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CNTO 95, A fully human monoclonal antibody to integrins alpha v β3 and alpha v β5 has direct anti-tumor and antiangiogenic activity

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Integrins alpha v β3 and alpha v β5 are implicated in tumor-induced angiogenesis, but their role in tumor growth has not been fully explored. These receptors are upregulated on angiogenic endothelial cells, and previous studies have demonstrated that inhibition of endothelial alpha v β;3 and alpha v β5 integrins can inhibit angiogenesis. These receptors may also directly contribute to tumor cell growth and invasion. We have developed a fully human antibody, CNTO 95, to integrins alpha v β3 and alpha v β5. Since CNTO 95 is a fully human antibody, it should be considerably less immunogenic in humans compared with chimeric or humanized antibodies. CNTO 95 bound to purified alpha v β3 and alpha v β5 with a K_D of ~200 pM. It did not bind to other integrin receptors such as alpha 5 β1 and alpha IIb β3. *In vitro* angiogenesis and anti-tumor assays demonstrated that CNTO 95 was a potent inhibitor of alpha v β3- and alpha v β5-mediated cell adhesion, invasion, and proliferation. In contrast, monospecific antibodies that blocked only alpha v β3 or alpha v β 5 were not as effective as CNTO 95. CNTO 95 inhibited human melanoma cell adhesion, migration and invasion at doses ranging from 7-20 nM. In a rat aortic ring sprouting assay CNTO 95 (~70 nM) completely inhibited sprouting. In order to determine if CNTO 95 had direct anti-tumor activity *in vivo*, we developed a human melanoma xenograft model in nude mice. In this model, CNTO 95 bound and inhibited alpha v β3 and alpha v β5 on the human tumors but did not inhibit mouse angiogenic integrins. CNTO 95 (10 mg/kg, 3x/week) inhibited growth of human melanoma tumors in nude mice by ~ 80% (P=0.0005). These data demonstrate that direct blockade of human tumor cells expressing alpha v β3 and alpha v β5 integrins by CNTO 95 can inhibit tumor growth independent of its antiangiogenic activity. These findings suggest that alpha v β3 or alpha v β5 in addition to participating in angiogenesis can also directly contribute to tumor growth. In conclusion, CNTO 95, a fully human monoclonal antibody to alpha v β3 and alpha v β5, has potent anti-tumor and antiangiogenic properties. CNTO 95 simultaneously inhibits angiogenesis and tumor growth and may offer substantial clinical advantage over agents that only have either antiangiogenic or anti-tumor properties.

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A phase I study of S-3304 a matrix metalloproteinase inhibitor in patients with solid tumors

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Background: S-3304 is a potent, orally active, non-cytotoxic inhibitor of matrix metalloproteinases (MMP) primarily MMP-2, -9 and -12. It prolonged survival in mice bearing B16-BL6 melanoma and the MA44 human lung carcinoma. It was well tolerated in healthy volunteers receiving 2400 mg b.i.d.
Objectives: To determine the maximum tolerated dose (MTD) dose limiting and other toxicities, the pharmacokinetic (PK) profile and the activity of S-3304 in inhibiting MMP in tumor from patients receiving the drug. Study Design: Patients (pts) with advanced solid tumors who have failed standard therapy or for whom no standard therapy exists are entered on study. All pts have a biopsy of accessible tumor. Following 28 days of twice daily oral administration of S-3304 (1 course), a second biopsy is performed. Tumor biopsies are evaluated by film in-situ zymography which has been shown to be useful in the evaluation of MMP inhibition *in vivo* in pre-clinical studies (Ikeda, M., et al. Clin Cancer Res 2000, 6:3290-6). Samples for PK are drawn on days 1 & 28. Treatment is continued until progressive disease or unacceptable toxicity.
Results and Discussion: A total of 19 patients completed at least 1 course, 6 at 800 mg b.i.d.; 6 at 1200 mg b.i.d. and 7 at 2400 mg b.i.d. Toxicities have been mild with grade I nausea and vomiting and grade I fatigue being the most common. A grade I rise in CPK has been noted in 2 pts. No clearly drug-related toxicity > grade II has been seen. Possibly drug related toxicities >grade II (each in 1 pt) include, grade III: - nausea, vomiting; grade II: - nausea, anorexia, myalgia, light headedness, proctitis and peripheral neuropathy. Steady-state conditions for the 12 patients on 800 mg b.i.d and 1600 mg b.i.d were reached by day 8 of the study with individual $C_{max,ss}$ ranging from 38 to 84 μg mL-1 and individual AUC_{ss} ranging from 177 to 592 μg mL^{-1}. h. Data for 2400 mg b.i.d. are pending. Film in-situ zymography of tumor biopsies demonstrated moderate-strong inhibition of MMP at both the 800 and 1600 mg b.i.d. dose levels at 28 days. Data for 2400 mg b.i.d. are pending. The study is continuing with recruitment at the final dose level of 3200 mg b.i.d.
Conclusion: S-3304 is a very well tolerated, oral MMP inhibitor which shows activity in tumor tissue from pts at doses which give minimal toxicity. Film in-situ zymography is an effective technique for monitoring MMP inhibition in patients with biopsiable tumor.

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A phase I study of the oral vascular endothelial growth factor (VEGF) receptor tyrosine kinase inhibitor PTK787/ZK 222584 on a twice daily schedule in patients with advanced cancer

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PTK787/ZK 222584 (PTK/ZK) is an oral inhibitor of VEGF-mediated KDR/flk-1 and flt-1 receptor tyrosine kinases. Patients (pts) with histologically confirmed and measurable advanced malignancy were recruited onto this phase I dose-escalating study. PTK/ZK was dosed on a twice daily schedule to exploit the theoretical advantage of maintaining constant drug levels above a threshold known to interfere with VEGF receptor signalling. Cohorts of pts (3 evaluable at each dose) were treated with total daily doses of 300 mg, 500 mg, 1000 mg, 1500 mg and 2000 mg. Dynamic contrast enhanced magnetic resonance imaging (DCE-MRI) was performed prior to PTK/ZK administration, on day 2 and after every 28 day cycle. The transfer constant Ki was calculated and tumour volumes measured for assessment by SWOG criteria. Full pharmacokinetic (PK) profiles were obtained on day 1 and day 28. 23 pts have been entered on to the study and 5 are ongoing. Tumour types treated include breast (1 pt), colorectal (15 pts), sarcoma (2 pts), gastric (2 pts), renal (2 pts) and carcinoid (1 pt). All were heavily pretreated. To date the range of cycles completed is 1-13 (median 3 cycles). 6 pts did not complete 1 cycle and were replaced, and 1 pt is too early for evaluation. DCE-MRI demonstrates reduction in Ki at all doses in a dose-dependent manner. In the 13 pts with evaluable disease, 4 had minor responses and 6 had stable disease (5/10 lasting * 6 months). Treatment has been well-tolerated. Transient grade 3 elevation in transaminases has been seen in 4 pts, and grade 3 hypertension in 2 pts, both with pre-existing hypertension. In addition transient grade 2 and 3 light-headedness and ataxia has been documented in 6 pts at total daily doses *1000 mg. Grade 3 nausea and vomiting has been seen in 4 pts, and grade 3 lethargy in 7 pts. PTK/ZK appears to reduce tumour perfusion/vascular permeability; moreover impressive stabilisation of disease and minor response * 6 months has been seen in 26% pts. Full PK and further MRI data will be presented demonstrating how these pharmacodynamic endpoints are guiding dose optimisation in future studies.

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Prognostic and predictive value of vascular endothelial growth factor (VEGF) in patients with non small cell lung cancer (NSCLC)

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VEGF is a regulator of angiogenesis and its production is stimulated by hypoxia. The circulating levels of VEGF could reflect the overall angiogenetic activity. The aim of our study was to investigate the prognostic value of VEGF on survival and response to chemotherapy in patients (pts) with NSCLC. 109 consecutive untreated NSCLC pts were enrolled in this prospective follow-up study from 4/1997 to 11/2001. Exclusion criteria were intra-vascular coagulation, recent transfusions (< 6 months), renal insufficiency, erythropoietin or anti-coagulant treatment. VEGF was measured in serum at baseline and at 2 hours after blood draw using a quantitative immunoassay kit for human VEGF 165 (Quantikine, R&D Systems, Minneapo-

lis, MN). Statistical analysis was performed by Cox's proportional hazard model. The pts population included 34 squamous, 26 adenocarcinomas, 12 large cell and 37 unspecified NSCLC. Nineteen pts had stage I-IIIA (94.7% underwent surgery), 29 had stage IIIB (55.2% underwent chemo-radiotherapy) and 61 had stage IV (65.6% pts received chemotherapy). At baseline, mean VEGF was 571 pg/ml (SD= 449). A difference was found for VEGF levels when measured at baseline or at 2 hours in a cohort of 68 samples (p=0.000009). VEGF positively correlated with white blood cells and platelets (r=0.24, p<0.0003; r=0.17, p=0.008) and negatively with Hb (r=-0.16, p=0.016). For stage, lymph nodes, metastasis (lung/brain) and co-morbidity (diabetes, arteriosclerosis, deep vein thrombosis) no association was detected. In 42 pts who were treated with Cisplatin and Gemcitabine, there was a trend for a better response in those with higher VEGF (61% vs 38%). Survival analysis was performed at a median follow-up time of 9 months and after 69 deaths. After adjusting for treatment, VEGF was associated with increased mortality risk (p=0.0004). Poor survival and high mortality risk were also associated with decreasing of Hb (p=0.0019) and low albumin levels (p=0.0004). VEGF was predictor of poor survival also in a multivariate model including treatment, Hb and albumin (Hazard Ratio 1.77, 95%CI from 1.01 to 3.10 for a variation of 1000 pg/ml, p<0.044).
Conclusions: A direct association between VEGF and mortality and an inverse correlation between Hb and VEGF were detected. The role of VEGF in response to chemotherapy could be due to its vascular permeability function. Measurements of serum VEGF should be performed at the same time point in order to reduce variability.

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Biomarkers (VEGF, BFGF) for assessing the biological activity of PTK787/zk222584 (PTK/ZK), a vascular endothelial growth factor (VEGF) receptor inhibitor, in tumours known to overexpress VEGF

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PTK/ZK is an orally active inhibitor of the VEGF receptor tyrosine kinases (flt-1/KDR), inhibiting VEGF-induced angiogenesis. It is known that such agents inhibit tumor growth, but not necessarily induce tumor regression. Thus, it is increasingly important to identify biomarkers that demonstrate the required drug-target interaction and the desired downstream biological effects. Two proangiogenic factors, plasma VEGF and bFGF (basic fibroblast growth factor) were assessed. Patients received a continuous daily dose of 50, 150, 300, 500, 750, 1000, 1200, 1500, or 2000 mg until progressive disease or toxicity. Samples were taken at predose, 10 hrs, day 8, day 15, day 15 + 10 hrs, day 22, and day 28 for each cycle. A total of 65 patients, with predominantly advanced colorectal cancer and glioblastoma, from 3 Phase I studies were evaluable for plasma VEGF and bFGF analysis. Using the SWOG criteria, non-progressive disease was defined as >= 2 months stable disease. PTK/ZK was rapidly absorbed with T_{max} of 1 to 2.5 hours. At steady-state (day 15), the exposure (AUC) was 30% lower than on day 1. Dose proportional increase in exposure was observed up to 1000 mg. The terminal half-life was 3-6 hours. No dose-limiting toxicity was observed up to 2000 mg. The extent of rise for both soluble biomarkers, plasma VEGF and bFGF, was evaulated by: 1) the concentration at 10 hours post-dose ($VEGF_{10\,hrs}$, $bFGF_{10\,hrs}$) and 2) the maximum concentration within 28 days ($VEGF_{max0-28}$, $bFGF_{max0-28}$). An dose-dependent rise in both plasma VEGF and bFGF levels was observed within the first 28 days of treatment. The rise was more prominent in non-progressors than progressors. In non-progressors, plasma VEGF and bFGF levels increased, respectively, by 5 and 3 fold at doses >= 1000 mg. The rise in plasma VEGF and bFGF would be consistent with an increased expression of VEGF and bFGF by tumor cells in response to hypoxia induced by the reduction in tumor vascular permeability and vascularization induced by PTK7ZK treatment. The observed decline in plasma VEGF and bFGF is attributed to the death of tumor cells. These results are supportive of previous DCE-MRI results which showed a reduction in tumor vascular permeability and vascularization within 36 hours post first dose of PTK/ZK treatment. The soluble biomarkers, plasma VEGF and bFGF, may be useful as indicators for biological activity of anti-angiogenesis agents and consequently tumor response.

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Generation and characterization of monoclonal antibodies that antagonize the binding of VEGF-C to VEGFR-3 (Flt-4)

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VEGFR-3, a member of the vascular endothelial growth factor family of receptors, has been shown to be involved in the proliferation and survival of lymphatic endothelial cells. Experimental over-expression of its ligands, VEGF-C and VEGF-D, by tumor cells results in increased rates of tumor metastasis. Although VEGFR-3 is absent from normal vascular endothelium in adults, its expression has been reported in actively forming blood vessels in tumors. Thus, VEGFR-3 is a potential target for both anti-metastatic and anti-angiogenic therapy. We used phage display to generate fully human monoclonal antibodies to human VEGFR-3. One such antibody, HF4-3C5, demonstrates strong inhibition of soluble VEGFR-3 binding to immobilized VEGF-C. BiaCore analysis shows the K_D of VEGF-C binding to VEGFR-3 to be approximately 3.5 nM. Using the same technique, the apparent K_D of HF4-3C5 for VEGFR-3 is 56 pM, exceeding that of VEGF-C by about 100-fold. Deletion studies show that both VEGF-C and HF4-3C5 bind to the three N-terminal immunoglobulin-like domains of VEGFR-3. NIH-3T3 cells were transfected with expression vectors encoding either full-length human VEGFR-3 or a chimeric receptor in which the extracellular domain of human VEGFR-3 was fused to the transmembrane and kinase domains of cFms. Resulting stable cell lines responded strongly to VEGF-C but not VEGF-A in a mitogenic assay. This response was inhibited by greater than 90 % by HF4-3C5 but not isotype-matched control antibodies. Because HF4-3C5 does not bind to murine VEGFR-3, monoclonal antibodies to the mouse receptor are being generated for the purpose of conducting proof-of-principle studies. These experiments will evaluate anti-angiogenic and anti-metastatic efficacy of blocking the activation of VEGFR-3 in mouse tumor models.

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A phase I dose escalating study of the angiogenesis inhibitor thrombospondin-1 mimetic (abt-510) in patients with advanced cancer

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Thrombospondin-1 (TSP-1) is a naturally occurring protein inhibitor of angiogenesis. ABT-510, a thrombospondin-mimetic peptide that exhibits antiangiogenic activity in preclinical models, inhibits tumor growth in animal studies at concentrations * 200 ng/mL for 3 hours per day. We determined safety and pharmacology of subcutaneously (SC) administered ABT-510, either given as daily continuous infusion (CI) or as bolus injections once or twice daily (QD and BID) in a phase I study. Plasma samples for PK obtained on days 1 and 22 were analyzed by LC/MS/MS. In selected patients, PET-scans with $H_2{}^{15}O$ and ^{18}F-FDG were performed at days 1 and 22. Response was assessed after every 2 cycles of 28 days each. To date, 26 patients (pts) are treated with CI 100 mg/day (4 pts), bolus 50 mg BID (6 pts), 100 mg QD (6 pts), 200 mg QD (5 pts) and 260 mg QD (5 pts). Local CTC grade 2 skin infiltration at injection sites occurred in all pts of the CI cohort. CI dosing was stopped, while SC dosing continues. The most commonly observed adverse events include grade 1 and 2 fatigue, anorexia, insomnia, headache and nausea. One patient (NSCLC) with progressive disease had a hemorrhage in an unknown cerebellar metastasis after 32 days of ABT-510 treatment at 100 mg QD. Another patient (leiomyosarcoma) had a TIA after 21 days at 260 mg QD. Both SAEs were determined to be possibly related to ABT-510. No other clinically significant, treatment-related toxicities, nor cumulative toxicities have been observed to date. PK analysis on day 1 revealed rapid absorption with T1/2 of approx. 1 h, C_{max} of 955 $\pm$ 350, 1793 $\pm$ 549 and 3293 $\pm$ 1105 ng/mL and AUC of 2734 $\pm$ 728, 4168 $\pm$ 1335 and 8636 $\pm$ 1331 ng*h/mL for the 50 mg BID (N=5), 100 mg QD (N =3) and 200 mg QD (N=5) cohorts, respectively. PK data on day 22 showed similar results. Serial PET-scans have been performed in 4 patients to date. Stable disease according to RECIST-criteria was seen in 9 out of 23 evaluable patients for more than 2 cycles or 8 weeks. Five patients had stable disease > 16 weeks (different tumor types).