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.