Biomarker discovery: Driving technologies
28th February 2013

The Royal College of Pathologists, London

Biomarkers identifying biological and physiological entities associated with disease are taking an increasingly important place at the tables of drug discovery and personalised medicine. Their discovery in biosamples requires the combined use of genomics, proteomics and bioinformatics platforms. Whilst for their application, robust techniques combining exquisite sensitivity and specificity must be developed. This conference is focused on technologies that are driving advances in this area and their application at gene and expression level in solid and fluid biosamples. As such this conference is targeted to provide leading edge information to researchers within the academic, biotechnology and pharmaceutical sectors.

We include a panel session in this event, so that delegates can discuss their work directly with a panel of experts.

This event has CPD accreditation

9:00 – 9:45 **Registration**

9:45 – 10:00 **Introduction by the Chair:** *Dr Peter H Bach,* Director: BioPharmaLogic LLC, Cambridge

10:00 – 10:30 **Begin with biomarkers**
Dr Peter H Bach, Director: BioPharmaLogic LLC, Cambridge.
Biomarkers are key to drug development and personalised medicine. The identification of the "right" fit-for-purpose biomarker is complex and resource intensive. Small companies have modest potentials to implement a biomarker strategy, but can still ensure that they build their development programs around potential biomarkers. A biomarkers strategy should be a core to the development of each molecule, and not a bolt on addition to clinical development. If undertaken as part of discovery-development transition there is a potential to help shape the assessment of possible biomarkers as part of nonclinical development so that they are credible when needed.

10:30 – 11:00 **Identification and quantification of cancer biomarkers using liquid chromatography-mass spectrometry**
Dr David J. Britton, Proteome Sciences plc, Institute of Psychiatry, London
Liquid chromatography-mass spectrometry based proteomics can identify and quantify a multitude of proteins and post translational modifications from many different sample types (cell culture, tissue, plasma, etc). We have used this technology to identify new biomarkers and developed quantitative assays to measure the abundance of known biomarkers

11:00 – 11:15 **Discovery of novel liver fibrosis biomarkers using proteomics**
Dr Bevin Gangadharan, Oxford Antiviral Drug Discovery Unit, Oxford Glycobiology Institute, Department of Biochemistry, University of Oxford, UK
Two-dimensional gel electrophoresis (2-DE) is often used to separate plasma or serum proteins in an attempt to identify novel biomarkers. A major problem with this approach is the presence of high abundant plasma/serum proteins which limit the detection of low abundance features. We used two proteomics approaches to identify new fibrosis biomarkers in patients with different stages of liver fibrosis. Plasma samples from healthy individuals and patients with hepatitis C virus (HCV) induced cirrhosis were analysed using 2-DE over a narrow pH 3-5.6 range, a range outside the pH of highly abundant albumin, transferrin and immunoglobulins. Novel markers identified by this approach were validated across all fibrosis stages by Western blotting. 44 candidate biomarkers were revealed of which 20 were novel. Western blot analysis with newly identified biomarkers showed a consistent change with increasing fibrosis stage and were promising when compared to the markers used in established fibrosis tests. This is the first time the pH 3−5.6 range has been used to separate plasma by 2-DE and this pH range could be useful for discovering novel biomarkers in other diseases. In addition, we used in-solution isoelectric focusing followed by SDS-PAGE to find biomarkers in HCV-induced liver cirrhosis and this approach was found to be beneficial for analyzing basic, high molecular weight proteins. The novel fibrosis markers identified by these proteomics approach may help to assess hepatic fibrosis and decrease the need for invasive liver biopsies.

11:15 – 11:40 **Speakers' photo then mid-morning break and trade show**
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11:40 – 12:10 **Quantitative Liver Proteomics for Biomarker Discovery in Non-alcoholic Fatty Liver Disease**
Dr Bernadette Moore, University of Surrey, UK
Non-alcoholic fatty liver disease (NAFLD) is now the most common liver disease worldwide. Given that NAFLD can progress from steatosis to non-alcoholic steatohepatitis (NASH), fibrosis and potentially hepatocellular carcinoma,

early diagnosis and accurate disease staging are primary clinical concerns. We have applied a relative quantitative proteomic approach utilizing isobaric tags for relative and absolute quantitation labeling combined with nano-liquid chromatography and tandem mass spectrometry to identify liver protein biomarkers in a mouse model of NASH. Novel candidate biomarkers for NAFLD, identified by proteomics and independently confirmed in these experiments, have now been confirmed in human clinical biopsy samples.

12:10 – 12:40 **DNA and RNA biomarker demonstration in solid tissues**

Dr Anthony Warford, University of Westminster, London, UK

Tissue donated from surgical procedures represents an important resource for biomarker identification. In ideal circumstances 'fresh' or frozen tissue will provide high quality DNA and RNA for analysis in situ or after extraction. However, most samples are held in the vast repositories of formalin fixed paraffin wax embedded (FFPE) tissues. In these preparations nucleic acids are degraded, principally through the fixation process. Practically this means that extraction methods have to be refined, amplicons are size limited and the possibility of the generation of spurious gene signatures needs to be considered. However, in spite of these limitations FFPE preparations provide very valuable information for the development and assessment of nucleic acid based biomarkers.

12:40 – 12:55 **Tracing antigenic impurities in serum controls of agglutination test kits by qs-ELISA assay and evaluation of immunological cross reactivity *In-silico***

Dr Surajit Debnath, Department of Medical Laboratory Technology, Women's Polytechnic , Hapania, India

Biomarkers trace out informative hotspots on a variety of samples so that investigators can surmise a correlation. The fundamental principle mostly involves either a Watson –Crick base pairing, as in nucleic acid based technologies or Antigen-Antibody compatibility. We generally attempt to replicate the stringent specificity *in-vitro* as in physiological systems. We take soaring effort to avoid cross reactivity. Coupling wet lab findings with Computational algorithms can predict for us , how much stringency control will be enough for a given set of problems in our labs.

12:55 – 13:45 **Lunch and trade show**

13:45 – 14:30 **Question and Answer Session**

Delegates will be asked to submit questions to a panel of experts. Questions can be submitted before the event or on the day

14:30 -15:00 **Proteomic analysis of uveal melanoma for biomarker discovery**

Dr. Paula Meleady, Dublin City University, Ireland.

Uveal melanoma is the most common intraocular malignancy in adults. The survival rate of uveal melanoma patients who develop metastatic disease is very poor. We have used both two dimensional difference gel electrophoresis (2D DIGE) and quantitative label-free liquid chromatography mass spectrometry (LC-MS) approaches for biomarker discovery in uveal melanoma tissue specimens (minimum follow-up of seven years), comparing uveal melanoma tumours from patients who developed metastatic disease with those who did not. Selected differentially expressed potential protein biomarkers of uveal melanoma metastatic disease were further followed-up by immunohistochemistry and functional validation assays of cellular invasion in vitro using siRNA.

15:00 – 15:15 **ERG immunohistochemistry is not predictive for PSA recurrence, local recurrence or overall survival after radical prostatectomy for prostate cancer**

A Marije Hoogland, Department of Pathology, Erasmus Medical Center, Rotterdam, The Netherlands

15:15 - 15:30 **Engrailed-2 (EN2): a biomarker for the detection of clinically 'significant' prostate cancer**

Richard Morgan and *Hardev Pandha, Oncology, Faculty of Health and Medical Sciences, University of Surrey, Guildford, UK*

Achieving cure for men with prostate cancer depends almost entirely on early detection, when the cancer is localized. It is also accepted that small volume prostate cancer may be carefully monitored (active surveillance) and only a small proportion of these cancers are 'significant' and will progress and need treatment. This spares the remaining men considerable morbidity from treatment and has obvious health economic benefits. To date the prostate specific antigen (PSA) test has been a useful biomarker for diagnosis and monitoring, but has marked limitations as its not cancer specific and cannot indicate the size and extent of the tumour. There is an urgent unmet need not only for new prostate cancer biomarkers, but more importantly those that can differentiate significant versus non-significant disease. EN2 is a homeodomain-containing transcription factor which, unusually for this type of protein, can be secreted from cells. It has a key role in early neural and limb development, but it is not expressed in adult cells. We have shown that EN2 can be released from prostatic cancer acini and ducts and detected in the urine of prostate cancer patients. We demonstrated that EN2 was not present in the urine of men with non-malignant conditions of the prostate such as prostatitis, and that EN2 was thus a potentially useful diagnostic marker for prostate cancer, with a reported sensitivity of 66% and a specificity of 88.2% (corroborated by a second independent centre) [1]. In a recent study, we found a strong positive correlation between pre-surgical levels of urinary EN2 and cancer volume in prostatectomy specimens in a retrospective series of 125 men who had undergone prostatectomy, and that higher EN2 levels correlated with tumour stage T2 versus T3 [2]. This has now been corroborated further in an equivalent

prospective study of 57 men undergoing prostatectomy. The urinary EN2 levels strongly correlated with the tumour volume (but not total prostatic volume) in a linear regression analysis ($p < 0.0001$, $r2 = 0.454$). EN2 levels also significantly correlated with increasing pathological T stage and margin positivity. Using any 3 different published 'cutoff levels' of tumour volume (0.5ml, 1.3ml and 2.5ml) used in published studies to define ', significant disease', men with 'significant disease' had markedly higher levels of urinary EN2 ($p<0.001$, $p<0.001$ and $p<0.001$ respectively) [3]. EN2 has potential utility as a biomarker to both detect prostate cancer and also indicated diseas volume, which in turn may aid the decision to treat versus observation and monitoring. EN2 is measured in first pass urine without the requirement for preceding prostatic massage, is stable at ambient temperature for up to 4 days and is detected by a simple ELISA, and is currently being developed as a lateral flow point of care test.

15:30– 16:00 **Afternoon Tea**

16:00 – 16:30 **Quantification of HER family Dimerisation Status in Primary Invasive Breast Cancer**
Mr Fabricio Barros, University of Nottingham¸UK
HER2 status is currently assessed in routine breast cancer reporting using immunohistochemistry (IHC) in addition to in situ hybridisation (ISH) in borderline cases. The ability of HER2 gene status to predict response to targeted therapy (Trastuzumab) is well documented. However, prognostic information provided by IHC expression categories and prognostic value added by using ISH in borderline cases remains unclear. Patients may acquire resistance to this drug after a period of treatment, which indicates that other molecular mechanisms might influence success of this therapy. Dimerisation between members of the HER family may contribute to resistance against treatments due to different combinations that trigger different downstream pathways. Further quantification analysis of HER family dimers using in situ Proximity Ligation Assay (PLA) may be crucial to identify subset of patients associated with distinct outcome, response to treatment and relationships with HER signalling related biomarkers

16:30 - 17:00 **Chairman's summing up**

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About the Chair
Peter Bach has worked in Academia, for National and International Medicine Safety Agencies, and Biotech companies on a spectrum of novel small molecules to third generation biologicals. Peter has a specialist interest in mechanistic pathology and toxicology, which allows a deeper understanding of the processes associated with disease and intervention, and identifying biomarkers that help establish efficacy and safety. This helps better match individual patients to maximised treatment.

About the Speakers
Anthony (Tony) Warford expertise is in molecular histopathology. He has set up and managed laboratories in the UK health service, academic institutions, biotechnology and Pharmaceutical companies. Technology developments he has spearheaded include the introduction of diagnostic immunohistochemical methods, validation of antibodies for use as biomarkers, production of probes and methods for in situ hybridisation and supervision and interpretation of GLP tissue based safety studies of potential therapeutic antibodies. Concurrently he has championed quality assurance programmes in histopathology and automation of

immunohistochemistry coupled with image capture and analysis. He has also run laboratory safety and human bio-banking programmes. He has published in these fields and shared experience with fellow scientists by organising wet workshops, chairing symposia and lecturing in many countries.

Bernadette Moore is research scientist and a Lecturer in Molecular Nutrition within the Faculty of Health and Medical Sciences at the University of Surrey. Her research primarily focuses on the dietary, genetic and immune factors that influence non-alcoholic fatty liver disease development. Utilizing discovery-based proteomics, genomics and systems biology approaches alongside molecular cell biology techniques, her group aims both to identify disease biomarkers and characterize molecular mechanisms of NAFLD progression.

Paula Meleady PhD (1997) in Cell Biology from Dublin City University. Senior Scientist at the National Institute for Cellular Biotechnology (NICB) since 2001 and currently Programme Leader of the Proteomics and Mass Spectrometry Core Facility at the NICB which is equipped with state-of-the-art proteomics and mass spectrometry equipment. Research interests include proteomic analysis of uveal melanoma, for biomarker discovery. Also have research interests in proteomics of recombinant mammalian cell lines in order to gain insights to improving productivity of biopharmaceuticals. Co-authored over 40 peer-reviewed publications, 6 book chapters and 2 patents to date in research areas related to proteomics, cancer and bioprocessing.

David J. Britton Degree; Pharmacology, University of Bristol (1998-2001). PhD; Cancer Pharmacology, Tenovus labs, Pharmacy Dept, Cardiff University (2001-2005). Post Doc; Cancer Proteomics, Benz/Gibson Lab, Buck Institute, California (2005-2008). Training Instructor; Proteomics, Thermo Fisher Scientific (2008-2010). Senior Research Scientist; Cancer Proteomics, Proteome Sciences plc, UK (2010-current).

Julian Beesley has over 22 years' experience as a research scientist in discovery research with a major pharmaceutical company and over 11 years' involvement in developing biotech businesses, operating widely throughout Europe, the USA, Asia and Japan.

Surajit Debnath, obtained PhD in the field of Animal Physiology (Tripura University, Govt. of India) after majoring with Biotechnology. As an Assistant Professor in the Department of Medical Laboratory Technology, Women's Polytechnic Tripura, India Dr. Debnath is extensively using Biomarker tools for basic research. He received Govt of India fellowship to Hongkong (2011) & Denmark (2012) & have visited Lab of Evolutionary Biology in the J.W.Goethe University, Frankfurt, Germany. Dr. Debnath has published several research papers and is serving as editorial board member of International research journals of repute.

Fabricio Barros just concluded his Phd based on examination of expression amongst the HER family in a large series of breast cancer cases examining the clinical effect of co expression of the receptors and respective ligands. This research study is being developed using not only the standard histopathological techniques as immunohistochemistry and in situ Hybridisation but also using a novel approach (in situ Proximity Ligation Assay) to characterise HER2+ invasive breast tumours. This study is being performed at University of Nottingham within the Breast Cancer Research Group leaded by Prof. Ian Ellis and Dr. Andrew Green.

Bevin Gangadharan obtained his DPhil under the supervision of Prof. Nicole Zitzmann at the University of Oxford where he carried out the first ever gel-based proteomics study to discover novel biomarkers for liver fibrosis. He has more than a decade of experience in proteomics and biomarker discovery and first started in this field in 2000 at Smithkline Beecham looking at depletion of albumin in plasma, an important approach in biomarker discovery. He has published in several peer-reviewed journals and has two patents on novel biomarkers for liver fibrosis. He is on the editorial board for *Biomarker Research*.

Key words: immunohistochemistry, biomarkers, personalised health care, oncology, drug development, mass spectroscopy, proteomics, bioinformatics, DNA, RNA,Biorepositories, biomarkers, drug discovery, Histochemistry, Metabolism, Biologicals, Safety, Efficacy, predictive PharmDx, pharmacodiagnostics,Oncology, biomarker identification/quantification, quantifying biomarkers, clinical tissues, automated-analysis, ELISA, TSH, hFillotropin, hepatitis C patients; single nucleotide polymorphisms; Il-10; IL-28, *MicroRNAs; Toll-like receptors;lung cancer patients,* ox-LDL Ab; Insulin resistance; atherosclerosis; IMT, Adrenomedullin; SLE; nephritis, uveal melanoma, metastasis, 2D-DIGE, label-free LC-MS, biomarker, Nonalcoholic fatty liver disease; proteomics; iTRAQ; Breast Cancer, HER2, Dimerisation, PLA, PSA, prostate, ERG, : FFPET, DLBCL, microRNA, qRT-PCR, biomarkers

Registration Web Site:
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TRACING ANTIGENIC IMPURITIES IN SERUM CONTROLS OF AGGLUTINATION TEST KITS BY qs-ELISA ASSAY AND EVALUATION OF IMMUNOLOGICAL CROSS REACTIVITY *In-silico*

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Dr. Soma Addya, House Physician, CMRI, Khidderpore, Kolkata, West Bengal, INDIA

Serum controls of immunological test kits are heterogeneous in terms of antigenicity. The objective of the study was to assess, the traces of antigenic glycoproteins in positive & negative control serum samples of immunological test kits by Quantitative Sandwich ELISA. hTSH trace was evaluated from control serums of RA & ASO immunological test kits by 96 well quantitative sandwich Enzyme Linked Immunosorbent Assay (ELISA) using anti-TSH ab-HRP conjugate with TMB chromogenic marker. Assessment of the possible cross reactive species was done using computational biology.

TSH was selected for the trace analysis by Quantitative Sandwich ELISA of the control serums for its high molecular weight (28 kda) and thus of a probably higher antigenicity in an heterogeneous immunologic environment as in serological studies. Peptide sequences of human TSH beta subunit, h fillotropin, hCG and human TSH alfa subunit were aligned by CLUSTAL W and percentage of identity of the sequences was evaluated by the NEEDLE program (http://www.ebi.ac.uk/emboss/align/) of the Pasteur institute. Antigenic domains of the peptides were identified using ANTIGENIC program (http://bioweb.pasteur.fr/seqanal/interfaces/antigenic.html).

Positive control serum of RA had highest concentration of hTSH traces (1.221 to 1.803 mIU/ml) with intra assay Coefficient of Variation (CV) 0.147 % (n=36) and lowest traces in ASO Positive control serum, estimated to be 0.224 to 0.270 mIU/ml with intra assay Coefficient of Variation (CV) 0.006 % (n=27). Themcombinatorialmapproach00of00using00theooimmunoinformatics revealed that highest structural homology of the peptides is present in the N-t segment (N -terminal ends, highest with hFillotropin, NEEDLE Score 191.5 with reference hTSH b) of the peptide and highest antigenicity (highest with hCG, ANTIGENIC Score 145 in a 17 residue segment, at 141->157) was present in the C-t segment (C -terminal end).

POLYMORPHISM IN INTERLEUKIN-10 AND INTERLEUKIN-28B GENES IN CHRONIC HEPATITIS C VIRUS EGYPTIAN PATIENTS AND THEIR EFFECT ON RESPONSE TO PEGYLATED INTERFERON/RIBAVIRIN-THERAPY

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Recently, It has been suggested that single nucleotide polymorphisms (SNPs) in some cytokine genes may influence the production of the associated cytokine that affect the host immune response to pegylated interferon–α (Peg-IFN–α) with ribavirin(RBV) in hepatitis C virus (HCV) patients. The present study aimed to investigate possible association of SNPs of IL-10 and Il-28B along with their serum levels in predicting treatment response of HCV-4 Egyptian patients to a Peg-IFN–α/ RBV. A total of 100 HCV genotype 4-infected patients and 80 healthy control subjects were included in the present study. SNPs in (IL-10592-rs1800872 A/C and -819-rs3021097 T/C) and IL-28B (rs8099917 T/G and rs12979860 C/T) genes and their serum levels were assessed. IL-10-592-CC, IL-28-rs8099917-TT and- rs12979860-CC genotypes were significantly higher in responders as compared to non-responders. Interestingly, serum IL-10 levels showed significant increase and in contrast serum Il-28B showed significant decrease in HCV patients compared to normal. Polymorphisms in IL-28B are more sensitive (P<0.001) than IL-10-592(P=0.03). However, serum IL-10 level is more superior to IL-28 level as a prognostic marker using receiver operator characteristic (ROC) analysis. It can be concluded that SNPs in IL-28B along with Il-10 and 28 serum levels may be promising predictors for HCV therapy.

SERUM MICRORNAS AS DIAGNOSTIC BIOMARKERS IN LUNG CANCER PATIENTS

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MicroRNAs (miRNAs) are a class of small non-coding RNAs that regulates gene expression by inducing RNA degradation or interfering with translation. Aberrant miRNA expression has been described for several human malignancies. Most recently, miRNA expression profiles have been proposed as potential biomarkers for cancer diagnosis and treatment monitoring. The Toll-like receptors

(TLRs) represent a group of single-pass transmembrane receptors conserved from drosophila to mammals; they are expressed on cells that are central to innate immune responses. Therefore, the aim of the present study is to evaluate the over-expression of certain miRNAs and TLRs in sera of lung cancer patients. A total of 150 individuals were enrolled in this study and were divided into the following groups: Group I: lung cancer patients; Group II: tuberculous patients; Group III: pneumonic patients with bacterial infection; Group IV: patients with transudative PE;Group V:normal healthy individuals. Biochemical, cytological, histopathological and microbiological examinations were performed for diagnosis. Serum gene expression levels of miRNA-21, miRNA-155, miRNA-197 and miRNA-182 were determined by microarray technique. Serum levels of TLR3, TLR4 and TLR7 by sandwich enzyme-linked immunosorbent assay (ELISA). Results revealed that increased levels of studied miRNAs but not TLRs in sera of lung cancer patients are considered as promising candidates as non-invasive diagnostic tools for lung cancer patients.

CAROTID INTIMA-MEDIA THICKNESS AND OXIDIZED LOW-DENSITY LIPOPROTEIN ANTIBODIES AS MARKERS PREDICTING CARDIOVASCULAR EVENTS IN ABSENCE OF CVD SYMPTOMS IN SYSTEMIC LUPUS ERYTHEMATOSIS PATIENTS

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We aim to investigate whether serum ox-LDL antibodies levels are altered in Systemic Lupus Erythematosis (SLE) patients and whether these alterations are related to insulin resistance and atherosclerosis. The study included 30 SLE women and ten age and sex matched healthy volunteers as controls. Measurement of insulin, glucose, creatinine, total cholesterol and ox-LDL antibodies were done. Intima-media thickness (IMT) of the carotid artery was measured in all subjects by ultrasonography. Patients had significantly higher mean values of ox-LDL Ab and IMT compared to controls. IMT significantly positively correlated with the SLEDAI and SLICC/DI respectively. We can conclude that oxLDL antibodies are altered in SLE patients especially with metabolic syndrome and correlated with insulin resistance and atherosclerosis as well as SLE activity and damage indices. Ox-LDL antibodies level and IMT measurement are recommended in SLE patients and could be used as markers predicting cardiovascular events in a symptomatic CVD.

PROTECTIVE ROLE OF ADRENOMEDULLIN IN LUPUS NEPHRITIS PATIENTS

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Tumor necrosis factor-α and interleukin-1ß, which have been implicated in systemic lupus erythematosus (SLE) to stimulate the secretion of human adrenomedullin (AM) in vitro, suggesting that AM has a role in the pathogenesis of SLE. We aim to find a possible role of plasma AM in patients with lupus nephritis and its relation to the severity of nephritis. Forty patients diagnosed to have SLE and twenty control subjects were enrolled in this study. All patients with renal involvement had percutaneous renal biopsy. Clinical and laboratory data and the SLE Disease Activity Index (SLEDAI) were recorded. Plasma AM was measured by enzyme linked immunosorbent assay (ELISA). Subjects were classified into three groups: SLE patients with nephritis, SLE patients without nephritis and normal controls. Twenty seven patients had SLEDAI >10 (moderate and severe activity) and 13 had SLEDAI ≤10 (mild activity). Twenty four of the SLE patients had nephritis. Plasma AM concentration was significantly higher in patients with SLE than in controls. Patients with SLEDAI >10 had significantly higher plasma AM level than patients with SLEDAI ≤10. Plasma AM level correlated with SLE disease activity (P=0.03). Patients with lupus nephritis had significantly higher plasma AM level than those without renal involvement and the plasma AM levels negatively correlated with 24h urinary protein levels. We conclude that plasma AM level is a potential candidate of disease activity marker for SLE patients and it probably provides a protective role in reducing the severity of lupus nephritis.

ERG IMMUNOHISTOCHEMISTRY IS NOT PREDICTIVE FOR PSA RECURRENCE, LOCAL RECURRENCE OR OVERALL SURVIVALAFTER RADICAL PROSTATECTOMY FOR PROSTATE CANCER

A Marije Hoogland[1], Guido Jenster[2], Wytske M van Weerden[2], Jan Trapman[1], Theo van der Kwast[3], Monique J Roobol[2], Fritz H Schröder[2], Mark F Wildhagen[2] and Geert JLH van Leenders[1]

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In prostate cancer genomic rearrangements involving genes encoding ETS transcription factors are commonly present, with *TMPRSS2-ERG* gene fusion occurring in 40-70%. Studies on the predictive value of *ERG* rearrangement as detected by in-situ hybridization or PCR have resulted in varying outcomes. The objective of this study was to correlate immunohistochemical ERG protein expression with clinico-pathologic parameters at radical prostatectomy specimens, and to determine its predictive value for post-operative disease recurrence and progression in a prostate cancer screening cohort. Since androgen receptor is down-regulated by ERG in cell lines, we also compared expression of respective proteins.

We selected 481 participants from the European Randomized Study of Screening for Prostate Cancer treated by radical prostatectomy for prostate adenocarcinoma. A tissue microarray was constructed containing representative cores of all prostate cancer specimens as well as 22 xenografts and seven cell lines. Immunohistochemical expression of ERG and androgen receptor was correlated with Prostate Specific Antigen (PSA), Gleason sum, pT-stage, surgical margins, biochemical recurrence, local recurrence, overall death and disease-specific death.

ERG expression was detected in 284 patients (65%). Expression occurred significantly more frequent in patients with PSA ≤ 10 ng/mL (p=0.024). There was no significant association between ERG and Gleason sum, pT-stage or surgical margin status. PSA (p=0.011), Gleason sum (p=0.003), pT-stage (p=0.001) and surgical margin status (p<0.001) all had independent value for post-operative biochemical recurrence, while positive surgical margin (p=0.021) was the only independent predictor for local recurrence. ERG protein expression did not have prognostic value for the clinical endpoints in uni- and multivariate analyses. A positive correlation existed between ERG and androgen receptor expression in single tissue cores (p<0.001).

In conclusion, immunohistochemical ERG expression has no predictive value for prostate cancer recurrence or progression after radical prostatectomy. Increasing ERG levels are associated with up-regulation of androgen receptor expression in clinical specimens.

EXPRESSION OF STEM CELL MARKERS IN HUMAN PROSTATE CANCER

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Various markers have been postulated to be specific for, or over-expressed in normal epithelial and prostate cancer (PC) stem cells, among which α2-integrin, α6-integrin, CD117, CD133, EZH2 and OCT3/4. Analysis of cancer stem cells (CSC) has predominantly been performed using cell lines, xenografts or short-term cultured prostate tissue. Little is known on the expression of proposed stem cell markers in human PC tissues. The objective of this study was to investigate the immunohistochemical expression of the stem cell markers α2-integrin, α6-integrin, CD117, CD133, EZH2 and OCT3/4 in a large set of clinical PC samples.

We included 481 participants from the European Randomized Study of Screening for Prostate Cancer (ERSPC) treated by radical prostatectomy for PC. A tissue microarray was constructed containing 3 representative cores of all PC specimens. Immunohistochemical marker expression was correlated with Prostate Specific Antigen (PSA) level, Gleason score (GS), pT-stage, surgical margins, biochemical recurrence, local recurrence, overall death and disease-specific death.

Expression of α2-integrin was identified in a subpopulation (<5%) of tumor cells in 94.7% of cases, while more abundant expression (≥5%) occurred in 5.3%. We find a significant association between expression of α2-integrin and PSA at time of diagnosis (p=.039). The majority of cases (71.6%) demonstrated abundant expressed of α6-integrin (≥5%), while sporadic expression was found in 28.4% of patients. Expression of α6-integrin was significantly associated with low PSA levels (≤10 ng/ml)(p=.006), low Gleason score (GS<7)(p=.000) and organ-confined disease (pT2)(p=.033). Absence of low α6-integrin expression was correlated with both biochemical (p=.028) and local recurrence (p=.021).

Expression of EZH2 was generally low with ≥ 1% positive tumor cells in 2.6% of cases, <1% in 39.1% and complete absence of expression in 58.3%. EZH2 expression was significantly associated with high Gleason score (GS ≥7) (p=.009) and biochemical recurrence (p=.003).

We did not identify expression of CD117 and OCT3/4 in normal epithelium and prostate cancer (positive controls: mast cells and seminoma). CD133 expression was expressed at the apical side of atrophic luminal prostate epithelium, but could not be identified in PC.
In conclusion, the sporadic expression of α2-integrin and EZH2 in human PC tissues is in line with their putative role in CSC's. The high expression of α6-integrin together with the absence of CD117, CD133 and OCT3/4 does not support their proposed role as CSC markers in patients' PC. The higher expression of α2-integrin and EZH2 in GS≥7 indicates that CSC's occur more frequently in high-grade PC. In contrast, α6-integrin expression is associated with low-grade PC and reduced recurrence rates.

ENVIRONMENTAL CONTAMINANT BISPHENOL A TOXICITY STUDY IN MICE MODEL

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Introduction- Bisphenol A (BPA) is a monomer of polycarbonate plastic used to manufacture plastic baby bottles and lining of food cans. It has endocrine-disrupting potential and exerts both toxic and estrogenic effects on mammalian cells.
Aims- The aim of this study was to investigate if BPA induced oxidative stress and toxicity in the testicular mitochondria of adult male Swiss albino mice.
Methodology- The mice were divided into four groups of 6 animals each, orally exposed to standardized doses of BPA (5, 10, 100 mg/kg body weight) for 14 days and changes in antioxidant enzyme were checked. Apoptosis marker proteins (ctochrome c and caspase 3) levels were estimated by immunohistochemistry.
Results- BPA treated group showed increased expression of apoptosis marker proteins as compared to control group which received no BPA treatment. The BPA-treated mice showed significant increase in lipid peroxidation (LPO) and decrease in reduced glutathione (GSH) content in their testicular mitochondria when compared to controls ($P < 0.01$). The activities of antioxidant enzymes such as superoxide dismutase, glutathione reductase and glutathione peroxidase were decreased.
Discussion- Based on this study, we can conclude that BPA induces oxidative stress in the testicular mitochondria of exposed mice. Apoptosis marker protein levels elevation confirms that mitochondrial intrinsic pathway of apoptosis is involved in the apoptosis of germ cells in the testes of BPA exposed mice.

SYNTHESIS OF BENZIMIDAZOLES BEARING OXADIAZOLE AND TRIAZOLO-THIADIAZOLES NUCLEUS: AS NEW ANTICANCER AGENTS

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Cancer is a disease of the cell cycle, in which abnormal cells divide mitosis without control and being one of the major health problems in the world from decades. there is an urgent need for novel effective drug regimens for the treatment of cancer because the current chemotherapy suffers from a slim therapeutic index, with significant toxicity A lot of chemical classes of heterocyclic and fused heterocyclic compounds have been identified through molecular biology, empirical screening and rational drug development for evaluation of antitumor agents during the past decades.

Due to of above view, it was thought worthwhile to prepare a new hybrids that comprises benzimidazole nucleus with oxadiazole as well as thiadiazoles ring to produce new promising anticancer agents. The structures assigned to all the synthesized hybrids were supported by the results of elemental analysis as well as IR, ^{1}H NMR, ^{13}C NMR and Mass spectral data and found in full agreement with the proposed structures. The anticancer activity of synthetic hybrids were screened at National Cancer Institute (NCI), USA against full NCI 60 cell lines panel representing on full nine human systems as leukemia, melanoma and cancers of lung, colon, brain, breast, ovary, kidney and prostate in accordance with their applied protocol (used SRB assay). The result is given in three calculated response parameters, GI_{50} (Growth inhibitory activity), TGI (Cytostatic activity) and LC_{50} (Cytotoxic activity).

The hybrid (**Ib-A3k**), showed remarkable growth inhibition at a single dose (10μM) and further selected for anticancer screening at five dose concentrations (0.01, 0.1, 1, 10 and 100μM) against full NCI 60 cell panel with GI_{50} values between 0.49 to 48.0μM except HCT-15 (GI_{50}>100) under sensitive range an outstanding activity on all the tested cell lines representing nine different subpanels. With regard to the sensitivity against some individual cell lines the compound showed high activity against Non-Small Cell Lung Cancer cell lines like HOP-92 (GI_{50} 0.49, TGI 19.9, LC_{50} >100 and $Log_{10}GI_{50}$ -6.30, Log_{10}TGI -4.70, $Log_{10}LC_{50}$ > -4.00). The compound proved to be sensitive towards all the tested Leukemia cancer cell lines with not more than 4.94μM concentrations. All the tested prostate cancer cell lines were sensitive with not more than GI_{50} 5.40μM concentrations of the tested compound. The highest growth inhibitory activity was observed against the Non-Small Cell Lung Cancer HOP-92 cell line with GI_{50} value 0.49μM and minimum growth inhibitory activity against Renal Cancer ACHN cell line with GI_{50} value 48.0μM.
Based on these observations, compound (**Ib-A3k**) could be a subject of further investigations for producing potential antitumor agents. Finally it's conceivable that further derivatization of such compounds will be of interest with the hope to produce more selective and potential antitumor agents.

MICRORNA EXPRESSION CAN BE RELIABLY ASSESSED FROM FORMALIN FIXED PARAFFIN EMBEDDED TISSUE AND MIR-24 REPRESENTS A SUITABLE REFERENCE MICRORNA FOR DIFFUSE LARGE B-CELL LYMPHOMA STUDIES.

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Recently, much interest has emerged in the study of microRNAs; small single-stranded RNAs that repress translation of mRNA into protein and thus, form an additional level of transcriptional regulation. MicroRNAs are involved in the pathogenesis of cancer, including lymphoma, by targeting genes known to be associated with carcinogenesis and demonstrate promise for use as diagnostic, prognostic and therapeutic markers, as they have the potential for reliable recovery from formalin fixed paraffin embedded tissue (FFPET).

Biopsies preserved as FFPET are widely available in the clinical setting and represent a wealth of archival material for research, which has been preserved in optimal histological condition. However, in contrast to cryogen preserved tissues, the fixation and embedding process is reported to compromise nucleic acid quality and integrity. Conversely, the short size of microRNAs makes them less vulnerable to degradation and thus, most likely well preserved in FFPET.

This study aimed to validate microRNAs as reliable and reproducible markers for assessment in FFPET biopsies. We used tissue from patients with diffuse large B-cell lymphoma (DLBCL), as there is still the need to identify new biomarkers for this disease. Initial studies showed that microRNA expression is comparable between matched fresh-frozen vs. FFPET samples (miR-17-5p: $p=0.01$, miR-92: $p=0.003$) and that no significant deterioration in expression occurs over prolonged FFPET storage ($p=0.06$). Furthermore, microRNA expression is equivalent irrespective of RNA extraction method ($p<0.001$) or DNAse treatment of total RNA ($p<0.001$). Finally, we validate miR-24 as a suitable reference microRNA for DLBCL FFPET studies.

DNA FROM URINE AS SOURCE FOR BIOMARKERS IN PUBLIC HEALTH

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Biomarkers play a key role in public health because they are indicators of hazard, exposure, disease and population risk. They provide information for early detection, prediction, prevention, prognosis, diagnosis and response to therapy of diseases. Biomarkers can therefore be used to make group and individual risk assessments to support a pro-active public health policy.

There are different types of biomarkers. The most commonly used types of biomarkers are obtained from body fluids, of which blood is traditionally the most used although it requires a certain expertise for sampling and some target groups might be reluctant to providing samples (e.g. difficult to get permission of the parents of young children). Urine might therefore be a valuable alternative as a source of biomarkers, because it is readily available and can be obtained by a noninvasive collection method, which is an advantage for large scale population studies. Until now, urine is mainly used as a source of protein biomarkers, although it also contains metabolites and nucleic acids. However, until now, urine-derived nucleic acids have not been used very often as biomarkers or for genotyping purposes.

A comparison of six commercial DNA extraction kits has been performed to select the most appropriate kit allowing the extraction of high-quality DNA from urine samples (from fresh and frozen urine), based on the following criteria: yield, PCR amplificability, presence of remaining downstream amplification inhibitors, price and time per extraction. The extracted DNA using all six kits was amplifiable by PCR but only two kits gave a good yield. The presence of remaining PCR inhibitors in the DNA extracts was evaluated by a qPCR inhibition test using the SYBR® green method. On the basis on these results, one kit has been retained, suitable to our needs, giving the highest yield and with the absence of remaining PCR inhibitors in the DNA extract.

In a next step, the DNA extracted from urine will be evaluated as source for biomarkers of public health, e.g. methylation sites, especially in large scale population/epidemiological studies, where urine collection is more convenient than blood collection.

PROTEOMIC ANALYSIS OF THE INTERACTION BETWEEN APHIDS AND PEA PLANTS

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Pea aphids *Acyrthosiphon pisum* is one of many insect species nutritionally dependent on plant sap. They cause damage directly by feeding on plant sap and indirectly as a pathogen vector. This study was divided into two parts: 1) to study the impact of mechanically wounding leaves with a mounted needle; 2) to compare the effect of wounding with the aphid infestation. For initial proteomic analysis, 500 mg samples of protein were extracted from healthy and wounded plants. The proteins were separated by isoelectric focusing (IEF) on 24 cm immobilised pH gradient (pH 3-11) strips.After equilibration, the strips were loaded onto 12.5 % SDS-polyacralamide gels for separation according to molecular weight. Separated proteins were detected by Coomassie Brilliant Blue staining, and analysed using PDQuest software.This identified 604 protein spots in gels of proteins from healthy plants versus 559 protein spots from wounded plants. Five proteins were detected in aphid infested plants and not in healthy plants. These proteins are beingidentified using Q-TOF Mass Spectrometry. We will be comparing proteins altered in abundance by aphid infestation with the proteins in wounded plants.

AMINOBENZANTHRONE DERIVATIVE ABM-FLUORESCENT BIOMARKER OF IMMUNE STATE OF PATIENTS

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In different pathologies membrane damage in immune cells and blood plasma albumin envolves as a consequence of alterations in a immune state of patients and persons exposed to ionizing radiation during Chernobyl clean-up works. It is important for clinicians to receive information on the biophysical status of these cells via quick, reliable, reproducible methods. In this regard, fluorescent probes have shown to be excellent tools for use in such protocols. Such an analysis has a great potential for not only for helping to comprehed mechanisms of immunomodulation associated with the induction/progression of pathologies, but might serve as a very important prognostic indicator of long- term survival among patients. ABM was approved in non-malignant diseases (advanced lung tuberculosis, rheumatoid arthritis, cardio-vascular diseases, type 2 diabetes mellitus etc.) ; malignant diseases (gastrointestinal cancer, advanced cancer), and in people exposed to ionizing radiation during Chernobyl clean-up works.

Spectral characteristics of aminobenzanthrones (e.g. ABM) satisfy all requirements for an ideal tracer (bright fluorescence, high extinction coefficient, photo-, thermo-and chemical stability, non-toxicity for cells).

Spectral characteristics of this probe correlate with a number of important parameters of artificial and cellular membranes such as physicochemical state, microviscosity, proliferating and lipid metabolic activities of cells, distribution of lymphoid subset, etc.

The choise to examined albumin among the myriad of constituents in plasma, is that this protein is practically the single source of ABM binding and subsequent fluorescence in plasma. ABM can be used as a probe sensitive to conformation changes in protein (the most prominent changes in fluorescence characteristics occured at pH values known to cause conformation transitions of proteins, such as accidic expansion, N-F transition etc.). Only fluorescent probes allow to detect the „effective" concentration of albumin in blood plasma („effective" concentration- equivalent in blood plasma of healthy albumin and reflects it's binding and carrier functions). The total albumin concentration is more conservative. Data correlates with albumin auto-fluorescence data and ABM binding sites characteristics (binding sites count, affinity for probe, polarity).

ABM possess highly potential in identification of fibrillar proteins aggregates and uncovering their structural peculiarities.

ABM spectral parameters in lymphocytes and blood plasma are coupled with alterations in cellular mechanisms of immune regulation in the patients with different pathologies.Interestingly, ABM in lymphocytes and plasma was found to correlate with select immunological parameters (CD4+: CD8+ ratios, lymphocyte count etc.) in patients. The results obtained showed strong strong agreement between changes in probe spectral characteristics and both clinical and pathological estimates of disease. Measures of ABM spectral characteristics in plasma albumin and/or especially those of lymphocytes (total and among different subtypes) could potentially be a useful tool in clinical immunilogical screenings to estimate the immune state of patients. Compared to many other commonly used diagnostics protocols the fluorescence-based methods is less expensive and not very time-consuming, technically simpleand 100 times more sensitivethat standart absorbance-based methods.