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), clinical 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 markers has been modest. In the recent past, 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 clinical impact limited.

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 greater content, DNA, RNA, protein and metabolite detection. Besides finding new markers, these technologies also unmask the use of small amounts and new types of patient samples such as circulating tumor cells (CTC), 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 novel using 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 features (e.g. patients on treatment with (new) blood 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 patients per group.

The variability in serum/urine content, tumor mutational variation (heterogeneity), levels of RNA and protein in tissue between men is 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 transcription factors) might have a chance for success. Once markers have been validated and the assays optimized and CE-marked/FDA-approved, there is no longer any need for external funding and implementation support. Many issues affect the use of an assay, including the proven added-value, cost and reimbursement, ease of use, lack of enthusiasm 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 2).

For risk assessment, single nucleotide polymorphisms (SNPs) can be identified in large genome-wide association studies (GWAS). 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 even with very old (or more) deleterious mutations. However, hereafter cancer-associated mutations are very rare.

Marker identification and validation has mainly concentrated on the diagnosis and prognosis of PCA. The development of disease biomarkers and overexpression 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 substantially or are close to independent validation are listed in Table 1. Although often not clearly yet, their added value to clinical practice is not expected to be at the breakpoint level. Combined however, several of the proposed marker profiles certainly deserve our attention.

Of interest are RNA profile assays for biopsies available from Genomic Health and Myriad© and based on radical prostatectomy samples from GenomicHealth. Their prognostic significance 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 markers and imaging©. Much research is directed at biological developments in radiotracers, model systems and PET/CT/SPECT scanning drive progress in this field. Competitive strategies to implement 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 aberrations and mutations in castration resistant PCA (CRPC) have been known for many years.

More recently 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, these AR variants in CTCs is a marker for the lack of response to enzalutamide and abiraterone®. A second example is the association between tumor-associated mutations in DNA repair genes such as BRCA2 and PALB2 and the increased sensitivity of the tumor to PARP inhibitors.

In normal cells, the inhibition of part of the DNA repair machinery by PARP inhibitors can result in the accumulation of double-strand breaks and eventual death. Paradoxically, the loss of expression of multiple DNA repair-associated genes and therefore markers for the sensitivity of tumors to PARP inhibitors.

Table 1: Common and emerging molecular markers for prostate cancer

New possibilities.

Technological developments, particularly in next generation sequencing, drive biomarker research and create new possibilities to detect more factors in limited amount 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/PECT) 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 short. The need for well-annotated biorepositories for marker validation is marked. Importantly, researchers, clinicians, funding agencies, health insurance companies and private partners should invest more in assessing promising markers in retrospective tissue samples and in daily routine.

References