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A phase I dose-escalation study with oral LY317615 (L) in combination with capecitabine (C) in advanced cancer patients

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Protein kinase C (PKC) is a key enzyme in the signal transduction cascade induced by vascular endothelial growth factor (VEGF) binding its primary receptor, VEGF-R2. L, an acyclic bisindolylmaleimide, is a potent, selective inhibitor of PKCβ, with preclinical demonstration of antiangiogenic activity. A single phase I study of single-agent L has been completed; its principal toxicity is fatigue. Capecitabine, an oral prodrug of 5-fluorouracil (FU), has activity in colon and breast cancer with principal toxicities of diarrhea and hand-foot syndrome (HFS). Interestingly, the final step in the conversion of capecitabine to FU, requires the pro-angiogenic enzyme, thymidine phosphorylase (PDEGF), suggesting that sensitive tumors may exhibit an angiogenic phenotype. The rationale for the combination of L and C was based upon non-overlapping toxicities, mechanism of action, and potentially targeting angiogenesis by L in tumors sensitive to the effects of C. This phase I study is designed to evaluate the safety and pharmacokinetic (PK) behavior of L (350–700 mg/day po d1–21) and C (750–1000 mg/m^2/day po bid d1–14) given in 3-week courses (crs) to pts with advanced cancer. To date, 15 pts (M: F 9:6, median age 61 [range 34–71]; all PS 0–1) have received 47 crs (range 1–9). Fourteen pts are evaluable for toxicity. Two of 6 pts experienced DLT (grade [gr] 3 QT_c prolongation and gr 3 chest pain due to FU-induced coronary vasospasm) at the 350/1000 dose level. No other significant QT_c prolongation has been observed. The protocol has been amended to expand that cohort since the idiosyncratic reaction to FU may not represent a true dose-limiting event. Other non-hematologic toxicity has been quite mild and includes HFS (gr 1–2, 9 crs; gr 3–4, 0 crs), diarrhea (gr 1–2, 7 crs; gr 3–4, 1 crs), and nausea (gr 1–2, 12 crs; gr 3–4, 0 crs). No visual toxicity or gr 3–4 hematologic toxicity has been encountered. Of 13 pts evaluable for response, 1 pt with pancreatic cancer maintained SD for 9 crs, 3 pts (colon, lung, sarcoma) have maintained SD for 4 crs and remain on study. One pt is too early to evaluate; one pt came off study after Cycle 2 for toxicity (QT_c prolongation). PK analysis is pending in addition to biological analysis of *ex vivo* whole blood stimulation, both of which will be presented. These results indicate that this schedule of L and C is well-tolerated. Accrual is ongoing to establish the MTD of the combination, after which phase II studies are planned.

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Phase I trial of an antisense to vascular endothelial growth factor (VEGF-AS, Veglin) in relapsed and refractory malignancies

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Background: Vascular endothelial growth factor (VEGF) is critical for tumor angiogenesis. Elevated tumor or serum VEGF levels predict for poor survival in cancer patients (pts). A novel VEGF-antisense (VEGF-AS, Veglin) compound has been developed which targets VEGF-A, -C and -D. In vitro and in vivo studies of VEGF-AS have shown inhibition of VEGF expression, with inhibition of VEGF binding to its receptor, resulting in growth inhibition.
Methods: In the initial 4 dose levels of this phase I dose escalation and pharmacokinetic trial, pts received a single course of Veglin given as an intravenous infusion over 2 hours for 5 consecutive days at doses of 15, 22.5, 30 and 37.5 mg/m^2. Subsequent pts were approved to receive repeat cycles of Veglin given for 5 days every 2 weeks for up to 8 cycles at doses of 47, 59, 74, 85, 96, 125 and 150 mg/m^2. Cohorts of 3 patients were accrued to each dose level.
Results: To date, 21 male and 14 female pts, median age 57 years, (range 19–84) have been accrued. All failed standard conventional therapy including systemic chemotherapy in all 30 (85%), biologics or immunotherapy in 17 (49%), and radiation in 12 (34%). Tumor types accrued: non-Hodgkin's lymphoma in 5; sarcoma in 5; renal cell cancer in 6 and AIDS-related Kaposi's sarcoma in 3; colon and lung in 3 each; melanoma in 2 and myeloma, pancreatic, myoepithelioma, thyroid, adenoid cystic, prostate, mesothelioma and malignant gastrinoma in 1 each. All patients completed the first planned 5 days of Veglin. Veglin infusions were well tolerated and no dose limiting toxicities have been observed at doses up to 150 mg/m^2. No dose limiting toxicity has been defined at the 11 dose levels studies. Two cases of grade 3 anemia have been reported; no neutropenia or thrombocytopenia has been observed. No perturbation in coagulation or complement profiles have been seen at any dose level. Non-hematologic side effects were all grade 1 or 2 in severity and included diarrhea, fatigue, hypotension, and perioral numbness, and seen in less than 20% of pts. The maximum tolerated dose has not yet been reached. There has been evidence of clinical activity in 2 patients with AIDS-related KS (one an objective CR at the first dose level), in a patient with renal cell cancer, and one pt with nodular cutaneous T-cell NHL. Plasma VEGF levels studied sequentially declined in 56% of pts and was unchanged in 24% of pts.
Conclusions: Veglin is well tolerated at doses at up to 150 mg/m^2. No dose limiting toxicities have yet been observed. Veglin has shown evidence of anti-tumor activity, even at the lowest dose studied. Updated clinical and pharmacokinetic information will be presented.

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BIBF1120 a novel, small molecule triple angiokinase inhibitor: profiling as a clinical candidate for cancer therapy

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The inhibition of tumor angiogenesis has been at the forefront of oncology drug discovery for several years, with encouraging clinical research results for antibody and small-molecule drugs. Among the small-molecule drug candidates, receptor kinase inhibitors are of special interest, with most efforts being directed at the vascular endothelial growth factor receptor 2 (VEGFR-2) as the primary driver of endothelial cell proliferation, survival and migration. There is additional preclinical evidence concerning the importance of the role of the fibroblast growth factor receptor (FGFR) and platelet-derived growth factor receptor (PDGFR) kinase pathways acting on endothelial cells or perivascular cells and contributing to tumor angiogenesis. To fully exploit these three angiogenesis kinase targets, a chemical synthesis programme was conducted to design potent and drug-like "triple angiokinase inhibitors". One novel compound, BIBF1120, has been selected for development as an orally active drug and is in early clinical studies in cancer patients. The biochemical profile of BIBF1120 shows potent inhibition of VEGFR, FGFR, and PDGFR (IC_{50} 20–70nM), and little if any inhibition of many other signaling pathways or receptor classes. The compound blocks proliferation of VEGF-stimulated, cultured human endothelial cells (IC_{50} 10nM) with down-stream effects in the MAP kinase pathway and increased apoptosis; by contrast, it has no direct effect on epithelial cancer cell proliferation *in vitro*. BIBF1120 shows acceptable tolerability and suitable pharmacokinetic behavior in animals, and potent and long-lasting growth suppression and tumor regressions are achieved in diverse human cancer xenograft models, including the FaDu head-and-neck squamous cell carcinoma, Caki-1 renal cell carcinoma, and GS9L syngeneic rat glioma treated once daily with 25–100mg/kg BIBF1120 per os. In the FaDu model, antitumor activity is correlated with ~80% decrease in tumor vessel density, as measured by CD31 immunotyping, starting as early as 5 days after initiation of treatment. A distinct feature of BIBF1120 in cell culture studies is a long lasting inhibition of VEGFR-2 activation of up to 32h after 1h exposure to the drug. *In vivo* tumor regression can be achieved even with intermittent dose schedules.
In conclusion, BIBF1120 is a potent, orally bioavailable triple angiokinase inhibitor which holds promise for the ongoing clinical development.

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Blocking VEGF and EGF receptor signaling with ZD6474 sensitizes human non-small cell lung cancer to chemotherapy with paclitaxel

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Background: Lung cancer is the leading cause of human cancer death worldwide and treatment options for patients with advanced disease are limited. Vascular endothelial growth factor (VEGF) and epidermal growth factor (EGF) are critical survival factors for tumor-associated endothelial cells and tumor cells, respectively, and act by upregulating the Akt pathway and producing anti-apoptotic molecules. The role of VEGF receptor and EGF receptor signaling in the sensitivity of human lung cancer to treatment with paclitaxel has therefore been investigated.
Methods: The effects of ZD6474 (a small molecule inhibitor of VEGFR-2 tyrosine kinase with additional activity against EGFR tyrosine kinase) plus paclitaxel was assessed *in vitro* using human lung adenocarcinoma cells, NCI-H441, and mouse lung endothelial cells (MLECs). *In vivo* assessments were performed using an orthotopic NCI-H441 mouse model, which closely mimics the patterns of growth and metastasis observed in the clinic. Treatment with ZD6474 alone (12.5 mg/kg, p.o. daily), paclitaxel alone (150 μg/mouse, i.p. weekly), or a combination of the two agents was initiated on day 5 post-implantation.

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.