performing tumor tissue microarray analysis of clinical lung cancer materials.

Preliminary results: Gene expression microarray study: RNA from 28 primary NSCLC, 8 samples of normal lung that were taken from the same patients, seven independent brain metastases and one specimen of normal brain (commercial RNA that is derived from a pool of normal brains) were hybridized to Affymetrix U95 Chips (containing 12625 probe sets). Of the 28 primary NSCLC cases 6 developed brain metastases and 7 extra-cranial metastases during a minimal follow-up of three years. Limited space precludes a detailed description of the analysis). The microarray results were confirmed by RQ-PCR of selected genes. ADAM8 and N-cadherin are according to these analyse genes associated with brain metastasi in NSCLC patiens screned above. After werrification on symplex from independent NSCLC patient files, collected both in Israel and Czech republic we found a significant association between n-Cadherin expression and brain metastasis (p= 0,008).

Conclusion: n-Cadherin is a wery strong predictor of brain metastasis in NSCLC patiens.

**340** Poster

**Pilot study of neo-adjuvant intra-arterial (i/a) chemotherapy in patients with sarcomas of a head and neck**

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Purpose. Reduction the volume of operation in patients with sarcomas of head and neck.

Methods. 19 pts (6 female, 13 male) with various histological types of head and neck sarcomas, age 19-59 years (middle age 39 years). Primary sarcomas were diagnosed at 15 people, recurrent at 4. Sarcomas of soft-tissue (SST) G1-G2 were diagnosed for 6 pts, the primary bone sarcomas G1-G2 at 13. Treatment regimen were: doxorubicin 45 mg/m2 i/a for 1 hour 1-2 days, cisplatin 75 mg/m2 i/a for hour, 1-2 days with one-stage intravenous hydratation, cyclophosphamide 800 mg/m2 intravenously 3 day. Interval between cycles was 14 days; number of cycles were 2-4. For i/a chemotherapy was used a. carotis externe.

Results. Clinical efficacy was registered at 15 pts (SST 4, bone sarcomas 11). According to beam methods of diagnostic, partial response was noted at 10 pts, stabilization at 5. All of them were underwent operation. Follow-up period in this group was from 5 till 22 months. At 4 pts treatment was inefficient. After 4 month the progress of disease was noted at 3 people and local recurrence of tumor in the same time at 1. Medical pathomorphosis III was noted in 4 cases (including bone sarcomas G1-G2), accordingly, changes of II and I wers noted in 8 and 7 cases.

Conclusion. Preoperative intra-arterial chemotherapy allows to reduce the volume of surgery, in the patients with widespreaded sarcomas of head and neck.

**341** Poster

**A functional link between the MRN complex and the Gcn2p kinase uncovered by the antitumour drug beta-lapachone**

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Background: Beta-lapachone (b-lap) is an anticancer agent that selectively induces cell death in several human cancer cells. We previously reported that, in budding yeast, b-lap was cytotoxic, induced DNA damage and activated a G1/S Mre11-Tel1p checkpoint pathway preceding death. Our aim was to gain further insights into the mechanism of b-lap action and identify the molecular targets of b-lap action.

Materials and methods: We compared the gene expression profile of the b-lap treated yeast cells with that obtained from untreated cells using cDNA microarrays. We used Significance Analysis of Microarrays to identify differentially expressed genes between untreated/b-lap treated cells. The data obtained after analysis of the microarray was validated by standard yeast genetics and molecular biology approaches.

Results: Interestingly, numerous amino acid biosynthesis genes were found to be regulated by the drug, suggesting that b-lap might activate the General Control of Nutrients (GCN) pathway in yeast. Accordingly, b-lap treatment incremented phosphorylation of the eIF2 alpha subunit in a GCN1, GCN2 and GCN20-dependent manner. Suriprisingly, phsophorylation of eIF2alpha was fully dependent on the MRN complex. Furthermore, Gcn2p kinase modulated i) checkpoint responses triggered by b-lap treatment, and ii) cell viability in response to b-lap exposure. finally, we found that Gcn2p regulated checkpoint function by mechanisms other than eIF2$\alpha$ phosphorylation.

Conclusions: These data uncover a functional link between the Gcn2p kinase and the MRN complex and suggest that Gcn2p may have additional functions besides regulating translation.

**342** Poster

**Sensitization of breast cancer cells to anthracyclines by docosahexaenoic acid through loss of glutathione peroxidase response**

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We found long-chain n-3 polyunsaturated fatty acids, such as docosahexaenoic acid (DHA), to enhance the sensitivity of human breast cancer cell lines (Germain 1998) and rat mammary tumors to chemotherapy containing agents that induce an oxidative stress (Colas 2004), such as doxorubicin. Since DHA, with its 6 double-bonds, is very prone to oxidation, its membrane incorporation would provide more abundant targets for ROS generated by doxorubicin. To examine the relation between chemo-sensitization by DHA and tumour cells oxidative and antioxidant status, we used two breast cancer cell lines: MDA-MB-231, in which DHA increases sensitivity to doxorubicin, and MCF-7, which is not chemosensitizable by DHA. Upon anthracycline treatment, reactive oxygen species (ROS) and lipid peroxidation levels were enhanced only in MDA-MB-231 under DHA supplementation (30 $\mu$M). This was concomitant with a decrease of cytosolic glutathione peroxidase (GPx1) activity by 30%, a crucial enzyme for protection against hydrogen and lipid peroxides, and an accumulation of glutathione, the GPx co-substrate. This lack of GPx response resulted from a decreased amount of GPx protein.

We used an autochthonous rat mammary tumour model to investigate in vivo the DHA effect on GPx1 activity and on anthracyclines treatment efficacy. Rats were fed a control diet and a DHA-enriched diet (3.6 % of DHA in the diet). When the tumour reached 1.5 cm², rats received 1 injection of epirubicin (2.5 mg/kg via intraperitoneal route) per week during 6 weeks. We found that dietary DHA enhanced tumour sensitivity to epirubicin and this effect was associated to a decrease of GPx1 activity by 20%. Furthermore we found an inverse correlation ($r^2$=0.488) between epirubicin efficacy and GPx activity. Conversely, when antioxidant vitamin E was added, tumour GPx1 activity was restored and the DHA effect on chemosensitization was abolished.

Thus, loss of GPx response to an oxidative stress in tumour cells may account for the ability of peroxidizable targets such as DHA to enhance tumour sensitivity to ROS-generating anticancer drugs.

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**343** Poster

**The G-quadruplex ligand RHPS4 interferes with telomere replication leading to ATR-dependent DNA damage response**

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Functional telomeres are required for the replicability of cancer cells. The G-rich strand of telomeric DNA can fold into a 4-stranded structure known as G-quadruplex (G4), whose stabilization by specific ligands, can limit telomere function and cancer cell growth. RHPS4 is a telomere-interactive molecule possessing antitumoral activity because of its ability to rapidly induce telomere dysfunction and cell death [1].

Here, we show that RHPS4 induces a potent DNA damage response specifically in S-phase cells. In particular, we show that in S-arrested cells treated by RHPS4 ATR, but not ATM, is required for the formation of phospho-H2AX foci colocalizing with proliferating cell nuclear antigen (PCNA), BRCA1 and 53BP1. Interestingly, ATM is phosporylated at Ser1981 but in contrast to ionizing radiations, this activation of ATM is strictly ATR dependent, suggesting that the cellular response to pharmacological telomere deprotection follows a pathway that, most likely, represents ATR activation in response to replicational stress. By combining BrdU incorporation with CHIPs assay we clearly demonstrated that RHPS4 interferes with the replication of the telomeres, altering the dynamic association of the telomeric proteins TRF1, TRF2 and POT1. Interestingly, RHPS4 does not induce a specific DNA damage at an interstitial telomeric sequence, suggesting that it interferes with a terminal event of telomere

replication without altering the passage of the replication fork through telomeric DNA. Overall, these data are in agreement with the possibility that RHPS4 triggers a replication stress at telomeres, leading to an ATR-dependent DNA damage response.

[1] Salvati E, Leonetti C, Rizzo A, et al. Telomere damage induced by the G-quadruplex ligand RHPS4 has an antitumor effect. J Clin Invest 2007.

**344** Poster

**Biological activities of newly synthesized polyamine derivatives as potential anticancer agents**

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Natural polyamines are nitrogen-bearing aliphatic chains that play an essential role in cell growth and differentiation and represent physiological sources of polycations necessary for stabilization of DNA topologies. Polyamine content in the cells is tightly regulated: in addition to synthesis, mammalian cells are equipped with an efficient polyamine uptake system, whose activity is proportional to cell proliferation.

Polyamines analogues and derivatives can suppress proliferation of cancer cells by inhibition of the biosynthesis of natural polyamines and can exert cytotoxic activity due to their DNA-binding properties. Many tumour types have been shown to contain elevated level of an active polyamine transporter (PAT) for importing exogenous polyamines.

In the present study, a bis(benzyl)polyamine analogue (MDL 27695), known to exert antiproliferative activity, has been used as a template where to inserte a well-known DNA-intercalator group (aromatic core) to combine the ability to use the polyamine uptake system with the property to intercale and bind tightly the double-stranded DNA. For this purpose two groups of polyamine derivatives (symmetric or non-symmetric) were synthesized: each group includes 9 compounds which differ for the number of C atom (n=2-10) in the lateral chain to verify the best lenght of the amino-alkyl chain. All derivatives were tested for antiproliferative activity in human breast cancer (SKBR-3) and leukemia (CEM) in vitro in a range of concentrations beetween 0.1 and 10 μM. The MTT assay was used to determine growth inhibition after up 72 h of treatment.

Results indicate that the symmetric derivatives are more effective than the corresponding non-symmetric ones in both cell lines. All the symmetric compounds cause a significant dose- and time-dependent growth inhibition in the range of concentration tested. The compound with n=3 emerges as the most potent among the symmetric derivatives, with an $IC_{50}$ (72h) of 0.35 and 0.17 μM in leukemic and breast cancer cells respectively.

The present preliminary results document that some of these polyamine derivatives are more active than polyamine DNA-Intercalator conjugates against human leukemia or than oxa-polyamines derivatives against breast cancer. The pharmacokinetics and pharmacodynamics of the active compounds are now under study.

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**POSTER SESSION**

# Signalling pathways 2

**345** Poster

**Combination of cetuximab with cisplatin and radiation therapy may have useful applications for the treatment of cervical cancer**

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Background: Cervical cancer kills 288,000 women each year, therefore, new therapeutic strategies are necessary. Here we examine the effects of cetuximab alone or in combination with cisplatin and radiation therapy (RxT) on cell cycle and proliferation, VEGF expression and antibody-dependent cellular cytotoxicity (ADCC) on the carcinoma cell lines A431 (vulva), Caski and C33A (cervical) and how these treatments influence downstream signaling pathways.

Methods: Cell lines were treated with cetuximab alone or in combination with CDDP or RxT, or with both, at different conditions. Cytotoxicity was assessed by clonogenic assays (CA) and cell cycle analysis by flow cytometry (FACS) using PI staining. Western blotting (WB) analysis was performed with antibodies against: EGFR (Tyr 845, 992, 1045 and 1068), AKT, HER2 and MAPK (total and phosphorylated). In vitro ADCC assay was done using the Cyto Tox 96® kit. VEGF expression was determined by real time RT-PCR and ELISA.

Results: A431 cells express $10^6$ EGF receptors on the membrane while Caski and C33A cells express 20% and 1% of that, respectively (WB). Combination of cetuximab with RxT or cisplatin led to a stronger inhibition (n=3, $P<0.05$) of cell survival of A431, Caski and C33A cell lines than each treatment alone. Indeed, we observed at least an additive effect following the addition of cetuximab to RxT and cisplatin ($P<0.05$) due to an arrest at the G0/G1 phase of the cell cycle (FACS analysis) for all three cell lines tested. We performed WB to analyze the effects of cetuximab on phosphorylated EGFR, HER2, MAPK and AKT. Cetuximab could decrease the phosphorylation status of almost all residues in A431 and Caski cell lines. In the C33A cell line, which has low EGFR but high HER2 expression, cetuximab inhibits HER2 and MAPK phosphorylation, suggesting it has a large dependency on these pathways. Two factors leading to in vivo antitumor activity of anti-EGFR antibodies are the induction of ADCC and inhibition of angiogenesis. Cetuximab induced ADCC in 26.40% (A431), 15.1% (Caski) and 1.75% (C33A) of the cells at effector/target ratio of 20:1. Furthermore, cetuximab inhibited VEGF expression in all three cell lines.

Conclusions: We observed that cetuximab treatment plus cisplatin/RxT decreased A431, Caski and C33A cell survival, inhibited VEGF expression and induced ADCC. Our data suggest that cetuximab combined with cisplatin and RxT has useful applications for the treatment of cervical cancer.

**346** Poster

**Celecoxib-induced apoptosis involves signaling through the Mcl-1/Noxa axis and can be blocked by overexpression of Bxl-xL but not Bcl-2**

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Introduction: The non-steroidal anti-inflammatory drug Celecoxib is known as a specific inhibitor of Cyclooxygenase-2 (Cox-2). Cox-2 has been shown to be overexpressed in many tumors and can contribute to an enhanced resistance towards apoptosis induction by certain apoptotic stimuli. Recent experiments in Cox-2 negative cell lines, however, suggest that Celecoxib is able to induce apoptosis in a Cox-2-independent manner, probably through the mitochondrial pathway. Mitochondrial integrity is controlled by the pro- and anti-apoptotic members of the Bcl-2 family. To date, however, it remains unclear which specific members are needed to be activated and neutralized for a successful induction of apoptosis, especially in response to Celecoxib.

Methods: Regulation of Celecoxib-induced apoptosis was analyzed in Jurkat T cell lymphoma cells which were transfected with Bcl-2, Bcl-xL, or the empty vector. The implication of Mcl-1 and Noxa was determined by transfecting the cells with the respective siRNA. Induction of apoptosis was determined by flow cytometry and fluorescence microscopy. Downregulation of protein expression and caspase activation during apoptosis was verified by Western blot analysis. cytochrome c release was assayed by fluorescence microscopy and cellular fractionation with subsequent Western blot analysis.

Results: Celecoxib induced apoptosis in the Cox-2 negative Jurkat T-cell lymphoma cell line through the mitochondrial pathway that involves the breakdown of the $\Delta\psi m$. During Celecoxib-induced apoptosis the pro-apoptotic protein Bak was activated and cytochrome c was released into the cytosol.

Examination of the members of the Bcl-2 family revealed that the anti-apoptotic Mcl-1 was down-regulated whereas the expression level of the pro-apoptotic BH3-only protein Noxa remained unchanged. Mcl-1 co-immunoprecipitated with Noxa. However, this interaction was diminished 6h after treatment with Celecoxib due to the downregulation of Mcl-1.

Mcl-1 downregulation by siRNA was sufficient to induce apoptosis within 3 hours in Jurkat T cells. Although the downregulation of Noxa by siRNA did not induce apoptosis it made the cells more resistant towards Celecoxib-induced apotosis.

The induction of apoptosis by Celecoxib could be inhibited by overexpression of the anti-apoptotic Bcl-xL but not by the closely related Bcl-2 although Mcl-1 downregulation was not inhibited by these two anti-apoptotic proteins.

Conclusions: Celecoxib induced apoptosis through the Mcl-1/Noxa axis in Jurkat T-cells. An early step in the induction of apoptosis by Celecoxib was the downregulation of Mcl-1. Despite the neutralization of Mcl-1, apoptosis induction could be blocked by overexpression Bcl-xL but not Bcl-2 implying a different role of Bcl-2 and Bcl-xL in Celecoxib-induced apoptosis.