ties with IC_{50} values in low nanomolar range as evaluated by MTT assays in various cancer cells such as breast cancer, prostate cancer, pancreatic cancer and non-small-cell lung cancer cells. The *in vivo* anti-tumor effects of this group of compounds are being investigated in animal models.

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Activation of c-Jun-N-terminal-kinase by R- and S-flurbiprofen results in cell cycle arrest in human colon carcinoma cells

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The unspecific cyclooxygenase inhibitor S-flurbiprofen and its "inactive" enantiomer R-flurbiprofen have been previously found to inhibit tumor development and growth in APC min mice, TRAMP mice as well as in rats. The mechanisms underlying the antiproliferative effects of R- and S-flurbiprofen are unknown. In the present study we show that both R- and S-flurbiprofen inhibit survival of three colon cancer cell lines which differ in the expression of COX-2 (HCT-15: no COX-2, Caco-2: inducible COX-2 and HT-29: constitutive COX-2). The IC_{50}s for S- and R-flurbiprofen ranged from 250-450μM. Both flurbiprofen enantiomers induced apoptosis in all three cell lines as indicated by DNA- and PARP-cleavage. In addition, R- and S-flurbiprofen treatment resulted in a G1-cell cycle block. These effects were associated with an activation of c-Jun N-terminal kinase (JNK), an increase of the DNA binding activity of the transcription factor AP-1 and downregulation of cyclin D1 expression. Supershift experiments indicated that R- and S-flurbiprofen-induced AP-1 activation was associated with a shift in its Jun-protein composition from c-Jun towards JunB. The latter is known to repress cyclin D1 expression. Inhibition of JNK activity prevented the R- and S-flurbiprofen-induced AP-1 DNA binding activity, the repression of cyclin D1 expression and the G1-cell cycle block. However, JNK inhibition had no effect on flurbiprofen-induced apoptosis. These data suggest that the cell cycle inhibitory effects of R- and S-flurbiprofen are mediated at least in part through activation of JNK and subsequent down-regulation of cyclin D1 whereas R- and S-flurbiprofen-induced apoptosis is largely independent of JNK activation. Although *in vitro* effects of R- and S-flurbiprofen were indistinguishable, only R-flurbiprofen inhibited HCT-15 tumor growth in nude mice, suggesting that additional anti-tumoral effects which are specific for R-flurbiprofen only become operative *in vivo*.

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Cks1, a subunit of cyclin-dependent kinases, as a novel target for the treatment of colon cancer

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The level of p27Kip1 protein, a cyclin-dependent kinase inhibitor, is indicative for tumor progression and tightly regulated by several mechansims during the cell cycle-one of them is degradation mediated by the ubiquitin conjugating enzyme complex SCFSKP2 during G1-phase of the cell cycle. Recently, it was demonstrated that Skp2 is overexpressed in various transformed cell lines and in human cancer and that Skp2 overexpression correlates directly with grade of malignancy and inversely with p27 levels in human lymphomas. Here, we report that Cks1, a subunit of cyclin-dependent kinases, is directly affecting p27Kip1 protein levels in human tumor cells while protein levels of cyclin B1, which needs to be degradated by the proteasome after ubiquitination in order to allow the cells to exit mitosis, remain unaltered. Microarray analyses revealed that Cks1 mRNA is more abundant in human colon cancer tissue samples than in normal tissue samples. Further we demonstrated that depletion of Cks1 does affect cell proliferation as well as anchorage independent growth in tumor cell lines indicating that Cks1 in involved in maintaining a transformed cell phenotype. These data provide some evidence that Cks1 could be a novel target for treatment of colon cancer and suggest regulation of the tumorsuppressor p27Kip1 as a possible mechanism.

Signal transduction modulators

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Pharmacogenomic expression profiling of renal cell carcinoma in a phase II trial of CCI-779: identification of surrogate markers of disease and predictors of outcome in the compartment of peripheral blood

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While it is evident that the transcriptomes of primary malignancies differ considerably from corresponding normal tissue, it is currently unknown whether in the context of active solid tumor burden there exist correspondingly distinct markers of gene expression in the peripheral blood of affected individuals. One of the main objectives of clinical pharmacogenomic studies is to determine whether easily obtained (often non-target) tissues such as peripheral blood can be used to identify surrogate markers of disease and predictors of outcome *in vivo*. In the present study we identified pharmacogenomic markers of advanced renal cell carcinoma (RCC) in peripheral blood mononuclear cells (PBMCs) from patients during a phase II trial of the investigational drug CCI-779 which targets the mTOR pathway. RNA samples of PBMCs from RCC patients at baseline (n=45) and normal volunteers (n=20) were hybridized to oligonucleotide arrays containing 12,626 unique transcripts and statistical analyses identified a subset of disease genes significantly changed between the groups. A supervised learning approach identified minimal sets of genes expressed in PBMCs capable of accurately predicting RCC versus normal state. To explore the molecular basis for this differentially expressed gene set, RCC PBMC expression profiles were compared with RCC tumor profiles in silico, with PHA-stimulated PBMC expression profiles ex vivo, and with PBMC expression profiles from end-stage renal failure patients. At the conclusion of the clinical trial, the original baseline expression data in RCC PBMCs were reassessed to determine whether biomarkers eventually predictive of outcome were present in blood samples prior to initiation of CCI-779 therapy. Both unsupervised and supervised approaches identified gene sets in RCC PBMCs that resulted in stratification of responders and non-responders into groups with significant differences in time to disease progression. The present disease gene set lays the foundation for biomarkers that will be explored in larger phase III clinical trials and may eventually aid in the diagnosis and treatment of renal cell carcinoma. Of equal importance, the pattern of gene expression within the easily obtained compartment of peripheral blood correlated with longer time to disease progression could eventually assist in the stratification of patients with higher likelihood for positive responses to CCI-779 therapy in this disease setting.

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Cellular and molecular markers of metastatic potential as targets for micrometastasis detection in gastrointestinal cancer

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Background: Tumor-cell dissemination and metastasis are complex processes whose outcome depend upon cancer cells properties and host-tumor interactions. Metastatic phenotype included activation of growth factors signalling pathways (G), differentiation (D), deregulated adhesion (DAd), epithelial-mesenchymal transition (EMT), invasiveness (Inv) and angiogenesis (A). In order to target micrometastasis (MM) in patients with gastrointestinal cancer (GC) we have developed a model system based on multi parametric immunocytochemistry (IC) and molecular analysis of metastasis-related (MR) markers.
Methods: As surrogate model of GC the following human tumor cell-lines (CL) were used: colorectal Gp5d, LoVo, DLD1, LS513, HT29; gastroesophageal OE19 and pancreatic MBQ-OJC1. In addition hematopoietic (H) CL were analysed. Monoclonal antibodies (mAb) against the following MR antigens (Ag) were selected for IC: EGFR, cytokeratins types I-II (CK), Ep-CAM and N-cadherin. Ag-mAb reaction was developed with avidin-biotin-complex and alkaline phosphatase. Molecular analysis were performed us-

ing reverse transcriptase(RT)-PCR. mRNA targets were EGFR (G), CK19 and CK20 (D), Beta-1,6-N-acetyl-glucosaminyltransferase V (GNT-V) related to DAd and pituitary-tumor transforming gene-1 (PTTG1) associated with Inv and A.

Results: IC revealed high expression for EGFR, CK and EpCAM in all the GCCL tested. N-cadherin (EMT-marker) staining was found only in a few number of Gp5d cells. No signal for any of these Ag was detected in normal blood mononuclear cells. Although CK and EpCAM are presumed to be epithelial-specific, IC staining found both on K562 HCL. RT-PCR showed specific amplicons for EGFR and CK20 in 7 and 6 GCCL respectively but not on HCL. PTTG1 mRNA was found in 6 GCCL but also in 2 out 3 HCL tested. GNT-V mRNA was also amplified in all GCCL and K562 cells. PCR amplification of cDNA from normal lymph nodes (LN) and bone marrows (BM) were negative for EGFR, CK20 and GNT-V but PTTG1 transcript was found on BM. CK19 was highly unspecific due to illegitimate transcription and/or pseudogene.

Conclusions: EGFR, CK and EpCAM seem to be sensitive targets for GC cells detection by IC. Multi parametric RT-PCR for EGFR, CK20, and GNT-V could serve as sensitive and specific method for targeting MM in LN and BM. Although high level of PTTG1 transcripts in GCCL was demonstrated our results suggest that PTTG1 is not specific enough for MM analysis.

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Combination of the heat shock protein 90 (HSP90) chaperone inhibitor 17-allylamino, 17-demethoxygeldanamycin (17AAG) and conventional cytotoxic agents in an ovarian cancer cell line model

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17-allylamino,17-demethoxygeldanamycin (17AAG) is a benzoquinone ansamycin that inhibits the HSP90 chaperone complex. This prevents folding of client proteins such as c-Raf-1, Akt, Src and Cdk4, leading to their subsequent degradation by the ubiquitin proteasome pathway. Paclitaxel (Pac), cisplatin (CDDP) and topotecan (Topo) are agents currently used to treat ovarian cancer and act by microtubular stabilization, DNA adduct formation and topoisomerase I inhibition respectively. We have investigated the interactions of 17AAG with these agents *in vitro*. Initial studies included treatment of HT29 and HCT116 human colon cancer cells with equitoxic doses of 17AAG, Pac, CDDP and Topo and studying client protein depletion and co-chaperone induction by western blot analysis. We then studied the potential synergy or antagonism of these agents used in combination with 17AAG in a human ovarian cell line (A2780) model. Sulforhodamine (SRB) growth inhibition assays were carried out and results analysed by median effect analysis as described by Chu and Talalay. Synergy was defined as a combination index (CI) < 0.9, antagonism as CI > 1.1 and additivity as 0.9-1.1). Western blot analysis revealed depletion of the client proteins c-Raf-1/ Cdk4 and the induction of the co-chaperone HSP70 when the HT29 and HCT116 cells were treated with 17AAG but not when treated with Pac, CDDP or Topo indicating that Pac, CDDP and Topo did not inhibit HSP90. Results of the combination studies in A2780 cells revealed 17AAG was antagonistic to Pac, and Topo (CI = 2.0 and 1.4 respectively), and was additive to CDDP (CI = 1.0) during simultaneous exposure. Based on this we chose to explore sequence dependency of 17AAG and CDDP. When cells were exposed to 17AAG 24 hrs prior to CDDP, the combination was antagonistic (CI = 1.6) while a sequence of 24hr pre-treatment with CDDP followed by 17AAG proved additive (CI = 1.0). It is possible that 17AAG alters intracellular stress response to DNA damaging agents and we are currently investigating this. We have previously shown promising activity of 17AAG in an A2780 ovarian cancer xenograft model and plan to follow this up with experiments combining 17AAG and CDDP in this model. 17AAG and CDDP have nonoverlapping toxicity profiles and it should be possible to combine these in a clinical setting.

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Final results of a clinical and pharmacokinetic (PK) phase I study of the Raf kinase inhibitor BAY 43-9006 in refractory solid cancers: a promising anti-tumor agent

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Raf-1 is a protein kinase that acts as a downstream effector of the Ras signal transduction pathway. BAY 43-9006 (BAY) is an inhibitor of Raf-1. This phase I study was initiated to determine the MTD, DLT, PK, pharmacodynamics (inhibition of ERK phosphorylation in peripheral blood lymphocytes) and recommended phase II dose of BAY given orally in an intermittent schedule 3 weeks out of 4 weeks. To date, 38 evaluable patients [colorectal 15, breast 7, renal 6, head and neck 3, melanoma 2, others 5; median age 58 (42-76); PS (0/1/2) 10/26/2] received BAY at 8 dose levels (DL); DL1: 50 mg OD, on days 1, 5, 10, 15 and 20 (3 patients); DL2: 50 mg OD on days 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21 (4 patients); DL3: 100 mg BID (4 patients), daily; DL4: 200 mg BID (4 patients), daily; DL5: 300 mg BID (4 patients), daily; DL6: 600 mg BID (12 patients), daily; DL7: 800 mg BID (3 patients), daily; DL8: 400 mg BID (4 patients), daily. BAY was administered for 3 weeks with a 1-week rest period. Dose escalation and schedule were decided based on clinical and PK results from this and other ongoing phase I studies. Anorexia, fatigue, alopecia, diarrhoea, and mainly skin toxicity (rash, hand and foot syndrome, folliculitis and dryness of skin) have been reported. Skin toxicity limited dose escalation and reduced dose intensity of BAY at the highest dose levels (600 and 800 mg). All toxicities were rapidly reversible and no myelosuppression was seen. The median time (days) on BAY for all patients and for patients started at 600 mg BID was 48+ (12 $\pm$ 356) and 84+ (19-196+) respectively. PK was evaluated on day 1, 7, and 21 at all dose levels. Steady state was achieved at day 7. After linear increase in C max and AUC up to 300 mg BID, further increase was modest. T 1/2 of the terminal phase beyond day 21 was between 30 and 45h and did not change between dose steps. Tumor shrinkage * 20% occurred in 3 patients (renal 2, rectum 1) entered at 600 mg BID, with 1 renal patient achieving a confirmed partial response. Three patients (colon 2, head and neck 1) had stable disease > 4 months. In summary, these phase I data suggest that BAY 43-9006 is a promising antitumor agent that warrants further clinical study. Accrual in this study is ongoing at 400 BID up to a total of 10 patients and full analysis of this cohort will be presented. Phase II studies with BAY 43-9006 are planned.

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Inhibition of ERK phosphorylation in patients treated with the Raf kinase inhibitor BAY 43-9006

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The Ras-Raf pathway is involved in the abnormally elevated signaling of many common solid tumors. The extracellular signal-regulated kinase (ERK) serves as a downstream shuttle protein into the nucleus and thus mediates cell proliferation. BAY 43-9006 is a novel potent and orally active inhibitor of Raf kinase and the first compound of this class to enter clinical trials. It was the purpose of this study to develop a method for the quantification of the inhibitory potency of this new compound by measuring phosphorylated (activated) ERK as a biomarker. Peripheral blood lymphocytes (PBLs) collected from patients with advanced cancers treated at various dose levels of BAY 43-9006 as part of a clinical trial were monitored for BAY 43-9006-dependent inhibition of PMA-stimulated ERK phosphorylation by flow cytometry. Western-blot analyses using the same phospho-specific antibody were performed for validation of the results. Blood samples were collected before treatment and on days 1, 2 and 10-21 between 10 am and 2 pm to allow comparisons among patients at different dose levels. We observed substantial inhibition of PMA-stimulated ERK phosphorylation in 2/6 patients following continuous treatment for 10-14 days starting at dose level (DL) 9 (200 mg bid continuous), as well as 4/6 patients treated at DL 10 (400 mg bid continuous) and all patients (6/6) treated at DL 11 (800 mg/ bid continuous). The time course and extent of ERK inhibition in PBLs tended to parallel the DL of BAY 43-9006 administered. Inhibition of stimulated ERK phosphorylation was measured in 1 patient with hepatocellular carcinoma who attained a sustained partial response and in 3 patients