Conclusion: P targets T matrix, resulting in T-associated HA reduction. Ph1 studies have shown in vivo activity of P as reflected by elevated plasma HA catabolites and increased T water diffusion and T perfusion by ADC/DCE-MRI. The use of histochemistry as a predictor of P efficacy as well as HA plasma catabolites, histochemistry and ADC/DCE-MRI to monitor response to P will be investigated Phase 2 trials.

PP 19

Highly sensitive detection of microRNA and mRNA from FFPE tissue and blood samples by expression microarray

H. Sudo, T. Satoko, U. Yoji, K. Toshihiko, I. Makiko, N. Osamu, A. Hideo, N. Hitoshi. *Toray Industries, Inc., Kanagawa, Japan*

Background: Gene expression profiling of readily available clinical samples, such as blood or FFPE tissue, is a promising method to discover novel diagnostic markers. As RNA is subjected to degradation even in properly-collected tissue samples, it is more difficult to obtain intact RNA from FFPE or body fluid samples for diagnostic analysis. 3D-Gene™ is highly sensitive gene expression microarray, featuring the unique micro-columnar structure on the platform substrate and the beads agitation system during the hybridization reaction. Using 3D-Gene™, we achieved highly sensitive and reproducible detection of mRNA or miRNA from FFPE tissue samples.
Materials and Methods: Total RNA was extracted from human serum, plasma and frozen or FFPE tissue samples, with the recommended protocol for each sample. For mRNA detection, total RNA was reverse-transcribed to cDNA and labeled with fluorescent dye directly or after the amplification. For miRNA detection, total RNA was labeled with fluorescent dye directly. These pretreated target nucleotides were hybridized to 3D-Gene™ while the hybridized buffer containing target nucleotides was agitated by beads during hybridization. The hybridized microarrays were washed and scanned for image acquisition.
Results: The result was highly correlated with the expression profiles from frozen tissue samples. Furthermore, exosomal miRNA from serum or plasma was also detected with high sensitivity and reproducibility. From these analyses of FFPE tissue or blood samples, we found potential miRNA biomarkers for various cancers. (i) Using 3D-Gene™, we detected mRNA expression profile from FFPE samples with high reproducibility. We also showed high correlation of the expression profiles between FFPE and frozen tissue samples. Furthermore, microRNA obtained from frozen as well as FFPE tissue samples was reproducibly detected at atto-molar level. Some miRNA biomarkers for various cancers were found from FFPE samples. (ii) Serum and plasma are suggested to contain microsomes in which miRNA is enclosed. miRNA from serum and plasma samples were detected with high sensitivity and reproducibility with 3D-Gene™. Some miRNA biomarkers for various cancers were found from patients' sera.
Conclusion: The Application of our 3D-Gene™ for the gene expression analysis of clinical samples could bring a formally unexplored venue in the biomarker discovery and diagnostic field.

PP 105

Multiple gene signatures: some putative answers on the why and the how

W. Talloen, L. Bijnens. *Janssen Pharmaceuticals group, Beerse, Belgium*

Background: With the completion of the sequencing of the human genome and with the emergence of biotechnologies such as microarrays, we have entered the post-genomic era with much hope to harvest some of the fruits hidden in the genomic text. At the same time, the current difficulties faced by pharma research to discover generally applicable block-buster drugs have lead to think in terms of personalized medicine. Consequently, high hopes are on clinical opportunities for gene-based prediction of illness or drug response using post-genomic tools. The -omics revolution was also warmly welcomed by statisticians as its data properties imposed new and interesting statistical challenges. For example, the quest for biomarkers in the context of personalized medicine has made many statisticians think about classification models that are robust against overfitting for generation of molecular signatures.
Materials and Methods: Here we propose three biological scenarios where multiple gene signatures may outperform single gene markers; (1) inhibition or catalyzation, (2) downstream effects and (3) upstream effects. A simulation study is set up to mimic three different biological scenarios, and each of the three datasets is analyzed using various algorithms including PAM, Random Forest, Support Vector Machines, CART, etc.
Results: The algorithms under study perform clearly differently between the three scenarios.
Conclusion: This presentation discusses how genes can be aggregated into one composite index (i.e., the marker) so as to reflect the underlying biology, as categorized using the three previously proposed biological scenarios.

PP 58

Plasma microRNAs in breast cancer detection

C. Thompson, H. Heneghan, L. Li, R. Leidner. *Case Western Reserve University, Cleveland, OH, USA*

Background: Circulating microRNA (miRNA) expression levels have been proposed as a potential biomarker for cancer detection due to their key role in cancer and stability in the circulation. In fact, a circulating miRNA-based test for colorectal cancer detection is already in clinical trials. However, the field is much less advanced in breast cancer.
Materials and Methods: Using the Illumina human miRNA microarray, we interrogated the expression level of 1145 miRNAs in the plasma of 18 breast cancer patients prior to tumor resection, 17 patients after tumor resection and 20 mammography-screened controls. Controls were matched to pre-resection cases on age and race. We excluded 245 miRNAs due to low expression across all samples. Differences in expression levels between pre-resection cases and controls were assessed via a pooled t-test.
Results: Thirty six of the remaining miRNAs were differentially expressed between pre-resection breast cancer cases and controls ($p < 0.01$). Using a single ratio-normalized miRNA level, with 100% specificity we were able to correctly identify 13% of the cases. Increasing the signature to 2 miRNAs ratios allowed us to correctly classify 50% of cases. Further signature modeling using 6 ratios yielded a test with 89% sensitivity at 100% specificity. This signature held up to random permutation testing ($p < 0.01$). We noted that this signature was better at detecting ER+ breast cancers, where it correctly identified 100% of the ER+ cases. Furthermore, the expression levels were highly correlated with stage (lowest in in-situ cases and highest in stages 3 and 4), and returned to baseline levels in post-resection samples.
Conclusion: Overall, our data provides compelling evidence of the potential of miRNAs to be used as a minimally invasive screening test for breast cancer, conceivably as an adjunct to improve mammographic accuracy. Confirmation of preliminary results in a larger sample size is underway.

PP 76

Biomarker discovery using multiplexed in solution proximity extension assays: a case–control study for early detection of colorectal cancer

S.B. Thorsen, M. Lundberg, E. Assarsson, N. Gee, B.S. Nielsen, I.J. Christensen, H.-J. Nielsen, N. Brünner, J. Stenvang, S. Fredriksson. *University of Copenhagen, IVS, Patobiology, Frederiksberg, Denmark*

Background: One of the challenges in early detection of colorectal cancer (CRC) is the limited success with screening. We here report a study, focusing on discovery of biomarkers for early detection of CRC, using EDTA plasma samples from a case–control group collected from a larger endoscopy study. We demonstrate that the combination of biomarker discovery and molecular technique development is one lead to discover new diagnostic biomarkers for CRC.
Materials and Methods: We have established and validated a high throughput multiplex in solution proximity extension assay (PEA) platform and demonstrate simultaneous quantification of 96 different proteins in 1 μL sample. The PEA employs two primary antibodies, linked to two different DNA strands; upon simultaneous and proximal binding to a target protein the two strands can be connected. The DNA strands now form a PCR amplicon detectable by real-time qPCR. The amplification ability of the DNA strands drive the sensitivity and lowers sample consumption, while supporting multiplexing capabilities based on the oligonucleotide design. The PEA technology possesses all the required qualities for a biomarker discovery tool. From a literature study investigating interesting molecular pathways relevant for CRC, we designed four biomarker panels. In total, we measure 150 different protein markers, of which many have never been reported in human plasma. Using this multiplex PEA discovery tool, we test the biomarker potential for each protein. Our case–control study consists of four groups: 74 stage I-IV CRC patients, 74 adenoma patients, 74 patients with other diseases, and 74 healthy individuals. All patients and individuals have been age and gender matched.
Results: We have previously applied an earlier version of the assay (PLA) and successfully demonstrated detection and quantification of 74 different protein markers in CRC and matched healthy individuals. This demonstrated the feasibility and potential for the assay and identified putative biomarkers [1]. We will present assay validation results and biomarker potential for all panels included in the new PEA high-throughput assays.

Conclusion: The technical as well as the biological aspects of this high throughput setup is very interesting.

References

[1] Lundberg, M., Thorsen, S.B., et al. Multiplexed homogeneous proximity ligation assays for high throughput protein biomarker research in serological material. Mol Cell Proteomics 2011 Apr;10(4).

PP 79

Low levels of cleaved urokinase receptor in plasma from healthy individuals

T. Thurison, I.J. Christensen, I.K. Lund, G. Høyer-Hansen, H.J. Nielsen. *The Finsen Laboratory, Copenhagen University Hospital, BRIC, Copenhagen N, Denmark*

Background: The involvement of the urokinase plasminogen activator, uPA, and its cellular receptor uPAR, in cancer invasion is well-established. uPA can cleave intact uPAR, uPAR(I-III) in the linker region between domains I and II, whereby uPAR domain I (uPAR(I)) is released and the cleaved uPAR(II-III) is left on the cell surface. Cleavage of uPAR(I-III) thus reflects the activity of uPA and possibly the aggressiveness of the cancer. uPAR can be shed from the cell surface and all uPAR forms have been identified in tumor tissue and blood. The cleaved uPAR forms are strong prognostic markers in colorectal cancer (CRC) (1). In order to determine a reference interval of the uPAR forms in blood from healthy individuals, we measured the uPAR forms in plasma from 200 men and 200 women, all without registered medication and co-morbidities and with no findings by colonoscopy.
Materials and Methods: Citrate plasma samples were collected before colonoscopy. The individual uPAR forms were measured by time-resolved fluorescence immunoassays specific for the three different uPAR forms.
Results: The median age of the included individuals was 48 (21−85) years. The mean level (geometric mean, male age 60 years) of uPAR(I-III) was 36.02 pmol/L with an upper normal limit of 55.06 pmol/L. Women had 22% higher levels and the level increased by 3.8% per 10 years. The mean level of uPAR(I-III)+uPAR(II-III) was 58.74 pmol/L and the upper normal limit was 94.15 pmol/L. Women had 18% higher levels and an increase of the level by 5.6% per 10 years was found. The mean level of uPAR(I) was 12.91 pmol/L and the upper normal limit was 36.90 pmol/L. Females had 25% higher levels and an upper limit of 42.13 pmol/L. The level of uPAR(I) was independent of age. The corresponding levels measured in citrate plasma from colorectal cancer patients (1) showed that 9% of the patients had elevated levels of uPAR(I-III), 23% of uPAR(I-III)+uPAR(II-III) and 32% of uPAR(I), as compared to the normal upper limit.
Conclusion: We have determined a reference interval for the three uPAR forms in citrate plasma. Women have significantly higher levels of all uPAR forms and the levels increase slightly with age in both genders for uPAR(I-III) and uPAR(I-III)+uPAR(II-III). Comparing the normal upper limits with the levels measured in the CRC patients reveal a greater proportion of patients with elevated levels of the cleaved uPAR forms compared to intact uPAR(I-III).

References

[1] Thurison et al., Clin Chem 2010; 56: 1636−40.

PP 2

Generation of a panel of "actionable" cancer genes for molecular profiling (MP) in a feasibility study of targeted and genome wide sequencing (TGWS)

B. Tran, J. Dancey, S. Kamel-Reid, J.D. McPherson, L. Stein, T. Petrocelli, P. Shaw, L.L. Siu. *Princess Margaret Hospital, Toronto, Canada*

Background: Increasing identification of genetic aberrations in cancers and the growing inventory of molecularly targeted agents (MTA) with potential predictive biomarkers (BM) are driving personalized cancer medicine (PCM). However, in clinical practice few MTA have had their regulatory approval predicated on specific predictive BM. Real time MP of tumors has potential to enable PCM but validation of this approach is necessary. Development of a recognized panel of cancer genes to enhance MP prioritization in clinical trial patients is relevant. This study utilizes the knowledge of cancer drug developers (DD) and genomic scientists (GS) in generating a gene panel for MP in a feasibility study of TGWS.
Materials and Methods: A survey exploring the perceived importance of 194 genes with aberrations proven or suspected to be tumorigenic was distributed to 29 DD and GS. Respondents were asked to assign importance to each gene, based on its likelihood to impact treatment recommendations for predictive or prognostic reasons: (1) highest; (2) intermediate; (3) lowest; (4) unknown. Genes were then ranked by mean score. Genes with aberrations targeted by established or investigational agents were identified. Subgroup analyses identified significant differences in scores assigned by DD and GS using chi-square.
Results: A total of 19 (73%) invitees, 10 (53%) DD and 9 (47%) GS completed the survey. Of the 194 genes, aberrations in 58 are targeted by established or investigational agents and a further 48 are within targeted pathways. The top 10 ranked genes include EGFR, BRAF, KIT, BRCA1, BRCA2, ErbB2, KRAS, ALK, ABL-1, BCR; all have aberrations predictive of efficacy with established or investigational agents. When compared to GS, DD are more likely to assign highest priority to genes where aberrations have MTA (37% v 31%, $p = 0.036$) and less likely to identify genes as unknown (21% v 33%, $p < 0.001$).
Conclusion: The ranked gene list generated by our survey allows generation of a prioritized panel of "actionable" genes for MP. This survey demonstrates the importance of utilizing expert knowledge of both DD and GS in both design of clinical trials using MP and successful translation of cancer genomics to PCM.

PP 86

Immunohistochemistry and molecular biology of the PI3K pathway did not correlate with treatment efficacy of everolimus as second line or third line treatment of advanced endometrial carcinoma

O. Tredan, D. Pissaloux, I. Treilleux, Q. Wanq, N. Bonichon-Lamichhane, V. Mari, G. Freyer, E. Pujade-Lauraine, I. Ray-Coquard. *Centre Leon Berard, Lyon, France*

Background: Recent evidence suggests the particular importance of the phosphatidylinositol 3-kinase (PI3K) pathway in patients (pts) who have recurrent or metastatic endometrial cancer [Oza et al. Abstract 5009, 2011 ASCO Meeting]. Activating mutations of the PI3K pathway is common, as well as mutation/loss of the tumor suppressor gene PTEN. Thus, mTOR (mammalian target of rapamycin) is activated and pS6K is a downstream marker of mTOR activation. Everolimus is an oral rapamycin analog that selectively inhibits mTOR. In the ENDORAD trial, we evaluated everolimus as a single agent for second- or third-line treatment of endometrial cancer pts [Ray-Coquard et al. Abstract P-8046, 2010 ESMO Meeting].
Materials and Methods: In the ENDORAD trial, pts received everolimus (10 mg PO daily) until progression or toxicity and were evaluated at 3 and 6 months for response and toxicity. Among 44 pts, 36 tumor blocks, mostly from primary tumor, were available to determine whether expression of biomarkers in the mTOR pathway would predict tumor response. Correlative studies evaluating ER, PR, HER2, LKB1, PI3K, PTEN, pAKT, 4EBP1, S6K and pS6K expression by immunohistochemistry (IHC) were performed. PTEN deletion (by FISH analysis) and mutational status of K-RAS, PI3KCA, PTEN and AKT1 genes were analyzed.
Results: 12 of 34 (35%) evaluable patients had partial response or stable disease (PR, SD) according to RECIST criteria, 22 pts had disease progression. Expression of ER, PR, LKB1, PI3K, pAKT, 4EBP1, S6K and pS6K using IHC did not predict response to everolimus (respectively: 8/12, 9/12, 3/12, 9/12, 6/12, 11/12, 11/12, 8/12 for responders, and 15/22, 13/22, 3/22, 11/22, 8/22, 18/22, 21/22, 19/22 for non-responders). Neither loss of PTEN expression (8/12 for responders and 13/22 for non-responders, $p = 0.6$), nor PTEN deletion, nor PTEN mutation (5/12 for responders and 7/22 for non-responders) predict pts outcomes. 31 specimens were evaluable for K-RAS mutations (10 for responders and 21 for non-responders). None of the pts with PR or SD had K-RAS mutation, whereas 4 mutations (19%) were identified in tumors that had progressed on everolimus.
Conclusion: None of the protein from the PI3K pathway tested in this study could predict response to everolimus. Interestingly, K-RAS mutational status correlated with response to everolimus. Other studies presented at the 2011 ASCO Meeting suggested also that K-RAS mutations were associated with resistance to everolimus.

PP 39

Multi-determinants analysis of molecular alterations as predictor of resistance to cetuximab in metastatic colorectal cancer

P. Ulivi, A. Passardi, L. Capelli, E. Chiadini, S. Bravaccini, M. Valgiusti, E. Scarpi, C. Molinari, V. Casadio, W. Zoli. *Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (I.R.S.T.), Meldola, Italy*

Background: KRAS mutations negatively affect outcome after cetuximab (CTX) in metastatic colorectal cancer (mCRC). As only 20% of KRAS wild-type (WT) patients respond it is possible that other mutations, constitutively activating the EGFR pathway, are present in the non-responding WT patients. We retrospectively correlated progression-free survival (PFS) with the mutational status of KRAS, BRAF, PIK3CA and expression of PTEN in 64 mCRC patients treated with Cetuximab, with the aim to clarifying the relative contribution of these molecular alterations to resistance.