Commentary

Prospecting for prostate cancer with precision medicine

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With the high false positive rate of prostate-specific antigen (PSA) for detection of prostate cancer, new reliable biomarkers for prostate cancer diagnosis and prognosis have been the topic of extensive research efforts. Preferably, the biomarkers should be present in biofluids that can be sampled with non- or minimally-invasive methods, to allow repeated analysis. In addition to many and varied analyses applied to blood specimens, three prostate-relevant body fluids have been investigated, namely, urine (1-3), seminal fluid (4,5) and expressed prostatic secretion (EPS) (6-8).

In parallel to the ongoing quest for predictive molecular biomarkers, management has progressed to differentiating indolent prostate cancer from significant disease through multi-parametric magnetic resonance imaging (mpMRI). mpMRI has been developed to a stage so that it is now being used in clinical practice to triage men with an elevated PSA with only those with PI-RADS scores of 3–5 proceeding to biopsy in the first instance (9). Approximately 90% of moderate to high risk lesions, are able to be detected by mpMRI although this approach is less reliable for detecting small (<0.5 cc) and lower risk tumours (10,11). However, mpMRI is expensive and as a majority of patients presenting with an increased serum PSA do not have significant prostate cancer diagnosed, the need for inexpensive and discriminating markers remains an imperative.

Ideally markers should not just be able to discriminate between those with minimal tumour burdens who do and do not have significant prostate cancer, but also between those patients whose disease is localized to the prostate and curable with localized treatments as opposed to the minority presenting with cancers that have spread beyond the gland. In a review of clinical and pathological data of 2,900 patients who underwent radical prostatectomy between 2008 and 2012, Samaratunga *et al*. reported that 2,681 cases (92.4%) had a final Gleason score of ≥7,669 (23.1%) had a tumour volume of >3 cc and 1,144 (39.4%) had extraprostatic extension (12). Although a finding of extraprostatic extension in the prostatectomy specimen may not be as devastating a finding prognostically as previously considered (13), this finding correlates strongly with high grade diseases and is likely to be associated with more extensive local treatment than otherwise with the attendant risk of further morbidity for these patients. Consequently, the prospect of markers that can distinguish between tumours confined to the prostate compared with those extending beyond the gland such as indicated in a recent paper by Kim *et al*. (14) is of particular interest.

The Kislinger group has previously reported biomarker discovery using EPS collected before radical prostatectomy to identify biomarkers for detecting extracapsular disease (8). The previous study identified 133 candidate biomarker proteins in EPS. The new study made use of the quantitative and high throughput nature of multiple reaction monitoring mass spectrometry (MRM-MS) to validate a subset of the candidates. MRM-MS allows the multiplex quantitation of hundreds of peptides within a single run (15). Accurate quantitation is achieved by spiking a known amount of stable-isotope standard peptides to all samples, which also

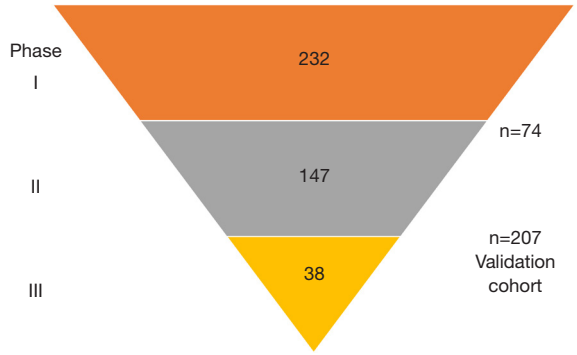

Figure 1 Phased biomarker study design as applied in Kim *et al.* (14). Each phase used increasingly larger cohorts to eliminate poor-performing candidate peptide biomarkers. Numbers of candidate biomarker are indicated by the inverted pyramid.

will account for any analytical variability between runs (15). Establishing a reproducible and quantitative MRM-MS assay and sample preparation pipeline is critical for accurate quantitation. This was successfully performed by Kim *et al.* through several phases (*Figure 1*) progressively reducing the number of candidate peptides using increasing large size validation cohorts.

Statistical analysis revealed that no single peptide biomarker had sufficient power for prostate cancer diagnosis or prognosis. Hence the authors applied a machine learning approach to develop the final quantitative data into signatures that can predict patient risk groups, cancer versus control, or organ confined versus extracapsular prostate cancer. In addition to these candidate biomarkers, an interesting finding from the study is the reduced level of the majority of proteins including PSA in EPS and urine in extracapsular disease. The authors speculate that deterioration of the prostate integrity may lead to increased leakage into the circulation, or loss of secretory function of the prostate gland (8).

Proteomics is a popular technique for biomarker discovery, presumably due to the broad acceptance of immunoassays in clinical diagnosis. However, few protein biomarkers have been developed into clinical assays, due to failure of the researchers to perform validation studies, or failure of the biomarkers to validate in a different biological sample type or independent cohorts. While this study has identified urine peptides that are potential biomarkers for prostate cancer diagnosis and prognosis, further validation in larger independent cohorts is required, as stated by the authors. Although MRM-MS assays allow high throughput multiplex quantitation of numerous peptides, implementation of this technology in the clinical diagnostic laboratory requires streamlining and automating the complex sample preparation procedures to ensure reproducibility. The potential large variability introduced at the trypsin digest step to generate the peptides, and during peptide clean-up steps will need to be systematically managed in a clinical diagnostics setting. Future development of an automated proteomics sample prep system coupled with MRM-MS assays will enable the translation of peptide signatures for prostate cancer diagnosis and prognosis.

As pointed out by the authors, a single biomarker was not expected to provide sufficient diagnostic value, hence their application of machine learning method to develop a signature. While biomarker discovery and validation studies necessarily focus on single molecular types using specific technology, the final diagnostic algorithm can combine multiple molecular types together with clinical parameters to increase the diagnostic power. This approach was recently applied to combine traditional clinical risk factors with a urine mRNA signature to produce predictive models for prostate cancer risk stratification (16). Future studies may evaluate the addition of urine peptide, protein and/or metabolite biomarkers to the signature to increase the predictive value. The investments in biomarker discovery and validation research, coupled with multivariate statistics and/or machine-learning methods may ultimately fulfil the lofty goal of precision medicine in prostate cancer management.

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Footnote

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