

# An interim report on the P-MARK project



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## P-MARK biorepository

The availability of well-defined clinical samples from multiple centres is a prerequisite for the identification of new markers and the evaluation of recently developed promising markers. For this purpose a P-MARK biorepository - with central storage at the Sheffield School of Medicine Biorepository Facilities - was set up by the principal investigators from Rotterdam, Malmö, Nijmegen and Sheffield. A collection of more than 1,900 historical serum samples from the ERSPC and the ProtecT studies and the previous European BIOMED project is now in place, divided over three Collections:

- Collection A: Diagnostic markers (patients with PSA < 3 ng/ml or PSA ≥ 3 - ≤ 10 ng/ml and healthy men with PSA < 3 ng/ml or PSA ≥ 3 - ≤ 10 ng/ml)
- Collection B: Predictive markers (patients with nadir PSA ≤ 1 ng/ml after radical prostatectomy and patients with advanced untreated disease)
- Collection C: Prognostic markers (clinical follow-up > 5 years, various groups ranging from healthy men to patients with metastatic disease at presentation)

The P-MARK biorepository is currently augmented with longitudinal serum, plasma, DNA and RNA samples from patients diagnosed with PCa. Prospective collection of these biomaterials - according to a standardized blood collection protocol - is ongoing in Sheffield and Rotterdam, and is soon to be initiated in Malmö and Nijmegen. A urine collection, now comprising 400 historical urine samples collected in Nijmegen, will be enlarged with samples obtained by prospective collection.

## Marker discovery for PCa

In order to be able to identify markers in serum the Rotterdam investigators finalized the development and evaluation of a mass spectrometry profiling method (2). In short, serum is depleted from the most abundant proteins and then digested by trypsin. Peptide profiles are generated using matrix-assisted laser desorption ionization Fourier-transform mass spectrometry (MALDI FT-MS) and PCa-specific peaks are identified based on the peak mass. Using this method, a discovery set - composed of 27 controls and 27 metastasized patients and a test set consisting of a case-mix of 250 samples from patients with PSA between 3 and 10 ng/ml, all derived from the P-MARK biorepository, has been studied. A number of promising candidate markers could be identified and the development of an immunoassay for one of these markers is currently ongoing at Fujirebio Diagnostics AB. In the final year of P-MARK, new candidate markers will be further evaluated in various patient groups.

A method for marker discovery in urine consisting of sample prepurification followed by nano LC tandem mass spectrometry (Q-TOF) was developed in Helsinki. This method has been used to study naturally occurring peptides in a discovery set of 10 controls and 10 patients with PCa. A total of 47 differentially expressed peptides were found. Ten of these peptides have been identified and identification of the remaining ones is ongoing. The utility of these will need to be evaluated in an extended urine sample set. Preliminary results indicate that tumour-associated trypsin inhibitor (TATI) is a candidate marker. TATI was previously discovered as a marker for ovarian cancer and is also reported to be associated with urological cancers like bladder and kidney cancer (3).



PSA. This was confirmed in a multicentre study including 534 patients, from which data will be published in due time (4). A commercial variant of the PCA3 RT-PCR assay has been developed by the Genprobe company (San Diego, USA) and its diagnostic value is now being tested in patients with an elevated PSA and a negative biopsy result in a European multicentre study. Within P-Mark, preparations for a study on the utility of PCA3 in the ERSPC screening cohort are ongoing.

## Bone morphogenetic protein-6 (BMP-6)

BMP-6 is a protein that has been shown to be overexpressed in patients with advanced stage PCa and with skeletal metastases, and may therefore be a promising prognostic marker. However, due to a combination of poor initial clinical evaluation results and technical assay difficulties, the P-MARK consortium decided to terminate the further development of this potential marker.

## Osteoprotegerin (OPG)

A second promising prognostic marker is OPG, a protein that regulates bone turnover. In a study on 389 patients with histologically confirmed PCa and 69 controls, serum OPG levels at baseline were significantly elevated in men who developed disease progression compared to those who had stable disease. In contrast, PSA levels at baseline did not differ between the two groups. This applied equally to men with or without metastasis at presentation. These findings suggest that serum OPG levels at diagnosis could be an important prognostic marker and indicator of the aggressive tumour phenotype, warranting early and aggressive treatment. For the analysis of OPG, a commercial immunoassay is available (Biovendor, Czech Republic). There are plans to extend the validation of OPG in a multicentre study.

## Human kallikrein 2 (hK2)

hK2 is a serine protease similar to PSA. The potential value of hK2 as a predictor of tumour aggressiveness was evaluated in Turku. Free and total hK2 and free and total PSA were measured before treatment in 188 PCa patients who underwent radical prostatectomy (5). Results demonstrated that neither parameter is useful in predicting cancer stage or grade on an individual basis. Nevertheless, changes in the hK2 and PSA concentrations occurred at different points in cancer progression, which warrant follow-up studies. Another study performed at the Memorial Sloan-Kettering Cancer Center in New York, USA by Prof. Lilja from Malmö, demonstrated that the pre-treatment level of hK2 significantly enhances the prediction of cancer recurrence after radical prostatectomy (6). In a subsequent study on 867 patients, elevated levels of hK2 were found to be significantly associated with unfavourable features of PCa, like extracapsular extension, seminal vesicle invasion and biochemical recurrence (7). These promising results on hK2 will now be confirmed in multicentre studies using investigational immunoassays optimised by Turku and Innorac.

## nicked PSA

Nicked PSA is an internally cleaved, enzymatically inactive isoform of PSA that may increase the diagnostic specificity of PSA. Preliminary evaluation by Malmö and Turku of a cohort of 1,100 biopsied patients from four rounds of screening of the Göteborg screening study has indicated that nicked free PSA, measured by an investigational immunoassay as the difference between total free PSA and intact free PSA, provides a statistically significantly better separation of cancer and benign cases, especially in the low PSA range (unpublished data). Nicked PSA may also be a valuable prognostic marker, since the nicked to total PSA ratio significantly differed between pathological stages T2B and T3A as well as between WHO grades 2 and 3 in 160 patients who underwent radical prostatectomy (unpublished data). Multicentre studies are now being designed to further study the diagnostic and prognostic value of nicked PSA.

## Cytochrome P450 3A5\*3 polymorphism (Cyp3A5\*3)

Genetic polymorphisms in the cytochrome P450 3A (CYP3A) family of drug metabolising enzymes have been reported to be associated with PCa. Rotterdam focused on Cyp3A5\*3 as a prognostic marker, but evaluation results show that its value as a single marker is limited. Further development will focus on the combination of Cyp3A5\*3 with other genetic polymorphisms in the CYP3A family.

## Alternative markers

The P-MARK consortium is open to any promising alternative marker for PCa. Currently, the potential



(ECAM members are: Chris Bangma, Anders Bjartell, Zoran Culig, Freddie Hamdy, Norman Maitland, Jack Schalken and George Thalmann)

diagnostic or prognostic value of the fusion transcript TMPRSS2-erg, cysteine-rich secretory protein 3 (CRISP-3), beta-MicroSeminoProtein (MSP) and urokinase plasminogen activator receptor (uPAR) is being studied. Any results will be made available in this final year of the project.

## Risk groups in the general population

The identification of risk groups in the general population is of pivotal importance to reduce overdiagnosis and overtreatment of PCa. The international screening data generated in the ERSPC will be used by P-MARK to contribute to the development of risk stratification. Based on the risk analysis of 6,300 men from the first screening round, a risk indicator was developed in Rotterdam which may be used to guide men who are considering having their PSA levels tested. At present, activities are ongoing to make the risk indicator available via the internet and as a mechanical tool.

Furthermore, in a study performed by Steyerberg et al. using ERSPC data indolent tumours were more often demonstrated in patients with screen-detected PCa compared to patients with clinically detected PCa (8). Based on these observations, an updated predicting nomogram for indolent PCa was generated (8) and a decision model for active surveillance developed, aiming to address overtreatment. This model is now being validated in a multicentre study called PRIAS (Prostate cancer Research International: Active Surveillance).

## Concluding remarks

The P-MARK project is on track and has achieved a number of important milestones, including the establishment of a P-MARK biorepository and the selection of four promising markers (PCA3, OPG, hK2 and nicked PSA) for further evaluation and validation. In the final year of the project, marker discovery by proteomics will continue and studies will be initiated to reproduce the initial evaluation results on OPG, hK2 and nicked PSA in a multicentre setting and in independent patient cohorts. Furthermore, a comparative assessment of markers that are likely to overlap in clinical utility - for instance PCA3 versus nicked PSA for diagnostic purposes and OPG versus hK2 for prognostic purposes - will be started. It is quite clear that such studies cannot be finalized within the time-frame of P-MARK. Activities are now ongoing to continue the progressions made in P-MARK, for instance via the Seventh Framework Programme (FP7). In addition, a P-MARK Biobank Foundation will be set up in order to consolidate and expand the P-MARK biorepository and to make these unique biomaterials available to the urological scientific community.

**For more information on P-MARK please contact project manager Dr. Ellen Schenk (e.schenk-braat@erasmusmc.nl) or visit the P-MARK website (www.p-mark.org).**

## References

1. van Gils MP, Stenman UH, Schalken JA, Schroder FH, Luider TM, Lilja H, Bjartell A, Hamdy FC, Pettersson KS, Bischoff R, Takalo H, Nilsson O, Mulders PF, Bangma CH. Innovations in serum and urine markers in prostate cancer current European research in the P-Mark project. *Eur Urol* 2005;48:1031-1041.
2. Dekker LJ, Bosman J, Burgers PC, van Rijswijk A, Freije R, Luider T, Bischoff R. Depletion of high-abundance proteins from serum by immunoaffinity chromatography: A MALDI-FT-MS study. *J Chromatogr B Analyt Technol Biomed Life Sci* 2006, in press.
3. Stenman UH. Tumour-associated trypsin inhibitor. *Clin Chem* 2002;48:1206-9.
4. Van Gils MPMQ, Hessels D, van Hooij O, Jannink SA, Peelen WP, Hanssen SLJ, Witjes JA, Cornel EB, Karthaus HFM, Smits GAHJ, Dijkman GA, Mulders PFA, Schalken JA. The time resolved fluorescence-based PCA3 test on urinary sediments after digital rectal examination; a

## Introduction

P-MARK is a European project funded by the European Commission Sixth Framework Programme (FP6). This project addresses the growing need for new and improved diagnostic and prognostic markers that can discriminate men with clinically irrelevant prostate cancer (PCa), curable PCa or life threatening PCa (1).

In P-MARK novel markers are explored using innovative mass spectrometry tools. Recently developed promising markers include urine marker PCA3 and serum markers bone morphogenetic protein-6 (BMP-6), osteoprotegerin (OPG), nicked PSA, human kallikrein 2 (hK2) and cytochrome P450 3A5\*3 polymorphism (Cyp3A5\*3) all of which are now being evaluated. For this purpose, a P-MARK biorepository composed of well-defined serum and urine samples is being constructed. Eventually, the markers arising from this project will be offered to the industry for commercialisation and to ongoing large European clinical studies like the ERSPC (European Randomized Study of Screening for Prostate Cancer) and the ProtecT (Prostate testing for cancer and Treatment) study for clinical implementation.

## The P-MARK consortium is composed of the following seven academic and two industrial partners:

1. Erasmus MC, Rotterdam, the Netherlands (Principal investigators Prof. Chris Bangma, Prof. Fritz Schröder and Dr. Theo Luider)
2. Lund University, Malmö, Sweden (Prof. Hans Lilja and Prof. Anders Bjartell)
3. University Medical Centre Nijmegen, The Netherlands (Prof. Jack Schalken)
4. University of Sheffield, United Kingdom (Prof. Freddie Hamdy)
5. University of Helsinki, Finland (Prof. Ulf-Håkan Stenman)
6. University of Turku, Finland (Prof. Kim Pettersson)
7. Rijksuniversiteit Groningen, The Netherlands (Prof. Rainer Bischoff)
8. Innorac Diagnostics Oy, Turku, Finland (Dr. Harri Takalo)
9. Fujirebio Diagnostics AB (formerly known as CanAg Diagnostics AB), Göteborg, Sweden (Dr. Olle Nilsson).

The three-year P-MARK project which started on 1 November 2004 has now entered its final stage. This article provides an interim report on the results obtained to date.



The P-Mark consortium at the P-Mark meeting held on 28-29 April 2006 in Sheffield, UK.

## Recently developed promising markers for PCa: PCA3, BMP-6, OPG, hK2, nicked PSA, Cyp3A5\*3 and alternative markers

**PCA3** (formerly known as DD3 and DD3<sup>PCA3</sup>) is a prostate-specific gene that is overexpressed in PCa. In a first study on 108 patients the PCA3 level in urinary sediments obtained after prostatic massage, measured by RT-PCR, was found to have additional diagnostic value next to

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Dutch multicenter validation of the diagnostic performance. *Clin Cancer Res* 2007 *in press*

5. Väisänen V, Pettersson K, Alanen K, Viitanen T, Nurmi M. Free and total human glandular kallikrein 2 in patients with prostate cancer. *Urology* 2006;68:219-25.
6. Steuber T, Vickers AJ, Haese A, Becker C, Pettersson K, Chun FK, Kattan MW, Eastham JA, Scardino PT, Huland H, Lilja H. Risk assessment for biochemical recurrence prior to radical prostatectomy: Significant enhancement contributed by human glandular kallikrein 2 (hK2) and free prostate specific antigen (PSA) in men with moderate PSA-elevation in serum. *Int J Cancer* 2006;118:1234-40.
7. Steuber T, Vickers AJ, Serio AM, Vaisanen V, Haese A, Pettersson K, Eastham JA, Scardino PT, Huland H, Lilja H. Comparison of free and total forms of serum human kallikrein 2 and prostate-specific antigen for prediction of locally advanced and recurrent prostate cancer. *Clin Chem in press*.
8. Steyerberg EW, Roobol MJ, Kattan MW, van der Kwast TH, de Koning HJ, Schroder FH. Prediction of indolent prostate cancer: validation and updating of a prognostic nomogram. *J Urol* 2007;177:107-12.