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