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 ⩾G3 in 7 cases (14%); neutropenia ⩾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