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Phase I safety, pharmacokinetic and pharmacodynamic study of recombinant human anti-VEGF antibody HuMV833 in patients with advanced cancer

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HuMV833 is a monoclonal humanised IgG4K anti-VEGF antibody (Ab) that is active against a broad range of human tumours *in vitro*. The study aims were to establish the toxicity, maximum tolerated dose (MTD) and optimum biologically active dose (OBD) of HuMV833. Cohorts of patients (pts) were treated with 0.3, 1.0, 3, and 10 mg/kg of HuMV833, administered as an I.V. infusion over sixty minutes on days 1, 15, 22, and 29. Serum samples for pharmacokinetic (PK) analysis were obtained on days 1, 15, 22, 29, 43, and at 3 months. Studies to ascertain the OBD included MR measurements of vascular permeability performed on days 0, 2 and 28 and 124I-labelled HuMV833 PET images taken on days 0, 2 and 3. A total of 20 pts (F=11, M=9) were recruited. The median age was 51 years (range: 31-70), and the median ECOG performance status was 1. A total of 229 doses were administered, with a median of 4 per pt. No grade IV toxicities attributable to the Ab were observed. There were no serious haemorrhagic events, although asymptomatic grade III elevation of APTT developed in 3 pts (dose levels 2,3 and 4) that resolved on discontinuation of the Ab. Two pts discontinued treatment because of grade III elevation of liver function tests secondary to progression of intra-hepatic metastases. Grade I or II toxicities, possibly or probably related to HuMV833, included coryza, cramp, dyspnoea and epistaxis. HuMV833 follows biphasic clearance kinetics. Plasma total VEGF (free and HuMV833-bound) concentrations undergo a significant sustained increase in response to HuMV833, but there remains a minimum of a 7 fold molar excess of Ab at all times. There is one ongoing partial response of 12 months duration in a patient with ovarian carcinoma. Stable disease was noted in 6 further pts with a median duration of 4.3 months (range: 1.4 -11). HuMV833 Ab is well tolerated at doses up to 10 mg/kg. The MTD was not reached but as the maximum amount of VEGF was chelated by doses of 3 mg/kg and as clearance was saturated at 10 mg/kg we recommend that doses of 1 or 3 mg/kg should be used for phase II evaluation.

Dose group (mg/kg)	N	Cmax (μg/L)	AUC (μg hr/L)	V1 (L/kg)	Vss (L/kg)	CL (L/hr/kg)	$t_{1/2}$ (hr)
0.3	4	3748.4	187664.6	0.0680	0.3046	0.00162	196
		±1009.5	±25133.6	±0.0367	±0.0775	±0.0002	±72
1.0	6	6432.7	438903.5	0.1584	0.7009	0.00239	326
		±1517.3	±109278.7	±0.0284	±0.1585	±0.0005	±97
3.0	6	46510.0	1949261.8	0.0690	0.3688	0.00171	333
		±15424	±596591.8	±0.0222	±0.0998	±0.0007	±157
10.0	5	401101	32597417.9	0.0268	0.1681	0.00045	448
		±148022	±25984532.7	±0.0094	±0.1942	±0.0003	±515

Biological activity was observed at the initial dose of 0.3 mg/kg but returned to pre-treatment levels within a week of completion of therapy, suggesting that the dose of 0.3 mg/kg is too low for further study. Overall, this anti-VEGF Ab is well tolerated and shows biological and clinical activity.

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PTEN suppresses hyaluronic acid induced matrix metalloproteinase-9 expression in U87MG glioblastoma cells through focal adhesion kinase dephosphorylation

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To investigate the role of PTEN in the regulation of hyaluronic acid (HA)-induced invasion of glioblastoma cells, the cells were treated with HA and it was found that matrix metalloproteinase (MMP)-9 secretion in glioblstoma cells lacking functional PTEN was induced, but not in wild type (wt)-PTEN-harboring cells. Introduction of wt-PTEN into U87MG cells reduced secretion of HA-induced MMP-9 and basal levels of MMP-2. Furthermore, the secretion levels of TIMP-1 and -2 were increased in PTEN-transfected cells. PTEN inhibited the activation of focal adhesion kinase and extracellularly regulated kinase 1/2, and then secretion of MMP-9 induced by HA. Infection of adenoviral wt-PTEN and lipid phosphatase-deficient PTEN, but not both protein and lipid phosphatase-deficient PTEN, reduced MMP-9 secretion and invasion by HA, thus protein phosphatase activity was crucial in these events.

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SU11248 and STI-571, small molecule inhibitors of Kit and PDGFR inhibit growth of SCLC preclinical models

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Coexpression of the receptor tyrosine kinase Kit and its ligand SCF has been reported in at least 30-70% of small cell lung cancer (SCLC) cell lines and tumor specimens. However, the role of Kit activity in SCLC is not well understood. We have evaluated the ability of two structurally distinct tyrosine kinase inhibitors with overlapping selectivity for their effects on Kit activity in SCLC. The indolinone kinase inhibitor SU11248 is a selective inhibitor of the angiogenic receptor tyrosine kinases Flk-1/KDR, Flt-1 and PDGFR, with ~10nM potency in cellular autophosphorylation assays. The aminopyrimidine STI-571 (Gleevec) inhibits Bcr-Abl and PDGFR, with ~100nM potency in cellular assays. To evaluate the potency of these two compounds against Kit in cell-based assays, the Kit-positive SCLC cell line NCI-H526 was utilized. Treatment of NCI-H526 cells with SU11248 or STI-571 resulted in a dose-dependent inhibition of SCF-stimulated Kit tyrosine phosphorylation, with IC_{50} values of ~10 nM and ~100 nM, respectively. SU11248 and STI-571 also inhibited SCF-stimulated *in vitro* proliferation of NCI-H526 cells at similar IC_{50} values to above. To examine the effects of these compounds on Kit phosphorylation *in vivo*, established tumors of NCI-H526 in athymic mice were treated with a single dose of each compound and samples were taken at a time point corresponding to approximate Cmax plasma concentrations. Consistent with the increased potency of SU11248 over STI-571 in the cellular assays, lower plasma levels of SU11248 were required to inhibit Kit phosphotyrosine levels *in vivo*. Additionally, PDGFR phosphotyrosine levels, presumably contributed by tumor stroma, were strongly inhibited by SU11248, and somewhat less so by STI-571. Repeated dosing of SU11248 in mice harboring NCI-H526 tumor xenografts resulted in inhibition of tumor growth. Interestingly, SU11248 also inhibited growth of tumor xenografts of the Kit-negative SCLC line NCI-H82, likely due to its anti-angiogenic activity. STI-571 is being evaluated in these models. Our data suggest that SCLC tumor growth *in vivo* is affected by multiple signaling pathways, including those of Kit, PDGFR, and VEGFR. SU11248 may have therapeutic clinical potential both as an angiogenesis inhibitor and in diseases that involve abnormal activation of Kit.

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Non-clinical therapeutic studies of S-3304, a novel matrix metalloproteinase inhibitor

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Among matrix metalloproteinase (MMP) family, MMP-2 and -9 are most strongly implicated, based on expression data and knock-out studies, as being important in tumor progression. S-3304 showed inhibitory activity against MMP-2 and -9. In the present study, the *in vivo* antitumor efficacy of S-3304 was examined using various tumor models. Daily oral administration of S-3304 (2-200 mg/kg) resulted in potent inhibition of metastatic lung colonization of Lewis murine lung carcinoma injected via tail vein and liver metastasis of C-1H human colon cancer implanted into the spleen. Daily administration of S-3304 also resulted in prolonged survival of mice given intraperitoneal implantation of Ma44 human lung cancer cells. When compared with other MMP inhibitors with broad inhibitory spectrum, the antitumor activity of S-3304 was as effective as that of AG-3340, and more potent than Marimastat and BAY12-9566. The inhibitory activity of orally administered S-3304 on gelatinolysis was investigated with Film In-situ Zymography (FIZ) using gelatin-coated films and tumor tissue from Ma44-bearing mice. Thus, oral administration of S-3304 successfully inhibited the MMP activity localized in Ma44 tumor tissues in both dose-dependent and time-dependent manner. In the hepatic metastasis of C-1H, the combination therapy with S-3304 and CPT-11 was examined. The results showed that S-3304 augmented the survival effect of CPT-11. The underlying mechanism for this combined antitumor effect of two drugs remains unclear. In order to explore whether S-3304 and CPT-11 interact with each other or not, S-3304, CPT-11 and SN-38, which was the active metabolite of CPT-11, were pharmacokinetically investigated. The results indicated that these drugs did not pharmacokinetically interact with each other even when S-3304 and CPT-

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-