C and receptor signaling

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Background: Metastasis is the primary cause of mortality in patients with solid tumors and peritumor lymphatic vessel density correlates with rates of metastasis and clinical outcome. The receptor tyrosine kinase (RTK) VEGFR-3 has been shown to regulate lymphangiogenesis and is frequently expressed in tumor but not normal adult blood vessels. The ligands for VEGFR-3, VEGF-C and VEGF-D, are expressed by diverse types of tumors. Thus, inhibitors of this RTK may be efficacious as anti-tumor angiogenesis agents as well as modulators of metastasis. We now report on the production of a novel rat monoclonal antibody mF4–31C1 antagonizes the murine VEGFR-3. Initial effects of mF4–31C1 in pre-clinical in vivo models will be described.
Methods: A) Antibody characterization. eEnd endothelioma cells were incubated with mF4–31C1 or controls prior to stimulation with VEGF-C. Phosphorylation of receptor proteins was detected by Western blotting. Mitogenic stimulation was measured in NIH 3T3 cells transfected with a chimeric mouse VEGFR-3/cFMS receptor. B) Lymphatic regeneration. A circumferential band of skin was removed from the tails of mice and replaced with a collagen scaffold. Lymphatic and blood vessel regeneration were observed over time with or without systemic treatment with mF4–31C1. C) Tumor growth and metastasis models. Human tumor lines were implanted into nude mice and the growth of the tumor was followed during treatment with mF4–31C1 or control antibodies. Immunohistological analysis of the tumors was also performed.
Results: mF4–31C1 antagonizes VEGFR-3 activation in eEnd-1 cells and strongly inhibits (IC_{50} of 2–3 nM) VEGF-C-mediated mitogenic stimulation of cells that express a chimeric VEGFR-3/cFMS RTK. In normal mice or in nude mice implanted with VEGF-C overexpressing cells, treatment with mF4–31C1 completely blocked lymphatic regeneration. Pre-existing lymphatics and blood angiogenesis were not affected. In preliminary tumor xenograft and syngeneic mouse studies, mF4–31C1 inhibited the growth of primary tumors in a dose-dependent manner. This effect was most likely due to an inhibition of tumor angiogenesis.
Conclusions: Our results support the notion that targeting VEGFR-3 with antagonist antibodies may reduce tumor metastasis with an additional anti-angiogenic effect on tumor growth.