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Genomic Predictors of Outcome in Prostate Cancer

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Abstract

Context: Given the highly variable behavior and clinical course of prostate cancer (PCa) and the multiple available treatment options, a personalized approach to oncologic risk stratification is important. Novel genetic approaches offer additional information to improve clinical decision making.

Objective: To review the use of genomic biomarkers in the prognostication of PCa outcome and prediction of therapeutic response.

Evidence acquisition: Systematic literature review focused on human clinical studies reporting outcome measures with external validation. The literature search included all Medline, Embase, and Scopus articles from inception through July 2014.

Evidence synthesis: An improved understanding of the genetic basis of prostate carcinogenesis has produced an increasing number of potential prognostic and predictive tools, such as transmembrane protease, serine2:v-ets avian erythroblastosis virus E26 oncogene homolog (TMPRSS2:ERG) gene fusion status, loss of the phosphatase and tensin homolog (PTEN) gene, and gene expression signatures utilizing messenger RNA from tumor tissue. Several commercially available gene panels with external validation are now available, although most have yet to be widely used. The most studied commercially available gene panels, Prolaris, Oncotype DX Genomic Prostate Score, and Decipher, may be used to estimate disease outcome in addition to clinical parameters or clinical nomograms. ConfirmMDx is an epigenetic test used to predict the results of repeat prostate biopsy after an initial negative biopsy. Additional future strategies include using genetic information from circulating tumor cells in the peripheral blood to guide treatment decisions at the initial diagnosis and at subsequent decision points.

Conclusions: Major advances have been made in our understanding of PCa biology in recent years. Our field is currently exploring the early stages of a personalized approach to augment

traditional clinical decision making using commercially available genomic tools. A more comprehensive appreciation of value, limitations, and cost is important.

Patient summary: We summarized current advances in genomic testing in prostate cancer with a special focus on the estimation of disease outcome. Several commercial tests are currently available, but further understanding is needed to appreciate the potential benefits and limitations of these novel tests.

1. Introduction

Prostate cancer (PCa) is the most common urologic malignancy and the second leading cause of male cancer-related deaths in many developed countries [1]. A personalized approach, including the prediction of individual patient outcomes and therapeutic responses, is important in all cancers but especially for PCa, given the variability in disease behavior, the diversity of treatment options, and the risk of treatment-related impairment of quality of life [2]. Novel genomic technologies, such as microarray analyses and next-generation sequencing, have improved our understanding of the biology of PCa. Consequently, the scientific community is faced with an explosion of data, new challenges, and opportunities in biomarker discovery and validation [3]. With improved approaches to biomarker research, combined with lower cost and more efficient techniques, the potential of a personalized genomic approach for clinical decision making has recently been made possible.

Among the most prominent topic in PCa genetics is the characterization of somatic genomic alterations in tumor tissue for the prognosis and prediction of treatment response. Novel approaches include genetic analyses from peripheral blood, either germline analyses or characterization of DNA/RNA from circulating tumor cells (CTCs), or free circulating nucleic acids. The genetic landscape, key genetic alterations, epigenetic events, and microRNAs (miRNAs) in PCa have been reviewed [4–7].

In this paper, we focus on the value of genomic markers in the personalized prediction of PCa outcome and response to various therapeutic interventions. Due to the breadth of the topic and recent high-quality reviews, we have specifically focused on genomic tests that are already available or approaching the point of clinical use [4–10].

2. Evidence acquisition

A literature review was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-analysis criteria. Figure 1 presents the process of identifying references

[11]. The first author performed a Medline, Embase, and Scopus search of all articles from inception through July 2014 using the keywords prostate cancer and genetics and prognostic. Genetic PCa outcome studies with the following criteria were prioritized: human clinical studies, clinical outcome end points (biochemical progression, clinical progression, disease-specific survival [DSS], and overall survival), and external validation cohorts. Articles of interest and review articles were surveyed and verified for any missed reports. All authors oversaw and approved the final literature review and selection.

3. Evidence synthesis

3.1. Clinically relevant genes and genetic pathways in prostate cancer

3.1.1. TMPRSS2:ERG fusion

In 2005, Tomlins and coworkers reported a novel frequent chromosomal rearrangement in PCa, a fusion between transmembrane protease, serine 2 (TMPRSS2) gene and v-ets avian erythroblastosis virus E26 oncogene homolog (ERG) gene or other ETS (E26 transformation specific) transcription factors, until now recognized as the most frequent gene-specific alterations in PCa [12,13]. ETS fusion–type cancers are believed to represent a genetically distinct subset of PCa characterized by deletions of the phosphatase and tensin homolog (PTEN) gene and of chromosome 3p, whereas deletions of 5q and 6q prevail in fusion-negative cancers [14–17]. Although gene fusions in general, and specifically ETS fusions, have been associated with the early onset of PCa [18,19], the clinical utility of the gene fusion as a prognostic or predictive tool is still unclear.

Many studies have investigated the association of TMPRSS2:ERG fusion status and outcome in PCa (Table 1). Ten studies reported the prognostic value of the gene fusion in radical prostatectomy (RP) cohorts [19–28]. In 6 of the 10 studies, TMPRSS2:ERG fusion status was not associated with outcome after surgery [19,21,22,24,27,29]. In one study, patients with rearrangement had an 8.6-fold increased risk for biochemical recurrence (BCR), and in

another study, fusion status was predictive of BCR risk in a small selected cohort of Gleason 7 cases [23,28]. In contrast, one study demonstrated lower BCR risk after RP among patients with the TMPRSS2:ERG fusion [25]. Overall, a meta-analysis including 5074 men following RP found no significant association with BCR or lethal disease [24]. One study investigated the outcome after intensity-modulated radiation therapy but found no association between fusion status and BCR.

Nevertheless, when investigated beyond the gene fusion status, some additional prognostic information has been reported. FitzGerald and coworkers did not observe a significant association between TMPRSS2:ERG fusion status and outcome, but patients with increased copy numbers of the fusion gene showed poorer survival [20]. Furthermore, Boormans and coworkers reported fusion gene transcript-specific data; that is, TMPRSS2:ERG (Exon0)–ERG fusion was associated with a lower risk of BCR compared with Exon1 fusion [26].

In contrast to studies in cohorts treated with curative intent, the presence of TMPRSS2:ERG fusion had an independent negative impact on outcome in four watchful waiting (WW) cohorts and on a cohort of patients with castration-resistant PCa (CRPC) undergoing palliative transurethral resection of the prostate (TURP) [30–34]. Therefore, one could speculate that TMPRSS2:ERG fusion status is a predictor of response to androgen-deprivation therapy (ADT). However, this hypothesis was not supported by Boormans and coworkers. They found no association between TMPRSS2:ERG fusion status with ADT response or outcome in PCa patients with lymph node metastases (N1) treated with ADT [35]. Similarly, Leinonen and coworkers found no association between TMPRSS2:ERG fusion status and outcome among ADT-treated patients [36]. A recent study investigated TMPRSS2:ERG fusion status from biopsies of 265 active surveillance (AS) patients and found that TMPRSS2:ERG fusion-positive patients had a significantly higher risk of disease progression (hazard ratio: 2.45) compared with fusion-negative patients [37]. However, another study of

PCa patients on AS showed that urinary TMPRSS2:ERG and the prostate cancer antigen 3 (PCA3) gene were not significant independent predictors of biopsy reclassification on multivariable analysis [38].

In addition to its own potential prognostic value, TMPRSS2:ERG fusion status may modify the interpretation of other PCa biomarkers in outcome prediction. Barwick and coworkers noted that the expression of several genes was affected by TMPRSS2:ERG fusion status [39]. In fusion-positive cases, upregulated genes were related to mismatch base repair and histone deacetylation, whereas genes involved in insulinlike growth factor (IGF) and Janus kinase/signal transducers and activators of transcription (JAK-STAT) signaling were downregulated [39]. In addition Brase et al showed the TMPRSS2-ERG gene fusion results in the modulation of certain transcriptional patterns and well-known PCa biomarkers like CRISP3 and TDRD1 that were found to be associated with the gene fusions [40]. Karnes and coworkers did not detect a direct association between TMPRSS2:ERG fusion status and outcome, but classifying the cohort according to TMPRSS2:ERG fusion status had a significant impact on the predictive value of other investigated markers [41]. Similarly, TMPRSS2:ERG fusion status was noted to significantly affect the prognostic value of a 36-gene expression panel [42]. Taken together, although the true prognostic value of TMPRSS2:ERG fusion status itself has not been proven, fusion status is a key genomic event and should be taken into consideration when the prognostic value of other genomic events is investigated.

3.1.2. PTEN

PTEN deleted on chromosome 10 is one of the most frequently mutated genes in human cancer. It dephosphorylates lipid-signaling intermediates, resulting in deactivation of PI3K signaling, and thus controls proliferation and growth [43]. In a landmark study by Saal and coworkers in 2007, PTEN loss was associated with poor outcome in a variety of cancers

including PCa and cancer of the urinary bladder [44]. The prognostic value of PTEN in PCa was investigated in a few studies (Table 2). In 649 PCa patients, Leinonen and coworkers demonstrated a higher frequency of PTEN loss in more advanced cases (CRPC compared with RP cases) and that PTEN loss was associated with shorter progression-free survival time but notably only in ERG-positive cases [45]. Similarly, in another study the prognostic value of PTEN was clearly associated with TMPRSS2:ERG fusion status [46]. In a large cohort including 4699 RP specimens and 57 CRPC cases, Krohn and coworkers also demonstrated that PTEN loss was associated with adverse clinicopathologic factors and a higher risk of BCR [47]. Contrary to the findings of Leinonen et al, PTEN had similar prognostic utility in ERG-positive and -negative cases. In a study among conservatively managed PCa patients by Reid et al, PTEN loss without TMPRSS2:ERG fusion was associated with poor cancer-specific survival, which is in contrast to other studies where PTEN loss and TMPRSS2:ERG fusion defined the patients with the worst survival [48].

As yet, the predictive role of PTEN status in castration-sensitive and resistant cancers has only been evaluated in one study [49]. McCall et al investigated PTEN status by fluorescent in situ hybridization and immunohistochemistry in matched tumor pairs (one before and one after ADT relapse). They noted that loss of PTEN expression in the nucleus was independently associated with poor DSS but only in the castration-sensitive tumor specimens. PTEN-negative tumors were recently shown to have shorter survival in the post-docetaxel abiraterone treatment setting compared with cases with preserved PTEN expression [50].

3.2. Gene/expression panels

Cancer is a complex disease, and it is unlikely a single genetic abnormality will sufficiently reflect events in a tumor to give enough prognostic information for clinical decisions. Most authors suggest that a combination of multiple genetic markers will be necessary. Panels evaluate differential expression of multiple genes between patient groups of interest (eg,

biochemical relapse vs no relapse after RP). These panels may be selected using prior knowledge by including key carcinogenic pathways in PCa (eg, cell cycle regulation, apoptosis) [51] or filtered from thousands of unselected genes to distinguish gene-phenotype correlates [52–54].

These studies face many challenges including the risk of chance associations given the quantity of data. Therefore experienced biostatistical support and appropriate external validations are essential before widespread clinical applications can be considered. Approved principles of study design include blinded marker analyses and randomly selected cases (in retrospective studies) [55]. Study reporting may be negatively affected by several potential biases, and therefore adherence to standard criteria, such as Reporting Recommendations for Tumor Marker Prognostic Studies (REMARK), is essential for providing evidence on the clinical utility of biomarkers in oncology [56]. The biomarkers to be included in clinical decision making have to provide additional independent prognostic information or additive value together with established clinical and pathologic variables in a multivariate setting like the Memorial Sloan Kettering Cancer Center or Cleveland Clinic nomograms or Cancer of the Prostate Risk Assessment Postsurgical (CAPRA-S) risk stratification for PCa.

3.2.1. Discovery studies

Table 3 lists studies reporting the prognostic value of gene/expression panels for clinically significant end points. Most have investigated prediction of outcome after RP using different end points, such as risk for biochemical failure [42,51,52,57,58], metastatic progression [52,53,59,60], and DSS [54,60,61]. A few studies investigated TURP tissue to predict the outcome of men undergoing conservative treatment [51,62,63].

The design of discovery studies included several approaches: single- and multicenter studies and correlation of gene panel data to outcomes of the full cohort or selected subgroups [42,51,52,57,58,62] or a case-control population selected on a particular outcome

[53,59,60,63]. All except one study reported that the applied expression panel offered significant prognostic information in the particular study cohort. Sboner and coworkers studied TURP tissue from WW patients, but the gene signature failed to improve the prognostic value of a model including clinicopathologic parameters [63]. Studies have utilized different methodological approaches to assess the value of genomic tests. These approaches included traditional statistical methods (survival analyses, multivariable models with other clinicopathologic variables, and receiver operating characteristic analysis) [42,51–53,59,60,62–64]. In some studies, results from expression panels were combined with other variables or a nomogram to determine if genomic data added prognostic information above the baseline models [54,57,58,61,65].

3.2.2. External validation studies

A 46-gene expression panel (31 cell-cycle progression genes and 15 housekeeping genes) initially reported by Cuzick and coworkers in 2011 was validated in four studies and is commercially available as the Prolaris test (Myriad Genetics, Salt Lake City, UT, USA). The test was first validated from biopsy and TURP specimens in a conservatively managed cohort, and the gene panel significantly predicted PCa death in a multivariate model [66]. The panel was externally validated in two RP studies (one analyzing pre-RP biopsy tissue and one using RP tissue) including a total of >1300 patients and was noted to be an independent prognostic factor for BCR and metastatic progression [65,67]. When added to a multivariable score reflecting post-RP clinical and pathologic risk (CAPRA-S score) [68], the gene classifier provided incremental prognostic value beyond standard clinical models (concordance index for combined genetic/clinical model was 0.77 versus 0.73 for the clinical model alone) [65].

A combined model incorporating CAPRA-S and a cell cycle progression score also performed better than either alone on decision-curve analysis. Similarly, in an external-beam

radiation therapy (EBRT) cohort, the panel was an independent prognostic factor after adjusting for clinical variables [69]. The potential impact of Prolaris was investigated in one study where physicians were surveyed about treatment recommendations in 305 men with newly diagnosed PCa [70]. In 65% of the cases, the treatment recommendation changed after the genetic test, and in 40% there was reduction in treatment burden (interventional treatment changed to noninterventional). Although this study shows genomic tests can have a significant impact on treatment decisions, follow-up data were not reported to determine the long-term impact of these changes in management. Furthermore, the test remains very expensive (approximately $3400), and available data on cost effectiveness are limited.

In 2013 Erho et al reported in a case-control study that a 22-gene panel predicted survival after RP [53]. This panel has also been externally validated in multiple cohorts and is commercially available as the Decipher genetic test (GenomeDX Biosciences, Vancouver, BC, Canada). Four studies reported the utilization of this gene panel to predict BCR, metastatic progression, or DSS after RP plus or minus EBRT [61,71–73]. The prognostic accuracy was highest when the genomic classifier and clinical models (CAPRA-S) were combined [61]. In another study, including 85 high-risk RP patients, the 22-gene panel was the only variable associated with metastatic progression in a multivariable model and had a favorable net benefit compared with clinical models (CAPRA-S and Stephenson postoperative nomogram) [68,74] in decision-curve analysis [71,72]. The test also improved prediction of BCR and metastatic progression risk in a cohort of 139 men undergoing EBRT after RP [73]. The impact of Decipher was evaluated in a clinical utility study where 21 uro-oncologists were presented 24 patient cases (12 potential candidates for adjuvant and 12 for salvage EBRT) and were asked for treatment recommendations with and without information from the genetic test [75]. The recommendation changed in 43% of the adjuvant cases and

53% in the salvage setting, suggesting a potentially significant impact on treatment decisions after RP. However, the long-term impact of these changes in management is unknown.

Another commercially available test, Oncotype DX Genomic Prostate Score (GPS; Genomic Health Inc, Redwood City, CA, USA), is a 17-gene expression panel that has been investigated as a predictor for the risk of recurrence, PCa death, and especially adverse pathology at RP [54]. For the latter, biopsy tissue was used to derive a gene panel and estimate the risk of high-grade (Gleason ≥4 + 3) and/or high-stage disease (pT3 or higher). The panel was validated in a cohort of 395 RP patients, and the Genomic Prostate Score was an independent predictor of unfavorable pathology in models including individual clinical parameters (age, prostate-specific antigen [PSA], clinical stage, and biopsy Gleason score) or a multivariable pretreatment clinical risk model (CAPRA score) [54]. The test was further recently validated on biopsies from 431 patients with very low-, low-, or intermediate-risk PCa. The test was significantly associated with adverse pathologic features and also independently predicted time to BCR after adjusting for risk as well as time to metastases [76].

It should be noted that although these three PCa expression panels include a total of 85 genes, there is virtually no overlap between the tests. The panels in Prolaris and Decipher have only one gene in common. Importantly, as yet there are no comparative data testing these panels in the same patient cohort.

3.3. Epigenetic signature

A comprehensive next-generation sequencing study of Gu and coworkers recently underscored the prognostic value of global- and gene-specific epigenetic alterations in PCa [77]. A methylation marker genetic test, ConfirmMDx (MDxHealth), utilizes methylation analysis of glutathione S-transferase pi 1 (GSTP1), adenomatous polyposis coli (APC), and Ras association (RalGDS/AF-6) domain family member 1 (RASSF1) genes from negative

biopsies to estimate the likelihood of a repeat biopsy also being negative [78]. The test achieved a 90% negative predictive value (NPV) within 30 mo of the initial biopsy. In a recent validation trial, 88% NPV was reported, and the test was the most significant predictor of biopsy results [79]. The impact of the epigenetic test on rebiopsy rates was recently surveyed in five centers, and among 138 patients with a negative ConfirmMDx assay, only six patients (4%) underwent repeat biopsies [80].

3.4. Copy number variation

Copy number variation (CNV) refers to gains or losses of certain areas of somatic DNA that potentially have carcinogenic consequences (eg, activation of oncogenes or inactivation of tumor suppressor genes) [4]. Overall, PCa is characterized by loss of genomic material [81]. The prognostic role of CNV may be analyzed with different approaches, by either investigating specific genetic gains or deletions, or by analyzing the overall burden of CNV. For example, Tsuchiya et al investigated specific chromosome 8 abnormalities, and loss of 8p22 was associated with an increased risk of BCR and metastatic progression [82]. Liu et al studied the 20 most significant CNVs (15 deletions, 5 amplifications) in two RP cohorts and noted two CNVs (gain of area of v-myc avian myelocytomatosis viral oncogene homolog [MYC], deletion of PTEN) were significantly associated with PCa death [83]. Similar findings were reported in patients undergoing radiation therapy [84].

Recent advances in high-throughput methodology have allowed investigations of the overall CNV burden and outcome. Taylor and coworkers analyzed RP cohorts for CNV utilizing unsupervised clustering and identified six patient clusters according to the degree of CNV. When analyzed for risk of BCR, CNV clusters had a significant association with outcome in univariate analysis [81]. The “simplest” approach was reported by Hieronymus and coworkers, who studied the association between percentage of CNV from intact somatic DNA and outcome after RP [85]. A significant difference was noted for BCR and metastatic

progression risks in patients with ≥5.4% altered tumor DNA. The degree of altered DNA was also an independent predictor of BCR on multivariable analysis of the whole cohort and a subcohort of Gleason 7 tumors.

Paris and coworkers utilized array comparative genomic hybridization to identify specific DNA-based biomarkers (eg, loss at 8p23.2 and gain at 11q13.1). They suggested a combined set of 39 loci termed Genomic Evaluators of Metastatic Prostate Cancer (GEMCaP). In the discovery study, the GEMCaP set of markers was associated with disease recurrence and metastasis [86]. Later the GEMCaP was demonstrated to offer additional prognostic information above the Kattan nomogram for disease recurrence in high-risk node-negative PCa cases after RP (nomogram accuracy 65% vs accuracy of nomogram and GEMCaP 78%) [87].

According to these studies, CNV analyses may have a prognostic role in PCa patients, but standardization of methods and additional validation studies are required before clinical applications may be planned.

3.5. Genetic information from nucleic acids in peripheral blood and circulating tumor cells

In addition to genetic information available from germline DNA and tumor tissue–derived DNA and RNA, peripheral blood is a potential source for genomic tumor characterization using free circulating nucleic acids, whole blood transcripts, or CTCs.

In 2007 Bastian and coworkers reported an increasing quantity of circulating cell-free DNA was independently associated with the risk of BCR after RP [88]. In November 2012, two separate studies reported on gene expression profiling from blood RNA in patients with CRPC. Ross and coworkers examined a six-gene panel in CRPC patients with significantly improved prognostic value compared with a clinical model alone [89]. Olmos et al used a similar approach but divided the CRPC cohort into four groups according to microarray data analyzed from blood messenger RNA (mRNA) [90]. One patient group had a significantly

poorer survival, identified by a nine-gene panel. Specific miRNAs are found, not only in tumor tissue, but also in the plasma of PCa patients; miRNA-375 and miRNA-141 are reported to be associated with advanced disease [91].

Recently Danila et al investigated the detection of CTCs and the expression of five genes frequently detected in PCa cells (but not in peripheral mononuclear cells) utilizing reverse transcriptase-polymerase chain reaction (RT-PCR) to detect transcripts from peripheral blood [92]. Both unfavorable CTC count (five or more cells) and detection of two or more gene transcripts had similar significant prognostic value for risk of PCa death, and when combined, additional prognostic value was demonstrated. With a similar approach, kallikrein-related peptidase 3 (KLK3), PCA3, and TMPRSS2:ERG mRNA could be detected in the peripheral blood of CRPC patients but not in healthy controls [93]. Also, decreased expression levels of these genes were noted after docetaxel treatment, suggesting a potential role for treatment monitoring.

Peripheral blood genetic information may also be useful to predict therapeutic response in CRPC. Recently, Antonarakis et al reported that a splice variant of the androgen receptor (AR-V7) could be detected in CTCs, and AR-V7–positive patients were less likely to respond to abiraterone or enzalutamide and had a poorer survival [94]. Confirmatory studies are awaited. In addition to specific genetic changes found in CTCs, the pretherapy CTC count has been demonstrated to predict response, and a decrease in the number of CTCs after therapy has greater predictive value than the classic 50% PSA decrease. This was observed after treatment with both docetaxel and abiraterone [95].

3.6. Discussion

After years of intense research, we are finally witnessing progress in the field of PCa genomics and the emergence of commercially available genetic tests to assist clinical decision making. Because information on these tests is available not only to PCa specialists

but to all physicians and patients, it is important to understand their potential implications, optimal use, and limitations. Genetic prediction tools may also add significant costs to the PCa diagnostic and therapeutic algorithms, but these costs might be justified if indeed they lead to a reduction in unnecessary treatments for localized disease or a more appropriate selection of therapy for advanced disease.

An important aspect of biomarker and genetics research is the heterogeneity of PCa both within a single tumor locus (intrafocal heterogeneity) and between different tumor deposits (interfocal heterogeneity) [96–98]. In addition to intra/interfocal heterogeneity, a field effect of genetic changes should also be considered because cancer-related genetic changes are also detected in benign areas of the same prostate [99]. This is the underlying premise of new tests designed to predict the risk of finding cancer on repeat biopsy for men with a negative biopsy [79], as well as biopsy-based tissue tests designed to predict whole-gland pathologic features. Genomic analysis of tumor tissue may aid in overcoming the challenges of sampling error and the variability of traditional pathologic grading. Standard pathologic evaluation, such as Gleason grading, is subjective and associated with significant inter- (and also intra-) observer variability that may have a significant impact on an individual patient's treatment recommendations [100].

Genetic prognostication has potential applications in every step of PCa care. Commercially available epigenetic ConfirmMDx may be of value when repeat biopsies are considered after negative initial prostate biopsies. One of the most important is the appropriate selection of men to AS versus treatments with curative intent. To offer AS safely, the risk of underestimating the metastatic and local invasive potential of the individual tumor has to be minimized. In addition to improved biopsy techniques and imaging, genomic tests may be used to estimate the potential of tumor progression. The Oncotype DX Genomic Prostate Score was investigated in this setting and found to provide additional information to clinical

parameters and nomograms. Even after RP, the risk of recurrence and metastatic progression is highly variable, and the addition of genomic information to traditional variables appears to improve prognostic accuracy modestly.

All three commercially available gene panels described in detail in this review (Prolaris, Decipher, and Oncotype DX Genomic Prostate Score) have been evaluated in terms of potential prognostic value after RP. The future will tell if this additional information is considered sufficient by the urologic community and PCa patients to change practice. Although clinical studies have suggested potential benefits with these tests, real clinical use and long-term data are needed to judge the added value.

In addition to general prognostic information, prediction of response to specific treatment modalities (eg, adjuvant/salvage radiation, ADT, novel systemic agents) is of great importance. Due to an ever-expanding number of treatment options in CRPC, involving very different mechanisms and significant costs, there is a great need for markers to predict therapeutic response, typically seen in a minority of patients. With multiple sequentially delivered treatments, longitudinal monitoring of disease status is needed. In this setting, promise exists for sampling free circulating DNA and RNA or CTCs in peripheral blood, but further work is necessary to validate the findings before widespread clinical use. The issue of tumor cell heterogeneity in CTCs has yet to be explored.

4. Conclusions

Major advances in PCa genetics have occurred in recent years, and in the near future personalized genetic profiling of primary and metastatic tumor cells may become readily available for routine clinical decision making. Many new genetic-based tests are newly available or in late stages of clinical development, with potential applications in PCa decisions ranging from the need for repeat biopsy to initial treatment selection, decisions about secondary therapy, and selection of treatment for advanced disease. Greater

understanding of the potential long-term benefits and limitations of these tests is important, and how exactly they should be used in clinical practice to optimize decision making must be the subject of future prospective studies.

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Study concept and design: Boström, Cooperberg.

Acquisition of data: Boström, Bjartell, Catto, Eggener, Lilja, Loeb, Schalken, Schlomm, Cooperberg.

Analysis and interpretation of data: Boström, Bjartell, Catto, Eggener, Lilja, Loeb, Schalken, Schlomm, Cooperberg.

Drafting of the manuscript: Boström.

Critical revision of the manuscript for important intellectual content: Boström, Bjartell, Catto, Eggener, Lilja, Loeb, Schalken, Schlomm, Cooperberg.

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Figure legend

Fig. 1 – Preferred Reporting Items for Systematic Reviews and Meta-analysis flow diagram presenting the steps of the literature search and the selection process of the articles.

Table

Table 1 – Studies reporting *TMPRSS2:ERG* fusion status and outcome after various treatment modalities

Study	No. of cases	Tissue type	Detection method	Intervention	*ERG* rearrangement rate, %	*ERG* rearrangement association with clinical parameters	Main results
Radical prostatectomy and external-beam radiation therapy cohorts							
Steurer et al [19]	9567	RP	FISH/IHC	RP	54	NR	Rearrangement associated with low-grade tumors in younger patients. *ERG* not associated with outcome.
FitzGerald et al [20]	214	RP	FISH, SNP genotyping	RP	36	No	Rearrangement not predictive of DSS, but cases with multiple fusion copies had trend toward poorer survival
Gopalan et al [21]	521	RP	FISH	RP	46	Lower Gleason score	Rearrangement not associated with outcome
Hoogland et al [22]	509	RP	IHC	RP	55	Lower PSA	*ERG* staining not associated with BCR or local recurrence risk
Nam et al [23]	165	RP	RT-PCR	RP	49	No	Rearrangement independently predictive of BCR (HR: 8.6)
Pettersson et al [24]	1292	RP	IHC	RP	49	Higher stage, lower PSA	Rearrangement not associated with outcome
Saramäki et al [25]	150	RP	FISH	RP	33	No	Rearrangement independently associated with lower BCR risk
Boormans et al [26]	112	RP	RT-PCR	RP	42	No	*TMPRSS2:ERG* (Exon0)–*ERG* fusion associated with lower risk of BCR compared with Exon1 fusion
Minner et al [27]	2891	RP	FISH/IHC	RP	52	No	*ERG* IHC positivity not predictive of BCR risk
Nam et al [28]	26	RP	RT-PCR	RP	42	NA	Rearrangement independently associated with recurrence risk
Dal Pra et al [29]	118 (IHC) 126 (aCGH)	Biopsy	IHC aCGH	IMRT	21 (aCGH), 50 (IHC)	Higher T stage	Rearrangement not associated with BCR risk after IMRT
Watchful waiting, active surveillance, and ADT cohorts							
Attard et al [30]	445	TURP	FISH	WW	30	Higher Gleason score, higher stage, higher PSA	Rearrangement independent predictor of poor DSS and OS
Demichelis et al [31]	111	TURP	FISH	WW	15	Higher Gleason score	Rearrangement associated with higher risk of metastatic progression and PCa death in univariate analysis
Hägglöf et al [32]	350	TURP	IHC	WW	40	Higher Gleason score and higher PSA	*ERG* IHC positivity independently predictive of poor DSS
Qi et al[33]	224	TURP	FISH/IHC	WW	23	Higher PSA	Rearrangement/*ERG* IHC positivity independently associated with PCa death risk (HR: 2.1)
Bismar et al [34]	152 (no.	TURP	IHC	AS/RP/EBRT	26	Higher Gleason score and higher	*ERG* IHC positivity associated with longer time to CRPC

	1); 160 (no. 2)			(no. 1), ADT (no. 2)		tumor volume	among androgen-deprived patients
Boormans et al [35]	85	Node metastasis is TURP	RT-PCR	ADT	59	No	Rearrangement was not associated with duration of ADT response or outcome
Leinonen et al [36]	178	Biopsy	FISH	ADT	34	Ki-67 proliferation index, age, and tumor volume	Rearrangement not associated with disease progression
Berg et al [37]	265	Biopsy	IHC	AS	38	Higher tumor volume in biopsies and higher clinical stage	*ERG* positivity independently associated with progression risk (HR: 2.45)
Lin et al [38]	387	Urine	RT-PCR	AS	NA	Higher Gleason score and higher tumor volume	Urine-detected rearrangement associated with positive repeat biopsy

aCGH = array comparative genomic hybridization; ADT = androgen-deprivation therapy; AS = active surveillance; BCR = biochemical recurrence; CRPC = castration-resistant prostate cancer; DSS = disease-specific survival; FISH = fluorescence in situ hybridization; HR = hazard ratio; IHC = immunohistochemistry; IMRT = intensity-modulated radiation therapy; NA = not applicable; NR = not reported; OS = overall survival; PCa = prostate cancer; PSA = prostate-specific antigen; RT-PCR = reverse transcriptase polymerase chain reaction; RP = radical prostatectomy; SNP = single nucleotide polymorphism; TURP = transurethral resection of prostate; WW = watchful waiting.

Table

Table 2 – Studies investigating association of *PTEN* mutations and outcome of prostate cancer

Study	No. of cases	Tissue type	Detection method	Mutation/Negative IHC staining rate, %	PTEN mutation and association with clinical parameters	Main results
Leinonen et al [45]	326 (RP), 166 (ADT), 177 (CRPC), 32 (CRPC mets)	RP, biopsy, TURP, autopsy	IHC	15 (RP) 45 (CRPC) 67 (CRPC mets)	NR	*PTEN* loss more frequent in CRPC than RP specimens. *PTEN* loss associated with shorter PFS in *ERG*-positive cases
Yoshimoto et al [46]	125	RP	FISH	45	NR	Homozygous *PTEN* deletion independently associated with BCR risk. Significant prognostic association between *ERG* and *PTEN*
Krohn et al [47]	4699 (RP), 57 (CRPC)	RP, CRPC (TURP)	FISH/IHC	Deletion (20) Negative/Weak IHC (30)	Advanced stage, high Gleason score, lymph node metastasis, and positive surgical margin	*PTEN* loss independent predictor of poorer PFS. *ERG* status did not affect predictive value of PTEN
Reid et al [48]	308 (conservative management)	TURP	FISH	17	Advanced stage, high Gleason score, lymph node metastasis, and positive surgical margin	*PTEN* loss alone not predictive, but patients with *PTEN* loss and normal *ERG* status had significantly poorer PCa survival
McCall et al [49]	68 matched castration sensitive and resistant	TURP	FISH/IHC	23 (castration sensitive) 52 (CRPC)	No	Low *PTEN* staining in IHC associated with poor PCa-specific survival among castration-sensitive cases

ADT = androgen deprivation therapy; BCR = biochemical recurrence; CRPC = castration-resistant prostate cancer; FISH = fluorescence in situ hybridization; IHC = immunohistochemistry; mets = metastases; NR = not reported; PCa = prostate cancer; PFS = progression-free survival; RP = radical prostatectomy; TURP = transurethral resection of prostate.

Table

Table 3 – Studies investigating predictive value of gene/expression panels in prostate cancer

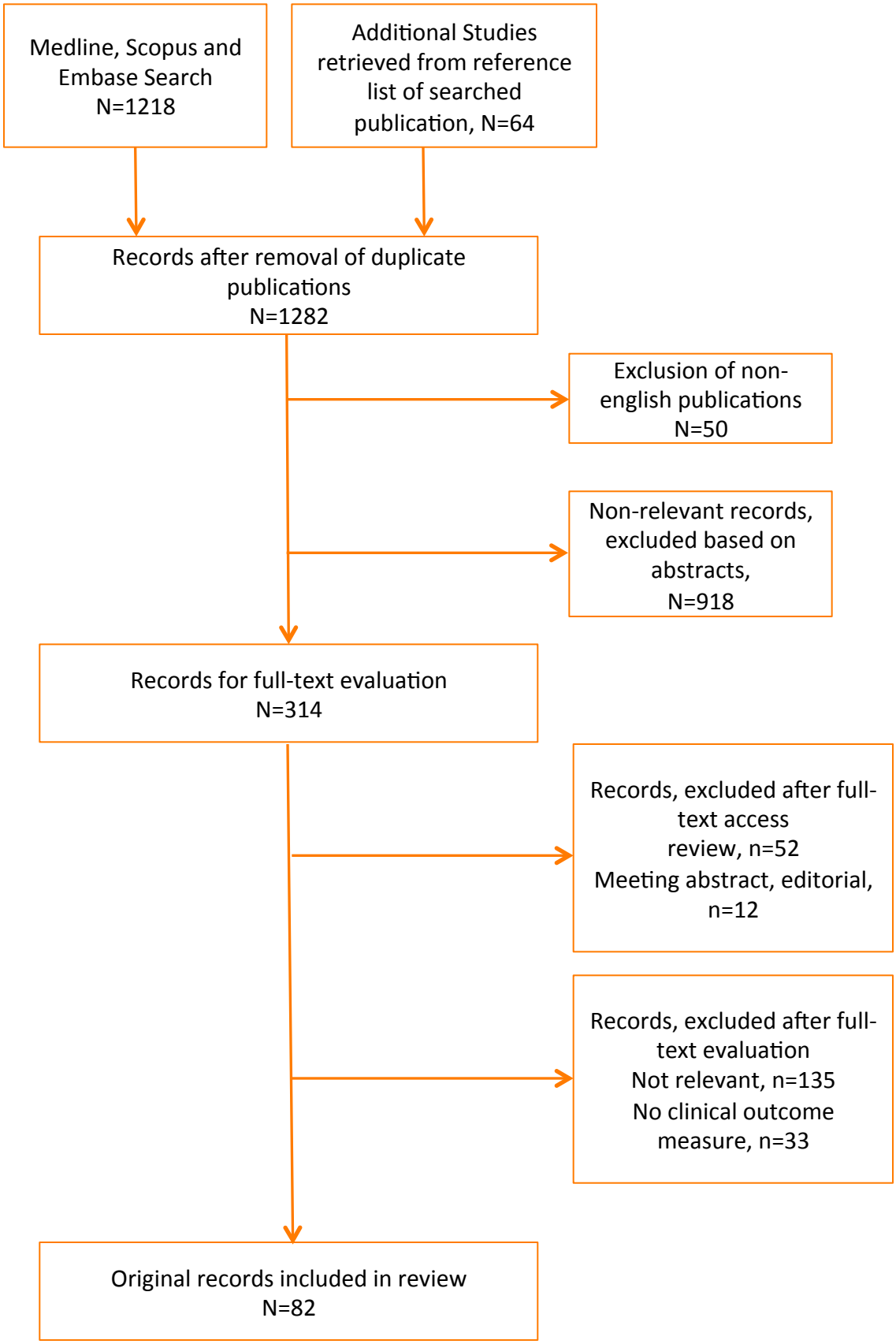
Study	No. of cases	Tissue type	No. of genes analyzed	No. of genes in final set	End point	Mean follow-up, yr	Main results	Commercial application
Discovery studies								
Cuzick et al [51]	366 (RP) 337 (TURP)	RP TURP	126	31	BCR (RP) DSS (TURP)	9.4/9.8	Expression panel independently predictive for BCR (RP) or PCa mortality (TURP, conservative management)	Prolaris
Erho et al [53]	Discovery (*n* = 359) Validation (*n* = 186)	RP	NR	22	Metastatic PFS	16.9	Nested case-control study (cases with metastasis, controls with or without PSA relapse after RP). Expression panel had AUC of 0.75 (validation) for prediction of metastasis	Decipher
Talantov et al [57]	Discovery (*n* = 138) Validation (*n* = 158)	RP	1200	3	BCR	6.0	BCR risk after RP. Predictive value of combined expression and Kattan postoperative nomogram better than clinical nomogram alone (AUC 0.77 vs 0.67)	No
Sboner et al [63]	Discovery (*n* = 186) Validation (*n* = 90)	TURP	6100	18	DSS	≥10	Case-control study (indolent vs lethal PCa in WW cohort). Expression panel not better than clinical model predicting outcome	No
Irshad et al [62]	Discovery, 2 sets (*n* = 29/25) Validation, 2 sets (*n* = 131/28)	Various	377	3	BCR	NA	Several discovery cohorts validated in TURP WW cohorts. Three-gene model had better prediction (AUC: 0.86) than Gleason (0.82) or D'Amico classification (AUC: 0.72)	No
Gasi Tandefelt et al [42]	Discovery (*n* = 48) Validation (*n* = 127)	RP	NR	36	BCR	10	BCR risk analysis after RP. Expression panel predictive for BCR risk, but only in subgroup of *ERG* fusion-positive cases	No
Penney et al [64]	358 (TURP) 109 (RP/TURP)	TURP RP	6100	157	DSS	≥10	Expression panel improved prediction of PCa mortality among Gleason 7 cases after conservative management or RP	No
Nakagawa et al [59]	Three sets; *n* = 213 in each	RP	1021	17	MFS, DSS	NR	Case-control study (systemic progression vs PSA relapse only vs no evidence of disease after RP). Expression panel predictive of systemic progression and DSS	No
Wu et al [52]	Discovery (*n* = 209) Validation (*n* = 306)	RP	1536	32	BCR, MFS	12.7	Expression panel offered independent predictive value and improved postoperative nomograms in prediction of BCR and freedom from metastasis after RP	No

Chen et al [58]	Discovery (*n* = 78) Validation (*n* = 79)	RP	22 283	7	BCR	4.3	Seven-gene panel predictive of BCR in univariate analysis	No
Cheville et al [60]	*n* = 157	RP	38	2 (with *ERG* and aneuploidy)	MFS and DSS	NR	Case-control study (metastasis/PCa death within 5 yr after RP vs no events, matched for Gleason/TNM/PSA/SM status). Expression panel had AUC of 0.81 (validation: 0.79) for prediction of metastasis or PCa death	No
External validation studies								
Cooperberg et al [65]	413 353 (second validation)	RP	NA	31	BCR	7.1	CCP score independent predictor of BCR after RP. Combined genetic and clinical CAPRA score outperformed individual scores	Prolaris
Bishoff et al [67]	Set 1 (283) Set 2 (176) Set 3 (123)	Bx	NA	31	BCR MFS	5.1 (1) 7.3 (2) 11.0 (3)	Validation of expression panel from biopsies among patients undergoing RP. Panel independent predictor of BCR and strongest predictor of metastatic progression in univariate analyses	Prolaris
Cuzick et al [66]	349	Bx	NA	31	DSS	11.8	Conservatively managed cohort. Expression panel strongest predictor of DSS when compared with clinical parameters	Prolaris
Freedland et al [69]	141	Bx	NA	31	BCR DSS	4.8	From biopsies to predict failure after EBRT. Gene panel improved predictive value when added to clinical parameters	Prolaris
Cooperberg et al [61]	185	RP	NA	22	DSS	6.4	Case-control study (high risk PCa, PCa death vs no PCa death). Combined high CAPRA and CCP scores predict high risk for PCa death	Decipher
Ross et al [71]	85	RP	NA	22	MFS	NR	Case-control study (BCR after RP, followed by metastasis vs no metastasis). Expression panel more predictive than clinical nomograms both in ROC analysis (AUC: 0.82) and decision-curve analysis	Decipher
Karnes et al [72]	219	RP	NA	22	MFS	6.7	Case-cohort study to validate 22-gene expression panel for high-risk RP patients. AUC: 0.79 for 5-yr metastasis risk	Decipher
Den et al [73]	139	RP	NA	22	BCR, MFS	7.4	Radiation patients after RP (pT3 or positive SMs). Expression panel independent predictor of BCR and metastasis risk. Additive predictive value when genetic and clinical models combined	Decipher
Klein et al [54]	Set 1(*n* = 441) Set 2 (*n* = 167) Set 3 (*n* = 395)	RP (1 and 3) Bx (2)	727	17	MFS (1), adverse RP pathology (2 and 3)	NR	Expression panel independently predicted adverse RP pathology (high grade/high stage) from biopsies. Inclusion of expression panel improved net benefit when combined with CAPRA in decision-curve analysis	Oncotype DX

Cullen et al [76]	431	Bx	NA	17	Adverse RP pathology BCR	5.2	Test associated with BCR risk in univariate analysis and after adjusting for NCCN risk groups	Oncotype DX

AUC = area under the curve; BCR = biochemical recurrence; Bx = biopsy; CAPRA = Cancer of the Prostate Risk Assessment; CCP = cell cycle progression; DSS = disease-specific survival; EBRT = external beam radiation; MFS = metastasis-free survival; NA = not applicable; NCCN = National Comprehensive Cancer Network; NR = not reported; OS = overall survival; PCa = prostate cancer; PSA = prostate-specific antigen; RP = radical prostatectomy; SM = surgical margin; TURP = transurethral resection of prostate; WW = watchful waiting.

Illustration



Instructions to typesetter for EURUROL-D-15-00217

Figure 1
-Lowercase and italicize n, insert space before and after equals signs (eg, n = 1218)
-Insert comma after Scopus
-Change Additional Studies to: Additional studies; change publication to: publications
-Change non-english to: non-English
-Change Non-relevant to: Nonrelevant
-Change Records, excluded [twice] to: Records excluded