As housekeeping we use 18S (Primer Design Co., Ltd.), such as was previously depicted.
Results: We have selected for this study three T-UCRs: uc.277, uc.301, and uc.440. We have found that the amount of these T-UCRs is generally low. T-UCR uc.440 was found over-expressed (nearly 16 times) in MDAMB231 breast cancer cell line versus healthy tissue ($p < 0.01$). T-UCR uc.277 is also slightly over-expressed in MDAMB231 ($p < 0.05$). In addition, T-UCR uc.440 was found slightly over-expressed in HT29 colon cancer cell line regarding to the others analyzed colon cancer cell lines ($p < 0.05$), but there weren't found differences regarding to healthy tissue. It could not be detected significant expression of the T-UCR uc.301 in any tumour cell line. In melanoma cell lines could not be detected the expression of any assayed T-UCRs.
Conclusion: Our goal was to find a T-UCR with a constant and high expression in tumour cell lines regarding to healthy controls. We have found that T-UCR uc.440 is significantly upregulated in MDAMB231 breast cancer cell line regarding to healthy control, but also regarding to the others breast cancer cell lines analyzed. No relevant changes in the expression for the others T-UCRs tested were observed. For our knowledge, this is the first study where T-UCRs are assayed in colon, breast and melanoma tumour cell lines.

P99

Rho C in melanoma: possible target for statin treatment?

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Background: There is large interest in statins as agents in cancer prevention and treatment. In more than 1300 melanoma cases statin use was associated with reduced Breslow thickness. Gene expression profiling experiments in melanoma cell lines revealed RhoC to be upregulated in cell lines with high metastatic capacity. We demonstrated that RhoC immunohistochemical expression in primary cutaneous melanoma was strongly associated with thicker and ulcerated tumors. Specific inhibition of Rho C in the A375M melanoma cell line reversed migration and invasion. Statins can block activation of RhoC by blocking geranylgeranyl pyrophosphate and farnesyl pyrophosphate which are important for isoprenylation of Rho proteins. Atorvastatin reverted the metastatic phenotype of several human melanoma cell lines and inhibited in vivo metastasis in SCID mice injected with A375M melanoma cells.
We explored if the statin simvastatin can influence the growth rate of melanoma cells of the BLM melanoma cell line.
Materials and Methods: The effect of different concentrations simvastatin (50, 500 and 5000 nM) on the global growth rate of BLM melanoma cell lines was investigated and compared to untreated BLM cells (control). By using 12 semi-automatic phase-contrast microscopes the relative increase of BLM cells was measured at 24, 48 and 72 hours.
Results: The simvastatin 5000 nM concentration significantly reduced global growth rate of the BLM cells after 48 and 72 hours ($p < 0.001$) with stabilization of the cell population. There were no significant differences in global growth rate of the 50 nM and 500 nM simvastatin groups compared to control.
Conclusion: These observations support a growth inhibitory effect of high concentrations of simvastatin on the BLM melanoma cell line. Further research will focus on the determination of growth inhibitory concentrations of different statins on different melanoma cell lines.

P10

Detection of circulating tumor cells (CTCs) in Stage cT3−4 or N+ rectal cancer patients (pts) undergoing combined neoadjuvant therapy plus curative surgery. Preliminary data

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Background: CTCs detected at baseline and at disease-evaluation time-point during treatment seem to be an independent prognostic factor in metastatic colorectal cancer (Cohen SJ JCO, 2008). CTCs' role as predictive marker in early stages after radical surgery is under investigation, while no data are available in locally advanced rectal cancer suitable for neoadjuvant chemoradiotherapy. Aim of the study is to investigate the role of CTCs in local advanced rectal cancer pts undergoing neo-adjuvant chemo-radiotherapy (CT-RT).
Materials and Methods: In a prospective single institution study, cT3−4 or N+ rectal cancer pts staged by transrectal ultrasound and/or pelvic MRI and chest-abdomen CT scan, are submitted to capecitabine (825 mg/mq, orally, twice daily continuous) with concomitant radiotherapy (50.4 Gy/fractions to the primary tumor and perirectal nodes), followed by two cycles of capecitabine (1250 mg/mq, orally, tid 14/21 days). Primary endpoint is evaluation of CTCs at baseline (t0), after neoadjuvant therapy, before surgery (t1), after surgery (t2), and at 6-month follow-up (t3) and its correlation with survival parameters. CTCs are enumerated with immunomagnetic separation in 7.5 ml peripheral blood at over-mentioned time-points (CellSearch System, Veridex Inc).
Results: Twenty-six pts (16M; 10F; median age:63$\pm$13 yrs; range: 44–83 yrs) underwent t0 sampling, 8 pts completed CT/RT and therefore underwent t1 and t2 sampling. At baseline (t0) three pts presented 1 CTC (12%), one 2 CTCs (3.5%), one 27 CTCs (3.5%) while in twenty-one (81%) no CTCs were detected. At t1 and t2 none of the eight pts analyzed showed CTCs.
Conclusion: CTCs $\geqslant$1 are present in 15% of our patients, but the sample is too small for statistical analysis. The study is still ongoing; more data will allow to assess prognostic and predictive significance of CTCs during treatment in this setting.

P30

Assessment of oxidative stress in tumor cells and normal mucosa cells from head and neck squamous cell carcinoma patients

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Background: One of the cancers particularly linked to oxidative damage and oxidative stress is the head and neck squamous cell carcinoma (HNSCC). Tobacco and alcohol are well defined etiological factors.
Concurrent radiation and chemotherapy, a recognized alternative treatment to surgery for patients with advanced HNSCC, can induce a systemic oxidative stress. Oxidative damage is the main mechanism mediating the clinical effect of radiotherapy, and an increased resistance to oxidative stress by malignant cells is associated with treatment failure. Response to (chemo) radiation treatment varies from patient to patient.
The purpose of this study was to compare the tissue levels of glutathione in HNSCC tumoral tissue (Tum) and adjacent normal mucosa (Muc) biopsies as a potential factor of variability in (chemo) radiosensitivity.
Materials and Methods: 27 newly diagnosed HNSCC patients were prospectively studied. All were current smokers. 27 tumoral biopsies and an equal number of biopsies from normal mucosa were analysed. The oxidised/reduced glutathione ratio was measured with the capillary electrophoresis Ceofix GSH/GSSG kit (Analis, Namur, Belgium). Two hundred µl of whole blood, normal and tumoral tissues were immediately grinded with 600 µl of 5% metaphosphoric acid. After centrifugation (within 3 hours), 100 µl of the supernatant was mixed with 400 µl of the kit diluent containing naphthalene sulfonic acid as an internal standard. Analysis was done on a P/ACE 5000 series with a 37 cm and 75 µm i.d. capillary maintained at 25ºC. The separation was realized at 8 kV with a pH 8.2 borate buffer containing SDS. The glutathione peaks were detected at 200 nm and integrated as under-the-curve areas (AUC). The results are expressed as the ratio of oxidised GSSG AUC to the reduced GSH AUC. Clinico-pathological parameters were also considered as potential factors of variability in oxidative stress status of HNSCC tumoral tissue.
Results: The GSSG to GSH ratio was higher in the tumoral tissue than in the adjacent normal tissue in 12/27 of the cases.
Conclusion: In 44% of our cases, HNSCC tumoral tissue from untreated patients had a GSSG/GSH ratio different than that found in the normal adjacent cancer free mucosa. This difference was not related with clinico-pathological parameters. Heterogeneity in HNSC cancer is emphasized. The ability of the GSSG/GSH ratio to predict differential chemoradiosensitivity will be evaluated by long-term survival data.

P34

Reduced p63 expression in myoepithelium correlating with increased invasiveness in epithelium

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Background: Our previous studies revealed that pregnancy associated breast cancer (PABC) had significantly reduced nuclear p63 expression

in the myoepithelium, while intense cytoplasmic p63 expression in the associated normal or hyperplastic epithelium.
Materials and Methods: Our current study attempted to further define these epithelial structures using immunohistochemistry with a panel of aggressiveness and invasiveness related markers and comparative genomic hybridization (array-CGH) with over 30,000 DNA probes.
Results: Our study revealed a number of unique alterations in these structures, including: (1) significantly reduced nuclear p63 expression in the myoepithelium of terminal ducts, (2) immunohistochemical and morphological resemblance to adjacent invasive cancer cells, (3) significant gain in the copy number of DNA coding genes for morphogenesis, angiogenesis, and metastasis, and (4) significant loss in the copy number of DNA coding genes for tumor suppressors, cell adhesion, and macromolecular complex assembly or intra-cellular trafficking. Detected array-CGH alterations correlated well with in vivo expression of a number of corresponding proteins tested.
Conclusion: Our findings suggest that reduced p63 expression in the myoepithelium may result in increased invasiveness in the associated epithelium, and that normal or hyperplastic epithelial cells with cytoplasmic p63 expression may represent a biologically more aggressive population that may progress to invasive lesions without undergoing through the stage of in situ cancer.
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P75

A promising method for visualization of immune responses in immunoproteomics

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Background: Breast cancer is the most common diagnosed cancer type in women worldwide. The early detection of this disease is a key factor for its successful treatment. The immunogenicity of cancer has been decribed in the last decade. Although the importance of the biomarkers CEA and CA 15-3 has been showed, the new specific protein biomarkers for the early detection of breast cancer are still missing. A new tool for visualisation of humoral responses and the following de novo sequencing of the involved proteins would be of great benefit.
Materials and Methods: Different protein extracts which were obtained from a healthy breast tissue or from a carcinoma were separated via sodium dodecylsulfate polyacrylamide gel electrophoresis (1D SDS-PAGE). The proteins were then cut out of the gel, digested with trypsin and spotted on nitrocellulose microarray slides. Each subarray was incubated either with sera of breast cancer patient or with control sera and afterwards labelled with a cyanine 5-labelled human anti-immunoglobulin G antibody (IgG).
Results: Ater the incubation of digested proteins from the breast tissue with different sera and labelling with anti-IgG antibody we have detected the antibody profiles as a part of the immune response.
Conclusion: This method enables the visualization of antibody profiles in the presence of breast cancer. Further subsequent de novo screening of the involved proteins could help to understand their role in the emergence, development and pathogenesis of this complex disease.

P115

Green tea catechins mixture (Polyphenon E) is an equally potent proteasome-inhibitor as purified EGCG

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Background: Among the constituents of green tea catechins, Epigallocatechin gallate (EGCG) found in green tea is the most potent chemopreventive agent that appears to affect a number of molecular processes including to potently and selectively inhibit the proteasome activity in intact human prostate cancer cells and consequently accumulates IkBα and p27 proteins, leading to growth arrest. Constitutive activation of NFkB has been reported in many tumors, associating it with progression of epithelial cells, including prostate, toward malignancy. However, since EGCG has poor bioavailability and stability to be used in chemoprevention trials, the purpose of this study is to see if a mixture of green tea polyphenols is equally potent inhibiting the proteasome activity as purified EGCG.
Materials and Methods: The effects of a mixture of green tea catechins (Polyphenon-E) on the PGPH-like and trypsin-like activities using a cell-free proteasome assay with a purified rabbit 20S proteasome was determined. To observe change in the levels of proteasome target proteins, human multiple myeloma U266 and prostate cancer LNCaP cells were treated with different concentrations of Polyphenon-E for 24 hours, followed by measurement of levels of the cyclin-dependent kinase inhibitor p27Kip1, a well known target protein of the proteasome.
Results: Similar to purified EGCG, Polyphenon E significantly inhibits the chymotrypsin-like activity of the purified rabbit 20S proteasome with an IC50 value of 0.88 μM. Polyphenon-E inhibited PGPH-like activity of the purified rabbit 20S proteasome with an IC50 value of 7 μM. The IC50 value for trypsin-like activity was above 100 μM, thus demonstrating that Polyphenon-E preferentially inhibits the proteasomal chymotrypsin-like activities over other activities. Polyphenon-E inhibits proteasome activity in intact cells in a concentration-dependent manner and treatment of Polyphenon-E, at all used concentrations in both in human multiple myeloma and prostate cancer cells lines increased accumulation of the proteasome target Protein p27Kip1. Levels of actin were found to be relatively unchanged during the Polyphenon E treatment, which was used as a loading control
Conclusion: The proteasome is a prostate cancer-related molecular target of a green tea catechin mixture, Polyphenon E similar to observations with purified EGCG and has significant potential to be validated in tissue biomarkers obtained in Phase II chemoprevention trials.

P71

Vascular endothelial growth factor (VEGF) inhibition and erythropoiesis − a missing link?

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Background: Sustained angiogenesis is one of six proposed hallmark characteristics acquired by normal cells to attain a malignant phenotype. Among the plethora of intricately regulated factors involved in maintaining angiogenic homeostasis, the pro-angiogenic factor VEGF, has been identified as a key protein in enabling and sustaining angiogenesis, thereby promoting tumor progression. AZD2171, a small molecule tyrosine kinase inhibitor of VEGF receptors, is being evaluated in clinical trials. Some patients who were treated with this agent at our center, were noted to develop erythrocytosis − a peculiar finding in a population that is otherwise prone to anemia. The objective of our project was to look for similarities amongst this cluster of patients and arrive at a hypothesis regarding the cause for this effect.
Materials and Methods: The charts of four patients consulted consecutively to the hematology department at the Juravinski Cancer Center for unexplained erythrocytosis on AZD2171, were reviewed. Detailed histories and physical examinations were performed to rule out secondary causes and complications of erythrocytosis. Erythropoietin levels, RBC scans and CT scans were some of the investigations done to rule out secondary causes of absolute erythrocytosis as well as the entity of relative polycythemia. JAK2 mutation analyses via Polymerase Chain Reaction (PCR) technology were performed to rule out primary Polycythemia. A literature search was conducted to evaluate a plausible biologic rationale for this phenomenon.
Results: Three of the four patients included in the review showed evidence of inappropriately elevated erythropoietin levels in the absence of anemia during the course of treatment with AZD2171. One patient showed inappropriately elevated erythropoietin in the presence of erythrocytosis. One patient was noted to have inappropriately normal erythropoietin in the presence of erythrocytosis suggesting a defect in the homeostatic function of erythrocytosis induced suppression of erythropoietin. A review of the literature confirmed the occurrence of this phenomenon in pre-clinical models. Of note, VEGF inhibition seemed to be selective for erythrocytosis. There seemed to be a differential effect amongst the different routes of VEGF inhibition.
Conclusion: VEGF inhibition may trigger erythrocytosis via an EPO dependant mechanism. This effect needs to be validated through prospective trials. Clinical implications of this effect need to be further evaluated.

P39

Response of non small cell lung cancer xenografts to targeted therapies is not related to epithelial-to-mesenchymal transition

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Background: Epithelial-to-mesenchymal transition may play a crucial role in the sensitivity of established non small cell lung cancer (NSCLC) cell lines to epidermal growth factor receptor (EGFR) inhibitors, such as erlotinib and cetuximab. It has been described that cell lines with epithelial