EUROPEAN UROLOGY FOCUS 4 (2018) 376–384

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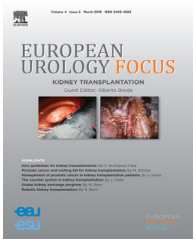
journal homepage: www.europeanurology.com/eufocus



Prostate Cancer

Validation of Cyclic Adenosine Monophosphate Phosphodiesterase-4D7 for its Independent Contribution to Risk Stratification in a Prostate Cancer Patient Cohort with Longitudinal Biological Outcomes

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Article info

Article history:
Accepted May 23, 2017

Associate Editor: James Catto

Keywords:
Androgen receptor
PKA
cAMP
Phosphodiesterase
Prostate cancer
Risk stratification
Prognosis
Postsurgical outcome prediction

Abstract

Background: The clinical metrics used to date to assess the progression risk of newly diagnosed prostate cancer patients only partly represent the true biological aggressiveness of the underlying disease.
Objective: Validation of the prognostic biomarker phosphodiesterase-4D7 (PDE4D7) in predicting longitudinal biological outcomes in a historical surgery cohort to improve postsurgical risk stratification.
Design, patients, and methods: RNA was extracted from biopsy punches of resected tumors from 550 patients. PDE4D7 was quantified using one-step quantitative reverse transcription-polymerase chain reaction. PDE4D7 scores were calculated by normalization of PDE4D7 to reference genes. Multivariate analyses were adjusted for clinical prognostic variables. Outcomes tested were: prostate-specific antigen relapse, start of salvage treatment, progression to metastases, overall mortality, and prostate cancer-specific mortality. The PDE4D7 score was combined with the clinical risk model Cancer of the Prostate Risk Assessment Postsurgical Score (CAPRA-S) using multivariate regression modeling; the combined score was tested in post-treatment progression free survival prediction.
Outcome measurements and statistical analysis: Correlations with outcomes were analyzed using multivariate Cox regression and logistic regression statistics.
Results and limitations: The PDE4D7 score was significantly associated with time-to-prostate specific antigen failure after prostatectomy (hazard ratio [HR]: 0.53, 95% confidence interval [CI]: 0.41–0.67 for each unit increase, $p < 0.0001$). After adjustment for postsurgical prognostic variables the HR was 0.56 (95% CI: 0.43–0.73, $p < 0.0001$). The PDE4D7 score remained significant after adjusting the multi-variate analysis for the CAPRA-S model categories (HR = 0.54, 95% CI = 0.42–0.69, $p < 0.0001$). Combination of the PDE4D7 score with the CAPRA-S demonstrated a significant incremental value of 4–6% in 2-yr ($p = 0.004$) or 5-yr ($p = 0.003$) prediction of progression free survival after

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http://dx.doi.org/10.1016/j.euf.2017.05.010

surgery. The combined model of PDE4D7 and CAPRA-S improves patient selection with very high risk of fast disease relapse after primary intervention.
Conclusions: The PDE4D7 score has the potential to provide independent risk information and to restratify patients with clinical intermediate- to high-risk characteristics to a very low-risk profile.
Patient summary: In this report, we studied the potential of a novel biomarker to predict outcomes of a cohort of prostate cancer patients who underwent surgery more than 10 yr ago. We found that a gene called *phosphodiesterase-4D7* added extra information to the available clinical data. We conclude that the measurement of this gene in tumor tissue may contribute to more effective treatment decisions.

1. Introduction

Prostate cancer displays as a heterogeneous disease with varying potential to develop progressively to deadly forms of the disease. Of the estimated 417 000 annual new cases in Europe, around 92 000 will die from their disease [1].

Accurate risk stratification of prostate cancer patients is essential to identify men who benefit from adjuvant or multi-modality treatments while sparing those with low risk from the adverse effects of aggressive therapy. Various clinic metrics have been developed in the past to predict disease progression after primary treatments such as surgery or radiation therapy [2]. Typically, predictions are based on the combination of clinical variables that are available at the moment of clinical need. However, clinical risk descriptors are limited by the information content of their clinical input variables and do not necessarily describe effectively either the extent of the disease or its aggressiveness for all patients [3–5]. Thus, there is a need for better patient stratification in order to optimize disease management. Additional molecular information representing the biology of the disease offers the potential to achieve this [6].

The cyclic adenosine monophosphate (cAMP) signaling pathway is known to play an important role in both the development and progression of prostate cancer [7]. While a family of adenylate cyclases is responsible for the synthesis of cAMP, cyclic nucleotide phosphodiesterases (PDEs) represent the only cellular mechanism for its destruction. PDEs provide both signal termination and, importantly, the compartmentalization of cAMP signaling within the three-dimensional matrix of cells. This is achieved through the spatially discrete destruction of cAMP via subpopulations of distinct PDE isoforms sequestered by localized anchor proteins/signalosomes [8–10]. Thus, changes in the expression and/or activity of distinct PDE isoforms can alter downstream signaling pathways during disease development and progression, providing potential targets for novel biomarkers and for targeted therapeutic intervention.

In this study, we demonstrate that PDE4D7 transcript levels correlate to the longitudinal outcome of prostate cancer after primary treatment. We further hypothesized that PDE4D7 independently adds to clinical risk stratification metrics like the Cancer of the Prostate Risk Assessment Postsurgical (CAPRA-S) score [11], which may add value for clinical decision-making.

2. Patients and methods

2.1. Patient cohorts and samples

Two small adjacent biopsy punches (approximately 1×2 mm) of a representative resected area of the largest volume tumor of 550 patients operated on between 2000 and 2004 at Martini Klinik (Hamburg, Germany) were collected (Table 1) with local Institutional Review Board approval. Patients with adjuvant hormone therapy were removed from the analysis. After data quality control based on predefined criteria, 503 patient samples were eligible for statistical analysis (Supplementary Figs. 1 and 2). For independent validation purposes, we selected a prostate cancer data set published previously [12]. All men with primary prostate cancer with available postsurgical outcome data as well as PDE4D7 expression data based on exon array measurements were selected for the validation ($n = 130$; Table 1).

2.2. Laboratory methods

Quantitative real-time polymerase chain reaction (RT-qPCR) primers and probes are outlined in Supplementary Table 1. RNA extraction, RT-qPCR assay development and protocols are described in the Supplementary data. The limit of detection for RT-qPCR quantification cycle value (Cq) was determined and was used as a predefined quality threshold to discard samples from the statistical analysis (Supplementary data).

2.3. Data analysis and statistics

Normalized PDE4D7 expression was calculated by subtracting the Cq of the respective PDE4D transcript from the averaged Cq of the reference genes [13] and transformed to the PDE4D7 score (Supplementary Fig. 3). The CAPRA-S risk score and corresponding low- (1), intermediate- (2), high-risk (3) categories were calculated as described previously [11]. Uni- and multivariate Cox regression analysis and modeling was applied to correlate biochemical recurrence progression free survival (BCR PFS) to the PDE4D7 score in the study cohort ($n = 503$) and the validation cohort ($n = 130$). The PDE4D7 score was either used as a continuous or as a categorical variable. The multi-variate analyses were adjusted for postsurgical clinical variables.

Logistic regression models to predict 2-yr or 5-yr BCR PFS were created using the CAPRA-S categories and the continuous PDE4D7 score as variables in the study cohort with complete 5-yr follow-up ($n = 469$ and $n = 449$, respectively). For statistical analysis, the software packages MedCalc (MedCalc Software BVBA, Ostend, Belgium) or R (R Foundation for Statistical Computing, Vienna, Austria) were used. All p values are two-sided; statistical significance is based on alpha level ≤ 0.05.

Analysis of GSE21034 [12]: raw CEL files were downloaded from Gene Expression Omnibus. Data processing and robust multichip average

Table 1 – Patient demographics of the analyzed patient surgery study and validation cohorts.

	Parameter	Study cohort (n = 503)	Validation cohort (n = 130)
Demographic & clinical characteristics, range (median; IQR)	Age range	41.3–74.5 (62.6; 7.4)	37.3–83 (58.0; 8.7)
	Preoperative PSA range	0.18–73.16 (6.7; 5.5)	1.15–46.4 (5.9; 4.6)
	Percent tumor in biopsy range	0.2–79.7 (10.3; 16.0)	NA
	Prostate volume range	9–148 (42; 22.5)	NA
	PSA density range	0.18–73.2 (6.7; 5.5)	NA
NCCN risk category, *N* (%)	Very low risk	67 (13.3)	58 (44.6)
	Low risk	144 (28.6)	
	Intermediate favorable risk	128 (25.4)	32 (24.6)
	Intermediate unfavorable risk	120 (23.9)	21 (16.2)
	High risk	44 (8.7)	19 (14.6)
Presurgery pathology, *N* (%)	Biopsy Gleason 3 + 3	316 (62.8)	78 (60.0)
	Biopsy Gleason 3 + 4	149 (29.6)	29 (22.3)
	Biopsy Gleason 4 + 3	25 (5.0)	12 (9.2)
	Biopsy Gleason ≥4 + 4	13 (2.6)	11 (8.5)
	cT1	342 (68)	74 (56.9)
	cT2	150 (29.8)	51 (39.2)
	cT3	11 (2.2)	5 (3.8)
Postsurgery pathology, *N* (%)	Pathology Gleason 3 + 3	201 (40)	42 (32.3)
	Pathology Gleason 3 + 4	257 (51.1)	53 (40.8)
	Pathology Gleason 4 + 3	41 (8.2)	21 (16.2)
	Pathology Gleason ≥4 + 4	4 (0.8)	14 (10.8)
	pT2	331 (65.8)	85 (65.4)
	pT3	172 (34.2)	39 (30.0)
	pT4	0 (0)	6 (4.6)
	Positive surgical margins	120 (23.9)	29 (22.3)
	Extracapsular extension (T3a)	113 (22.5)	87 (66.9)
	Seminal vesicle invasion	60 (11.9)	13 (10.0)
	Lymph node invasion	5 (1)	5 (3.7)
Follow-up, mo (IQR follow-up)	Mean	123.6	58.2
	Median	141.8 (60.1)	54.2 (35.6)
Outcome, no. of events/total patient no. (percentage; follow-up; IQR)	BCR within 5 yr	92/446 (20.6; 121.2; 87.5)	26/130 (20.0; 46.9; 33.6)
	BCR within 10 yr	134/347 (38.6; 134.0; 95.6)	
	CR within 5 yr	5/441 (1.1; 144.4; 37.8)	8/130 (6.2; 52.3; 34.9)
	CR within 10 yr	13/306 (4.2; 154.7; 32.85)	
Salvage treatment, no. events/total patient no. (percentage; follow-up; IQR)	SRT within 5 yr	53/439 (12.1; 120.4; 53.5)	13/130 (10.0; NA; NA)
	SRT within 10 yr	83/320 (25.9; 132.3; 39.6)	
	SADT within 5 yr	27/441 (6.1; 120.7; 46.6)	14/130 (10.8; NA; NA)
	SADT within 10 yr	54/312 (17.3; 132.4; 24.2)	
Survival, no. events/total patient no. (percentage; follow-up; IQR)	PCSM within 5 yr	17/453 (1.1; 144.4; 37.7)	0/130 (0; NA; NA)
	PCSM within 10 yr	38/330 (2.6; 154.8; 30.3)	
	OM within 5 yr	5/441 (3.7; 144.4; 45.1)	NA
	OM within 10 yr	10/302 (11.2; 146.0; 35.4)	

BCR = biochemical recurrence; CR = clinical recurrence; IQR = interquartile range; OM = overall mortality; NA = not applicable; PCSM = prostate cancer-specific mortality; PSA = prostate-specific antigen; SADT = salvage androgen deprivation therapy; SRT = salvage radiotherapy.

normalization were performed using the aroma.affymetrix R-package (Affymetrix Inc, CA, USA) [14] and transcript expression was measured by averaging log2-transformed intensity values of the following isoform-specific probe sets: PDE4D7 (2858406, 2858407, and 2858408). The normalized PDE4D7 expression values were transformed into a PDE4D7 score equivalent to the qPCR expression data.

3. Results

3.1. Correlation of PDE4D7 score to longitudinal clinical outcomes

We set out to correlate the expression of the putative prostate cancer biomarker PDE4D7, which has recently been initially described by us [13], to the longitudinal patient outcome of BCR after surgery. Although prostate-specific antigen (PSA) relapse is regarded as a surrogate for more severe clinical outcomes like metastases or cancer-specific death, it is nevertheless very often used as a trigger to start secondary treatments.

Univariate and multi-variate Cox regression analysis demonstrated a significant correlation of the continuous PDE4D7 score to time to BCR (hazard ratio: 0.53, 95% confidence interval [CI]: 0.41–0.67, $p < 0.0001$, and hazard ratio: 0.56, 95% CI: 0.43–0.73, $p \leq 0.0001$, respectively; Table 2, Supplementary Fig. 4A). Adjusting the multi-variate Cox regression analysis for the prognostic CAPRA-S score resulted in a significant independent contribution to the

Table 2 – Uni- and multivariate Cox regression analysis of the biochemical recurrence (BCR) free survival of the phosphodiesterase-4D7 (PDE4D7) score in the patient surgery cohort adjusted for postsurgical clinical variables in the study cohort (n = 503).

Postsurgical clinical parameters	Uni-variate			Multi-variate		
Endpoint BCR (#503/#148; 29.4%)	p value	HR	95% CI of HR	p value	HR	95% CI of HR
Pathology Gleason score 3 + 3 (n = 201); reference						
Pathology Gleason score 3 + 4 (n = 257)	0.003	2.10	1.4–3.1	0.24	0.77	0.5–1.2
Pathology Gleason score 4 + 3 (n = 41)	<0.0001	8.60	5.2–14.2	<0.0001	2.6	1.7–4.2
Pathology Gleason score ≥4 + 4 (n = 4)	<0.0001	27.7	9.7–79.2	<0.0001	13.3	4.5–39.4
Pathology Stage pT2 (n = 331); reference						
Pathology Stage pT3 (n = 172)	<0.0001	4.30	3.1–6.0	0.0001	2.4	1.6–3.6
Surgical margin status	<0.0001	2.60	1.8–3.6	0.0006	1.0	1.3–2.7
Seminal vesicle invasion	<0.0001	4.50	3.1–6.4	0.027	1.6	1.1–2.5
Lymph node invasion	<0.0001	55.7	20.9–148.6	0.0015	5.5	1.9–15.6
PDE4D7 score (continuous)	<0.0001	0.53	0.41–0.67	<0.0001	0.56	0.43–0.73

CI = confidence interval; HR = hazard ratio.

Table 3 – Uni- and multi-variate Cox regression analysis of the biochemical recurrence (BCR) free survival of the phosphodiesterase-4D7 (PDE4D7) score in the patient surgery cohort adjusted for the Cancer of the Prostate Risk Assessment Postsurgical Score (CAPRA-S) in the study cohort (n = 503).

Postsurgical clinical parameters	Uni-variate (enter)			Multi-variate (enter)		
Endpoint BCR (#503/#148; 29.4%)	p value	HR	95% CI of HR	p value	HR	95% CI of HR
CAPRA-S score category (1; n = 288); reference						
CAPRA-S score category (2; n = 173)	<0.0001	2.9	2.0–4.2	<0.0001	2.9	2.0–4.2
CAPRA-S score category (3; n = 42)	<0.0001	8.7	5.5–13.7	<0.0001	8.4	5.3–13.3
PDE4D7 (continuous)	<0.0001	0.53	0.41–0.67	<0.0001	0.54	0.42–0.69

CI = confidence interval; HR = hazard ratio.

Table 4 – Uni- and multi-variate Cox regression analysis of the biochemical recurrence (BCR) free survival of the phosphodiesterase-4D7 (PDE4D7) score categories in the patient surgery cohort adjusted for the Cancer of the Prostate Risk Assessment Postsurgical Score (CAPRA-S) in the study cohort (n = 503).

Postsurgical clinical parameters	Uni-variate (enter)			Multi-variate (enter)		
Endpoint BCR (#503/#148; 29.4%)	p value	HR	95% CI of HR	p value	HR	95% CI of HR
CAPRA-S score category (1; n = 288); reference						
CAPRA-S score category (2; n = 173)	<0.0001	2.9	2.0–4.2	<0.0001	2.9	2.0–4.2
CAPRA-S score category (3; n = 42)	<0.0001	8.7	5.5–13.7	<0.0001	8.2	5.2–13.1
PDE4D7 score (4–5; n = 85); reference						
PDE4D7 score (3–4; n = 289)	0.02	2.1	1.1–3.8	0.02	2.1	1.2–3.9
PDE4D7 score (2–3; n = 118)	<0.0001	3.8	2.0–7.1	0.0001	3.5	1.8–6.5
PDE4D7 score (1–2; n = 11)	0.0005	5.7	2.1–15.2	0.0007	5.5	2.1–14.7

CI = confidence interval; HR = hazard ratio.

prediction of postsurgical BCR for the continuous as well as the categorical PDE4D7 score (Tables 3 and 4, Supplementary Figs. 4B and 4C).

Next, we investigated the hazard of biochemical progression across the continuous PDE4D7 score (Fig. 1A). It is evident that patients with the highest PDE4D7 scores (>4) demonstrated virtually no risk of postsurgical progression. We observed a linear increase of the hazard between PDE4D7 scores 2–5 while a steep increase occurred when the PDE4D7 scores decreases below a value of 2. Although the number of men in this lowest PDE4D7 score category (1–2) is small (n = 11) it seems that this group of men harbor a distinct form of prostate cancer as their risk clearly deviates from the observed linear increase of any PDE4D7 score >2.

Kaplan-Meier analysis revealed that the PDE4D7 score category (4–5) includes men with a <5% probability of 5-yr BCR while the chance to experience this endpoint increased to >50% for patients in PDE4D7 score category (1–2) with all events occurring within 3.5 yr after surgery (logrank $p < 0.0001$; Fig. 1B, Supplementary Table 4).

When comparing the patient characteristics of the validation population to the study cohort (Table 1) we noticed a similar distribution of clinical risks for the low- (41.9% vs 44.6%, $p = 0.73$) and intermediate favorable-risk groups (25.4% vs 24.6%, $p = 0.91$) in both cohorts while there is a nonsignificant trend towards less unfavorable intermediate-risk (16.2% vs 23.9%, $p = 0.16$) but more high-risk cases (8.7% vs 14.6%, $p = 0.08$) in the study versus the validation

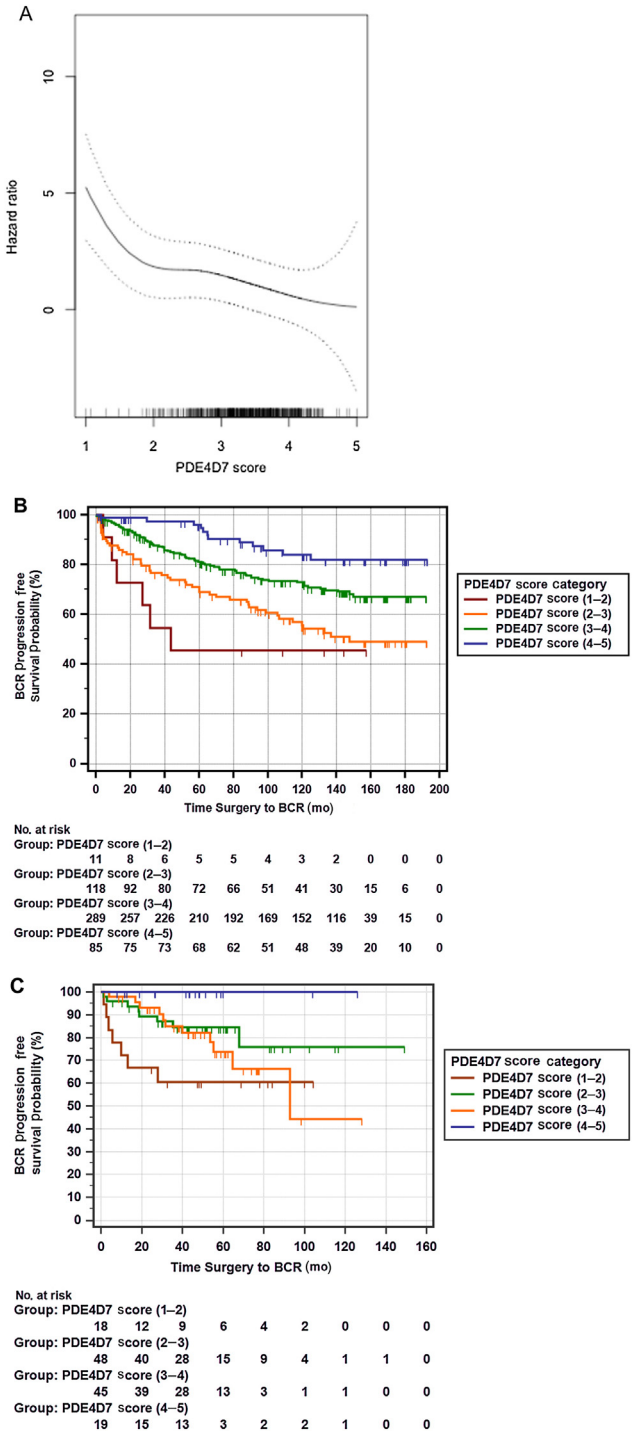

Fig. 1 – (A) Regression terms for the phosphodiesterase-4D7 (PDE4D7) scores calculated from Cox proportional hazard model predicting biochemical recurrence (BCR) free survival in the study cohort (n = 503). (B) Kaplan-Meier analysis of the PDE4D7 score with BCR free survival in the study cohort (n = 503). (C) Kaplan-Meier analysis of the PDE4D7 score with BCR free survival in the in the validation cohort (n = 130).

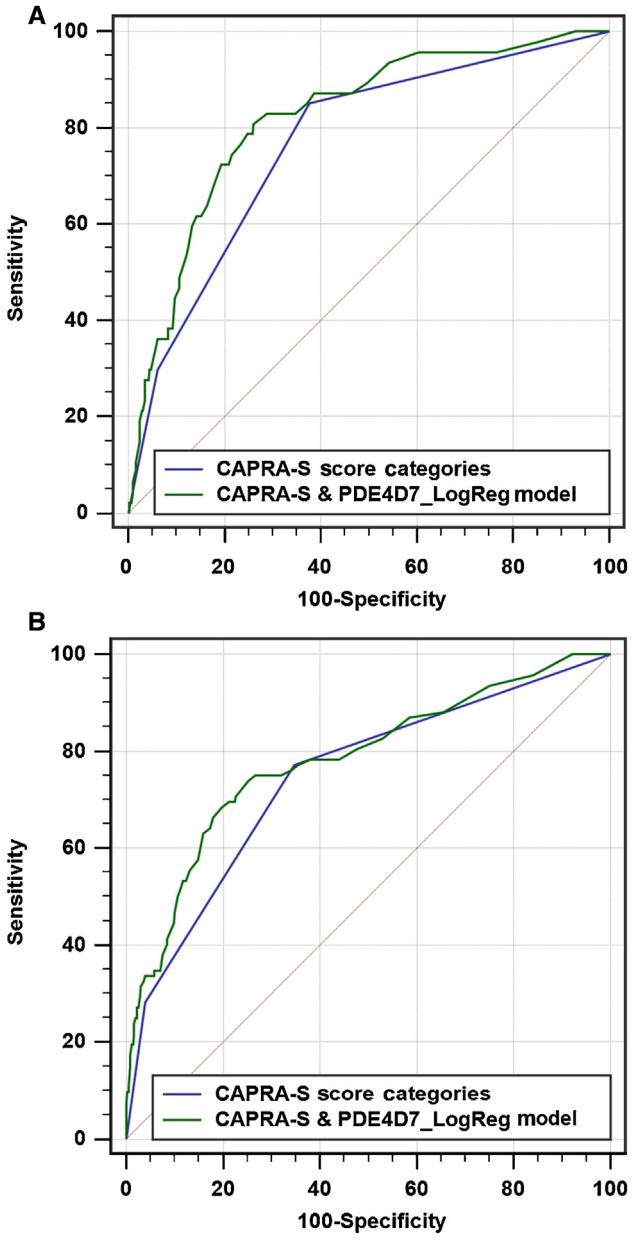

Fig. 2 – (A) Receiver operating characteristic cuve analysis of 2-yr biochemical recurrence for the incremental value of the phosphodiesterase-4D7 (PDE4D7) score added to the Cancer of the Prostate Risk Assessment Postsurgical Score (CAPRA-S) categories by logistic regression analysis in the study cohort (n = 469). (B) Receiver operating characteristic cuve analysis of 5-yr biochemical recurrence for the incremental value of the PDE4D7 score added to the CAPRA-S score categories by logistic regression analysis in the study cohort (n = 449).

cohort (Table 1). Kaplan-Meier survival analysis to correlate the four PDE4D7 score categories with BCR PFS showed very similar results as found in the study cohort (logrank p = 0.036; Fig. 1C, Supplementary Table 5).

Testing of other clinical endpoints other than BCR demonstrated equivalent association of PDE4D7 in the Cox regression analysis (Supplementary Fig. 4D, Supplementary Table 3).

3.2. Outcome modeling of combined clinical and PDE4D7 score categories

The data presented here indicate that the risk of disease progression provided by PDE4D7 scores offers a novel

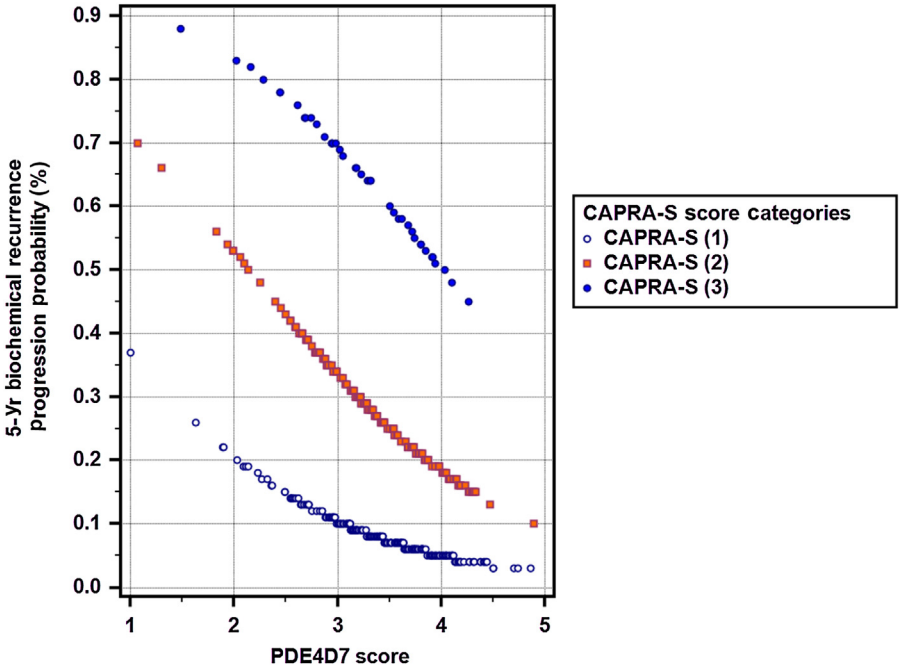

Fig. 3 – Analysis of 5-yr biochemical recurrence free survival predicted by a logistic regression model of the Cancer of the Prostate Risk Assessment Postsurgical Score (CAPRA-S) categories and the phosphodiesterase-4D7 (PDE4D7) scores in a patient subcohort with complete 5-yr follow-up in the study cohort (n = 449). CAPRA-S (1) = CAPRA-S Scores 0–2; CAPRA-S (2) = CAPRA-S Scores 3–5; CAPRA-S (3) = CAPRA-S Scores ≥6.

insight into prostate cancer risk assessment and thus is set to be complementary to the risks provided by clinical practice criteria. Thus, we hypothesized that the combination with clinical risk scores by computational modeling might predict long-term disease outcomes more effectively compared with using any single score alone. To evaluate this hypothesis, we selected a subcohort of 449 patients (92 events; 20.5%) with complete 5-yr outcome histories and generated a logistic regression model to combine the established clinical metric of the CAPRA-S score categories with the PDE4D7 score in order to predict the 5-yr risk of BCR after surgery. The modeling proved the independent predictive value of the PDE4D7 scores (odds ratio: 0.45, 95% CI: 0.29–0.67, p = 0.0001; data not shown). Receiver operating characteristic analysis calculated the 2-yr, 5-yr area under the curves (AUC) as 0.82 and 0.78, respectively. The PDE4D7 score showed incremental value to the AUC of CAPRA-S categories alone of 6% (p = 0.0004) and 4% (p = 0.003) to the 2-yr and 5-yr postsurgical progression prediction, respectively (Fig. 2A and 2B). Cross-validation of the logistic regression model in the validation cohort showed AUCs of 0.77 and 0.74 for the 2-yr and 5-yr outcome prediction, respectively. The logit function of the regression model was used to predict the individual 5-yr BCR PFS. Predicted risk analysis per clinical risk group as a function of PDE4D7 scores revealed heterogeneous 5-yr progression risk distribution even within the lowest CAPRA-S risk category (Fig. 3).

To evaluate this further we modeled the probability of BCR in the study cohort after surgery by Cox regression using the CAPRA-S risk categories as well as the continuous PDE4D7 score as inputs. The regression function was used to calculate the probability of biochemical progression after surgery for the patient study cohort. Based on the progression probability we defined four risk groups of PSA failure and compared this with survival prediction of the CAPRA-S score categories alone (Fig. 4A and 4B). When using probability group (0 to <0.1) as a reference in the Kaplan-Meier analysis the hazard ratios for probability groups (0.1 to <0.25), (0.25 to <0.5), and (0.5–1.0) were 2.0 (95% CI: 1.4–2.9), 5.7 (95% CI: 3.5–9.1), and 17.2 (95% CI: 6.0–49.2) compared with hazard ratios of 2.9 (95% CI: 2.0–4.1) and 8.4 (95% CI: 3.9–17.8) for the CARPA-S score category model (Supplementary Tables 6 and 7). We tested the CAPRA-S and PDE4D7 Cox regression model in the validation cohort to confirm that the four probability groups represent patient cohorts with significant difference in postsurgical risk of disease progression (Fig. 4C, Supplementary Table 8). We finally applied Kaplan-Meier survival analysis to demonstrate the ability of PDE4D7 to restratify the risk in particular the CAPRA-S (2) and (3) patients into groups of patients with high BCR PFS (>70%) versus those with low BCR PFS (<20%; Supplementary Table 10).

We have illustrated that the measurement of the prostate cancer biomarker PDE4D7 in a tumor sample provides risk stratification information to predict long-term clinical outcomes. Moreover, PDE4D7 provided independent and incremental value to the existing risk prediction algorithm CAPRA-S.

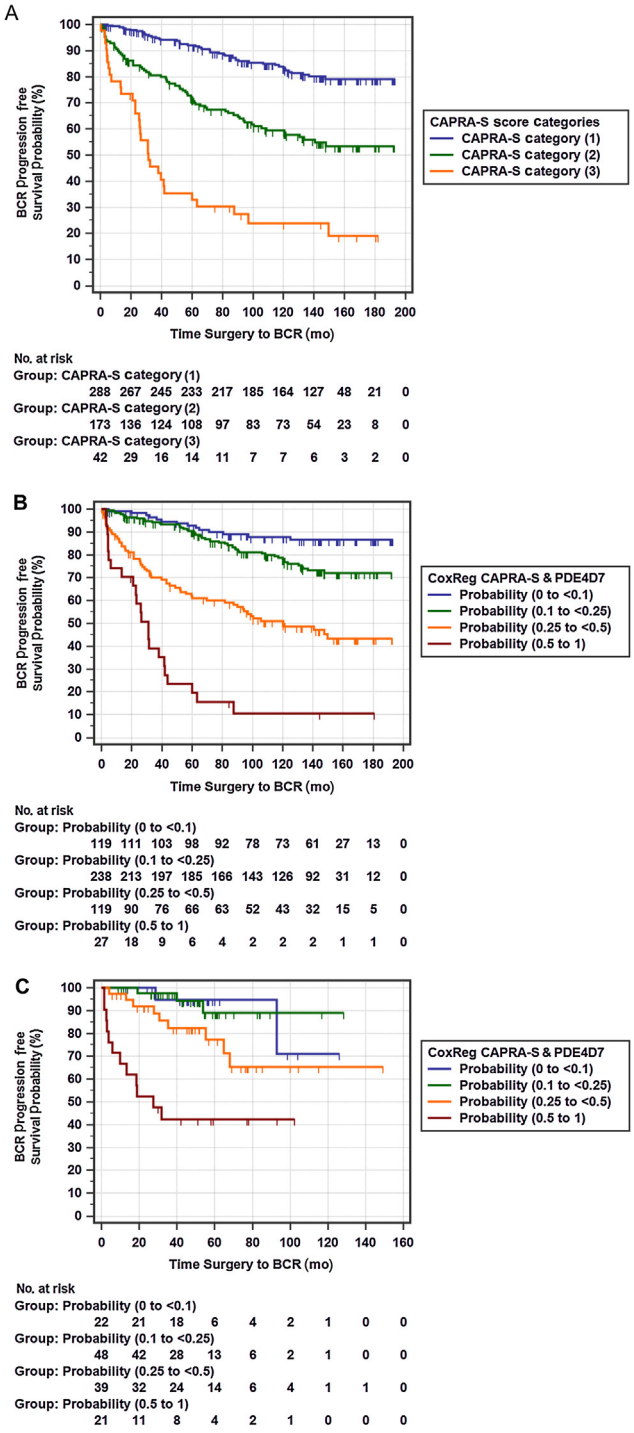

Fig. 4 – (A) Kaplan-Meier analysis of the biochemical recurrence (BCR) free survival predicted by the Cancer of the Prostate Risk Assessment Postsurgical Score (CAPRA-S) categories in the study cohort (n = 503). (B) Kaplan-Meier analysis of the BCR free survival predicted by a Cox regression model of the CAPRA-S categories and the phosphodiesterase-4D7 (PDE4D7) score in the study cohort (n = 503). (C) Kaplan-Meier analysis of the BCR free survival predicted by a Cox regression model of the CAPRA-S categories and the PDE4D7 score in the validation cohort (n = 130).

4. Discussion

The management of prostate cancer patients is strongly dependent on risk profiling. Several risk metrics based on clinical inputs have been developed to predict either before or after surgery the risk of PSA progression [2]. Cooperberg and colleagues [11] presented the postsurgical prediction algorithm, CAPRA-S, which is based on a weighted sum of scores for the clinical variables preoperative PSA, pathology Gleason score, pathology status of extracapsular extension, seminal vesicle invasion, surgical margins, and lymph node invasion. The resulting CAPRA-S score is categorized into three groups representing low- (CAPRA-S 0–2), intermediate- (CARPA-S 3–5), and high- (CAPRA-S $\geq$ 6) risk of disease progression after surgery. The c-index was initially reported to be 0.77 for 5-yr BCR PFS [11]. In a large validation study using external data, a c-index of 0.73 for the prediction of BCR at 5 yr after surgery was published [15]. The CAPRA-S score is one of the most extensively validated clinical risk metric.

Alterations in the expression of members of the cAMP-degrading PDE4 family are associated with several diseases, including stroke [16], acrodysostosis [17,18], schizophrenia [19], and chronic obstructive pulmonary disease [20]. Functionally, PDE4D provides part of the cellular desensitization system to cAMP and enables cross-talk between signaling pathways that lead to the activation of extracellular signal-regulated kinase and AMP-activated protein kinase [21], for example. Recently, we have shown that down-regulation of a particular PDE4 isoform (PDE4D7) may impact on prostate cancer [13,22,23].

Therefore, we investigated the incremental value of PDE4D7 to CAPRA-S to predict postsurgical PSA relapse.

To test this, we generated logistic and Cox regression models for BCR PFS to combine the CAPRA-S categorical score with the continuous PDE4D7 score. Both regression models showed better performance in predicting fast relapse within 24 mo after the primary treatment. The incremental value of PDE4D7 to the CARPA-S was larger in this setting compared with the 5-yr outcome prediction (6% vs 4%, respectively); however, in both settings the incremental contribution of the PDE4D7 score was statistically significant.

Testing the PDE4D7 score in Cox regression modeling with CAPRA-S to model time-to-PSA recurrence indicated the impact of PDE4D7 for patients at high risk of postsurgical PSA relapse within 24–36 mo after operation. The Kaplan-Meier analysis showed that patients with the highest probability of relapse after surgery had a mean PFS of 44.6 mo when stratified with the CAPRA-S and PDE4D7 combined score versus 64.3 mo for the CARPA-S model alone.

Not all patients with PSA failure after local therapy have the same prognostic pathway in terms of future disease progression. Fast PSA doubling times and fast PSA recurrence after surgery have been significantly associated with progression to metastases and prostate cancer-specific mortality [24]. In our study cohort, we confirmed the correlation between the mean time to BCR and prostate cancer-specific death. The mean BCR PFS of 44.6 mo in the highest CAPRA-S and PDE4D7 risk group corresponds to a 22.2% risk of prostate cancer specific in this cohort. In contrast to a risk of 11.9% to die from prostate cancer in the CAPRA-S category

(3), is a patient group with a mean of 64.3 mo BCR PFS. These data support the view that lowest level of PDE4D7 scores contribute to higher risk of rapid recurrence after primary intervention. Several retrospective studies have provided evidence that stratification of patients to salvage radiation therapy after local failure based on time-to-PSA recurrence may increase cancer-specific survival [25,26]. Thus, accurate stratification of patients is essential to provide most optimal therapeutic strategies for patients.

Our study is limited by the retrospective design. Due to the long-term follow-up of 10–15 yr as well as the chosen design to investigate a consecutively managed patient cohort the clinical risk distribution may not completely represent the risk of a contemporary surgery cohort. The study population includes a limited number of high-risk cases which may limit the generalizability of our presented results to high-risk populations. This is important to note as one of our main findings is that PDE4D7might support improved stratification of patients at high risk of post-surgical disease recurrence. Thus, PDE4D7 may have a role in the selection of secondary treatments for this patient group. However, to confirm this further research in clinically high-risk patient cohorts is required.

5. Conclusions

We have validated the prostate cancer biomarker PDE4D7 in a historic patient cohort consecutively managed by radical prostatectomy. We confirmed the independent prognostic and incremental value of PDE4D7 score next to the established clinical risk metric CAPRA-S score. The PDE4D7 score may support the risk stratification of patients after local treatment to select the right timing for the start of secondary therapy for men at very high-risk of rapid disease recurrence.

Author contributions: Ralf Hoffmann had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
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Obtaining funding: None.
Administrative, technical, or material support: None.
Supervision: Schlomm, Hoffman.
Other: None.

Financial disclosures: Ralf Hoffmann certifies that all conflicts of interest, including specific financial interests and relationships and affiliations relevant to the subject matter or materials discussed in the manuscript (eg, employment/affiliation, grants or funding, consultancies, honoraria, stock ownership or options, expert testimony, royalties, or patents filed, received, or pending), are the following: de Inda, van Strijp, den Biezen-Timmermans, van Brussel, Wrobel, van Zon, Vos, and Hoffmann are employees of Philips Research Europe and partly filed patents in the area and own company stocks; Houslay has ownership interest in the company Mironid Ltd. and declares honoraria and travel support by Celgene Inc.; Schlomm discloses funding support from Philips, honoraria and advisory role with Ipsen Pharma and Myriad Genetics.

Funding/Support and role of the sponsor: This project was supported by the framework of The Center for Translational Molecular Medicine, The Netherlands, PCMM project (grant 03O-203)

Acknowledgments: We gratefully thank Dr. G. Sauter and Ms. C. Koop for providing the surgical biopsy punches.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.euf.2017.05.010.

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