# A first catch, non-DRE urine exosome gene signature to predict Gleason 7 prostate cancer on an initial prostate needle biopsy.

Abstract #45 (141579)
Poster #C12

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## Introduction:

Prostate cancer (PCa) is the second leading cause of cancer death among men in the United States, with an anticipated 233,000 new cases and nearly 29,480 deaths in 2014. The definitive diagnostic for PCa is the prostate needle biopsy, typically recommended for men with elevated serum PSA levels and/or a suspicious digital rectal exam (DRE) with added indication from family history, age and race. The majority of prostate cancers remain indolent, infrequently resulting in death. Thus, there is a major risk for detecting cancers that are clinically insignificant and do not require treatment. Unfortunately, due to the low positive predictive value (PPV) of PSA and the high prevalence of low risk PCa, approximately 70% to 80% of men will undergo an unnecessary biopsy. There is a critical need to reduce initial prostate biopsies (PB) in the PSA 'gray zone' of 2-10 ng/mL. Non-invasive screening tools that add predictive value for identifying high-grade, Gleason score (GS) ≥7 should impact the current diagnostic paradigm. We have developed standardized processes to isolate exosomal RNA (exoRNA) from first-catch urine specimens and sought to identify a gene signature that reliably differentiates GS7+ from GS6 and benign disease.

## Methods:

The study population consisted of men aged ≥40 years scheduled for an initial or repeat prostate needle biopsy, due to a suspicious DRE and/or PSA levels, and met the eligibility criteria. The "Intended Use" population was comprised only of men who were undergoing their initial PB and had an equivocal 'gray zone' serum PSA levels (>2 and <10 ng/ml). First-catch non-DRE urine specimens were collected at six sites in standard collection vessels without preservative, stored at 2-8C (up to two weeks) and shipped on ice to a central laboratory (Exosome Diagnostics, St. Paul, MN). Upon receipt, samples were filtered (0.8um), and exosome isolation and RNA extraction performed. RT-qPCR RNA copy numbers of ERG and PCA3, normalized to SPDEF, were measured to generate a three-gene signature, defined to yield a score S between 0 and 30, where S>10 predicts GS7+ vs. GS6 and benign lesions with optimal negative predictive value (NPV), Sensitivity and Specificity. The performance of the EXO Score using PSA RNA (encoded by kallikrein-related peptidase 3 [KLK3]) was also evaluated using receiver operating characteristics (ROC) analysis. The area under the curve (AUC) resulting from the use of KLK3 as a normalizer were comparable, with that of SPDEF being slightly improved.

## Results:

Urine samples from 170 men (PSA 2-10 ng/mL, first biopsy, 40 mL; median age 62 years; median PSA 5.1 ng/mL; 70% negative DRE; 77% no family history; 82% Caucasian) of 453 total, with comparable demographics, were evaluated. With 46% and 21% prevalence of PB for any cancer or GS7+ disease, respectively, a dichotomous gene signature demonstrated good clinical performance in predicting biopsy results. For GS7+, the NPV and PPV were 98.6% and 34.7%, respectively. A continuous score alone had an AUC of 0.76 for discriminating GS7+ from GS6 and benign disease, and the results were significantly better than the prostate cancer prevention trial risk calculator (PCPTRC), AUC 0.60.

**TABLE 1: A dichotomous EXO106 Score is predictive of initial biopsy results.**

| Performance Parameters (N=170) | | | | | | |
|---|---|---|---|---|---|---|
| | Sensitivity % (95% CI) | Specificity % (95% CI) | NPV% (95% CI) | PPV% (95% CI) | NLR | PLR |
| **ANY CANCER** | 76.9 (67.6 – 86.3) | 55.4 (45.3 – 65.6) | 73.9 (63.6 – 84.3) | 59.4 (49.8 – 69.0) | 0.416 | 1.726 |
| **HIGH-GRADE CANCER** | 97.2 (91.9 – 102.6) | 50.7 (42.3 – 59.2) | 98.6 (95.7 – 101.4) | 34.7 (25.4 – 43.9) | 0.055 | 1.974 |

NPV=negative predictive value, NLR=negative likelihood ratio, PLR=positive likelihood ratio, PPV=positive predictive value

**FIGURE 1: The EXO106 Score is predictive of biopsy result for any cancer (Fig. 1A) and high-grade disease (Fig. 1B) and adds significant predictive value to that of the PCPTRC alone. For each cohort, the AUC was determined for ROC analyses of multivariable models that included the gene predictors of the EXO106 Score with and without the PCPTRC parameters, as well as for PCPTRC parameters alone.**

Fig. 1A

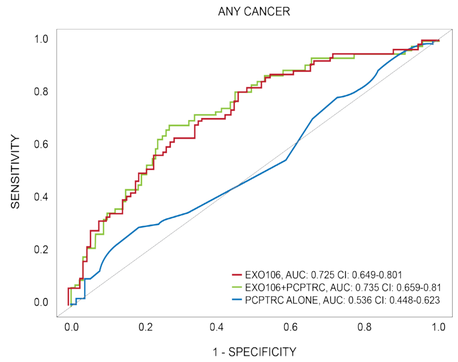



Fig. 1B

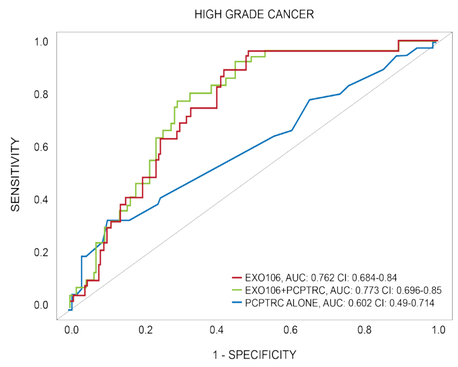
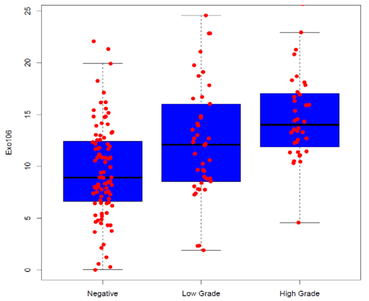



**FIGURE 2: Consistent with the predictive accuracy for high-grade disease, a statistically significant direct correlation (p<0.001; Spearman's rank-order) between the EXO106 Score and Gleason Score was observed, as determined by a box plot.**




## Conclusions:

In summary, this study presents a novel, non-invasive molecular assay utilizing a first-catch, non-DRE urine exoRNA signature as a prognostic tool for predicting high-grade PCa among men presenting for an initial biopsy with serum PSA levels within an expanded gray zone (>2 and <10 ng/mL). Once validated in a larger cohort, we anticipate that this assay could be used along with PSA and other prognostic factors to inform and guide current biopsy decision models and reduce unnecessary biopsies.

## References:

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