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.

**182** POSTER

**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***

A. Malek[1], L.E. Núñez[2], N. Menéndez[2], F. Morís[2], G.M. Carbone[1], J. Rohr[3], C. Méndez[4], J.A. Salas[4], C.V. Catapano[1]. [1]*Oncology Institute of Southern Switzerland, Experimental Oncology, Bellinzona, Switzerland;* [2]*EntreChem SL, Oncology, Oviedo, Spain;* [3]*University of Kentucky, Pharmaceutical Sciences, Lexington, USA;* [4]*Universidad de Oviedo, Biologia Funcional and IUOPA, Oviedo, Spain*

**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.

**183** POSTER

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

J. Stanslas[1], G. Bagalkotkar[1], S.C. Tang[2], A.S. Hamzah[3], K. Shaari[4], N.H. Lajis[4], M.S. Saad[5]. [1]*Universiti Putra Malaysia, Faculty of Medicine and Health Sciences/Institute of Bioscience, Serdang, Malaysia;* [2]*Universiti Putra Malaysia, Faculty of Medicine and Health Sciences, Serdang, Malaysia;* [3]*Universiti Technology MARA, Faculty of Applied Science, Shah Alam, Malaysia;* [4]*Universiti Putra Malaysia, Institute of Bioscience, Serdang, Malaysia;* [5]*Universiti Putra Malaysia, Faculty of Agriculture, Serdang, Malaysia*

**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.

**184** POSTER

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

J. Stanslas[1], G. Bagalkotkar[2], S.C. Tang[2], A.S. Hamzah[3], K. Shaari[4], N.H. Lajis[4], M.S. Saad[5]. [1]*Universiti Putra Malaysia, Faculty of Medicine and Health Sciences/Institute of Bioscience, Serdang, Malaysia;* [2]*Universiti Putra Malaysia, Faculty of Medicine and Health Sciences, Serdang, Malaysia;* [3]*Universiti Technology MARA, Faculty of Applied Science, Shah Alam, Malaysia;* [4]*Universiti Putra Malaysia, Institute of Bioscience, Serdang, Malaysia;* [5]*Universiti Putra Malaysia, Faculty of Agriculture, Serdang, Malaysia*

**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

other known compounds including 3α-acetoxy-25-hydroxyolean-12-en-28-oic acid (**10**), glochidone (**11**) and glochidonol (**12**). Among the cytotoxic compounds, **1**, **5**, **10** and **12** were selective toward MCF-7 cells ($IC_{50}$ 17.1–69.2 microM), whereas compound **7** was more active against DU-145 cells ($IC_{50}$ 20.5 microM).
**Conclusions:** Ability of some of the compounds in exhibiting selective growth inhibition of breast and prostate cancer cells suggests these agents maybe beneficial in the treatment of human breast and prostate cancers.

**185** POSTER

**Chlamydocin, a HDAC inhibitor identified by Compare analyses in a cellular screen**

H.H. Fiebig[1], T. Metz[1], T. Beckers[1], A. Korrat[1], G. Kelter[1]. [1]*Oncotest GmbH, Institute for experimental Oncology, Freiburg, Germany*

**Background:** Inhibitors of histone deacetylases (HDAC) were shown to be potent anti-proliferative and pro-apoptotic agents. At Oncotest we have developed a cellular profiling screen in which new compounds are being tested in a standard cell line panel consisting of 36 cell lines from all major solid tumor types with a subsequently Compare Analyses. In the search for novel HDAC inhibitors we have screened a collection of 2000 pure compounds derived from natural products.
**Methods:** 4,000 to 10,000 adherent cells were seeded in 96 well plates, compounds were added at 5 different concentrations one day later and left over for 4 days. The read-out of the assay is propidium iodide-based fluorescence, which is a measure of viable cell number. Based on $IC_{50}$ and $IC_{70}$ values tumor selectivity of test compounds were analysed. In the Compare Analysis the $IC_{50}$ and $IC_{70}$ pattern of the new compounds are compared with the corresponding patterns of about 100 agents with known mechanism of action using Spearman Correlations.
**Results:** The known HDAC inhibitors show distinct $IC_{50}$ and $IC_{70}$ activity profile in the Oncotest 36 cell line panel. Concentration-dependent antitumor activity was detected for the 5 structurally diverse HDAC inhibitors Depsipeptide (mean $IC_{70}$ = 0.009 μM), M344 (1.7 μM), SAHA (3.9 μM), acetyldinaline (22 μM) and SBHA (61 μM). The benzamide analog acetyldinaline and M344, as well as the three hydroxamic acids M344, SAHA and SBHA showed similar activity patterns. We used this cellular activity pattern to screen pure natural compounds isolated from bacteria and fungi. Amoung 2000 compounds tested, Chlamydocin showed the closest match with HDAC inhibitors. Chlamydocin was originally isolated from the fungus Diheterospora chlamydosporia. Chemically it belongs to a family of hydrophobic cyclic tetrapeptides. Potent anticancer activity was reported in-vitro. Compare analysis revealed significant correlations of Chlamydocin to M344, SAHA, SBHA and acetyldinaline, the spearman rho ranked between 0.75 and 0.61. Chlamydocin was potent with a mean $IC_{70}$ of 0.018 μg/ml. It showed selective activity in 3/4 prostate, in 3/5 NSCLC, 2/3 ovarian cancer cell lines as well as in 2/5 melanomas.
**Conclusion:** In conclusion, the evaluation of 5 structurally diverse HDAC inhibitors revealed closely related activity profiles in a panel of 36 cell lines. In the Oncotest cell line screen Chlamydocin was found to be highly potent and selective, and that it act as an HDAC inhibitor a property which was published by Scheper et al (JPET 304:881, 2003). This finding demonstrates that our cellular screen with the subsequent Compare Analysis is able to identify inhibitors against targets of high interest for cancer therapy.

**186** POSTER

**Screening for the inhibitor against filopodia protrusion**

M. Kitagawa[1], E. Tashiro[1], T. Soga[2], M. Imoto[1]. [1]*Keio University, Department of Biosciences and Informatics, Yokohama, Japan;* [2]*Keio University, Institute for Advanced Biosciences, Tsuruoka, Japan*

**Background:** Filopodia, a rod-like cell membrane protrusion, is a morphological marker of metastatic tumor cells. On the other hand, the use of small molecular inhibitors has potential benefit to dissect the underlying cellular processes in cancer. In this study, we attempted to obtain the inhibitor against filopodia protrusion as analytical tool of metastatic tumor cells.
**Material and Methods:** Filopodia protrusion: filopodia was induced by Epidermal Growth Factor (EGF) stimulus in human epidermis carcinoma A431 cells. After 30 minutes, filopodia protrusion was observed under microscopy. Screening source or compounds were treated 30 minutes prior to the EGF stimulus. Intracellular ATP: cellular ATP levels were quantitated by ATP assay kit (Sigma). Metabolome analysis: metabolites in cells were collected by methanol extraction. Amounts of each metabolite were quantitatively analyzed by CE-MS system. Glucose uptake: cells were treated with 2-[$^3$H]deoxyglucose, washed and lysed. Radioactivity was counted by Tri-Carb (Perkin-Elmer). And following compounds are additionally used: rotenone, antimycins, and oligomycins.
**Results:** In the course of screening, we found that glucopiericidin A (GPA) strongly inhibited filopodia in combination with the inhibitors of mitochondrial respiratory chain complexes (MRCIs). Under this condition, we also found that cellular ATP levels were dramatically decreased. Since the process of actin polymerization in filopodia depends on the ATP-energy, it is likely that the decrease of cellular ATP levels caused the inhibition of filopodia protrusion in cells co-treated with GPA and MRCIs. On the other hand, it is well known that inhibition of both glycolysis and mitochondrial oxidative phosphorylation processes results in marked decrease in ATP levels. Thus, we hypothesized that GPA would be a glycolysis inhibitor. To examine this possibility, we conducted metabolome analysis and found that cellular levels of lactate and pyruvate were decreased by the treatment with GPA. Moreover, we found that GPA inhibited cellular incorporation of glucose, indicating that GPA inhibits glycolysis. Meanwhile, malignant tumor cells located within solid tumors possess higher glycolytic capacity because tumors in this region are distant from blood vessels and lack of oxygen, and thereby, mitochondria respiration is limited. This forces them to activate glycolysis to survive. Therefore, we examined whether GPA affects tumor cell viability when mitochondria is suppressed by MRCI. As a result, GPA synergistically induced cell death in A431 cells with MRCI. Therefore, it is likely that GPA, an inhibitor of glycolysis would be effective against the viability in tumor cells.
**Conclusions:** We identified GPA as a glycolysis inhibitor and suggested that GPA would be a potential candidate for cancer chemotherapy.

**187** POSTER

**Fusicoccin derivative (ISIR-005) suppresses anchorage-independent growth of cancer cells through anoikis activation**

Y. Honma[1], M. Akimoto[1], T. Sassa[2], N. Kato[2]. [1]*Shimane University School of Medicine, Life Science, Izumo, Japan;* [2]*Osaka University, Institute of Scientific and Industrial Research, Osaka, Japan*

Cotylenin A, which has a diterpenoid tricarbocyclic skeleton, was isolated as a plant growth regulator, and has been shown to affect several physiological processes of higher plants and to have differentiation-inducing activity in several myeloid leukemia cell lines. Cotylenin A also affected the differentiation of leukemic cells that were freshly isolated from acute myeloid leukemia patients in primary culture. Injection of the human promyelocytic leukemia cell line NB4 into mice with severe combined immunodeficiency resulted in the death of all mice due to leukemia. Administration of cotylenin A significantly prolonged the survival of mice inoculated with retinoid-sensitive and -resistant NB4 cells without no appreciable adverse effects. Combined treatment with interferon-alpha and cotylenin A significantly inhibited the growth of human lung cancer cells as xenografts without apparent adverse effects. These results suggest that cotylenin A is useful in therapy for leukemia and some other malignancies. However, cotylenin A is difficult to apply to clinical study, since the supply is very limited and it has an epoxide-ring. For clinical application, in the present study, we aimed to synthesize various derivatives from fusicoccins, which are closely related to cotylenin A and are able to be supplied in a large amount as metabolites of phytopathogenic fungus (and examined their differentiation-inducing effects). Although natural fusicoccins did not induce differentiation of myelomonocytic leukemia cells, we synthesized several fusicoccin derivatives with differentiation-inducing activity and without epoxide-ring, based on the structure–activity relationship of cotylenin derivatives. We found some effective derivatives and ISIR-005 was the most potent at inducing differentiation of leukemia cells. Although a low concentration of ISIR-005 hardly affected cell proliferation of lung carcinoma A549 cells, it effectively inhibits anchorage-independent growth and migration of the cells. The drug restored the sensitivity of cancer cells to anoikis. Enhanced anoikis appears to be mediated in part by modulated function of Bcl-2 family proteins.

**188** POSTER

**Leucinostatins suppress prostate cancer cell growth through the tumour-stromal cell interactions**

M. Kawada[1], H. Inoue[1], I. Momose[1], T. Masuda[1], D. IKeda[1]. [1]*Microbial Chemistry Research Center, Numazu Bio-Medical Research Institute, Shizuoka, Japan*

The tumor-stromal cell interactions play an important role in the growth and metastasis of tumors through diffusible factors and cell-cell adhesion. Modulation of the tumor-stromal cell interactions could result in the suppression of tumor growth and metastasis. We have therefore been studying the tumor-stromal cell interactions of prostate cancer and searching for the modulators of the interactions. We designed a coculture system of prostate cancer cells and prostate stromal cells (PrSC) and we recently found that IGF-I secreted from PrSC regulates the growth of prostate cancer. The small molecules that inhibit the growth of prostate cancer cells in coculture with PrSC will become new type anticancer