them (including diarrhoea) were grade 1 or 2, except for leukopenia. There was no clear relationship between flavopiridol dose level and the occurrence or severity of these adverse events. One subject at 85 mg/m^2 died of cardiac arrest and myocardial infarction during the first cycle and was judged to be possibly related to administration of flavopiridol. Pharmacokinetics of flavopiridol showed a non-linear increase in concentrations at the end of the infusion. The mean half-life of flavopiridol was 27 hr [range 7-66 hr]. Most patients exhibited a secondary peak in plasma concentrations between 24 hr and 48 hr after initiation of dosing. Of the 12 patients (evaluable for response), 6 had an overall partial response and 6 had stable disease (WHO criteria). The results of this study indicate that flavopiridol in combination with paclitaxel and carboplatin can be given to cancer patients safely up to a dose of 70 mg/m^2 as a 24-hr infusion. Future clinical trials will investigate whether 1-hr infusion of flavopiridol in combination with chemotherapy is superior to chemotherapy alone in the treatment of advanced NSCLC.

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Phase II study of E7070 in patients with metastatic melanoma (stage IV)

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Background: E7070 (N-(3-chloro-7-indolyl)-1,4-benzenedisulfonamide) is a novel sulphonamide derivative which delays the G1/S transition by inhibiting cyclin E expression and cdk2 phosphorylation.
Patients and methods: Patients received 700 mg/m^2 of E7070 as a 60-min infusion every 3 weeks. A two stage Gehan study design was applied on the basis that if the results of the trial were compatible with a response rate of 15% in the studied population, the drug should be further investigated in malignant melanoma. Blood samples were taken during cycles 1 and 3 for population pharmacokinetic analysis. Paired skin biopsies were collected from 3 patients for micro-array analysis within 14 days prior to the 1st administration and 48h (±6h) following the 1st administration of E7070. Results The study was conducted in 8 EORTC institutions in 7 countries. 24 patients were eligible out of 28 recruited in 6.5 months. Demographic data were as follows: median age 56 years (range 26-75); M/F - 18/10; PS 0/1/2-15/12/1. 25 patients showed normal ECG at baseline with only 3 judged to be abnormal with no clinical significance. 27 patients were chemo-naive and one had received (neo)adjuvant chemotherapy. 6 patients had prior immuno/BRM/vaccination treatment. Site of the primary was leg (11), head (7), trunk (5), neck (1), arm (1) and 3 unknown. Data are available for 28 patients who received 71 cycles (median-2; range: 1-14). Safety profile: 3 patients experienced Grade (G) 3 leucocytopenia; 4-G3 neutropenia; 3-G3 anemia and 3-G3 thrombocytopenia. Non-hematological G3 toxicity was: supraventricular arrhythmia; infection; thromboembolism; injection site reaction; rash; vomiting; (one case each); fatigue (3 cases) and G4: dyspnea. Reasons for stopping treatment were PD (22), toxicity (2) and other reason (4). Central objective response review was performed and, in eligible patients, 5 NC, 13 PD, 1 early death were validated and 5 not assessable.
Conclusion: E7070 at the dose of 700 mg/m^2 as a 60-min infusion does not produce objective responses in metastatic melanoma stage IV. Toxicity profile was as expected from previous preclinical and Phase I clinical data and appears to be acceptable, reversible and easily manageable. Full data analysis will be available at the time of presentation.

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Gene expression profiling of the cyclin-dependent kinase inhibitor CYC202 (R-Roscovitine)

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Components of the cell cycle are crucial for regulating proliferation and are frequently deregulated in cancer. In particular, loss of negative regulators of the cell cycle, such as p16INK4A or amplification of positive regulators such as cyclin D1, leads to an increased proliferative capacity. Therefore, inhibitors of cell cycle progression are of interest as anticancer therapeutics. The cyclin-dependent kinases phosphorylate the retinoblastoma protein (RB), displacing E2F transcription factors and lead to progression through the cell cycle. Inhibition of CDK activity blocks the phosphorylation of RB and prevents cell cycle progression, retarding cellular proliferation. In addition, specific inhibition of CDK2 has been shown to induce tumour cell-specific apoptosis. As a consequence, CYC202, a selective inhibitor of CDK2 is currently undergoing clinical trials. Gene expression profiling of human colon adenocarcinoma cell lines (KM12 and HT29) treated with CYC202 was performed in order to further explore the mechanism of action of the compound and how it may be utilised most effectively. Cells were treated with 20-50uM CYC202 for time course experiments of up to 72h, concentrations that inhibit RB phosphorylation and block cellular proliferation. Analysis of treated cells by flow cytometry showed that CYC202 caused a loss of cells in G1 phase and an increase in the number of cells in G2/M. RNase protection assay demonstrated a loss of cyclins D1, B1 and A which was confirmed by Western blotting. The levels of CDK1, 2 and 4 protein were unchanged in response to CYC202. Loss of the cyclins is a potential mechanism through which cell cycle arrest is maintained in addition to inhibition of CDK activity by CYC202. CYC202 was compared with equitoxic doses of flavopiridol and alsterpaullone to determine any common trends associated with CDK inhibition. Preliminary analysis indicates that CYC202 induced changes in the expression of genes involved in cell cycle control and apoptosis. The significance of these responses is to be investigated and results will be discussed.

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A novel class of pyridopyrimidine tyrosine kinase inhibitors blocks cancer cells in the S-phase of the cell cycle

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A novel family of pyrido [2,3-d]pyrimidines has been identified on the basis of their selective inhibition of Src-related tyrosine kinases (TKs) (Kraker A.J. et al, 2000, Biochem. Pharmacol. Oct. 1; 60(7):885-98). Further work has shown that individual members of the family also have activity against PDGF receptor and c-Abl. We have examined the effects of this family on cancer cell lines and have previously shown that a subset of compounds, including PD173955, block cells in the prophase stage of mitosis. We now report that another subset inhibits S-phase progression. The M-phase and S-phase activities are mutually exclusive in that individual compounds inhibit progression of the cell through mitosis or S phase, but not both. Paradoxically, the S-phase inhibitor PD179483 caused activation of cyclin A, cdk2 and cdc2-associated kinase activity. No such activation was elicited by the M-phase inhibitors. Phosphorylation of the Tyr-15 residue of cyclin-dependent kinases (cdks) results in their inhibition and is catalyzed by the Myt1 and Wee1 tyrosine kinases. Activation of cdk kinases in PD179483 treated cells was associated with dephosphorylation of Tyr-15 and with failure to rephosphorylate this tyrosine in cells progressing from mitosis to early G1. Furthermore, PD179483 causes the accumulation of both Wee1 and Myt1 kinases in hyperphosphorylated state that correlates with their inhibition. These data suggest that PD179483 selectively affects a target upstream of Wee1 and Myt1 that is required for their activation. This results in inability to phosphorylate Tyr-15, dysregulated cdk activation and is associated with S-phase arrest.

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A novel class of cdc25 phosphatase inhibitors shows potent anticancer activity by blocking cell cycle and inducing apoptosis

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The dual specificity phosphatase cdc25 regulates cell cycle progression through activation of cyclin/cyclin-dependent kinase (cdk) complexes by removing inhibitory phosphate groups from cdks. Of three human cdc25 homologs, cdc25A and cdc25B are considered potential oncogenes because overexpression of these genes is found in up to 50% of all major human cancers and is associated with oncogenic transformation. MAXIA has developed a new class of small molecule compounds that inhibit cdc25. MX7306 and other compounds of this series exhibited selective inhibition of cdc25A activity in *in vitro* phosphatase assays as compared to the dual specificity phosphatase MKP-1 and the tyrosine phosphatase PTP1B. Treatment of PC-3 prostate cancer cells with cdc25 inhibitors enhanced cdk2 phosphorylation at Tyr15 and diminished the cdk2 kinase activity without changes in protein levels of cdks 1, 2 and 4 as well as p21 (waf1) and p27 (kip1), which is consistent with the direct inhibition of cdc25. Cell cycle analysis revealed that the phosphatase inhibitors blocked G1/S transition in synchronized PC-3 cells. MX7306 or its analogs also inhibited DNA replication and S phase progression of asynchronously growing cells as assessed by BrdU incorporation. Furthermore, extended exposure to inhibitors led to cell apoptosis. MX7306 and its analogs exhibited efficacious and broad anticancer activi-

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-