11 were given in combination. Moreover, the therapeutic effect of S-3304 in combination with carboplatin or paclitaxel was demonstrated in the solid tumor model of B16-BL6 murine melanoma cells. In conclusion, S-3304 has a potential for clinical use. All animal studies were approved by the Animal Care and Use Committee prior to initiation of the studies.

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A phase I study of the novel high affinity VEGF blocker VEGF trap in patients with refractory solid tumors and lymphoma

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VEGF Trap is a fusion protein consisting of portions of the human Vascular Endothelial Growth Factor (VEGF) receptor VEGFR1 (flt-1) and VEGFR2 (KDR) extracellular domains fused in series to the Fc portion of human IgG1. It acts by binding and inactivating VEGF in the circulation and in tissues. VEGF Trap has substantially greater (1-5 pM) affinity for the VEGF ligand than monoclonal antibodies. Preclinical studies indicate that subcutaneously (sc) administered VEGF Trap can substantially inhibit the growth of a variety of tumors implanted in mice. Preclinical pharmacokinetics predicted a half-life compatible with weekly dosing in humans. In this open-label, dose-escalation phase 1 study, a single sc dose of VEGF Trap is given to patients with relapsed and refractory solid tumors and lymphoma followed 4 weeks later by 6 weekly (sc) doses of the drug. Samples for pharmacokinetic analysis are collected both after the single dose and during chronic treatment. Patients are monitored for the development of anti-VEGF Trap antibodies. Anti-tumor efficacy is assessed by measuring changes in tumor mass clinically and/or by MRI. Tumor perfusion and water content is assessed in a subset of patients by dynamic contrast (Gadolinium)- enhanced MRI techniques. To date 6 patients have been treated on two dose levels: 25mcg/kg and 50mcg/kg. Early data reveals that the VEGF Trap complexes to circulating VEGF in plasma. To date, no anti-VEGF Trap antibodies have been detected in any of the patients treated. Longer term results for a larger number of patients and the pharmacokinetics of the Trap and Trap:VEGF complexes will be discussed.

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In vitro pharmacological profiles and *in vivo* anti-angiogenesis activity of S-3304, a novel matrix metalloproteinase inhibitor

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S-3304, a Na-[[2-[5-[[4-methylphenyl]ethynyl]thienyl]]sulfonyl]-D-tryptophan, which is synthesized through a few steps from commercially available compounds, is an orally-active and non-cytotoxic inhibitor of matrix metalloproteinase (MMP). The inhibitory effect of S-3304 against various human MMPs was examined in *in vitro* enzyme assay. S-3304 most potently inhibited the activities of MMP-2, -8, -9, -12, -13, weakly inhibits MMP-3-CD, -10, -14, -15, and -16, but does not inhibit MMP-1, -3 or -7. Crystal structure of DeltaFND-MMP-9 complexed with S-3304 was solved (Space group: P212121, Cell constants(Å): a=37.04, b=52.01, c=69.14,). The electron density map with 1.8 Å resolution revealed the interaction between S-3304 and the active site of the protein. In crystallographic data, it was clear that S-3304 sits in the S1' pocket deeply and nicely as a drug compound. We next examined the MMP inhibitory activity of S-3304 using gelatin zymography. The result showed that the gelatinase activity of MMP-2 and -9, derived from human tumor cells, was completely inhibited by S-3304. Furthermore, the effect of S-3304 on tumor-induced angiogenesis was investigated by the dorsal air-sac method. 107 cells of HT1080 human fibrosarcoma cells, which produce angiogenesis factors including VEGF, were filled into a chamber. The chamber was subcutaneously implanted into the dorsal side of mice. S-3304 was orally administered to the chamber-implanted mice twice a day at a dose of 20 and 200 mg/kg. Four days after implantation, the skin on the chamber was removed and fixed. The number and vascular area of vessels beneath the musculi cutaneous were histologically analyzed. Treatment with S-3304 resulted in reduction of the number and vascular area of vessels. Thus, S-3304 significantly inhibited the tumor-induced angiogenesis. All animal studies were approved by the Animal Care and Use Committee prior to initiation.

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Anti-angiogenic activity of the VEGF receptor tyrosine kinase inhibitor ZD6474

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ZD6474 is a small molecular weight inhibitor of KDR tyrosine kinase and a potent inhibitor of VEGF-induced human umbilical vein endothelial cell (HUVEC) proliferation (IC_{50} = 60 nM) that is in clinical development. Consistent with anti-angiogenic activity, the compound has demonstrated broad-spectrum activity in pre-clinical tumour models following chronic oral administration. ZD6474 has also been shown to inhibit ossification in the femoral growth plate of young rats; a physiological process which is dependent upon angiogenesis. For further confirmation of anti-angiogenic activity, ZD6474 was examined in two additional preclinical models. An *in vitro* model of endothelial cell tube formation was first used to determine the effect of ZD6474 on tubule growth and morphology. HUVEC and human fibroblasts were obtained as commercial co-cultures (AngioKit, TCS Cellworks, UK). Cells were maintained in MCDB131 media with or without ZD6474 for 11 days. To quantify tubule growth a novel whole-well method was developed using a Zeiss KS400 3.0 image analyser (Imaging Associates Ltd). Tubule formation was examined at day 11 following fixation and staining of tubules for CD31. Morphological parameters measured were total number of branch points, total tubule length and total area of tubule growth: ZD6474 inhibited each parameter significantly, with IC_{50} values of 33nM, 61nM and 93 nM respectively. An intradermal (i.d.) model of tumour-induced angiogenesis was then used to assess the effects of ZD6474 treatment *in vivo*. Male nude mice were implanted intradermally with A549 human lung tumour cells (1x107 cells/implant, 2 implant sites per mouse). Two additional injections of phosphate buffered saline (50 ul) were administered to each mouse as a control. ZD6474 (50 or 100mg/kg) or vehicle was administered orally for 5 days. Following treatment (day 6) the total number of blood vessels (major vessels and branching points) was determined within a 1cm^2 area around each implant site by light microscopy. A549 tumour cells induced significant angiogenesis, i.e. 152 $\pm$ 6.5 vessels compared with a background count of 27 $\pm$ 1.2 vessels in vehicle implants (mean $\pm$ S.E.). Treatment with 50 or 100 mg/kg/day ZD6474 inhibited the tumour-induced blood vessel formation by 63% and 79% respectively ($P<0.001$). These additional data are confirmatory of the anti-angiogenic activity of ZD6474.

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A phase I, double-blind, randomized, placebo-controlled study to investigate the safety tolerability and pharmacokinetic profile of S-3304, a matrix metalloproteinase inhibitor, when given in multiple doses with high doses for 4 weeks to healthy volunteers

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Objectives: This study was conducted to define maximum tolerated dose of S-3304 in normal healthy volunteers prior to initiation of a patient phase I study with solid tumors.
Study design: Eight subjects were randomized, 6 subjects to receive S-3304 and 2 subjects placebo at the following dose levels: 800 mg bid, 1600 mg bid, 2400 mg bid and 3200 mg bid. Subjects were to take study drug orally after meals once on Day 1, twice daily on Day 3 - 27 and once on Day 28. Safety assessment was based upon symptoms, signs, clinical laboratory tests and ECG. Dose escalation or study treatment was to stop, if three or more subjects at one dose level either: (1) experienced a Dose Limiting Toxicity defined as > grade 2 toxicity (NCI CTC); (2) had a hepatic transaminase of > 2.5 times upper limit of normal reference range; or (3) were withdrawn from further dosing due to symptoms interfering with normal daily activities. The protocol was approved by the local ethics committee prior to the study.
Results and Discussion: 4 male and 4 female subjects were enrolled to each dose group. All subjects at 800 mg bid completed the treatment. Two subjects were discontinued from treatment with 1600 mg bid, due to increased transaminases (grade 1 toxicity) and increased creatinine phosphokinase (grade 3 toxicity) respectively. One subject was discontinued from treatment at 2400 mg bid due to transient hair loss. Five subjects were withdrawn from treatment with 3200 mg bid due to: raised hepatic transam-