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

pediatric malignancies. It is composed of 35 pediatric oncology clinical centres in five European countries for early clinical trials and of 9 laboratories for preclinical evaluation of targeted anti-cancer compounds in pediatric cancer models. The aim of the preclinical ITCC biology program is to prioritize compounds for clinical development on the basis of in vitro/in vivo activity and relevance of biological targets. PM02734 is depsipeptide produced by chemical synthesis, which has in vitro growth-inhibitory properties against several adult tumor types in the low micromolar range, and low nanomolar range for prostate cancer. PM02734 is currently being evaluated in two phase I clinical trials with different dosing schedules. The objective of our study was to assess the in vitro efficacy of PM02734 in pediatric tumour models.
Material and Methods: The in vitro cytotoxicity of PM02734 was screened by the MTS-assay on a panel of 24 pediatric tumor cell lines, composed of 4 cell lines for each of the following tumor types: Ewing sarcoma, acute lymphocytic leukemia, medulloblastoma, neuroblastoma, osteosarcoma, and rhabdomyosarcoma. Cells were exposed for 72 h to PM02734 concentrations ranging from 1.26 pmol/l to 12.6 μmol/l. Experiments were performed thrice and in triplicate. GI50 was considered as parameter of growth inhibition, whereas LC50 represents cytotoxicity.
Results: PM02734 significantly though moderately reduced the growth and cell viability of all cell lines in a dose-dependent manner. The most sensitive lines were within osteosarcoma and rhabdomyosarcoma with some cell lines showing GI50s below 1 μmol/l. The LC50 values ranged from 3.0 to 15.4 μM. The mean±SD LC50 values were 10.2±3.0 μM in Ewing sarcoma, 11.9±1.3 μM in ALL, 10.9±3.7 μM in medulloblastoma, 11.0±0.8 μM in neuroblastoma, 10.9±5.5 μM in osteosarcoma and 9.6±2.0 μM in rhabdomyosarcoma, respectively.
Conclusions: PM02734 is cytostatic and cytotoxic against pediatric tumor cell lines in vitro at micromolar concentrations, with osteosarcoma and rhabdomyosarcomas being the most sensitive cell lines.

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In vitro and in vivo antitumor activity of novel aureolic acid analogues generated by metabolic engineering of the biosynthetic pathways in *Streptomyces argillaceus* and *Streptomyces griseus* subsp. *griseus*

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Background: Aureolic acids, like mithramycin (MTM) and chromomycin (CMM), are bacterial natural glycosylated polyketides that interact in a non-intercalative manner with DNA at GC-rich sites, inhibit binding of the GC-rich DNA binding Sp1 transcription factor, and have potent antitumor activity. MTM and CMM are interesting leads for discovery of new compounds that might be active against tumors in which Sp1 is overexpressed or overactive. Genetic engineering of the aureolic acid metabolic pathway in the producer strains *S. argillaceus* and *S. griseus* subsp. *griseus* can yield derivatives with modified polyketide-derived or deoxysugar side chains that might have improved anti-Sp1 activity and better pharmacological and toxicological properties.
Methods: MTM and CMM derivatives were produced by targeted inactivation of key genes in the producer strains and purified by HPLC. Biological activity of the new compounds was assessed in vitro in a panel of human cancer cell lines and normal cells using cell proliferation and viability assays (e.g., MTT and clonogenic assays). In vivo antitumor activity was assessed in subcutaneously implanted human tumor xenografts in nude mice following i.v. injections of the compounds using different doses and schedules of administration. Toxicity and pharmacokinetics was evaluated in CD1 mice.
Results: In vitro assays identified MTM and CMM analogues with potency comparable or superior to the parent compounds. New analogues (i.e., CMM-SK, CMM-SDK and DMC-A3, MTM SK and MTM-SDK) inhibited cancer cell growth and viability with IC50 $\leqslant$25 nM. Other derivatives (e.g., DDAC-A3, PC-A4, PC-A4C and PC-A3) exhibited antiproliferative activity only at >10-fold higher concentrations (IC50, $\geqslant$250–500 nM). Active analogues were generally less toxic in vitro to normal fibroblasts than cancer cells, suggesting an improved therapeutic index compared to the parent compounds. Selected compounds (i.e., MTM-SK and MTM-SDK) were tested in human tumor xenografts in nude mice and induced delayed tumor growth or tumor regression in different tumor models.
Conclusions: Metabolic engineering of the biosynthetic pathway of aureolic acids is a powerful approach to generate new "unnatural" compounds with diverse structures and improved properties. Using this approach we have identified MTM and CMM analogues with promising activity in a variety of in vitro and in vivo models exhibiting antitumor activity and low toxicity. These new analogues might be very effective agents to treat cancer and other conditions with abnormal activity of Sp1 and GC-rich DNA binding transcription factors.

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New clerodane diterpenes from *Casearia capitellata* as potential antitumour agents

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Background: *Casearia capitellata*, a medicinal herb was investigated for its anticancer phytochemicals owing to the fact that an extract of the plant displayed potent cytotoxicity against *in vitro* tumour cell lines.
Materials and Methods: Silica column chromatography was used to isolate and purify bioactive compounds from the crude extracts. Various spectroscopic techniques (^{1}H/^{13}C NMR, FT-IR, LC-MS, HRMS) were used to elucidate the structures of the isolated compounds. MTT cytotoxicity assay was performed to assess the *in vitro* growth inhibitory properties of extracts and compounds.
Results: Cytotoxicity-guided fractionation of sequentially extracted dichloromethane, ethyl acetate and methanol extracts of *C. capitellata* resulted in the isolation of one pentacyclic terpenoid 7α-acetoxy-hop-12(13)-en-11-one (**1**), three coumarin derivatives, 5-methoxy-7-hydroxycoumarin (**2**), 5-methoxy-7-β-D-glucopyranosylcoumarin (**3**), 5,7-dimethoxycoumarin (**4**), and two new clerodane diterpenes, casearine-A (**5**), and casearine-B (**6**). The isolation of **5** and **6** has never been reported from natural products before, whereas the isolation of **1**, **2**, **3**, and **4** is the first report from this genus. The isolated compounds were tested for cytotoxic effect against breast (MCF-7), lung (NCI-H460) and prostate (DU-145) cancer cell lines. Clerodane diterpenes **5** and **6** exhibited strong antitumour activity against MCF-7 and DU-145 cell lines with IC_{50} values ranging 2.0–4.2 microM. The compounds also exhibited cytotoxic activity against NCI-H460 cells with the IC_{50} values of 27.2 and 16.9 microM, respectively.
Conclusions: Compounds **5** and **6** were more selective towards breast and prostate cancer cells as compared with lung cancer cells. Therefore, these compounds are potential lead molecules for future antitumour studies to discover prospective clinical candidates for the treatment of breast and prostate cancers.

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New antitumour agents from *Phyllanthus pulcher*, a tropical medicinal plant

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Background: In a previous study to identify local herbs with *in vitro* antitumour properties, *Phyllanthus pulcher* was found to display a remarkable cytotoxic activity against various tumour cell lines. In this study the bioactive compounds were isolated and purified from the dried aerial parts and roots of the plant.
Materials and Methods: The plant parts were sequentially extracted with dichloromethane (DCM) and methanol (MeOH). Silica column chromatography was used to isolate and purify the bioactive compounds. Various spectroscopic techniques (1H/13C NMR, FT-IR, LC-MS, HRMS) were used to elucidate the structures of the compounds. The extracts and compounds were tested for cytotoxic effect against three human tumour cell lines representing tumours of the breast (MCF-7), lung (NCI-H460) and prostate (DU-145) using MTT assay.
Results: The DCM extract of the aerial parts exhibited potent cytotoxic activity as compared with the MeOH extract. Stigmast-5-en-3-ol-oleate (**1**), diisobutyl adipate (**2**), β-sitosterol (**3**), 7-tridecanone (**4**), sitosterol-3-O-β-D-glucopyranoside (**5**), a new coumarin derivative, 3,4-dihydroxy-5-methoxy-3′,4′,5′-trihydroxyoxepino-chromene-2-one (**6**) and a new diterpene lactone, phyllanthal-A (**7**) were isolated from the DCM fraction. Investigation on the active DCM extract of *P. pulcher* roots resulted in the isolation of two new pentacyclic triterpenes, 12(13)-dehydro-3α-acetoxyolean-28-oic acid (**8**) and lupanol acetate (**9**) and three