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

K1183R, E1038G, S1613G, P871L, Q356R in BRCA1 gene and 203G>A 5′UTR, N372H and IVS21−66T>C in BRCA2 gene. BRCA1 5382insC pathological mutation resulted significantly associated with 2 BRCA2 polymorphisms: 203 G>A 5′UTR and IVS21−66T>C ($p < 0.05$), while BRCA1 P871L variant resulted significantly associated to both BRCA2 203 5′UTR and IVS21−66 variants ($p < 0.05$). In order to investigate the role of IVSs found in both BRCA1 and BRCA2 in determining an alternative splicing, the mutated sequences have been analyzed by the neural network splicing prediction model [http://www.fruitfly.org/seq_tools/splice.html]. Only BRCA1 IVS23+2T2C and BRCA2 IVS14–1087C>T seemed to determine alternative site of splicing. The BRCA1 S1613G and BRCA2 N372H polymorphisms are associated with older age ($p < 0.05$). BRCA1 P871L and BRCA2 IVS21−66 T>C and 203 G>A 5′UTR resulted associated with high proliferation rate ($p < 0.05$).
Conclusions: In conclusion, SNPs profile are an ideal platform for identifying germline genetic variants that lead to cancer. They provide a basis for DNA-based cancer risk classification and help to define the gene alterations that could influence biochemistry activity protein or could modify drug sensitivity.

P24

Telomerase (h-TERT) and targeting EGFR in NSCLC: A combined immunohistochemistry and chromogenic in situ hybridyzation study based on tissue microarrays

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Background: EGFR overexpression is observed in significant proportions of non-small cell lung carcinomas (NSCLC). Furthermore, overactivation of telomerase leads to cell immortalization during carcinogenesis. Our aim was the evaluation of EGFR gene and protein alterations in NSCLC and the potential role of telomerase in the regulation of its expression.
Methods: Using tissue microarray technology (ATA 100), forty ($n = 40$) paraffin embedded histologically confirmed primary NSCLCs were cored twice at a diameter of 1 mm and re-embedded into a recipient block. Immunohistochemistry was performed by the use of monoclonal antibodies anti-EGFR (31G7), and anti-telomerase/h-TERT (44F12). Also, a chromogenic in situ hybridization (CISH – SPOTLIGHT) protocol was applied based on the use of EGFR gene and chromosome 7 centromeric probes. Computerized Image Analysis was performed for the evaluation of immunohistochemistry results. Statistical analysis was based on SPSS (v 11.0).
Results: EGFR overexpression was observed in 23/40 (57.5%) cases correlating to stage ($p = 0.001$) and histological type ($p = 0.04$). Telomerase was overexpressed in all examined cases (high and moderate levels) correlating to stage ($p = 0.001$). A significant value of concordance (kappa = 0.686, 0.677−0.695) was assessed comparing telomerase and EGFR protein expression. EGFR gene amplification was identified in 2/40 (5%) cases associating to histological type ($p = 0.027$) and chromosome 7 aneuploidy in 7/40 (17.5%) cases.
Conclusions: NSCLC is characterized by rare cases of EGFR gene amplification and this genetic event maybe affect the efficacy of targeted therapeutic strategies based on monoclonal antibodies. Also, the strong concordance between EGFR and telomerase overexpression demonstrates that the enzyme is potentially involved in the growth-controlling gene expression.

P87

Dynamic contrast-enhanced MRI (DCE-MRI) as imaging biomarker in non-small cell lung cancer (NSCLC)

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Background: Compared to traditional anticancer agents, the anti-neoplastic effect of targeted agents may be cytostatic rather than cytotoxic. Therefore, determining the activity of targeted drugs by RECIST criteria may underestimate the actual activity of the agents.
The aim of the current study was to evaluate the feasibility of DCE-MRI as an imaging biomarker to assess the combined biological activity of bevacizumab and erlotinib in advanced NSCLC patients.
Methods: A total number of 46 patients were enrolled in a multicenter phase II trial with bevacizumab (15 mg/kg q 3 week) and erlotinib (150 mg daily) as first line treatment. Complete DCE-MRI data are available from 17 patients (37%). DCE-MRI was used to measure changes in endothelial transfer (Kps) within the tumor.
DCE-MRI was performed at baseline, week 3 and week 6. A 1.5 Tesla MRI scanner was used. DCE-MRI included five pre-contrast T1-weighted measurements (3D fast spoiled gradient echo, TR 4.5 ms, echo time 2 ms, 5 transversal slices (slice thickness 10 mm), FOV 350 mm, matrix 144×256) with different flip angles (FA) to determine the T1 relaxation time in the blood and tissue before contrast arrival. This was followed by the DCE series using the same sequence, but with an FA of 35, containing 30−35 scans of 2 seconds each. We used a fast data acquisition period (2 s) to freeze breathing motion.
A pharmacokinetic two-compartment, bidirectional exchange model was used to determine the tumour endothelial transfer coefficient (Kps) by a region of interest (ROI) that covers the whole tumour cross-section. The value of Kps in each individual voxel was also determined to assess intratumour heterogeneity by the 95th percentile tumour values. The analysis was performed by using a functional form of AIF.
Due to considerable variability in the baseline enhancement of tumours, the relative rather than the absolute Kps was used.
Results: The relative Kps was significantly decreased at week 6, with an average relative Kps of 87% compared to baseline Kps ($p < 0.05$). The intratumor heterogeneity assessed by the 95th percentile tumor values was also significantly altered at week 6 ($p = 0.001$). These results reflect an alteration in tumor perfusion and permeability during treatment. Correlation with response data as determined by RECIST criteria will follow.
Conclusions: These preliminary results show that DCE-MRI is a feasible technique to detect early effects of combined biological agents in advanced NSCLC patients.

P63

A multiplex XP-PCR gene expression assay for monitoring efficacy of inhibitors of heat shock protein 90

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Background: Heat shock protein 90 (Hsp90) is part of a protein complex that facilitates the maturation and increased stability of many physiologically important proteins including those critical for tumor cells. These proteins, also called clients of Hsp90, include proteins associated with breast cancer like Her-2, EGF-R and ER. Inhibition of Hsp90 results in disruption of the "chaperone complex" and the degradation of the client proteins in a proteasome-dependent manner. Because of their critical role in producing functional proteins critical to oncogenesis inhibitors of Hsp90 have been studied as possible therapeutics for the treatment of cancer, including breast cancer. In fact, Hsp90 proteins in tumor cells have found to be preferentially targeted by their inhibitors. There are many HSP inhibitors being tested in clinical trials including 17-allylamino-17-demethoxygeldanamycin (AAG) and others but the search continues for Hsp90 inhibitors that are more potent, more specific, and less toxic. Because of this there is a need for an early screen to determine which candidate compounds are impacting Hsp90 and so it's associated complex in order to eliminate the large scale production and testing of molecules that will not inhibit Hsp90 in a safe effective manner. Inhibition results in alteration of the expression of many genes associated with Hsp90 and expression changes of these molecules can be used to monitor the impact a putative Hsp90 inhibitor is having on its target protein as well as those associated with it.
Methods: We have carried out extensive literature searches, obtained opinions from experts in the field, and carried out data mining of the connectivity map of Hsp90 inhibitors to obtain a 30-gene set to monitor the impact of Hsp90 inhibitor candidates. A single multiplex RT-PCR assay was then produced that can monitor the expression of these 30 genes in one reaction. To test the power of this gene set RNA from an Hsp90 inhibitor in vitro study was analyzed. Briefly MCF-7 cells were treated with multiple concentrations of radicicol, geldanamycin and novobiocin. The RNA was then extracted and analyzed using the multiplex XP-PCR assay.
Results: Gene expression data for approximately 30 genes will be presented for MCF-7 cells at multiple time points that have been treated with 3 HSP90 inhibitors at multiple concentrations. The relevance of these gene expression changes will be discussed.
Conclusions: Significant and relevant gene expression changes were produced by the Hsp90 inhibitors and were successfully monitored in a single assay. In conclusion we have developed an assay that can be used for evaluation of potential Hsp90 candidates and could also be used to monitor lead inhibitor candidates in the clinic.