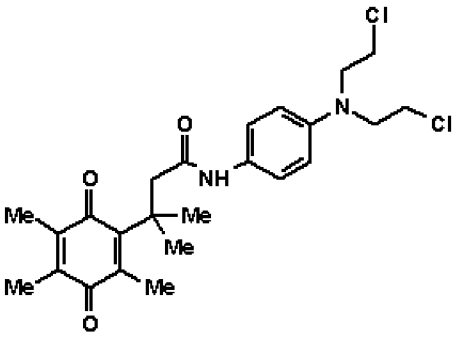
assay. MUP 03/704 exhibited a Km of approximately 940 μM and a Vmax of 5.77 mmol/min/mg of NQO1. A 5-fold difference in IC_{50} was obtained between the two cell lines, with 20 μM in H460 cells and >100 μM in BE cells, after 1h exposure to the drug. Analysis of DNA ICLs in cell lines showed differences both in terms of extend of DNA damage induced and repaired. At a dose of 20 μM, 10% more ICLs were obtained in H460 cells compared to BE cells, with respectively around 40% and 30% of DNA crosslinked. But, after just 6h of recovery, BE cells repaired approximately 78% of the damage whereas H460 cells repaired only around 45%. In addition, treating H460 cells with flavone-8-acetic acid (a known inhibitor of NQO1) prior and during the drug treatment did not significantly reduce either the drug cytotoxicity or the ICLs formation.



MUP 03/704 chemical structure.

In conclusion, the results of this study indicate MUP 03/704 could be effectively reduced by NQO1 in cell free system and induced formation of ICLs in cancer cells. The results in interstrand crosslinks induction and repair may explain the variation in cell line sensitivity to the drug. In addition, other reductases, such as cytochrome P450 reductase could be activating the prodrug. More studies will be carried out to further characterise its pharmacological features and to investigate its bioactivation mechanisms.

Topoisomerase I inhibitors

501 POSTER

Prospective UGT1A1 genotyping in a phase I study of safety and pharmacokinetics of liposome encapsulated SN-38 (LE-SN38)

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Polymorphisms in the promoter region of the hepatic enzyme UGT1A1 are associated with increased risk of irinotecan (CPT-11) toxicity. These variant alleles affect expression of this enzyme, which glucuronidates SN-38, the active moiety of CPT-11. This Phase I study is assessing safety and pharmacokinetics of liposomal SN-38 (LE-SN38) in patients with advanced cancer who have failed prior therapies. To establish safe dose levels for patients with and without the common *UGT1A1*28* variant allele, patients are stratified prospectively according to their *UGT1A1* genotype, defined by the number of TA repeats in the $A(TA)_nTAA$ promoter sequence. Strata consist of homozygous wild-type (6/6), homozygous variant (7/7), and heterozygous (6/7) patients, who are expected to have normal, low, and intermediate levels of glucuronidation activity, respectively. LE-SN38 is infused intravenously over 90 minutes every 21 days until disease progression or unacceptable toxicity occurs. Dose escalation is planned with separate patient cohorts receiving 2.5 to 90 mg/m^2 of LE-SN38. As of May 2004, genotype frequencies of 152 screened patients were 43% homozygous wild-type, 44% heterozygous, 11% homozygous variant, and 2% other; 58 of these patients were enrolled in the study. Dose escalation has reached 40 mg/m^2 for the wild-type and heterozygous strata, and 20 mg/m^2 for the homozygous variant stratum. Best response has been stable disease for up to 15 treatment cycles. Pharmacokinetic (PK) data indicate that drug exposure is greatest in homozygous variant patients, where the rate of conversion to SN-38 glucuronide is greatly reduced. PK differences between the wild-type and heterozygous strata are less pronounced. At a dose of 40 mg/m^2 LE-SN38, preliminary mean AUC_{0-8} values for plasma SN-38 in the latter groups were 3223 and 6498 ng·hr/mL, respectively, exceeding the value of 1120 ng·hr/mL reported for the approved CPT-11 dose of 350 mg/m^2. Severe diarrhea, which can occur with CPT-11 treatment, has not been observed. However, neutropenia appears to be dose limiting, with 2 wild-type patients experiencing dose-limiting toxicity at 40 mg/m^2. One of these 2 patients was heavily pre-treated with 9 prior chemotherapeutic regimens. To bring the best possible dose into Phase II second and third line patient populations, the study has been amended to continue dose escalation in the wild-type and heterozygous strata by enrolling only minimally pre-treated patients (⩽3 prior regimens). Dose escalation and accrual also continues in the homozygous variant stratum. Greater drug exposure observed in homozygous variant patients suggests that prospective genotyping is warranted to prevent overdosing of these patients. It remains to be determined whether wild-type and heterozygous patients will exhibit clinically significant differences in safety profiles that would require differential dosing for these patients.

502 POSTER

SUMO conjugation and proteolysis regulate cell sensitivity to DNA topoisomerase I poisons

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Camptothecin (CPT) reversibly stabilizes a covalent DNA toposiomerase I (Top1)-DNA complex, which is converted into lethal lesions during S phase. Despite intense investigation, cellular processes required for the repair of CPT-induced DNA lesions remain poorly characterized. To address this, a yeast genetic screen was used to define genes that protect cells from self-poisoning *top1* mutants: Top1T722A mimics CPT by inhibiting DNA religation, while Top1N726H exhibits increased rates of DNA cleavage. A *UBC9* mutant (*ubc9P123L*) was isolated with enhanced sensitivity to Top1 poisons and DNA damaging agents at 35ºC. *UBC9* encodes a highly conserved E2 enzyme that conjugates the ubiquitin-like protein SUMO to lysine residues in substrate proteins. Sumoylation alters protein activity, subcellular localization and/or complex formation. SUMO is also recycled by the Ulp1 and Ulp2 proteases. In *ubc9P123L*, a Pro123 to Lys substitution reduces SUMO conjugates at 35ºC. This suggests a higher threshold of Ubc9 activity is required to maintain cell viability in the presence of genotoxic agents. Supporting this model, *ubc9P123L* complemented the essential function of the Ulp2 protease at 35ºC, but not cell hypersensitivity to hydroxyurea (HU) and Top1 poisons. Further, overexpression of human *UBC9* restored the viability of yeast strains deleted for *UBC9*, yet did not restore *ubc9P123L* cell resistance to Top1 poisons or HU. In human Ubc9, Pro123 lies in a loop over the catalytic cysteine, and is immediately N-terminal to residues implicated in substrate binding. As a Pro123 to Ala mutation had no effect on Ubc9 activity, structural perturbations in Ubc9P123L seem unlikely. Rather chimeric human-yeast Ubc9 enzymes indicate substrate binding and/or E3 ligase interactions are critical determinants of Ubc9 function in yeast. This is consistent with our identification of the *Siz1* E3 ligase as a dosage suppressor of *ubc9P123L* cell sensitivity to Top1 poisons. In contrast, cells deleted for *ULP2* (*ulp2Δ*) were extremely sensitive to Top1 protein levels. *ulp2Δ*, *top1Δ* cells were hypersensitive to HU at all temperatures and rapidly acquired compensatory mutations, allowing growth at 35ºC. HU sensitivity at 26–30ºC and the genetic instability were suppressed in wild-type *TOP1*, *ulp2Δ* strains. Yet, these cells did not tolerate increased levels of Top1. Thus, diverse effects on SUMO conjugation, induced by defects in Ubc9 or SUMO proteases, alter the cytotoxic consequences of Top1 activity.

503 POSTER

First results of diflomotecan, a new topoisomerase 1 inhibitor, as oral soft-gel capsules in a phase I dose escalation study in patients with advanced malignant solid tumours

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Diflomotecan is a new generation topo 1 inhibitor, being an E-ring modified camptothecin analogue. It was tested versus irinotecan and topotecan in xenograft models, both as oral and intravenous (iv) administration. In terms of both tumour growth inhibition and survival time, diflomotecan was more active than irinotecan and topotecan in most models. As oral bioavailability of diflomotecan in the preclinical setting was high (65%), it entered in a phase I dose escalation study.
A total of 18 patients were enrolled, 10 men and 8 women. The median age was 56 years (33–70), and the median WHO PS 1 (0–1). Patients

had a median of 3 (0–5) prior chemotherapy lines. Colorectal cancer was diagnosed in 4 patients, breast and prostate cancer in 2 patients each, and 10 patients had other solid tumours. The starting dose level was 0.15 mg per day for 5 consecutive days once every 3 weeks, preceded by the same dose as iv infusion 2 weeks before the first oral administration in order to assess the absolute bioavailability of oral diflomotecan. The increases were done in 0.05 mg steps. Up to the dose of 0.35 mg 3 patients per dose level were treated for 1–8 cycles per patient (median 4+). At the maximum tolerated dose (MTD) of 0.35 mg, 2 patients experienced dose limiting toxicities (DLT): one grade 4 neutropenia for more than 7 days, and one cycle delay due to prolonged neutropenia. At the recommended dose (RD) of 0.30 mg, the cohort was extended up to 6 patients and no DLT was observed. One patient treated at the dose of 0.25 mg experienced a toxic death due to infection with grade 4 neutropenia at cycle 2. Five serious adverse events related to diflomotecan were reported in 3 patients: 1 patient had grade 3 fatigue, 1 patient grade 4 infection with neutropenia and 1 patient experienced 3 episodes of anaemia (two grade 2 and one grade 3) In18 patients, grade 3/4 haematotoxicity was reported as follows: neutropenia (8 patients), anaemia (6 patients) and thrombocytopenia (2 patients). Out of 16 patients, study drug related grade 3/4 adverse events were infection with grade 4 neutropenia, vomiting and fatigue (1 patient each). Study drug related grade 1/2 toxicities were fatigue (8 patients), vomiting (7 patients), nausea (6 patients), anorexia and alopecia (5 patients each), infection (4 patients), dypsnea (2 patients), diarrhoea, constipation, abdominal pain, weight loss, depression and cardiac pain (1 patients each)..

Regarding the pharmacokinetic results at the oral RD, the Tmax at day 1 was 1.06 hour and at day 5 1.50. $T_{1/2}$ was around 3 hours at both days, and the AUC was 9.69±3.63 at day 1 and 6.94±3.50 at day 5. Bioavailability was 95% at the RD of 0.30 mg.

One patient with breast cancer treated at RD achieved a partial response, 11 patients had stable disease (5 at RD), and 5 patients had progressive disease after 2 cycles.

These preliminary results are promising, and the second part of the study which investigates food interaction is ongoing. The oral route of diflomotecan administration may be a more convenient way for patients to receive chemotherapy.

504 POSTER

Phase I study of CT-2106 (polyglutamate camptothecin) in patients with advanced malignancies

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Background: CT-2106 is a novel camptothecin (CPT) conjugate in which CPT is bound to a biodegradable water-soluble poly-L-glutamic acid-glycine polymer. CPT-polymer conjugation allows for greater stability of CPT in circulation and enhanced permeability and retention in tumor tissue. CT-2106 has demonstrated anti-tumor activity in several human tumor cell lines in vivo. **Methods:** To determine the maximum tolerated dose (MTD) and evaluate the pharmacokinetics (PK) of CT-2106, 31 pts were treated with a 10-minute IV infusion every 21 days. Toxicity was assessed according to NCI CTC v2. PK samples (cycles 1 and 2) were analyzed for conjugated and unconjugated CPT levels by validated HPLC/FD methods. Cohorts of pts received conjugated CPT doses of 12, 25, 50, 75, 90, or 105 mg/m^2. **Results:** Dose-limiting toxicities (DLTs) included: grade (g) 3/4 neutropenia, thrombocytopenia, and mucositis. One pt experienced a g4 cholinergic reaction and esophageal spasm; this pt had previously experienced a severe reaction to irinotecan. Other related toxicities were g3 increased ALT and ⩽g2 anemia, anorexia, dysgeusia, peripheral sensory neuropathy, fatigue, nausea, diarrhea, vomiting, abdominal pain, alopecia, rash, decreased hemoglobin, and hematuria. No g3/4 hematuria or diarrhea was observed. Using standard response criteria, 1 pt with metastatic pancreatic cancer had a partial response, 2 pts with NSCLC had stable disease (SD) for >35 weeks, and 2 pts with colon cancer had SD for >9 weeks. Preliminary PK parameters calculated from 18 pts treated at 25, 50, 75, or 105 mg/m^2 demonstrated sustained levels of conjugated CPT in systemic circulation, with mean elimination half-life from 16.6 to 50.8 hrs. Cmax and AUC of conjugated CPT increased linearly with dose, suggesting PK linearity. Unconjugated CPT levels suggest that this active form of the compound is generated by a slow, progressive release from the polymer following the distribution of conjugated CPT to tissues. The PK profile of unconjugated CPT is dependent on the disposition profile of the conjugated drug; unconjugated CPT elimination is formation rate limited. Unconjugated CPT half-life ranged from 31.9 to 60.4 hours. Five days after the 1st administration, mean cumulative urinary excretion of conjugated and unconjugated CPT accounted for 27.9% and 5.1% of the administered dose, respectively. A major conjugated CPT species in urine was glu-gly-CPT (6.9% of dose). Accumulation of conjugated or unconjugated CPT was not observed with repeated dose administration. Plasma and urine PK parameters were nearly identical in cycles 1 and 2. The MTD has been established at 75 mg/m^2. **Conclusion:** CT-2106 has been well tolerated with easily manageable toxicities while generating prolonged systemic exposures to free CPT in plasma. Since clinical activity has been observed, phase I/II single-agent and combination trials are planned.

505 POSTER

Human carboxylesterase isoform 2 (hCE2) mRNA expression in peripheral blood lymphocytes as a predictive marker of irinotecan activation rate in vivo

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Background: Irinotecan {7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyl-oxycamptothecin} is a pro-drug used in cancer therapy as topoisomerase I inhibitor. Its activation occurs mainly by the action of the human carboxylesterase–converting enzyme isoform 2 hCE2 that cleaves the bulky piperidino side chain and generate the metabolite SN38 which is the biologically active molecule responsible for the therapeutic effect as well as for the toxic reactions associated with the drug. The pharmacological inter-patient variability of irinotecan gives rise to unpredictable toxicity in certain individuals. This could be due also to the highly variable extent of irinotecan activation found among patients. In an attempt to identify a marker to predict irinotecan activation in cancer patients we consider the hCE2 mRNA expression in lymphocytes to correlate it with in vivo activation rate of irinotecan to SN38. hCE2 was the isoform considered in this study since it shows the higher affinity for irinotecan among the human hCEs.
Materials and methods: Twenty-one gastro-intestinal cancer patients treated with irinotecan including schemes have been analysed for hCE2 mRNA expression. Total RNA was extracted from peripheral lymphocytes. hCE2 mRNA was relatively quantified using specific primers by RT-PCR associated with the Real Time technology and the SYBR Green chemistry. Irinotecan pharmacokinetic analysis was performed in each single patient. Irinotecan, SN38 and SN38-glucuronide plasmatic concentrations were determined by HPLC at 2, 6, 10 and 50 hours after the beginning of drug infusion.
Results: A high inter-individual variability was found in terms of mRNA expression. The median value of expression in relative units is 1.235 (range: 0.01–14.07). The activation rate was described as the concentration ratio of total SN38 (free and glucuronide) to irinotecan. The median values found among the patients are: 0.048 (0.013–0.126) at 2 hours, 0.100 (0.031–0.294) at 6 hours, 0.136 (0.047–1.774) at 10 hours and 0.544 (0.257–2.303) at 50 hours. A significant correlation was found between the relative hCE2 mRNA expression and the irinotecan activation rate at 2 (R=0.631, p=0.0022), 6 (R=0.553, p=0.0093) and 50 hours (R=0.591, p=0.0048) by the linear regression analysis.
Conclusion: Though these results should be confirmed by further investigation in a larger population the preliminary data support a predictive power of hCE2 mRNA expression in peripheral lymphocytes for the activation

506 POSTER

Durable disease stabilization and antitumor activity with rubitecan, an orally administered topoisomerase I (topo-I) inhibitor, in combination with gemcitabine: a phase I and pharmacokinetic study in patients with advanced cancer

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Background: Rubitecan, an oral camptothecin analogue and potent inhibitor of topo-I, has demonstrated clinical activity in gemcitabine-sensitive malignancies, such as pancreas, breast, ovarian and urothelial tumors, as well as gemcitabine-resistant cancers. Preclinical synergism between topo-I inhibitors and gemcitabine as well as the presence of nonoverlapping toxicities, provide a sound rationale for their evaluation in combination.
Patients (pts) and Methods: Escalating oral doses of rubitecan from a starting dose level of 1.0 mg/m^2/day × 5 days every 7 days × 3 weeks, every 28 days, with a fixed dose of gemcitabine 1000 mg/m^2 IV on days 1,