As for the combination treatment, pretreatment by P inhibited the induction of C-induced HSP27 expression in both C-sensitive and resistant cells. Relative HSP27 expression for the PTC sequence was 1.3 for sensitive cells and 1.1 for resistant cells, whereas that for the CTP sequence was 1.9 for sensitive cells and 2.4 for resistant cells.

Conclusion: Pre-treatment by P appeared to strengthen the cytotoxic effect of C by inhibiting HSP27 expression in both C-sensitive and resistant cells. This observation suggested that inhibition of HSP27 by P play an important role in reduction of the C resistance.

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Screening of beta tubulin mutations in serous and clear cell ovarian carcinoma

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Purpose: Beta tubulin mutations have been implicated in resistance to chemotherapeutic treatment with paclitaxel. The aim of the present study was to screen for beta tubulin mutations in a group of serous and clear cell ovarian carcinoma patients treated with paclitaxel/cisplatin combination.

Methods: We selected 34 ovarian carcinoma patients (26 serous ovarian carcinomas and 8 clear cell ovarian carcinomas), classified as invasive or borderline, treated after surgery with paclitaxel and cisplatinum, for at least 4 cycles. These patients were radio and chemotherapy naïve. DNA was extracted from paraffin embedded tumours, after microdissection, amplified with specific primers for exon 4 of beta tubulin gene, and finally sequenced. 10 patients (29,4%) were chemo-resistant.

Results: None of the cases revealed beta tubulin mutations.

Conclusion: In the studied ovarian carcinoma patients the paclitaxel resistance mechanism was not associated with exon 4 of beta tubulin gene mutations.

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Daunomycin-polypetide conjugates: in vitro antitumour effect in sensitive and multidrug resistant cell lines

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Purpose: Anthracyclines, like daunomycin (Dau) are widely used in the therapy of cancer. Side effects (e.g. immunosuppression, cardiotoxicity) and multidrug resistance developed during the treatment, seriously limit their therapeutic efficiency. Earlier results from our group have demonstrated that coupling of daunomycin with an acid labile spacer (cAD) to an amphoteric branched chain polypeptide, EAK, significantly increased the survival of L1210 bearing mice (1). cAD-EAK conjugate contains two isomers of the cAD, namely a-and b-cis-aconytil-daunomycin. Since isomers might have different biological effects, we have prepared both isomers and their polypeptide derivatives. The aim of these studies was to compare their antitumour effect in vitro.

Methods: The in vitro antitumour effect of isomers and isomer containing conjugates was investigated in the following tumour cell lines: C26H colon carcinoma, MDA-MB 435P breast carcinoma, HL-60/sensitive and HL-60/MDR1-HL-60/MRP1 resistant human leukemia cell lines. In HL-60/sensitive, as well as in HL-60/MDR1 and HL-60/MRP1 resistant cell lines, we have compared the uptake and effect of daunomycin with those of cAD-EAK conjugate, since daunomycin is the substrate of the MDR1 and MRP1 membrane proteins.

Results, Conclusion: Data suggest that one of the isomer (cAD1, IC50~5 μmol; cAD2, IC50~250 μmol) and one isomer-conjugate (cAD1-EAK, IC50~8 μmol; cAD2-EAK, IC50~300 μmol) was more effective in tumour cell lines studied. In addition we have observed that the cAD-EAK polypeptide conjugate enters not only the sensitive, but also the MDR resistant human leukemia cells.

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Pharmacokinetics

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Ecteinascidin (ET-743) pharmacokinetics(PK) -overview of phase i and advanced phase II results

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ET-743 derives from the tunicate Ecteinascidia turbinata. It showed activity during Phase I and II in several tumor types, such as sarcoma, breast and ovary. Its potency (IC50 pM-low nM) required a sensitive analytical method, liquid chromatography-tandem mass spectrometry (LC-MS/MS). The dose-limiting toxicity (DLT) is myelosuppression. Other toxicities include transaminitis, increased alkaline phosphatase (AP) or bilirubin (bil) and rare (0.9%) cases of rhabdomyolysis. ET-743 is eliminated by the liver, probably by metabolism by CYP3A4 and probably CYP2C9/10 (but not 2C8), with contribution of CYP2E1 and 2D6. Urinary recovery is <2%. ET-743 PK features are: dose linearity after 3 or 24 h infusion, but not 1 or 72 h infusion, wide distribution (Vss 800-5500 L/m2). slow elimination (t1/2 40-300 h) and high clearance (Cl) (mean values at the recommended dose (RD) 22-50 L/(hr*m2) -50% to 90% of expected liver blood flow-). Most profiles after 24 h infusion are best fit by a 2-compartment model (median (M) half lives alpha and β; 0.5 h and 88.8 in Phase I patients (pts); 0.5 and 45.6 h in Phase II pts). In single stage population PK (NONMEM) the best fit is provided by a 3-compartment model (M half lives: alpha 0.3 h, β 2.6 h, gamma 135.9 h). After 3-h infusion, most profiles are best fitted by a 3-compartment model (M half lives: alpha 0.2 h, β 2.2 h, gamma 67.6 h). ET-743 toxicity is exposure related. AUC is the only PK parameter predictive for hematological toxicity in the 24 h schedule. In the 3 h schedule Cmax is predictive too. The proportion of treatment courses with DLT increases from 8.5% to 40% (p=0.009) when comparing courses with AUCs≤ and >70 h*mcg/L in the 24 h schedule. The pts with rhabdomyolysis had AUCs >100 h*mcg/L. AUC in responding and non-responding pts were similar: M(range) 41.6(17.1-76.4 h*mcg/L) in responders vs. 38.8(16.7-178.8 h*mcg/L) in the other Phase II sarcoma pts treated with 24 h infusion. Biliary function is determinant for ET-743 PK. The best predictive parameter is increased AP (at baseline or during treatment). ET-743 Cl decreases by 50% with relatively low increases in AP (10% over the upper limit of normality). Bil increases occur less frequently but cause a greater decrease in Cl. Colorectal cancer pts had decreased ET-743 Cl; mean(SD) 20.7(6.8) in colorectal vs. 33.0(21.3) L/h*m2; p=0.006 in Phase II sarcoma pts treated with ET-743 as a 3-h infusion. ET-743 PK offers opportunities to optimize its therapeutic range.

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Pharmacokinetics (PK) of irofulven using three different intermittent 5-minutes (MIN) infusion dosing schedules (sch) in advanced solid tumors (AST): Final results

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Irofulven, an acylfulvene analog of illudin S, has shown promising anti-tumor activity during preclinical/clinical development, with delayed thrombocytopenia (T), asthenia and nausea/vomiting as treatment-limiting toxicities. A new phase I study exploring 3 different intermittent dosing sch with a 5-min infusion (A: D1–8–15 q4 weeks (w), B: D1–8 q3w, C: D1–15 q4w) is ongoing in patients (pts) with AST. Starting dose intensity (DI) was 10 mg/m^2/w (i.e. 13.3, 16 and 18 mg/m^2/dose in sch A, B, C respectively). Planned DI (PDI)

Dose (mg/m^2)	N eval. 1 pts-cycle 1	D1 Cmax (ng/ml)	AUC (ng/mlxh)	Clt (l/h/m^2)	$T_{1/2}$ beta (min)
13.3	5	190 ± 99	25.1 ± 12.8	654 ± 319	5.6 ± 1.8
15	3	211 ± 30	29.1 ± 4.4	522 ± 72	5.6 ± 2.9
16	7	300 ± 307	38.9 ± 38.4	855 ± 750	5.2 ± 2.5
18	8	306 ± 160	36.4 ± 17.0	645 ± 424	4.3 ± 1.9
18.6	6	413 ± 335	45.8 ± 27.2	509 ± 240	3.7 ± 1.4
20	2	334 ± 74	60.0 ± 10.3	339 ± 59	4.1 ± 1.8
21	6	599 ± 345	66.1 ± 38.6	493 ± 423	6.6 ± 2.1
24	6	586 ± 562	78.5 ± 68.4	431 ± 186	4.1 ± 1.9
28	7	754 ± 268	92.2 ± 48.7	380 ± 174	6.0 ± 1.2

was increased by 2 mg/m²/w at successive dose levels (DL). A total of 60 pts were included in the first 3 DLs of each sch. During each pt's first 3 infusions, 10 plasma samples were collected up to 6 hours post-infusion. We report on the first 50 pts evaluable for day 1 PK analysis. Doses were (mg/m²): 13.3 (6 pts); 15 (3 pts); 16 (9 pts) 18 (9 pts); 18.6 (6 pt); 20 (3 pts); 21 (7 pts); 24 (9 pts), 28 (8 pts). The PK parameters exhibited high inter-pt variability. The PK of irofulven was characterized by a short terminal half-life (3.7–6.6 min). Cmax and AUC increased dose proportionally; the coefficients of correlation (R) were 0.350 and 0.543. There were no dose-related effects on total body clearance (Clt) through the full dose range studied. Initial analysis fails to show any relationship between the AUC at D1 and T nadir. Fully updated PK analysis will be presented.

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Pharmacokinetics of single and multiple oral doses of zd1839 ('Iressa'), a specific epidermal growth factor receptor tyrosine kinase inhibitor (egfr-tki), in healthy male volunteers

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Aims: ZD1839 ('Iressa') is an orally active, selective EGFR-TKI which blocks signal transduction pathways involved in the proliferation and survival of cancer cells and has shown activity in Phase I trials. Two studies have been conducted to (1) assess the dose proportionality of ZD1839 and (2) evaluate multiple-dose pharmacokinetics.

Methods: In study 1, 15 male volunteers were randomised to receive single ZD1839 doses (50, 100 and 500 mg or 50, 250 and 500 mg) to assess ZD1839 linearity across a dose range that encompassed the doses being used in clinical efficacy trials. Doses of ZD1839 were administered orally in random order with a minimum 2-week washout period between each dose. In study 2, 12 male volunteers received a single oral dose of ZD1839 100 mg, followed by a washout period of at least 2 weeks, before receiving 100 mg twice at 12-hour intervals for 1 day, followed by 13 daily doses of 100 mg.

Results: In study 1, following a single oral dose of ZD1839 Cmax was reached in 3-5 hours and plasma concentrations declined in a biexponential manner. Dose proportionality was observed at 50, 100 and 250 mg dose levels with respective mean Cmax values of 11.8, 32.7 and 82.1 ng/ml and mean AUC(0-24) values of 143, 388 and 1088 ng.h/ml. Mean AUC and Cmax values for ZD1839 50, 250 and 500 mg were not dose-proportional as a greater than proportional increase in both parameters was observed for the 500 mg dose.

In study 2, Cmax, t1/2 and AUC(0-24) ranged from 23.6-57.8 ng/ml, 13.7-42.2 hours and 308-927 ng.h/ml, respectively, following a single 100 mg dose of ZD1839. Steady-state ZD1839 plasma concentrations were reached within 3-5 days of starting multiple dosing using a loading dose regimen. The multiple-dose pharmacokinetics of ZD1839 were generally not well predicted from the single-dose parameters. As expected from the ZD1839 t1/2, there was evidence of accumulation (approximately 3-fold) during the 14-day multiple-dosing period.

Conclusion: Overall, ZD1839 was well tolerated when administered as a single dose or a multiple-dose regimen to healthy male volunteers. Events that were considered possibly related to treatment with multiple doses of ZD1839 included mild rash, acne and dry skin, which have been reported previously. The good tolerability profile of ZD1839 confirms the feasibility of such studies in healthy volunteers.

'Iressa' is a trade mark of the AstraZeneca group of companies

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Pharmacokinetics of Irinotecan (CPT-11) in wildtype- and P-glycoprotein- (P-gp) knockout (mdr1a/1b (-/-)) mice

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Purpose: In vitro studies have demonstrated that CPT-11 is a substrate of the transmembrane efflux pump P-gp. Previously we have shown in a pharmacokinetic study in mice, that the co-administration of a P-gp inhibitor could be an approach to reduce CPT-11 induced diarrhoea by blocking biliary secretion of CPT-11 and its metabolite SN-38 into the intestinal lumen.. To further investigate the in vivo influence of P-gp on the pharmacokinetic behaviour of CPT-11 and SN-38, we have administered an i.v. bolus of CPT-11 to wildtype- and mdr1a/1b (-/-) mice, focussing on the drug distribution to the brain and the intestinal excretion of CPT-11.

Methods: CPT-11 was given as an i.v. bolus at a dose of 10 mg/g bodyweight. At fixed time points mice were sacrificed and organs were prepared for analysis. Intestinal contents have been collected in specific metabolic cages. Standard High Pressure Liquid Chromatography (HPLC) was used for the detection of CPT-11 and metabolites.

Results: 4h after an i.v. bolus 3-4 fold higher levels of CPT-11 have been found in brain tissue of mdr1a/1b (-/-) mice compared to wildtype mice. In contrast significantly more CPT-11 was detected in the intestinal contents of wildtype mice. For the pharmacodynamic active metabolite SN-38 no differences have been found both in brain tissue (negligible levels of SN-38) and intestinal contents between wildtype and mdr1a/1b (-/-) mice.

Conclusions: A lack of functional P-gp increases the penetration of CPT-11 into the brain in vivo. By co-administering certain P-gp inhibitors (e.g. PSC833) + CPT-11 an increased CNS-toxicity should be taken into account.

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Pharmacokinetics of amifostine after subcutaneous administration in head and neck cancer patient during postoperative radiotherapy

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Purpose: Amifostine (Ethyol®) is the prodrug for the dephosphorylated metabolite WR-1065, which is formed intracellular dependent on alkaline phosphatase. The drug serves as a protector against chemotherapy- and radiation-induced toxicities. Determination of amifostine and WR-1065 as well as the resulting disulfid-metabolites in plasma and urine was done after subcutaneous (s.c.) or intravenous (i.v.) application of 500 mg amifostine in patients with head and neck cancer receiving radiotherapy.

Methods: Blood and urine samples (n= 752) of 5 patients were enrolled into this study. Amifostine was given 30 min prior to daily fractionated irradiation, five days a week during post-operative radiotherapy. Plasma and urine analysis was performed on day 1, 4, and 5 of week one, and for the first days of the following weeks of treatment. For the quantification of amifostine, WR-1065 and the disulfides a RP-HPLC method was performed. The detection was done electrochemically with a Pt-working electrode and an Ag/AgCl counter electrode. Amifostine was converted to WR-1065 by acidic hydrolysis, the disulfides were reduced to WR-1065 by the use of DTT. Calculation of amifostine and the disulfides was performed after conversion, by substracting the measured concentrations of the active metabolite WR-1065.

Results: The limit of quantification was 10 ng/ml WR-1065. The within- and between-day accuracy of the assay for WR-1065 varied from 5% to 15% (from 100 μg/ml to 10 ng/ml, respectively), the precision was in the range of 8%. The s.c. administration led to comparable AUCs as was obtained after i.v. dosage. There is evidence for an accumulation of the disulfides and the active metabolite WR-1065 in plasma, leading to an increase of cmax as well as AUC after repeated s.c. administrations. The mean terminal half life for the parent compound was calculated in a one compartmental analysis to 60 min, for WR-1065 to 30 min. Tmax for amifostine and WR-1065 was in the range of 30 min after s.c. application, and in the range of 5 min after i.v. administration. The peak plasma level for the dithiols after s.c. formulation was observed after 75 min. The disulfides were cleared much slower from the plasma than amifostine and WR-1065, leading to an increase of the peak plasma levels of WR-1065.

Conclusion: The subcutaneous route of administration seems to be an attractive choice and offers a more practical route of administration for cancer patients during radiotherapy.

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Pharmacokinetics (P) of Tamoxifen (T) and related biological variables during long-term adjuvant T therapy in M_0 primary breast cancer (M_0PBC)

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The antiestrogen T is currently used for breast cancer but TP is very seldom studied and the induced biological variables (ibv) by the estrogen-like T activity have not sufficiently related to the TP. Moreover, the ibv were studied regardless the presence of cancer, inherited or acquired lipid disorders, chemotherapy and the T day dose. No relation is clear between TP, vaginal epithelial maturation and endometrial behaviour.