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

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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

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**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

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**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

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**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.