Materials and Methods: Patients were treated orally, given the drug each day for five days for two weeks at 4–5 mg/m^2 of total dose per cycle. To date, pharmacokinetic were evaluated in twelve patients: three at 5 mg/m^2 and nine at 4 mg/m^2. Blood samples were collected after the last administration on day 12, (0, 1, 2, 3, 6, 12 and 24 h), after 24, 48, 72 h and on day 22 and 29. Plasma levels of gimatecan and its metabolite ST1698 were determined by HPLC with fluorescence detector.
Results: Gimatecan was mainly present in plasma as the intact lactone form, i.e. the active form as DNA-topoisomerase I poison. The drug shows high plasma levels and long half-life. The pharmacokinetic parameters obtained in the nine patients treated at 4 mg/m^2 were: C_{max} 64.2±17.7 ng/ml (CV 27.5%), T_{max} 1.7±0.9 h (CV 51.9%), AUC_{72h} 2853±915 ng/ml h (CV 32.1%), AUC_{inf} 7554±2671 ng/ml h (CV 35.4%) and half-life 102±40 h (CV 40%). ST1698 AUC amounted to 5–15% of the AUC of the parent drug. As previously indicated from pharmacokinetic data obtained during phase I study, a significant linear relationship between gimatecan $AUC_{72h,}$ and the α1-acid glycoprotein plasma levels ($p = 0.0031$) was found in the investigated patients.
Conclusions: Gimatecan is a new camptothecin with good oral absorption, unique stability of the active lactone form and long T1/2.

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hMLH1 protein sensitizes colon carcinoma cells to topoisomerase I inhibitor SN-38

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Introduction: The cellular mismatch repair (MMR) system plays an important role in surveillance and repair of damaged DNA. The protein hMLH1, a crucial component of the MMR system is mutated in approximately 50% of MMR-defective tumours. Our previous work showed that the hMLH1 status influences the extent of CPT-11-induced tetraploid cell cycle arrest in colon carcinoma cells (Magrini et al., Int. J. Cancer. 2002). Here, we generated an isogenic system of HCT116 cells differing only in hMLH1 status. Our aim was to investigate the effect of hMLH1 on the response of colon carcinoma cells to the CPT-11 and its metabolite SN-38.
Materials and Methods: Stable mock- and hMLH1-transfectants were generated by introducing the hMLH1 cDNA into MMR-deficient HCT116 cells. Sensitivity to the MMR-activating compound methyl nitrosourea, (MNU) or to SN-38 was determined by clonogenic assay. The cell cycle distribution was analysed by FACS. To determine the response *in vivo*, tumour xenografts were generated from hMLH1 transfectants and mock-transfectants in nude mice and their growth was monitored in time intervals.
Results: The hMLH1-expressing clones were more sensitive to MNU than the hMLH1-deficient ones thus showing that the MMR system in the transfectant clones was functional. Treatment with MNU led to a G2/M arrest only in the hMLH1-expressing cells. In response to SN-38, the hMLH1-transfectants underwent a long-term tetraploid cell cycle arrest and showed a slower rate of cell proliferation as compared to the mock-transfectants. Treatment of tumour xenografts with CPT-11 led to a longer delay in the exponential growth phase of tumours derived from transfectants as compared to the tumours derived from mock transfectants.
Conclusions: 1. The MMR system is functional in the HCT116 transfectants which we have generated. 2. The presence of hMLH1 sensitizes cells to SN-38. 3. hMLH1 lowers the rate of cell proliferation in response to SN-38 treatment. 4. The experiments with tumour xenografts confirm the *in vitro* data and show that hMLH1-deficient tumours are more resistant to CPT-11 than hMLH1-proficient tumours. This isogenic system is suitable for the detailed investigation of the role of hMLH1 protein in the mechanism of response to irinotecan and other chemotherapeutic agents.

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Pharmacogenomic association between genetic polymorphism of UGT1A1 and serious toxicities occurring in the cancer patients receiving irinotecan-containing chemotherapy

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Background: Irinotecan (CPT-11) is one of the widely used anti-cancer drugs, especially for colorectal and lung cancers, whereas it causes severe neutropenia and diarrhea limiting dose escalation in clinical practice. It undergoes drug metabolism to form an active SN-38, which is further converted to its beta-glucuronide by UDP-glucuronosyltransferase (UGT) 1A1. Pharmacogenomic associations between genetic variants on several specific sites including UGT1A1*28 and toxicities of irinotecan have been reported.
The aim of our study was to evaluate interethnic differences allelic frequency and haplotype of UGT1A1 gene in healthy Koreans and to determine the significance of UGT1A1 variants (−3279T>G, −3156G>A, promoter TA indel, 211G>A, 686C>A) on the serious toxicities induced by irinotecan in a prospective pharmacogenomic study.
Material and Method: Genotypes were identified by gene scan analysis on the ABI3730XL sequencer for variants in UGT1A1 (−3279T>G, −3156G>A, −53TA5−8, 211G>A, 686C>A) in blood samples from 218 healthy volunteers and 50 patients (pts) with advanced colorectal and lung cancer receiving irinotecan-containing chemotherapy. Toxicities have been graded according to NCI common toxicity criteria (ver 3.0).
Results: In 218 healthy Koreans, the allelic frequencies of −3279T>G, −3156G>A, TATA indel, 211G>A, and 686C>A were 26%, 12%, 12%, 15% and 1%, respectively. The median age of cancer pts was 58 years (range: 43−73 years). There were 34 Colorectal cancers and 16 Lung cancers. The number of patients who received prior chemotherapy were 36. Serious gastrointestinal or/and hematological toxicities were observed in 19 of 50 pts: diarrhea $\geqslant$G3 in 7 cases (14%); neutropenia $\geqslant$G3 in 14 cases (28%). There was no evidence that gender, age, primary disease and prior chemotherapy history could affect on neutropenia or diarrhea induced by CPT-11. Interestingly, it was identified that genetic polymorphisms of three different promotor sites, −3279T>G, −3156G>A, and (TA) indel existed simultaneously. Of five of UGT1A1 variants, there was a significant association between the variants for −3279T>G and the occurrence of severe neutropenia (OR 2.85; 95% CI: 0.31−26.7).
Conclusion: These results indicate that genotyping of 211G>A polymorphism as well as the heterozygotes (TA)7 and promoter (−3279, −3156) polymorphisms might predict the occurrence of serious toxicities by irinotecan in genetically predisposed cancer pts.

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Gene expression profiling of patient-derived colon xenograft tumors following treatment with irinotecan

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Background: Irinotecan (CPT-11) is one of the most active drugs in the first- and second-line treatment of metastatic colorectal cancer. Irinotecan belongs to the topoisomerase I interactive class of anticancer agents, which target the DNA-topoisomerase I complex and prevent the reannealing of the nicked DNA strand. Although the mechanism of topoisomerase I poisoning is clearly established, the exact response of tumor cells to this DNA damage is still poorly understood, since tumor response is generally not correlated to the target gene expression status *in vivo*. To better characterize the pathways associated with *in vivo* tumor growth inhibition, we analyzed the entire transcriptome of human colon tumors after *in vivo* treatment with irinotecan.
Material and Methods: Irinotecan (40 mg/kg ip q5dx5) was tested on 7 patient-derived colon xenograft tumors established subcutaneously in nude mice. Gene expression profiles in xenografts of control and treated mice were determined using Affymetrix Human Genome U133 Plus 2.0 micro arrays. Data were analyzed using previously published oligonucleotide probe masks that allow to efficiently measure human-specific transcriptional profiles in chimeric human-mouse samples.
Results: As expected, we observed that irinotecan significantly inhibited tumor growth in all xenografts tested. The predominant effect was a stabilization of the tumor to its initial size. Gene expression analysis showed that among significant changes in transcript abundance, the expression of VEGF and several other hypoxia-inducible factor (HIF)-1 target genes was systematically reduced in xenografts of irinotecan-treated mice. Since previous reports have shown that topotecan, another camptothecin analogue, is able to inhibit hypoxia-induced HIF-1 protein accumulation, we are currently investigating whether some of the *in vivo* transcriptional changes observed in colon cancer xenografts treated with irinotecan could be attributed to a similar mechanism.
Conclusion: Preliminary analysis of irinotecan-induced transcriptional changes associated with tumor growth inhibition raises the hypothesis that

part of the *in vivo* therapeutic response could be due to a direct anti-angiogenic effect of irinotecan. This hypothesis is in agreement with the predominant cytostatic effect we observed *in vivo*.

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ST1968: a new camptothecin analogue endowed with distinctive pharmacological properties

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Camptothecins isolated from the Chinese tree *Camptotheca acuminata* are effective antitumor drugs acting as topoisomerase I poisons. Although camptothecin derivatives topotecan and irinotecan are currently used in clinic, their narrow therapeutic index as well as the percentage of "naïve resistant patients" have generated interest in developing new camptothecin analogues with an improved pharmacological profile. A preliminary screening of various new camptothecin derivatives in a yeast model of Saccharomyces cerevisiae expressing wild type or different camptothecin-resistant mutants of human DNA topo I, allowed us to characterize a molecule, ST1968, able to inhibit the viability of yeasts transformed with wild type and mutated DNA topo I. ST1968 revealed *in vitro* an antiproliferative activity at nanomolar doses for lung (NCI-H460), ovarian (A2780) and prostate (DU145) carcinomas, and at micromolar doses for squamous oral carcinoma (KB) and other cancers. Flow cytometry showed that ST1968 exerted on tumor cells mainly a cytostatic activity by arresting cells in G_2/M upon a 2h exposure, and both cytostatic and cytotoxic activities with cell cycle block in G_2/M and induction of apoptosis upon 72h. In all *in vitro* experiments, the irinotecan metabolite SN38 appeared to be more cytotoxic than ST1968. Conversely, in *in vivo* studies, ST1968 delivered intravenously according to the schedule q4dx4, revealed a high efficacy against rapidly growing tumors (A2780, KB) as well as a camptothecin-resistant slowly growing human solid tumor (DU145-RC1). Interestingly, the efficacy of ST1968 in terms of tumor volume inhibition (TVI%) or log10 cell kill (LCK), complete response (CR) and Long Term Survivors (LTS) was substantially improved compared to irinotecan. *In vitro* uptake and release studies exhibited a direct correlation between cytotoxicity and intracellular concentrations of ST1968 in A2780 and DU145 cells, while in KB cells a very low cytotoxicity was associated to very high drug concentrations. *In vivo*, the highest AUCinf was found in A2780 and KB tumor lesions and a correlation between tumor uptake and antitumor activity of ST1968 was observed.
In conclusion, the high ST1968 antitumor activity toward a broad range of tumors, including camptothecin-resistant tumor expressing mutated topoisomerase I, and its higher therapeutic index compared to irinotecan support the clinical investigation of this new drug.

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The impact of UGT1A1*28 and UGT1A1*6 on irinotecan-induced neutropenia in Asian cancer patients receiving weekly and three weekly irinotecan regimens

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Background: Irinotecan is a topoisomerase I inhibitor and its pharmacokinetics and pharmacodynamics are greatly influenced by several polymorphic variants in genes responsible for encoding the various drug metabolizing enzymes and drug transporters involved in its disposition. Severe diarrhoea and neutropenia are two dose-limiting toxicities of irinotecan and its incidence varies in cancer patients of different ethnic background and depends on the *UGT1A1* polymorphic status. The purpose of this study was to investigate the influence of *UGT1A1*28* and *UGT1A1*6* polymorphisms on toxicity in Asian cancer patients receiving either the weekly or three weekly schedules of irinotecan.
Materials and Methods: Patients received irinotecan infusion either at 100 mg/m^2 on days 1, 8 and 15 and the regimen was repeated every 28 days (N = 28) or at 375 mg/m^2 once every three weeks (N = 46) over 90-minutes. A total of 19 and 18 serial blood samples were collected from cancer patients in the weekly and three weekly schedules, respectively, on the first day of irinotecan administration. The promoter (1.5 kb) and exon 1 of the *UGT1A1* gene was screened for polymorphic variants (N = 73) and correlated with irinotecan's pharmacodynamic parameters.
Results: A significantly higher exposure levels to irinotecan ($AUC_{0-\infty}$/dose/BSA: 42.1$\pm$18.8 vs 14.5$\pm$6.4; P = 0.0001) and SN-38 ($AUC_{0-\infty}$/dose/BSA: 1.2$\pm$0.63 vs 0.71$\pm$0.39; P = 0.0002) and lower exposure levels to SN-38G ($AUC_{0-\infty}$/dose/BSA: 4.2 $\pm$ 2.4 vs 10.1 $\pm$ 4.0; P = 0.0001) were observed in cancer patients receiving the three weekly regimen compared with patients on the weekly regimen. The relative extent of glucuronidation (REG) was approximately 5-fold lower in cancer patients on the three weekly regimen compared with those on the weekly regimen (REG: 3.9$\pm$2.2 vs 18.3$\pm$13.9; P = 0.0001). The *UGT1A1*28* and *UGT1A1*6* alleles were present in 20% and 13% of the cancer patients, respectively. Diarrhoea was uncommon in cancer patients receiving either schedules of irinotecan. Grade 4 neutropenia (ANC $\leqslant$ 500/μL) occurred in 26% (N = 12) of cancer patients on the three weekly schedule. None of these patients were homozygous for the *UGT1A1*28* allele. One patient was heterozygous and one was homozygous for the *UGT1A1*6* allele while the rest carried the reference genotype.
Conclusion: This study showed that neutropenia was more common in cancer patients receiving the three weekly irinotecan regimen compared with patients on the weekly regimen and this effect may be attributed to the high systemic exposure levels to SN-38 in patients receiving the three weekly regimen. The *UGT1A1*28* and *UGT1A1*6* variants did not have significant impact on the incidence of neutropenia in the Asian cancer patients and questions the need for *UGT1A1* testing in Asian cancer patients receiving irinotecan. Perhaps the less toxic but effective weekly schedule of irinotecan should be adopted in Asian cancer patients.

Topoisomerase II inhibitors

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Common over-expressions of TOP2A in hepatocellular carcinoma: a potential therapeutic target

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Genomic amplification is common in human cancers and represents an important mechanism in the activation of proto-oncogenes. In many instances, over-expressed oncogenes induced from genomic gains hold clinical implications both as prognostic markers and targets for therapeutic design. In this study, regional genomic gains that are commonly found in hepatocellular carcinoma (HCC) were investigated for underlying over-expressed genes by transcriptional mapping. Using a high-density cDNA microarray, a series of 22 HCC cell lines was screened for candidate genes at 8 loci. A subset of consistently over-expressed genes was indicated in each of the genomic locus, which included hepatoma derived growth factor (at 1q21.3), C-MYC (at 8q24.2) and aurora kinase A (at 20q13.2). Distinctively, common over-expressions of the DNA topoisomerase II alpha gene (TOP2A; at 17q21.2) ranked the highest with an average induction of 18.5-fold. By FISH analysis, a concordant copy gain of TOP2A was confirmed in most cell lines (~80%). Quantitative RT-PCR revealed the presence of frequent TOP2A up-regulations in HCC tumours compared to paired adjacent non-malignant liver tissues (p = 0.0018). Since Etoposide (a topoisomerase II binding agent) can interrupt the activity of DNA topoisomerase II by suppressing the enzyme mediated DNA cleavage, we investigated the potential therapeutic value in targeting TOP2A by Etoposide, as a single agent, and in combination with Doxorubicin (a DNA intercalator), which is currently the first-line chemotherapeutic agent for HCC patients. *In-vitro* cytotoxic studies on HCC cell lines suggested a potent synergistic effect in the combinatory application of Etoposide with Doxorubicin. Etoposide applied at IC20 concentrations readily reduced the IC50 concentrations of Doxorubicin by 3–5 folds when compared to Doxorubicin alone. Our study is the first to demonstrate a synergistic effect in the combinatory application of Etoposide with Doxorubicin and highlights the potential use of TOP2A reactive agents in the clinical treatment for patients with HCC.