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)

T. Fortin[1], A. Salvador[2], J.P. Charrier[1], C. Lenz[3], G. Lacoux[1], G. Choquet-Kastylevsky[1], J. Lemoine[2]
[1]bioMérieux, Biomarker Research and Validation, Marcy l'Etoile, France; [2] Université Claude Bernard, UMR 5180 Sciences Analytiques, Villeurbanne, France; [3] Applied Biosystems, Applied Biosystems, Darmstadt, Germany

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

S. Van Laere[1], I. Van der Auwera[1], G. Van den Eynden[1], X.B. Trinh[1], P. van Dam[1], E. Van Marck[1], P. Vermeulen[1], L. Dirix[1]
[1]Translational Cancer Research Group, (Lab Pathology University Antwerp and Oncology Center General Hospital Sint-Augustinus), Wilrijk, Belgium

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

S.M. Tovey[1], J. Edwards[1], S.B. Brown[1], E. Mallon[1], T.G. Cooke[1]
[1]University of Glasgow, Section of Translational Research, Glasgow, United Kingdom

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