Biomarcadores urinarios para la vigilancia en cáncer de vejiga no músculo-invasivo

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Conceptos básicos elementales de Biomarcadores

Diagnóstico: detección de la enfermedad
Pronóstico: Resultado probable de la evolución de una enfermedad; la probabilidad de recuperación o de que la enfermedad reaparezca. Directamente asociado a características patológicas de la enfermedad

Predictivo (de respuesta): probabilidad de que el paciente responda a una cierta terapia

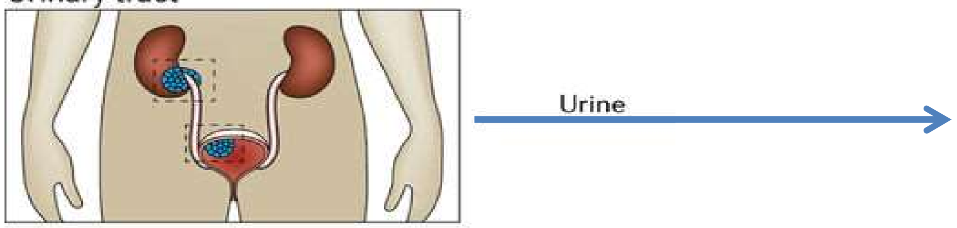
Sensibilidad: describe qué tan bien una prueba puede detectar una enfermedad o afección determinada en las personas que tienen la enfermedad

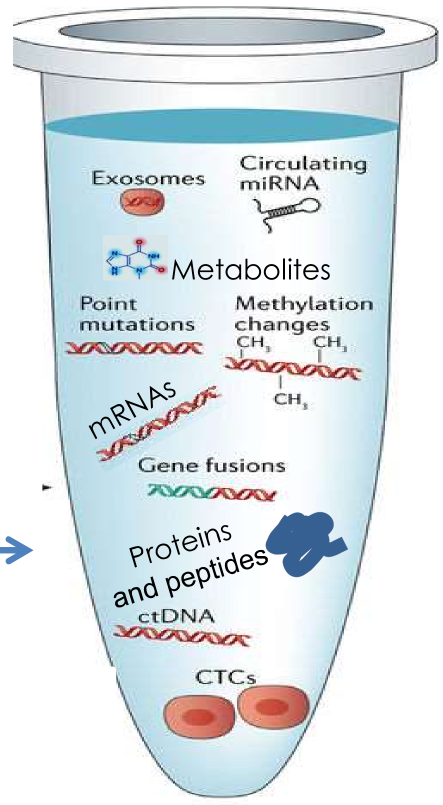
Especificidad: Frecuencia en la que una prueba produce resultados negativos verdaderos en las personas que no tienen la enfermedad o la variante genética en estudio.

Valor predictivo negativo: Probabilidad de que una persona con un resultado negativo en una prueba verdaderamente no esté afectada o no tenga la mutación génica en estudio

Valor predictivo positivo: Probabilidad de que un individuo con prueba positiva tenga la enfermedad.

Liquid Biopsy for Detection, Prognosis and Monitoring Bladder Cancer: Urine





Nature Reviews | Clinical Oncology

Urine is an ideal biofluid to analize multiple biomarkers for GU tumors

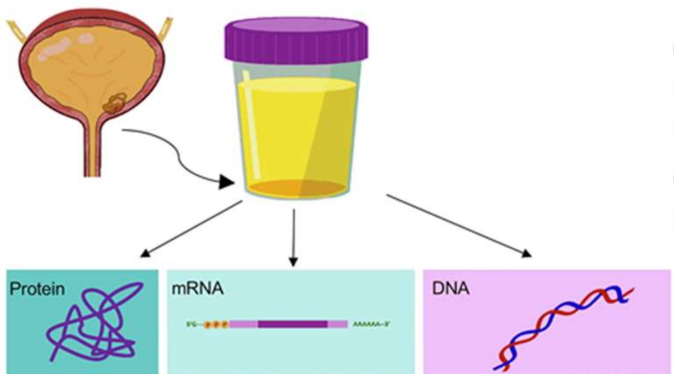
Optimal Biomarker:

- Performance over previous diagnostic (prognostic, predictive) procedures
- Simplicity of the analysis (including costs)

Novel Urinary Biomarker Tests in Non–muscle-invasive Bladder Cancer

Xpert utilizes the Cepheid GeneXpert Instrument Systems to measure the expression of five mRNA targets in a voided urine sample in a self-contained cartridge.

ADXBladder is an ELISA of urine MCM5 levels



	ADXBLADDER	Xpert bladder cancer	Cxbladder Monitor	Uromonitor	Bladder EpiCheck
Cystoscopies avoided (FN + TN)	579	689	500	706	740
Recurrence missed (FN)	78	51	15	10	47
Unnecessary cystoscopies (FP)	319	182	335	124	127
Recurrence diagnosed (TP)	102	129	165	170	133

Per 1000 patients with any-grade NMIBC.
Calculated with the pooled recurrence rate of 18%

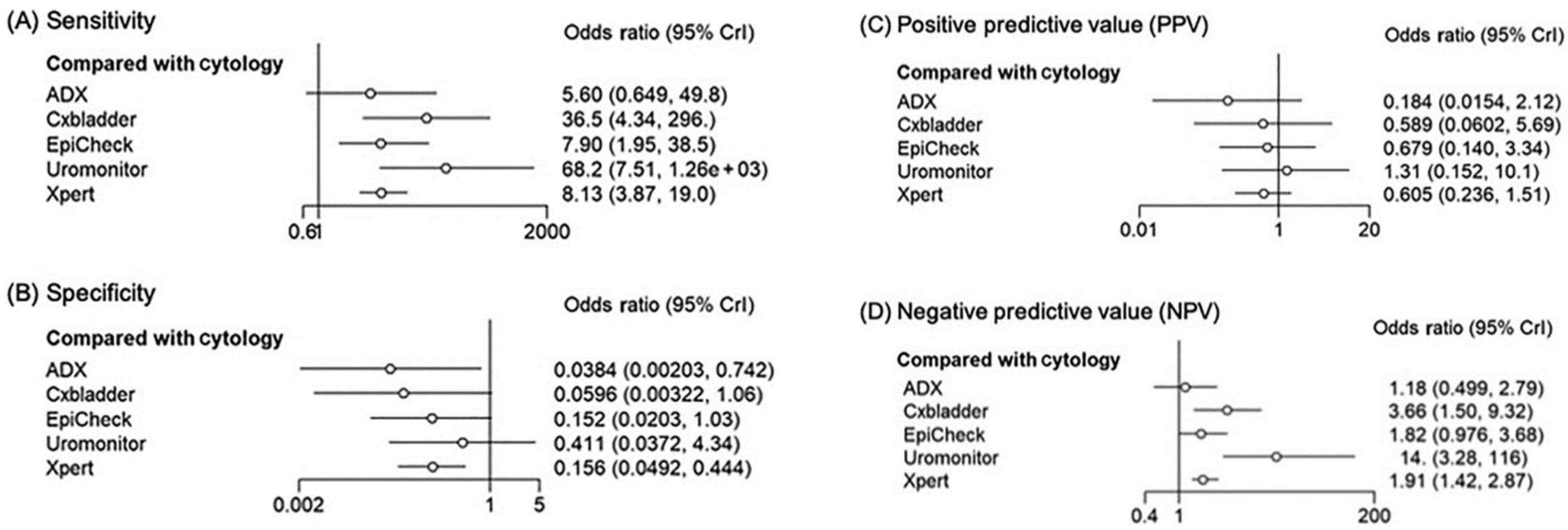
Cxbladder combines clinical risk factor markers with genetic information, measuring five biomarker genes to detect the presence or absence of bladder cancer.

Uromonitor is an urine test for FGFR3 and Tert promoter mutations

Epicheck is a methyl PCR test analyzing 15 probes and integrating clincal data. Not for LG Ta tumors

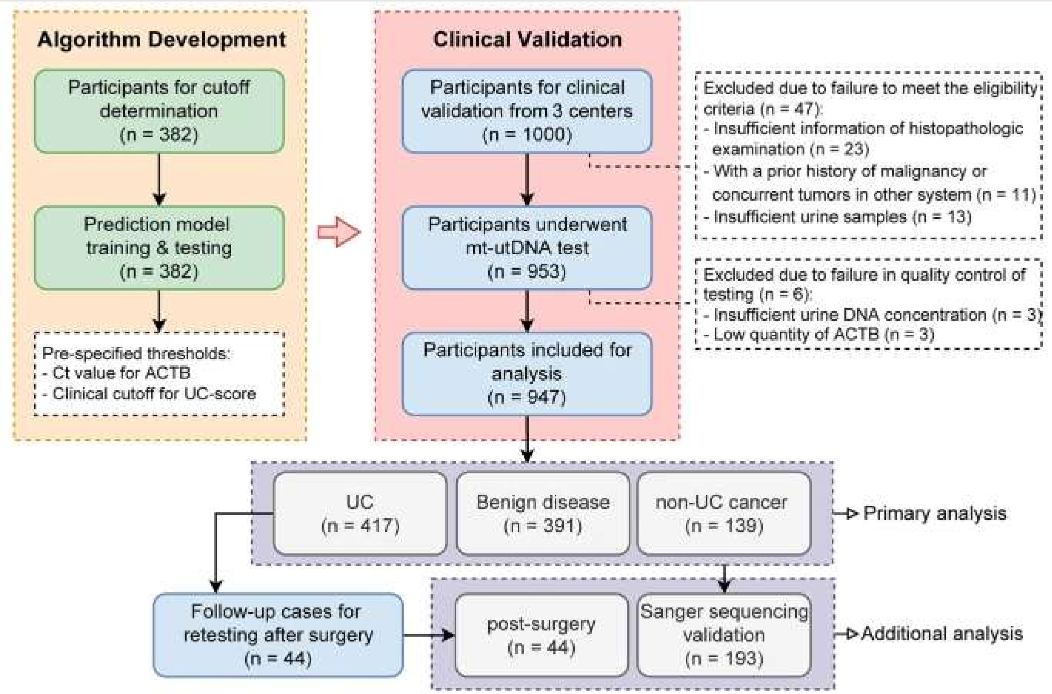
Twenty-one studies, comprising 7330 patients

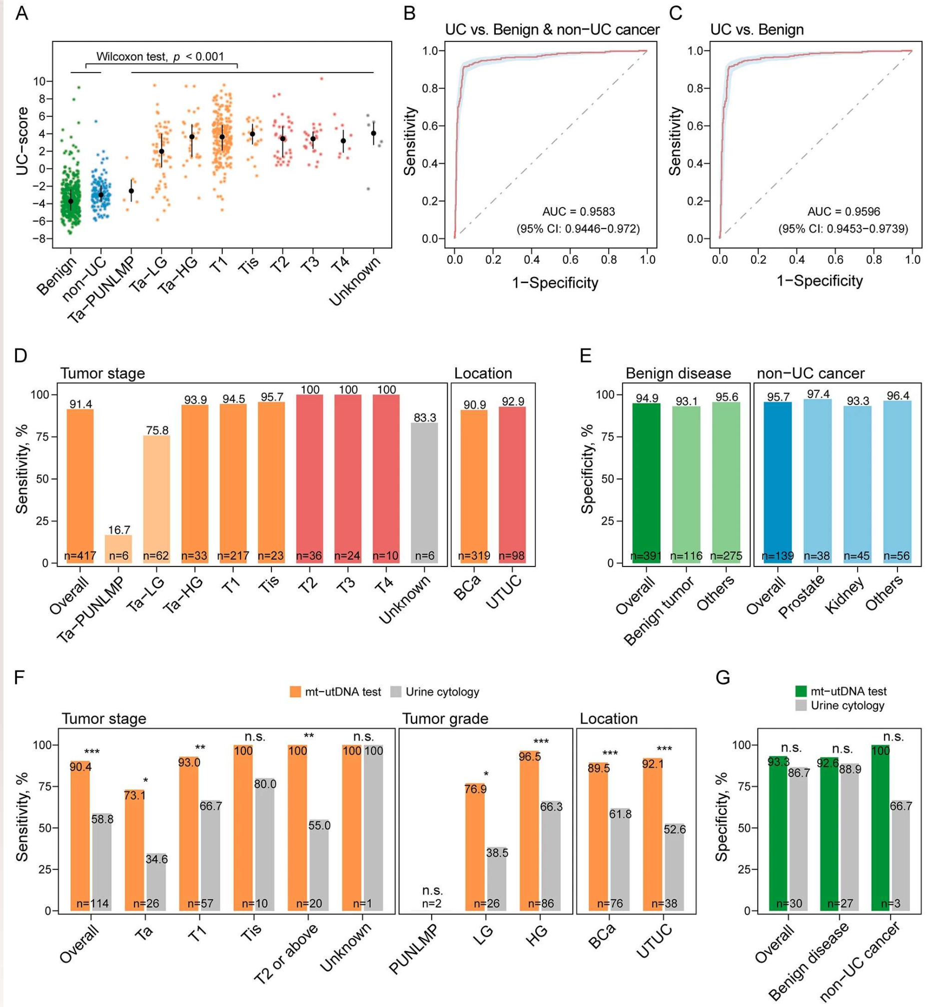
Laukhtina et al., European Urology Oncology 2021 4927-942



Better biomarkers are needed!!!

A novel multitarget utDNA (mt-utDNA) test involving assays of FGFR3 and TERT mutations and methylation of ONECUT2 and VIM was developed to facilitate the early detection of UC





Clinical effectiveness of a multitarget urine DNA test for urothelial carcinoma detection: a double-blinded, multicenter, prospective trial Junlong Wu, et al. Molecular Cancer 23 : 57 (2024)

MiRNAs

- Very stable and present in every tissue sample.
- It could be purified from frozen and fresh tissue, plasma, Serum, Saliva, Urine, FFPE.
- Can efficiently classify different tumors (better sensibility than mRNA profiles)
- It shows low inter-individual variability.

Drawback.

Low expression levels.

Study 1. Compare RNA molecules in urine as good biomarkers.

✓ Evaluate mRNA, RNU and miRNA.

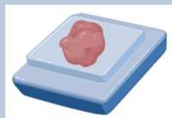
✓ Use three mRNAs (normalizers), three RNU (normalizers), three miRNAs non associated to BC.

miRNA are stable and homogeneous in urine

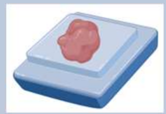
Donors	25
Age	52,9 (19-69)
Gender	14 Male
	11 Female (3 pregnant)
Smoker	4
Chronic Disease	11

Martinez et al., J.Mol.Diag 2016

Phase I. Identification of differentially expressed miRNAs in tissue samples by microarrays expression data (n=38)



Phase II. Validation of differentially expressed miRNAs by RT-qPCR using an extended cohort (n=96)



Phase III. Validation of differentially expressed miRNAs by RT-qPCR in urine samples (n=232)

Suárez-Cabrera et al., Eur Urol 2022

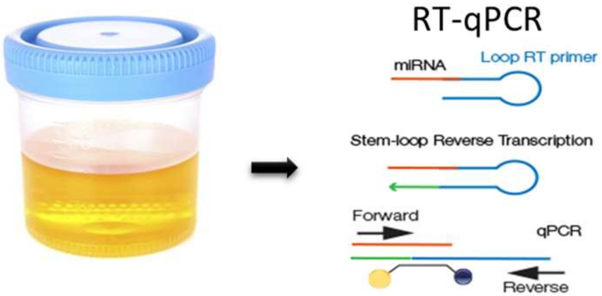
Discovery cohort of tissue samples (n = 38)	
10 normal tissues	28 tumor tissues

Validation cohort of paired tissue samples (n = 82)	
41 normal urothelium	41 tumor samples

Proof of concept cohort of urine samples (n = 148)	
24 healthy donors	124 BC patients

Blinded validation cohort of urine samples (n = 84)			
42 urological controls		42 BC patients	
21 incidents	21 recurrents	21 incidents	21 recurrents

Cohort 3: Proof-of-concept cohort of urine samples (n = 143)			
Age	**Gender**	**Tumor Stage**	**Tumor Grade**
24 healthy donors			
61 (49-78)	M (n=20) F (n=4)		
119 BC patients			
64 (53-83)	M (n=93) F (n=26)	Ta (n=80) T1 (n=20)	HG (n=50) LG (n=69)



Non-invasive

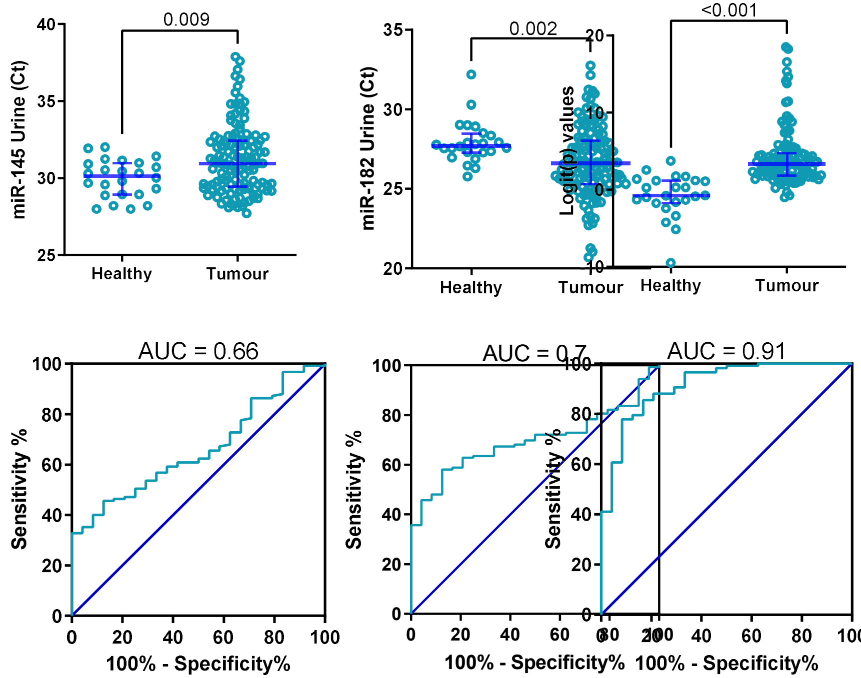
BlaDimiR

Suppl. Figure 1. Logistic regression analysis of 5 best miRNA predictors of BC in urine (cohort 3)

Logistic regression analysis of selected miRNA predictors of BC in urine (cohort 3)

Variables	β coefficient	SE β coef.	t	P	OR (95% CI)
miR-145	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
miR-125b	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
miR-182	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
miR-99a	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Constant	-0.48	0.63	-3.3	<0.01	0.046 (0.0058-0.37)
miR-96	-0.42	0.25	-1.7	0.093	0.54 (0.28-1.03)

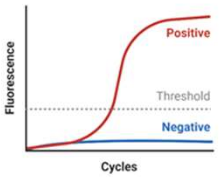
SE = standard error; t = t-statistic; P = *p-value*; OR = odds ratio; CI = confidence interval

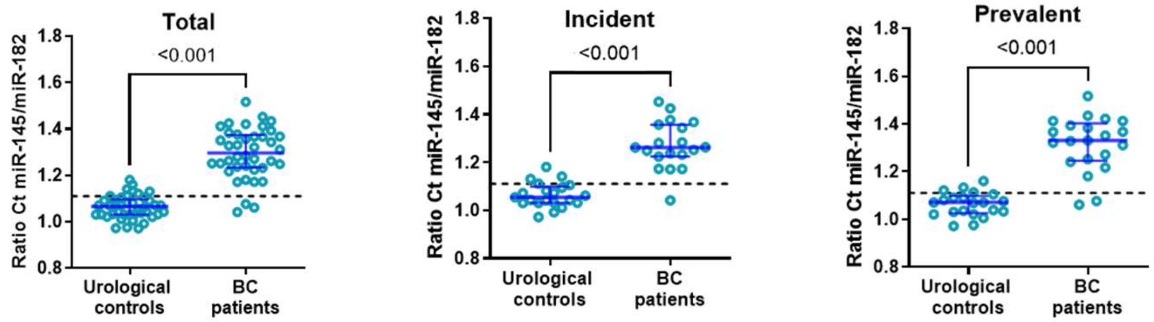


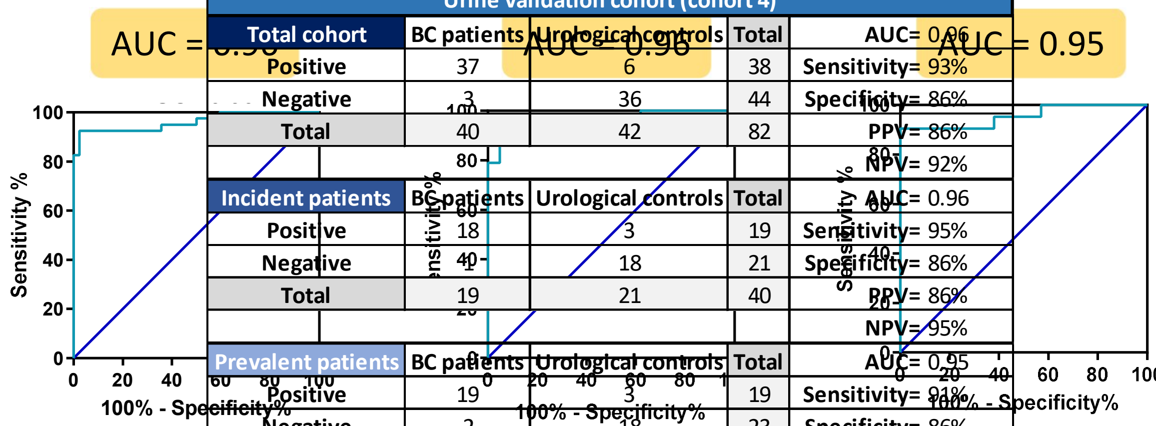
Suárez-Cabrera et al., EurUrol 2022

Cohort 3: Proof-of-concept cohort of urine samples (n = 143)			
Age	**Gender**	**Tumor Stage**	**Tumor Grade**
24 healthy donors			
61 (49-78)	M (n=20) F (n=4)		
119 BC patients			
64 (53-83)	M (n=93) F (n=26)	Ta (n=80) T1 (n=20)	HG (n=50) LG (n=69)

Cohort 4: Blinded validation cohort of urine samples (n = 82)			
Age	**Gender**	**Tumor Stage**	**Tumor Grade**
42 urological controls			
21 incidents and 21 prevalents			
69.5 (41-89)	M(n=31) F(n=11)		
40 BC patients			
19 incidents and 21 prevalents			
Age	**Gender**	**Tumor Stage**	**Tumor Grade**
72.50 (35-95)	M(n=36) F(n=4)	Ta (n=18) T1 (n=6) T2 (n=5) T4 (n=1) pTIS (n=2) Tx (n=7) NA (n=1)	HG (n=15) LG (n=22) NA (n=3)



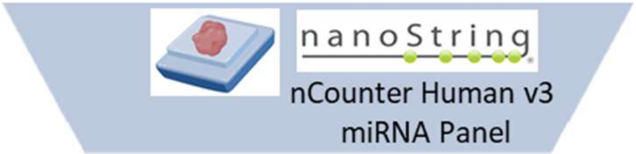




Urine validation cohort (cohort 4)				
Total cohort	**BC patients**	**Urological controls**	**Total**	**AUC= 0.96**
Positive	37	6	38	**Sensitivity= 93%**
Negative	3	36	44	**Specificity= 86%**
Total	40	42	82	**PPV= 86%**
				NPV= 92%
Incident patients	**BC patients**	**Urological controls**	**Total**	**AUC= 0.96**
Positive	18	3	19	**Sensitivity= 95%**
Negative	1	18	21	**Specificity= 86%**
Total	19	21	40	**PPV= 86%**
				NPV= 95%
Prevalent patients	**BC patients**	**Urological controls**	**Total**	**AUC= 0.95**
Positive	19	3	19	**Sensitivity= 91%**
Negative	2	18	23	**Specificity= 86%**
Total	21	21	42	**PPV= 86%**
				NPV= 90%

Suárez-Cabrera *et al.* 2022. Euro. Urology

Phase I. Identification of differentially expressed miRNAs in tissue samples by Nanostring miRNA panel (n=39)



Phase II. Validation of differentially expressed miRNAs by RT-qPCR (n=43)

Phase III. Proof of concept validation of differentially expressed miRNAs (PreTURBT) (n=18)

Phase IV. Validation of differentially expressed miRNAs (PreTURBT and PreBCG) (n=143)

BCG Response Predictor

Discovery cohort on tissue samples (n = 39)

14 Tumour (non-responders)	25 Tumour (responders)

Validation cohort on tissue samples (n = 43)

11 Tumour (non-responderse)	32 Tumour (responders)

Proof-of-concept cohort on urine samples (pre-TURBT; n = 18)

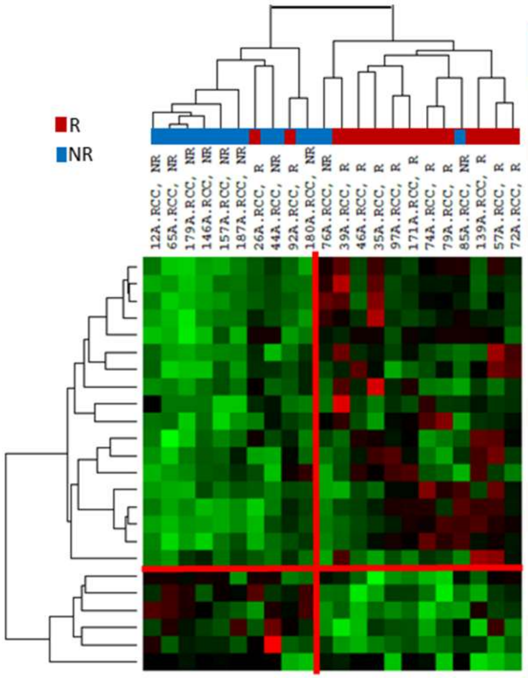
8 Non-responders	10 Responders

Validation cohort on urine samples (n = 143)

Pre-TURBT	Pre-treatment
28 Non-responders 56 Responders	17 Non-responders 42 Responders

BCG Response Predictor.

Discovery & tissue validation



NR: recurrence in 2 yrs

Validation

Differentially Expressed miRNAs	Fold Change R *vs* NR	*p*-value *NanoString*	*p*-value RT-qPCR
hsa-let-7c-5p	-2.11	0.00109	0.013
hsa-miR-29a-3p	-2.05	0.00230	0.050
hsa-miR-222-3p	-1.74	0.00517	0.015
hsa-miR-199a-3p/miR-199b-3p	-2.28	0.00601	0.039
hsa-miR-23a-3p	-1.72	0.00642	0.032
hsa-miR-223-3p	-4.16	0.00942	0.006
hsa-miR-199b-5p	-2.26	0.01039	0.033
hsa-miR-218-5p	-3.20	0.01091	NS
hsa-miR-125b-5p	-3.34	0.01321	NS
hsa-miR-23b-3p	-1.77	0.01353	0.033
hsa-miR-199a-5p	-1.84	0.01443	0.041
hsa-miR-1-3p	-7.28	0.01735	0.048
hsa-miR-483-3p	6.72	0.01885	0.025
hsa-miR-145-5p	-3.12	0.02327	0.030
hsa-miR-106a-5p/miR-17-5p	2.06	0.02724	0.036
hsa-miR-4443	1.79	0.02726	0.024
hsa-miR-874-5p	3.37	0.02841	0.025
hsa-miR-497-5p	-2.24	0.03099	NS
hsa-miR-143-3p	-3.14	0.03452	0.046
hsa-miR-574-3p	-2.91	0.03465	NS
hsa-miR-579-3p	20.37	0.03616	0.027
hsa-miR-342-3p	-1.65	0.03671	NS
hsa-miR-182-5p	2.03	0.03870	0.042
hsa-miR-21-5p	-2.34	0.03730	0.023
hsa-miR-429	2.24	0.04484	0.050
hsa-miR-99a-5p	-5.30	0.04592	0.047

Validation: 21 DE miRNAs (7 upregulated and 14 downregulated)

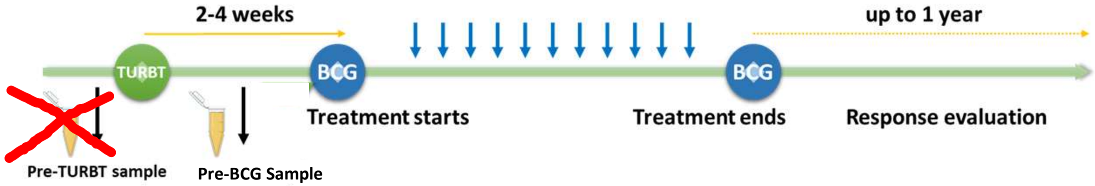
Discovery

26 miARNs DE
7 induced
19 repressed

Validation

21 miARNs
7 induced
14 repressed

National patent (ES2856232)
PCT/ES2021/070204



Best ratios

Variable	AUC	Sensitivity (%)	Specificity (%)	*p* -value
miR-223-3p	0.71	87.5	70.0	0.131
miR-483-3p	0.79	62.5	90.0	0.041
miR-579-3p	0.83	75.0	90.0	0.021
miR-223-3p/miR-483-3p ratio	0.90	87.5	80.0	0.005
miR-223-3p/miR-579-3p ratio	0.90	100.0	80.0	0.005

Logistic Regression combined ratios

Variable	β coefficient	SE β coefficient	*p* -value
miR-223-3p/miR-182-5p ratio	18.015	11.486	0.117
miR-223-3p/miR-483-3p ratio	-53.331	32.959	0.106
Constant	30.771		

AUC = 0.95; *p* -value = 0.0004

Estudio clínico prospectivo multicéntrico

Norma ISO13485, SpinOff, Marcado CE, marketing y comercialización

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And patients and their families!!!