Materials and Methods: In order to identify tumor cells in urine, we loaded tumoral or normal proliferating cells in a urine sample of healthy volunteers. The cells were recovered by centrifugation, fixed and attached to positively charged slides. We performed a double FISH technique using specific ODN probes for each one of the ncmtRNAs; the SncmtRNA was identified with a specific probe labeled with Alexa fluor 488, and the ASncmtRNA with a Texas red labeled probe. The same set of experiments was performed in suspension cells, and analyzed by flow cytometry. Finally, patients' urine samples were analyzed in the same way.
Results: The double FISH approach developed here showed high sensitivity and specificity in the differentiation of tumor from normal cells in a mixture obtained from human fluids, constituting a promising new and highly accurate cancer diagnostic method.
Conclusion: We developed a double FISH approach with specific ODN probes complementary to SncmtRNA and ASncmtRNA which can identify normal or tumoral status in single cells. The same results were corroborated by flow cytometry. This approach, based on visualization of the expression of these new cancer biomarkers can be used as a novel approach for the non invasive diagnostis of bladder and prostate cancer.

PP23

Immunohistochemical marker profile in colorectal cancer: Our experience

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Background: Our aim was to examine whether certain immunohistohemical molecular markers, specifically PCNA, Ki-67 and p53, could be used to predict the tumor response of rectal cancer to determine the overall and disease-free survival rates of patients following adjuvant therapy.
Materials and Methods: In "Sveti Vracevi" Hospital in Bijeljina 301 patients suffering from colon cancer received treatment from 1st January 2000 to 31st December 2008. We analyzed the prognostic value of PCNA, Ki-67, and p53 by immunohistochemistry on formalin-fixed, wax-embedded sections in a series (n = 153) of stage III (Dukes C) colorectal cancers. An immunohistochemical score based on the intensity of immunoreactivity and, where relevant, the proportion of immunoreactive cells was established for each marker. We elected to investigate PCNA, Ki-67 as a marker of cell proliferation indices and p53 oncogenes/tumor suppressor gen because these markers have been demonstrated in a number of studies to have potential value in defining populations of individuals who either may or may not benefit from the use of adjuvant chemotherapy.
Results: Using 9 years of follow-up data, our retrospective analysis demonstrated an association between PCNA intensity (relapse-free survival [RFS]: risk ratio [RR] = 1.47, P = 0.01; overall survival [OS]: RR = 1.49, P = 0.002), Ki-67 (RFS: RR = 0.71, P = 0.05; OS: RR = 0.6, P = 0.05), and p53 (RFS: RR = 1.42, P = 0.01; OS: RR = 1.19, P = 0.0013) for RFS and OS. High PCNA intensity levels and positive p53 staining were associated with a worse outcome. Tumors containing a high percentage of Ki-67-positive cells enjoyed an improved outcome compared with those patients whose tumors contained relatively few positive cells. An interaction with treatment was not identified for any of the markers.
Conclusion: Immunohistochemical analysis is not used in the routine analysis of colon cancer. This retrospective investigation demonstrated that PCNA, and p53 staining each had significant prognostic value for patients colon carcinoma. There was not statistically significant difference in the survival rate of patients with positive immunohistochemical Ki-67 values in relation to the patients with the negative values.

PP84

Na+/H+ exchanger regulatory factor 1 (NHERF1) and angiogenesis in familial breast cancer

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Background: NHERF1 is a scaffolding protein that recruits membrane and cytoplasmic proteins into functional complexes. Our recent evidences demonstrate that in breast cancer NHERF1 overexpression is associated with increased tumor hypoxia and poor prognosis. Hypoxia is implicated in tumor proliferation and angiogenesis that interests neoplastic regions. In fact, the hypoxia-inducible factor-1 (HIF-1α), mediating trascriptional activation of vascular endothelial growth factor (VEGF) gene, is considerated the central initiator of angiogenic activity in tumor. Our aim was to determine NHERF1 expression on a series of familial and sporadic breast cancer patients and examine the relationship with other progression markers (HIF-1α, VEGFR 1 and HER2/neu).
Materials and Methods: NHERF1, VEGFR1, HIF-1α and HER2/neu proteins expression were analysed by immunohistochemistry on a tissue microarray, including 94 familial and 93 sporadic breast tumors. Cytoplasmic, membrane and nuclear NHERF1 reactivity was analysed.
Results: Membrane NHERF1 expression was significantly higher in sporadic than familial patients (p = 0.000). Familial cancers showed high levels not statistically significant of cytoplasmic NHERF1 expression compared with sporadic cancers. In familial breast patients, cytoplasmic NHERF1 overexpression was related with VEGFR1 positivity, in 48.3% of cases (p = 0.009). Furthermore, high levels of nuclear NHERF1 in familial cancers were associated with positive HIF1α tumors (p = 0.003). No significant correlation was found between NHERF1 and HER2/neu. In contrast, 48% of overexpressing HER2/neu sporadic tumors, showed a significant association with high cytoplasmic NHERF1 levels (p = 0.007). Moreover, in these tumors, nuclear NHERF1 protein is significantly correlated with HIF1α expression (p = 0.019). Any NHERF1 significant association between both VEGFR1 and HIF-1α was found.
Conclusion: In familial breast cancer, NHERF1 resulted strongly related with VEGFR1 and HIF-1α proteins with respect to sporadic tumors. In this context, we suggest an emerging role of NHERF1 in angiogenesis.

PP19

Randomised phase III clinical trial of 5 different arms of treatment for patients with cancer-related anorexia/cachexia syndrome (CACS)

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Background: Cancer-related anorexia/cachexia syndrome (CACS) is a multifactorial syndrome characterized by tissue wasting, loss of body weight, particularly of lean body mass (LBM), metabolic alterations, fatigue, reduced performance status, very often accompanied by anorexia.
Materials and Methods: In April 2005 we started a phase III randomised study to establish the most effective and safest treatment of CACS addressing as primary endpoints: LBM, resting energy expenditure (REE), total daily physical activity, serum IL-6, TNF-α, and fatigue evaluated by the Multidimensional Fatigue Symptom Inventory − Short Form (MFSI-SF). The sample size was 475 patients (pts). Eligibility criteria: hystologically confirmed tumors of any site; weight loss $\geqslant$5% in the last 3 months and/or abnormal values of proinflammatory cytokines and oxidative stress parameters predictive of the onset of CACS; life expectancy >4 months. Patients could be treated with either antineoplastic therapy with palliative intent or supportive care. All pts enrolled received as basic oral treatment: polyphenols + alpha lipoic acid + carbocysteine + Vitamins ACE. Pts were then randomised to one of the following 5 arms: (1) Medroxyprogesterone Acetate (MPA)/Megestrol Acetate (MA); (2) Pharmaco-nutritional support containing EPA; (3) L-carnitine; (4) Thalidomide; (5) MPA/MA + Pharmaco-nutritional support + L-carnitine + Thalidomide. Treatment duration was 4 months. Interim analyses were planned after every 100 randomized pts.
Results: At April 2009, 332 pts were randomized and 310 were evaluable: M/F 180/130, mean age 62 yrs (range 30−84), 96% were stage IV. A first interim analysis on all 125 pts enrolled showed a significant worsening of LBM, REE and fatigue in arm 2 (Pharmaco-nutritional support containing EPA) in comparison to the others and it was withdrawn from the study. A second interim analysis after the enrolment of 204 pts showed arm 1 (MPA/MA) significantly less effective than the others for primary efficacy endpoints: it was withdrawn from the study.
Statistical analysis at April 2009 showed a significant increase of LBM (by DEXA) and decrease of REE, IL-6 and fatigue in arm 5. As for safety, the treatment was overall well tolerated and patient compliance was very good.
Conclusion: The results so far seem to suggest that the most effective treatment for cancer pts with CACS should be the combination regimen.

PP106

DNA promoter methylation in breast cancer as possible biomarkers for screening breast cancer and association with molecular breast cancer subtypes

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Background: Aberrant DNA methylation has been found in breast cancers associated with the loss of expression of regulatory genes for growth.
Purpose: To investigate the association between DNA methylation as possible biomarkers for screening breast cancer and association with clinico-pathological and molecular breast cancer subtypes.
Materials and Methods: We quantified methylation levels of genes; APC, RAR-Beta, E-Cadherin, ESR1 and 14-3-3σ gene in 107 women with breast cancer and 108 control subjects. A sensitive PCR quantitative technique was used to analyze the utility of hypermethylation gene promoter regions.

Tumours were classified as phenotype basal, luminal A, luminal B and phenotype HER2+.
Results: The mean serum values of methylated gene promoters significantly differed between breast cancer patients and healthy controls ($p = 0.0112$ for ESR1 and $p = 0.0047$ for 14-3-3σ). When their results were combined, it was found that hypermethylation of these two genes differentiated between breast cancer patients and healthy controls ($p < 0.0001$) with a sensitivity of 81% (95% CI: 72−88%) and specificity of 88% (95% CI: 78−94%). Presence of methylated ESR1 in serum of breast cancer pts was associated with ER-negative phenotype ($p = 0.0179$) and presence of methylated 14-3-3σ was associated with T3−4 stage (OMS) ($p < 0.05$) and nodal positive status ($p < 0.05$). We observed that methylated ERS1 was preferably associated with phenotype Basal Like and worse interval progression free and survival global though $p > 0.05$ and HER2+ subtype was correlation with significant more frequent methylation gene ($p < 0.05$). With a median follow up of 6 years, we found that patients with a significant decrease of sera methylated levels of both genes after surgery had better time to progression an overall survival respect patients without this observation
Conclusion: Our study identifies the presence of variations in global levels of methylation promoters genes in healthy controls and breast cancer with different phenotype classes and shows that these differences have clinical significance. In the future this panel of genes detected could be useful as markers for early detection of breast carcinoma and perhaps as a prognostic and predictor factor response to treatment. Although numerous issues remain to be resolved, the quantitative measurement of circulating methylated DNA is a promising tool for cancer risk assessment even this may be of significance in the assessment of targeted therapy resistance related to ER and HER2 status in breast cancer patients

PP83

Implementation and cost effectiveness of intra-operative qRT-PCR analysis of sentinel lymph nodes (SLN) in breast cancer

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Background: Accurate intra-operative SLN assessment enables axillary clearance to be completed immediately in node positive breast cancer patients.
Materials and Methods: An analysis of 254 consecutive patients with core biopsy proven breast cancer who underwent SLN biopsy in a single centre. 99mTc and Patent blue dye guided localisation with lymphoscintigraphy were used to identify SLN's which were harvested. The nodes were sectioned at 2 mm intervals and alternate slices were analysed using an FDA-approved and CE-marked commercial diagnostic RT-PCR assay (GeneSearchTM BLN Assay, Veridex, LLC) for mammaglobin (MG) and cytokeratin 19 (CK19). Remaining slices of node were sent for histological analysis, which included CK19 immunohistochemistry at multiple levels. Whilst the assay was being carried out, the surgeon performed the breast tumour resection. Operative time and hospital bed stay was analysed as part of a health economic evaluation.
Results: A total of 255 SLN in 250 patients including 5 with bilateral breast cancer were evaluated. There were no localization failures. The intra-operative assay showed positive SLN in 72 patients. There was 100% detection of macrometastases within sentinel nodes analysed by GeneSearchTM. Overall concordance between histological nodal status, including micrometastases, and GeneSearchTM analysis was 95% (Sensitivity 96%, Specificity 95%). The assay takes 40 minutes. A health economic evaluation suggests that this assay is cost neutral to the NHS, with substantial benefit
Conclusion: Intra-operative assessment of SLN in breast cancer, using an RT-PCR based assay is a safe, acceptable and accurate technique. This should allow a reduction in the frequency of delayed axillary clearance surgery with minimum unnecessary morbidity and no additional overall cost to the healthcare provider.

PP44

Development of a quantitative scoring algorithm for a Dual-Hapten, Dual-Color ISH assay (DDISH) to determine HER2 gene status

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Background: The HER2 gene, located on chromosome 17, is amplified in ~15−20% of patients diagnosed with invasive breast carcinoma. Determination of HER2 gene status is critical for selecting an appropriate therapeutic course of action, and several assays are commercially available for in situ hybridization-based quantification of the HER2 gene. However, essential data on developing scoring algorithms that maximize precision and efficiency are lacking. A new, fully automated assay (INFORM HER2 Dual ISH DNA Probe Assay) uses Red ISH to detect chromosome 17 and silver in situ hybridization (SISH) to detect the HER2 gene: the staining result is obtained in <12 hours and interpreted using standard brightfield microscopy. This study's goal was to develop a precise yet efficient quantitative scoring algorithm for the HER2 Dual ISH assay.
Materials and Methods: 24 specimens representing the dynamic range of HER2 status were stained with the Dual ISH assay: the cohort was spiked with borderline and low-level amplified cases. Raw counts from 100 nuclei/specimen were obtained and the HER2 status obtained from the 100 nuclei was considered "truth". Multi-stage Monte Carlo statistical methods were used to: (1) vary the distance from the cut-off value of 2.0 and (2) vary the number of nuclei counted. Three goals were to determine: (1) the number of nuclei needed to obtain a "stable" diagnosis; (2) the necessary "grey" zone window; and (3) the number of additional tumor nuclei that should be counted within that window.
Results: Quantification of as few as 10 nuclei resulted in a precise HER2 status diagnosis in cases outside of the 1.8−2.2 equivocal zone. Little statistical gain was achieved by expanding the window beyond the 1.8−2.2 range. Initially counting 20 nuclei for all cases, then counting 20 additional nuclei for equivocal cases resulted in a consistent classification rate of 96%.
Conclusion: The HER2 Dual ISH assay algorithm results in a stable and precise HER2 gene status determination. A combination of 20 nuclei chosen initially followed by an additional 20 nuclei for cases whose HER2/CHR17 ratio falls in the range of 1.8−2.2 is recommended. These data support the clinical need and significance for an equivocal zone within the 1.8−2.2 window.

PP58

Novel potential therapeutic targets for cholangiocarcinoma identified by array comparative hybridization

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Background: Cholangiocarcinoma (CC) is a devastating malignancy, with surgery presently offering the only chance of cure. Conventional chemotherapy is ineffective in CC, with a dismal 5-year survival. We evaluated DNA copy number alterations between intrahepatic (ICC), perihilar (PHCC), and extrahepatic (ECC) cholangiocarcinoma, to identify novel potential therapeutic targets.
Materials and Methods: 22 cases were analysed (7 ICC, 5 PHCC, 10 ECC) using DNA from FFPE archival specimens. Array CGH was performed using 1Mb BAC arrays. Spatial normalisation, circular binary segmentation, calling of gains and losses using the CGHcall method as well as secondary analyses were done within the R statistical language environment.
Results: This study found gains at 17p13.3-q21.33, 17q22-q25.3, and 22q11.1-q13.3 in all cases. Common gains were found in all cases of ICC and PHCC at 11q12.2-q13.4, 19p13.11-p13.3, and 19q13.11-q13.43. Alterations were least frequent among ECC, with no alteration being exclusively seen in all cases of ECC alone. Common regions of alteration detected among ICC and perihilar CC, but not ECC supports the hypothesis that carcinogenesis in these tumours is different, and may potentially require different targeted therapies. The clone covering c-erb-B2 at 17q12 was amplified in all cases of CC, potentially highlighting a role for monoclonal antibodies such as Trastuzumab, or small molecule tyrosine kinase inhibitors such as lapatinib. All cases of ICC and PHCC, and 77.7% ECC showed gain at 11q12.2-q13.4 containing VEGF-B (Vascular endothelial growth factor B) which is known to be overexpressed in ICC and PHCC and therefore may be an attractive target for treatment in these types of CC, possibly being less effective in ECC with this region affected slightly less frequently. This could be achieved using a global VEGF inhibitor such as Cediranib (AZD2171). EGFR is located at 7p11.2, and this region showed gain in 42.9% ICC and 20% PHCC, however no gain was detected in ECC. Alterations of EGFR in ICC and PHCC highlights a potential therapeutic target, for example with Getifinib and Erlotinib, or EGFR/ERB2 inhibitor lapatinib, for tailoring treatment in these patients. This will also enable the identification of patients that will not benefit from this treatment, such as those with ECC, and therefore not be subjected to negative side effects with no benefit.
Conclusion: In this study c-erb-B2, VEGF-B and EGFR have been identified by array CGH as attractive novel therapeutic targets in cholangiocarcinoma.