hypermethylation of tumour suppressor genes, is a hallmark of cancer. Information about which methylation events are disease specific has a great potential in diagnostics and drug development. The aim of this study was to investigate the methylation status of the tumour suppressor genes SHP1, SOCS1 and STAT1, their counterparts SOCS3 and SHP2, as well as the drug resistance gene MGMT, and their effect on protein expression and cytotoxic drug sensitivity in glioblastoma cell lines. A further aim was to investigate the possibility to increase cytotoxic drug sensitivity in the glioblastoma lines by demethylation treatment.
Methods: To study methylation patterns, bisulfite treatment of total DNA followed by PCR amplification and Pyrosequencing® analysis was employed. Protein expression of total lysates was evaluated by Western blot analysis. Cytotoxic drug sensitivity was analysed by the fluorometric microculture cytotoxicity assay. Demethylation was obtained by treatment with the drug decitabine. Six glioblastoma cell lines were used in the studies.
Results: MGMT, SHP1 and SOCS1 were methylated at varying levels in the analyzed gene regions, whereas SHP2, SOCS3 and STAT1 were not methylated. The observed methylation levels in MGMT and SHP1 were associated with a reduction of protein expression. In addition, a low degree of methylation and a high protein level of MGMT were related to a decreased sensitivity to the cytotoxic drugs 5-fluorouracil, 17-AAG, bortezomib, and picropodophyllin. Finally, it was possible to increase the sensitivity in the glioblastoma cells lines to several cytotoxic drugs by demethylation treatment with the drug decitabine.
Conclusions: Epigenetic regulation of MGMT and SHP1 appear to affect tumor phenotype in glioblastoma. The correlation between a low degree of methylation of MGMT and SHP1, a high protein level and low sensitivity to several cytotoxic drugs constitutes a potential predictive marker for chemotherapy of glioblastoma. Finally, the possibility to increase cytotoxic drug sensitivity by demethylation treatment points to novel therapeutic strategies in combination drug therapy of glioblastoma.

Natural products and marine compounds

175 POSTER

Role of ERK activation in triptolide-induced apoptosis in MDA-MB-231 human breast cancer cells

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Background: Triptolide (PG490), a compound isolated from *Trypterygium wilfordii*, has been shown to have potent activity in a variety of xenograft tumor models. However, very little is known about the molecular mechanism by which triptolide acts in cancer cells. Therefore, the aim of this study was to investigate the role of extracellular signal-regulated protein kinase (ERK), a member of the mitogen-activated protein kinase family, in triptolide-induced cell death using the human breast cancer cell line MDA-MB-231.
Materials and Methods: MTT assay was used to determine cell viability upon treatment with 0−40 ng/mL triptolide. Apoptosis was assessed by annexin-V/7AAD staining, and caspase 3/7 activity was measured by a fluorescence-based assay kit. To assess the involvement of ERK and caspases, phosphorylated ERK and cleaved PARP were probed by western blot, respectively, as well as by the use of a MEK inhibitor, U0126, and the pan-caspase inhibitor, Z-VAD-FMK. Expression of phosphorylated eIF2α was determined by western blot.
Results: Dose-dependent reduction in MDA-MB-231 cell viability was observed upon a 72-hour exposure to triptolide, with an IC50 value of 1.9 ng/mL. A 3.2-fold increase in annexin-V+/7AAD− cells was observed when cells were treated with 4 ng/mL triptolide for 48 hours, indicating induction of apoptosis. Triptolide-induced apoptosis was caspase-dependent, as supported by significant increases in caspase 3/7 activity, PARP cleavage and cell viability in the presence of caspase inhibitor Z-VAD-FMK. ERK was activated as early as 2 hour post triptolide treatment, and remained activated for 48 hours. eIF2α was also activated in a time-dependent manner in triptolide-treated cells. The concomitant use of MEK inhibitor, U0126, attenuated triptolide-induced caspase 3/7 activation, PARP cleavage, and significantly increased cell viability from 49% to 98%, indicating that ERK activation acts upstream of caspase activation.
Conclusion: Our data demonstrated for the first time that ERK activation played an important role in triptolide-induced apoptosis, in contrast to the general view that ERK activation contributes to cancer cell survival and proliferation. Furthermore, the sustained activation of ERK, together with eIF2α activation, suggested a possible link of triptolide-induced apoptosis to endoplasmic reticulum stress which warrants further characterization.

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Outcome of three Phase I trials of the marine compound ES-285 (3 hour infusion) in patients with refractory solid tumors

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Background: ES-285 is a marine compound originating from the mollusc *Spisula polynyma*, an edible clam, which is also known as the Stimpson or Atlantic surf clam. The drug has cytotoxic properties by disrupting actin fibers and interacting with the ceramide pathway. The agent has a broad antitumor spectrum in vitro, in vivo and in xenograft models. ES-285 was subject to 4 parallel Phase I studies in patients (pts) with solid tumors, three of them reported here, all of them using 3-hour infusions of the compound.
Material and Methods: Pts had advanced malignancies, good performance status (ECOG PS 0−2) and adequate organ function. The following intravenous schedules of ES-285 were tested: (A) 3 h d1 qwk, (B 3 h d1−5 q3wk, and (C) 3 h d1 q3wk.
Results: The dose of ES-285 per administration was ranging from 2–256 mg/m^2, depending on the study. 117 pts were entered (25−61 per trial), their median age was ranging from 52−59 yrs per trial, and there was a male predominance. The most common tumor types were colorectal, renal and prostate cancer and melanoma. Pts received a median of 2 cycles of treatment in all studies, ranging from 1−18 per patient. Less than 15% of treatment cycles were delayed. More than 80% of pts went off study due to disease progression (83−88%). Only 8.0–13.4% of patients discontinued due to toxicity. The most common clinical adverse events were nausea, vomiting, asthenia, pyrexia (all schedules) and injection site reactions. Anemia, lymphocytopenia and increases of serum liver enzymes were frequently seen, independent of treatment scheme. Ten dose-limiting events were observed, mainly consisting of grade 3/4 (CTC version 2.0) reversible increases in serum ALT, AST and reversible neurotoxicity. Only in schedule C the maximum tolerated dose (200 mg/m^2) and recommended dose (160 mg/m^2) could be established. Among 117 pts, one melanoma patient had a non-confirmed partial response (RECIST) and 29 pts had disease stabilization as best response.
Conclusions: After thorough review of the risk/benefit outcomes of the Phase I program the clinical studies with ES-285 were discontinued.

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Antiproliferative effects of fluoro-chalcone derivatives in human melanoma A375 cells and peripheral blood mononuclear cells

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Background: At present, no treatment options are available for patients with advanced melanoma providing either sufficient response rates or a significant prolongation of overall survival. Chalcones are included in fruits and vegetables, and are suggested to be cancer-preventive. In this study, we reported the effects of synthetic chalcone derivatives on proliferation of human melanoma cells and peripheral blood mononuclear cells (PBMCs).
Material and Methods: Twelve synthetic derivatives of methoxy-and/or fluoro-chalcones were included in this study. To measure the effect of chalcone derivatives on cells of a human melanoma A375 cell line, we used the 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) assay procedures. Effects of chalcones on the proliferation of PBMCs in response to a T cell mitogen concanavalin A was assessed by [^{3}H] thymidine incorporation. PBMCs were isolated from seven healthy subjects. Cell cycle and apoptosis was detected by TUNEL assay and PI staining of the cells, using flow cytometric analysis.
Results: Four out of the 12 chalcone derivatives: 4-trifluoromethyl-4′-methoxychalcone (CH-1), 4-trifluoromethyl-2′-methoxychalcone (CH-3), 3-trifluoromethyl-2′, 4′-dimethoxychalcone (CH-4) and 3-trifluoromethyl-4′-methoxychalcone (CH-7) exhibited the strongest antiproliferative effects on the melanoma cells with IC_{50} values of 9.6, 5.7, 5.8 and 7.2 μM, respectively. Then, we studied the effects of CH-1, CH-3 and CH-4 on apoptosis and cell cycle of A375 cells. 10 μM CH-3 induced apoptosis in 0.15, 15.3 and 54.05% of A375 cells at 24, 48, and 72 hr of culture, respectively. Percent of G2/M phase cells in control wells was 31.2, whereas CH-3and CH-4 caused accumulation of cells in the G2/M phase to be 70.1% and 90.55%, respectively. On the other hand, CH-1 reduced the G1 phase cells, as

compared to control. 10 μM CH-1 slightly induced apoptosis at 72 hr. All chalcone derivatives inhibit proliferation of PBMCs dose-dependently. The IC_{50} values of these derivatives on PBMCs were 0.7–16.3 μM.
Conclusion: Our results suggest that some methoxy- and/or fluoro-chalcone derivatives have anti-melanoma cell efficacy with less suppression against human immune system. The data also suggest that the molecular mechanisms of the chalcone derivatives on the human melanoma cells involve the induction of apoptosis and blockade of cell cycle. These chalcone derivatives may be useful as lead molecules for developing new anti-melanoma agents.

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Eph receptor A2 modulation in human glioma cell lines by the natural product, Schweinfurthin A

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Eph kinases, the largest group of transmembrane receptor tyrosine kinases, bind to ephrin ligands and initiate bidirectional signaling impacting a wide variety of cellular processes including actin cytoskeletal organization, cell shape, motility, adhesion, growth, survival, and differentiation. The EphA2 receptor has been reported to be overexpressed in multiple cancers, including glioblastoma and astrocytomas, and is an attractive target for the treatment of brain tumors. Schweinfurthin A (SA) is a small molecule natural product isolated from a tree in Cameroon, Africa with a unique growth inhibitory fingerprint in the NCI 60 cell-lines, and potent activity against the CNS subpanel. COMPARE analysis of the pattern of toxicity of this highly active agent was unable to identify any putative mechanism of action. In an effort to understand the underlying molecular mechanism for the CNS specificity, microarray studies in the drug sensitive glioma cell line, SF-295, were used to identify candidate genes linked to the activity of the molecule. A group of SA-regulated genes were identified, including several related to the cytoskeleton, which was in accord with a dynamic change in the actin cytoskeleton observed in SA-treated sensitive cells. In particular, we identified changes in EPHA2 and EFA1 genes which code for the EphA2 receptor tyrosine kinase and its cognate ligand EphrinA1 respectively. In SF295, SA treatment led to down regulation of the receptor concurrent with an increase in the expression of the ligand, and these results were confirmed using PCR, Western Blotting and immunofluorescence. When RNAi was used to knock down EPHA2 receptor expression in the human glioma cell line U251 the consequences on phenotype, morphology and actin organization were similar to those observed following SA treatment. As EphA2 has been identified as a potential chemotherapeutic target, and as an important marker and determinant of aggressive, metastatic gliomas, functional studies are ongoing to confirm an SA-mediated effect on ephrin signaling and phenotypes such as migration and invasion.
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Phase I study of the novel anti-cancer drug PM00104 as a 1-hour weekly infusion resting every fourth week in patients with advanced solid tumors or lymphoma

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Background: PM00104 (ZALYPSIS®) is a novel synthetic alkaloid related to the marine compounds jorumycin and renieramycins. Preliminary analyses point to changes in cell cycle and DNA binding properties, as well as to transcriptional inhibition as main mechanisms of action. ZALYPSIS® has shown anti-tumor activity in vitro ($IC50 \leqslant 10^{-8}$ M) and in xenografts models, and an acceptable toxicological profile.
Methods: Patients (pts) with advanced cancers or lymphoma were enrolled to determine the safety, tolerability, maximum tolerated dose (MTD), recommended dose (RD), pharmacokinetics (PK), relationship between PK and pharmacodynamics (PD) and anti-tumor activity of ZALYPSIS® administered as a 1-hour i.v. infusion weekly and resting every fourth week. Sequential cohorts of 3−6 pts have received the following doses: 75, 150, 300, 600, 900, 1350, 2025, 2500 and 3037 μg/m^2.
Results: Thirty seven pts have been treated (22M; median age: 57, range: 36−73; ECOG PS $\leqslant$ 2). Six dose-limiting toxicities (DLT) have been reported, two at 3037 μg/m^2, three at 2500 μg/m^2 and one at 2025 μg/m^2, respectively. The DLTs were grade 3−4 asthenia, grade 3 nausea and grade 3−4 hematological toxicity (neutropenia, thrombocytopenia and anemia), delay in the administration of the dose due to hematological toxicity, and reversible grade 4 lipase increase. The MTD was reached at 2500 μg/m^2 and the RD at 2025 μg/m^2. At the RD nine more pts have been included in order to evaluate the safety and the anti-tumor activity. Other toxicities were the majority of grade $\leqslant$2 and included: transaminase increases, anorexia, diarrhea, constipation, asthenia and nausea, and vomiting (that augmented at doses >600 μg/m^2). Seven pts have had stable disease (SD) lasting >3 months, two of them with pleural mesothelioma. PD analysis is being performed in tumor samples in pts treated at 2500 μg/m^2. PK of ZALYPSIS® in this study is been characterized by a half life of 30−40 hours at the RD, wide volume of distribution (around 800 L) and a moderate to high inter-patient variability. The dose proportionality is been maintained in terms of Cmax and AUC. The presence of DLT has been found to be more related to total AUC than to Cmax.
Conclusions: this trial has shown an acceptable tolerability profile for ZALYPSIS® with limited anti-tumor efficacy. The usefulness of ZALYPSIS® in combination with other anti-tumor compounds shall be explored.

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The novel taxane derivative, IDN6140, crosses the Blood Brain Barrier and has a promising activity in CNS tumors

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Background: IDN6140 is a new paclitaxel (PTX) analogue derived from 14-β-hydroxy-10-deacetylbaccatin III, that was selected for further preclinical evaluation based on its high cytotoxic activity in human tumor cell lines, being about 40 fold more potent than PTX. Previous pharmacokinetic studies indicate that IDN6140 is characterized by good and rapid absorption, high distribution and long half-life allowing to achieve and maintain for long time plasma concentrations higher than the IC50 values (Marangon et al., Abstract No C140, 2007 AACR-NCI-EORTC Annual Meeting San Francisco).
The aims of this study were to evaluate the brain distribution of IDN 6140 and its antitumor activity against an orthotopically growing human glioma in nude mice.
Methods: The U-87 MG human glioma cell line was xenografted into the brain of CD1-nude mice. IDN 6140 was administered i.v. three times every fourth day at the dose of 5.4 mg/kg, and antitumor efficacy was assessed by examining mouse survival time and by MRI. Pharmacokinetic study was conducted on CD1 mice treated with single i.v. or oral dose of IDN 6140, 5.4 mg/kg. Drug levels in plasma and brain were determined according to HPLC/MS/MS method.
Results: IDN6140 was effective in increasing the survival time of mice orthotopically injected with U-87 MG cells achieving 53% ILS ($P < 0.05$ vs controls). The results were supported by the pharmacokinetic data where, after both oral or i.v. administration, IDN 6140 was rapidly distributed to mouse brain (Tmax $\leqslant$2 hr), achieving Cmax of 0.14 and 4.00 μg/mL, respectively. After both treatments, the compound disappeared from brain with a higher half-life (more than 30 hours) than the half-life determined in plasma (about 20 hours), causing accumulation in brain tissue. The ratios brain-AUC/plasma-AUC were 1.1 and 3.7 after oral and i.v. administration respectively, indicating high distribution of the compound in the organ.
Conclusions: The study provides evidence of good pharmacological properties of IDN 6140, i.e. high and prolonged brain distribution, which was reflected in the ability to affect the growth of intracranial tumors. These data suggest that IDN6140 deserves further investigations as a potential new drug for the therapy of CNS tumors and metastases.

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Evaluation of the marine compound PM02734 against a pediatric tumor cell line panel by ITCC preclinical drug evaluation program

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Background: The Innovative Therapies for Children with Cancer (ITCC) European consortium aims to develop new drugs for the treatment of