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