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 know 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 MZ2 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 μM GGTI-2418 was added to the apical side of six Caco-2-containing and six empty transwells. 282 nM 3H-mannitol (2 μCi) was also added to the apical side of six Caco-2-containing and six empty transwells. The plates were then placed in a 37ºC, 5% CO_2 incubator. The plates were removed from the incubator and 60 μL 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 $\leqslant$20 nm/s using approximately 4 μCi/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 ± 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

treatment appears to be related with a response and associated with disease-free and overall survival. We recently demonstrated that circulating mitochondrial (mt) plasma DNA and RNA is a strong prognostic marker for survival in patients with prostate cancer (Clin Cancer Res 2007, Mehra et al.). The basis for this study was to assess whether these findings may be translated to other tumor types, and whether mtDNA/RNA can be seen as a pan-tumor marker.
Methods: We collected plasma from 198 cancer patients (prostate, head-and-neck, renal, and colorectal cancer) and 40 healthy subjects. Nucleic acids were isolated and mitochondrial and genomic nucleic acids were quantified using a PCR-based real-time detection and quantification method. The amplified mtDNA transcript encodes 16s rRNA, and the mtRNA transcript encodes cytochrome c oxidase subunit 1 (COX1). Using standardized cut-off points, mt nucleic acids were assessed as discriminatory marker for cancer, and as prognostic marker based on 2-year survival data.
Results: We demonstrate that mtRNA copies are increased in plasma of cancer patients compared to healthy subjects ($p = 0.001$). Patients with mtRNA copies above the normal range found in healthy controls, showed a trend to poorer survival after two-year follow-up (Log rank 3.21 with $P = 0.07$). The patients with highest mtRNA copies (above 50th and 75th percentile) showed significantly decreased survival, when compared to the patients with lower copy number (Log rank 5.05 with $P = 0.02$ for 75th percentile). We found no significant differences in survival based on mtDNA copies.
Conclusions: mtRNA copies in plasma of 198 cancer patients are increased compared to healthy controls. Patients with mtRNA copies above the normal range found in healthy controls, showed poorer survival. Standardized cutoffs for mtRNA could significantly discriminate between good and poor prognosis cancer patients, independent of cancer type. Plasma mtRNA is a prognostic factor that deserves further study as a pan-tumor marker.

P15

BRCA1 mRNA expression levels are associated with clinical responses to front-line docetaxel/gemcitabine in patients with lung adenocarcinomas in an expanded multicentre phase II study

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Background: Cis-platin based chemotherapy improves survival and symptoms control but its toxicity cannot be easily managed or prevented. Non-platinum-containing combinations offer similar survival times to the corresponding platinum-containing combinations. RRM1 plays a central role in the metabolism of gemcitabine and its overexpression in the tumor cell seems to offer resistance to the drug. BRCA1, a regulator of mitotic spindle assembly, is also associated with sensitivity to taxane. The efficacy of the docetaxel–gemcitabine (DG) regimen in patients with advanced lung adenocarcinomas in correlation with the expression of these two genes in the tumor cells was investigated.
Methods: Chemotherapy-naive patients, with locally advanced or metastatic lung adenocarcinomas and performance status (PS) $\leqslant 2$ (ECOG) received gemcitabine 1100 mg/m^2 (days 1 + 8) and docetaxel 100 mg/m^2 (day 8). rhG-CSF was given from day 9 to day 15. BRAC1 and RMM1 mRNA levels were determined by a quantitative Real-Time PCR, after RNA isolation from microdissected cells from the patients' primary tumors.
Results: Fifty-three patients (45 men and 8 women; median age 60 years) were enrolled. Amplification of at least one gene could be performed in 44. High levels of BRCA1 mRNA were significantly associated with response to treatment ($p = 0.024$), but not TTP and OS. For patients with BRCA1 mRNA levels in the upper quartile of expression a higher response rate ($p = 0.022$) and TTP ($p = 0.048$) but not OS ($p = 0.139$) was observed. Only patients with RRM1 mRNA levels in the bottom quartile experienced a benefit for the treatment with significantly prolonged TTP ($p = 0.044$) and OS ($p = 0.02$) and a trend for higher RR ($p = 0.62$). Response rate was, also, significantly higher for patients with high BRCA1/low RRM1 expression level in comparison with patients with low BRCA1/high RRM1 expression of both genes ($p = 0.016$).
Conclusions: BRCA1 and RRM1 expression is potentially an important tool for use in the management of patients with NSCLC and prospective studies are needed for the evaluation of their role for predicting differential chemosensitivity and tailoring chemotherapy in these patients.

P76

Clinical experiences with therapeutic derivatives of the anti-ED-B fibronectin immunoprotein L19

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Background: One avenue towards the development of more selective anti-cancer drugs consists of the targeted delivery of bioactive molecules to the tumor environment by means of binding molecules specific for tumor-associated markers. The use of antibodies specific to markers of neoangiogenesis is particularly attractive, in view of the ready accessibility of these structures within the solid tumor mass and the pathophysiological relevance of angiogenesis in cancer. The human monoclonal antibody L19 is specific to the extra-domain B (EDB) of fibronectin, which represents one of the best characterized and validated antigens associated with neoangiogenesis. This antibody has been produced in several formats (e.g., scFv, scFv fusions, SIP, IgG), which have been shown to preferentially localize at neoplastic sites in rodent tumor models and in patients with cancer using nuclear medicine techniques.
Methods: Phase I-II trials with L19IL2 (a fusion protein consisting of scFv(L19) and of recombinant human interleukin-2) and 131I-L19-SIP (a radioiodinated version of the L19 antibody in SIP format) have been conducted in European countries in patients with solid tumors.
Results: From November 2005 to March 2007 a Phase I trial of L19IL2 has been carried out in 21 patient with solid tumor. We explored five dose levels (5, 10, 15, 22.5, 30 Mio IU IL2 equivalent) in a modified Fibonacci dose-escalation study. L19IL2 was safely administered in an outpatient modality. All toxicity was manageable and reversible. Two dose-limiting toxicities occurred in the upper level dose (30 Mio IL2 equivalent): a Grade 2 increase of creatinine level during the first cycle and hypotension requiring vasopressor support. We identified 22.5 Mio IU IL2 equivalent as the recommend dose for further phase II study. Seven patients experienced disease stabilization (confirmed in two cases): 4 patients with renal cell carcinoma (RCC), one patient with biliary tract adenocarcinoma, one with peritoneal mesothelioma. A disease-oriented study in patients with renal cell carcinoma is still ongoing. The L19 antibody both in scFv and SIP formats has been studied in more than 70 patients with cancer in a clinical trial featuring the administration of radiolabeled product for dosimetric calculation. The SIP format showed a clearly superior targeting capability and therefore has been chosen for radioimmunotherapy in those patients featuring a tumor radiation dose which was at least ten-fold higher compared to the dose delivered to the bone marrow. The study is ongoing at the European Institute of Oncology and at two additional centers.
Conclusions: The human immunoprotein L19 represents a good-quality validated agent, which can be used for the construction of innovative anti-cancer therapeutic agents selectively directed against tumors by targeting markers of neoangiogensis. Studies using L19IL2 in combination with gemcitabine for patients with pancreatic cancer and of L19 fused to TNFa for a variety of different malignancies are ongoing.

P49

BRCA1 and BRCA2 polymorphisms and intronic variants: which pathological role?

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Background: Genetic polymorphisms are variants in individual genomes which could contribute to variability in both pharmacokinetic and pharmacodynamic drugs. The aim of our study was to evidence the possible pathological role of polymorphisms and intronic variants of BRCA1 and BRCA2 genes in familial breast cancer of Apulia population.
Methods: 110 patients affected by familial breast and/or ovarian cancer have been consecutively enrolled according to pathological features, family history and BRCA mutation risk. All of them came from Genetic Counselling Program of National Cancer Institute of Bari and were nominated for BRCA1 and BRCA2 genes genetic testing. DNA extracted from blood sample was amplified and used in pre-screening analysis by dHPLC. DNA sequencing was performed on both strands of two independent PCR products by cycle sequencing.
Results: In the present series, BRCA1 resulted mutated in 14% (15/110) while BRCA2 in 4% (5/110) of cases. We have found four different type of BRCA1 mutations: 5382insC, 4647delA, 172delC and R1495M, and five different mutations in BRCA2 gene: 2024del5, 6024delTA, 6714delACAA, Lys3326Stop and 6696delTC. We also studied the most frequent polymorphic alterations identified in both genes: in particular