Development of a Whole-Urine, Next Generation Sequencing-Based Assay for Early Detection of Aggressive Prostate Cancer

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Background: Despite advances in biomarker development, early detection of aggressive prostate cancer (PCa) remains challenging. We previously developed the Michigan Prostate Score (MiPS) for individualized risk prediction of aggressive prostate cancer. MiPS uses transcription-mediated amplification to quantify expression of *TMPRSS2:ERG* (*T2:ERG*) and *PCA3* from whole urine obtained after a digital rectal exam (DRE), combined with serum PSA. To improve upon MiPS, herein we describe the pre-clinical development and validation of a targeted RNA next generation sequencing assay (NGS-MiPS) to detect aggressive prostate cancer.
Methods: Patients with urine collected prior to prostate biopsy or prostatectomy were identified. A case control study designed compare patients with benign or Gleason 6 prostate cancer vs. Gleason ≥ 4+3=7 disease. We isolated mRNA from 2.5 mL of post-DRE whole urine. Next generation sequencing was performed to asses ~90 PCa transcriptomic biomarkers: including T2:ERG, PCA3, and additional isoforms of common PCa gene fusions, mRNAs, lncRNAs, and expressed mutations.
Results: NGS-MiPS showed a 98% informative sample rate, high technical reproducibility, robustness and concordance with orthogonal methods (TMA and RT-qPCR), and was able to detect expressed *HOXB13* p.G84E variant expression. NGS-MiPS accurately recapitulated clinical MiPS-measured risk scores for the presence of PCa or high-grade PCa (Gleason Score >6) on biopsy as determined by clinical MiPS vs. the same model but with NGS-MiPS data. In an extreme design cohort (benign or Gleason 6 vs. Gleason ≥ 4+3=7 cancer) NGS-MiPS showed expected differences in the levels of *T2:ERG* T1E4 ($p<0.00001$) and *PCA3* ($p=0.02$), with additional T2:ERG splice isoforms and other biomarkers also showing significantly different expression between low vs. high grade cancer. We used a machine learning approach trained on a subset of the extreme design cohort ($n=73$) to generate a 29-transcript model that outperformed MiPS and serum PSA in two validation cohorts: 1. A held-out set from the extreme design cohort $n=36$, (AUC 0.82 vs. 0.73 and 0.69, respectively); 2. A separate PCa active surveillance cohort n=45, (AUC 0.66 vs. 0.58 and 0.53, respectively).
Conclusions: These results support the potential utility and continued development of our urine based targeted NGS assay to supplement serum PSA for improved early detection of aggressive prostate cancer.

Conflict of Interest: Sumin Han's Current affiliation: Research Scientist, Bristol-Myers Squibb, Redwood City, CA. Scott A. Tomlins is a cofounder of Strata Oncology, Ann Arbor, MI.

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