p53-mutated human colon adenocarcinoma cell line SW480 (proliferation, clonogenicity, cell cycle, different schedulings) and *in vivo* with the combination of EPO906 and a minimally fractionated treatment schedule of IR (4×3Gy) in an nude mice xenograft tumor model.
Results: The paclitaxel-refractory colon cancer cell line SW480 was sensitive to treatment with subnanomolar concentrations of EP0906. Combined treatment with EPO906 followed by clinically relevant doses of IR (2 and 5 Gy) further resulted in a supraadditive cytotoxic effect in the low dose range (0.1 nM EPO906). Cell cycle analysis revealed a G2/M-related mechanism of radiosensitization by EPO906. Based on the supraadditive *in vitro* effects in this radioresistant cell line, combined treatment with EPO906 and fractionated irradiation was tested *in vivo* against nude mice tumor xenografts. Combined treatment resulted in an at least additive tumor growth delay.
Conclusions: EPO906 retains full activity in multidrug-resistant human colon cancer cell line *in vitro* and *in vivo* alone and in combination with IR. Thus Epothilone might be a promising alternative in Paclitaxel-resistant, PgP-overexpressing tumors for a combined treatment regimen using IR and microtubule inhibitors.

538 POSTER

New synthetic Epothilone Derivative ZK-EPO inhibits tumors generally resistant to chemotherapy

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Based on a broad fully synthetic drug optimization program with more than 350 synthesized Epothilone analogs, we have developed ZK-EPO, a new derivative with outstanding preclinical efficacy.
In contrast to other tubulin targeting drugs (i.e. paclitaxel), ZK-EPO is rapidly taken up by the tumor cells and preferentially accumulates in the cell nucleus.
ZK-EPO inhibits the growth of a wide range of different human cancer cell lines, and, unlike paclitaxel, also suppresses the growth of cell lines that over-express P-glycoprotein at sub-nanomolar concentrations. We have shown that this epothilone is not recognized by cellular efflux mechanisms. Dose response studies with *in vivo* xenograft cancer models either sensitive or intrinsically resistant to paclitaxel demonstrated strong antiproliferative activity and a large therapeutic window of ZK-EPO.
To identify further indications for clinical development we have tested ZK-EPO in a broad range of tumor models. Beside to the classical indications for tubulin stabilizing drugs as breast, ovarian, and lung cancer, we have observed strong antiproliferative activity in pancreatic and colorectal cancer, as well as in melanomas.
In pancreatic cancer models, ZK-EPO has clearly demonstrated antitumor activity that is superior to Gemcitabine in all five tumors evaluated in this study (four cell lines and one clinically derived tumor). Against paclitaxel- or dacarbacine-resistant human melanoma models, ZK-EPO produced strong antiproliferative activity: *i.e.* SK-Mel-28 and A375.
This broad preclinical activity spectrum provides strong evidence, that the novel epothilone analog ZK-EPO may have antitumor efficacy in a variety of rather chemoresistant cancer indications and recommends an extended evaluation of this compound in clinical trials.
The potential of the new derivative is currently being investigated in patients with different solid tumors.

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Comparative pharmacokinetic (PK) study of a cremophor-free, protein stabilized, nanoparticle formulation (ABI-007) and a cremophor-based formulation of paclitaxel (P) in patients with advanced solid tumors

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Background: Abraxane™ (ABI-007 or ABX), a novel, albumin-bound nanoparticle P, was developed to eliminate solvents from the 1st-generation formulation of P (Taxol® or TAX). The absence of Cremophor-EL (CrEL) and alcohol allowed ABX to be administered with a shorter infusion (30 minutes) using standard IV tubing without steroid and antihistamine premedication. A phase 3 trial of ABX vs TAX in patients with metastatic breast cancer demonstrated superior antitumor activity for ABX as measured by response rate and time to disease progression (O'Shaughnessy, SABCS 2003). The present study compared the PK of P following administration of ABX and TAX at the doses and schedules used in the phase 3 trial.
Patients and Methods: Patients with advanced solid tumors were randomly assigned to receive either ABX 260 mg/m^2 (n=14) or TAX 175 mg/m^2 (n=12), both IV q3w. Whole blood samples (12 scheduled for ABX; 13 for TAX) from the first dose cycle were analyzed using a validated LC-MS/MS method (lower limit of quantitation: 5 ng/mL). Noncompartmental PK parameters were estimated using WinNonlin 4.1 (Pharsight, Cary NC).
Results: For both ABX and TAX, P displayed multiphasic disposition. AUC_{inf}, λ_z, and $T_{1/2}$ were similar for ABX and TAX (see Table). Plasma clearances and volumes of distribution were clinically different and reached statistical significance for CL and V_z. Differences in T_{max}, C_{max}, and dose adjusted C_{max} were attributed to differences in dose and duration of administration. When analyzed with data from other clinical trials, ABX AUCs were linear with respect to dose from 80 to 300 mg/m^2. The observed parameters were similar to those reported for TAX and to previous clinical trials for ABX.
Conclusion: Nonlinear pharmacokinetics of TAX have been attributed to the formation of CrEL micelles which sequester P in the intravascular compartment. This study suggests that CrEL micelles also decrease P clearance by prolonging circulation in the intravascular space. In animals bearing the MX-1 mammary tumor, ABX resulted in 30–40% higher intratumor P concentrations compared to equal doses of TAX. This difference may be due to in part to sequestration of P by CrEL micelles which reduced the bioavailability of TAX compared to ABX. In addition, the use of albumin as the delivery vehicle may enhance plasma clearance and drug transport into tumors by taking advantage of albumin receptor (gp60)-mediated transcytosis across endothelial cells (Desai, SABCS 2003).

Parameter	Abraxane (260 mg/m^2 IV over 30 minutes) Mean (%CV)		Taxol (175 mg/m^2 IV over 3 hours) Mean (%CV)		p-value
CL (L/h•m^2)	21.13	(43.8)	14.76	(31.8)	0.048
Vd_{ss} (L/m^2)	230.7	(54.3)	156.3	(43.2)	0.211
V_z (L/m^2)	663.8	(48.1)	433.4	(31.1)	0.040
AUC_{inf} (ng•h/mL)	14,788.6	(45.3)	12,602.7	(21.0)	0.524
Dose adjusted AUC_{inf} (ng•h/mL)	56.84	(46.3)	71.90	(21.1)	0.049
C_{max} (ng/mL)	22,968.6	(112.5)	3,543.3	(57.2)	< 0.001
Dose adjusted C_{max} (ng/mL)	88.69	(114.2)	20.14	(55.8)	< 0.001
T_{max} (h)	0.36	(45.2)	2.65	(27.6)	< 0.001
λ_z (h^{-1})	0.033	(16.9)	0.034	(13.0)	0.477
$T_{1/2}$ (h)	21.6	(17.2)	20.5	(14.6)	0.479
$AUC_{\%extrap}$ (%)	2.8	(41.3)	2.8	(52.6)	0.983

DNA-interactive agents

540 POSTER

Clofarabine administered weekly to adult patients with advanced solid tumors in a phase I dose-finding study

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Background: Clofarabine, a next-generation nucleoside analogue that inhibits DNA synthesis, has demonstrated activity in acute leukemia in Phase I & II trials. The agent has also shown potent cytotoxic activity in a wide range of solid tumor cell lines and therapeutic activity in murine tumor models.
Methods: A Phase I dose-finding study is ongoing to determine the maximum tolerated dose (MTD) of clofarabine in patients with advanced solid tumors. To avoid myelosuppression observed with the daily × 5 administration used for hematologic malignancies but yet to achieve high plasma concentrations, clofarabine is administered IV on days 1, 8, and 15 of a 28-day cycle. Patients are treated with escalating doses starting at 4 mg/m^2 until MTD is determined.
Results: Preliminary data are available for 32 patients, 17 males and 15 females with a median age of 66 years (range 48 to 78). The patients were treated in 8 cohorts; 3 each at 4, 6, 10, 14, and 27.5 mg/m^2, 5 at 18 mg/m^2, 8 at 22 mg/m^2, and 4 at 34 mg/m^2. Tumor types include lung (7), colorectal (7), pancreas (3), prostate (3), SCC larynx (2), transitional cell bladder (2), cholangiocarcinoma (2) and one each of melanoma, ovarian, gallbladder, SCC esophagus, SCCHN, and leiomysarcoma. All patients received at least one cycle of therapy, 8 pts completed ⩾3 cycles of treatment with 5 pts completing 4 cycles. Adverse events occurring in >30% of patients include fatigue, nausea, vomiting, weakness, anorexia, and dyspnea. Available hematologic data on 30 patients indicate 4 (13%) experienced transient grade 3 or 4 neutropenia; no febrile neutropenia was observed. Twenty-six of 30 patients (87%) experienced grade 3 lymphopenia; however 18 (60%)

had grade 2 or 3 lymphopenia at baseline. Data on 23 of the 32 patients show that 9 had stable disease and 14 had progressive disease as best response; 1 prostate cancer patient had a drop in PSA level of 88% from baseline. PK data available from 35 patients up to 42.5 mg/m^2 indicate that Cmax and AUC (0–6) appeared to be dose-proportional. Clearance (~19 L/h/m^2), half-life (~5 h), and volume of distribution at steady state (~91 L/m^2) were consistent with the PK seen in adult leukemia patients.
Conclusions: Clofarabine has been administered weekly for 3 weeks (days 1, 8, and 15) every 28 days to adult pts with solid tumors. Patients have been treated with doses up to 53 mg/m^2 and MTD has not been reached. Enrollment is ongoing in the 66 mg/m^2 cohort.

541 POSTER

Effects of bisintercalating DNA threading agents on global gene expression

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We are investigating the capacity of 12 novel DNA bisintercalating threading agents and related compounds, together with recognized transcription poisons (including actinomycin D, echinomycin and nogalamycin), to affect gene expression. The novel agents are dimers of 9-aminoacridine carboxamide in which the linker is attached at the 9 position and bearing different 4-carboxamide threading side chains (Wakelin LPG, *J Med Chem* 46: 5790–5802, 2003). We studied the effect of each of the available agents on the expression of 6000 sequence-verified human genes by cDNA microarray analysis following treatment of cultured CEM cells using a 5 × IC50 concentration for 24h. Cube root plots of array fluorescence intensity values indicated that changes in global gene expression could be represented by three separate populations of genes which respond differently to the various agents. The largest population was comprised of genes which were expressed in control preparations, and whose expression was altered by treatment. Ratiometric analysis, involving comparison of the distribution of log2 of the ratio of fluorescence from both channels on each cDNA microarray, indicated that, for each agent, this population exhibited a near-Guassian distribution and it was further invesigated using heirarchical clustering and Significance Analysis of Microarray (SAM) procedures. Within this set of genes, expression profiles suggestive of common effects attributable to the various agents were not immediately apparent. Thus, despite similarities of structure and DNA interaction, in the context of a common cytotoxic response, a state of 'transciptional chaos' was indicated 24h after toxic insult by these agents. However, expression of a separate gene population (>1000 in each treatment) was eliminated completely in response to the respective agents, despite expression of these genes in control cells. A major proportion of genes in this cluster appear to be common between the agents used and the response will be discussed with reference to mechanisms of action of the DNA interactive agents. The third population of genes, which are silent in control preparations, is expressed following treatment with the various agents. Initial examination using Gene Ontology database searches of the latter population indicates a considerable proportion of these genes is associated with stress response and apoptotic mechanisms.

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Synthesis, lipophilicity and cytotoxicity of new oxaliplatin derivatives

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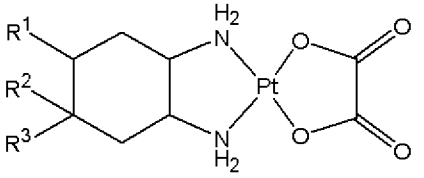
Introduction: Oxaliplatin has been the first platinum drug to prove clinical activity in an inherently cisplatin-resistant malignancy, i. e. colorectal cancer. Although the genuine pharmacodynamic effects of oxaliplatin and the specific properties of its DNA adducts apparently result from the presence of the sterically demanding, hydrophobic cyclohexane ring, structure-activity relationships with regard to modifications of this part of the molecule have not been systematically investigated. In order to fill this gap and to explore possibilities of improving antitumor activity, we have synthesized ring-substituted cyclohexanediamine derivatives and prepared the oxalatoplatinum complexes depicted in the figure.
Methods: Lipophilicity of these complexes has been estimated by means of microemulsion electrokinetic chromatography (MEEKC), and their cytotoxicity in human colon carcinoma and other tumor cell lines has been determined in colorimetric microculture assays (resazurin assay, MTT assay).
Results: The following structure-activity relationships can be deduced from these studies: (1) Compared to oxaliplatin, potency is increased in subsets of cell lines, particularly in leukemia and some colon carcinoma cells, by introduction of small substituents (methyl, ethyl) on C4 of cyclohexanediamine, but tremendously affected in all cell lines by bigger substituents (propyl, tert-butyl, phenyl). (2) Within a panel of five colon carcinoma cell lines, the activity profile of the 4,4-dimethyl-substituted complex most closely resembles that of oxaliplatin, while that of the *cis*-4,5-dimethyl-substituted complex, which on average exhibits a lower potency, contrasts sharply. (3) No simple correlation is found between lipophilicity and cytotoxicity.
Conclusions: These findings warrant testing in a greater panel of cell lines in order to further explore the possibility of improving antitumor activity and of altering the spectrum of activity compared to oxaliplatin.



R^1	R^2	R^3
H	H	CH_3 (methyl)
H	H	C_2H_5 (ethyl)
H	H	C_3H_7 (propyl)
H	H	C_4H_9 (tert-butyl)
H	H	C_6H_5 (phenyl)
H	CH_3 (methyl)	CH_3 (methyl)
CH_3 (methyl)	H	CH_3 (methyl)

543 POSTER

In vitro evidences on the role of the halogenoacrylic moiety in modulating brostallicin mechanism of action

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Brostallicin (PNU-166196) is a α-bromoacrylic distamycin-like derivative DNA minor groove binder (MGB), currently in Phase II clinical evaluation. Unlike other cytotoxics, this drug has the peculiarity of showing enhanced antitumor activity in cells with high glutathione-S-transferase (GST) and/or glutathione (GSH) content. In order to better characterize its mechanism of action, molecules with different acrylic moieties have been synthesized and tested for in vitro cytotoxic activity on tumor cells, chemical reactivity *vs* nucleophiles and in vitro DNA binding mechanism.
The in vitro cytotoxicity of brostallicin and its analogs was tested against murine L1210 leukemia. Results showed that the Cl-acrylic analog (PNU-248427) is only 8 times less cytotoxic than brostallicin (IC_{50} = 14.95 nM and IC_{50} = 1.85 nM, respectively) while F-acrylic (PNU-248482) and acrylic (PNU-230858) derivatives were not cytotoxic (IC_{50} = >7000 nM and IC_{50} = 4382.29 nM, respectively).
The chemical reactivity of these compounds against nucleophiles such as GSH, amines and thiols correlates with their in vitro activity. In fact, while brostallicin and the Cl-acrylic derivative react with nucleophiles giving the corresponding adducts, F-acrylic and acrylic analogs do not. Thus, suggesting that the α-halogenoacrylic moiety plays a crucial role in the cytotoxic activity of these new MGBs and supporting the hypothesis that a reactive adduct between brostallicin and a biological nuclephile eg. GSH could lead its antitumor activity.
Finally, to verify the correlation between chemical reactivity and a possible covalent DNA binding, experiments on the interaction of brostallicin and the inactive F-acrylic derivative with plasmid DNA (pUC18) were performed. Both molecules did not interact covalently with DNA by themselves. Conversely, upon incubation with GSH only brostallicin showed a change of the DNA topology from the supercoiled to the circular form (nicking).
In order to better characterize the brostallicin-DNA binding mechanism, Taq Stop assay on topoisomerase IIα cDNA was performed with or without GSH/GST. Brostallicin was tested in comparison with a synthetic GSH-halogenoacrylic-adduct model (PNU-571077) and tallimustine. Data confirmed the ability of brostallicin to bind covalently to DNA only upon