MANCHESTER
1824

The University of Manchester

UNIVERSITY OF Southampton

Monday, 18 March 2013

Trevor Kelham
Standard Chartered Trust (Guernsey) Ltd
PO Box 620
Bordeaux Court
Les Echelons
South Esplenade
St Peter Port
Guernsey GY1 4PX

Dear Mr Kelham

Please find attached our application to fund a -80°C freezer and blood processing centrifuge to be situated at the MSG. This funding has been requested to enable the collection of blood samples from prostate cancer patients at presentation. By having this equipment at MUG, the samples will be able to be processed immediately and stored until sufficient numbers are there for transportation back to Southampton for further research into prostate cancer biomarkers. To date, we have collected over 550 samples from the Guernsey Islanders, catalogued and stored, and have recently completed the deep proteomic mining of the serum proteome. We are currently working to functionally validate 13 (from a potential 1039) statistically and reproducible biomarkers using ELISAs. All of the samples we have collected thus far are from patients at varying stages of prostate cancer treatment and we would like to continue our work on patient samples prior to any treatment to remove any confounding by treatment associated effects.

Thank you for your consideration.

Yours sincerely

Professor Paul A. Townsend
Associate Dean
p.a.townsend@manchester.ac.uk

Tel: +44(0)161 701 6923 (PA)

Please reply to:
Professor Paul A. Townsend
Associate Dean, Faculty Institute for Cancer Sciences, University of Manchester, Manchester Academic Health Science Centre, Research Floor, St Mary's Hospital, Oxford Road, Manchester M13 9WL, UK

Title: MudPIT and iTRAQ LC-MS/MS prostate cancer proteomic biomarker discovery – *continuation – validation of the novel prostate cancer biomarkers*

Applicant:

- Professor Paul A. Townsend, BSc, CertEd, PhD, Professor in Molecular Cell Biology, Cancer Sciences Unit, School of Medicine, University of Southampton.

Co-applicants:

- Dr Samantha Larkin, BSc, PhD, Postdoctoral Researcher, Cancer Sciences Unit, University of Southampton.
- Dr Spiros Garbis, BA, PhD, Lecturer, Institute for Life Sciences/Cancer Sciences Unit, University of Southampton

Collaborators:

- Dr Catherine Chinyama, MB, ChB (Hons), FRCPath, LLM. Consultant Pathologist, Princess Elizabeth Hospital, Guernsey.
- Mr Owen Cole, Urology Consultant, Princess Elizabeth Hospital, Guernsey.
- Professor Hardev Pandha, MB, ChB, MRCP, FRACP, PhD. Head of Oncology, Postgraduate Medical School, University of Surrey.
- Dr Claire Aukim-Hastie, DipHE, BSc, PhD, Lecturer in Health Sciences, University of Surrey.

Background:

Despite the high mortality rate associated with prostate cancer, a far more indolent and asymptomatic form is also found in the majority of men over 80 years of age[1]. Currently, raised levels of the biomarker prostate specific antigen (PSA) along with a suspicious digital rectal examination (DRE) are used to identify patients who require a trans-rectal ultrasound (TRUS) guided biopsy of the prostate to look for prostate cancer[2]. However, PSA lacks both sensitivity[3] and specificity[4], DRE is inherently subjective, and even TRUS guided biopsy is imperfect[5]. This means that patients with aggressive forms of prostate cancer may be missed, and those with indolent disease may be subjected to unnecessary radical therapy and the subsequent treatment-associated morbidity. There are guidelines in place to attempt to categorise patients and thereby limit over treatment of low risk patients[2]. However, meta-analyses of such patients cast doubt on the prognostic significance of the 'low risk' definition, showing that a large proportion of these individuals had a more advanced form of the disease than predicted when their radical prostatectomy specimen was examined[6]. Thus novel biomarkers are needed that can better predict prognosis of prostate cancer to help guide management. In terms of patient benefit and quality of life, biomarkers indicative of a more aggressive prostate cancer phenotype would limit invasive treatment (i.e. those with associated side effects) for patients with indolent disease and target radical treatment strategies to those patients with faster growing aggressive disease.

Proteomics is a group of techniques that may well be able to deliver such biomarkers. The approach involves studying the protein element of a biological sample ("the proteome"), and has seen a rapid expansion over the past few years so that it is now possible to quantify and identify the proteomes of multiple samples very quickly. This implies that samples from large cohorts of patients with cancer can be compared with those from controls to identify proteins which are differentially expressed and may therefore be useful as future disease biomarkers.

We hypothesised that robust serum proteomic analysis and quantification could accurately detect, monitor and stage prostate cancer. Using advanced proteomic biomarker discovery platforms, we aimed to employ untargeted screening and targeted proteomic approaches to identify predictive and/or prognostic prostate cancer biomarkers.

Status of prostate cancer proteomics:

Serum sample collection from Guernsey Islanders for use in this study.

We collected have collected a total of 558 samples from Guernsey Islanders in 3 sampling sessions. Of the samples taken, we have 96 from confirmed prostate cancer sufferers (mean 71.9 years old), 12 with confirmed benign disease (mean 71.8 years old), 26 with putative benign disease (mean 71.8 years old) and 135 controls (mean 63.5 years old). The remaining 289 are currently unallocated as we are awaiting information from the Urology team on Guernsey.

Discover potential novel biomarkers of prostate cancer diagnosis and progression.

Using a multidimensional approach to separate, identify and quantify the thousands of protein within the serum proteome, we have identified over 1000 proteins that were differentially expressed between the control group and BPH, T1-T2 stage prostate cancer and T3-T4 stage prostate cancer. Using our prostate cancer and molecular cell biology expertise, we have identified 29 proteins that are most promising based on peptide representation, fold difference in expression between the subgroups, and cellular function. We have selected 13 of these proteins for initial ELISA validation. Of note, PSA (prostate specific antigen) was identified in our study and found to be virtually unchanged in BPH and low stage prostate cancer but significantly elevated in later stage prostate cancer. This is in agreement with current views on PSA.

Validate potential novel biomarkers using a geographically distinct cohort.

We anticipated that this would be performed using either targeted mass spectrometry (multiple reaction monitoring; MRM) or by ELISA. We decided to utilise ELISA as this seems to be the most clinically applicable method currently. We have just ordered the reagents for this stage of the study and hope to have the first 13 biomarkers validated and data analysed by the end of March 2013. Further validation of the remaining 16 biomarkers will then be complete by the end of August 2013.

Moving on from this...

The current, accepted 'gold standard' prostate cancer markers lack sensitivity and specificity, and risk calculators have limited value. Thus, any improvement is likely to have a considerable impact on clinical practice. By applying our novel proteomic methodology, reliable bed-side markers are highly likely to be revealed and validated. Our current prostate cancer samples from Guernsey were taken from men with prostate cancer who were having treatment of some form at the time of sampling. We would now like to sample men at diagnosis, prior to any treatment, to limit and confounding that treatment may have on biomarker analysis. In order to do this, we would need to have a centrifuge and a -80°C freezer on the Island to enable processing of the samples and adequate storage for processed samples prior to shipping over to Southampton for analysis.

Funding request:

We are applying for funding to finance a -80°C freezer and a centrifuge to enable the collection of samples for a prospective study. In collaboration with Owen Cole, we aim to collect samples from prostate cancer patients at diagnosis, have them processed at the MSG or Princess Elizabeth Hospital and then ship them back to Southampton in larger batches. The centrifuge will enable processing of the samples to obtain serum for analysis and the freezer will enable medium-long term storage of samples so that cost-effective shipping can occur once we have enough samples to batch and ship. A centrifuge will cost £3090 and a small -80°C freezer will cost £4,700, a total of £7,790.

The Future:

Following on from this work, we hope to have a panel of markers that can be utilised to diagnose and chart the course of prostate cancer progression. We expect to have a greater understanding of how they contribute to the progression of the disease and to also have some markers that can be targeted by therapeutics. Once this work is completed we will then focus on the pre-treatment samples that we will soon be collecting with Owen Cole to further test these markers, and to dissect and understand treatment pathways. In addition, we will continue to study the function of any markers identified to better understand the process of prostate carcinogenesis using cell models. In order to do this, we are currently writing large national bids to the Biotechnology and Biological Sciences Research Council (BBSRC), Cancer Research UK (CRUK) and Prostate Cancer Charity (PCC); applications that would not have been possible without the support of Wessex Medical Research. Further, we believe that our work to date has raised public awareness of prostate cancer and research into the disease through our work with MUG (Men Uprising in Guernsey), the Orchideans and our work with the Guernsey healthcare professionals. We hope that this will continue in our further work and applications.

References:

1. Bostwick, D.G., et al., *Human prostate cancer risk factors.* Cancer, 2004. **101**(10 Suppl): p. 2371-490.
2. NICE. *Prostate Cancer Diagnosis and Treatment.* 2008 [cited 2012; Available from: http://www.nice.org.uk/nicemedia/pdf/CG58NICEGuideline.pdf.
3. Thompson, I.M., et al., *Prevalence of prostate cancer among men with a prostate-specific antigen level < or =4.0 ng per milliliter.* N Engl J Med, 2004. **350**(22): p. 2239-46.
4. Thompson, I.M., et al., *Operating characteristics of prostate-specific antigen in men with an initial PSA level of 3.0 ng/ml or lower.* JAMA, 2005. **294**(1): p. 66-70.
5. Selley, S., et al., *Diagnosis, management and screening of early localised prostate cancer.* Health Technol Assess, 1997. **1**(2): p. i, 1-96.
6. Harnden, P., et al., *The clinical management of patients with a small volume of prostatic cancer on biopsy: what are the risks of progression? A systematic review and meta-analysis.* Cancer, 2008. **112**(5): p. 971-81.