machine with high resolution melting capacity[2]. Reconstruction experiments showed that by manipulating the annealing temperature, methylation could be detected at levels as low as 0.1%. Moreover MS-HRM allowed estimation of the methylation level by using standards with a known unmethylated to methylated template ratio. We used MS-HRM for the determination of the methylation status of the promoter region of a panel of DNA stability and tumour suppressor genes such as BRCA1 and MGMT in cell lines of known methylation status and in panels of cancer specimens. Furthermore we have developed a MS-HRM assay for diagnostic testing of the H19/IGF2 imprinting centre. The changes in methylation status of H19/IGF2 imprinting centre are implicated in etiology of the Beckwith Wiedemann and the Russel Silver syndromes, which clinically demonstrate growth abnormalities and high cancer incidence. The utility of new assay was tested in a blinded study and 100% concordance of MS-HRM assay was obtained with Southern blot analyses (the current diagnostic procedure) of the same locus [3].MS-HRM proved to be highly sensitive, specific and robust for methylation detection. The simplicity and high reproducibility of the MS-HRM protocol has made MS-HRM the method of choice for methylation assessment in our laboratory. It is suitable for both research and diagnostic settings and will be of special utility in multi-centre trials where a reproducible method for methylation analysis is required.References:1. Wojdacz TK, Hansen LL: Reversal of PCR bias for improved sensitivity of the DNA methylation melting curve assay. Biotechniques 2006, 41(3):274, 276, 278.2. Wojdacz TK, Dobrovic A: Methylation-sensitive high resolution melting (MS-HRM): a new approach for sensitive and high-throughput assessment of methylation. Nucleic acids research 2007, 35(6):e41.3. Wojdacz TK, Dobrovic A, Algar E: Rapid detection of methylation change at H19 in human imprinting disorders using methylation sensitive high resolution melting. submitted.

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Robust and absolute quantitation of PSA in clinical human sera using Protein Reaction Monitoring (PRM)

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The field of proteomics has led to the discovery of numerous protein biomarkers that subsequently need to be verified and validated to evaluate their clinical use with a statistically significant number of patients. At this stage, ELISA test development is a bottleneck as antibody design and generation is time-consuming. To overcome this barrier, we propose to use an alternative assay, called Protein Reaction Monitoring (PRM). PRM associates a robust and automated sample preparation and a mass spectrometry-based detection. Briefly, crude human sera are reproducibly depleted, fragmented and fractionated using a robot. Peptides resulting from specific protein fragmentation are subsequently separated using a robust micro LC column and quantitated using a triple quadrupole mass spectrometer in selected reaction monitoring (SRM) mode. As a proof of concept, we demonstrated the absolute quantitation of a biomarker model, the Prostate Specific Antigen (PSA). In patient sera, PRM doses were compared to automated ELISA quantitation (Vidas TPSA). Between 4 to 30 ng/ml, PRM and ELISA presented an excellent correlation (r2 = 0.94(with similar accuracies and precisions. As a consequence, PRM-based assays can now be considered as valuable alternative assays for proteomic biomarker validation.

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An inflammatory breast carcinoma signature is associated with reduced relapse free survival in patients with non-inflammatory breast cancer

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Background. We hypothesize that a gene expression profile characteristic for Inflammatory Breast Cancer (IBC), an agressive form of breast cancer associated with poor patient survival, might be related to tumour aggressiveness in non-IBC (nIBC).

Materials and methods. RNA from 19 IBC samples and 40 nIBC samples was hybridized onto Affymetrix chips. A gene signature predictive of IBC was identified and applied onto 7 publicly available gene expression data sets (1157 nIBC samples) with survival data of 881 nIBC samples (4 data sets). Samples were classified as "IBC-like" or "nIBC-like". Relapse Free Survival (RFS) was compared between these groups by the Kaplan-Meyer method. We classified the 1157 nIBC breast cancer samples according to other prognostically relevant gene signatures and compared these classifications with the IBC signature classification. Cox regression analysis was performed to identify the most predictive signature with respect to RFS.

Results. Patients with an "IBC-like" phenotype demonstrate a shorter RFS interval in all 4 data sets (p=0.049, p=0.032, p<0.0001, p=0.0005). Classification according to the IBC signature is significantly (p<0.0001) associated with the cell-of-origin subtypes-, the Wound Healing Response (WHR)-, the Invasive Gene Signature (IGS)-, the Genomic Grade Index (GGI)- and the Fibroblastic Neoplasm Signatures (DTF/SFT). Breast tumours having an "IBC-like" phenotype generally belong to the Basal-like (32.8%), ErbB2-Overexpressing (22.6%) or Luminal B (29.6%) subtypes, have an activated WHR (71.6%), express the IGS (75.7%), are less frequently of the DTF phenotype (44.7%) and have a GGI of 3 (71.1%). Significant associations (p<0.0001) were found between the IBC signature and tumour grade, ER status, ErbB2 status and patient age at diagnosis. Cox regression analysis on the entire data set of 881 nIBC samples identified the IBC signature as an independent predictor of RFS (RR=1.532, C.I.=1.100–2.133, p=0.012), together with the WHR and GGI.

Conclusions. We demonstrate that nIBC breast tumours having an "IBC-like" phenotype have a reduced RFS interval. This suggests that IBC and nIBC tumours demonstrate the same phenotypic traits with respect to aggressive tumour cell behaviour. Gene signatures related to tumour stroma and tumour grade add information regarding patient survival. Hence the IBC signature represents a different aspect of aggressive tumour behaviour.

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Poor survival outcomes in HER2 positive breast cancer patients with low grade, node negative tumours

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Background: HER2 status has long been established as a poor prognostic marker for survival in breast cancer and more recently has been validated in numerous adjuvant trials as a predictive marker for response to trastuzumab. However, there remains a small subset of low grade, node negative HER2 positive patients who are currently ineligible for trastuzumab treatment as have been deemed to have no requirement for standard adjuvant chemotherapy.

Methods: We used a cohort of 367, grade 1/2, node negative patients diagnosed between 1980-2002 with full follow-up (median 6.2yrs) and clinicopathological details to assess the impact of HER2 positivity (IHC

Table 1 (Poster 407)

		Events				95.0% CI	
	number in group	HER2 pos	HER2neg	Sig.	Hazard Ratio	Lower	Upper
whole cohort	367	7/19	27/348	<0.001	6.78	2.93	15.69
ER positive	286	3/11	18/275	0.004	6.05	1.76	20.77
ER negative	34	3/5	6/29	0.012	7.97	1.58	40.22
Age<50	66	2/7	3/59	0.030	8.82	1.24	62.69
Age 50-65	170	3/7	11/163	0.001	8.79	2.44	31.70
Age>56	131	2/5	13/126	0.032	5.12	1.15	22.78
Size<20mm	233	5/13	9/220	<0.001	11.75	3.92	35.27
size>20mm	94	2/5	13/89	0.015	7.02	1.45	33.90

Herceptest 3+ or FISH positive) on survival in this otherwise very good prognostic group. The group were 89% ER positive, with 72% smaller than 20mm. 80% were aged over 50 and 10% received chemotherapy and 91% received endocrine therapy (tamoxifen).

Results: The overall hazard ratio (HR) for HER2 positivity was 6.78 (95% CI 2.9-15.7, p<0.001) with 5yr breast cancer specific survival rates of 96% (HER2 negative) and 68% (HER2 positive). This reduction in survival in HER2 positive cases persisted when patients were split into subgroups by ER status, tumour size and age (table 1).

Conclusion: These results provide support for the use of adjuvant trastuzumab in this group of patients who are typically classified as very good prognosis, not routinely offered standard chemotherapy, and as such do not fit current prescribing guidelines for trastuzumab. A clinical trial to assess the benefit of adjuvant trastuzumab alone within this subgroup of HER2 patients would resolve this. These results are in keeping with those from HERA trial that suggested that patients with the best prognosis tumours (node negative and size 1-2cm) had benefit similar to the overall cohort.

The persistence of a reduction in survival in our ER positive subgroup despite endocrine therapy confirms the recent trans-ATAC analysis based on HER2 status and suggests that we cannot not rely solely on adjuvant endocrine therapy in these largely ER positive patients.

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Structure-guided design of inhibitors of the eukaryotic initiation factor 4E (eIF4E) mRNA-cap interaction as anti-cancer agents

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Protein synthesis in eukaryotes is regulated by eIF4E together with the other components of the translation initiation complex eIF4F. eIF4E anchors the mRNA by recognition of the 5' cap structure m7GpppN (where N is the first transcribed nucleotide and p represents phosphates), which contains a N-7-methylated guanine base. The role of eIF4E in cell proliferation and tumour progression is well documented, thus making eIF4E an attractive cancer drug target.

eIF4E recognizes the 5' cap structure through a characteristic cation-pi interaction involving the delocalized charge of the at N-7-modified guanine in the cap and two tryptophan residues in the eIF4E binding site, along with H-bonding interactions of the guanine base and electrostatic interactions with the phosphates. Our work aims to find non-nucleotidic cap-binding antagonists, and we look mainly for moieties in such inhibitors that could maintain the critical cation-pi interaction and would avoid the need for phosphate groups that render compounds membrane-impermeable and metabolically labile.

We follow a structure-guided drug design approach that consists of defining binding site constraints and performing in silico docking of small ligands into the eIF4E cap-binding site. We find that most of the scoring functions used to rank docked ligands fail to reward for the cation-pi interaction and we have implemented a quantum mechanical (QM) scoring strategy for the scoring of docked ligands. Hits from these virtual docking and scoring approaches were screened for binding eIF4E by Electrospray Ionization mass spectrometry (ESI-MS).

Several small molecules have been identified that bind to eIF4E and the results from these binding studies, as well as the effects of hit compounds on protein translation will be discussed.

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Gene expression profiling in formalin-fixed paraffin-embedded primary melanomas

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Melanoma is an aggressive highly metastatic disease arising from epidermal melanocytes. Diagnosis and prognosis of this disease is currently limited to histological factors such as measurement of tumour invasion by Breslow Thickness and few successful treatments are available due to our poor understanding of the metastatic phenotype.Studies investigating the molecular basis of melanoma initiation and progression have been limited in the past due to the heterogeneous nature of melanoma and limited availability of fresh primary tumour. New techniques such as whole genome gene expression profiling are improving knowledge of many human cancers, however this is often a DNA intensive method resulting in most studies of this kind on melanomas involving ether small numbers of fresh tissues or melanoma cell lines. We have used a novel method for gene expression profiling of 500 cancer genes in formalin-fixed paraffin-embedded primary melanomas. For this study, we selected 27 (FFPE) primary tumours from 27 patients; 15 of who had relapsed from their primary tumour within 5 years and 12 who had not relapsed after 5 years. The deepest, most invasive part of the tumours was sampled with a core biopsy needle and total RNA was isolated for analysis with the cDNA-mediated annealing selection, extension and ligation (DASL) assay (Illumina®). This assay is designed for use on partially degraded RNA for measurement of relative gene expression levels of up to 1536 sequence targets using as little as 25ng of total RNA. We used a Cancer Panel to target 502 genes commonly altered in cancer with three probes per gene. The results were visualised with Beadstudio analysis software (Illumina®) and normalisation was carried out using cubic spline methods, prior to export of results into STATA for further analysis.All tumours produced satisfactory results for the 502 genes in the Cancer Panel after quality control tests for average fragment length and amplification ability by qPCR. Genes found to have significantly different levels of expression between the group who had relapsed and the group that had not relapsed were SKI, PAI-1, BRCA2, WT1, MLLT4, NFKBIA and FGF8 (t-test and wilcoxon p

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Diagnosis of thyroid cancer by gene expression profiling on thyroid nodule biopsy

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The diagnosis of thyroid cancer relies on cytological examination of material collected from nodules by fine-needle aspiration biopsies (FNAB). Due to the absence of markers, it is difficult, even for experienced cytologists, to discriminate benign from malignant thyroid tumors. Thus, only 35% of patients undergoing thyroidectomy for cancer or suspicion of cancer, actually have a cancer. The diagnostic procedure must be improved to reduce the number of thyroid ablation subsequently proved to be unjustified (about 10,000 per year in France). With the aim of identifying marker genes capable to discriminate benign from malignant thyroid tumors, we designed an oligonucleotide-based nylon macroarray formed from 200-potentially informative genes. Gene expression profiles of normal and tumoral (adenomas, carcinomas) thyroid tissue were generated with the macroarray and validated by real-time PCR. In this study, we built tumor classifiers from macroarray data and we tested their performances on a series of samples corresponding to FNAB. Gene expression data deriving from samples of the Lyon Thyroid Tumor Bank, representing the "training set", were subjected to a weighted voting algorithm to generate prediction models or classifiers capable of assigning a sample to one of the two classes: benign or malignant. Three prediction models were built by considering either all thyroid carcinomas (the commun classifier) or only follicular carcinomas (the F classifier) or only papillary thyroid carcinomas (the P classifier). The classifiers were composed of 9 to 12 genes and brought into play a total of 19 "marker" genes which were used to compose a fourth predictor, the global classifier. The capacity of the 4 classifiers to discriminate benign from malignant tumors was tested on a series of FNAB (carried out on nodules after surgical resection) used as "validation set". In 23 out of 26 FNAB, the 4 classifiers gave a diagnosis similar to that of the pathologist used as "gold standard"; in the 3 other cases, the correct diagnosis was given by 3 of 4 classifiers. Thus, the combination of classifiers identified benign and malignant tumors with very high sensitivity and specificity. In conclusion, we developed a procedure of molecular diagnosis of benign versus malignant tumors applicable to the material collected by FNAB. This molecular test which complied with a pre-clinical validation stage is now subjected to a prospective, large-scale (800 patients) evaluation study.

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Identification of drug-sensitive prediction genes by an epigenetic reactivation screen of cisplatin-resistant NSCLC cell lines

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Non-small cell lung cancer (NSCLC) shows resistance in tumors that are initially chemo-sensitive, which is a serious problem in cisplatin-based adjuvant chemotherapy. CDDP is the paradigm of cytotoxic drugs in NSCLC treatment, however, it also induces, de novo DNA hypermethylation in vivo. Histone deacetylation and aberrant promoter hypermethylation are common epigenetic mechanisms for the silencing of