KP1019 accumulation in P-gp positive cells which could be partly reversed by co-administration of P-gp modulators. KP1019 inhibited P-gp ATPase activity with an K_i of approximately 31 μM. Selection of KB-3−1 cells against increasing KP1019 concentrations for more than year led to only an around 2-fold resistance (KB-1019 cells), and unexpectedly no P-gp expression. Accordingly KB-1019 cells displayed no drug accumulation defect and a unique cross-resistance pattern, indicating an ABC-transporter-independent acquired resistance phenotype.
Conclusion: In summary P-gp has to be considered as significant but weak intrinsic resistance mechanism against KP1019. Acquisition of resistance against KP1019 during chemotherapy seems to be relatively unlikely and acquired resistance based on ABC-transporter overexpression has not to be expected.

631 POSTER

Tumor associated fibroblasts have a profound impact on drug sensitivity of gastric cancer cells

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Background: In most tumor types, including gastric cancer, one of the main obstacles in anti-cancer therapy is the development of drug resistance. Some of the molecular mechanisms involved in acquired drug resistance, such as MDR, are well-characterized. In contrast, cellular mechanisms, i.e. cell-to-cell interactions between the cancer cells and the surrounding stromal cells, are poorly understood. Therefore, the purpose of the present study was to investigate the impact of benign and tumor associated fibroblasts on drug sensitivity of gastric cancer cells. Material and Methods: Fibroblast cultures were originated from benign gastric mucosa and the corresponding primary gastric carcinomas obtained from eight gastric cancer patients. Characterization of the fibroblasts using a panel of cell type specific antibodies confirmed the connective tissue origin. Two gastric cancer cell lines, namely MKN-28 representing the intestinal type and Hs746T indicating the diffuse type of gastric cancer, were used for co-culture experiments using the multicellular spheroid model. Homotypic spheroids consisting of either cancer cells or fibroblasts and heterotypic spheroids consisting of both cell types were established and treated with a variety of clinically relevant drugs. Treatment effects were measured using apoptotic (TUNEL, nucleosome ELISA) and metabolic (MTS) assays. Changes in the protein profiling were identified using 2D-gel electrophoresis followed by MALDI-TOF analysis. Results: Homotypic and heterotypic multicellular spheroids imitated a number of features observed in gastric carcinomas, such as the original differentiation phenotype and a slow proliferation activity. In contrast to homotypic spheroids and heterotypic spheroids containing benign fibroblasts, heterotypic spheroids with tumor associated fibroblasts were less sensitive to most of the drugs tested. Two-dimensional gel electrophoresis revealed that the decreased drug sensitivity of the heterotypic spheroids was associated with changes in the protein expression profile detected in both the gastric cancer cells and the tumor related fibroblasts. Most often, quantitative changes of the proteins were found. In addition, de novo expression of distinct proteins also could be identified. Conclusion: Tumor related fibroblasts, but not their benign counterparts, modulate drug sensitivity of gastric cancer cells. This is associated with profound changes in the protein profile.

632 POSTER

Down-regulation of mitochondrial F1F0-ATP synthase in human colon cancer cells with induced 5-fluorouracil resistance

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5-Fluorouracil (5-FU) is widely used for treatment of advanced colorectal cancer. Unfortunately it is common for such patients to ultimately develop resistance to 5-FU, creating a major problem for chemotherapy. The mechanisms underlying this resistance are largely unknown. To screen for proteins possibly responsible for 5-FU resistance, cells resistant to 5-FU were derived from human colon cancer cell lines, and two-dimensional gel electrophoresis (2-DE)-based comparative proteomics was performed. 2-DE data showed there was lower expression of the alpha subunit of mitochondrial F_1F_0-ATP synthase (ATP synthase) in 5-FU-resistant cells compared to parent cells. Western blotting showed expression of other ATP synthase complex subunits was also lower in 5-FU-resistant cell lines, and that these resistant cells also showed decreased ATP synthase activity and reduced intracellular ATP content. The ATP synthase inhibitor, oligomycin A, strongly antagonized 5-FU-induced suppression of cell proliferation. W hen 5-FU sensitivity was compared to ATP synthase activity in six different human colon cancer cell lines, the positive correlation has been found. Bioenergetic dysfunction of mitochondria has been reported as a hallmark of many types of cancers, i.e., down-regulation of ATP synthase β-subunit expression in liver, kidney, colon, squamous oesophageal and lung carcinomas, as well as in breast and gastric adenocarcinomas. Our findings demonstrate that ATP synthase down-regulation may not only be a bioenergetic signature of colorectal carcinomas, but may also lead to cellular events responsible for 5-FU resistance.

633 POSTER

Genetic variation in P-glycoprotein gene (ABCB1) and tipifarnib exposure

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Background: Farnesyl transferase inhibition is a novel approach to cancer chemotherapy in both solid and hematologic tumors. Tipifarnib (Zarnestra™; R115777) is a potent farnesyl transferase inhibitor currently under clinical development as a monotherapy or as a combination therapy with other antitumor agents. P-glycoprotein (P-gp) is an efflux transporter that contributes to transport of drugs from intracellular to extracellular compartments. Hence, interindividual variations in P-gp function may influence drug bioavailability, predisposition to treatment resistance as well as drug-drug interactions among compounds subject to drug efflux mediated by P-gp. The aim of the present study was to evaluate the influence of functional genetic variations in the P-gp gene (*ABCB1*) in relation to clinical pharmacokinetics of tipifarnib.
Material and Methods: 24 healthy volunteers who participated in a food-effect study with a single 100 mg oral dose of tipifarnib were included in the present study. Pharmacokinetic data from the unfed state were utilized for all association analyses. Three synonymous but functional single nucleotide polymorphisms (SNP) in the coding region of the *ABCB1* (C1236T, G2677T, C3435T) were genotyped. Additionally, the key functional C3435T SNP in exon 26 of the *ABCB1* was characterized in a patient sample (N= 29) with advanced solid tumors administered multiple oral doses of tipifarnib (200 mg b.i.d, 4 days).
Results: A high degree of linkage disequilibrium (LD) was observed among the three *ABCB1* SNPs with p-values for all pair-wise LD <0.002. There was no deviation from the Hardy-Weinberg equilibrium in the sample (p values: 0.23–0.69). No significant association was found between haplotypes consisting of any combination of one to three of the *ABCB1* SNPs and tipifarnib C_{max} and AUC_{0-72h} (p values: 0.26–0.99). These observations were consistent with the analysis of the C3435T SNP in relation to tipifarnib C_{max} and AUC_{0-10h} in the patient sample (p values: 0.28–0.57).
Conclusions: Tipifarnib plasma exposure is not appreciably influenced by common genetic variants in *ABCB1*. These preliminary data suggest that P-gp is not involved in tipifarnib absorption in humans.

634 POSTER

E2F-1 induction and MEK inactivation coordinates with p53-generated signals to switch chemotherapy-induced growth arrest to apoptosis in human colorectal HCT116 cancer cells

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Cancer chemotherapeutic agents exert their cytotoxic effect by inducing DNA damage and activating apoptosis. The tumour suppressor protein p53 is an important modulator of apoptosis and whose mutation often affects the sensitivity of cancer cells to chemotherapy. In human colon cancer cell HCT116, anti-metabolite anticancer drug 5-FU triggers a p53-dependent apoptosis, whereas DNA damaging agent adriamycin results in growth arrest albeit both agents are strong p53 activators. To investigate the molecular mechanisms leading to the differential outcomes of DNA damage, we compared the gene expression profiles induced by 5-FU and ADR. We found that 5-FU and ADR induced a similar expression profiles in p53 responsive genes, indicating that differential cellular response to 5-FU and ADR is not due to differential activation of p53 target genes but depends on additional molecular events. Further analysis revealed the activation of E2F-1 pathway in response to 5-FU treatment, which was not observed in ADR-treated cells. Over-expression of E2F-1 in HCT116 cells resulted in apoptosis and partially abrogated the G2/M arrest induced by ADR, which mimics a 5-FU-like phenotype. In addition, signaling pathway analysis indicates that 5-FU treatment results in inactivation of MEK/ERK pathway but ADR did not. Inhibition of this pathway by MEK inhibitor U0126 resulted in a significant apoptosis, suggesting that MEK/ERK pathway is required for the survival of HCT116 cells. Thus, our data suggest that

multiple molecular events contribute to the apoptotic effect of 5-FU, by which E2F-1 activation and MEK inactivation coordinate with p53 generated signals to induce efficient apoptosis.

635 POSTER

Inflammatory response might influence the pharmacokinetics (PK) and pharmacodynamics (PD) of Imatinib and CGP 74588 in patients with advanced gastro-intestinal-sarcoma (GIST)

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Background: Ten percent of patients with advanced GIST presented primary resistance and 20% developed secondary resistance per year of Imatinib (Gleevec) treatment. Possible mechanism for resistance might be low drug exposure. Prior PK analyses of Imatinib showed large inter-individual variability in patients. This study was designed to explore the factors affecting PK variability of Imatinib and its main metabolite, CGP 74588, along with PK-PD correlations.
Methods: Thirty-five patients (26 males; median age 55 yrs, range 28–84 yrs) with advanced GIST, registered in the French Sarcoma Group phase III study (BFR14 trial), received 400 mg/d of Imatinib. Five blood samples were obtained before intake, between 1 and 3 hours and 6 and 9 hours on day 1, prior to next dose on day 2 and at steady state on days 30 and 60. Imatinib and CGP 74588 plasma levels were quantitated by reverse-phase HPLC coupled with tandem mass spectometry, and analysed by population PK using NONMEM program. We examined the influence of 17 covariates on Imatinib clearance (CL) and apparent CGP 74 588 clearance (CLM/fm, with fm = fraction of Imatinib converted to CGP 74588). These covariates included age, weight, gender, alpha-1-acid glycoprotein (AAG), renal, hematological and liver biological values at baseline along with oedema, liver metastasis and occasion (OCC = 0 if PK data obtained at day 1, or = 1 at day * 30).
Results: Both clearances (CL and CLM) decreased in case of elevated AAG, probably due to higher plasma protein binding with a best regression formulas of: CL = 17.2/(1 + 0.961*AAG), and CLM/fm = 164*(1 − 0.46*OCC)/(1 + 1.52*AAG) (AAG in g/L). A significant time-dependent decrease in CLM/fm was evidenced with a mean+SD CGP/Imatinib AUC ratio of 0.25+0.07 at steady state, compared to 0.14+0.03 on day 1. Hematological toxicity, measured by the relative decrease in absolute neutrophil count (ANC) [ΔANC = (ANC nadir − ANC on day 1)/ANC on day 1) and in Δ platelets, was significantly correlated with high exposure to Imatinib on day 1 and at steady state, particularly if considering unbound plasma Imatinib concentration at steady state. Significant correlation between ΔANC and AAG was observed on day 1 ($p<0.0001$). Response and oedema occurrence were not correlated with any PK parameters.
Conclusion: Inflammatory response might influence the metabolism, the drug disposition and the hematological toxicity of Imatinib in with advanced GIST.

636 POSTER

Promoter hypermethylation of the DNA repair gene mgmt is more frequent in secondary glioblastomas and is independent from other prognostic factors

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O^6-methylguanine-DNA methyltransferase (MGMT) is a DNA repair protein that specifically removes promutagenic alkyl groups from the O^6 position of guanine in DNA. Repair of cytotoxic DNA damage by MGMT is a potentially important factor of resistance to alkylating chemotherapeutic agents, commonly used in the treatment of glioblastoma multiforme (GBM) since it reduces the cytotoxicity of these drugs.
We assessed the inactivation of the DNA-repair gene MGMT by promoter hypermethylation using Methylation-Specific PCR (MSP) in 45 GBM obtained from patients subsequently treated by conventional radiotherapy and CDDP+BCNU. We observed that the MGMT gene was methylated in 15 patients (33%). This finding was associated with prolonged overall survival (25 versus 14 months; log-rank p=0.026) and with a longer Progression Free Survival (PFS) (11 versus 7 months; log-rank p=0.037). Secondary GBMs had prolonged overall survival (30 versus 11 months; log-rank p=0.0030) than *de novo* tumors, whereas other prognostic factors were not statistically associated with ST or PFS. Moreover, methylation status was more frequent in secondary than in primary GBMs (70% versus 23%, p=0.0091), but was not associated with other clinical parameters. Other genetic markers as EGFR amplification, p53 mutations and microsatellites analysis for loss of heterozigosity are under study to assess their influence on the treatment response and overall survival of patients with GBM.

637 POSTER

Effective combinations of carboplatin with low doses of TRAIL, HGS-ETR1 and HGS-ETR2 in the TRAIL-sensitive HX62 human ovarian tumour cell line

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TNF-related apoptosis inducing ligand (TRAIL) has the ability to induce apoptosis in cancer cells, with minimal toxicity in normal cells, in pre-clinical models, via the extrinsic pathway. A strategy that may overcome drug resistance in ovarian cancer is to combine cytotoxic agents with TRAIL, engendering a partnership between the extrinsic and intrinsic pathways of apoptosis. Ovarian cancer cell lines are often resistant to TRAIL, as we have observed in 5 of our panel of 7 lines (IC50s >3 μg/ml). The expression levels of receptors DR4, DR5 and DcR2, caspase-8 and XIAP are similar in these lines. We evaluated the effect of combining TRAIL with carboplatin in the TRAIL sensitive, carboplatin resistant cell line, HX62 (TRAIL IC50 = 387±117ng/ml) using an MTT growth inhibition assay as the endpoint and analysed using the Median Effect equation as described by Chou and Talalay. An ~IC25 dose of TRAIL (150ng/ml) was shown to be additive (CI_{ED50} value = 1.0) when combined with increasing doses of carboplatin (CI_{ED50} = 1.1±0.16). We performed similar studies with the agonistic antibodies to TRAIL receptors, HGS-ETR1 and HGS-ETR2. HX62 cells were sensitive to both agents (IC50 = 0.16 and 1.8 μg/ml respectively). HGS-ETR1, at 0.05 μg/ml (minimally growth inhibitory) sensitised HX62 cells to carboplatin, shifting the IC50 from 60±13 μM to 24±13 μM (p= 0.029) and the data suggest this is a synergistic interaction (CI_{ED50}<1.0); CI_{ED50} = 0.85±0.31. Reducing the HGS-ETR1 dose to 0.01 μg/ml (~6% of IC50; non-growth inhibitory) also resulted in sensitisation (CI_{ED50} = 0.79; n=2). A non-growth inhibitory dose of HGS-ETR2 (0.1 μg/ml; ~5% of IC50) induced similar sensitisation; CI_{ED50}= 0.75; n=2. SKOV-3 cells, in comparison, are resistant to TRAIL (no growth inhibition at 3 μg/ml) and also resistant to HGS-ETR1 and HGS-ETR2 (no inhibition at 10 μg/ml). Preliminary studies show no sensitisation when TRAIL (500ng/ml) was combined with carboplatin. This work will be extended to other cell lines and the reasons for TRAIL resistance in cell lines such as SKOV-3 will be investigated. In conclusion, the agonistic antibodies, HGS-ETR1 and HGS-ETR2 are effective alone and in combination with carboplatin in a TRAIL-sensitive ovarian tumour cell line.
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638 POSTER

Acquired resistance to EGF receptor-targeted cancer therapy

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Pharmacological approaches to target the epidermal growth factor receptor (EGFR) in cancer cells include monoclonal antibodies that block ligand binding to the receptor and small-molecule inhibitors that compete for the ATP binding site on the receptor. Two leading agents, Iressa (gefitinib/ZD1839) and Erbitux (cetuximab/C225) were recently approved by the US Food and Drug Administration for the treatment of patients with chemorefractory lung cancers and colon cancers, respectively. With the incorporation of this novel anti-cancer therapy into standard practice, it is anticipated that acquired resistance to the treatment may occur. The purpose of this study is to develop experimental models to explore potential molecular changes associated with the acquired resistance. We developed two types of resistant sublines from the DiFi colon cancer cells, which have an innate sensitivity to EGFR inhibition, by exposing the cells to sub-effective doses of C225 (DiFi5 cells) or AG1478 (DiFi-AG cells) for extended time periods. Compared with parental DiFi cells (DiFi-P), DiFi5 cells exhibit remarkable reduction in the level of EGFR (approximately equal to 10% of the EGFR in DiFi-P cells) and slight reduction in growth rate, and become insensitive to C225 or AG1478. In contrast, DiFi-AG cells showed similar level of EGFR and slightly increased growth rate, and are resistant