Development and validation of a Multigenomic Liquid Biopsy (PROSTest) for Prostate Cancer Detection

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Background:
A crucial requirement in prostate cancer (PCa) management is an accurate, easily measurable, liquid biopsy that can define the molecular pathology of an individual PCa. We report the development and clinical validation of a novel PCa-specific, multi-genomic biomarker.

Methods:
We identified candidate mRNA biomarkers in PCa-Adeno transcriptomes (n=1,159) using several strategies: co-expression networks, differential expression, and functional enrichment. mRNA transcripts were screened in an independent tumor tissue (n=50) set and validated as biomarkers in the TCGA-PRAD (n=500) dataset. An amalgam of Random Forest, Gradient Boosted Machines and Support Vector Machines, all standard machine learning classifiers, was used to develop a classification algorithm and probability score in a peripheral blood gene expression test cohort (n=430). This multigene biomarker was validated in two independent clinical blood sample sets (Set I: PCa n=77, controls n=54; Set II: PCa n=132, controls/BPH n=99) to determine as PCa-specificity and diagnostic efficacy Clinical utility was evaluated versus Gleason scores, T-staging and PSA (n=209) and in a prostatectomy cohort (n=47).

Results:
The pipeline identified 27 of PCa gene markers in the tumor tissue set and TCGA-PRAD dataset. Gene expression was significantly correlated ($r=0.72$, $p<0.0001$) in matched tissue/blood samples. The PROSTest (scale: 0-100) ensemble algorithm (developed in blood) had a sensitivity for PCa of 92.2% (95% CI: 83.8-97.1%; Set I) and 95.0% (95% CI: 89.9-98%. Set II). The specificity was 100% for Set I (95% CI: 93.4-100%) and 100% for Set II (95% CI: 96.3-100%). PCa scores were significantly

($p<0.0001$) lower for controls (Set I: 17±4; Set II: 18±4) and BPH (19±6) to PCa; 82±19 (Set I) and 80±19 (Set II). The AUROC was 0.98±0.01. PROSTest scores were elevated ($p<0.05$) in T2-4 and were significantly correlated with Gleason ($r=0.93$, $p<0.02$). In contrast, PSA from matched samples was not associated (p=NS) with clinically significant disease (Gleason 7-10 or T2-4 tumors). In head-to-head comparisons, the PROSTest was considerably more accurate than PSA for detecting significant disease (z-statistic: 2.43, $p=0.015$). In the R0 prostatectomy cohort, all scores were elevated (72±7) and significantly decreased post-surgery (26±8, $p<0.0001$, $n=37$). Individuals with residual disease ($n=10$) exhibited elevated (60±4) post-surgical scores.

Conclusion:
The PROSTest is a multigenomic blood-based PCR tool that accurately (>90%) identifies prostate cancer. It is significantly more accurate than PSA for the detection and stratification of clinically significant prostate disease. A multigenomic liquid biopsy for PCA provides a real-time, non-invasive method for the detection of a PCa and may facilitate the early identification of residual/recurrent disease.

About Wren Laboratories:
Founded in 2014, Branford, CT-based Wren Laboratories is a CLIA-certified and CAP-accredited liquid biopsy molecular diagnostic laboratory intent on developing unique, minimally invasive, and highly accurate fluid-based testing solutions.

A subsidiary of Clifton Life Sciences, Wren Laboratories is driven to develop liquid biopsy diagnostic and monitoring tests for various cancers including Neuroendocrine, Prostate, Lung, Melanoma, Myeloma, Colorectal, and Breast Cancer as well as Endometriosis.

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