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

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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

D. Zips[1], M. Krause[1], J. Westphal[1], K. Bruechner[1,2], W. Eicheler[1,2], A. Doerfler[1], C. Hoinkis[1], R. Grenman[3], C. Petersen[1], M. Baumann[1,2]. *[1]Radiation Oncology, [2]Experimental Center, Medical Faculty of the TU Dresden, Dresden, Germany; [3]University of Turku, Head and Neck Surgery, Turku, Finland*

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.

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

J.S. Lindsey, B. Puckett, T.M. Phillips. *Texas Tech University Health Science Center, Pharmaceutical Sciences, Amarillo, USA*

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.

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

R. Bagley, J. Walter-Yohrling, C. Rouleau, T. Lodie, B. Simons, A. Dash, B. Teicher. *Genzyme Molecular Oncology, Tumor Biology, Framingham, USA*

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.

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

R.J. Dirschinger[1,2], R. Schwab[1,2], F. Faber[1,2], C. Buske[1,2], S. Schnittger[2], L.M. Kelly[3], D.G. Gilliland[3], W. Hiddemann[1,2], K. Spiekermann[1,2]. *[1]GSF National Research Center for Environment and He, Clinical Cooperative Group Leukemia, Munich, Germany; [2]University Hospital Grosshadern, Department of Medicine III, Munich, Germany; [3]Howard Hughes Medical Institute and Brigham and Wom, Harvard Instit, Harvard Medical School, USA*

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

activity in AML cells which endogenously express an activated FLT3 receptor. The selective and potent cytotoxicty of FLT3 PTK inhibitors support a clinical strategy of targeting FLT3 as a new molecular treatment option for patients with FLT3ITD/D835 positive AML.

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Tumour associated cellular and molecular changes induced in endothelial cells

N.N. Khodarev[1], J. Yu[1], E. Nodzenski[1], T. Darga[1], C.K. Brown[1], H.J. Mauceri[1], R. Yassari[2], N. Gupta[3], R. Weichselbaum[1]. [1]*University of Chicago, Radiation and Cellular Oncology, Chicago, USA;* [2]*University of Chicago, Section of Neurosurgery, Chicago, USA;* [3]*University of California San Francisco, Department of Neurosurgery, San Francisco, USA*

Tumour angiogenesis is a complex process based upon a sequence of interactions between tumour cells and endothelial cells. To model tumour/endothelial cell interactions, we co-cultured U87 human glioma cells with human umbilical vein endothelial cells (HUVEC). U87 cells induced an "activated" phenotype in HUVEC including an increase in proliferation, migration, tube formation and protection from radiation-induced apoptosis. Activation was observed in co-cultures where cells were in direct contact and physically separated, suggesting an important role for soluble factor(s) in the phenotypic and genotypic changes observed. Expressional profiling of tumour-activated endothelial cells was evaluated using cDNA arrays and confirmed by quantitative PCR. Four major functional groups of genes have been shown to be induced in endothelial cells in the result of their interactions with tumor cells. These groups were 1. growth factors, cytokines and chemokines, 2. receptors of growth factors and cytokines, 3. cell structure/motility/extracellular matrix and 4. DNA repair/recombination. Matching pairs of receptors/ligands were found to be coordinately expressed, including TGFbRII with TGFb3, FGFRII and CRF-1 with FGF7 and FGF12, CCR1, CCR3, CCR5 with RANTES and CGRP type 1 receptor with adrenomedullin. Consistent with cDNA array data immunohistochemical staining of expressed proteins revealed the up-regulation of Tie-2 receptor *in vitro* and *in vivo*. Our data suggest that tumour-induced activation of quiescent endothelial cells involves the expression of angiogenesis-related receptors and the induction of autocrine growth loops. Combination of co-culture system with large scale expressional profiling of both counterparts (tumor cells and endothelial cells) may lead to identification of new molecular targets for tumor therapeutics.

Chemoprevention

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Risk targeting and strategy for chemoprevention of head and neck cancer

J. Sudbo[1], J.E. Sondresen[2], A. Reith[1], M. Bryne[2]. [1]*The Norwegain Radium Hospital, Department of Oncology, OSLO, Norway;* [2]*University of Oslo, Department of Oral Biology, OSLO, Norway*

Emerging data indicate a link between genetic instability and up-regulation of cyclooxygenase-2 (COX-2). To see if individuals at high risk of oral cancer are candidates for treatment with selective COX-2 inhibitors (coxibs), levels of COX-2 expression in healthy, premalignant and cancerous oral mucosa were compared to the occurrence of DNA ploidy status as a genetic risk marker of oral cancer. COX-2 gene product was detected immunohistochemically in 30 healthy persons, in 22 patients with dysplastic lesions without previous or concomitant carcinomas, and in 29 patients with oral carcinomas. The immunohistochemical findings were verified by in situ hybridization of COX-2 mRNA and Western blotting. COX-2 expression was correlated to DNA content as a genetic risk marker of oral cancer. COX-2 was up-regulated from healthy to premalignant to cancerous oral mucosa. Thus, COX-2 expression was found in 1 case of healthy oral mucosa (3 percent). All specimens from healthy mucosa had a normal DNA content. In patients with premalignancies. In 29 patients with oral carcinomas, cyclooxygenase-2 expression was observed in 26 (88 percent), and aneuploidy was observed in 25 cases (94 percent, P = 0.04). Notably, Of 22 patients with dysplastic lesions, COX-2 was exclusively expressed in a subgroup of 9 patients (41 percent) identified to be at high risk of cancer by the aberrant DNA content of their lesions. Seven of these patients were followed for 5 years or more. An oral carcinoma developed in 6 of them (85 percent; P=0.02). These findings emphasize the need to determine whether coxibs can reduce the risk of oral cancer in patients with high-risk precancerous lesions.

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A phase II trial of an attenuated adenovirus, ONYX-015, as mouthwash therapy for premalignant dysplastic oral lesions

E.E.W. Cohen[1], V.A. Papadimitrakopoulou[2], S.J. Silverman[3], W. Recant[4], A. El-Naggar[5], W.K. Hong[2], S. Freeman[6], E.E. Vokes[1], C.M. Rudin[1]. [1]*The University of Chicago, Medicine, Chicago, USA;* [2]*MD Anderson Cancer Center, Thoracic/Head and Neck Medical Oncology, Houston, USA;* [3]*The University of California San Francisco, School of Dentistry, San Francisco, USA;* [4]*The University of Chicago, Pathology, Chicago, USA;* [5]*MD Anderson Cancer Center, Pathology, Houston, USA;* [6]*Onyx Pharmaceuticals, Richmond, USA*

Oral leukoplakia and erythroplakia are measurable lesions of the oral mucosa that can progress to carcinoma, especially when harboring histologic characteristics of severe dysplasia. It is estimated that 30-40% of these dysplastic lesions contain inactivating mutations of the p53 gene, while a significant proportion of the remainder are likely to have functional defects in p53 response pathways. Surgery can eradicate these lesions but does not reduce cancer incidence. ONYX-015 is an adenovirus lacking the gene encoding E1B 55kd. Since this protein binds to and inactivates cellular p53, and is necessary for efficient viral replication in cells, ONYX-015 should be selectively cytotoxic against p53 deficient cancerous and precancerous cells. The current study sought to establish the feasibility and activity of ONYX-015, administered topically as a mouthwash, to patients with clinically apparent lesions and histologic dysplasia. The trial endpoint was the degree of histologic improvement. ONYX-015 was administered in three different schedules to consecutive cohorts (TABLE). Biopsies of the involved mucosa were performed to evaluate histologic response and changes in expression of p53, cyclin D1, and Ki-67 by immunohistochemistry. Serology was also performed to measure anti-adenoviral titers. A total of 22 (19 evaluable) patients were enrolled on study from August 1998 to January 2002. Complete histologic resolution of dysplasia was seen in 4 (21%) of 19 patients, while the grade of dysplasia improved in an additional 3 patients. Of the patients who had complete responses, 2 have gone on to develop worsening histological features at the same or a new site. No toxicity greater than grade 2 (febrile episode in one patient) and no increase in anti-adenoviral antibodies were seen. A comprehensive analysis of biologic correlates to clinical and histologic responses will be presented. This approach to cancer prevention is tolerable, feasible, and has demonstrable activity.

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COX189 (PrexigeTM), a novel, selective cyclooxygenase-2 inhibitor, totally inhibits formation of intestinal polyps in C57BL/6J-APCmin mouse model of human adenomatous polyposis coli, and reduces neovascularization *in vivo*

P. Lassota[1], K. LaMontagne[1], A.W. Wood[1], R. Kashi[1], H. Wang[1], J. Butler[1], C. Schnell[1], J. Wood[1], R. Fujimoto[2], Y.P. Chen[1]. [1]*Novartis Institute for Biomedical Research, Oncology, Summit, USA;* [2]*Novartis Institute for Biomedical Research, Arthritis and Bone Metabolism, Summit, USA*

Levels of cyclooxygenase-2 (COX-2) are elevated in various types of cancers, like adenocarcinomas of the colon, breast and pancreas, and squamous cell carcinomas of the head and neck. Inhibition of COX-2 activity delays growth of tumors in animal models, and inhibits neovascularization. Here we describe the antineoplastic activity of a novel, selective, COX-2 inhibitor, COX189 (PrexigeTM, lumiracoxib). The compound, administered as a dietary admixture at 125 and 250 ppm, totally inhibited formation of intestinal polyps in the C57BL/6J-APCmin mouse model of human adenomatous polyposis coli. At higher doses (500, and 1000 ppm) COX189 also reduced the number of existing polyps without lowering the platelet thromboxane B2 levels, indicating that COX-1 was not inhibited under these conditions. In the mouse corneal micropocket assay the compound administered at 250 ppm caused statistically significant reduction of bFGF-induced neovascularization by 66%. The above results confirm that selective COX-2 inhibition, without inhibiting COX-1 activity, result in a potent antitumor activity, which, in part, may result from the inhibition of neovascularization.