A urinary exosome assay interrogating small non-coding RNAs accurately identifies and classifies prostate cancer into low-, intermediate-, or high-risk disease

Laurence Klotz, Alvin Lopez Pujals, Carl Olsson, Deepak Kapoor, Ann Anderson, Federico Corica, Alberto Corica, James Libby, Rebecca DeVaux, Gregory DiRienzo, Winnie Wang, Martin Tenniswood

Conflicts of Interest

- Laurence Klotz is Chief Medical Officer of miR Scientific LLC
- Martin Tenniswood is Chief Scientific Officer of miR Scientific LLC
- Greg DiRienzio is Director of Biostatistics of miR Scientific LLC
- Winnie Wang is Director of Research of miR Scientific LLC

The miR Sentinel™ Prostate Cancer Test

Measures the expression levels of 442 sncRNAs (221 miRNAs, 221 snoRNAs) and spike-in controls isolated from urinary exosomes using RT-qPCR on a customized OpenArray platform using Quant Studio 12 K flex system.

An individual's sncRNA expression profile is input to a statistical classification system that classifies the patient into one of four groups:

- **No molecular evidence of prostate cancer (Mol-NR)**
- **Molecular evidence of Low-Risk (Mol-LR)**
- **Molecular evidence of Intermediate-Risk (Mol-IR)**
- **Molecular evidence of High-Risk (Mol-HR)**

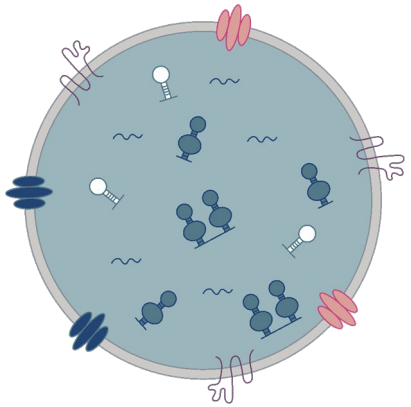
These molecular risk groups do not simply recapitulate pathological risk groups, rather identify underlying molecular processes involved in tumor progression.

miR Sentinel™ is **NON-INVASIVE** (no DRE)
It was awarded Breakthrough Status by the FDA for the diagnosis and prognosis of patients with suspicion of prostate cancer

Basis of the miR Sentinel™ Disease Management Platform

miR Sentinel™ Prostate Disease Management Platform

- based on analysis of urine exosomal sncRNAs (miRNAs and snoRNAs)
- provides a molecular assessment of the health of the whole prostate
- molecular characterization of tumor status throughout the prostate
- does not distinguish between monofocal and multifocal disease
- *includes contribution of **small potentially aggressive tumors** that can be missed by TRUS or MRI-guided biopsies and/or not recorded on pathology 10^{-4} cc (0.1 mm^3)*



Small membrane bound vesicles released from into the extracellular milieu from all cells in the prostate and can be isolated from the blood, seminal fluid and urine

Pre-miRNA

C/D Box snoRNA

miRNA

H/ACA Box snoRNA

mRNA stability and translation

mRNA translation and primary transcript splicing

Demographics of training dataset by pathology biopsy grade group

	Total (n = 1100)	NPEPC (n = 502)	cGG1 (n = 253)	cGG2 (n = 153)	cGG3 (n = 92)	cGG4 (n = 48)	cGG5 (n = 52)
Race							
NHW	570 (52%)	259 (52%)	137 (54%)	69 (45%)	54 (59%)	25 (52%)	26 (50%)
NHB	106 (9.6%)	48 (9.6%)	23 (9.1%)	10 (6.5%)	9 (9.8%)	8 (17%)	8 (15%)
Hispanic	331 (30%)	155 (31%)	67 (26%)	62 (40%)	21 (23%)	13 (27%)	13 (25%)
Asian	22 (2.0%)	9 (1.8%)	6 (2.4%)	2 (1.3%)	3 (3.3%)	1 (2.1%)	1 (1.9%)
Others	71 (6.5%)	31 (6.2%)	20 (7.9%)	10 (6.5%)	5 (5.4%)	1 (2.1%)	4 (7.7%)
Age							
Range	36 – 90	36 – 90	40 – 86	46 – 89	52 – 87	54 – 81	50 – 88
Mean ± SD	65 ± 8.2	64 ± 8.5	65 ± 7.7	67 ± 7.7	68 ± 7.5	69 ± 6.4	69 ± 8.5
PSA (ng/mL)							
Range	0.2 – 1467	0.2 – 163	0.8 – 411	2.3 – 71	2.2 – 38.2	2.5 – 722	3.9 – 1467
Mean ± SD	12 ± 62	6.7 ± 8.5	7.7 ± 26	8.1 ± 8.5	9.8 ± 7.1	30 ± 106	81 ± 250
≥3 (%)	981 (89%)	423 (84%)	225 (89%)	148 (97%)	88 (96%)	46 (96%)	51 (98%)
<3 (%)	76 (6.9%)	41 (8.2%)	27 (11%)	4 (2.6%)	2 (2.2%)	2 (4.2%)	0 (0%)

*Note: ~ 30% of the cohort were a group of high risk men from Puerto Rico not previously screened.

miR Sentinel™ v1.0.4.4 cross validation study

Pathology-based Classification	Total Participants (row %)	Sentinel Test Classification v1.0.4.4 Frequency Counts			
		Mol-NR	Mol-LR	Mol-IR	Mol-HR
Biopsy Negative	502 (46)	51	113	245	93
GG1	253 (23)	6	87	133	27
GG2	153 (14)	1	27	103	22
GG3	92 (8)	2	17	44	29
GG4	48 (4)	0	7	22	19
GG5	52 (5)	0	5	17	30
TOTAL (column %)	1100	**60 (6)**	**256 (23)**	**564 (51)**	**220 (20)**
TOTAL (excluding NMEPC) (column %)		**--**	**143 (24)**	**319 (54)**	**127 (22)**

Performance Metrics

- For 755 men with Bx neg or GG1
  - 34% had Mol NR/LR
  - 66% had Mol IR/HR result
- Diagnostic Sensitivity:
  Prob(LR/IR/HR with Bx GG1-5)
  589/598 = 98.5%
- Prognostic Sensitivity:
  Prob(Mol-IR or HR with Bx GG2-5)
  286/345 = 83%

Comparison of miR Sentinel™ v.1.0.4.4 versus systematic-guided or MRI-targeted biopsy

miR Sentinel™ v1.0.4.4 Classification Frequency Counts					
	Number	Mol-NR	Mol-LR	Mol-IR	Mol-HR
A. Systematic negative; MRI-targeted positive					
GG1	21	0	6	14	1
GG2	12	0	1	7	4
GG3-GG5	12	0	2	4	6
TOTAL	45	0	9	25	11
B. Systematic positive; MRI-targeted negative					
GG1	21	1	6	11	3
GG2	3	0	0	3	0
GG3-GG5	3	0	0	2	1
TOTAL	27	1	6	16	4

- 356/1100 = 32% of patients in the training dataset had TRUS-guided **and** MRI-targeted biopsies
- 72/(356-122) = 31% were biopsy negative with one technique and positive with the other
- 71/72 = 99% observed misses by either TRUS or MRI identified by miR Sentinel™

This suggests that, in high-risk patients, a positive Mol-HR test result in the face of a negative biopsy accurately predicts for the presence of significant cancer.

Comparison between biopsy GG and miR Sentinel™ in serum PSA < 3 ng/mL stratum

	N	Mol-NR	Mol-LR	Mol-IR	Mol-HR
PSA < 3 ng/mL					
NPEPC	**41**	3	10	22	6
GG1	**27**	0	11	12	4
GG2	**4**	0	1	1	2
GG3-GG5	**4**	0	0	3	1
Total	**76**	**3**	**22**	**38**	**13**

These data demonstrate that miR Sentinel™ identifies patients with high-risk disease that would otherwise be missed using the threshold of PSA > 3.0 ng/mL for triggering a biopsy

This confirms that miR Sentinel™ is independent of PSA level.

- 8/76 = 11% of patients with PSA levels < 3.0 ng/mL have pathological evidence of GG2-GG5

- 7/8 cases with PSA < 3.0 ng/mL and cGG2-GG5 have miR Sentinel™ classification of Mol-IR or Mol-HR.

Summary

- The miR Sentinel™ Prostate Cancer Test offers a non-invasive test for accurately identifying, classifying and monitoring patients
- Validation data based substantially on a large high risk Puerto Rican population
- miR Sentinel™ identifies patients at risk for prostate cancer who may be unsuspected due to PSA < 3.0, or who had a negative biopsy
- In this study, < 2% of prostate cancers identified by biopsy were missed by miR Sentinel™
- 87% of patients with PSA < 3 ultimately found to have GG2-5 were identified by miR Sentinel™ as intermediate or high-risk
- Further studies in progress to clarify the significance of the high proportion of men with negative biopsies and positive test results