Are there useable molecular markers for prostate cancer?

There is an urgent need for well-annotated biorepositories for marker validation

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There is no doubt that we need more and better markers to determine risk, presence, aggressiveness and therapy response of prostate cancer (PCa). Besides physical symptoms such as palpable irregularities upon digital rectal examination (DRE), current indicators range from molecular (e.g. PSA), histo-pathological (e.g. Gleason grade) to imaging markers (e.g. CT, bone scan).

From the clinical point of view, progress in all these marker areas has been modest. In the past decade, many new promising markers and assays have been published, but few made it into the clinic. The funnel from marker discovery to validation and clinical implementation is ruthless. Particularly for molecular marker research in the last decade, a feeling of continuous excitement for the novel developments contrasts their limited clinical impact.

Challenges for molecular markers

For the molecular markers, it has become clear that we are very good at discovering novel candidates. Technological progress, particularly in the –omics area of next-generation sequencing and mass spectrometry, continuously provides new options for higher throughput and higher content DNA, RNA, protein and metabolite detection. Besides finding new markers, these technologies also unlock the use of small amounts and new types of patient samples such as circulating tumor cells (CTCs), extracellular vesicles, circulating tumor RNA/DNA in urine and serum and formalin-fixed paraffin-embedded (FFPE) cancer biopsy sections.

Unfortunately, the enthusiasm from technology development and promising novel markers does not encompass the validation phase. The major challenge we currently encounter is that candidate markers are not or cannot be validated in independent cohorts. Reasons for not being able to perform a validation include the lack of samples with the right clinical follow-up, robust detection assays (e.g. lack of good antibodies) and finances to validate all candidate markers. The reason why most of the candidates fail in the validation phase varies. Very often, the discovery phase is performed on a high number of molecules (all genes or thousands of peptides and metabolites) with a limited number of samples per group.

The variability in serum/urine content, tumor mutational variation (heterogeneity) and expression levels of RNA and protein in tissue between men is simply too high to escape the 'noise in the system.' Preventing this problem is not easy, but can be reduced by using larger cohorts of samples during discovery and focusing on cancer-derived and cancer-specific markers. A generic search for protein or metabolite changes in urine or serum among a hundred men is futile.

However, focusing on cancer-derived extracellular vesicles (e.g. exosomes), CTCs, cancer tissue or on DNA mutations or RNA expression unique to cancer (fusion genes, deletions, cancer-associated transcripts, etc.), might have a chance of success. Once markers have been validated and the assays optimized and CE-marked/FDA-approved, there is no guarantee they will be implemented in the clinic. Many issues affect the use of an assay, including the proven added-value, cost and reimbursement, ease of use, marketing and enthusiasm of the clinicians. If the proven added-value is very strong, worries about the other issues dissolve. If the added-value is limited, all other factors that play a role in adopting a new marker test, will determine its fate.

Molecular markers

Molecular markers used in common clinical practice are few, but their impact is high. Particularly prostate-specific antigen (PSA) is a major determinant in the diagnosis and monitoring of prostate cancer. The majority of validated molecular markers however, are not frequently used in clinical practice (Table 1).

For risk assessment, single nucleotide polymorphisms (SNPs) have been identified in large genome-wide association studies (GWAS)[1]. Typically, each individual SNP has a limited discriminatory value, but as a panel might reach useful hazard ratios. In contrast, high hazard ratios are observed with BRCA2 and HOXB13 germline mutations. However, these hereditary cancer-associated mutations are very rare.

Marker identification and validation has mainly concentrated on the diagnosis and prognosis of PCa. Unnecessary biopsies and overtreatment rightfully remain the main objectives. An overwhelming number of proteins, metabolites and RNAs have been investigated and proposed as markers. As mentioned above, most are not fully validated or failed to be independently validated by others.

A selection of the markers that have been substantiated or are close to independent validation are listed in Table 1. Although often not fully clear yet, their added value to clinical practice is not expected to be at the breakthrough level. Combined however, some of the proposed marker profiles certainly deserve our attention[2,3].

Of interest are RNA profile assays for biopsies available from Genomic Health and Myriad[4,5] and based on radical prostatectomy samples from GenomeDX Biosciences[6]. Their prognostic value needs to be further established in daily practice but their basis of gene expression differences directly measured in PCa tissue is solid.

Of interest are established and emerging molecular agents for nuclear imaging[7]. Again, technological developments in radiotracers, model systems and PET/SPECT/CT scanning drive progress in this field and an increase in their implementation to detect and monitor PCa metastases is expected.

The lack of robust predictive markers is a sign of our limited knowledge on the detailed molecular changes of therapy resistance. Only recently, some clear examples demonstrate progress in this field. The androgen receptor (AR) is the major target of hormone therapy and presence of AR amplification and mutations in castration resistant PCa (CRPC) have been known for many years.

More recent is the discovery that splice variation between exons 3 and 4 can result in constitutively active receptors that are unaffected by hormonal therapies. As could be expected, the presence of AR variants in CTCs is a marker for the lack of response to enzalutamide and abiraterone[8]. A second example is the association between tumor-associated mutations in DNA repair genes such as BRCA1/2 and PALB2 and the increased sensitivity of the tumor to PARP inhibitors[9].

In normal cells, the inhibition of part of the DNA repair mechanism by PARP inhibitors is compensated by their slow growth and other DNA repair systems. When these are inactive through specific mutations in BRCA1/2 or PALB2, the cancer cells cannot cope with the accumulation of double-strand breaks and eventually perish. Mutations and loss of expression of various DNA repair-associated genes are therefore markers for the sensitivity of tumors to PARP inhibitors.

Future of molecular markers

The powerful technology developments are and will remain the main driver for progress in the field of marker research. Technological improvements further result in our ability to measure more parameters using less patient material. This allows us to perform marker analysis on the most limited material such as cell free or circulating tumor DNA (cf/ctDNA), CTCs and extracellular vesicles isolated from blood and urine.

Taking biopsies of primary or metastasized PCa to establish tumor status is expected to be supplemented or even replaced by investigating the tumor-released cells (CTCs) and vesicles. Different types of vesicles are produced by (cancer) cells and the larger apoptotic vesicles contain DNA fragments (cf/ctDNA) while small exosomes carry cytoplasmic proteins and RNA[10]. Much research is directed towards the isolation of PCa-derived vesicles from blood and urine to determine the tumor status by the DNA, RNA and proteins they release.

Dependent on cost developments of next generation sequencing (NGS), the measurement of single or panels of markers will be replaced by sequencing of the whole genome and transcriptome. The future in which recurrent tumors are sequenced to determine optimal treatment is near. This will be followed by sequencing of diagnostic biopsies, ctDNA and exosomal RNA for the diagnosis, prognosis, therapy response and monitoring of disease. For discovery and validation of novel markers, these efforts are established and ongoing. Clinical implementation, as mentioned, is mainly dependent on the added clinical value, costs and reimbursement.

With respect to the type of markers, there is a clear bias towards DNA and RNA as compared to protein and metabolites. DNA and RNA can be easily and highly amplified and specific detection using PCR and NGS is straight forward. Although mass spectrometry is undergoing similar technological breakthroughs as NGS, implementing this technology in a clinical setting for protein and metabolite detection is more cumbersome. Protein detection using classical methods such as ELISA and immunohistochemistry (IHC) depend on specific antibodies, which are still not always available and laborious to generate.

With the assay technologies in place, the limiting factor remains the molecular markers. It is expected that diagnostic and monitoring markers will be identified that have high specificity and sensitivity. Since our knowledge of DNA mutations in PCa is exponentially increasing through the many sequencing efforts, detecting cancer from changes in DNA and RNA extracted from urine or blood is feasible. Knowing the patient-specific DNA mutations, allows for the monitoring of disease via cf/ctDNA and CTC-DNA in blood.

Much harder is resolving risk assessment, prognosis and therapy response predictions. If the majority of PCa tumors are caused by random mutations (just bad luck), informative risk markers will not exist for most men. For prognostic and predictive markers, knowledge on the biology of the disease is essential, but far from comprehensive. In addition, heterogeneity within and among tumors is a problem for the identification of common markers and, therefore, panels of markers have to be designed.

Technology developments, particularly in next generation sequencing, drive biomarker research

New possibilities

Technology developments, particularly in next generation sequencing, drive biomarker research and create new possibilities to detect more factors in limited amounts of patient sample. For example, changes in cell-free DNA and RNA from extracellular vesicles extracted from urine and blood will become important future personalized diagnostic and monitoring markers.

A proportion of the new generation prognostic, predictive and imaging (PET/SPECT) markers will be based on our increasing understanding of PCa biology and therapy resistance. Due to the high variability among tumors, sensitive markers will not consist of a single factor, but will be a panel of parameters.

Although many novel candidate markers have been discovered, their validation and implementation fall behind. The need for well-annotated biorepositories for marker validation is high. Importantly, researchers, clinicians, funding agencies, health insurance companies and private partners should invest more in assessing promising markers in retrospective tissue banks and in daily routine.

Table 1: Common and emerging molecular markers for prostate cancer

Type of marker	Most common current markers	Less commonly used and emerging molecular markers	Future molecular markers
Risk	Family history, urinary symptoms, age, race	SNPs, rare germline BRCA2 and HOXB13 mutations, PSA at age 40-50	SNPs, other polymorphisms and germline mutations determined by genome sequencing after birth
Diagnostic	PSA, DRE, biopsy histo-pathology and in case of doubt AMACR/p63 or ERG IHC, imaging (MRI, CT, TRUS)	Prostate Health Index (PHI), PCA3, TMPRSS2-ERG, marker profiles of differentially expressed and mutated genes in urine and blood	DNA mutations and RNA expression (e.g. PCa-associated transcripts) determined by NGS of DNA/RNA from urine or biopsies
Prognostic	Gleason score (biopsy), extend of disease in biopsies, imaging (CT, MRI bone scan), after prostatectomy pT stage, Gleason grade and surgical margins	Oncotype DX (Genomic Health), Polaris (Myriad), Decipher (GenomeDX), PTEN, cMYC and expression of other oncogenes and tumor suppressor genes in tissue (biopsies), miRNA profiles in serum, urine and tissue, imaging (PSMA [ProstaScint], Bombesin, FDG, CXCR4)	DNA mutations and (small) RNA expression determined by NGS of DNA/RNA from blood, urine or biopsies. Novel imaging agents for PET/SPECT. IHC of protein profiles on biopsies
Predictive		Androgen receptor variants (indicating resistance to hormonal therapy), mutations in DNA repair genes (indicating PARP inhibitor sensitivity)	DNA mutations and RNA expression determined by RT-PCR or NGS of DNA/RNA from blood (CTCs, Exosomes, cell free), urine or biopsies
Monitoring	PSA, imaging (MRI, CT, bone scan)	EpCAM capture and molecular characterstics of CTCs, imaging (PSMA [ProstaScint], Bombesin, FDG, CXCR4)	Number and type of extracellular vescicles. DNA mutations and RNA expression from cell free DNA and exosomal RNA from blood. Novel functional imaging agents for PET/SPECT

*Abbreviations: IHC, immunohistochemistry; SNP, single nucleotide polymorphism; NGS, next generation sequencing; DRE, digital rectal examination

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Editorial Note: Due to space constraints the reference list has been shortened. Interested readers can email at EUT@uroweb.org for a complete listing.

Sunday, 22 March
07.30-10.55: Plenary Session 2
Prostate Cancer