therapies that augment intratumoral effector T lymphocytes recruitment, while inhibiting Treg accumulation, may be worthy of pursuit.

PP138

A whole blood RNA transcript-based model to predict biopsy Gleason score in newly diagnosed prostate cancer patients

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Background: Histologic grading of prostate cancer is a critical determinant of the biology of prostate cancer and is strongly associated with prognosis. Biopsy Gleason scores (GS) are typically assigned by pathologists, but are subject to variable interpretations. In addition, prostate needle biopsies may underestimate the true score in up to 15−20% of patients due to sampling error. Better methods are needed to assess Gleason score and, by extension, aggressiveness of prostate cancer.
Materials and Methods: From August 2006 to October 2008, a prospective cohort of 198 men with newly diagnosed, localized, untreated prostate cancer consented to the collection of whole blood in PAXgene™ Blood RNA tubes for gene expression analysis. 216 inflammation and cancer-related genes (Source MDx Precision Profiles™) were assayed using optimized Q-PCR technology and logistic regression and latent class (LC) methods were used to develop a 5-gene model which distinguished higher Gleason score (4+3 or higher) from lower Gleason score (3+4 or 3+3) cancers.
Results: In evaluating all 1-, 2- and 3-gene models based on 216 target genes, the best 3-gene model distinguishing higher versus lower GS cancers included CD4, TP53, and E2F1. The best 2-gene model which separated Gleason 6 cancers from all 7 or higher cancers included CASP9 and SOCS3. Together, the combined 5-gene model was able to predict GS 4+3 or higher versus 3+4 and 3+3 with sensitivity of 0.75 and specificity of 0.63, and an AUC of the ROC curve of 0.73 ($p = 8.2 \times 10^{-6}$). In an exploratory LC model which assumes that GS is an imperfect reference test for 'aggressiveness', the combined 5-gene model is able to accurately predict 'aggressive' cancers with sensitivity of 0.84 and 'non-aggressive' cancers with specificity of 0.83. ROC curves for the models predicting 'aggressiveness' of cancer demonstrated AUC 0.91 ($p < 8 \times 10^{-6}$).
Conclusion: Models distinguishing between higher and lower GS cancers were developed based on whole blood RNA transcript measurement of inflammation and cancer-related genes. Furthermore, LC models which assume that some prostate biopsies may miss underlying aggressive disease in some patients suggest that molecular tests could improve the diagnostic accuracy of currently available tests. Validation of this and other models predicting GS and 'aggressiveness' is planned.

PP90

Hereditary transmission of polymorphisms in familial breast cancer

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Background: Recently, it has been demonstrate that many SNPs could predispose people to disease. Genetic alterations in BRCA1 and BRCA2 genes lead to a higher predisposition to breast and ovarian cancer and confer a significantly higher risk of endometrial, pancreas, cervix and prostate cancers. Many studies are focalizing the attention about a possible biological interpretation of the unknown and polymorphic variants in BRCA1 and BRCA2 genes to understand if they have a pathogenic role. The aim of our study was to clarify the role of BRCA SNPs as susceptibility markers of risk studying the genealogic transmission of coding and non coding variants of BRCA1 and BRCA2 genes in family's members enrolled by the genetic counselling program.
Materials and Methods: 20 families, in which DNA from at least one first degree relative was available, have been studied for both pathological mutation and polymorphic variants transmission. BRCA1 and BRCA2 variants have been investigated by dHPLC and direct sequencing.
Results: As expected, pathological mutations were mendelian transmitted. BRCA1 5382insC mutation has been individuated in 7 patients but only 4 families showed a mendelian transmission in at least one first-degree relative while BRCA1 R1494M and BRCA2 2150insTA and 6710delACAA mutations have been found to be transmitted in different family members belonging to the same genealogic tree. Interestingly, polymorphic coding and non coding variants were present in relatives of the studied family while transmission for unknown variant was not evidenced. In particular in our series, the BRCA2 Lys3327Stop, an unknown variants, has been individuated only in a female 63 years old with familial breast cancer history but not in any analyzed relatives, while BRCA1 P871L, E1038G and K1183R segregated together and were all transmitted to first and second degree relatives. Patients with these clusterized SNPs seemed to have peculiar pathologic features as higher differentiated tumors (71% was G1−2, $p = 0.05$) and a trend for less probability to present mutation in BRCA1 or BRCA2 (74% of Myriad >10%, $p = 0.06$).
Conclusion: The significant association of some SNPs with tumor aggressiveness or susceptibility risk lead to underline possible polymorphism transmission pathological significance. SNP maps and modality of their transmission could help to identify further susceptibility markers and provide a basis for a better DNA-based cancer classification.

PP43

RCL2 fixation of neurosurgical specimens: well preserved histomorphology and DNA integrity

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Background: Neurosurgical tumour tissue specimens are usually fixed in formalin to allow optimal histopathological tumour typing. However, formalin fixation damages nucleic acids and impairs molecular biomarker research. RCL2 is a commercial alcohol-based fixative that has been described to preserve histomorphology and nucleic acid integrity in non-CNS neoplasms. In this study, we performed comparative evaluation of the effect of formalin- and RCL2-fixation on histomorphology and DNA integrity in neurosurgical specimens.
Materials and Methods: We included neurosurgical specimens of 13 brain tumours (2 diffuse astrocytomas, 1 anaplastic astrocytoma, 1 anaplastic oligoastrocytoma, 5 glioblastomas, 1 pleomorphic xanthoastrocytoma, 1 meningioma, 1 medulloblastoma, 1 metastasis). Of each patient, 1 tumour sample was fixed in standard 4.5% buffered formaldehyde solution (FOR) and 1 tumour sample was fixed in RCL2 solution. Fixation times ranged from 1 to 8 days before paraffin-embedding. Of each tissue block, we performed: (1) hematoxylin and eosin staining and neuropathological evaluation, (2) DNA extraction using the QIAamp DNA Mini Kit, (3) measurement of OD260nm to determine DNA quantity, (4) measurement of OD 260nm and 280nm to determine DNA quality, (5) polymerase chain reaction using primers for DNA fragments of 100, 200, 300, 400 and 600 base pairs (bp) followed by gel electrophoresis to evaluate suitability of the material for PCR amplification.
Results: Histomorphology was comparable between FOR- and RCL2-fixed tissue samples. DNA extraction from RCL2-fixed tissue specimens (DNA-RCL) resulted in significantly higher yield than DNA extraction from FOR-fixed tissue specimens (DNA-FOR) by a median factor of 2 (range 0.33 to 4.44) ($p = 0.006$, paired T-test). OD 260/280 ratio was $\geqslant$1.7 in 13/13 DNA-RCL and 11/13 DNA-FOR samples. DNA was amplifyable up to a length of 600bp in 12/13 DNA-RCL and 9/13 DNA-FOR specimens.
Conclusion: (1) In our hands, the histomorphology of RCL2-fixed neurosurgical specimens has no significant disadvantages compared to FOR-fixed tissue samples and allows tumour typing according to WHO criteria. (2) RCL2-fixation results in higher DNA yield and quality than FOR-fixation. Thus, RCL2-fixation may be of advantage for comprehensive characterization of neurosurgical specimens regarding both histopathology and molecular analyses.

PP98

High expression of hsa-miR-30a-3p, hsa-miR-30c and hsa-miR-182 predict favorable outcome on tamoxifen treatment in patients with recurrent breast cancer

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Background: Altered miRNAs expression levels have been described in breast cancer (BC) and reported to be associated with metastasis, prognosis and treatment response, suggesting that miRNAs play an important role in BC. We have explored the association of selected miRNAs and tamoxifen clinical response.
Materials and Methods: In a series of 246 ER+ recurrent BC patients treated with tamoxifen, five selected miRNAs, hsa-miR-30a-3p, hsa-miR-30c hsa-miR-182, hsa-miR-187 and hsa-miR-422a, were quantified by real time PCR.
Results: Univariate logistic regression analysis, using log-transformed continuous variables, showed that high levels of hsa-miR-30a-3p (odds ratio [OR]: 1.51, 95% confidence interval [95% CI]: 1.16−1.96; $P = 0.002$), hsa-miR-30c (OR: 3.87, 95% CI: 2.16−6.93; $P < 0.001$), and hsa-miR-182 (OR: 1.53, 95% CI: 1.09−2.16; $P = 0.013$), were associated with clinical benefit of tamoxifen therapy. In multivariate analysis, including traditional predictive factors, of the miRNAs tested, only hsa-miR-30c was significantly associated with clinical benefit (OR: 3.14, 95% CI: 1.61−6.12; $P = 0.001$).

In order to assess the progression free-survival (PFS) time, miRNA expression levels were categorized in quartiles. In analogy to their relationship with clinical benefit, the same three miRNAs were also associated with longer PFS: hsa-miR-30a-3p (hazard ratio [HR]: 0.51, 95% CI: 0.34−0.76; P = 0.001), hsa-miR-30c (HR: 0.47, 95% CI: 0.31−0.70; P < 0.001), and hsa-miR-182 (HR: 0.57, 95% CI: 0.37−0.86; P = 0.008). Global testing using available global gene expression data significantly associated the 3 predictive miRNAs with differential gene expression of HER-2, Rac-1 and Ceramide signaling pathways.
Conclusion: This study shows associations between hsa-miR-30c, hsa-miR-30a-3p and hsa-miR-182 expression levels and clinical benefit to treatment with first-line tamoxifen for recurrent BC and describes pathways putatively involved in these associations. Assessment of these miRNA levels and their pathways in primary tumors could help to improve treatment strategies for patients with recurrent ER+ breast cancers.

PP122

Intraoperative tissue fluorescence using 5-aminolevolinic acid (ALA) is more sensitive than contrast-MRI or amino acid (FET)-PET guided glioblastoma (GBM) surgery

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Background: The ability of 5-ALA to visualize white matter infiltration zones of GBM compared to MRI contrast or [18F]fluoroethyltyrosine positron emission tomography (PET) was investigated.
Materials and Methods: Fluorescence tissue margins were mapped intraoperatively by neuronavigation and compared to pre- and postoperative MRI and FET-PET scans in 3 glioblastoma patients (2 temporal, 1 fronto-central tumor).
Results: In all patients, the intraoperatively detected 5 ALA fluorescence exceeded the MRI contrast tumor areas and FET-PET uptake, verified by intraoperative neuronavigation. Furthermore, all patients received complete resection of contrast affine tumor parts, which was verified by contrast MRI scans within 24 hours postoperatively. Although intraoperative fluorescence tissue was generously left in place, because it was estimated as tissue at risk for neurological deterioration, no contrast affine tissue could be detected by postoperative MRI. Additionally, postoperative FET-PET uptake was demonstrated only in one patient as a small residual spot. FET-PET did not show any uptake at the intraoperatively mapped large marginal areas of 5 ALA fluorescence, left in place in account of neurological preservation
Conclusion: Our findings demonstrate that 5 ALA fluorescence is more sensitive than FET-PET and MRI contrast uptake in detecting glioblastoma multiforme white matter infiltration zones.

PP127

INHANCE (Iressa™ Novel Head and Neck Chemotherapy Evaluation) randomised phase II trial: clinical findings and associated translational research into EGFR-related biomarkers in tumour and skin biopsies

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Background: The INHANCE randomised phase II trial (1839IL0544) explored the feasibility and benefits of adding an EGFR tyrosine kinase inhibitor (gefitinib, AstraZeneca, Macclesfield, UK) to induction chemotherapy with cisplatin and 5-fluorouracil in patients with newly diagnosed squamous cell carcinoma of the head and neck. Associated translational research enabled the unique investigation of EGFR-related signalling changes induced by chemotherapy and a comparison with those elicited by chemotherapy plus gefitinib in randomised therapy-naïve patients.
Materials and Methods: Patients were openly randomised to two cycles (q21 days) of cisplatin (100 mg/m^2 day 1) and 5-fluorouracil (1g/m^2 days 1−4) with or without oral daily gefitinib (250 mg days 1−42). Tumour and skin biopsies were collected pre-treatment and after 14 days of therapy. Given the limitations of immunohistochemistry, we analysed EGFR-related signalling by western blotting and a novel electrochemiluminescent immunoassay (Meso Scale Discovery, Gaithersburg, MA, USA) that we have previously validated in vitro and in vivo.
Results: Thirty-eight patients were randomised. The combination was well tolerated. 53% and 71% of patients donated paired tumour and skin biopsies respectively. Each biopsy yielded sufficient lysate for two western blots and three immunoassays. Using the two independent techniques, there was good concordance between expression and activation of EGFR and AKT in 80% and 86% of tumour biopsies respectively. Signalling changes in skin biopsies only reflected those seen in tumour biopsies in 50% of patients. Two of three patients treated with chemotherapy plus gefitinib who developed a rash demonstrated a reduction in EGFR phosphorylation in tumour, but not skin, biopsies.
Conclusion: Correlation with western blotting shows that the electrochemiluminescent immunoassay is a useful method for quantifying signalling changes in small volume clinical samples but skin was not a reliable surrogate tissue for tumour. Cytotoxic chemotherapy alone elicited changes in EGFR phosphorylation that confounded the interpretation of gefitinib-induced alterations. We conclude that biomarkers optimised for the evaluation of targeted therapies as single agents may be compromised when combined with conventional therapy.

PP63

Predictive markers in patients with upper gastrointestinal (GI) cancers treated with erlotinib and bevacizumab in a multicenter phase II trial

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Background: We investigated the role of several growth factors, growth factor receptors, and markers of ischemia as predictors of response and survival in patients treated with VEGF and EGFR targeted therapy.
Materials and Methods: This exploratory study evaluated the predictive value of plasma levels of vascular endothelial growth factor A (VEGF-A), platelet derived growth factor AB and BB (PDGF-AB and PDGF-BB), soluble Fms-related tyrosine kinase 1 (sFlt-1 or sVEGFR-1), growth differentiation factor 15 (GDF15), hepatocyte growth factor (HGF), high sensitivity troponin T (hsTnT), and pro brain natriuretic peptide (proBNP) in patients with advanced upper GI cancer in progression after chemotherapy. Patients were treated with drugs targeting angiogenesis (bevacizumab) and the EGFR pathway (erlotinib) in a multicenter phase II trial (ASCO GI 2009, abstract #170). Plasma was collected at baseline and weekly during the first 4 weeks. Plasma was analysed using quantitative immunoassays. Results of baseline samples and changes in plasma levels of the markers were correlated to progression-free survival (PFS) and clinical benefit (CB), defined as stable disease (SD) or partial response (PR).
Results: Baseline plasma was available in 79 out of 100 patients (median age 62 [25−78]) with carcinoma in esophagus (36%) (adeno [30%], squamous [6%]), stomach (12%), pancreas (33%), and biliary tract (19%). Three patients had PR, 28 SD, 22 PD, and 26 were not evaluable. Patients with baseline PDGF-AB in the upper quartile had significantly longer PFS (HR: 0.45, 95% CI: 0.23–0.85). Patients with proBNP below median had significantly better PFS than patients with levels above (HR: 1.73, 95% CI: 1.06–2.83). The remaining markers failed to predict CB or PFS.
We observed a decrease of VEGF-A (11.36, 95% CI: 6.16–32.41), HGF (0.07, 95% CI: 0.01–0.28), and GDF15 (0.33, 95% CI: 0.08–1.31), and a trend towards decrease of proBNP (22.34, 95% CI: −0.68–159.29) between baseline and after 1 week of therapy. However, VEGF-A was in most cases undetectable in plasma after start of therapy.
Conclusion: Decrease in plasma levels of VEGF-A, HGF, proBNP, and GDF15 was observed during EGFR/VEGF-targeted therapy. The high frequency of undetectable VEGF-A after initiation of therapy could be attributed to its binding to bevacizumab.
High levels of PDGF-AB and low levels of proBNP seem to predict longer PFS. These findings are of biological and therapeutic relevance and warrants further investigations.

PP103

RT-PCR-based UCA1 expression detection in urine samples as non-invasive reliable method for urothelial cancer diagnosis

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Background: Bladder cancer is among the five most common malignancies in industrialized countries. There are currently no satisfactory markers