patients completed treatment with a total of 107 cycles (median: 3.5 cycles, range: 1-9). Four patients are still on treatment. One complete response (breast) and 2 partial responses (larynx and lung) were observed with 7 additional stable diseases. The main toxicity is neutropenia G3-4. The following DLTs were observed: At level 3 (D:80/MS:600): febrile neutropenia G4, infection G3 leading to death, At level 4 (D:80/MS:900): stomatitis G3, dysphagia G3, fatigue G3, At level 5 (D:80/MS:1200): 2 DLT: stomatitis G3/neutropenia G3 and stomatitis G4/fatigue G3 The MTD was reached at level 5. The recommended dose is then level 4 (D:80/MS:900). PK analysis did not demonstrate a strong PK interaction between the two compounds but at the highest dose levels, there is a trend to an increase of docetaxel AUC when this agent is given in combination with MS209. Complete data set and PK analysis will be presented.

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A phase I pharmacokinetic study of ONT-093 in combination with paclitaxel in patients with advanced cancer

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Background: ONT-093 (OC144-093) is a third generation, orally bioavailable inhibitor of P-glycoprotein (P-gp). In pre-clinical studies, inhibition of P-gp and reversal of multidrug resistance (MDR) could be achieved at nM concentrations. ONT-093 does not affect paclitaxel pharmacokinetics (PK) in rodents. Phase I clinical trials of single agent ONT-093 in normal human volunteers showed no dose-limiting toxicities at biologically active serum concentrations in doses ranging from 300 to 500 mg.
Methods: We performed a Phase I pharmacokinetic trial of ONT-093 in doses from 300 to 500 mg administered orally 14 h before, 2 h before and 10 h after intravenous paclitaxel doses of 150 to 225 mg/m^2 repeated every 21 days. All patients received paclitaxel alone for cycle 1 to allow intra-patient comparisons of toxicity and paclitaxel pharmacokinetics.
Results: To date, 18 patients have been enrolled into 4 dose levels and have received doses of ONT-093 up to 500 mg and paclitaxel up to 175 mg/m^2. Toxicities have mainly been attributable to paclitaxel, and included arthralgia, myalgia, neutropenia, and peripheral neuropathy. Toxicities possibly associated with ONT-093 include grade 1-2 headaches and transient grade 1 elevation of liver transaminases in 1 patient. Three patients have had higher-grade neutropenia with cycle 2. One of these patients, who was also heavily pre-treated and had extensive hepatic metastases, had febrile neutropenia on cycle 2, dose level 4 (ONT-093 500 mg and paclitaxel 175 mg/m^2). This cohort is being expanded. Cmax concentrations of ONT-093 given at 500 mg are $> 8\ \mu$M, well above that required in pre-clinical models to inhibit P-gp and completely reverse MDR. Plasma PK of paclitaxel are unchanged between cycle 1 and 2.
Conclusions: Biologically active doses of ONT-093 have been well tolerated in combination with standard doses of paclitaxel. There have been no alterations of paclitaxel PK parameters with the combination at the doses tested. These results support the continued clinical development of ONT-093 as an active, potent, non-toxic inhibitor of P-gp in conjunction with cytotoxic chemotherapy. Patient accrual continues, and final results will be presented.

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Hyperthermia and multidrug resistance: Impact on expression and regulation of MDR genes in human cancer cells

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Hyperthermia is used for the treatment of cancer patients in combination with chemotherapy, cytokines and/or radiotherapy. Since multidrug resistance (MDR)-associated genes are inducible by external stress factors such as heat and drugs used for chemotherapy, we investigated the influence of hyperthermia on expression, regulation and function of the MDR genes MDR1, MRP1, and MVP/LRP in *in vitro* models as well as in clinical specimens. In colon carcinoma cell lines, hyperthermia caused nuclear translocation of the transcription factor YB-1. Nuclear YB-1 interacts with the promoters of the MDR1 and MRP1 genes and is associated with increased MDR1 and MRP1 gene transcription, as well as strong efflux pump activity. However, a combination of hyperthermia and drug treatment effectively reduced cell survival demonstrating that activation of MDR1 and MRP1 gene expression and increased efflux pump activity after hyperthermia did not consequently lead to an increase in a MDR phenotype. The ability of hyperthermia to abrogate drug resistance in the presence of an increase in functional MDR proteins may provide an explanation for the efficacious results seen in the clinic in colon cancer patients treated with hyperthermia and chemotherapy. We investigated MDR1 expression in colon cancer specimens of patients who were treated by radio-chemo-thermo therapy. We find that the levels of MDR1 expression in colon cancer specimens before and 6 weeks after radio-chemo-thermo-therapy were not significantly different in the majority of cases. Since induction of MDR1 gene expression by external stress factors such as heat occur directly after exposure to hyperthermia, we conclude that this increase of MDR1 gene activity has ceased after 6 weeks, the time point of resection when the tumor specimens were analyzed. We then determined the levels of MDR genes sequentially before, during, and after isolated hyperthermic isolated limb perfusion (hILP) with rhTNFa/melphalan in patients with advanced soft tissue sarcoma and locoregional metastatic malignant melanoma. In the majority of patients (> 80%) MVP/LRP expression was induced during hILP, often parallelling the increase in temperature during hILP. This is the first study to investigate expression of MDR genes sequentially during hILP of patients. The result of this study demonstrates that hILP caused selective induction of MVP/LRP expression, whereas MDR1 and MRP1 expressions were rarely affected by the treatment regimen.

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Polymorphisms of MDR1 and MRP2/cMOAT in healthy North Eastern Italian subjects

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MDR1 and MRP2 belong to the ABC transporter genes superfamily encoding for integral membrane glycoproteins that function as ATP-dependent export pumps with substrate specificity. The increased expression of these proteins in cancer cells is associated with the development of cross resistance of tumors to many antiblastic agents. Their activity in normal tissue cells has a protective role towards xenobiotcs and prevents chemotherapy toxicity modulating pharmacokinetics of antineoplastic drugs. At least 15 single nucleotide polymorphisms (SNPs) have been described in a Caucasian population for MDR1, one of them resulted particularly interesting such as C3435T in exon 26, its pharmacological effect has been demonstrated and its frequency is quite variable among different ethnic groups. For MRP2 several mutations have been described in Dubin-Johnson Syndrome patients. Few of them have been detected also in Japanese and Jewish healthy subjects and described as SNPs. Their influence on the protein activity are not yet clarified but they could have a role on MRP2 expression or functionality. No population studies have been reported till now on them. We analysed the frequency of C3435T SNP for MDR1 gene and C24T SNP for MRP2 in 800 healthy blood donors from North East of Italy. Distribution of MDR1 C3435T genotype was as follows: C/C in 191 subjects (23.96%), C/T in 434 subjects (54.26%), and T/T in 175 subjects (21.78%). The allelic frequency was 48.91% for T allele,51.09% for C allele. Distribution of MRP2 C24T genotype was as follows: C/C in 494 subjects (61.78%), C/T in 271 subjects (33.86%), and T/T in 35 patients (4.36%). The allelic frequency was 78.7%1 for C allele, 21.29% for T allele was. In conclusion the population we analysed showed for MDR1 a frequency quite similar to the one described in literature for the Caucasian population. For MRP2 we found out a considerable allelic frequency for C24T in our geographic area and this encourages further investigations to evaluate its impact on pharmacokinetics of drugs excreted by this transporter protein.

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Co-dominance of cisplatin resistance in somatic cell hybrids

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Intrinsic or acquired resistance to cisplatin in cancer cells remains a major obstacle to successful chemotherapy. The relevant genetic and molecular mechanisms of resistance have not yet been identified. We have isolated cisplatin-resistant human KB epidermoid carcinoma cell lines resistant to varying levels of cisplatin after single and multiple selection steps.

Intraspecies hybrids of the sensitive and resistant KB cells (single step: KCP.5 resistant to 0.5 mg/mL of cisplatin, two steps: KCP1 resistant to 1 mg/mL of cisplatin) were fused with D98OR (HAT sensitive, ouabain resistant) to determine whether cisplatin resistance is a dominant or recessive trait. Cell-cell hybridization between the sensitive cells and single-step or two-step KB cisplatin resistant cells both indicated codominance of cisplatin resistance compared to hybrids between sensitive cell lines (D98ORxKB). The hybrids between sensitive cell lines (D98xKB) and a single-step cisplatin resistant KB cell line (D98xKCP.5) also were cross-resistant to carboplatin. In addition, based on the doubling times of hybrid cells, the relatively slower growth rate of cisplatin resistant cells appears to be dominant. Previous work in our lab has found membrane protein mislocalization in the KB cisplatin resistant cell lines, including intracellular accumulation of folate binding protein (FBP). Confocal microscopy imaging of the D98xKCP.5 hybrids for FBP showed the same mislocalization as the parental cisplatin resistant cell lines, further indicating that mislocalization of FBP is likely to be a dominant phenotype linked with cisplatin resistance, consistent with a molecular defect in inability of cisplatin binding/transport proteins to get to the cell surface. In the two-step cisplatin resistant KB cell line, KCP1, resistance is no more dominant than in the single-step cisplatin resistant KB cell line, KCP.5, suggesting that one of the two steps of resistance in KCP1 may not be dominant. These dominance data suggest that it might be possible to identify a gene or genes responsible for cisplatin resistance by gene transfer from a resistant cell line in a sensitive cell line.

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DNA hypermethylation and resistance to chemotherapy in ovarian cancer

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Aberrant DNA methylation is one of the hallmarks of tumours. Human cancers show altered patterns of CpG island (CGI) methylation at genes involved in essentially every facet of tumour development. We have shown that CGIs associated with genes known to be involved in drug sensitivity, such as hMLH1, can become methylated in ovarian tumours and that treatment of resistant cells with DNA methyltransferase (DNMT) inhibitors can sensitise tumour cells to a variety of cytotoxic chemotherapeutic drugs. In a pilot study we have identified patterns of increased gene methylation, using array-based differential methylation hybridisation (DMH), which predict poor progression-free survival in ovarian cancer (Wei et al 2002, Clin. Cancer Res., in press). Thus, ovarian tumours with such increased CGI methylation may help define patient populations for combination treatment of DNMT inhibitors with cytotoxics such as carboplatin. The MCJ gene, a member of the DNAJ (HSP40) protein superfamily, has been identified as a target for aberrant methylation and shown to play a role in sensitivity to cisplatin (Shridhar et al 2001, Cancer Res. 61:4258). We show that expression of the MCJ gene is lost in 8/10 cisplatin-resistant derivatives of the ovarian carcinoma cell line A2780. Furthermore, treatment of two of the resistant cell lines with the DNMT inhibitor 5-azacytidine, resulted in re-expression of MCJ, suggesting that loss of expression may be due to increased methylation. A CGI was identified beginning 164bp downstream of the transcriptional start site, within the first exon of the MCJ gene. Bisulfite sequencing of this region in normal ovarian tissue DNA determined that about 50% of clones were densely methylated and about 50% of clones largely unmethylated. MCJ expressing cell lines revealed a pattern of methylation similar to normal DNA. However, the cisplatin-resistant, non-expressing, cell lines exhibited dense methylation of 100% of clones sequenced. These results suggest that methylation of the intragenic CGI of MCJ can result in loss of gene expression. Sequencing of this region of the MCJ gene in a cohort of 32 ovarian tumour samples identified a subset of tumours (16%) that exhibited high levels of methylated clones (>90%). Furthermore this identified a possible link between high levels of MCJ methylation and poor response to chemotherapy following platinum based chemotherapy in ovarian cancer patients ($p=0.01$).

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Preclinical rationale for a combined treatment with irinotecan and the epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKI) ZD1839 (Iressa) in irinotecan-refractory human colon cancer

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EGFR over-expression and activation of its intrinsic TK are involved in malignant transformation, which is commonly associated with poor clinical prognosis. EGFR targeting agents have shown antiproliferative activity in clinical trials, eg monoclonal antibody IMC-C225 (cetuximab) in patients (pts) with irinotecan-refractory colorectal cancer (CRC) [ASCO 2001, abs 7] or small molecule EGFR-TKIs, eg ZD1839 (Iressa), in NSCLC (ASCO 2002, abs 1166, 1188). We investigated the effect of ZD1839 on cellular determinants of resistance to the active metabolite of irinotecan (SN-38) in drug-sensitive (HCT-8/wt) and resistant (HCT-8/SN-38) human colon cancer cells. Co-administration of ZD1839 at non-cytotoxic concentrations completely restored sensitivity to SN-38 in EGFR-expressing HCT-8/SN-38 cells, both in the presence or absence of EGF (1-100 ng/mL). ZD1839 did not affect topoisomerase I (Topo I), Topo II-b and general protein expression, but we observed a significant time- and dose-dependent downregulation of Topo II-a protein and inhibition of its enzymatic function, which corresponded to a G1-phase block in cell cycle analyses. These results were confirmed using quantitative RT-PCR. ZD1839 dose-dependently increased SN-38-mediated induction of protein-linked DNA single-strand breaks (for 10 μM SN-38: 5.1 $\pm$ 0.87% [IC_{50} ZD1839] and 15.9 $\pm$ 0.44% [4-fold IC_{50} ZD1839] vs 2.42 $\pm$ 0.29% [untreated] [$p=0.005$ and $p<0.0001$, respectively]), with no alteration of Topo I protein expression or unwinding activity of pBR322 plasmid DNA using nuclear extracts of HCT-8/SN-38. Neither induction of resistance to SN-38 nor the following exposure to ZD1839 showed an influence on EGFR expression, but there was a significant decrease in EGFR phosphorylation levels with ZD1839 in specific immunoblotting and immunocytochemical analyses. Cellular pharmacokinetics of the active SN-38 lactone revealed no significant differences of drug accumulation or retention by ZD1839 using HPLC. Analyses of membrane transporters, EGFR downstream signaling and differential gene expression in the resistant cell line with or without ZD1839 treatment will be presented. In conclusion, inhibiting EGFR-TK activation with ZD1839 reverses resistance to SN-38 in human colon cancer cells. These data support combination therapy with ZD1839 and irinotecan in patients with CRC that is refractory to irinotecan-based regimens. Iressa is a trademark of the AstraZeneca group of companies

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Pharmacogenetics of the human glutathione S-transferase P1 gene and tumor response to chemotherapy

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The polymorphic human GSTP1 gene locus encodes proteins that differ functionally in their metabolism of electrophilic compounds, including a number of anticancer agents. This study was designed to gain a better insight into the potential role of the GSTP1 genetic polymorphism in the outcome of cancer chemotherapy. Using a prokaryotic system and GSTP1-null human tumor cells genetically modified to express each of the GSTP1 alleles, we investigated the differential protection conferred by the GSTP1 alleles against four anticancer agents, namely, carboplatin, cisplatin, thiotepa, and 4-hydroperoxyifosfamide (HI). In the prokaryotic system, E. coli were transformed with expression vectors carrying cDNAs of the GSTP1 alleles and the cytoprotective effects examined in a clonogenic survival assay. Simultaneously, isogenic variants of the GSTP1-null human medulloblastoma cell line, engineered to stably express each of the GSTP1 alleles, were examined for altered resistance to cisplatin, and for the level of cisplatin-induced DNA damage and apoptosis. The results showed all GSTP1 alleles to be cytoprotective against the anticancer agents. For cisplatin and carboplatin, the GSTP1*C allele was most protective followed by GSTP1*B and GSTP1*A. In contrast, protection against thiotepa was highest for GSTP1*A followed by GSTP1*B and GSTP1*C. Protection against 4-HI was the same for both GSTP1*B and GSTP1*C and higher than GSTP1*A. In the medulloblastoma cells, the levels of cisplatin-induced DNA damage and apoptosis were decreased by all three GSTP1 alleles in the order GSTP1*C > GSTP1*B > GSTP1*A the same order as was observed for the increase in cisplatin resistance. Using HPLC and mass spectral anal-