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Project acronym: P-Mark

Validation of recently developed diagnostic and prognostic markers and identification of novel markers for prostate cancer using European databases

Instrument: Specific Targeted REsearch Project (STREP)

Thematic Priority: Sixth Framework Programme Priority 1 (Life sciences, genomics and biotechnology for health)

Final report for P-Mark

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P-Mark

A quest for new diagnostic and prognostic markers for prostate cancer

INTRODUCTION TO P-MARK

Prostate cancer is nowadays the most frequent cancer in European men and one of the major causes of cancer-related death. The incidence of this malignancy is increasing as a result of screening for prostate cancer via a test that measures the level of Prostate Specific Antigen (PSA) in blood. While screening for prostate cancer has become a common phenomenon for men above the age of 50, its value has not been established yet and is currently subject of investigation in the European Randomized Study of Screening for Prostate Cancer (ERSPC, www.erspc.org). If the amount of the blood marker PSA is elevated, a prostate biopsy is performed to determine the presence of tumor cells. Because PSA is not only increased in prostate cancer but also in other physical conditions such as inflammation and benign prostate enlargement, unnecessary biopsies are being performed in a significant number of men who are screened for prostate cancer. In addition, PSA is a poor predictor of the aggressiveness of a prostate tumor. It is known that more than half of the prostate cancers detected by screening are harmless and not life-threatening, and can therefore best be treated by active surveillance. Since PSA cannot discriminate well between these indolent tumors and relevant tumors that need invasive therapy, a large proportion of men diagnosed with prostate cancer is unnecessarily treated by complete surgical removal of the prostate or by radiotherapy. These treatments are associated with side effects that severely affect quality of life, including impotence and incontinence. It is clear that there is a strong clinical need for novel markers that can improve the diagnosis and prognosis of prostate cancer and that can stratify between patients who need active curative therapy and patients who are better off with active surveillance. For a period of four years (1 November 2004 - 31 October 2008), the members of the P-Mark project have searched for improved diagnostic and prognostic prostate cancer markers by the identification and evaluation of novel markers as well as the evaluation and validation of recently developed promising markers, as outlined below.

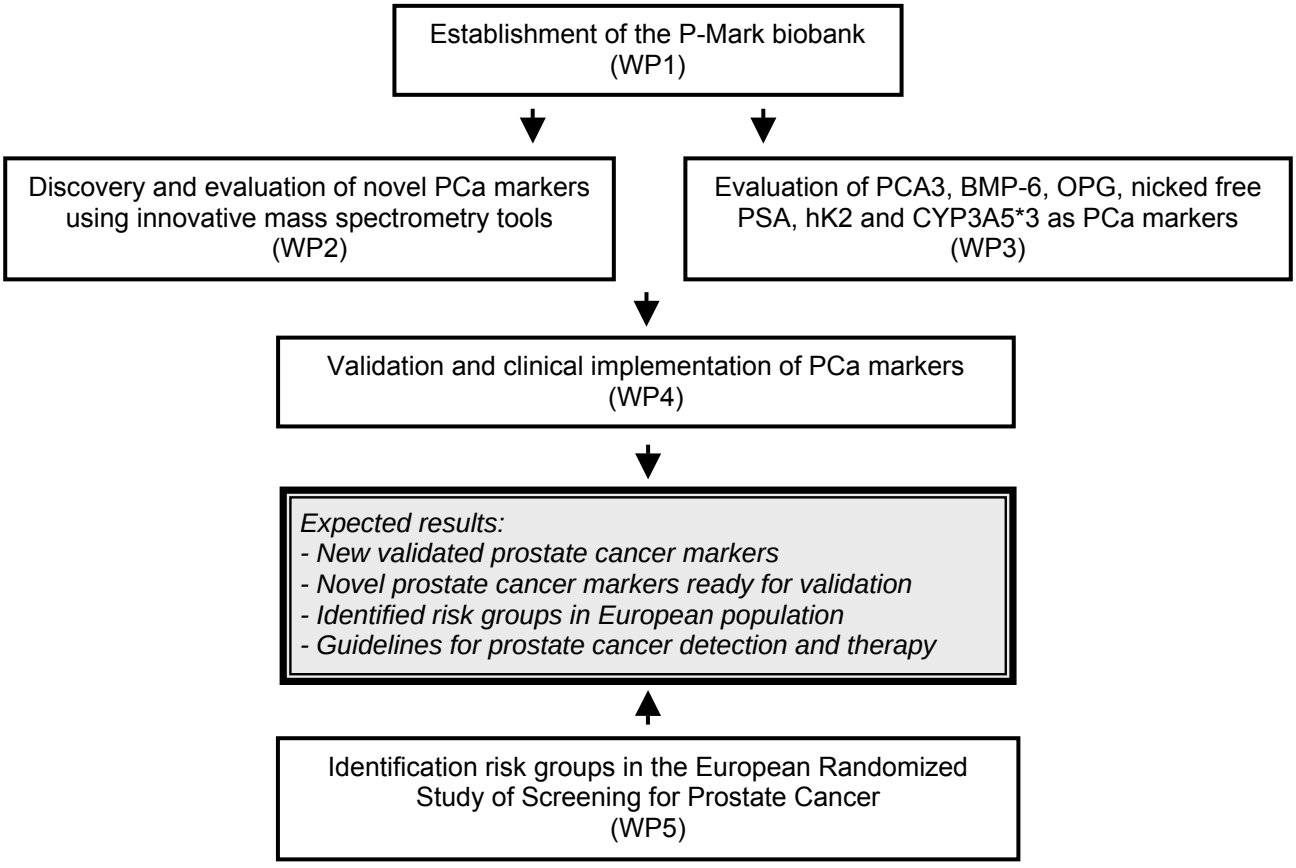
THE GOALS OF P-MARK

For the P-Mark project, the following five main objectives have been defined:

1. Establishment of a serum biorepository and a urine biorepository
2. Discovery of novel prostate cancer biomarkers in serum and urine by innovative mass spectrometry tools
3. Establishment of the clinical utility of recently developed promising prostate cancer markers
4. Validation of prostate cancer markers and identification of risk groups in the general population in Europe
5. Development of guidelines for cost-efficient strategies for prostate cancer detection and therapy

To address these objectives, P-Mark has been broken down into 5 subprojects, called workpackages (WP), as shown in Figure 1. In ***WP1***, a unique European biorepository composed of clinically well-defined biomaterials from healthy men and men with various stages of prostate cancer has been established. In ***WP2***, novel potential serum and urine markers were explored in sample sets from the P-Mark biobank using innovative mass spectrometry tools, and the most promising candidate markers were evaluated by antibody-based immunoassays. The recently developed promising markers PCA3, bone morphogenetic protein-6 (BMP-6), osteoprotegerin (OPG), nicked free PSA, human kallikrein 2 (hK2) and cytochrome P450 3A5*3 polymorphism (CYP3A5*3) available at the start of P-Mark were evaluated for their clinical value in ***WP3*** and the best markers were transferred to ***WP4*** for further validation in various sample sets derived from European studies. In ***WP5***, the international monitoring of the ongoing European Randomized Study of Screening for Prostate Cancer (ERSPC) aimed at the identification of risk groups in the general European population was supported, and the delivery of ERSPC serum samples to the P-Mark biobank was coordinated.

Figure 1. P-Mark project design



Abbreviations: BMP-6; bone morphogenetic protein-6, CYP3A5*3; cytochrome P450 3A5*3 polymorphism, hK2; human kallikrein 2, PCa; prostate cancer, OPG; osteoprotegerin, WP; workpackage.

THE P-MARK CONSORTIUM

The P-Mark consortium is composed of the following 7 top European research laboratories in the field of (urological) oncological marker development and 2 SMEs providing pivotal state-of-the-art technologies (principal investigator(s) in brackets):

- **Erasmus MC**, Rotterdam, The Netherlands
  (Prof. dr. Chris Bangma, Prof. dr. Fritz Schröder and Dr. Theo Luider)
- **Lund University**, Malmö, Sweden
  (Prof. dr. Hans Lilja[1] and Prof. dr. Anders Bjartell)
- **Radboud University Nijmegen Medical Centre**, The Netherlands
  (Prof. dr. Jack Schalken)
- **University of Sheffield**, United Kingdom
  (Prof. dr. Freddie Hamdy[2])
- **University of Helsinki**, Finland
  (Prof. dr. Ulf-Håkan Stenman)
- **University of Turku**, Finland
  (Prof. dr. Kim Pettersson)
- **University of Groningen**, The Netherlands
  (Prof. dr. Rainer Bischoff)
- **Innotrac Diagnostics Oy**, Turku, Finland
  (Dr. Harri Takalo)
- **Fujirebio Diagnostics AB**[3], Göteborg, Sweden
  (Dr. Olle Nilsson)

P-Mark is coordinated by Prof. dr. Chris Bangma together with the project manager Dr. Ellen Schenk (Erasmus MC, Rotterdam, The Netherlands).

The P-Mark consortium is advised by a **Scientific Advisory Board** consisting of the following experts in the field:

- Prof. dr. Georg Bartsch (Professor of Urology, Universitatsklinik Innsbruck, Austria)
- Prof. dr. Michael Fountoulakis (Proteomics expert, F. Hoffmann-La Roche Ltd, Basel, Switserland)
- Prof. dr. Lars Holmberg (Professor of Cancer Epidemiology, King's College London, United Kingdom)
- Dr. Marc Meyer (Clinical diagnostics marketing director, Beckman Coulter, Nyon, Switserland)
- Prof. dr. Axel Semjonow (Professor of Urology, University of Münster, Germany).

[1] Prof. Lilja is currently an Attending Research Clinical Chemist at the Departments of Clinical Laboratories, Surgery (Urology), and Medicine (GU-Oncology) of Memorial Sloan-Kettering Cancer Center in New York, USA, and Professor (adjunct/visiting) at the Department of Laboratory Medicine of Lund University.
[2] Prof. Hamdy is currently Nuffield Professor of Surgery, Professor of Urology, and Head of the Nuffield Department of Surgery of the University of Oxford, United Kingdom.
[3] Formerly known as CanAg Diagnostics AB, which was acquired by Fujirebio Diagnostics Inc. in September 2006.

THE RESULTS OF P-MARK

THE P-MARK BIOBANK

Partners involved: Erasmus MC, Lund University, Radboud University Nijmegen Medical Centre, University of Sheffield

The availability of well-characterized clinical specimens is often a barrier in biomarker development. Therefore, the clinical centers in P-Mark (Erasmus MC, Lund University, Radboud University Nijmegen Medical Centre and University of Sheffield) have joined efforts to establish a **European prostate cancer biorepository** with blood and urine specimens from controls and patients with various stages of prostate cancer. The P-Mark Biobank contains a collection of over 2000 historical serum samples, as specified in Appendix 1. This collection has been extensively used for the discovery of novel markers in serum within P-Mark. In addition, a **prospective multicenter collection** has been initiated that is composed of **longitudinal** blood (serum, plasma, DNA and RNA) and urine (urinary fluid and sediment) samples from men who are biopsied because of suspicion of having prostate cancer. The unique aspect of this prospective collection is that samples are being collected, processed and stored according to standardized protocols. This biobank therefore allows for the composition of large multicentre sample cohorts with a significant reduced risk of sampling bias. Furthermore, on the longer term this biobank of longitudinal specimens will be of high value to test biomarkers for their prognostic value or for their value in the assessment of response to treatment.

The P-Mark Biobank is situated at the Sheffield School of Medicine Biorepository Facilities managed by Prof. dr. Hamdy, and will move to the University of Oxford[4] in the beginning of 2009. Samples are treated anonymously by a bar coding system, and a web-based central database links clinical information to the samples.

The clinical centers have agreed to continue the prospective collection beyond P-Mark. In March 2008, the **P-Mark Biobank Foundation** was raised in order to consolidate and expand the P-Mark Biobank, and to make these unique biomaterials available to the urological scientific society.

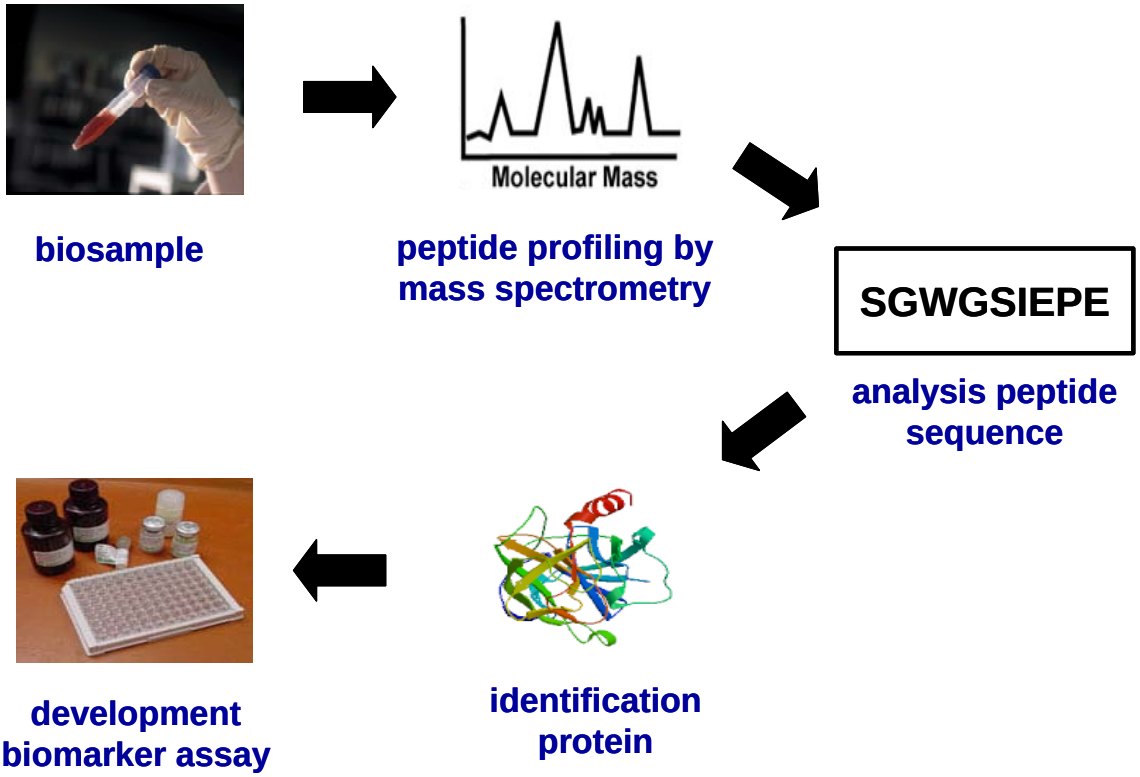
[4] Prof. Hamdy and his team have moved to the Nuffield Department of Surgery of the University of Oxford, United Kingdom in October 2008.

THE DISCOVERY OF NOVEL BIOMARKERS FOR PROSTATE CANCER IN SERUM AND URINE

Partners involved: Erasmus MC, Fujirebio Diagnostics AB, University of Groningen, University of Helsinki

The P-Mark consortium has focused on the discovery of novel biomarkers for prostate cancer in serum and urine, because this type of biomarkers can easily be applied in a clinical setting. For the biomarker discovery studies, innovative mass spectrometry approaches were used. Mass spectrometry allows for the profiling of peptides in serum or urine based of the molecular mass of these components. By comparing the profiles of patients and healthy individuals, differentially expressed peptides can be assessed. Identification of the full protein by analysis of the peptide sequences then allows for the development of a biomarker assay (for example an immunoassay), which can be used for further validation of the biomarker and eventual clinical implementation. The concept of the biomarker discovery strategy used in P-Mark is illustrated below.

Biomarker discovery strategy in P-Mark



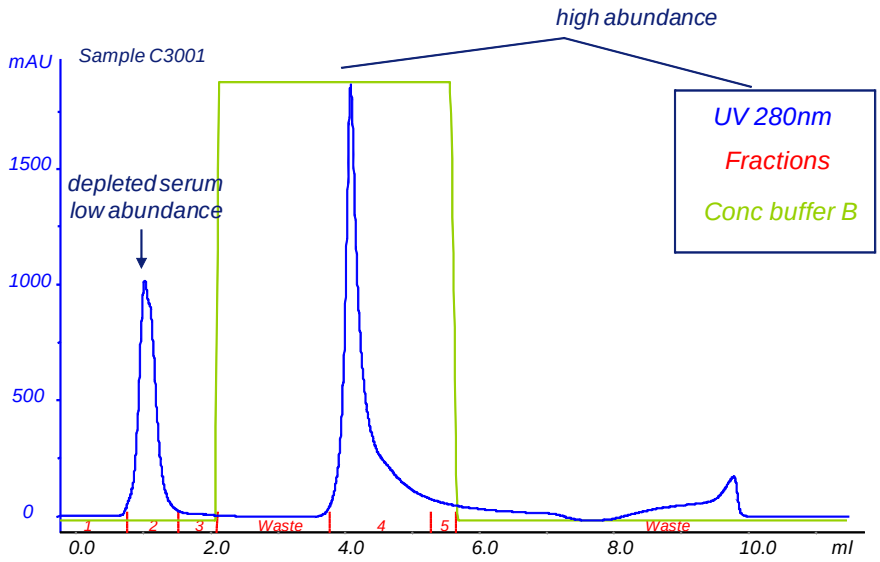
Biomarker discovery in serum

Partners involved: Erasmus MC, Fujirebio Diagnostics AB, University of Groningen

Step 1 Serum depletion and digestion

The major part of the serum proteins is composed of 7 abundant proteins. In order to remove these abundant proteins prior to mass spectrometry, a reproducible prepurification step was developed by the University of Groningen using immunoaffinity chromatography (Figure 1). Subsequently, the remaining proteins in the depleted serum were enzymatically digested into peptides by trypsin.

Figure 1. Depletion of high abundant serum proteins

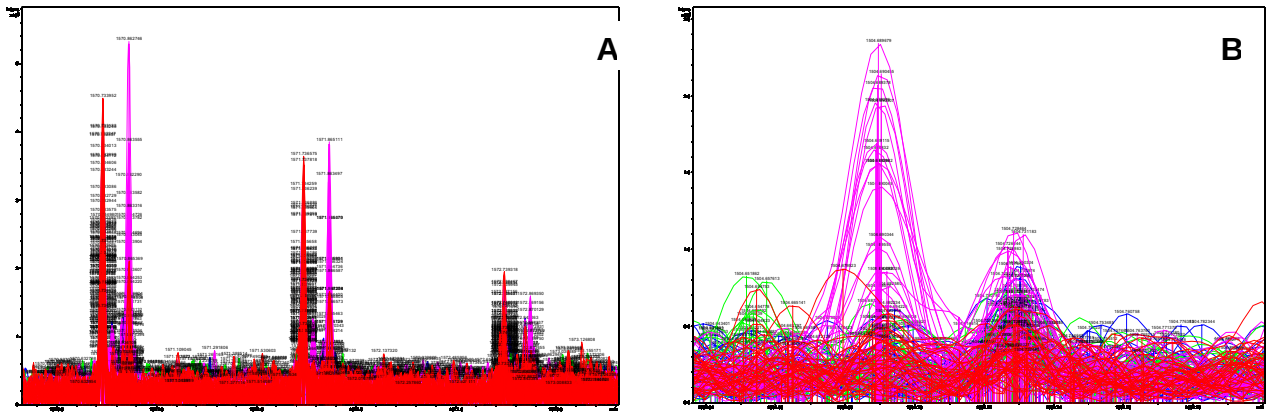


This method has been published by Dekker LJ, Bosman J, Burgers PC, van Rijswijk A, Freije R, Luider T, and Bischoff R (Depletion of high-abundance proteins from serum by immunoaffinity chromatography: A MALDI-FT-MS study. J Chromatogr B Analyt Technol Biomed Life Sci 2007;847:65-9).

Step 2 Peptide profiles by MALDI FT ICR mass spectrometry

In this step, the peptides were measured directly on a MALDI Fourier Transform (FT) ICR mass spectrometer by Erasmus MC. This advanced mass spectrometer has the possibility to reach mass accuracies below the 1 peptide per million peptides (ppm). By combining vast numbers of mass spectra, peptides could be discovered that were linked to different forms of prostate cancer. In Figure 2, a graphical view of such a measurement is given. On the x-axis the mass is indicated and on the y-axis the intensity is indicated for various peptides. The different colours relate to two different prostate cancer samples (red and purple) and control samples (green and blue).

Figure 2. Graphical view of zoom-ins of mass spectra of serum samples from prostate cancer patients and healthy men



Red and purple profiles represent prostate cancer patients, green and blue profiles represent healthy men. On the x-axis the mass is indicated and on the y-axis the intensity. Panel A shows a molecular mass range of 25 Dalton and Panel B show a molecular mass range of only 0.15 Dalton. Each colour line represents one serum sample.

Using this MALDI FT ICR approach, a quantitative analysis of serum proteins could be obtained in a reproducible way (CV below 10%) with a large dynamic range (three to four orders of magnitude). To this end, a label-free method was developed to quantify peptides in the FT mass spectrometer.

Step 3 Protein identification by nanoLC Orbitrap FT mass spectrometry

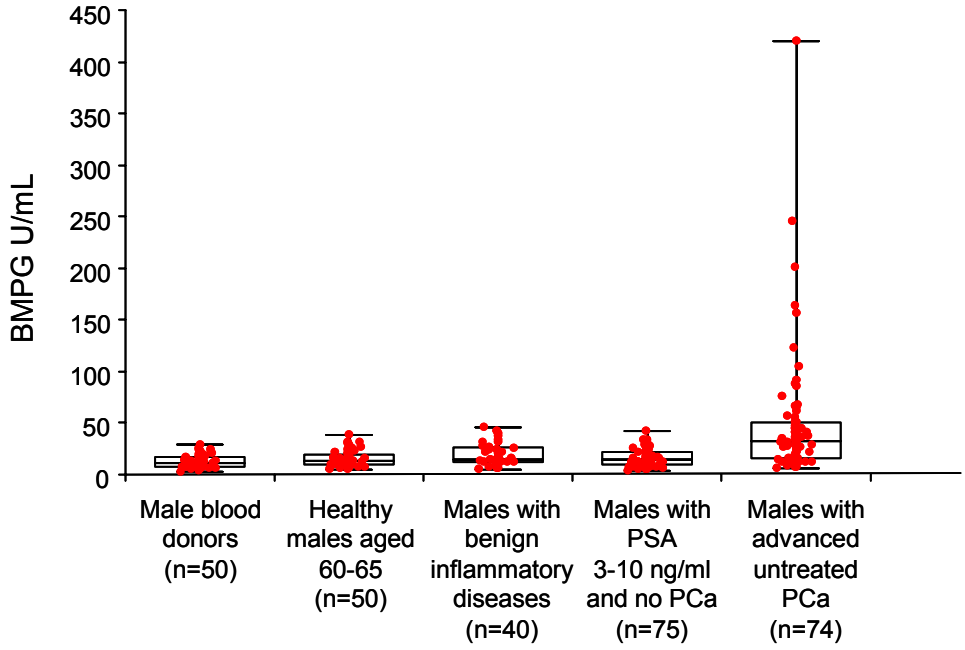
The prostate cancer-related peptides found in step 3 were subjected to nanoLC Orbitrap FT MS/MS mass spectrometry in order to asses the amino acid sequence of these peptides. This approach was successful because the mass accuracy of both the FT ICR mass spectrometer used for peptide profiling in step 2 and the Orbitrap FT mass spectrometer used for sequencing in step 3 is extremely high and therefore allows for comparisons between the two. Using the NCBI Mascot database, the masses and sequences of the prostate cancer-related peptides were then translated into proteins.

For the analysis of mass spectrometry data, sophisticated software is required that can deal with large size data. Therefore, different software packages were developed to analyze the data from the MALDI FT ICR and the Orbitrap FT mass spectrometers. In addition, software (Peptrix version 20) was developed that can handle both MALDI FT ICR and nanoLC Orbitrap FT data, and in this way enables comparative analyses.

Step 4 Immunoassay development for validation of mass spectrometry results

A series of proteins has been identified that relate to prostate cancer and therefore may be potential candidate serum biomarkers. Three of these proteins (Basement Membrane extracellular ProteoGlycan, gelsolin and prothrombin) were further validated in an independent way by immuno-assays. This is illustrated below for Basement Membrane extracellular ProteoGlycan (BMPG), which was found to be differentially expressed in the serum of patients with metastasised prostate cancer. BMPG is a heparin sulphate proteoglycan that is secreted into the extracellular matrix. The binding capacity of BMPG for growth factors suggests that this protein may act as a co-receptor for growth factors and is involved in the extracellular accessibility of growth factors. A research immuno-assay was developed and optimised by Fujirebio Diagnostics AB, and used for the assessment of BMPG in the serum of healthy male blood donors, healthy ageing men, men with benign inflammatory diseases, men with PSA 3-10 ng/ml and no prostate cancer, and men with advanced prostate cancer. As shown in Figure 3, the BMPG levels were significantly elevated in the advanced prostate cancer cohort compared to the healthy male subjects and patients with no evidence of disease. 34 out of 74 (46%) samples with advanced prostate cancer showed elevated BMPG levels. No correlation was found between BMPG and PSA in patients with no evidence of prostate cancer, while a high positive correlation was found in patients with advanced disease. BMPG might therefore have a clinical value as a relapse biomarker for anti-androgen therapy. No correlation between Gleason stage and BMPG levels was found. This validation study in an independent way confirms the proteomics biomarker discovery results from Erasmus MC showing that BMPG can be detected in elevated levels in advanced prostate cancer. Further studies are warranted to explore the value of BMPG as a diagnostic, prognostic or therapy-response biomarker for prostate cancer. The validation of the other two potential candidate serum biomarkers (gelsolin and prothrombin) is ongoing and preliminary results suggest that the mass spectrometry results are confirmed by the immuno-assay validation studies.

Figure 3. *Box plot of BMPG in healthy male subjects, males with benign inflammatory disease, men with PSA 3-10 ng/ml and no evidence of prostate cancer, and men with advanced untreated prostate cancer at the time of diagnosis*

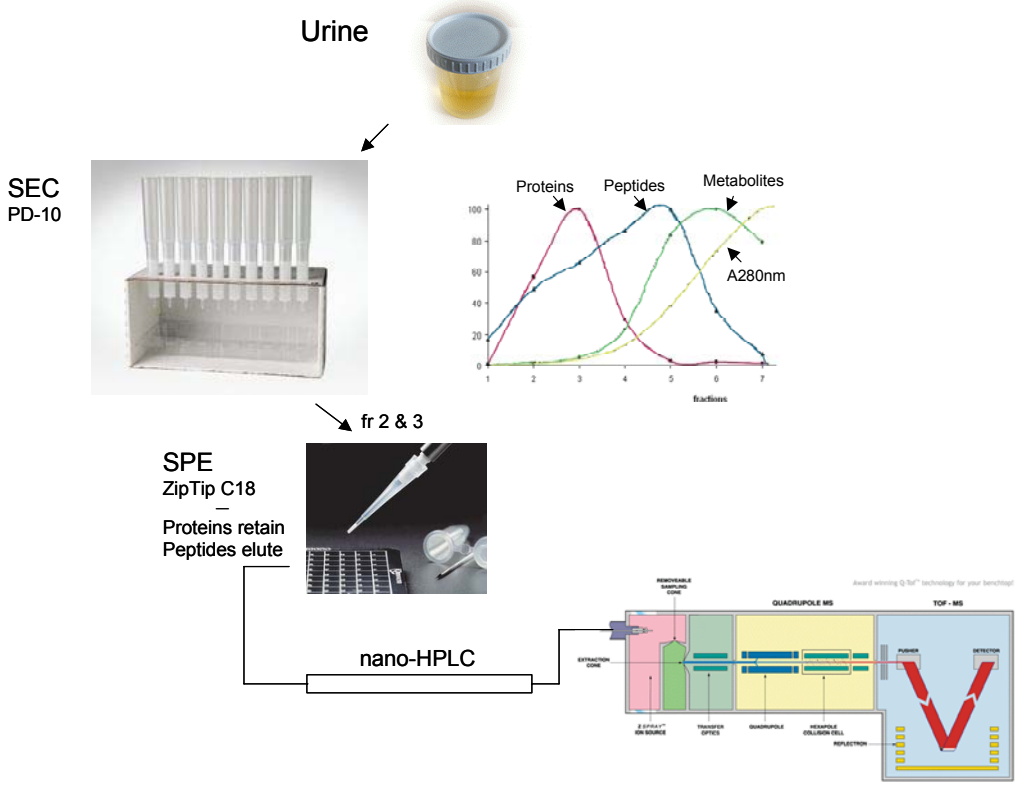


Biomarker discovery in urine

Partner involved: University of Helsinki

The University of Helsinki has focused on the discovery of naturally occurring urinary peptides as potential biomarkers for prostate cancer. The proteomics strategy developed for this purpose is shown in Figure 4. First, urine was pre-purified from proteins, metabolites and salts by size exclusion chromatography and solid phase extraction. In the next step, peptides in urine were separated and characterized using electrospray hybrid time-of-flight mass spectrometry (Q-TOF Micro, Waters) coupled to nanoLC. This method allows for the comparison of peptides patterns between patients and controls and for the identification of individual peptides by *de novo* sequencing. A major challenge in these proteomics studies was finding a software package suitable for the comparison of the peptide profiles and concentrations of individual peptides. The Progenesis LC-MS software appeared to provide the most reliable results. More than 20 peptides that are differently expressed in prostate cancer when compared to controls could be identified. Remarkably, one of the peptides is tumor-associated trypsin inhibitor (TATI), that was identified earlier by the University of Helsinki and was found to be associated with ovarian, bladder and kidney cancer (Stenman UH, Clin Chem 2002;48:1206-9). The association of TATI with prostate cancer is in agreement with a P-Mark study in which higher expression of TATI was observed in cancerous prostatic tissue compared to benign prostatic tissue (Paju A *et al*. Eur Urol 2007;52:1670-1681). Most of the other peptides found appear to be derived from fibrinogen. The excretion of fibrinogen-derived peptides is in line with earlier findings showing deposition of fibrin around tumors. In addition, differently processed peptides derived from alpha-1-antitrypsin were identified. These observations were confirmed by analysis on a state-of-the-art LTQ Orbitrap mass spectrometer. The development of immuno-assays for the validation of a selection of the most promising peptides on large sample cohorts is now ongoing. For TATI, a time-resolved immunofluorometric assay is already available.

Figure 4. Flow chart of biomarker discovery in urine



Urine is cleaned up from proteins, metabolites and salts by size exclusion chromatography (SEC) and solid phase extraction (SPE), and then subjected to nanoLC ESI Q-TOF.

Impact of biomarker discovery results on scientific, clinical and industrial stakeholders

The pre-purification and mass spectrometry methods developed within P-Mark for biomarker discovery in serum and urine are unique and will greatly contribute to the progress and innovations in this research area. In particular, significant bioinformatics experience has been obtained with respect to proteomics studies on large sample sets using FT mass spectrometers. In particular, software development for the comparison of profiles and the identification of differentially expressed peptides has been a major challenge. This knowledge is made available to the scientific society via publications and presentations, and is already applied by Erasmus MC for other types of diseases such as glioma, multiple sclerosis and pre-eclampsia, as well as by the University of Helsinki for biomarker discovery in urine. Various companies have already shown to be interested in the proteomics research and methods used, and a patent has been filed successfully (see section “Dissemination and use of P-Mark results”).

In P-Mark, a list of novel potential candidate serum protein and urine peptide biomarkers has been generated. These candidates will now need to be further explored in future validation studies. In order to decide and test if candidate biomarkers (such as BMPG, gelsolin and prothrombin in serum, and fibrinogen peptides and TATI in urine) are of clinical interest, important future steps will be:

- Exclusion of sampling bias caused by differences in conditions used for collection, processing and storage of samples from the various patient groups
- Determination of the clinical value of the potential candidate biomarkers prior to further evaluation studies
- Further evaluation using larger cohorts with proper groups, samples and assays
- Validation of the potential candidate biomarkers
- Commercialisation

EVALUATION OF RECENTLY DEVELOPED PROMISING MARKERS FOR PROSTATE CANCER

Partners involved: Erasmus MC, Lund University, Innotrac Diagnostics Oy, Radboud University Nijmegen Medical Centre, University of Sheffield, University of Turku

At the start of the P-Mark project, a panel of six promising candidate prostate cancer biomarkers recently developed by P-Mark partners was available for evaluation of their diagnostic or prognostic value. These involved the serum biomarkers **bone morphogenetic protein-6** (BMP-6), **osteoprotegerin** (OPG), **free PSA** (including **nicked free PSA, intact free PSA** and **total free PSA**), **human kallikrein 2** (hK2) and **cytochrome P450 3A5*3 polymorphism** (Cyp3A5*3), and the urine biomarker **PCA3**. The outcome of the evaluation studies is presented in Table 1.

Table 1. Evaluation outcome of 6 recently developed promising biomarkers for prostate cancer

Biomarker	Description	Assay type	Evaluation outcome
BMP-6 (UOS)	Inducer of bone formation	Immunoassay developed by the University of Turku	No proof found for prognostic value
Cyp3A5*3 (EMC)	Genetic polymorphisms in the cytochrome P450 3A family of drug metabolising enzymes	PCR-RFLP assay developed by Erasmus MC	No proof found for prognostic value
hK2 (ULUND, U.Turku)	Serine protease similar to PSA	Immunoassay developed by the University of Turku and Innotrac Diagnostics Oy	**Proof** found for increase diagnostic specificity PSA, prediction long-term prostate cancer risk, and prediction treatment failure
total, intact and nicked free PSA (ULUND, U.Turku)	PSA unbound to inhibitors, including total, intact and inactive, internally cleaved free PSA	Immunoassay developed by the University of Turku and Innotrac Diagnostics Oy	**Proof** found for increase diagnostic specificity PSA and prediction long-term prostate cancer risk
OPG (UOS)	Regulator of bone formation	Commercial immunoassay	**Proof** found for prediction progressive prostate cancer
PCA3 (RUNMC)	Prostate-specific non-coding RNA overexpressed in prostate cancer	RT-PCR assay developed by RUNMC	**Proof** found for increase diagnostic specificity PSA

EMC; Erasmus MC, ULUND; Lund University, RUNMC; Radboud University Nijmegen Medical Centre, UOS; University of Sheffield, U.Turku; University of Turku.

Based on this outcome, **hK2**, the **free PSA forms** (total free PSA, nicked free PSA and intact free PSA), **OPG** and **PCA3** were selected for further validation on large cohorts. From this point, hK2 and the free PSA forms (nicked, intact and total) are referred to as **multi-kallikreins**. The validation results are presented in the next paragraph.

VALIDATION OF MULTI-KALLIKREINS, OSTEOPROTEGERIN AND PCA3

Partners involved: Erasmus MC, Lund University, Innotrac Diagnostics Oy, Radboud University Nijmegen Medical Centre, University of Sheffield, University of Turku

The following major P-Mark validation studies on the multi-kallikreins (hK2, total free PSA, intact free PSA and nicked free PSA), OPG and PCA3 have been carried out or are still ongoing:

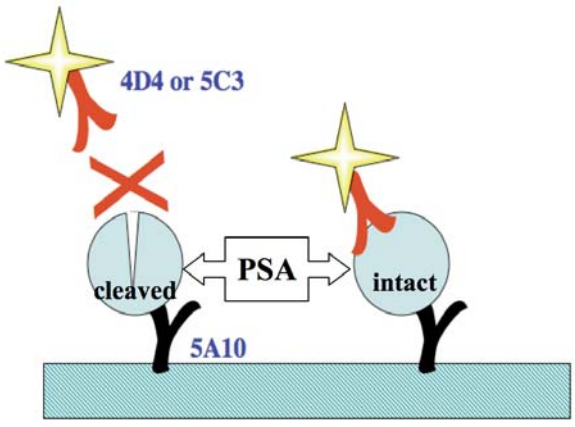
1. Validation of **OPG and multi-kallikreins** in a cohort of patients with untreated prostate cancer from the University of Sheffield
2. Validation of **OPG and multi-kallikreins** in a cohort of patients with prostate cancer treated by radical prostatectomy from the University of Hamburg
3. Validation of **multi-kallikreins** in first round and subsequent rounds screening cohorts from the Göteborg section of the ERSPC
4. Validation of **multi-kallikreins** in a first round and third round screening cohort from the Rotterdam section of the ERSPC
5. Validation of **PCA3 and multi-kallikreins** in a third/fourth round screening cohort from the Rotterdam section of the ERSPC

The multi-kallikreins measurements were performed at Lund University, the OPG measurements were performed at the University of Sheffield and Lund University, and the PCA3 measurements were performed at the Radboud University Nijmegen Medical Center.

Multi-kallikreins validation studies

As described in Table 1, the multi-kallikreins hK2, total free PSA, intact free PSA and nicked PSA are assessed by immunoassays developed and optimized by the University of Turku and Innotract Diagnostics Oy according to patented design principles. Since hK2 circulates at a level about two orders of magnitude lower than PSA, exquisite sensitivity and specificity was required. The immunoassay for intact free PSA was based on the patented discovery of specific antibodies to detect a previously unrecognized epitope of PSA, which is lost in the internally cleaved form of PSA. Combining assays for total free PSA and intact free PSA gives a quantitative measure of the cleaved PSA (Figure 5).

Figure 5. Principle for the detection of intact free PSA and nicked free PSA



Results from the first round screening cohort (ERSPC - Göteborg) validation study

The cohort comprised serum samples from 740 men undergoing biopsy in the first screening round of the Göteborg section (Sweden) of the ERSPC. The area-under-the-curve (AUC) was calculated for predicting prostate cancer at biopsy. AUCs for a model including age and PSA (the 'laboratory' model) and age, PSA and digital rectal exam (the 'clinical' model) were compared with those for models that also included additional

kallikreins. Addition of free, intact PSA and hK2 improved the AUC from 0.68 to 0.83 and from 0.72 to 0.84, for the laboratory and clinical models respectively (Table 2).

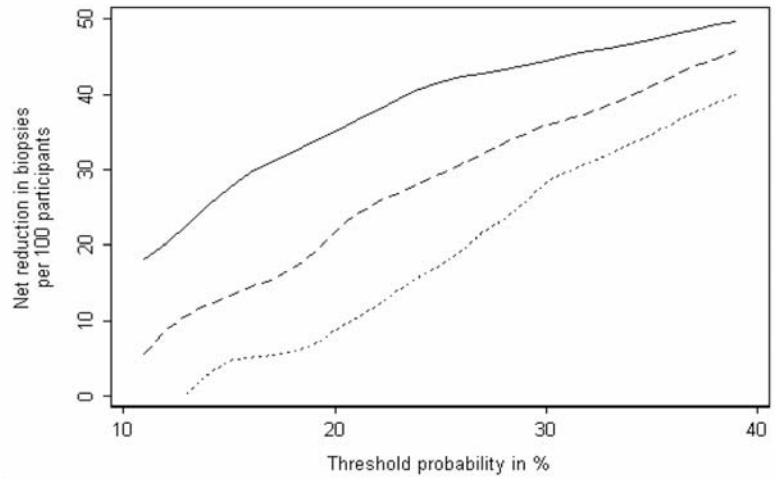
Table 2. Predictive accuracy (area-under-the-curve, AUC) of multi-kallikreins as a diagnostic biomarker

Predictor	Laboratory model		Clinical model	
	Any cancer	High-grade cancer	Any cancer	High-grade cancer
Base model (age, PSA ± DRE)	**0.680**	**0.816**	**0.724**	**0.868**
Base model + Free PSA	0.762	0.832	0.779	0.867
Full model	**0.832**	**0.870**	**0.836**	**0.903**

The base model for the laboratory model includes age and total PSA, and for the clinical model includes age, total PSA, and DRE result. The full model includes the base model plus free PSA, intact PSA, and hK2. Cancers with biopsy Gleason score 7 and higher were considered high grade.

It was concluded that multi-kallikreins measured in blood can importantly enhance the accuracy in predicting the result of biopsy in previously unscreened men with elevated PSA. A multivariable model can determine which men should be advised to undergo biopsy and which might be advised to continue screening, but defer biopsy until there was stronger evidence of malignancy (Figure 6). The results of this study recently were published by Vickers *et al.*, BMC Medicine 2008,6:1.

Figure 6. Prediction of biopsies by multi-kallikreins



The reduction in the rate of unnecessary biopsy keeping number of cases of prostate cancer identified constant. The dotted line is for base model (age and total prostate specific antigen (PSA)); the dashed line is for base model plus free PSA; the thin black solid line is for full model (age, total PSA, free PSA, intact PSA, and human kallikrein.

Results from the first round screening cohort (ERSPC - Rotterdam) validation study

The next step in the validation of the multi-kallikreins was to determine whether the predictive value of these biomarkers found in the ERSPC-Göteborg study described above could be replicated in a large, independent, representative, population-based cohort. For this purpose, a study was performed in a cohort of 2914 previously unscreened men undergoing biopsy due to elevated PSA levels (≥3 ng/ml) during the first round of the Rotterdam-section of the ERSPC. The results of this study demonstrate that the previously published finding was independently replicated in this very large and representative

population-based cohort. The findings were robust to the inclusion of cancers detected within four years of initial negative biopsy. It was concluded that use of the multi-kallikreins panel would dramatically reduce the biopsy rate with only a small number of men with cancer advised against immediate biopsy, very few of whom would have high grade disease. The results of this study have been submitted for publication.

Results from the subsequent round screening cohorts (ERSPC - Göteborg) validation study

The association between PSA and prostate cancer likely depends on a man's recent screening history. The effect of prior screening on prostate cancer risk prediction using the multi-kallikrein panel was studied in a cohort comprising 1241 men undergoing biopsy for elevated PSA during their second or later screening round in the Göteborg section of the ERSPC. In these men, PSA was not predictive of prostate cancer. From the results it was concluded that prior participation in PSA-screening significantly changes the performance of statistical models developed to predict cancer in unscreened men. A four-kallikrein panel including free PSA, intact free PSA, nicked free PSA and hK2 was found to predict prostate cancer in men with a recent screening history, reducing the number of biopsies by 413 per 1000 men with elevated PSA and missing only 60/216 low grade and 1/43 high-grade cancers. This study provides independent replication that multi-kallikreins have a significant diagnostic value. The results have been submitted for publication, and are currently being validated in a cohort from the third screening round of the Rotterdam section of the ERSPC. The outcome of this study will be submitted for publication mid 2009.

OPG validation studies

For the validation of OPG, a commercially available immunoassay (BioVendor, Czech Republic) has been used.

Results from the Sheffield cohort validation study

OPG was measured in serum samples from a cohort of 232 untreated patients, of which 185 had cancer (localized disease n=96; advanced disease n=89). In a univariate analysis OPG was significantly associated with advanced disease but not with the presence of cancer. Upon adjustment for total PSA, % free PSA and hK2, OPG was no longer an independent predictor of disease stage. In 47 untreated and 64 treated patients with locally advanced disease, OPG was significantly associated with time to recurrence (univariate analysis). With adjustment for a multivariate confounding score, OPG remained an independent predictor of recurrence among treated patients. Based on these results, it can be concluded that OPG may tentatively be applied as a relapse biomarker after therapy. Results will be submitted for publication.

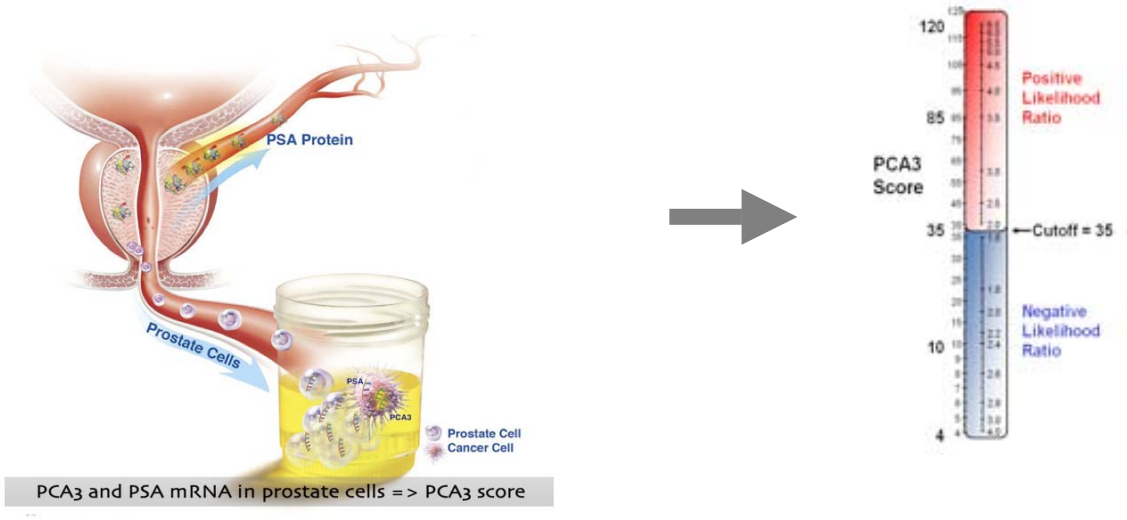
Results from the Hamburg cohort validation study

OPG was studied in combination with multi-kallikreins and urokinase receptor in a cohort of 905 patients undergoing radical prostatectomy for clinically localized prostate cancer to determine whether additional biomarkers to standard clinical predictors may importantly increase the accuracy to assess risk for biochemical recurrence (BCR). In univariate analysis, free PSA, hK2, and intact PSA, but not OPG or urokinase receptor were significantly associated with BCR. In the subset of patients with available OPG measurements, it was shown that OPG was independently associated with BCR in a multivariate setting. In conclusion, measurements of free PSA, hK2, and OPG may contribute marginally to enhanced predictive accuracy compared to that of the standard clinical predictors. The outcome of this study will be submitted for publication mid 2009.

PCA3 validation studies

PCA3 (formerly known as DD3 and DD3^{PCA3}) is a prostate cancer biomarker discovered by the group of Prof. dr. Jack Schalken at the Radboud University Nijmegen Medical Centre. This group has developed a RT-PCR for the assessment of PCA3 in urinary sediments obtained after prostatic massage. The principle of the PCA3 assay is shown in Figure 7.

Figure 7. Principle of the PCA3 assay



In a first study on a cohort of 108 patients biopsied for prostate cancer it was shown that the PCA3 level had additional diagnostic value next to PSA. This has been confirmed in 534 patients in a multicentre study supported by P-Mark, as published by van Gils *et al.*, Clin Cancer Res 2007. As a next step, a commercial variant of the PCA3 RT-PCR assay called the PROGENSA^{TM} PCA3 test, was developed by the company Gen-Probe Incorporated (San Diego, USA). Recently, the diagnostic value of the PROGENSA^{TM} PCA3 test for repeated biopsy was established in patients with an elevated PSA and a negative biopsy result in a European multicentre study (Haese *et al.*, Eur Urol 2008). The PROGENSA^{TM} PCA3 test has now entered the clinic as an **additional diagnostic tool** used to decide on a second biopsy in case of a negative first biopsy, and is made available as a service from designated laboratories in Europe (see www.pca3.org for further information).

Importantly, the applicability of PCA3 as a **first-line screening test** is presently being studied in men from the third or fourth screening round in the Rotterdam section of the ERSPC. The results of this study are still pending and might lead to new recommendations for PCA3. In an across-marker P-Mark validation study, the PCA3 results will be combined with the multi-kallikreins panel (hK2, total free PSA, intact free PSA and nicked free PSA). After completion of the PCA3 side study within the Rotterdam section of ERSPC, the serum samples will be sent to Lund University (Malmö General Hospital) for the determination of the multi-kallikreins. The outcome of this final clinical analysis is expected mid 2009.

Impact of biomarker validation results on scientific, clinical and industrial stakeholders

The P-Mark project has highly impacted the research on improving the diagnosis and prognosis of prostate cancer by biomarkers both from a scientific, clinical and commercial point of view by:

- demonstrating the great significance of **multi-kallikreins** (hK2, nicked free PSA, intact free PSA and total free PSA) as a diagnostic tool for prostate cancer next to PSA by large validation studies using prospectively enrolled, independent, randomly selected, representative population-based study cohorts with extensive clinical annotation and highly accurate follow-up.
- providing evidence on the association between **OPG** and advanced prostate cancer and recurrence after treatment, which justifies further research on the value of OPG as a relapse biomarker after therapy.
- supporting the validation of **PCA3**, which has led to the clinical implementation of the PROGENSA™ PCA3 test by the company Gen-Probe Incorporated. This test is used to decide on a second biopsy in case of a negative first biopsy in order to avoid unnecessary biopsies, thereby reducing healthcare costs.

It is expected that multi-kallikreins as a novel diagnostic biomarker will gain substantial scientific and commercial interest. The ultimate goal is to develop commercial multi-kallikreins assays and next to implement multi-kallikreins in the clinic through updates of current guidelines and recommendations, including the risk indicator developed by Erasmus MC (see next section). The design of the biomarker validation studies in P-Mark can be used as a model for future biomarker studies, not only for prostate cancer but also for other diseases.

IDENTIFICATION OF RISK GROUPS IN THE GENERAL POPULATION: THE ROLE OF THE ERSPC IN P-MARK

Partner involved: Erasmus MC (on behalf of the European Randomized Study of Screening for Prostate Cancer)

The P-Mark consortium has been closely collaborating with the coordination team of the European Randomized study of Screening for Prostate Cancer (ERSPC). The ERSPC study is a multi-centre study conducted in 8 European Countries. It has been going on since 1993 and has recruited more than 240,000 men, randomized between a screening and control arm. More than 10,000 cases of prostate cancer have been diagnosed. The biorepository of serum, tissue specimens and related clinical information stored in a large comprehensive database turned out to fit well into the goals of the P-Mark project. The ERSPC has substantially contributed to the establishment of the P-Mark retrospective serum biorepository described in Appendix 2.

Next to the delivery of serum samples and clinical information to facilitate the discovery, evaluation and validation of biomarkers, two other goals within P-Mark were defined for the ERSPC. First of all, in order to be able to provide essential information within the P-Mark project, the collection of biomaterials and data in the ERSPC project needed to be continued. In addition to that, the available materials were supposed to be used to identify the risk of having biopsy detectable prostate cancer in men aged 55-74. The findings of the group are documented in 20 publications published during the period of 01.11.2004-01.10.2008, including those that are still in press. An important finding was that the risk of having prostate cancer in men who have never been evaluated for this disease before strongly depends on the PSA level. In addition, rectal examination, the volume of the prostate determined by ultrasound and the presence or absence of a suspicious liaison on

ultrasound examination were significant predictors. Based on these observations, the prostate risk indicator was developed. The risk indicator is meant as a tool for men at risk, general practioners and urologists to assess the probability of having a biopsy detectable prostate cancer, and - if the diagnosis has been made - the probability of having an indolent prostate cancer. The first level of the risk indicator is a rough estimation of the probability of having a biopsy detectable prostate cancer using readily available information as age, micturation complaints and family history. The second level can be used after a man has decided to actually have a PSA test. With the information of the PSA test, the prediction of having a biopsy detectable prostate cancer becomes more accurate. The third level of the risk indicator is meant to be used by the urologist. For this risk assessment data on digital rectal examination, ultrasound examination and prostate volume must be available. The calculated probability can be of help in the decision to have a prostate biopsy yes or no. The fourth level of the risk indicator can be used to assess the probability of having an indolent prostate cancer and can be of help in the decision for treatment. The first version of the prostate risk indicator was made available to the general public in September 2007. The tool is freely accessible through the website of the European Association of Urology (EAU, www.uroweb.org) and via the www.prostate-riskindicator.com website. In September 2008, an extension of the prostate risk indicator was launched. It includes those risk modifiers that are identified in men who have been previously screened or who have previously undergone a biopsy of the prostate. A previous negative biopsy and prostatic volume are the most important negative predictors. Molecular subforms of PSA are of value to increase specificity (the prediction of a negative biopsy) more accurately.

The further improvement of the prostate risk indicator will continue beyond P-Mark, and will focus on the diagnostic value as well as the value of improving specificity of identification of prostate cancer in men who had been previously screened.

Impact of risk identification results on scientific, clinical and industrial stakeholders

Screening for prostate cancer nowadays has become common practice in the Western countries. Identification of men who are at risk of having prostate cancer requiring active treatment in order to prevent overdiagnosis and overtreatment is a major task. The ERSPC addresses this important issue and has now made its extensive data available to the general public by constructing a freely available prostate risk indicator. This tool helps in the decision making process by both men at risk and healthcare professionals. It is expected that the risk indicator will continuously be optimized by new data from the ERSPC. In addition, inclusion of new diagnostic and prognostic biomarkers such as the multi-kallikreins may significantly improve the predictive power of this tool in the future. A prerequisite in this respect will be the availability of assays for these biomarkers to clinical centers, for instance via service laboratories.

CONCLUSIONS

Based on the results described in this report, it can be concluded that the P-Mark project has successfully met four of the five main project objectives within the time-frame of the project:

- ***Establishment of a serum biorepository and a urine biorepository***
  The P-Mark Biobank has been established, consisting of an extensive retrospective serum collection and a unique multicentre prospective blood and urine collection.
- ***Discovery of novel prostate cancer biomarkers in serum and urine by innovative mass spectrometry tools***
  A list of novel potential candidate biomarkers has been delivered for further research beyond P-Mark.
- ***Establishment of the clinical utility of recently developed promising prostate cancer markers***
  The clinical utility was demonstrated for osteoprotegerin, multi-kallikreins and PCA3, warranting further validation studies on these biomarkers.
- ***Validation of prostate cancer markers and identification of risk groups in the general population in Europe***
  Osteoprotegerin, multi-kallikreins and PCA3 were extensively validation. PCA3 was introduced into the clinic. The research on the identification of risk groups in the general European population has resulted in the development of the prostate risk indicator.

The fifth objective relates to the development of guidelines for cost-efficient strategies for prostate cancer detection and therapy. In the nearby future, studies initiated within P-Mark may eventually result in new guidelines for prostate cancer. For instance, the ERSPC study on the applicability of PCA3 as a first line screening test might lead to novel recommendations for PCA3. Furthermore, commercial interest in the multi-kallikreins may result in the development of commercial assays and subsequently to the implementation of these biomarkers in the clinic through updates of current guidelines and recommendations, including the prostate risk indicator developed by Erasmus MC.

DISSEMINATION AND USE OF P-MARK RESULTS

DISSEMINATION OF P-MARK RESULTS

The P-Mark results have extensively been communicated to the scientific society by over 70 publications in peer-reviewed journals, over 18 publications submitted or in preparation, and presentations at national and international meetings. A total of 8 students have obtained or will obtain their PhD degree on P-Mark studies. In Appendix 2, an overview of P-Mark related publications is presented.

USE OF P-MARK RESULTS

P-Mark has significantly contributed to the improvement of the diagnosis and prognosis of prostate cancer, one of the major cancer-related health problems for men in Europe, and will continue to do this in the future in the following ways:

- The **P-Mark Biobank**, a unique European multicentre biorepository composed of retrospective serum samples (Appendix 1) and of longitudinal serum, plasma, DNA, RNA, and urine samples from patients with various stages of prostate cancer collected in a standardized way, is now available for the scientific society. This biobank will in the nearby future become of tremendous significance by allowing for the composition of large multicentre sample cohorts with a significantly reduced risk of sampling bias. On the longer term, the biorepository will also enable testing of biomarkers for their prognostic value or for their value in the assessment of response to treatment. The P-Mark Biobank is managed by the P-Mark Biobank Foundation (Dutch Chamber of Commerce 24432295).
- **Novel potential candidate biomarkers** are available for further evaluation and validation. This has already resulted in a **patent** (international publication number WO 2008/143494) by Erasmus MC on the use of differentially expressed peptides caused by low abundant specific proteases and their inhibitors, detected by mass spectrometry, for the diagnosis of prostate cancer using their enzyme activity. This invention can also be used for determining the efficacy of prostate cancer treatment by periodically performing this method in patients who receive therapy. Besides for prostate cancer, this principle is also of high interest for other diseases in which proteases have a function in the pathogenesis such as Alzheimer's disease.
- **The validated multi-kallikreins biomarkers** are ready for clinical implementation and commercialization. The P-Mark participants Lund University and University of Turku hold a number of pre-existing patents on the multi-kallikreins (hK2 and free PSA variants), thereby ensuring that clinical implementation and commercialization of these biomarkers is attractive for industry. The clinical introduction of **PCA3**, which was developed by the Radboud University Nijmegen Medical Centre holding various patents related to this biomarker, demonstrates that commercialization of a biomarker developed in an academic setting is feasible.
- The **risk indicator** developed by Erasmus MC with support from P-Mark is a tool for men at risk, general practioners and urologists to determine the risk on positive prostate biopsies, on indolency of diagnosed prostate cancer, and on the presence of a tumour four years after a previous PSA screen with or without a negative prostate biopsy. The calculator is available without costs to any man, patient and physician in the EU via www.prostate-riskindicator.com and through the website of the EAU (www.uroweb.org). The Dutch version ("Prostaatwijzer") is freely accessible to the general public via the SWOP website (www.prostaatwijzer.nl).

The P-Mark consortium is dedicated to continue the collaborations and the validation and clinical implementation of promising biomarkers beyond P-Mark. There are currently three spin-off collaboratives:

1. PROCABIO

PROCABIO (Tailored treatment of prostate cancer by biomarkers) is a European initiative that focuses on the use of biomarkers for indolent prostate cancer (www.erspc-media.org). Due to screening, a significant number of harmless indolent cancers in men aged ≥ 50 are nowadays detected besides relevant tumors that need invasive therapy. These indolent tumors are best managed by active surveillance but in case of disease progression delayed active therapy should be delivered. The value of active surveillance is currently being tested in a prospective program entitled Prostate Cancer Research International: Active Surveillance (PRIAS, www.prias-project.org) initiated at Erasmus MC. Treatment choices should at best be tailored to the individual based on prognostic biomarkers, but current biomarkers lack sufficient accuracy to predict individual disease outcome. The PROCABIO project, coordinated by Prof. dr. Chris Bangma (project manager Dr. Ellen Schenk), will address the need for targeted therapy through the assessment of biomarkers in the setting of a multicentred clinical trial on active surveillance as a treatment option for indolent prostate cancer. The P-Mark partners involved in PROCABIO are Erasmus MC, Lund University, Radboud University Nijmegen Medical Centre, University of Oxford and University of Turku.

2. TRANSMARK

Within the European Commission Seventh Framework Programme, an Initial Training Network called TRANSMARK has been recommended for funding. TRANSMARK will train young researchers to become an independent and all-round biomarker scientist and team leader by a European integrated, multi-disciplinary biomarker training programme. This will offer them a broad career prospect in translational biomarker research on every type of cancer as well as other diseases. The network, coordinated by Prof. dr. Chris Bangma (project manager Dr. Ellen Schenk), is driven by recognised and experienced academic and industrial scientists who are jointly dedicated to deliver novel biomarkers for prostate cancer that can improve the diagnosis and prognosis of this major European health problem, and that can aid in the development of tailored therapy. TRANSMARK will start mid 2009 and offers various PhD and post-doc research projects. The P-Mark partners involved in TRANSMARK are Erasmus MC, Fujirebio Diagnostics AB, Lund University, Radboud University Nijmegen Medical Centre, University of Oxford and University of Turku.

3. Prostate Cancer Molecular Medicine

Erasmus MC and Radboud University Medical Center Nijmegen are involved in the Dutch Prostate Cancer Molecular Medicine (PCMM) application that recently was submitted to the Dutch funding programme Center for Translational Molecular Medicine (www.ctmm.nl). In the PCMM programme, running for a period of 5 years and coordinated by Erasmus MC (Prof. Chris Bangma assisted by Dr. Ellen Schenk), novel biomarkers and innovative targeted imaging techniques will be developed and validated for their use in a more specific detection of prostate cancer and a better prediction of tumor aggressiveness. A unique Dutch prospective multi-centre biobank composed of blood, urine, tissue specimens and imaging data from patients with various stages of prostate cancer will be established, based on the format of the P-Mark biorepository. The peer-review by external referees was favorable and the final evaluation outcome is expected in Spring 2009.

APPENDIX 1: THE P-MARK BIOBANK RETROSPECTIVE SERUM COLLECTION

Group	Description	Samples
Collection for research on diagnostic biomarkers		
A1	Men with PCa and PSA 3-10 ng/ml	357
A2	Men with PCa and PSA < 3 ng/ml	50
A3/A4	Men without PCa and PSA 3-10 ng/ml (controls)	667
A5	Men without PCa and PSA < 3 ng/ml (controls)	50
Collection for research on diagnostic biomarkers		
B1	Men with PCa who have received radical prostatectomy with a post-operative PSA (nadir PSA) <= 1 ng/ml (preferably < 0.1ng/ml)	263
B2	Men with advanced, untreated disease and good staging imaging, including bone scans	81
Collection for research on prognostic biomarkers (clinical follow-up ≥ 5 years)		
C1	Normal individuals, PSA < 1ng/ml	65
C2	Men without PCa and PSA > 3ng/ml (2 sets of negative biopsies)	285
C3	Men with PCa (pT2a, tumour volume <=0.5cc, no single Gleason pattern of 4 or 5, organ confined, T1c clinically insignificant cancer) who underwent radprost	24
C4	Men with asymptomatic early stage PCa, selected after 2nd and 3rd round of screening in ERSPC with aggressive disease, >=cT2bN0M0, single Gleason pattern >=4)	17
C5	Men with significant cancers after removal of the prostate, without metastases during follow-up, pT2N0M0	62
C6	Men with significant cancers after removal of the prostate, and got metastases during follow-up, pT2-3N0M0 >> pT2-3N+M+ after radprost	5
C7	Men that became progressive with androgen-independent disease, pT2-3N0M0 >> pT2-3NxM+ after radprost	14
C8	Men with extracapsular disease who do not progress after radprost, pT3N0M0	105
C9	Men with metastatic disease at presentation (pT3-T4Nx-+M1)	78

ERSPC; European Randomized Study of Screening for Prostate Cancer, PCa; prostate cancer, radprost; radical prostatectomy.

APPENDIX 2: P-MARK PUBLICATIONS

P-MARK PUBLICATIONS IN PEER-REVIEWED JOURNALS (TILL 31 JANUARY 2009)

This list refers to publications in which P-Mark has been acknowledged. The total number of articles on studies related to P-Mark is far higher.

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P-MARK PUBLICATIONS SUBMITTED OR IN PREPARATION

1. Helo P, Cronin AM, Danila DC, Wenske S, Gonzalez-Espinoza R, Anand A, Väänänen RM, Pettersson K, Chun FKH, Steuber T, Huland H, Guillonneau BD, Eastham JA, Scardino PT, Fleisher M, Scher HI, Lilja H. Circulating prostate tumor cells detected by RT-PCR in men with localized or castration-refractory prostate cancer: concordance with CellSearch assay and association with bone metastases and with survival. *submitted*

2. Helo P, Cronin AM, Danila DC, Gonzalez-Espinoza R, Anand A, Nurmi J, Pettersson K, Scardino PT, Fleisher M, Scher HI, Lilja H. Detection of circulating tumor cells in patients with advanced prostate cancer: concordance of results of real-time RT-PCR assay for KLK2 and KLK3 (PSA) mRNAs and CellSearch assay. *in preparation*

3. Lenshof A, Ahmad-Tajudin A, Järås K, Swärd-Nilsson AM, Åberg L, Malm J, Lilja H, Laurell T. Whole blood plasma extraction using acoustopheresis. *in preparation*

4. O'Brien MF, Serio AM, Fearn PA, Smith B, Stasi J, Vickers AJ, Guillonneau BD, Scardino PT, Eastham JA, Lilja H. Both pretreatment PSA velocity and doubling time associate with outcome but neither improves prediction of outcome beyond pretreatment PSA alone in patients treated with radical prostatectomy. *submitted*

5. Robert Klein RJ, Halldén C, Cronin AM, Ploner A, Wiklund F, Bjartell AS, Stattin P, Xu J, Scardino PT, Vickers AJ, Grönberg H, Lilja H. Blood biomarker levels to aid discovery of cancer-related single nucleotide polymorphisms: kallikreins and prostate cancer. *in preparation*

6. Sävblom C, Halldén C, Cronin AM, Savage C, Wenske S, Klein R, Giwercman A, Lilja H. Allelic variation in the genes encoding kallikrein-related peptidases 2 and 3 influence levels of hK2 and PSA in seminal plasma and in blood in young healthy men.*in preparation*
7. Ulmert D, Cronin AM, Scher HI, Seandel M, Becker C, Franke D, Jensen JK, Vickers AJ, Olesen TK, Lilja H. Rapid elimination kinetics of free PSA and hK2 after initiation of GnRH-antagonist treatment of aggressive prostate cancer: potential for more efficient monitoring of treatment effects using uncomplexed kallikreins. *submitted*
8. Vickers AJ, Cronin AM, Aus G, Pihl CG, Becker C, Pettersson K, Scardino PT, Hugosson J, Lilja H. Impact of recent screening on the properties of a four-kallikrein panel for detection of prostate cancer: data from the European Randomized Study of Prostate Cancer Screening in Göteborg, Sweden. *submitted*
9. Vickers AJ, Wolters T, Savage CJ, Cronin AM, Pettersson K, Roobol MJ, Aus G, Scardino PT, Hugosson J, Schröder FH, Lilja H. Value of changes in prostate specific antigen levels for early detection of prostate cancer. *submitted*
10. Vickers AJ, Cronin AM, Roobol M, Savage CJ, Peltola M, Pettersson K, Scardino PT, Schröder FH, Lilja H. Independent replication of a four kallikrein panel to detect prostate cancer: data from the European Randomized Study of Prostate Cancer Screening in Rotterdam, Netherlands. *in preparation*
11. Wenske S, Steuber T, Cronin AC, Vickers AJ, Huland H, Pettersson, K, Høyer-Hansen G, Piironen T, Eastham JA, Scardino PT, Fleisher M, Lilja H. Clinical value of soluble forms of prostate-specific antigen, kallikrein-like peptidase 2, osteoprotegerin, and urokinase plasminogen activator receptor measured prior to prostatectomy in predicting prostate cancer recurrence. *in preparation*

TOPICS FOR JOINT P-MARK PUBLICATIONS TO BE SUBMITTED IN 2009

- P-Mark Biobank: protocols, contents, future
- Evaluation of proteomics methods evolved in P-Mark
- Discovery of novel markers from proteomics research
- Prognostic markers in blood, urine and tissue from P-Mark
- Challenges in assay development: experiences from P-Mark
- Validation of biomarkers in P-Mark and ERSPC
- P-Mark overall accomplishment and future research in Europe

ECAM PUBLICATIONS

In 2004, the coordinators of the three FP6 prostate cancer programs P-Mark, GIANT and PRIMA have joined efforts in order to inform urologists on the progress of these urological research projects. A team of EC-activity members (ECAM) was established that regularly reports in European Urology Today, the official newsletter of the European Association of Urology. Since then, ECAM was extended with representatives from new EU-funded projects related to prostate cancer. The following articles related to P-Mark have been published in European Urology Today:

1. Schenk E, Bangma C. Launch of the PROCABIO project: tailored treatment of prostate cancer. European Urology Today 2008;20(3):13.
2. Schalken J. The way forward on PCa prognosis and detection. European Urology Today 2008;20(1):27.
3. Schenk E, Bangma C. An interim report on the P-Mark project. Eur Urol Today 2007;19:13.
4. Titulaer M, Dekker L, van Rijswijk A, Bangma C, Luider T. Bioinformatics used in the EU P-Mark project for biomarker discovery. European Urology Today 2005;17(4):9-10.
5. Bjartell A. From urine and serum to peptide/protein identification. European Urology Today 2005;17(3):3+11.
6. Schalken JA. The establishment of prostate cancer biorepositories: “garbage in, garbage out”. European Urology Today 2005;17(1):11.

7. Rehman I, Azzouzi A-R, Catto JWF, Hamdy FC. Essential proteomics for urologists. European Urology Today 2004;16(4):14.
8. Bangma CH, Schenk E. P-Mark comes to life. European Urology Today 2004;16(4):3.
9. EC-activity members (ECAM: Bangma CH, Bjartell A, Culig Z, Hamdy F, Maitland N, Schalken J). European Community projects in urology. European Urology Today 2004;16(2):3.

P-MARK THESES

The following theses on P-Mark studies have been successfully defended:

- Measurement of human glandular kallikrein 2. Ville Vaisanen, University of Turku, Finland, thesis defended on 9 March 2007
- Assessments of PSA Forms and hK2 as Very Early Predictors of Prostate Cancer. David Ulmert, Lund University, Sweden, thesis defended on 15 May 2007
- Proteomics of body fluids. Lennard Dekker (Erasmus MC Departments of Neurology and Urology), Erasmus University Rotterdam, the Netherlands, thesis defended on 10 October 2007
- Screening for prostate cancer. Intermediate outcome measures and active surveillance. Stijn Roemeling (Erasmus MC Department of Urology), Erasmus University Rotterdam, the Netherlands, thesis defended on 7 November 2007
- Screening for prostate cancer. Digital rectal examination: outdated or still valuable? Claartje Gosselaar (Erasmus MC Department of Urology), Erasmus University Rotterdam, the Netherlands, thesis defended on 5 November 2008

In 2009/2010, the following graduations are planned:

- Suvi Ravela (University of Helsinki) will defend her thesis on urinary peptidomics
- Mari Peltola (University of Turku) will defend her thesis on the assessment of kallikreins as biomarkers for prostate cancer
- Riina-Minna Väänänen (University of Turku) will defend her thesis on the assessment of circulating tumor cells as biomarkers for prostate cancer