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THE CANCER THERANOSTIC COMPANY

International User Group Meeting

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Can DNA Biomarkers enhance Non-Muscle Invasive Bladder Cancer Surveillance? Evaluating the efficacy of Multimodal Genomic Profiling for bladder cancer recurrence and progression: A Prospective Longitudinal Study

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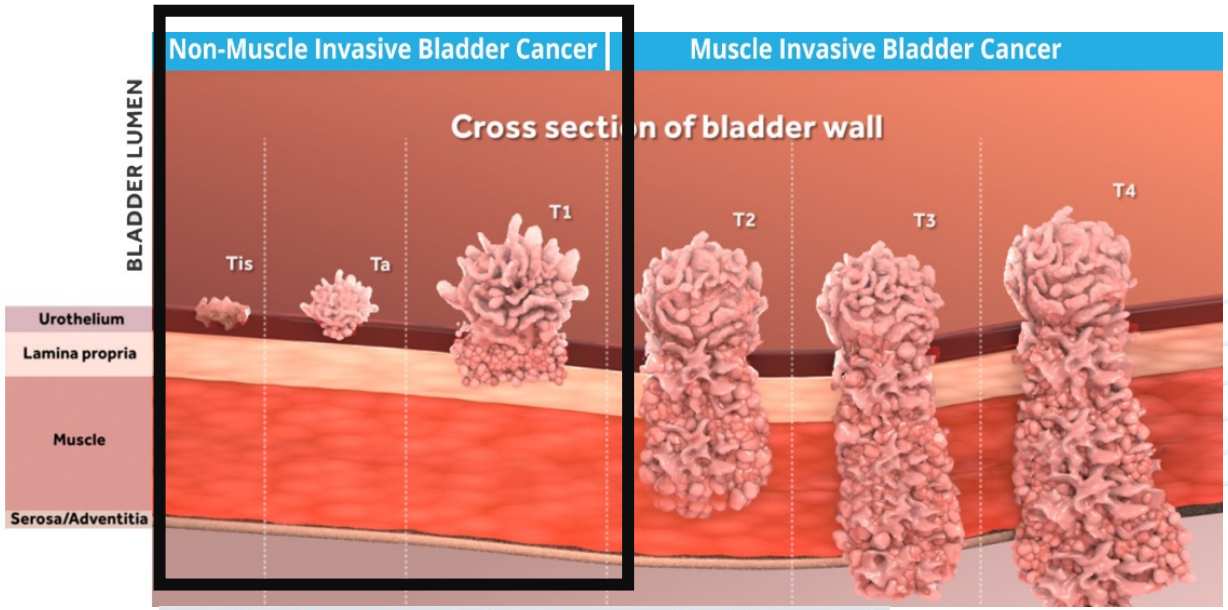
Overview

- Non-muscle invasive bladder cancer (NMIBC)
  - Diagnosis, management and monitoring
- Genomic landscape of NMIBC
- Summary of the project aims and objectives
- Preliminary data of urine samples sequenced using the OncoDEEP assay
- Comparison against PCR assays for bladder cancer surveillance

Bladder cancer

- 9th most common type of cancer in men and women, strongly associated with smoking
- Begins in the lining of the bladder
  - present with haematuria or lower urinary tract symptoms
- Non-muscle invasive bladder cancer (NMIBC) stays in the bladder lining
- Muscle invasive bladder cancer (MIBC) spreads into the muscle
- Transurethral resection bladder tumour (TURBT) – to remove the tumour(s)
- Radical cystectomy in high/very high risk



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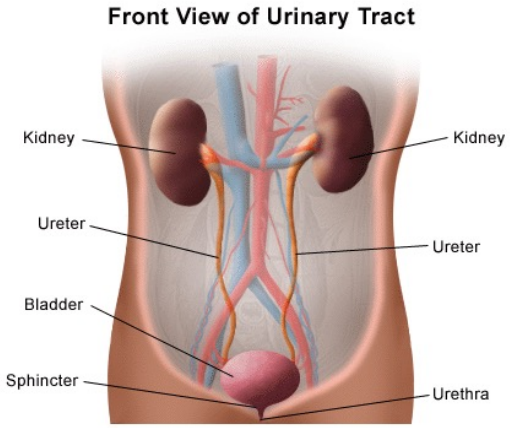
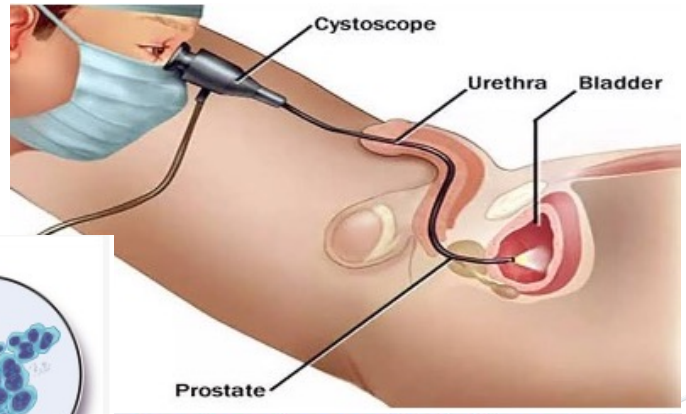


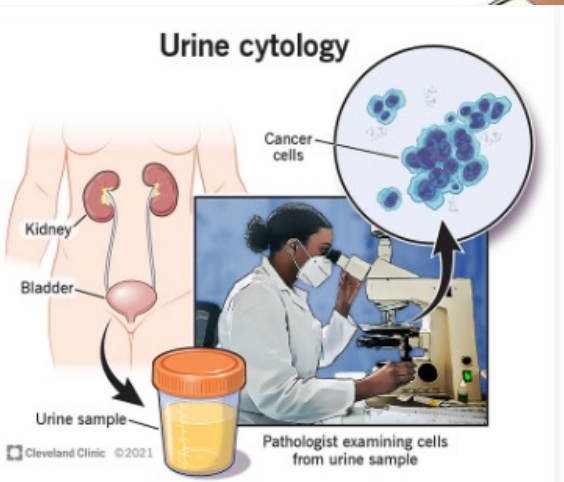
hopkinsmedicine.org/health/wellness-and-prevention/anatomy-of-the-urinary-system

Monitoring NMIBC patients after TURBT

- High chance of recurrence or progression (50%)
- Frequent check-ups needed every few months
- Up to 5 years
- Cystoscopy (standard of care)
  - Painful, infections, anaesthetic
  - Healthy vs. cancer tissue
- Urine cytology (standard of care
  - Some cancers can be missed
  - Easy to give urine
- Biopsy - Histology (SOC)



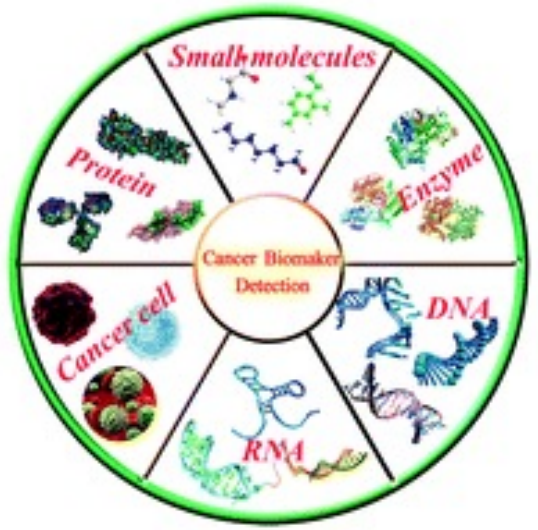
edgbastonwellness.co.uk/flexible-cystoscopy/



my.clevelandclinic.org/health/diagnostics/22942-urine-cytology

Monitoring NMIBC using biomarkers

- Many biomarkers for NMIBC available using urine
  - Proteins, **DNA**, RNA, enzymes
  - Some FDA approved
  - **None included in professional guidelines or as part of routine care**
  - Too sensitive – detect inflammation
- Unmet need
  - A test to predict recurrence of low-grade NMIBC
  - It needs to be easy and fit in the clinical workflow
  - Implement into NHS / Healthcare system
  - Cost effective
  - Reduce the number of cystoscopies



Why urine?

- Exfoliated urothelial cells from the bladder wall are shed into urine
- Malignant and normal cells
- Good quality DNA
- Non-invasive
- Patient already giving a urine sample in the clinic during check up
- Urine sample can be done at home and sent straight to GP/laboratory
- Reduce hospital visits

Research Aim and Objectives

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AIM

To address the unmet need of a DNA biomarker with adequate sensitivity and specificity

To predict which patients are likely / unlikely to have a recurrence using DNA biomarkers/assays

OBJECTIVES

- To discover the genomic profile of patients with NMIBC in urine and tissue
- To evaluate different DNA assays and compare to the current standard of care
  - qPCR assay; dPCR assay; OncoDEEP NGS panel

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Research Questions and Outcomes

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Research Questions

- Are biomarkers better than or equivalent to cystoscopy with cytology in detecting recurrence?
- Is there a place for NGS within the clinical pathway for NMIBC?

OUTCOMES

- A change in monitoring strategy can:
  - reduce inefficiencies – time and cost
  - reducing the number of cystoscopies and patient footfall in the clinic.

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Impact and added value for patients

- **Short-term benefits**
  - Improved early detection of NMIBC recurrence
  - Urine test is easy and doesn't hurt – less cystoscopies
  - Less trips to the hospital

- **Long-term benefits**
  - Form part of routine care
  - NHS Genomic Test Directory – NHSE long term plan
  - Treatment options and response to treatment
  - Certain genetic alterations could be used to assess severity of cancer

Most common mutations in NMIBC

- Highly heterogenous disease
- Studies varied greatly – testing time-point, assay used, genes tested, cohort size, grade and stage of tumour
- 70-80% of NMIBC harbour at least one somatic mutation

Mutually exclusive genes	Co-occurring mutations
FGFR3 (28%)	*TERT* promoter (35-80%)
RAS (NRAS, HRAS, KRAS) (10%)	*PIK3CA* (26%)
ERBB2 (10%)	*TP53* (45%)
MAP Kinase Pathway genes	*KDM6A, ARID1A* (chromatin modifying)
	BRCA1/2, ATM, CHEK1, FANCC, FANCA (DNA damage repair genes) *CDKN2A, CDKN2B, MDM2* (Cell cycle genes) – often more high risk or muscle

Targets detected by each assay

q PCR assay	d PCR - assay	OncoDEEP NGS
TERT promoter hotspots c.-124C>T; c.-146C>T **LOD: 6.25%**	*TERT* promoter hotspots c.-124C>T; c.-146C>T **LOD: AF 0.5**	*TERT* promoter, *FGFR3, KRAS* hotspots Alpha list – many oncogenic hotspots **LOD: >12 reads and >1% VAF**
FGFR3 hotspots Codons 248, 249, 372, 375 **LOD: 3.125%**	*FGFR3* hotspots Codons 248, 249, 372, 375 **LOD: AF 0.5**	638 Genes; CNVs, Fusions, TMB, HRD, MSI
KRAS hotspots Codons 12, 13, 61 **LOD: 6.25%**		All genes associated with NMIBC **LOD: >20 reads and >5% VAF**
Adv: Widely used, accessible in most laboratories, can multiplex, cheapest	Adv: Detect low abundance targets; MRD; ctDNA, Single targets, multiplex?	Adv: Can sequence large number of genes at once
Not sensitive!	Cheap	Costly compared to PCR

LOD: Limit of detection; AF: Allele fraction; VAF: Variant Allele Frequency

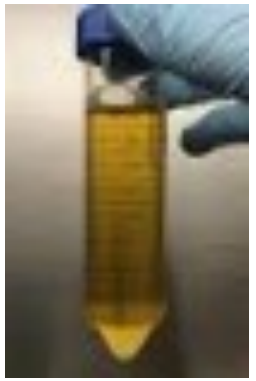
Methods – Patient cohort & sample size

- Preliminary data for OncoDEEP

- Verification cohort **n=14**
- interlaboratory comparison of qPCR and dPCR assays
- Orthogonal testing using OncoDEEP
- Urine only

- First set of Biobank patients **n= 16**
- Initial TURBT FFPE tissue (Histology) – Gives grade and stage of tumour
- Urine samples – taken at different stages often before cystoscopy or surgery for radical cystectomy
- Urine + FFPE

Methods



- Urine filtration kits (n=14)
- Biobank samples (n=16) Urine cell pellet

- DNA 30-80ng input
- Library prep – manual/automation
- Sequencing – Illumina Novoseq 6000

- Data analysis - OncoKDM

Urine NGS results N=30

- 32 Urine samples sequenced by OncoDEEP
- 2 samples failed outright with no data available
- 2 samples failed due to low coverage overall <150x
  - However key regions / hotpots in *TERT, FGFR3, KRAS, PIK3CA* >80x
  - Eligible for analysis*
- All cases had low TMB <7mut/Mb

UROMONITOR vs OncoDEEP

Patient	Tumour present at time of urine	qPCR results	Mutation	OncoDEEP results	Mutation	Digital PCR results	Mutations
1	Yes	Positive	*TERTp* c.-124C>T	Positive	TERTp c.-124C>T (27.7%)	Positive	TERTp c.-124C>T
2	No	Negative	-	Negative	-	Negative	
3	No	Negative	-	Negative	-	Negative	
4	No	Negative	-	Fail	*FGFR3* exon 7 83x NMD	Positive	c.746C>G, p.(S249C)
5	No	Negative	-	Positive	TERTp c.-124C>T (17.49)%	Positive	TERTp c.-124C>T
6	Yes	Positive	*FGFR3* c.1108G>T, p.(G370C)	Positive	TERTp c.-124C>T 7.42% FGFR3 c.1108G>T, p.(G370C) 8.71%	Positive	TERTp c.-124C>T FGFR3 c.1108G>T, p.(G370C)
7	No	Negative	-	Negative	-	Negative	-
8	Yes	Negative	-	Positive	TERTp c.-124C>T (3.58%)	Negative	-
9	No	Negative	-	Negative	-	Negative	-
10	No	Negative	-	Negative	-	Negative	-
11	No	Negative	-	Negative	-	Negative	-
12	Yes	Negative	-	Positive	TERTp c.-124C>T (3.74%)	Positive	TERTp c.-124C>T
13	Yes	Negative	-	Positive	FGFR3 c.1111A>T, p.(S371C) (38.95%)	Negative	
14	Yes	Negative	-	Positive	TERTp c.-124C>T (2.79%) FGFR3 c.1118A>G, p.(Y373C) (8.25%)	Positive	FGFR3 c.1118A>G, p.(Y373C)
15	NO	Negative		Negative		insufficien	DNA

Results – Assay comparison

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- N= 15 (including 1 failed sample)
- 6 Tumour present (histology)
- 9 No tumour present (cystoscopy)
- qPCR
  - Detected only 2/6 tumours (P1, P6)
- OncoDEEP
  - Detected mutations in all 6 confirmed tumours
  - One discordant (P5) – developed recurrence 14 months
  - One failed sample (P4) – see below
- Digital PCR
  - Detected 4/6 tumours
  - Patient 13 – FGFR3 S371C not covered by assay
  - Patient 8 – Did not pick up TERTp
  - ?False positive (P4) – FGFR3 S249C not detected by other assays

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Results – Positive urine samples by OncoDEEP

Tumour present at time of Urine	OKD result	*TERTp NM_198253.3*	*FGFR3 Canonical NM_000142.5*	*PIK3CA NM_006218.4*	Other oncogenic variants	Grade (WHO 1973)	Grade (WHO 2004)	Stage
Yes	Positive	c.-124C>T (27.7%)	-	-	*FANCA* (NM_000135.4) c.1917_1927delinsTAGGGCATAAG, p.(Glu642*) (24.71%)	G3	HG	pT2
Yes	Positive	c.-124C>T 7.42%	c.1108G>T 8.71%	-	*CDKN1A* (NM_001220778.2) c.146G>A, p.(Trp49*) (9.81%)	G2/3	HG	pT2
Yes	Positive	c.-124C>T (3.58%)	-	-	*FANCA* (NM_000135.4) c.2853-1G>A (6.06%)	G1	LG	pTa
Suspicious	Positive	c.-124C>T (3.74%)	-	-	None	No histology. Suspicious on cytology		
Yes	Positive	-	c.1111A>T (38.95%)	-	*FANCL, PTEN* - copy number loss (<1.6) *FRS2, MDM2* - copy number amplification (>6.0)	G3	HG	pTa
Yes	Positive	c.-124C>T (2.79%)	c.1118A>G (8.25%)	c.1624G>A, p.(Glu542Lys) (7.1%)	*KMT2D* (NM_003482.4) c.4219dup, p.(Tyr1407Leufs*25) (13.23%)	G2	LG	pTa
Yes	Positive	c.-124C>T (8.78%)	-	-	*TP53 c.524G>A, p.(Arg175His) 13.27%*	G3	HG	pT1
Yes	Positive*	c.-124C>T (10.85%)	-	-		G3	HG	pT1
Yes	Positive	-	-	c. p.(Glu545Lys) 1.36%		G3	HG	pT1
No	Positive	c.-124C>T (17.49)%	-	-		G2	HG	pTa
No (CIS)	Positive	c.-124C>T (7.52%)	-	-	BAP1 c.1547dup, p.(Thr517Aspfs*20) 18.87% RUNX1 c.1011dup, p.(Ala338Argfs*262) 40% SETD2 c.3790C>T, p.(Gln1264*) 14.42%	G3	HG	pT1
Frequency		9	3	2				

True positive = 9/10

- = hotspot driver mutation detected

Discordant = 2

- One with residual CIS

10 confirmed tumour present by histology at time of urine taken (or within 3 weeks)

Results – Tissue and urine by OncoDEEP

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Grade and stage at diagnosis				Tissue testing OnoDEEP				Urine testing OncoDEEP				
Sex	Grade (WHO 1973)	Grade (WHO 2004)	Stage	OKD tissue result	TERTp NM_198253.3	FGFR3 NM_000142.5	KRAS/ PIK3CA	OKD result	TERTp NM_198253.3	FGFR3 NM_000142.5	KRAS/ PIK3CA	Any confirmed tumour /suspi
Male	G1	LG	pTa	FAIL	FAIL	FAIL	FAIL	Fail	Fail	Fail	Fail	Yes
Male	G1	LG	pTa	Fail	fail	FAIL	fail	Positive	c.-124C>T (3.58%)			Yes
Male	G3	HG	pTa	Positive		S371S		Positive		S371S		Yes
M	G1	LG	Ta	Positive			KRAS G12V	Negative*				No
M	G3	HG	T1	Positive		R248C		Negative				No
M	G3	HG	T1	FAIL	FAIL	FAIL	FAIL	Negative				No (CIS)
M	G2	HG	T1	Negative				Negative				No (CIS)
M	G3	HG	T1	Positive	124			Negative				No
M	G2-3	HG	Ta	Positive	124	FGFR3::TACC3		Negative				Yes
M	G3	HG	T1	Positive	124	S249C & Y373C		Negative				No
F	G3	HG	T1	Positive	124		PIK3CA E545K	Positive			PIK3CA E545K	Yes
M	G3	HG	Ta	Positive	124			Negative				No
F	G3	HG	Ta	Positive	124	S249C		Negative				No

- N=13
  - Tissue + urine
- False negative = 1/10
  - Radical cystectomy 2 weeks later showed residual disease
  - Original sample had a FGFR3::TACC3 fusion and *TERT* promoter 124
- N=2
  - Same variant present in tissue and urine

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Statistical analysis

	OncoDEEP Verification		
	Positive	Negative	Total
TOTAL	11	19	**30**
No cancer	2	18	20
Cancer	9	1	10

Sensitivity	90%
Specificity	90%
PPV	82%
NPV	95%
Accuracy	90%

	Assay comparison			
	n= 104 q PCR	n=96 d PCR	n = 64 Cytology	**n = 30 OKD**
Sensitivity	36%	60%	52%	90%
Specificity	90%	90%	91%	90%
PPV	67%	78%	73%	82%
NPV	72%	80%	80%	95%
Accuracy	68%	79%	78%	90%

Conclusions

- Pathogenic hotspots in all urine were confined to
  - *TERT* promoter; *FGFR3; PIK3CA*
- Additional variants in DNA repair, chromatin modifying and cell cycle genes where frequently present in tissue
- Findings are consistent with the literature
- Preliminary data shows the OncoDEEP assay is superior to hotspot PCR assays in detecting tumour either at diagnosis or recurrence of NMIBC in urine

Limitations and barriers

- Small sample size for OncoDEEP (n=30)
- Limited number of low-grade samples available
- Ethics approval and consenting patients – unexpected delays
- Are we really reducing inefficiencies? Or are we taking them from one area and pushing them onto another?

Special thanks to

- Cancer Genetics, Synnovis, London
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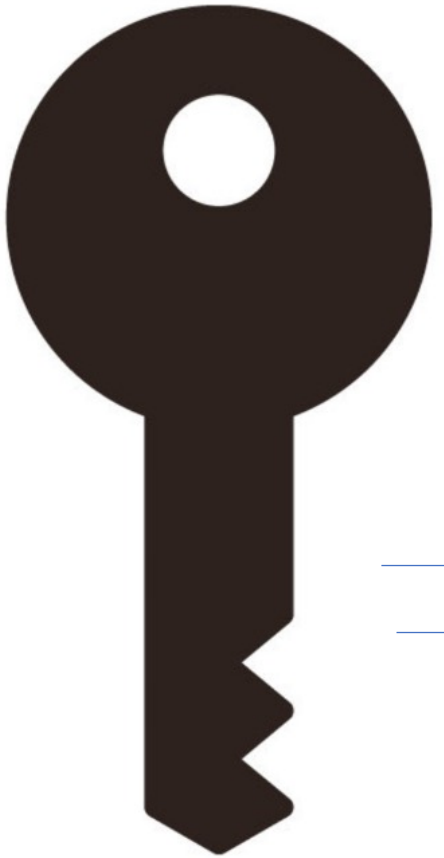
Key Take Away Slide



- DNA extracted from urine can be sequenced successfully using the OncoDEEP assay, producing excellent quality metrics and high concordance with standard of care
- Preliminary studies in a small cohort suggests OncoDEEP can detect the presence of tumour in new diagnosis, recurrence and progression of non-muscle invasive bladder cancer in urine samples
- More low-grade non-muscle invasive urine samples are needed for robust statistical analysis
- Strategic planning, regular engagement and ‘buy-in’ from clinicians, regulatory bodies, patients and other stakeholders is essential to deliver timely and successful research projects

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