dependent increase in acridine orange staining indicating the increase in autophagic cell death. The confirmation of autophagy (Type II programmed cell death) in U87 cells following 5 mM 2-DFG treatment was demonstrated using transmission electron microscopy (TEM) showing the presence of multilamellar structures, otherwise called autophagosomes. Our studies show that 2-DFG is an equally potent inhibitor of cell proliferation and a potent inducer of autophagic cell death in gliomas as are 2-DG, 2-FG, and 2-FM. Therefore, targeting the energetic metabolism of cancer cells and the autophagic survival response using inhibitors of glycolysis is a promising therapeutic approach to the treatment of cancers that are dependent on glycolysis for survival.

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Preliminary evidences for recruitment of innate responses to rectal cancer cell death elicited by neo-adjuvant radio-chemo therapy

A. Tamburini[1], A. Castiglioni[2], K. Bencardino[3], E. Orsenigo[1], V. Russo[4], L. Albarello[5], M. Ronzoni[3], P. Rovere Querini[2], A. Manfredi[2], C. Staudacher[1]. *[1]Vita-Salute San Raffaele University, Gastrointestinal Surgery, Milan, Italy; [2]Vita-Salute San Raffaele University, Clinical Immunology Laboratory Cancer Immunotherapy and Gene Therapy Programme (CIGTP), Milan, Italy; [3]San Raffaele Scientific Institute, Medical Oncology, Milan, Italy; [4]Vita-Salute San Raffaele University, Tumor Gene Therapy Laboratory Cancer Immunotherapy and Gene Therapy Programme (CIGTP), Milan, Italy; [5]Vita-Salute San Raffaele University, Pathology, Milan, Italy*

Colorectal cancer is the fourth cancer in the world with 1.023.000 new cases and 529.000 death per year, 27% are rectal and 73% colon cancer. 30% of rectal cancer patients with a T3N+M0 tumor stage (locally extended tumor with a lymph node involvement, but without metastasis) responds to the neo-adjuvant therapy, which causes necrosis and inflammation in situ. We cannot predict which patients will response. The aim of this study is to verify: (i) whether the pattern of innate response to synchronized death of the tumor cells elicited by the neo-adjuvant radio-chemotherapy is heterogeneous among patients and whether (ii) this information can be used to identify which patients will benefit from the treatment. We focused our attention on macrophages, which represent specialized sensors of injury in the midst of living tissues; in particular we assessed the expression of Heme Oxygenase (HO-1), CD68, CD163, CD206, Tie2, RAGE. Moreover, we assessed inflammatory molecules and soluble pattern recognition receptors. We are also verifying polymorphism of Tlr4 gene. We are analyzing at diagnosis, before the second cycle of chemo-radio therapy and after surgical resection the monocyte phenotype in the peripheral blood by flow cytometry, the infiltrating tumor macrophages by immunoistochemistry and immunofluorescence and the levels of inflammation molecules by ELISA assays.
The results so far obtained confirm a substantial involvement of macrophages and of innate molecules in coping with the neoplasm and with the effects of the therapy.

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Influence of 2-methoxyestradiol on cell numbers, metabolic activity, morphology, cell cycle progression and gene expression in a breast adenocarcinoma cell line

A.M. Joubert[1], B.A. Stander[1], S. Marais[1], J. Janse van Vuuren[1], F. Joubert[2]. *[1]University of Pretoria, Department of Physiology, Pretoria, South Africa; [2]University of Pretoria, BIoinformatics and Computational Biology Unit, Pretoria, South Africa*

It has been reported that the natural metabolite of estradiol (E2), namely 2-methoxyestradiol (2ME) exerts both antitumorigenic and antiangiogenic effects *in vitro* and *in vivo*. However, several questions regarding the action mechanism of 2ME remain to be answered. The aim of this study was to determine the influence of 2ME on cell numbers, metabolic activity, morphology, cell cycle progression and gene expression in a breast adenocarcinoma (MCF-7) cell line.
Influence on cell growth and metabolic activity was investigated spectrophotometrically. Effects of 2ME on morphology were determined by means of light- and fluorescent microscopy and transmission electron microscopy (TEM). Flow cytometry was conducted to assess cell cycle progression and the Annexin V kit to determine possible occurrence of apoptosis. Microarray slides containing 20,173 known human 60-mer oligonucleotide probes were subsequently employed to study the influence of 2ME on gene expression profiles in MCF-7 cells.
2ME (10^{-6} M) caused 50% decrease in cell number and metabolic activity after 24 hours of exposure. Light- and fluorescent microscopy revealed hallmarks of apoptosis including abnormal metaphase cells, membrane blebbing and apoptotic bodies. Annexin V indicated 3.8% cells to be in early apoptosis compared to 0.5% of the control cells after 24 hours exposure to 10^{-6} M 2ME. TEM revealed increased apoptotic bodies and large intracellular vacuoles in the 2ME-exposed MCF-7 cells suggesting the induction of apoptosis and autophagy. Fluorescent microscopy showed increased acidic lysosomes and cells with compromised membranes. Bioinformatics analysis conducted on microarray data identified 681 differentially expressed genes (B-value >2.5) when compared to vehicle-treated control cells including CALM2, BAK1 and AKT1S1. These genes are involved in the regulation of apoptosis, autophagy and the G_2/M-phase transition.
The above-mentioned study is currently also being conducted to assemble the possible mechanism of action of 2ME in a non-tumorigenic breast epithelial cell line (MCF-12A). These results will thus indicate differences in signal transduction *in vitro* exerted by 2ME in cancer and normal cells respectively. Research concerning unravelling the exact mechanism of action of 2ME will enable scientists to focus on affected cellular mechanisms, as well as the identification of possible new targets for therapeutic intervention.

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Platinum (IV) complex LA-12 induces cell cycle arrest and phase specific apoptosis in colon carcinoma cells HCT116

O. Blanarova[1], I. Jelinkova[2], R. Jendzelovsky[3], K. Soucek[1], J. Hofmanova[1], P. Sova[4], A. Kozubik[1]. *[1]Institute of Biophysics AS CR, Department of Cytokinetics, Brno, Czech Republic; [2]Masaryk University, Faculty of Science, Brno, Czech Republic; [3]P. J. Safarik University, Faculty of Science, Kosice, Slovak Republic; [4]Pliva-Lachema a.s., R&D, Brno, Czech Republic*

Background: Platinum antitumor drugs are employed in treatment of various tumor types. They create DNA adducts generating DNA damage signals resulting in cellular stress response, which forces the cell to slow down or arrest the cell cycle to allow time for DNA repair or undergo cell death. LA-12 is a novel platinum (IV) complex expressing cytotoxic effects in many cancer cell lines. It has been shown to overcome intrinsic and acquired resistance to cisplatin and oxaliplatin in ovarian and colon cancer cell lines.
Material and Methods: Colon carcinoma cells HCT116 were continually incubated with LA-12. DNA content, marker of DNA double strand breaks (gamma-H2A.X), mitotic (phospho-histone H3) and apoptotic markers and protein expression were analyzed by flow cytometry in individual cells. Cell cycle phase specific apoptosis was assessed by bivariate analysis of DNA content and presence of caspase-cleaved cytokeratin 18 (M30 antibody), as an early apoptotic marker.
Results: LA-12 induced histone H2A.X phosphorylation in S-phase cells and accumulation of the cells in G2 phase of the cell cycle, whereas the proportion of mitotic cells declined. In contrast, cells expressing cyclin B1 and active form of CDK1 were still present, even after 48 hours of incubation with LA-12, when apoptosis was massively induced. Bivariate analysis of caspases-cleaved cytokeratin 18 fragments and DNA content revealed increase in early apoptotic cells with G1 DNA content.
Conclusions: On the basis of our results, we hypothesize that in HCT116 cells, LA-12 activates DNA damage signaling by creating double strand breaks in S-phase cells by perturbation of DNA replication. This early event is followed by accumulation of the cells in G2 phase. However, certain part of the cells is somehow able to overcome this arrest and proceed to mitosis and G1 phase, where apoptotic cascade is activated and cells eventually die.
This work was supported by grants No. 1QS500040507 IGA ASCR and 301/07/1557 GACR.

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Expression of the genes involved in apoptosis, proliferation and endoplasmic reticulum stress in ionomycin/PMA treated Jurkat cells

K. Stankov[1], S. Tauszig-Delamasure[2], G. Bogdanovic[3], L. Popovic[3], S. Stankov[4], N. Sylvius[5], S. Popovic[6], P. Mehlen[2]. *[1]Clinical Centre Novi Sad, Department of Biochemistry, Novi Sad, Serbia; [2]Centre Léon Bérard, Cancer and Development CNRS FRE 2870, Lyon, France; [3]Institute of Oncology, Department of Oncology, Sremska Kamenica, Serbia; [4]Health Center, Department of General Medicine, Novi Sad, Serbia; [5]CEA/Institut de Génomique, Centre National de Génotypage, Evry, France; [6]Medical faculty Novi Sad, Department of Haematology, Novi Sad, Serbia*

The principal objective of our study was to test the regulation of gene expression of SCF/c-kit tyrosine kinase signaling pathway and the genes involved in apoptosis, proliferation and endoplasmic reticulum stress in ionomycin/PMA treated Jurkat cells.

By the means of quantitative real-time PCR, we measured the expression levels of 23 genes involved in apoptosis, proliferation and endoplasmic reticulum stress in Jurkat cells RNA, extracted from ionomycin/PMA treated and non-treated cells. The expression data were normalized to the expression levels of four housekeeping genes and cDNA concentration. Our preliminary results show that in the Jurkat cells, in the absence of exogenous SCF (c-kit ligand), ionomycin/PMA treatment down-regulates the expression c-kit receptor and induces the moderate up-regulation of both pro-apoptotic and pro-survival genes. The increased expression of IL-2, NFkB, JNK, ERK, XBP and GADD34 genes, together with the down-regulation of c-kit, show that the ionomycin/PMA treatment induces the proliferation, inflammation and differentiation processes, independently from c-kit activation.
According to our data, the up-regulation of the genes involved in Jurkat cells proliferation and endoplasmic reticulum stress, does not disturb the balance between pro- and anti-apoptotic Bcl-2 family genes upon the ionomycin/PMA treatment.

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New curcumin analogues show enhanced antitumour activity in malignant melanoma cells

M. Pisano[1], S. Cossu[1], I. Sassu[1], G. Pagnan[2], D. Fabbri[1], M.A. Dettori[1], G. Palmieri[1], G. Delogu[1], M. Ponzoni[2], C. Rozzo[1]. *[1]Istituto di Chimica Biomolecolare CNR, Progettazione Molecolare, Sassari, Italy; [2]Laboratorio Oncologia, Ospedale "G. Gaslini", Genova, Italy*

Background: Malignant Melanoma (MM) is one of the fastest growing cancer in western populations with the incidence having tripled in the last decades. Chemotherapy, immunotherapy and vaccines are still unsatisfactory thus new approaches for MM treatment are urgently needed. Curcumin, a natural spice extracted from the root of *Curcuma longa* L. and largely used in oriental cuisine and medicine, has recently been described as potential anticancer agent. We tested several curcumin-related compounds for their capability to inhibit cell growth on primary MM cell lines.
Material and Methods: Viability and antiproliferative assays together with dose and time-response assays have been carried out on MM cell lines to compare antitumour activity of curcumin to that of six related biphenyls. Cultured fibroblasts from healthy donors have been used as controls. DNA fragmentation with ELISA and TUNEL assays have been performed to assess apoptosis triggered by some of the treatments.
Results: Curcumin, a natural compound already known for its antitumour activity, showed to be a potent antiproliferative agent on our MM cells. We tested six curcumin-related hydroxylated biphenyls (D2-D7) on MM cells to assess their potential antitumour activity in comparison with that of curcumin: IC50 values established after 5 days of treatments showed the α,β-unsaturated keton (D6) the most efficient at concentrations around 1−2 μM, much lower than the IC50 values calculated for curcumin (about 10 μM). Fibroblasts proliferation rate was not affected in the same conditions. Wash-out experiments further demonstrated that the D6 action was more powerful and rapid in arresting MM cells growth than that of curcumin, giving rise to irreversible effects after only 2−4 hours of co-culture with MM cells. Clonogenic assays were performed to measure long-term effects of D6 on permanent cell growth arrest and cell death, showing a dose-dependent reduction in MM colony formation. ELISA and TUNEL assays on some of the MM cell lines allowed the detection of oligonucleosomes in the cytoplasm and apoptotic bodies in the nucleus, showing involvement of apoptosis in D6 activity.
Conclusions: Our results indicate this compound as good lead to develop new therapeutic agents against MM. D6 activity should be further investigated on in vivo melanoma models to assess the real anticancer effectiveness on such tumour.

Aurora kinase

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MLN8054, a selective inhibitor of Aurora A kinase: final results of a phase I clinical trial

A. Cervantes[1], T. Macarulla[2], S. Roselló[1], E. Rodríguez-Braun[1], J. Baselga[2], J. Tabernero[2], H. Liu[3], A. Chakravarty[4], D. Bowman[5], O. Eton[6]. *[1]Hosp. Clínico Universitario de Valencia, Oncology, Valencia, Spain; [2]Vall d'Hebron University Hospital, Medical Oncology, Barcelona, Spain; [3]Millennium Pharmaceutical Inc., Biostatistics, Cambridge, MA, USA; [4]Millennium Pharmaceutical Inc., Cancer Pharmacology, Cambridge, MA, USA; [5]Millennium Pharmaceutical Inc., Molecular and Cellular Oncology, Cambridge, MA, USA; [6]Millennium Pharmaceutical Inc., Oncology Clinical Research, Cambridge, MA, USA*

Background: MLN8054 is an oral, selective, small-molecule inhibitor of Aurora A kinase. This phase I clinical trial examined the safety, pharmacokinetics (PK), and pharmacodynamics (PD) of MLN8054 administered over two weeks in a 28-day cycle.
Materials and Methods: Patients (pts) with advanced solid tumors at 2 centers were enrolled. Cohorts of 3−6 pts received successively increasing doses until dose-limiting toxicity (DLT) was seen in $\geqslant$2 pts. The first 2 cohorts received 10 and 20 mg once daily (QD) on days 1−5 and 8−12. Subsequent cohorts were treated on days 1−14 with 25, 35, 45, 55, 60, 70, and 80 mg/day in four divided doses (QID) with the largest dose at night to mitigate against benzodiazepine-like effects, such as somnolence. Starting at the 45 mg dose level, oral methylphenidate (MP) 5−15 mg was also permitted during daytime dosing. Serial blood samples were collected to estimate PK. Skin and tumor biopsies were obtained before and after dosing to assess accumulation of mitotic cells as a measure of PD effects.
Results: Of the 44 pts enrolled, 43 were treated with MLN8054. Pts received a median of 1 cycle (range, 1−10). DLT included reversible Grade 3 benzodiazepine-like effects, primarily somnolence (n = 3), and reversible Grade 3 liver function test (LFT) elevations (n = 2). Dose-escalation was stopped at 80 mg/day because of DLTs of somnolence despite prophylactic therapy with MP (1 pt), and LFT elevation (1 pt). Grade 2 neutropenia and alopecia (1 pt) and mucositis (1 pt) were first observed at the highest dose level of 80 mg. Mean exposure levels were roughly linear with dose. The terminal half-life was 30−40 hours. Among skin biopsies evaluable pre- and post-treatment in 40 pts, there was sporadic evidence of accumulation of mitotic cells in basal epithelium within 24 hours after the first daily dose or at steady-state. Among tumor biopsy specimens evaluable pre- and post-treatment in 14 pts, there was evidence of Aurora A inhibition as measured by multiple mechanistic PD markers, especially at the higher doses.
Conclusions: MLN8054 dosing for up to 14 days of a 28-day cycle was feasible. Somnolence and LFT elevation were dose-limiting ahead of clinical anti-proliferative effects. Skin and tumor biopsy findings supported Aurora A kinase inhibition. MLN8054 has been replaced in clinical trials by MLN8237, a more potent second-generation Aurora A kinase inhibitor.

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Phase I study of the safety, pharmacokinetics (PK), and pharmacodynamics (PD) of MLN8237, a selective Aurora A kinase inhibitor, in the United States

J. Infante[1], E.C. Dees[2], R.B. Cohen[3], H. Burris[1], B. O'Neil[2], P. Murphy[3], Y. Lee[4], J. Pappas[5], J.A. Ecsedy[6], O. Eton[7]. *[1]Sarah Cannon Research Institute, Drug Development, Nashville, USA; [2]University of North Carolina, Hematology/Oncology, Chapel Hill, NC, USA; [3]Fox Chase Cancer Center, Medical Oncology, Philadelphia, USA; [4]Millennium Pharmaceuticals Inc., Clinical Pharmacology, Cambridge, MA, USA; [5]Millennium Pharmaceuticals Inc., Clinical Operations Oncology, Cambridge, MA, USA; [6]Millennium Pharmaceuticals Inc., Molecular and Cellular Oncology, Cambridge, MA, USA; [7]Millennium Pharmaceuticals Inc., Oncology Clinical Research, Cambridge, MA, USA*

Background: Preclinical studies suggest the selective Aurora A kinase inhibitor, MLN8237, is more potent than MLN8054 and less likely to cause benzodiazepine-like effects. This ongoing phase I clinical trial examined the safety, PK, and PD of MLN8237.
Materials and Methods: MLN8237 was given orally once daily (QD) for 7 days in 21-day cycles. Cohorts of 3 patients (pts) with advanced solid tumors were enrolled to increasing dose cohorts (5, 10, 20, 40, 80, and 150 mg/day) until dose-limiting toxicity (DLT) was seen in $\geqslant$2 of 6 pts. Serial blood samples were collected to estimate PK. PD effects on Aurora A kinase were inferred from accumulation of mitotic cells in the basal epithelial layer of skin biopsies.