

## P50

**Expression of estrogens, progesterone and c-erbB-2 in patients with recurrent breast cancer in Mexico**

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**Background:** Breast cancer is the most common form of malignancy occurring in women around the world. In Mexico, Breast cancer is the second cause of death in women. Some authors have reported a high concordance, ranging from 71% to 85% between estrogen receptor (ER) expression in primary breast carcinoma and its local, regional recurrence, or metastasis. No studies exist that estimate the frequency of breast cancer recurrence in Hispanic population from Mexico.

**Objective:** Compare the recurrence of breast cancer with the expression for estrogens, progesterone and c-erbB-2 in women of the northwest of Mexico.

**Methods:** Cross-sectional study in 397 cases of breast cancer. A correlation between clinical and pathological factors [estrogen receptor (ER), progesterone receptor (PGR) and c-erbB-2 in the primary tumor] including the recurrence of disease were evaluated. The immunohistochemical result for ER, PGR and c-erbB-2 was interpreted like (+) with a 10% or higher expression in the tumor cells. Those variables whose differences reached statistical significance were incorporated for logistic regression analysis to predict the biomarker's effect in disease recurrence. A p value <0.05 was considered statistically significant.

**Results:** Age average was  $52 \pm 12$  years old. Twenty-three percent presented recurrence of disease (95% CI 19–27) ( $p = 0.001$ ), 152 cases (38%, 95% CI 33–43; OR = 0.35;  $p = 0.001$ ) were in stage II, 201 cases (51%, 95% CI 46–56; OR = 0.64;  $p = 0.07$ ) were positive to estrogens, 170 (43%, 95% CI 38–48; OR = 0.76;  $p = 0.27$ ) to progesterone and 129 (32%, 95% CI 28–37; OR = 1.53;  $p = 0.08$ ) to c-erbB-2. Death due to the disease was in 13 cases (3%, 95% CI 1–5; OR = 21.23;  $p = 0.001$ ). Univariate analysis revealed that up to 9% of the recurrences are associated to the expression of receptors for estrogens ( $R = 0.09$ ;  $p = 0.03$ ).

**Conclusions:** Recurrence observed in our study is similar to the reported for other series, ER continuous being an excellent marker in the prognosis of breast cancer, the determination of PGR and c-erbB-2 receptors it does not add additional prognosis information to the recurrence risk in breast cancer.

## P57

**The PARP1 gene is over-expressed in triple negative breast cancer**

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**Background:** Poly(ADP-ribose) polymerase (PARP1) is a critical enzyme of cell proliferation and DNA repair. Inhibition of PARP1 leads to apoptosis in BRCA1 and BRCA2 deficient tumor cells and can facilitate the response to alkylating agents. Several PARP inhibitors are in clinical trials. We investigated PARP1 gene expression in major human primary cancers and corresponding morphologically normal tissues in order to direct clinical trial efforts toward cancers that over-express PARP1.

**Methods:** We used the Gene Logic BioExpress<sup>®</sup> System database to analyze PARP1 expression in human ovarian, breast, uterine, lung and prostate tumor samples in comparison to normal counterpart tissues. Gene expression was assessed on Affymetrix HU133AB microarrays and over-expression was defined by using upper confidence limits (UCL) calculated from the normal sample distribution for each tissue type. Samples from cancer subtypes were individually tested relative to the UCL and results tallied.

**Results:** The expression of PARP1 was above the 95% UCL of the corresponding normal tissue distribution in 25% of uterine cancers ( $n = 50$ ;  $nI = 23$ ), 75% of ovarian cancers ( $n = 64$ ;  $nI = 88$ ), 75% of lung cancers ( $n = 85$ ;  $nI = 122$ ) and 66% of infiltrating ductal breast cancers ( $n = 169$ ;  $nI = 68$ ). By contrast there was no difference in PARP1 expression in prostate cancer ( $n = 57$ ) and normal prostate ( $n = 57$ ). Mean PARP1 expression was 1.8-fold greater in breast cancers than in normals,  $p = 2 \times 10^{-27}$ , and was more pronounced in tumors that were negative when assayed for hormone and HER2 receptors by standard (non-microarray) diagnostic methods. PARP1 expression was above the 95% UCL in 88% (15/17) of patients whose tumors were ER-, 80% (15/19) that were PR- and 90% (8/9) that were HER2-. PARP1 expression was above the 99.9% UCL in 45% of all patients with infiltrating ductal breast cancer compared with 75% of ER-/PR- and 90% of HER2- patients. When all 169 infiltrating ductal breast cancer samples were categorized according to expression of the estrogen receptor1 transcript on the microarray, PARP1 gene expression was observed to be significantly higher in the ER- group ( $p = 0.009$ ).

**Conclusions:** The PARP1 gene is over-expressed in some but not all types of cancer. If PARP1 gene over-expression defines increased responsiveness to PARP1 inhibition, then these results imply that a substantial fraction of patients with cancer of the breast, ovary, uterus and lung would be rational candidates for therapy with a PARP inhibitor. Increased PARP1 expression in the subset of patients with breast cancer of the triple negative phenotype strengthens the link of this subgroup to patients with basal derived or BRCA-related disease, and makes them particularly attractive candidates for studies of PARP inhibitor therapy. This information has been incorporated into the design of a trial of BSI-201, a novel PARP inhibitor, in patients with triple negative breast cancer.

## P27

**Antiangiogenic activity of combining gefitinib and rapamycin in a panel of pancreas cancer cell lines**

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**Background:** Advances in molecular biology have greatly improved understanding of the pathogenesis and progression of pancreatic adenocarcinoma, generally considered resistant to common cytotoxic drugs. Our previous study showed that the combination of gefitinib plus rapamycin, acting at different levels of the EGFR cellular pathway, could induce a pronounced schedule-dependent antitumour effect in the pancreas cancer model; in fact, only the sequence gefitinib before rapamycin resulted in a synergistic cytotoxicity, through the strong inhibition of Akt and p70S6K phosphorylation. In this report, we investigated the antiangiogenic effects of the combination of gefitinib and rapamycin, acting on EGFR and mTor, respectively in the same panel of pancreas cancer cell lines (Panc-1, AsPC-1 and MiaPaCa-2).

**Methods:** The activity of the two drugs, both alone and in combination, were evaluated as modulation of HIFa by western blot analysis and as modulation of VEGF protein release by Elisa kit. According with rapamycin activity bound to a decrease in the transmission of proliferative signals, resulting in a cell cycle block in G0/G1 phase, we utilised, in the following characterization, 30 ng/ml rapamycin in Panc-1 cells and 100 ng/ml in the other cell lines for 3 days. Conversely, gefitinib was utilised at the IC50 values for 3 days. The 3 schedules utilised were the simultaneous and sequential ones.

**Results:** Preliminary results of the determination of VEGF release modulation showed that each drug reduced this growth factor release; this phenomenon was schedule dependent, with the max efficacy when gefitinib was given before rapamycin. In all cell lines, the Western Blot analysis of the modulation of HIFa in normoxia after exposure to gefitinib and rapamycin showed that gefitinib after 3 days exposure did not influence HIFa expression while 3-days-rapamycin slightly reduced its expression. The combination of the two drugs did not induce marked inhibition of HIFa expression, suggesting that this factor has not a relevant role in determining antiangiogenic effects of the two drugs.

**Conclusions:** Our results suggest that the combination of gefitinib plus rapamycin, acting at different levels of the EGFR cellular pathway, could induce a pronounced antitumour and antiangiogenic effect in the pancreas cancer model. Moreover, VEGF release is independent from HIFa inhibition in our pancreas cancer in vitro model.

Gefitinib is a trademark of the AstraZeneca group of companies.

## P10

**Identification of possible metastasis associated markers expressed on the surface of a breast cancer model**

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**Background:** Despite advances in detection and treatment of cancer, mortality still remains high. Antibodies have in recent years become a growing field in cancer therapy, and the results obtained with monoclonal antibodies in the treatment of various cancers, show the great potential in this avenue. There is still, however, a growing need for new targets and a better understanding of the mechanisms involved in immunotherapy.

As metastasis is the main cause of cancer mortalities, we are focusing on identifying antibodies against possible metastasis associated markers. As a metastatic model system we are using two isogenic cell lines, NM-2C5 and M-4A4, derived from the MDA-MB-435 breast carcinoma cell line. Although equally tumorigenic, these two breast cancer cell lines have diametrically opposite metastatic capabilities. In 74% of inoculated athymic mice clone M-4A4 metastasised to the lungs, whereas clone NM-2C5 did not metastasise to any distant sites.

**Methods:** For the identification of differentially expressed surface proteins on NM-2C5 and M-4A4 we used subtractive immunisation of mice. Mice

were immunised with NM-2C5 and, after 24 and 48 hour, treated with cyclophosphamide, rendering the mice tolerant to NM-2C5 antigens. On days 18 and 39 the mice were immunised with M-4A4 and on day 42 the spleens were extracted for hybridoma production. Hybridomas were screened for reactivity towards the two cell lines using cell based ELISA and FACS analysis. Clones with a marked preference for M-4A4 were tested for biological effects and further characterized by immunohistochemistry and FACS analysis.

**Results:** We isolated 4 monoclonal antibodies that bound exclusively, or preferentially, to the M-4A4 cell line. FACS analysis on an array of various breast cancer cell lines showed a promising expression pattern as 3 of the antibodies reacted preferentially with cell lines known to be metastatic in vivo. One clone exhibited no binding to 20 breast cancers or 30 different normal tissues but stained cancer cells in medullary thyroid carcinoma and lung carcinoid by immunohistochemistry. Another clone inhibited the growth of the melanoma cell line M22 in vitro.

**Conclusions:** We successfully identified 3 monoclonal antibodies capable of binding exclusively to cell lines shown to be metastatic in vivo. Further studies will evaluate if the profile observed in cell lines correlates with the metastatic process on resected patient tumours, and whether the isolated antibodies are capable of inhibiting metastasis formation.

#### P85

##### Investigation of mitochondrial common deletion (mtDNA4977) in breast cancer tumors

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**Background:** Breast cancer is the most common cancer and the second most common cause of cancer-related death among women. The breast cancer progression involves the accumulation of various genetic mutations, which are present in both nuclear genomes (nDNA) and mitochondrial genomes (mtDNA). Every human cell contains between 100–1000 mitochondria with many copies of mtDNA and mutation rate of mtDNA is at least 10 times higher than that of nuclear DNA. Mutation analysis of mitochondrial DNA is helpful in the determination of developmental potential, early diagnosis and gene therapy for breast cancer. In our study, we used multiplex PCR to analyze breast cancer patients for most common mitochondrial deletion (mtDNA4977) in tissue samples and blood samples using immunohistochemical features as criteria.

**Methods:** Patient samples were drawn from three medical centers in Iran. We retrieved formalin-fixed, paraffin-embedded tissue blocks from women with breast cancer diagnosed, the age of 25–80 years for the years 2004 and 2005. Forty-seven samples were used for multiplex PCR and immunohistochemical diagnosis from 34 formalin-fixed and paraffin-embedded samples and 9 blood samples. CINAGEN Inc.'s DNA Extraction Kit was used to isolate blood and tissue DNA. A simple and rapid method was used to detect the simultaneous detection of mtDNA4977 deletion. Morphological and immunohistochemical diagnoses of breast cancer were retrieved from their hospital records.

**Results:** Five-mtDNA4977 deletions were detected by multiplex PCR in 9 breast cancers in blood samples. Comparison presence of mtDNA4977 deletion in blood samples with ER (negative) and tissue samples with ER (negative) have shown that frequency of mtDNA4977 deletions was higher in blood samples ( $P < 0.001$ ) relatively tissue samples in breast cancer.

**Conclusions:** Cancer tissues are essentially free of mtDNA4977 deletions and the metabolic effect of it may be intolerable in cancer tissue but it may be minimal in blood (non-tumor) tissue. Our analysis shows testing of mtDNA4977 deletion in blood samples can be utilized as one of prognosis factors of breast cancer development risk in combination with ER.

#### P62

##### In vitro bioavailability testing of GGTI-2418 using the Caco-2 model

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**Background:** Protein prenylation is involved in the activation of a number of oncogene products. In particular, geranylgeranylated substrates of geranylgeranyl transferase I (GGTI) such as RhoA, RhoC, Rac1, R-Ras1 and R-Ras2 are known to promote tumorigenesis, metastases and invasion. On the contrary, inhibition of GGTI may impede the aberrant activation of Rho proteins and result in tumor growth inhibition and subsequent induction of apoptosis of human cancers with aberrant Rho function. GGTI-2418 (NSC 732082, MW 442) was found to be the most promising compound among the series of GGTI inhibitors, which inhibits GGTI potently (IC50 9.5 nM) and selectively over FTase (IC50 53 nM).

**Methods:** Caco-2 cells are commonly used as a model of mature intestinal cells. We therefore utilized an in vitro Caco-2 model to predict the bioavailability of the compound GGTI-2418. 8  $\mu$ M GGTI-2418 was added to

the apical side of six Caco-2-containing and six empty transwells. 282 nM 3H-mannitol (2  $\mu$ Ci) was also added to the apical side of six Caco-2-containing and six empty transwells. The plates were then placed in a 37°C, 5% CO<sub>2</sub> incubator. The plates were removed from the incubator and 60  $\mu$ L samples taken from the basolateral side of each transwell at 0.5, 1, 2, 4 and 8 hr in order to measure the levels of 3H-mannitol and GGTI-2418 that passed through the Caco-2 cells into the basolateral side of the transwells. The amount of GGTI-2418 that passed from the apical to the basolateral side of the transwell was measured using a HPLC method. The amount of 3H-mannitol passing from the apical to the basolateral side of the transwell was measured by liquid scintillation counting.

**Results:** A Caco-2 monolayer is considered intact and acceptable for transport studies if it exhibits a low mannitol permeability of  $\leq 20$  nm/s using approximately 4  $\mu$ Ci/mL in a 2 hr incubation (Caco-2 Product Sheet, In Vitro Technologies). Under these conditions, the permeability of the Caco-2 transwells used in this study was  $1.93 \pm 0.40$  nm/s, indicating that the Caco-2 monolayer was intact and had formed very tight junctions. Over the 8 hr period of study, 23% of 3H-mannitol passed from the apical to basolateral side of empty transwells, compared to 17% of GGTI-2418. During the same time period, only 0.2% of 3H-mannitol passed from the apical to basolateral side of Caco-2 transwells, compared to 0.7% of GGTI-2418.

**Conclusions:** These results suggest the poor oral bioavailability of GGTI-2418 in vivo.

#### P44

##### Non small cell lung cancer xenografts as preclinical models for investigations with tyrosine kinase inhibitors

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**Background:** The epidermal growth factor receptor (EGFR) plays a crucial role in human cancer. It is involved in tumor development and progression, cell proliferation and regulation of apoptotic cell death. In lung cancer the EGFR is frequently overexpressed in 50–80% of the patients. With the tyrosine kinase inhibitors (TKI) Gefitinib and Erlotinib as well as with the monoclonal antibody Cetuximab drugs are available for the treatment of patients with lung cancer. The evaluation of clinical trials using Erlotinib and Gefitinib revealed that only a small group (adenocarcinomas, women, never-smokers and people with asian origin) did benefit from the treatment with TKIs. In addition, patients with mutations in the exon 18–21 of the EGFR gene showed a better response to therapy with TKIs.

**Methods:** Fresh tumor material of patients with non small cell lung cancer (NSCLC) was subcutaneously transplanted in immunodeficient mice shortly after removal. Protein analysis was performed via Western Blot analysis and immunohistochemistry with optimized protocols. DHPLC was used for mutation analysis.

**Results:** Up to now 102 tumors have been transplanted from which 23 passagable models were generated. It could be demonstrated that the murine passages coincide with the original tumor regarding histology, the expression of the surface proteins E-Cadherin, EpCAM, the cell proliferation marker Ki-67 and in gene profiling. The analysis of the EGFR gene revealed no mutations relating to a better response to TKIs. With the exception of two models all express a wild type EGFR. Three K-ras mutations were found in the xenografts and eight different mutations could be located in the p53 gene. Furthermore, the sensitivity of the xenografts was tested against five clinically used cytotoxic agents (Etoposid, Carboplatin, Gemcitabine, Paclitaxel and Navelbine) and two EGFR inhibitors (Erlotinib and Cetuximab). It could be shown that there exist strong differences in responses among the xenografts.

**Conclusions:** In summary, we have available a panel of well characterized NSCLC xenografts correlating with the clinical situation and being able to identify biomarkers and their regulation after therapeutic interventions both at genetic and at protein level.

#### P13

##### Elevated, cell-free mitochondrial RNA in plasma identifies a poor prognosis in prostate, head and neck, kidney and colorectal cancer patients

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**Background:** Quantification of circulating plasma DNA and RNA has been studied as a diagnostic marker for cancer and as prognostic marker in cancer patients. Increased levels of cell-free nucleic acids prior to treatment were found to be associated with a poor prognosis, and a decrease after