The **aim** of this study was to investigate the anti-tumour effect of SAHA (suberoylanilide hydroxamic acid), a histone deacetylase inhibitor, in cell culture models.
Material and Methods: Human papillomaviruses (HPV) immortalized cell lines (CaSki and Hela) and two HPV positive cell lines obtained from cervical xenografts were treated with increasing doses of SAHA (0.25−5 μM). MSPCR and immunotechniques were approached for monitoring certain tumour suppressor genes epigenotype linked with their real time PCR expression analyses.
Results: Flow-citometry analyses revealed that SAHA has an anti-tumour activity by blocking cell proliferation and inducing tumour cell apoptosis in immortalized cell lines. At 2.5 μM/24h SAHA treatment mRNA levels of DNMT1 were slightly increased, as appreciated in real-time PCR (Taqman). In cell lines derived from xenografts DNMT1 activity increases at the same concentration of SAHA after 48 of treatment. In Hela and CaSki lines DNMT3b immunoreactivity presented a rather constant feature, while the only affected enzyme was DNMT3a whose immunoreactivity decreased significantly at high SAHA concentrations (3 μM/48h). The silent versus active state of the considered genes were also estimated by antibody targeting the modified (methylated) histone H3.
Conclusion: HDAC inhibitors may revert the silent heterochromatin to an active chromatin conformation and restore the normal function of silenced genes in cervical cancer. The obtained data suggests any changes in the modifications to either DNA or histone may influence the other.

279 AMPK activators act together with paclitaxel to block tumour growth

G. Zweig Rocha[1], M.M. Dias[1], E.R. Ropelle[1], F.O. Costa[1], J.B.C. Carvalheira[1]. [1]*University of Campinas, Internal Medicine, Campinas SP, Brazil*

AMP-activated protein kinase (AMPK) and mammalian Target of Rapamycin (mTOR) are key regulators of cellular growth and the aberrant activation of mTOR signaling promotes cell growth, and this underlies the pathophysiology of numerous cancers. Thus, drugs that selectively target AMPK pathway offer great promise for cancer treatment, particularly in combination with chemotherapy. Human tumours were xenografted in SCID mice and treated with low doses of paclitaxel alone, AMPK activators (2-deoxyglucose (2-DG) and metformin) alone, or combination of both drugs. The cellular effects of paclitaxel and AMPK activators were further characterized for breast adenocarcinoma (MCF-7) and lung carcinoma (A549). We observed that treatment with AMPK activators and paclitaxel resulted in an increase in the number of cells arrested in G2/M phase of the cell cycle and decreased tumour growth in mice when compared to individual drugs treatments and control. AMPK activators and paclitaxel alone are able to produce molecular activation of AMPK and inhibition of mTOR signaling in a time and dose dependent manner in MCF-7 and A549 cells. Combined treatment with 2-DG and paclitaxel as well as metformin and paclitaxel lead to quantitative potentialization of molecular signaling through the AMPK pathway by inhibiting mTOR signaling. These findings suggest that AMPK activators interact with paclitaxel in a synergistic manner in lung and breast cancer cells by inhibiting mTOR signaling. Therefore, AMPK activators are a promising therapeutic agent in combination with paclitaxel in lung and breast cancer.
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280 HIV protease inhibitor ritonavir increases heat sensitivity of renal cancer cells by inhibiting heat-induced NF-kappaB activation

A. Sato[1], T. Asano[1], J. Asakuma[1], A. Horiguchi[1], K. Ito[1], M. Sumitomo[1], T. Asano[1]. [1]*National Defense Medical College, Urology, Tokorozawa, Japan*

Background: Thermotherapy is one of the treatment modalities against renal cancer, but its efficacy is limited and the ways it affects renal cancer cell survival are not fully understood. In the present study we investigated the molecular mechanism of thermotherapy in renal cancer cells and tried to increase their heat sensitivity by using the HIV protease inhibitor ritonavir, which has recently been shown to inhibit NF-kappaB activity.
Material and Methods: After the heat sensitivity of renal cancer cells (769-P, 786-O, A498, ACHN, Caki-1) had been evaluated by incubating them at 42ºC for 0−60 minutes and assessing cell viability by MTS assay, cells were treated at 42ºC for 0−15 minutes in medium containing 0−50 μM ritonavir before their viability was assessed. Changes in the expression of phosphorylated retinoblastoma protein (Rb); cyclin D1; cyclin-dependent kinase 4 (CDK4); heat shock proteins (HSPs) 27, 70, and 90; NF-kappaB (p65); and phosphorylated p65 were examined by western blot analysis.
Results: In each cell line, treatment at 42ºC inhibited cell proliferation in a time-dependent fashion, especially after more than 15 min, and induced Rb dephosphorylation by suppressing the expression of cyclin D1 and CDK4. The treatment at 42ºC for 15 minutes in the presence of 50 μM ritonavir inhibited cell proliferation synergistically in all the cell lines tested. In Caki-1 cells the treatment at 42ºC for 15 minutes decreased the expression of HSP70, which acts as a suppressor of NF-kappaB, and thus activated NF-kappaB as shown by the increased expression of phosphorylated p65. Interestingly, administration of 50 μM ritonavir in combination with thermotherapy inhibited this increase in phosphorylated p65.
Conclusions: Thermotherapy inhibited renal cancer cell survival by suppressing the expression of cyclin D1 and CDK4. We have for the first time shown that ritonavir increases the heat sensitivity of renal cancer cells, and inhibition of heat-induced NF-kappaB activation is one mechanism of this action. Ritonavir may be used as a heat sensitizer when treating renal cancer by thermotherapy.

281 Cancer: a less depressing outlook? Using antidepressants to induce autophagic programmed cell death in a resistant strain of Burkitt's lymphoma

S. Cloonan[1], D.C. Williams[1]. [1]*Trinity College Dublin, School of Biochemsitry and Immunology, Dublin, Ireland*

Background: Burkitt's lymphoma (BL) accounts for 30−50% of lymphomas in children and 35−50% of HIV-associated non-Hodgkin lymphomas. Survival rates in response to standard chemotherapy are 60% in children but only 25% for older adults and HIV infected patients with reoccurrence and resistance common. Such resistance to chemotherapy is a major obstacle for the success of cancer therapy and is most commonly attributed to the inability of cancer cells to die by apoptosis, the archetypal programmed cell death (PCD) response. The development of anticancer drugs that can overcome this resistance to apoptosis and induce other forms of cell death, such as Type-II autophagic PCD is paramount for efficient cancer therapy and is becoming an increasingly popular alternative therapeutic approach.
Materials and Methods: Apoptotic morphologies in BL cells treated with antidepressants were investigated using Propidium Iodide FACS analysis for apoptotic body detection, agarose gel electrophoresis for the detection of DNA fragmentation, Western Blot analysis for detection of PARP cleavage and the use of the general caspase-inhibitor zVAD-fmk. Type-II autophagic cell death was confirmed by transmission electron microscopy, Western Blot analysis for the detection of the autophagic-specific protein, Beclin-I and the use of the autophagic inhibitors 3-methyladenine and Bafilomycin A1. Mechanisms of cell death were further investigated using confocal microscopy, Western Blot analysis for the detection of Bax and Bak and measuring cytoplasmic calcium levels using FURA-2.
Results: We report that the antidepressants maprotiline and fluoxetine induce autophagic PCD in the chemoresistant Burkitt's lymphoma cell line DG-75, that does not involve caspases, DNA fragmentation or PARP cleavage, but is associated with the development of cytoplasmic vacuoles, all consistent with an autophagic mode of PCD. Autophagic PCD was confirmed by transmission electron microscopy, up-regulation of Beclin-I and the extent of PCD being reduced by the autophagic inhibitor 3-MA. In contrast these compounds, induced apoptotic PCD in the biopsy-like chemo-sensitive BL MUTU-I cell line. We provide evidence that the chemoresistant DG-75 cells do not express the pro-apoptotic Bcl-2 proteins Bax and Bak, show diminished levels of stored intracellular calcium and display shortened rod-like mitochondria, all of which are known to be associated with a defective 'apoptotic' response in cancer cells. PCD in the two cell lines has different Ca^{2+} responses to maprotiline and fluoxetine which may also account for their differential PCD responses.
Conclusions: This study therefore supports a new mechanistic role for maprotiline and fluoxetine as novel pro-autophagic agents in the treatment of resistant Burkitt's lymphoma, and thus an alternative therapeutic application for these compounds.

282 3D-morphometry of squamous epithelium at different stages of malignization

S.S. Sukharev[1], I.V. Reshetov[1], V.I. Chissov[2], E.N. Slavnova[3], N.N. Volchenko[3]. [1]*P.A. Hertzen Cancer Research Institute, Microsurgery, Moscow, Russian Federation,* [2]*P.A. Hertzen Cancer Research Institute, Management, Moscow, Russian Federation,* [3]*P.A. Hertzen Cancer Research Institute, Oncocytology, Moscow, Russian Federation*

Background: It is quite common for cytological studies to take into account linear measurements and their ratios for cells in investigation. For example nuclear-cytoplasmic ratio, cell size, size of nucleus and so on. Sometimes 3D-morpfometry is useful as well. 3D-model of tissue based on histological specimens processed by image processing software could be created to improve the differentiation between benign and malignant epithelium specimens. Confocal microscopy is a common tool for 3D-imaging of intracellular structures. In contrast to other works, where the 3D-characteristics of cellular objects were created by computers as a result of 2D-images processing, in present work Atomic Force Microscopy (AFM) has been used for direct measurements of squamous epithelium at different stages of malignization: superficial cels of the cervical squamous epithelium, HPV infected cells (koilocytes), dysplasia, keratinizing squamous cell carcinoma, non-keratinizing squamous cell carcinoma.

Material and Methods: We have studied routine cervical smears stained by Leishman's stain. For each smear a definitive diagnosis was made by light microscopy. Then all specimens with known diagnoses were investigated by AFM (NTEGRA Prima, NT-MDT Co., Russia). We measured heights of cytoplasm and nucleus, nuclear-cytoplasmic ratio, the parameters of koilocytes perinuclear cavity in case of HPV infection.
Results: See the table.

	Nucl. height nm	Cytoplasm height nm	nuclear–cytoplasmic ratio (vertical)
Superficial cells of squamous epithelium	1290±488	511±169	2.56±0.53
Dysplasia	1153±433	381±157	3.33±1.45
Non-keratinizing squamous cell carcinoma	758±164	225±65	3.48±0.82
Keratinizing squamous cell carcinoma	1607±549	986±291	1.61±0.22

Koilocytes perinuclear cavity: depth – 212±69 nm; width – 3.78±1.86.

Conclusions: There are tendencies in 3D-parameters of cells according to malignization's progression: decrease in heights of nuclei and cytoplasm and increase in nuclear-cytoplasmic ratio. The only exclusion is keratinizing squamous cell carcinoma.
Some of differences in 3D-parameters of squamous epithelium of different nozologies are statisticaly significant ($P < 0.05$) and could have differential diagnostics meaning: nuclear-cytoplasmic ratio for superficial cells of squamous epithelium and squamous cell carcinomas (Keratinizing and Non-keratinizing).

283 The trifunctional antibody catumaxomab: mode of action

D. Seimetz[1], R. Linke[2], J. Atz[3], M. Essing[4], A. Klein[4]. [1]*Fresenius Biotech GmbH, CSO, München, Germany*, [2]*Fresenius Biotech GmbH, Clinical Development, München, Germany*, [3]*Fresenius Biotech GmbH, Preclinical Research and Development, München, Germany*, [4]*Fresenius Biotech GmbH, Medical Affairs, München, Germany*

Background: Catumaxomab (Removab®) is a trifunctional bispecific monoclonal antibody which is presently the only approved therapeutic antibody targeting EpCAM, a surface antigen that is widely expressed in the most frequent forms of human cancer. With its trifunctional mode of action, catumaxomab represents a new generation of antibodies activating the body's own immune system for tumour cell elimination.
Material and Methods: For preclinical testing a targeted program was designed taking into account that catumaxomab binds to human antigens. Non-clinical properties were assessed in vitro using human cells and using in-vivo models including a surrogate antibody. Several clinical findings support the proposed mode of action.
Results: Catumaxomab differs from other antibodies by the ability to bind three different cell types: One specific antigen-binding site binds T cells via CD3, the other site tumour cells via the EpCAM antigen. The Fc-region provides a third functional binding site that is able to bind and activate Fcγ receptor-positive accessory cells. Preclinical studies identified different mechanisms of action including T cell-mediated lysis, phagocytosis and ADCC mediated by accessory cells. These complex immune reactions lead to an effective activation of immune cells against the tumour.
The mode of action is underlined by several clinical studies. Patients had cytokine release related symptoms like fever, nausea or vomiting as a main side effect which also demonstrate an activation of the immune system. Moreover, a positive trend between cytokine-release-related symptoms and clinical outcome was observed.
Due to the fact that catumaxomab is a murine antibody the majority of patients develop anti-drug antibodies after the end of treatment. Anti-drug antibody-positive patients seemed to have an improved clinical outcome possibly due to their better immunological response which on the other hand is a precondition for the functioning of the mode of action of catumaxomab.
Conclusions: Catumaxomab simultaneously recruits and activates different types of immune cells resulting in an efficient destruction of tumour cells. Positive treatment effects led to an approval in the European Union for catumaxomab in April 2009 for the intraperitoneal treatment of malignant ascites in patients with EpCAM-positive cancer.

284 Withdrawn

285 Gene array analysis of anticancer agents in breast cancer cell lines

M. Alokail[1]. [1]*King Saud University, Biochemistry, Riyad, Saudi Arabia*

Taxotere belongs to a group of anti-cancer drugs known as taxanes. It is a recently developed taxoid representing a novel class of compounds with a unique mechanism of action on the cytoskeleton. Ukrain is a derivative of alkaloids from the greater celandine plant. It is a semi-synthetic compound made up of three molecules of the deravitve alkaloids and one molecule of Tri-ethylene thio-phosphoric acid trimide (thio TEPA). We investigated here the molecular regulation of taxotere and ukrain treatments in MCF-7, MDA-MB231 breast cancer cell lines compared with normal breast cell line, MCF-12A. Cell viability have shown in MCF-7 high sensitivity for both agents, whereas, MDA-MB231 was resistant to both drugs. Douwn regulation of cell cycle genes have shown in MDA-MB231 compared with MCF-7 in both treatments. In case of MCF-7 treated with taxotere, cyclins (B1 and D1), CDKs (CDK4 and CDK5) were suppressed. Regarding the anti-apoptotic proteins; Bag1 level was low in MCF-7, but it was high in MDA-MB231 and in MCF-12A. It was also noticed that taxotere treatment had no affect in Bcl2 level in MCF-7, while it was significantly repressed in MDA-MB231. However, the expression level of Bag1 and Bcl2 was significantly repressed in ukrain treated MDA-MB231. Gene array analysis of cell proliferation and cell cycle genes have show down regulation of some genes like cyclins A1, B, D1 and D3, and some transcription factors like E2F5, TFDp-1, TPp73 and inhibitor of DNA binding 1 in both MCF7 and MDA-MB231, but unaffected in MCF-12A. In conclusion, taxotere and ukrain as breast cancer chemotherapy have shown to be useful predictive for the gene markers associated with breast cancer and the induction of apoptosis.

286 In vitro susceptibility of triple negative breast cancer cells to docetaxel, epirubicin and carboplatin

M. Laranjo[1], M.J. Carvalho[2], A.M. Abrantes[1], A. Brito[1], I. Torgal[3], C.F. Oliveira[4], M.F. Botelho[1]. [1]*Biophysics and biomathematics Institute IBILI CIMAGO, Faculty of Medicine of Coimbra University, Coimbra, Portugal*, [2]*Gynaecology Service University Hospitals of Coimbra Biophysics and biomathematics Institute IBILI CIMAGO, Faculty of Medicine of Coimbra University, Coimbra, Portugal*, [3]*Gynaecology Service University Hospitals of Coimbra, Faculty of Medicine of Coimbra University, Coimbra, Portugal*, [4]*CIMAGO, Faculty of Medicine of Coimbra University, Coimbra, Portugal*

Background: Triple negative (TN) tumours represent 10 to 17% of all breast cancers. These tumours are more prevalent in young women (under 50), are diagnosed in the interval between screenings (interval tumours) and have a more aggressive clinical behavior. This group of tumours are challenging considering systemic therapy, as they have a poor prognosis despite responding to conventional chemotherapy. The interest in the study of TN was first based on the lack of targeting therapies and overlap with the profile of basal-like tumours. Recent studies considered a particular susceptibility of TN cells to platinum derivatives.
Material and Methods: Human breast cancer cell lines HCC1806 (non-expressing estrogens, progesterone and HER2) were purchased to ATCC and cultured according to recommended procedures. Cells were incubated in absence and presence of the docetaxel, epirubicin and carboplatin in several concentrations ranging from 50nM to 150μM. The sensitivity of the cell line to the drugs studied was analyzed using the MTT colorimetric assay, performed 24, 48 and 72 hours after incubation. Cytotoxicity was expressed as the percentage of inhibition of cell proliferation correlated with untreated cultures. Dose-response curves were established and the half maximal inhibitory concentration (IC50) was calculated in Origin7 software.
Results: The cytotoxic capacity of epirrubicin revealed a IC50 of 2.3 μM and 1.15 μM respectively at 24 and 72 hours. Considering the results for carboplatin, it was found higher IC50 than epirrubicin. The IC 50 values for the former at 24 hour and 72 hours were 224.4 μM and 8.6 μM respectively. The IC50 for docetaxel was lower than the other drugs evaluated, 0.03 μM at 24 hours.
Conclusions: TN cells seem to harbor more susceptibility to epirrubicin than to carboplatin, according with a higher IC 50 testing the last drug. The lowest IC50 was reported with taxotere, what emphasizes its importance in association adjuvant therapy.

287 In vitro study of the antitumour effect of Artemisia annua tea

Z. Juranic[1], I. Matic[1], P. Lutgen[2], M. Juranic[3]. [1]*Institute of Oncology & Radiology of Serbia, Experimental Oncology, Belgrade, Serbia*, [2]*IFBV, Hostert, Luxembourg*, [3]*Galenika-Phytopharmacy, Belgrade, Serbia*

Background: One of the main goals in modern cancer research is to find chemotherapeutic agents that selectively suppress survival of malignant cells, with minimal cytotoxic effect against healthy cells. Bioactive phytochemicals of Chinese medicinal plant species *Artemisia annua* have been demonstrated to perform antimalarial, bactericidal and anticancer effect. The aim of this *in vitro* cytotoxic study was to elucidate whether the *Artemisia annua* tea possess anticancer potential.
Material and Methods: *Artemisia annua* tea for the each experiment was prepared by adding 100 ml of boiling distilled water to 5 g of dry herb leaves. The mixture was covered, stayed for 10 min and the leaves were removed by filtration. After cooling at room temperature, the tea was filtered through Millipore filter, 0.22 μm, before use. Cytotoxicity of *Artemisia annua* tea was evaluated against malignant cell lines: human cervix adenocarcinoma HeLa, human malignant melanoma Fem-x and BG, human myelogenous leukemia K562, human breast adenocarcinoma MDA-MB-361, human colon carcinoma LS174, normal human immunocompetent peripheral blood mononuclear cells