and Cox proportional hazard models showed that miR-221 down-regulation was linked to tumor progression and recurrence.
Conclusion: Our results suggest that progressive miR1-221 down-regulation is a hallmark of metastasis and a novel prognostic marker in prostate carcinoma. This suggests that miR-221 has potential as a diagnostic marker and therapeutic target.

PP59

Prognostic significance of αB-crystallin, vimentin and HSP 27 association in primary breast cancer

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Background: αB-crystallin is a heat shock protein, which function as stress-induced molecular chaperones to inhibit the aggregation of denatured proteins. Previous studies have identified αB-crystallin as a marker of poor prognosis for breast cancer and have suggested that it is an excellent marker for tumours of basal origin. We have considered that αB-crystallin binding proteins, vimentin and HSP27 also show a similar association with poor prognosis.
Materials and Methods: Tissue Micro Arrays of 0.6 mm cores of 246 breast cancers were stained with antibodies to αB-crystallin, vimentin, HSP27 (antibody ERD5) and HSP27 82P and scored using the Quick Score Method. The results stored with the Aperio Pathology Database were then subsequently compared with clinical and pathological parameters.
Results: Expression of αB-crystallin was associated with vimentin [$P < 0.001$ Fishers exact test (FET)]. αB-crystallin expression was linked to a low expression of the estrogen receptor and reduced survival ($P < 0.001$ (FET), $P = 0.002$ Kaplan Meier Log Rank (KM) respectively). Vimentin expression was associated with estrogen receptor (ER) negative cancers and poor survival ($P < 0.001$ (FET), $P = 0.002$ (KM Log Rank) respectively). In contrast to αB-crystallin, low expression of HSP27 was associated with low ER and progesterone receptor (PGR).
Conclusion: Increased expression of the protein chaperon, αB-crystallin and its binding partner, vimentin were linked to reduced survival. A similar association was not found for HSP27 expression. The potential functional significance of this interaction for metastasis will be discussed in the context of other predictive markers for breast cancer.

PP118

Detection and quantification of EGF receptor phosphorylation in formalin-fixed tumor sections by selected/multiple reaction monitoring mass spectrometry

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Background: The epidermal growth factor receptor (EGFR) is a drug target in several cancers, but suffers from a lack of molecular biomarkers to facilitate the selection, monitoring and dosing of patients. Mass spectrometry (MS) has emerged as a sensitive method to track not only the EGFR, but to monitor specifically sites of phosphotyrosine (pY) on the EGFR and the protein components of its signaling network that may serve as biomarkers of EGFR expression and activity. Major challenges in the development and application of MS as a means to discover and assay biomarkers, and in particular phosphorylation-type protein features related to drug target modulation, include (i) the preservation of protein-phosphorylation in patient samples, and (ii) the detection and quantification of such features in minute, heterogeneous patient samples. To address these challenges we have combined Liquid Tissue technology, which enables solubilization of protein from cells obtained by laser microdissection of formalin fixed patient samples, with selected/multiple reaction monitoring (SRM or MRM) MS, which enables accurate relative and absolute quantification of proteins and their sites of phosphorylation.
Materials and Methods: Liquid Tissue technology was used to solubilize protein from formalin fixed tissue samples. Solubilized, with selected/multiple reaction monitoring (SRM or MRM) MS, which enables accurate relative and absolute quantification of proteins and their sites of phosphorylation. This approach was applied to measure features of the EGFR network in formalin fixed tissue culture cells, non-small cell lung carcinoma (NSCLC) xenografts and patient tumor samples.
Results: EGFR peptides were measured by direct SRM/MRM analysis of trypsin-digested, liquefied samples from formalin fixed cultured cells, non-small cell lung carcinoma (NSCLC) xenografts and patient tumor samples. Enrichment of phosphorylated peptides by using titanium dioxide resins enabled the measurement of EGFR phosphorylation sites reflecting activated EGFR.
Conclusion: These results provide proof of concept for a robust approach to monitor in tumors the EGFR and other phosphorylation-associated drug targets and biomarkers, and which may offer superior dynamic range and quantification over traditional immunohistochemistry-based methods.

PP94

The Chernobyl Tissue Bank − a model for integrating "omics" research on single blocks of tissue

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Background: The Chernobyl Tissue Bank (CTB) was established in 1998 to collect, store and distribute biological samples from patients resident in the regions of Ukraine and Russia contaminated by fallout from the Chernobyl accident and who developed thyroid cancer. Patients give generic (broad) informed consent for thyroid cancer research; access to biomaterials is approved by an external review panel. A sample of blood for extraction of DNA, serum and samples of both frozen and formalin fixed paraffin embedded (FFPE) tumour and normal thyroid tissue are provided from each patient.
Materials and Methods: The current collection includes 2493 cases of thyroid cancer and adenoma. RNA and DNA are extracted from the same frozen tissue block and are distributed to researchers in aliquots of 5 μg (RNA) and 3 μg (DNA), permitting multiple projects to have access to material from the same block of tissue. A frozen section is taken from each block prior to extraction and the relative proportions of epithelial, stromal, lymphoid cells are assessed. Quality assurance (QA) is carried out by Aglient Bioanlyser (RNA–RIN) and 10 kb gel (DNA), enabling samples only of the highest quality to be provided to projects that require this e.g. Affymetrix 3′ array.
Results: A recent QA audit showed that the average RIN was 8.5 (range 6.4−9.4). There was no significant degradation over a 10 year period of storage as a frozen block prior to extraction. 1631 aliquots of RNA and 703 of DNA from tissue, 136 aliquots of DNA from blood and 5921 sections from FFPE blocks have been issued to researchers worldwide. The research projects supported by the CTB range from single gene investigations to complex projects using a variety of array based platforms. One example is Genrisk-T, an EC funded project is currently combining mRNA array, bac array (on RNA and DNA extracted from a single frozen tissue block) and germline SNP data with miRNA (from FFPE material of the same case) and clinicopathological data on an age-matched series of 50 patients who were exposed to radiation and 50 who were born after 1/1/87 and have developed spontaneous thyroid cancer at a young age. The aim of the study is to identify radiation related changes and novel genes in thyroid cancer.
Conclusion: The CTB is being used by others (e.g. the Wales Cancer Bank) and by clinical trial groups as a paradigm for a tissue bank to support integrated "omics" research on other tumour types.

PP85

Genotyping of microsatellite alterations and EGFR somatic mutations in exhaled breath condensate of NSCLC patients

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Background: A common goal in treatment of NSCLC patients is to individualize genetic and epigenetic events which can be used as early diagnostic marker and which could be easily and time-saving investigated. We recently demonstrated the possibility of studying microsatellite alterations (MAs) in the DNA of exhaled breath condensate (EBC). The aim of the present study was to verify whether MA analyzed in DNA from EBC can be used to detect tumor susceptibility in high risk subjects studying microsatellite alterations and whether it can be useful to detect EGFR more common mutations in lung cancer.
Materials and Methods: 59 subjects entered the study: 41 with NSCLC and 18 with non-neoplastic diseases. All subjects underwent allelotyping on DNA from whole blood, EBC, and lung tissue removed for histologic diagnosis by analyzing a panel of five microsatellites (D3S2338, D3S1266, D3S1300, D3S1304, D3S1289) located in chromosomal region 3p. Among the overall series, 23 patients were also investigated for EGFR mutations in exons 18−21 on DNA from EBC and paraffin embedded tumor tissues.
Results: MAs in DNA from tumor tissues and EBC of each patient with cancer presented an overlapping profile of loss of heterozygosity (26%)

and microsatellite instability (26%). Furthermore, a significantly higher percentage of MAs was present in smoker patients compared with smoker control subjects ($P < 0.001$). One patient presented pathological mutation in exon 19, 745_750del, while 21 patients presented 3 polymorphisms in intron regions (rs17337100, rs2017000, rs10241451) and one synonymous in exon 2 (rs1050171). EBC-DNA was investigated in the patient who presented the pathological mutation and in nine other patients carrying polymorphisms in introns 19 and 20 and in exon 20. No alterations have been evidenced by sequencing these sites.
Conclusion: We demonstrated that MA in DNA from EBC of NSCLC patients are significantly more frequent than in control subjects and could be used as susceptibility markers for lung cancer risk. However, EBC cannot be used to investigate somatic alterations of epithelial growth factor receptor.

PP45

Analytical performance of a novel dual color dual hapten brightfield genotypic assay for determination of HER2 status in breast carcinoma (DDISH)

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Background: Silver In Situ Hybridization (SISH) results correlate well with fluorescence in situ hybridization (FISH), the current reference standard for assays of HER2 status in breast carcinoma. Endogenous positive stromal cell signal detection via SISH ensures accurate delineation of HER2 status for individual cases. A third-generation, fully automated, dual-color dual-hapten brightfield in situ hybridization method (DDISH) was developed and performance assessed using FISH as the reference standard.
Materials and Methods: 100 invasive breast carcinomas fixed in formalin were evaluated with fully automated DDISH and the results compared with non-equivocal and non-heterogeneous FISH results (Vysis PathVysion: Abbott/Molecular). The HER2 locus was visualized using SISH detection of dinitrophenyl (DNP)-labeled, repeat-depleted HER2 probe. Reference chromosome enumeration signals were generated using a digoxigenin labeled centromeric chromosome 17 probe, detected by an alkaline phosphatase driven reaction employing naphthol and fast red as chromogen. All procedural steps, from deparaffinization through counterstaining, were fully automated and required approximately 12 hours for completion of staining 30 slides. Cell-by-cell individual HER2 SISH and CHR17 red signals were enumerated using conventional light microscopy, the HER2/CHR17 ratio calculated, and the results compared to FISH.
Results: Overall agreement between DDISH and FISH was excellent (98.0%) (sensitivity 95.7%, specificity 100%). Discordance in two of 100 cases between DDISH and FISH was due to low level amplification (HER2/CEP17 ratio >2.2 but $\leqslant$2.5) identified by FISH but not by DDISH.
Conclusion: Dual-color, dual-hapten brightfield hybridization results for invasive breast carcinoma correlate well with FISH, are fully automated, and are readily evaluable with conventional brightfield microscopy.

PP93

Molecular heterogeneity in G3 N0 breast cancer − better treatment tailoring for patients of different ages?

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Background: It is already known that there is an age-related difference in the relative proportion of Grade 1 and 3 breast cancer (BC), with Grade 3 (G3) BC being more common in younger women. The aim of this study was to examine these differences more extensively, concentrating solely on G3, node negative (N0) BC in two distinct populations selected on the basis of age − one group aged under 43 and the other aged over 70 at diagnosis. In this study we used BAC array CGH to study genomic copy number alterations (CNA) of the tumours to investigate whether G3N0 BC shows significantly different patterns with respect to age.
Materials and Methods: Ethics approval for the study was obtained from the South West Wales Research Ethics Committee and sections from routine diagnostic formalin fixed paraffin embedded (FFPE) blocks were obtained from 39 patients with G3N0 BC; 18 were from patients aged under 43 at operation and 21 from patients aged over 70. All had invasive ductal BC. DNA was extracted using the QiAmp system for FFPE tissue, and the integrity of DNA assessed by multiplex PCR. We used 1Mb BAC array CGH to identify genomic copy number alterations. Spatial normalisation, circular binary segmentation and the CGHcall algorithm was used to generate CGH profiles. Unsupervised hierarchical clustering, supervised and correlation were carried out using packages and tests within the R statistical platform.
Results: Three distinct groups were identified on the basis of their CNA. One group of 12 patients and one of 13 were identified which significantly correlated ($p = 0.015$, Fisher's Exact test) with young (8/12) and old age (11/13) at diagnosis. The main CNAs that distinguished the two groups on age involved small regions on chromosomes 1, 9, 10, 14 and 20. The remaining patients formed a group which showed no correlation with age. There was no significant difference with respect to ER or Her2 status among these groups.
Conclusion: Our results show considerable heterogeneity in CNA in G3N0 breast cancer, some of which associated with younger and older groups of patients. Other studies have suggested that breast cancer in elderly women is more indolent than in younger patients, although few have dissected this as a function of histological grade. Further studies breaking down these differences may result in better targeting of therapy in pathologically similar BC, and may lead to differing treatment options based on age-associated changes in biology.

PP64

Prognostic relevance of isocitrate dehydrogenase I and II mutations and MGMT promoter hypermethylation in diffuse astrocytomas

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Background: O6 Methylguanine DNA methyltransferaseis (MGMT) is a DNA repair enzyme. Through removal of alkylating lesions on O6 of guanine it protects cells against mutagenesis and malignant transformations; however during chemotherapy it provides resistance to treatment with alkylating agents, removing selectively cytotoxic adducts from O6 guanine in DNA. Loss of MGMT expression due to promoter hypermethylation may occur in the pathway leading to secondary glioblastomas. However recent studies showed that that isocitrate dehydrogenase 1 (IDH1) and 2 (IDH2) somatic mutations are currently the most reliable genetic marker for secondary GBM.
Materials and Methods: Using Methylation-Specific PCR (MSP) we investigated the inactivation of the DNA-repair gene MGMT by promoter hypermethylation in 54 low grade diffuse astrocytomas (grade II WHO) obtained from patients who undergone surgery in our Institution. We screened for IDH1 and IDH2 mutations the same patients using PCR-SSCP analysis
Results: The MGMT gene was methylated in 22 patients (Meth+; 41%). IDH1 mutations were observed in 50 patients (90 %), while no IDH2 mutation were detected. No association between methylation status and IDH1 mutations was observed. After a median follow up of 53 months, 34 patients showed disease progression and 22 underwent a second surgery. Among Meth+ patients at primary tumor, 17 displayed recurrence (77%) versus 16 cases among Meth− patients (50%). MGMT methylation was signicantly associated with a shorter Progression Free Survival (PFS). Median PFS was 31 months in Meth+ patients and 71 months in Meth− patients (log-rank test $p = 0.02$).
During the follow-up 14 patients died of tumor recurrence. MGMTP Methylation resulted significantly associated with a higher mortality: 9 cases among Meth+ (41%) patients and 5 cases among Meth− patients (15%) ($p = 0.03$ Fisher exact test).
The median overall survival resulted significantly longer among Meth+ patients than in Meth− group: 68 months among Meth+ patients versus non calculable among Meth− patients ($p = 0.03$). 22 patients out of 34 with a recurring tumor had a second surgery, in no case we we observed the appearance of IDH1 or IDH2 mutations in the second tumor sample
Conclusion: Our results confirm that IDH1 mutations are an early event in glioma formation. While in low grade astrocytomas MGMT methylation is associated with tumor recurrence and is a significant predictor of risk decreased overall survival.

PP96

Tumor tissue profiling at the drug targeting level: kinase activity

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Background: Here we present the latest results obtained in the application of a novel biomarker discovery strategy. This approach is based on measuring kinase activities in tumor tissue extracts. Discovery of markers at this enzymatic level, i.e. at the biological level many of the new targeted drug therapies intervene, is different from many other strategies where DNA mutations/amplification, RNA or protein levels/modifications are the source of investigation. This activity-based approach is enabled by dynamic peptide microarrays. These biochips comprise peptides, which are known substrates for phosphorylation by protein tyrosine kinases. While the