223

Increased angiogenesis by bcl-2 in melanoma cells

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To evaluate the role of bcl-2 on melanoma-associated angiogenesis, we studied the effect of bcl-2 overexpression on *in vitro* and *in vivo* angiogenesis, as well as on vascular endothelial growth factor(VEGF) synthesis, mRNA stability and transcriptional activity of a human melanoma line. To this purpose the M14 cell line has been transfected with a bcl-2 expression vector and some clones overexpressing bcl-2 have been used for all the experiments. We demonstrated that bcl-2 overexpression enhances the synthesis of VEGF in hypoxic conditions, and that the conditioned media from bcl-2 transfectants exposed to hypoxic conditions increase endothelial cell proliferation and sprouting and angiogenesis in the chick chorioallantoic membrane. We also found that increased VEGF mRNA stability contributes to the induction of the VEGF message with bcl-2 overexpressing cells grown in hypoxia. Next, we evaluated HIF-1a expression at the mRNA and protein levels and HIF-1a DNA binding activity in hypoxic conditions. Although HIF-1a mRNA expression was shown to be similar in parental line and bcl-2 transfectants, HIF-1a protein expression and DNA binding activity were significantly increased after bcl-2 overexpression. Taken together our results indicate that bcl-2 plays an important role in melanoma angiogenesis, and that VEGF mRNA stabilization and HIF-1-mediated transcriptional activity are two important control points in bcl-2/hypoxia induced VEGF expression. Since VEGF is an early signal in the process of angiogenesis, the identification of the mechanism regulating VEGF induction by bcl-2 could be useful in clarifying the basis of pathological angiogenesis and could be useful for early diagnosis. In addition, therapy with molecules able to downregulate bcl-2 expression will be particularly indicated in those patients having solid malignancies with high vascularization and high levels of bcl-2 protein.

224

Non-clinical assessments of drug metabolism and pharmacokinetics of S-3304, a matrix metalloproteinase inhibitor

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S-3304, a potent, orally-active and non-cytotoxic inhibitor of matrix metalloproteinase-2, -9 and -12, has been developed by Shionogi Research Laboratories. Absorption, distribution, metabolism and excretion studies of S-3304 have been carried out in mice, rats and dogs. All animal studies were approved by the Shionogi Animal Care and Use Committee prior to initiation. Oral administration of [14C]-S-3304 showed that plasma radioactivity was rapidly increased with Tmax of 0.6 hr (mice) and 1.5 hr (rats), and eliminated with T1/2 of 5.7 hr (mice) and 3.5 hr (rats). After oral administration of S-3304, dose-linearity of AUC was observed at the doses up to 10 mg/kg in mice, 30 mg/kg in rats and dogs. The absolute bioavailability of S-3304 was 34% in mice, 32% in rats and 13% in dogs. Gender-related differences of the AUC and Cmax of S-3304 were observed in mice and rats. AUC was unchanged in fasted rats, but Cmax and MRT were increased. Following 14 days of repeated dosing in rats, PK parameters were essentially the same as those following a single dose. After oral administration of [14C]-S-3304 to rats (30 mg/kg), radioactivity was predominantly distributed to the liver, mesenteric lymph node and kidney with Tmax (liver) around 2 hr, and then almost all the radioactivity was eliminated within 24 hr. Most of the radioactivity (>94%) was excreted into feces within 24 hr after oral administration of [14C]-S-3304 to mice, rats and dogs, suggesting that the major excretion pathway of the radioactivity was fecal excretion via bile. In the plasma, 4-carboxy-S-3304 and 4-hydroxymethyl-S-3304 were found in mice, rats, dogs, monkeys and humans, while 6-hydroxy-S-3304 and 5-hydroxy-S-3304 were found in mice, rats, monkeys and humans, but not in dogs. When S-3304 was incubated with rat and human hepatic microsomes, 6-hydroxy-S-3304 and 4-hydroxymethyl-S-3304 were produced as major metabolites. In the case of rat hepatocytes, 4-carboxy-S-3304 was produced as an additional metabolite. S-3304 has weak inhibitory effect on human CYP2C9 and CYP3A4.

225

A Phase II Study of ANGIOZYME® in combination with 5-fluorouracil, leucovorin and irinotecan in the treatment of metastatic colorectal cancer patients

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Background: Vascular endothelial growth factor (VEGF) mediates angiogenesis through two endothelial cell surface receptors, VEGFR-1 and VEGFR-2. ANGIOZYME, a chemically stabilized ribozyme, specifically targets VEGFR-1 mRNA. ANGIOZYME demonstrated good preclinical activity in several xenograft models.
Goal: Evaluate the safety and efficacy of ANGIOZYME, an anti-angiogenic ribozyme, in combination with 5-fluororuacil (5-FU), leucovorin and irinotecan as compared to historical data from the Saltz trial (Saltz LB, et al., N Engl J Med 343, 905-14, 2000).
Study Design: Open-label Phase II trial of ANGIOZYME in combination with 5-FU, leucovorin, and irinotecan according to the Saltz regimen in 83 treatment naïve Stage IV metastatic colorectal cancer patients (pts). Patients receive daily subcutaneous injections of 100 mg/m^2 ANGIOZYME starting on day 4 of the trial.
Preliminary Safety Analysis (as of April 5, 2002): The all-cause 60-day mortality included only one patient. Patients enrolled in this trial had a similar number of risk factors for progression when compared to the patients enrolled in the Saltz study. Serious adverse events possibly related to the study medication include: febrile neutropenia (7 pts), deep venous thromboses (6 pts), pulmonary emboli (6 pts), abdominal pain (3 pts), dehydration (3 pts), neutropenia (2 pts), pneumonia (2 pts) and exertional dyspnea (1 pt). ANGIOZYME was generally well tolerated.
Initial Results: Preliminary analysis of 12-week follow-up data for the first 40 patients enrolled in this trial demonstrated that only 5 patients (12.5%) progressed by week 12. In the comparator study (Saltz regimen), 25% of patients had progressed by week 12. The results to date compare favorably to chemotherapy alone. Final evaluation will include response rate, stable disease, time to progression and survival.

226

The effect of human recombinant endostatin (rh-Endo) on blood flow and glucose metabolism in normal tissues

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rh-Endo decreases tumor blood flow and glucose metabolism in tumor deposits assessed in patients treated with doses of rh-Endo > 120 mg/m^2/day (Herbst RS, ASCO 20:3a, 2001 [abstr 9]). However, since the specificity of rh-Endo for tumor-related endothelial cells isunknown, evaluating its effects on normal tissue is important. We sought to analyze the PET scans performed on patients treated with daily intravenous bolus doses of rh-Endo to measure the changes in blood flow and glucose metabolism in normal tissues. Regions of interest were drawn in normal tissue imaged by the PET scan. Thirty-five tumors were analyzed from 25 diferent patients. Measurements of blood flow and glucose metabolism were made from non-tumor bearing regions of the lungs, liver, cardiac muscle, and skeletal muscle. Data were analyzed by computing the change in blood flow and metabolism from the baseline scan to the scan performed after 28 days of therapy. For this preliminary analysis, all doses and all the normal organ data were grouped together and analyzed in this fashion. There was no significant change in blood flow or glucose metabolism in the lungs, liver, cardiac muscle, or skeletal muscle following 28 days of treatment with rh-Endo. Additional studies are underway to evaluate the long-term effect of rh-Endo on these organs as function of of dose and will be presented at the meeting.