($p<0.001$), but RRM1 and p53R2 expression were not significantly correlated. There was a trend towards longer survival in patients with increased RRM1 expression.

266

Inhibition of VEGFR tyrosine kinase by ZK 222584/ ptk 787 (PTK/ZK) combined with fractionated radiotherapy (RT) in human squamous cell carcinoma (hSCC) in nude mice

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Purpose: To investigate the effect of the antiangiogenetic substance PTK/ZK, a specific inhibitor of VEGFR tyrosine kinases, on the growth rate of different hSCC and on the growth delay after fractionated RT of hSCC.
Materials and methods: Five hSCC lines (FaDu, UT-SCC-14, UT-SCC-33, UT-SCC-15, MKG7) were transplanted s.c. in NMRI nu/nu mice. Presence of murine VEGFR mRNA was confirmed by RT-PCR. At a mean tumor diameter of 6 mm animals were treated daily with PTK/ZK (joint development of Schering and Novartis; 50 mg/kg bodyweight per os) or with carrier (control). In a second set of experiments FaDu and UT-SCC-14 tumors were irradiated with 15 fractions of 2 Gy under ambient conditions (200 kV X-rays, 0.5 mm Cu, 1.2 Gy/min). PTK/ZK was given either before (4-8 days), during (15 days), or after (45 days) the course of fractionated RT.
Results: PTK/ZK was well tolerated. A significant decrease of growth rate in tumors treated with PTK/ZK was observed in 3 of the 5 hSCC. For the combination experiments with RT a non-responding (FaDu) and a responding (UT-SCC-14) tumor model were chosen. Short-term application of PTK/ZK before and during fractionated irradiation did not significantly change the growth delay of FaDu and UT-SCC-14 tumors. In both tumor models the longer application of PTK/ZK after fractionated RT showed a significant increased growth delay compared with irradiated controls. In UT-SCC-14 a significant increase in local tumor control was observed.
Conclusions: Short term neoadjuvant or simultaneous application of PTK/ZK did not decrease the efficacy of fractionated RT in non-responding FaDu and responding UT-SCC-14 tumors. Adjuvant application improved the effect of RT in both tumor models, i.e. also in FaDu tumors in which PTK/ZK alone had no effect. This might suggest enhanced sensitivity of irradiated tumor vessels to VEGFR-inhibition. Supported in part by Schering AG, Berlin, Germany.

267

Use of a novel, hepatocyte growth factor-induced transcript, Mig-7, as a marker for circulating and migrating cancer cells

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Although various molecular markers have been used for the detection of circulating cancer cells in the blood or of migrating cancer cells in tissue surrounding tumors, many have been found to be limited to certain types of cancers or not to be specific for cancer cells. Hepatocyte growth factor, also known as scatter factor (HGF/SF), has been shown to cause migration of many different types of cancer cells upon activation of the c-Met protooncogene receptor. HGF/SF has also been shown to cause epithelial to mesenchyme transition so that migrating cancer cells are difficult to detect in the stroma surrounding the tumor. Both HGF/SF and c-Met have been localized to the invasive edge of tumors. Because HGF/SF and c-Met are found in normal cells as well as in the bloodstream, they themselves do not make good markers for migrating and circulating cancer cells. Our laboratory has isolated a novel, HGF/SF-induced transcript, now called Mig-7 that is specific to migrating cancer cells. We hypothesized that circulating cancer cells could be detected in the blood using Mig-7 as a marker. Under Internal Review Board approval, we isolated total RNA from the blood of treated and untreated metastatic cancer patients (breast, endometrial, and lung) and compared transcripts to those from normal individuals. By RT-PCR, we detected Mig-7 mRNA in 66.7% of blood samples from untreated patients (n=3) and a complete absence of Mig-7 transcripts in treated (n=2) or normal individuals (n=3). Our second hypothesis was that Mig-7 is a marker for epithelial to mesenchyme transitioned migrating cancer cells in normal tissue surrounding tumors. We have tested tumor samples from metastatic cancer patients and were able to detect various levels of Mig-7 mRNA in 100% of the tumor samples (n=4). Negative control was negative for Mig-7 expression and positive controls showed that RNA was intact and that there was no DNA contamination. Results from a cancer-profiling array of various cancer types show that Mig-7 expression is detected in pathologist evaluated "normal" tissue surrounding tumors. In conclusion, Mig-7 may be used as a broad spectrum, cancer cell-specific marker to detect circulating and migrating cancer cells.

268

Endothelial precursor cells from human bone marrow: target for anti-angiogenesis therapy

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Tumor vasculature has been a potential target for anti-cancer therapy. While blood vessels can be derived from nearby existing vasculature, more recent evidence is suggesting that endothelial precursor cells (EPCs) derived from bone marrow or mobilized into peripheral blood may play a role in neoangiogenesis. Endothelial precursor cells from bone marrow expressing CD34 and AC133 markers of endothelial cell lineage were stimulated in culture with vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), and heparin on fibronectin-coated flasks. Within two weeks, cells that had been maintained in suspension became adherent and elongated. As EPCs continued to differentiate and proliferate, expression of AC133 and CD34 was downregulated while expression of VEGFR2/FLK-1 was upregulated. Cells also expressed CD105, a common stem cell marker and protein expressed in vascular endothelial cells. Expression of additional endothelial markers such as VE-cadherin, CD31, and von Willebrand factor was also investigated. In addition to stimulation with VEGF and bFGF, the roles of epidermal growth factor (EGF), platelet derived endothelial cell growth factor (PD-ECGF), and transforming growth factor beta (TGF-b) were subsequently explored to determine their effects on cellular differentiation. These endothelial progenitor cells can form tubule networks on Matrigel, and possess migratory and invasive properties *in vitro*. Lectin-binding and acetylated LDL uptake have also been investigated. Because EPCs may be involved in the development of tumor vasculature, the response of these precursor cells to cancer cells in various settings was demonstrated *in vitro* in a novel tumor spheroid assay. Endothelial precursor cells from bone marrow or mobilized into circulation with cytokines can be stimulated by pro-angiogenic factors into differentiating into a more mature cell type that possesses properties associated with well-defined endothelial cells such as HMVECs and HUVECs. The potential contribution of EPCs to tumor neovascularization defines them as an additional target for drug intervention therapy that may lead to a reduction in tumor vasculature or prevention of metastasis.

269

The protein tyrosine kinase inhibitor SU5614 inhibits FLT3 and induces growth arrest and apoptosis in AML cells expressing a constitutively activated FLT3

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Activating mutations of the protein tyrosine kinase (PTK) FLT3 can be found in approximately 30% of patients with acute myeloid leukemia (AML) thereby representing the most frequent genetic alterations in AML. These mutations occur in the juxtamembrane (FLT3ITD) and the catalytic domain (FLT3D835/836) of FLT3 and confer IL-3 independent growth to Ba/F3 cells. In the mouse BMT model, the FLT3ITD mutants induce a myeloproliferative syndrome stressing their transforming activity *in vivo*. In this study we analyzed the pro-proliferative and anti-apoptotic potential of FLT3 in FLT3ITD/D835 transformed Ba/F3 cells and AML cells expressing an endogenous activated FLT3 receptor by using the PTK inhibitor SU5614. SU5614 has inhibitory activity for FLT3 and induces growth arrest, apoptosis and cell cycle arrest in Ba/F3 and AML cells expressing a constitutively activated FLT3. No cytotoxic activity of SU5614 was found in leukemic cell lines which express a nonactivated FLT3 or no FLT3 protein. At the biochemical level, SU5614 downregulated the activity of the hyperphosphorylated FLT3 receptor and its downstream targets STAT3, STAT5 and MAPK and the STAT5 target genes BCL-XL and p21. Our results show that SU5614 is an PTK inhibitor of FLT3 and has potent anti-proliferative and pro-apoptotic