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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-