White Paper

Prostatype – White Paper

Background

Prostate Cancer is the most common type of cancer among men in Sweden as well as in many Western countries [1]. However, approximately 85% of prostate cancer tumors are not rapidly progressing and will not cause clinical symptoms at the time of diagnosis, while the remaining ~15% are more aggressive and potentially life-threatening [2].

Choosing the appropriate treatment for the individual patient is challenging, as prostate cancer risk assessment is a critical aspect of treatment decision-making. Current tools, such as the D'Amico system, divide patients into low-, intermediate- and high-risk patients. However, these methods have limited prediction accuracy and consequently, studies indicate that 7 out of 10 patients are being over- or undertreated [3]. A substantial proportion of patients are undergoing radical treatment, which is often associated with devastating physical and psychological side effects dramatically affecting quality of life. Thus, to safely avoid unnecessary radical treatment, there is an urgent need for novel tools that provide additional information to estimate the aggressiveness of prostate cancer. Simultaneously, preventing under-treatment of patients is equally important and a concern for many doctors.

Prostatype®

The Prostatype Test System is a gene expression test that provides prognosis of survival and decision support for various treatment options. Prostatype combines gene expression information with the currently used clinical parameters (PSA, Gleason Score, Tumor Stage, and age). The test is based on a unique, searchable database containing authentic information from approximately 600 historic prostate cancer patients. Based on these historic patients, the Prostatype P-Score has been developed. This score estimates aggressiveness of prostate cancer. Thereby, Prostatype provides decision support for patients and doctors when making a treatment decision with the potential to reduce the risk for over- or under-treatment and to postpone repeated biopsies.

Development

Dr. Chunde Li's research group at Cancer Center Karolinska started from whole-genome bioinformatics analyses based on the embryonic stem (ES) cell hypothesis. The group identified a list of 641 Embryonic Stem Cell Gene Predictors (ESCGPs) markers for cancer diagnosis and prognosis. It was hypothesized that gene signatures of embryonic stem (ES) cells may have prominent importance to determine tumor subtypes and may be associated with the prognosis of various cancers including prostate cancer (PCa). Using gene expression patterns of these 641 ESCGPs, tumor subtypes of different cancers can be stratified, particularly for prostate cancer [4].

Identification of significant ESCGPs can improve prostate cancer mortality prediction accuracy at the time of diagnosis. Using a step-wise way of gene selection process (Figure 1A), we measured the gene expression levels of selected ESCGP genes in fresh-frozen fine needle aspiration biopsy samples taken from a pilot cohort of 189 prostate cancer patients. These patients were diagnosed from 1989-1991. At the time of analyses, 98 patients had died from prostate cancer, 65 had died from other diseases, 22 were alive and 4 patients were lost to follow-up. As a result, a three-gene signature (VGLL3, IGFBP3 and F3) was identified and found sufficient to categorize the patients into high-risk, intermediate-risk and low-risk subtypes directly correlated with the overall and cancer-specific survival [5].

To ensure reliable results, it is critical to determine whether selection of FFPE core needle biopsy for gene expression analysis is dependent on the Gleason pattern of the respective biopsy. The 3-gene signature was identified from ESCGPs, which was hypothesized to capture the 'stemness' of aggressive cancer cells. We measured cancer tissues with different Gleason patterns as well as pathologically benign tissue to investigate the effect of the Gleason pattern on gene expression. In total, 112 samples from 41 patients were

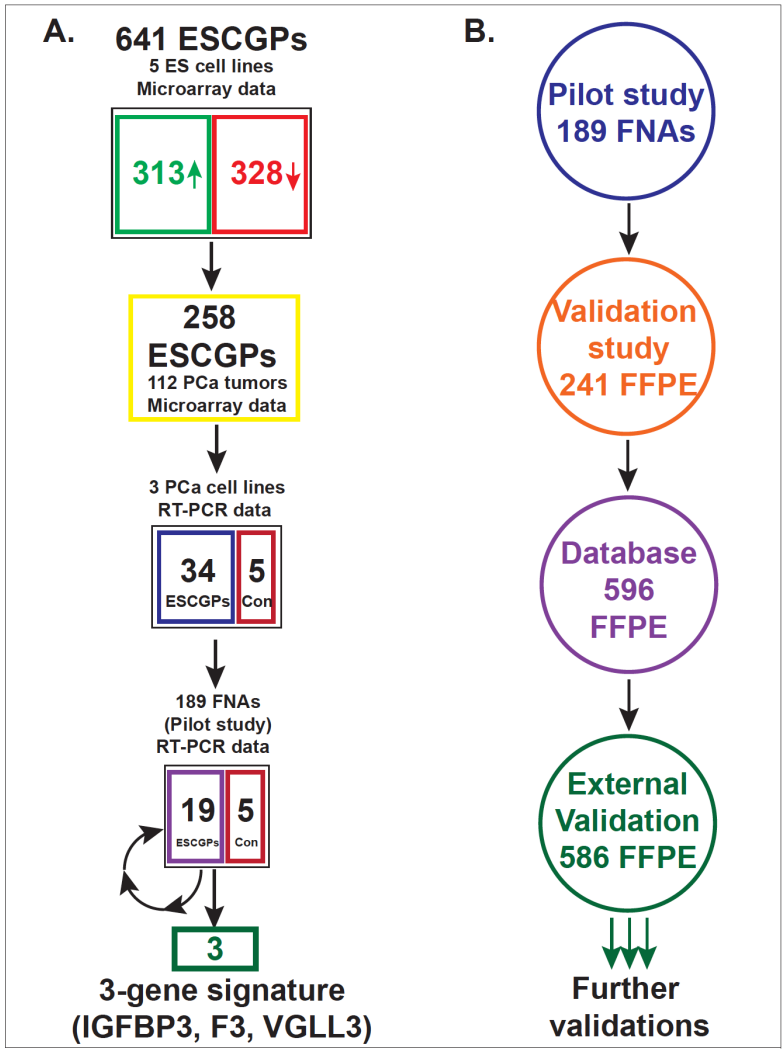

Figure 1: (A) Step-wise gene selection process; (B) Cohort studies used for estimation and validation of Prostatype.

measured. IGFBP3 and F3 had consistent gene expression levels regardless of different Gleason patterns as long as cancer tissues were taken from the same prostate gland. More interestingly, variations between cancer cells and pathologically benign tissue were also limited [6].

The prognostic value of the 3-gene signature was validated in a modern cohort of 241 prostate cancer patients (64 prostate cancer deaths, 60 died of other diseases, 117 alive) with 6-9 years follow-up by measuring FFPE diagnostic core needle biopsy samples [6-8]. This was the first validation study; thus, more retrospective cohorts were followed to further validate the prognostic value of gene signature (Figure 1B).

Prostatype RT-qPCR kit measures a 3-gene signature in prostate FFPE core needle biopsy samples, which is combined with clinical parameters into an algorithm (Classification of Prostatic Malignancy Algorithm, CPMA) to generate a prognostic statement. The CPMA software contains a database that includes 596 authentic, historical PCa patients with their gene signature data, four classic clinical parameters (age, PSA, Gleason score (GS) and tumor stage at diagnosis), and information on their treatment and overall survival time (123 prostate cancer deaths, 168 died of other diseases, 305 are still alive at last follow-up). In contrast to randomized selection, those authentic historical PCa patients included in the CPMA were selected aiming at the largest possible variety of clinical characteristics including death, COD, survival time, treatment, age, GS, clinical stage, and PSA value.

The patients mainly originate from Stockholm population with 8-12 years of follow-up. The CPMA is being extended continuously and will be updated in specific time intervals.

Besides the CPMA algorithm, a novel risk score (P-score) was developed and validated using the same 596 patients included in the CPMA database. P-score uses the 3-gene signature and clinical data at diagnosis to estimate the probability risk of prostate cancer- specific mortality. Compared to European Association Urology guideline's risk classification system (EAU risk groups), prediction accuracy of prostate cancer-specific mortality (Harrell's C index) using P-score is significantly improved. When looking at subgroup patients with Gleason score 7, P-score significantly outperformed EAU risk groups (Details are shown in performance description) [9].

An external validation study with a cohort of 586 prostate cancer patients was started in Q1 2018 in collaboration with the university hospital in Malmö, Sweden. These patients were diagnosed from 2008 to 2010 in southern Sweden, until the time of analyses there will be 8-10 years registry follow-up (Figure 1B). Further validation studies in other countries are being started as well, such as at the University Hospital Erlangen, Germany, and at the University Hospital Frankfurt, Germany. Prospective studies can ultimately prove Prostatype's clinical significant benefit in prostate cancer clinical management. A prospective study will therefore be started in parallel in the Stockholm area.

Performance

The 3-gene signature (IGFBP3, F3 and VGLL3) was identified and showed significantly improved overall and cancer specific survival prediction accuracy in the pilot FNA study [5]. By adding the 3-gene signature to four commonly used clinical parameters, the AUC for predicting cancer specific survival is significantly improved. (Figure 2).

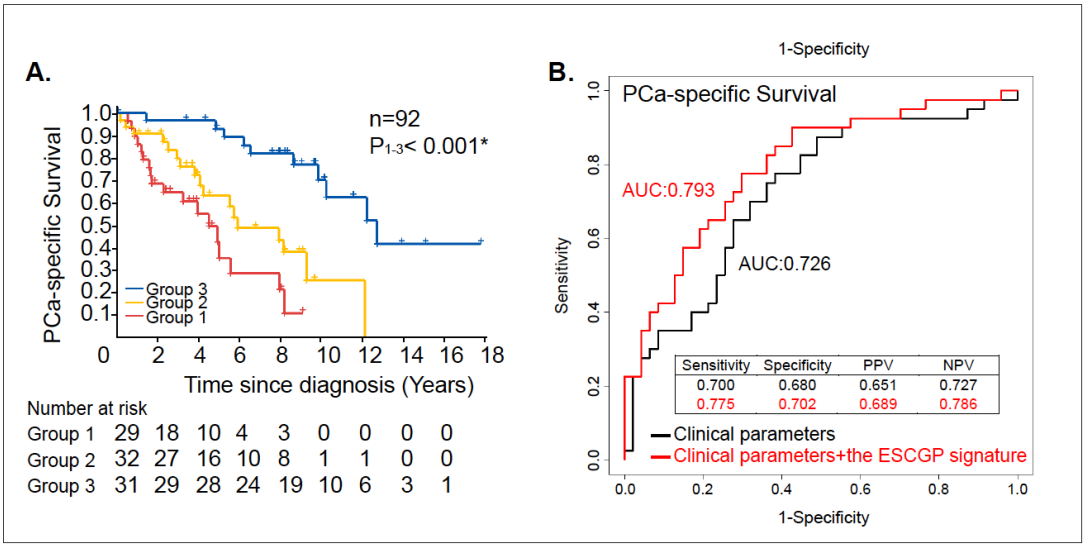

Figure 2: A 3-gene signature (IGFBP3, F3 and VGLL3) was identified and improved both overall and prostate cancer specific survival prediction accuracy. (A) Kaplan-Meier curves for different subgroups using the 3-gene signature. (B) ROC curves for 5-year survival prediction.

In FFPE core needle biopsy studies, CPMA parameters outperformed CAPRA and D'amico risk groups in terms of overall and cancer-specific survival prediction accuracy [8]. Particularly for intermediate-risk patients and patients with Gleason score 7, marked and significant improvements were evident (Figure 3 and Table 1).

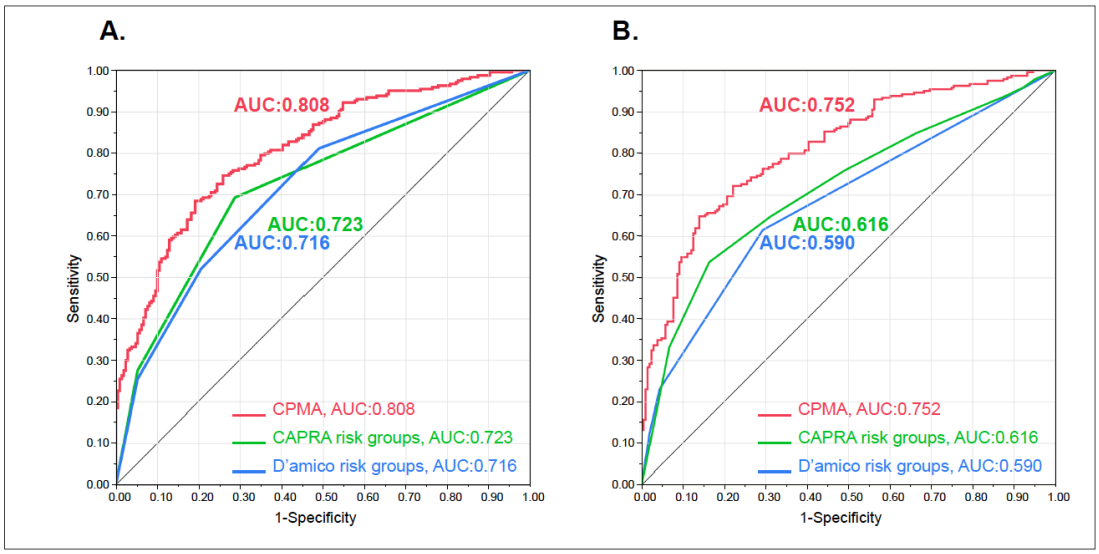

Figure 3: Receiver Operating Characteristics (ROC) curves for overall survival prediction, compared to CAPRA and D'Amico risk groups. (A) All patients. (B) Patients with Gleason Score 7.

Table 1. Overall survival prediction accuracy				
	Low-risk	Intermediate-risk	High-risk	Locally advanced
D'Amico	89%	54%	65%	72%
CPMA	89%	77%	76%	85%
	CAPRA 0-2	CAPRA3-5	CAPRA 6-10	
CAPRA	88%	56%	66%	
CPMA	88%	76%	83%	

The latest data generated from 596 prostate cancer patients (the same 596 patients filled in CPMA database and estimated P-score) further proves that the 3-gene signature significantly improves prediction accuracy of prostate cancer specific-mortality risk when used to supplement EUA risk groups. As confirmed, more prominent improvements were observed in subgroups of patients with Gleason score 7 or higher (Figure 4).

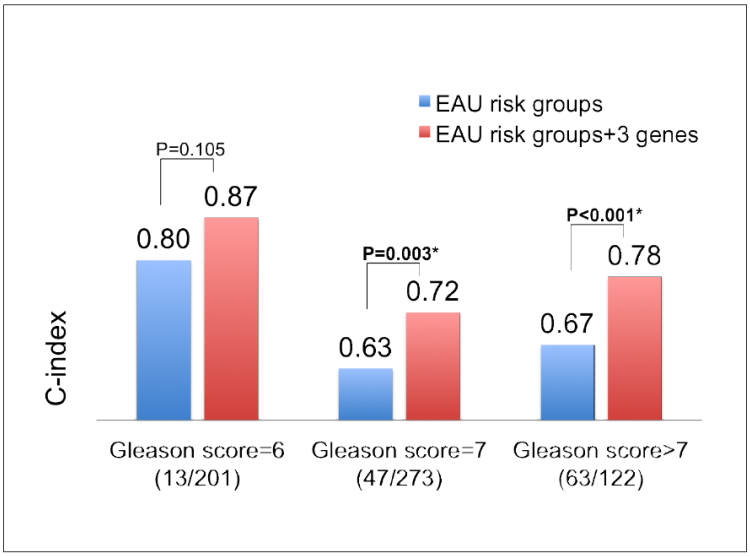

Figure 4: The 3-gene signature improves the prostate cancer specific survival prediction accuracy.

Based on the significant improvement by the 3-gene signature, a novel risk score system (P-score) using PSA, Gleason score and tumor stage at diagnosis combined with the 3-gene signature was identified and validated in independent cohorts. Out of 596 patients, 317 patients who were not treated with radical therapy were selected as the estimation cohort to develop P-score. The P-score was subsequently validated in an independent validation cohort of 279 patients with different treatments including all radical treated patients.

The P-score was modeled using the estimation dataset and has a range of 0 to 15 with 1 as the smallest unit. Multivariate analysis showed that P-score is associated with a higher HR than EAU risk groups (Table 2) and can therefore be considered a stronger predictor of prostate cancer-specific mortality [9].

Table 2. Multivariate cox proportional analyses				
	Estimation dataset (317/86)		Validation dataset (279/37)	
	HR (95%CL)	P value	HR (95%CL)	P value
P-score	1.42 (1.29-1.57)	<0.0001*	1.56 (1.33-1.84)	<0.0001*
EAU risk group	1.26 (0.86-1.84)	0.2458	1.18 (0.70-1.99)	0.3156

Prediction performance evaluation for prostate cancer-specific survival time led to a concordance index significantly improved both in estimation and validation datasets (Figure 5A). Again, even more significant improvements were found in the subgroup of patients with Gleason score 7 (Figure 5B).

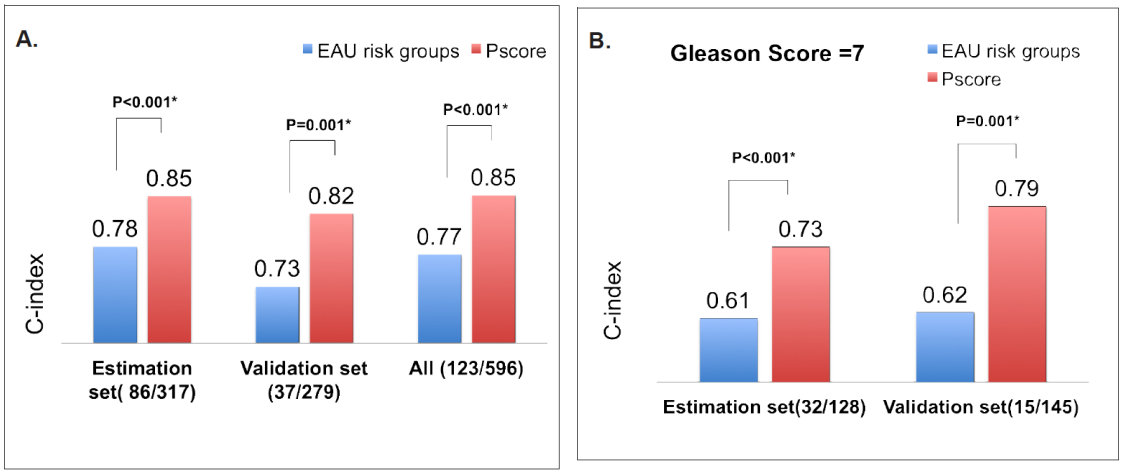

Figure 5: The P-score significantly outperforms EAU risk groups for prostate cancer specific survival prediction accuracy. (A) All patients. (B) Subgroup of patients with Gleason score 7.

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