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MKC-1 a novel cell cycle inhibitor: preclinical studies to support Phase 2 clinical trial evaluations in pancreatic and non-small cell lung cancers (NSCLC)

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MKC-1 (previously Ro 31–7453) was identified in a screen for cyclin dependent kinase (cdk) inhibitors. While it has weak affinity for cdks, treatment of cells with MKC-1 results in G2/M cell cycle arrest and broad antiproliferative activity in tumor cell lines. To determine if MKC-1 inhibits other kinases, we evaluated MKC-1 against a panel of 100 recombinant kinases. GSK-3beta was revealed as the only kinase significantly inhibited (IC50 7 nM). In vitro screening indicates pancreatic cell lines are particularly sensitive to MKC-1 activity, which may be due in part to the contribution of GSK-3beta to proliferation and survival in these lines (Ougolkov, et al). Several non-enzymatic targets for MKC-1 have been identified, including members of the importin β family, and the colchicine binding site of tubulin. These binding partners are consistent with effects observed in cells treated with MKC-1, such as aberrant mitotic spindle formation and cell cycle arrest. Functional consequences of the binding of MKC-1 to importin β are currently under investigation. A decrease in levels of HIF-1α, activation of pNF-κB and pStat3 and transcriptional activity of the three transcription factors is observed after 24 h treatment.
MKC-1 has demonstrated significant antitumor activity in several preclinical tumor models. Additionally, in Phase 1 and 2 clinical studies, this orally active agent has shown signs of efficacy with partial responses in NSCLC and breast cancer, and minor responses and tumor marker reductions in NSCLC, breast, pancreatic and ovarian cancer patients. The DLT was neutropenia, which is typical for this class of compounds. Importantly, no neurotoxicity, cardiotoxicity or secretory diarrhea was observed. Clinical studies are underway to further evaluate the recommended Phase 2 dose of 125 mg/m^2 bid × 14 days × q4 weeks. In vitro antiproliferative studies using H2122, a NSCLC cell line, have demonstrated the combination of MKC-1 and Tarceva results in enhanced activity (CI < 1). In a H2122 human tumor xenograft on day 42, tumor growth inhibition was observed with both MKC-1 and Tarceva, and the combination resulted in additive antitumor activity (100 mg/kg MKC-1 33% TGI, 50 mg/kg Tarceva 53% TGI, and the combination 72% TGI). Our preclinical mechanism of action studies and combination studies are being used to support clinical trials in several new indications.

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The proto-oncoprotein SYT activates transcription by interacting with the SWI/SNF chromatin remodelling protein BAF250, whilst the synovial sarcoma oncoprotein SYT-SSX acts as a repressor of transcription

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Synovial sarcoma is a highly malignant soft tissue sarcoma that accounts for about 7–10% of all human soft-tissue sarcomas and it is primarily seen in patients between the age of 15 and 40 years old. It can occur in any part of the body and nearly all synovial sarcomas have a specific t(X;18)(p11.2;q11.2) chromosomal abnormality that appears to be independent of tumour location. This translocation results in the fusion between the *SYT* gene on chromosome 18 and *SSX1*, *SSX2* or *SSX4* genes on chromosome X; the chimeric genes resulting from the translocation are *SYT-SSX1*, *SYT-SSX2* or *SYT-SSX4*. These gene products together with the fusion proteins are localised in the nucleus but excluded from the nucleoli. However, no DNA-binding domain has been identified in SYT or SSX. We have previously reported that both SYT and SYT-SSX proteins interact with the two DNA-dependent ATPases subunits of the chromatin remodelling complexes SWI/SNF named hBRM and BRG1. In this work we show that the transcriptional regulatory functions of SYT and SYT-SSX are exerted also through interaction with another chromatin remodelling protein called BAF250. BAF250 is the human homologue of the *Drosophila* Osa protein and BAF250 has been shown to mediate the interaction between the glucocorticoid receptor and the SWI/SNF remodelling complex. Here we identify the domains of interaction as the C-terminus domain of SYT, named QPGY, and the related repeat sequences present at the N-terminal end of BAF250. In both proteins the repeat sequences constitute very strong transcriptional activating domains. To further investigate the functionality of this interaction we have used a luciferase reporter construct containing glucocorticoid response elements transfected in T47D cell line (a breast carcinoma cell line which does not express BAF250). The SYT protein activates transcription only in presence of exogenous BAF250 and interestingly SYT-SSX acts as a repressor in the same reporter assay. We have also discovered that the transcriptional regulation involving SYT and SYT-SSX is hormone dependent. The interaction between BAF250 and SYT-SSX could be a potential new target area for the development of new therapeutic options for synovial sarcoma.

Topoisomerase I inhibitors

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Imatinib mesylate potentiates topotecan antitumor activity in rhabdomyosarcoma preclinical models

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Background: Imatinib mesylate (IM) is a protein tyrosine kinase inhibitor selective for bcr-abl, PDGFRα/β, c-kit, c-abl, ARG and c-fms. Recently, IM has also been shown to inhibit ABCG2 (breast cancer resistance protein, BCRP), a member of the ATP binding cassette (ABC) transporter superfamily that, extruding a variety of therapeutic compounds from tumor cells, can reverse drug resistance. In primary rhabdomyosarcoma (RMS), high levels of PDGFRα/β expression have been associated with disease progression. This study was aimed at investigating in RMS preclinical models the activity of IM as a single agent and in combination with topotecan (TPT), an ABCG2 substrate highly effective in advanced RMS.
Materials and Methods: Five human RMS cell lines of embryonal (RD, RD/18, CCA) and alveolar (RH30, RMZ-RC2) type were used. PDGFRα/β, c-kit, ABCG2 mRNA (real-time RT-PCR) and proteins (flow cytometry); PDGFRα/β and c-kit phosphorylation status (western blot); *in vitro* antitumor activity of IM and TPT alone and in combination (MTT assay); type of *in vitro* interaction of combined IM and TPT (Chou-Talalay combination index [CI] method); ABCG2-mediated Hoechst 33342 extrusion were evaluated. *In vivo* activity was assessed in RD and RH30 xenografts: IM (100 mg/kg/day), TPT (0.5 mg/kg/day), and the combination at the same dosage were given orally, 5 days a week, 3 consecutive weeks.
Results: PDGFRβ was expressed in all cell lines, with the highest levels in RD; PDGFRα and ABCG2 were detected at higher levels in RH30 and RMZ-RC2; c-kit was not expressed. Incubation with recombinant PDGF-BB showed an increase of PDGFRβ phosphorylation that was inhibited by co-incubation with IM. ABCG2-mediated Hoechst 33342 extrusion was effectively inhibited by IM in RH30 cells but only marginally in RD, and reflected the ABCG2 expression levels. *In vitro*, IM was active at concentrations not therapeutically achievable (IC_{50} = 22.6–29.0 μM) but showed significant synergism with TPT in a wide range of concentrations (CI: 0.47−0.79). Significant synergism between IM and TPT was confirmed in RD and RH30 xenografts. No obvious toxicity was observed.
Conclusions: IM as single agent is marginally active in RMS preclinical models, but significantly potentiates TPT activity *in vitro* and *in vivo* through at least two mechanisms, inhibition of ABCG2 and/or PDGFRβ. In RMS, the combination of TPT and IM warrants further study in the clinical setting, especially in ABCG2-expressing tumors.

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Pharmacokinetic of the novel oral camptothecin gimatecan in women with pre-treated advanced breast cancer

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Background: Gimatecan (ST1481;7-[(E)-tert-butyloxyminomethyl]-camptothecin) is a new topoisomerase I inhibitor with a lipophilic substitution in position 7 of the camptothecin molecule to stabilize the lactone ring, thus improving the pharmacological properties. It showed striking activity against human tumor xenografts after oral administration.
Aims: To determine the pharmacokinetics of gimatecan given orally to patients participating to the phase II study performed in women with pre-treated breast cancer.

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