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

with stabilization of previous progressive disease. Our results demonstrate that BAY 43-9006 administered at dose levels >200 mg bid inhibits PMA-stimulated ERK phosphorylation in treated patients and indicates that PBLs are suitable surrogate tissues for biomarker studies in future trials.

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Results of a phase I trial of the humanized anti epidermal growth factor receptor (EGFr) monoclonal antibody emd 72000 in patients with EGFr expressing solid tumors

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The epidermal growth factor receptor has been identified as an important target for anticancer therapy. EMD 72000 is a humanized monoclonal antibody that binds selectively to the EGFr and inhibits ligand mediated activation.
Study objective: To determine the maximum tolerated dose (MTD), dose-limiting toxicities (DLT) and the pharmacokinetic profile of EMD 72000.
Methods: Patients (pts) had to have EGFr-positive (confirmed by immunohistochemistry) tumors, measurable disease, refractory to standard chemotherapy (CTx). EMD 72000 was administered once a week as 1 h infusion without any routine premedication. The initial dose level (DL) of 400 mg (absolute dose)/week was escalated in 400 mg steps in cohorts of 3 patients until DLT was reached.
Results: 22 pts received EMD 72000 on 5 different DLs. Pts characteristics: Male 11/female 11; median age 58 years (range 29-71); median PS 90% (range 70-100%), primary tumors: upper oesophagus 2 pts, colorectal 11 pts, head and neck 4 pts, others 5 pts. The median number of prior CTx regimens was 3 (range 1-4) and all pts had progressive disease. The MTD was exceeded at DL5 (2000 mg of EMD 72000/week); DLTs were NCI-CTC grade 3 headache and fever after the first infusion. All 3 patients continued therapy with EMD 72000 at a reduced dose (1600 mg/week). Acneiforme skin reactions were mild with NCI-CTC grade 1 in 9 pts (41%) and grade 2 in 4 pts (18%). No other related adverse events especially no severe diarrhea and alterations in transaminases were seen. All pts are evaluable for tumor response: 5 out of 22 pts had a partial remission and 4 pts had stable disease. Responding pts have been treated for up to 25+ weeks without severe cumulative toxicities. Pharmacokinetic analysis showed a dose proportional increase of EMD 72000 in terms of Cmax and AUC, indicating that EMD 72000 exhibits predictable pharmacokinetics at the investigated dose range.
Conclusions: The MTD of a weekly schedule of EMD 72000 is 1600 mg per week with severe headache and fever being dose-limiting at higher doses. Objective remissions have been observed with EMD 72000 as single agent therapy.

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Thalidomide modulation of Irinotecan; an NF-kB dependent effect?

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Irinotecan (I) and thalidomide (T) in combination result in considerable antitumor activity in patients (pts) with colorectal cancer and attenuated toxicity. Preclinical evidence suggests T inhibits activation of the transcription factor NFkB, and decreases tumor resistance to I by inhibition of NFkB nuclear localization. We hypothesized that the mechanism for the favorable interactions of these agents is inhibition of NFkB activation. To test this hypothesis, we performed a phase I trial of I in combination with T. I, 125 mg/m^2 IV on days 1 and 8, every 3 weeks was administered in combination with daily oral T to pts with solid malignancies. To evaluate potential pharmacokinetic (PK) and biological interactions, T was started on day 3 of the first cycle. Plasma, serum and peripheral blood mononuclear cells (PBMC) lysates were obtained during days 1-3, 12-14 and 22-24, for evaluation of I and T Pks, as well as serum TNF alpha, bFGF and PBMC NFkB nuclear-localization (Trans-AM Active Motif ELISA). The starting T dose was 400 mg/day in a 10 pts cohort. De-escalation to 200 mg was planned in a second cohort of 10 pts if NFkB activation was inhibited in 80% of pts at 400 mg, for dose-effect evaluation. 33 cycles of the combination were given to the first 10 pts. Somnolence, nausea/vomiting and mild peripheral neuropathy were the most frequent side effects. No episodes of grade 3/4 diarrhea or myelosuppression occurred. 1 patient experienced a pulmonary embolism but was able to continue treatment while on oral anticoagulants. No differences in I Pks for the combination as compared to single agent were detected (SN-38+SN38G/I AUC metabolic ratio d1, 0.18±0.09; d22, 0.17±0.05). Although no changes in serum TNF or bFGF levels were detected on the first 4 pts, NFkB expression normalized to control increased after I alone (d1 baseline, 0.079±0.035mg; 48 h, 0.118±0.06 mg) (mean percentage increase, 44), but decreased after exposure to combined I/T (d22 baseline, 0.087±0.048 mg; 48 h, 0.06±0.024 mg) (mean decrease, 25%). Antitumor activity was observed in various refractory malignancies, including non-small cell lung, carcinoid, colorectal and thyroid carcinoma. In summary, T appears to improve the tolerability of I without interfering with its disposition and metabolism. Initial results suggest that I induces NFkB activation and that 400 mg of T daily can inhibit this activation. Data on additional patients receiving lower doses (200 mg) of T will be presented.

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Phase I, pharmacokinetic (PK), and biological studies of the epidermal growth factor receptor-tyrosine kinase (EGFR-TK) inhibitor OSI-774 (Erlotinib or Tarceva) in combination with docetaxel

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OSI-774, an oral quinazoline with potent and selective inhibitory effects on EGFR-TK, has demonstrated impressive activity in non-small cell lung carcinoma, and head/neck cancers in early clinical trials. This study was undertaken to evaluate the feasibility of administering OSI-774 with docetaxel, the propensity for PK interactions, and pertinent pharmacodynamic effects, in patients with advanced solid malignancies. From April 2001 to June 2002, 24 patients have received 84 total courses of OSI-774 (mg/day)/docetaxel (mg/m^2 every 3 weeks iv) at the: 100/60, 100/75, 125/75 and, 150/75 dose levels. Since docetaxel is begun 3 days before OSI-774 in course 1, docetaxel and OSI-774 PKs are being assessed alone (course 1) and in combination (course 2) to identify drug-drug interactions. To determine the effects of treatment on EGFR-TK phosphorylation, serial skin and tumor biopsies are being performed in selected patients. 150/75 and 125/75 dose levels were poorly tolerated due to a relatively high incidence of febrile neutropenia. Thus far, 100/75 dose level has been reasonably well tolerated in 11 patients and 100/75 appears to be the recommended dose for subsequent trials. The principal dose-limiting event has been fever associated with neutropenia. Anti-tumor activity observed includes a complete response (nasopharyngeal carcinoma), a minor response (non small cell lung cancer), and stable disease for 4-7+ months (bladder, ovary, stomach, skin, and non-small cell lung cancer). Paired analyses of docetaxel clearance values with and without OSI-774 indicates that OSI-774 does not significantly affect docetaxel clearance ($p = 0.67$, paired t-test). Pharmacodynamic studies assessing drug effects on EGFR-TK phosphorylation in normal skin (5 patients) and tumors (2 patients) have also been performed. Accrual of additional new patients is ongoing at the 100/75 dose level. In summary, the maximum tolerated and recommended dose of OSI-774 is projected to be 100 mg daily in combination with docetaxel 75 mg every 3 weeks for patients previously treated with chemotherapy. This study provides preliminary evidence of anti-tumor activity of this combination in head and neck, NSCLC, ovarian, and some other epithelial cancers. Subsequent phase II studies should be considered to evaluate the efficacy of this combination, especially in NSCLC where both OSI-774 and docetaxel have previously demonstrated activity.

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A clinical phase I and biomarker study of the Raf kinase inhibitor BAY 43-9006: preliminary evidence of activity in patients with advanced solid tumors

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Raf is a protein kinase that acts downstream of Ras, and is thus a significant contributor to the malignant phenotype driven by activated Ras signaling. BAY 43-9006 is a novel potent, orally active inhibitor of Raf and the first compound in this class to enter clinical trials. The primary objectives of the present study are to: define dose limiting toxicities (DLTs) and maximum