20 years of urinary markers: no nearer a reliable test?

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Cystoscopy is not a great gold standard

Accuracy

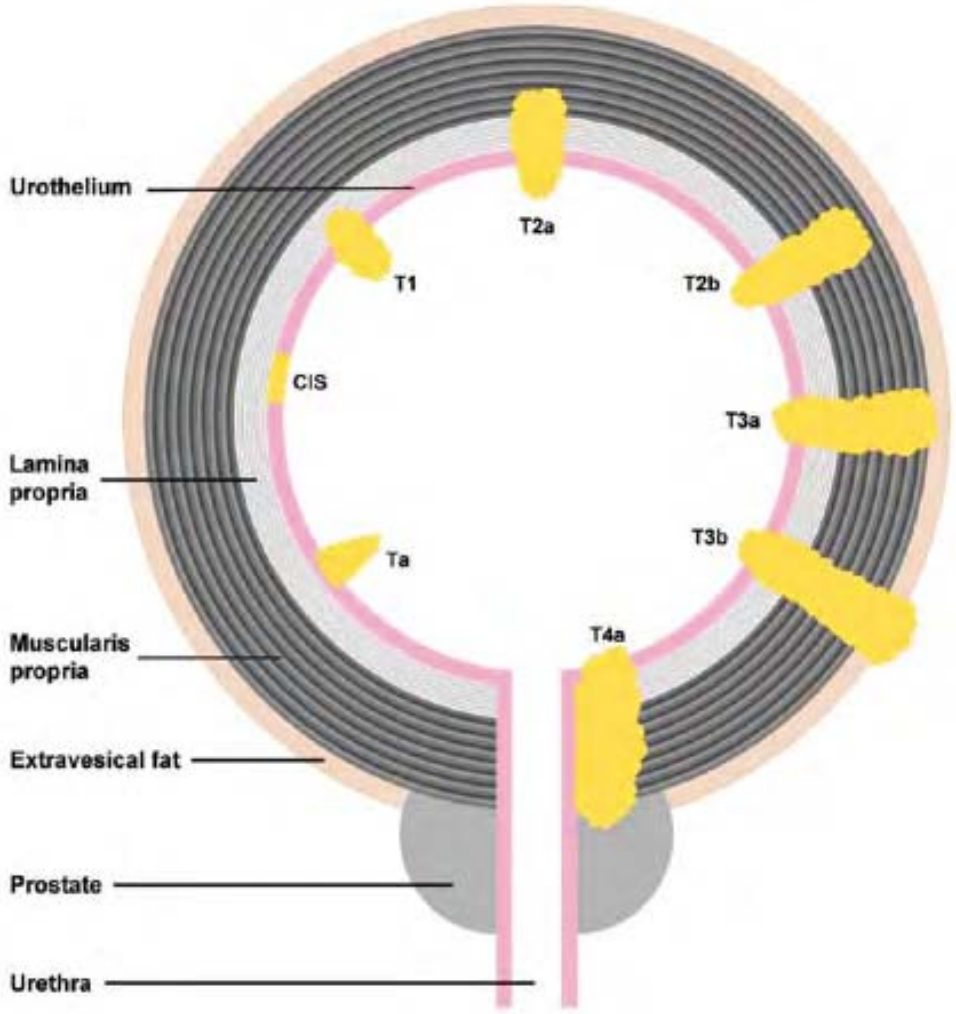
Invasive

Complications

Cost

Time

This makes a urinary marker attractive



We are asking too much

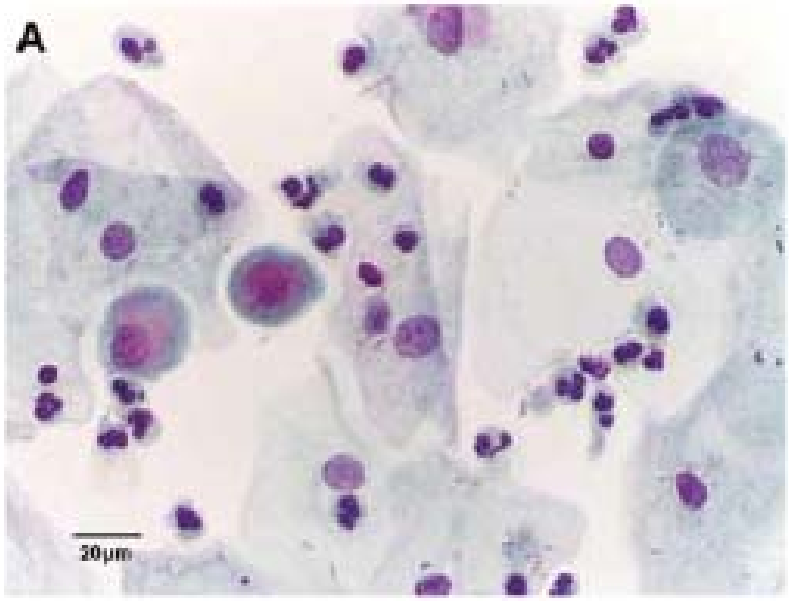
Haematuria screening

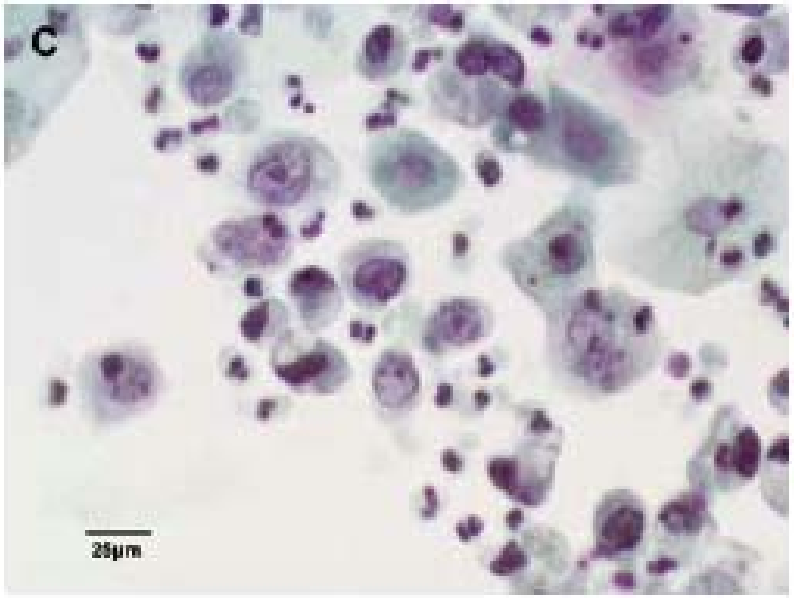
Solid v papillary

Recurrence

Response to treatment

Prognosis





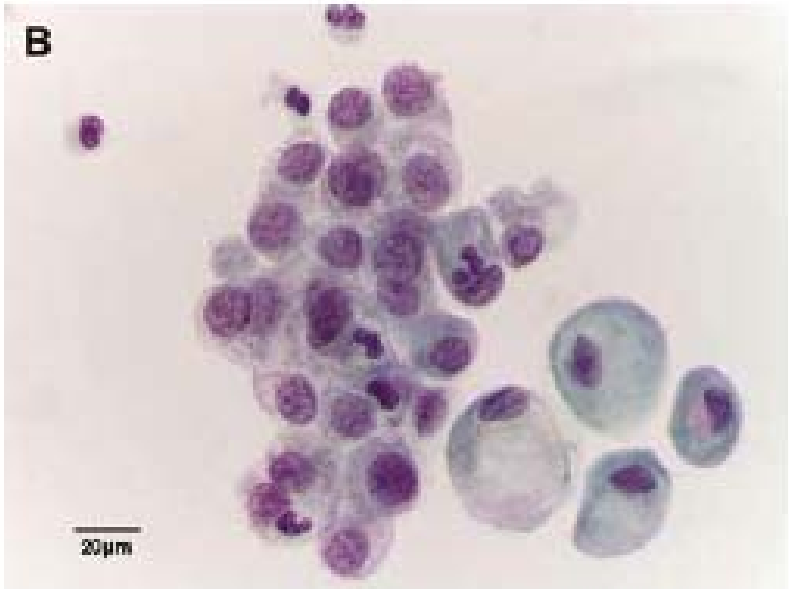


Figure 2. Urine cytology stained by the Papanicolaou procedure. (A) Normal cytology with no mitotic activity and normal nuclear-to-cytoplasmic ratio. (B) Urothelial cells showing slight atypia with increased nuclear-to-cytoplasmic ratio. (C) Severe urothelial atypia that is characteristic of bladder cancer, with varying cell size, increased nuclear-to-cytoplasmic ratio and an abnormal chromatin pattern. In all cases, note the presence of inflammatory cells in the field that can potentially interfere with the analysis. Slides at 400x magnification. Images courtesy of Alessandro Brollo (Hospital of Monfalcone, Italy).

Can we make cytology better?

- Improve the specimen
  - Ethanol preservative
  - Parylene membrane microfilter device

- Automated image cytometry
  - Quantitative cytology (Quanticyt)
  - Automated image analysis of cell nucleus for DNA content and nuclear morphometry.
  - Subsequent division into low, intermediate, and high risk for recurrence of bladder cancer.
  - Limited by the necessity of a bladder wash sample
  - Increases sensitivity at expense of specificity (van der Poel et al)

Potential markers

Cell morphology

Chromosomal/ gene alterations

Tumour receptor tyrosine kinases

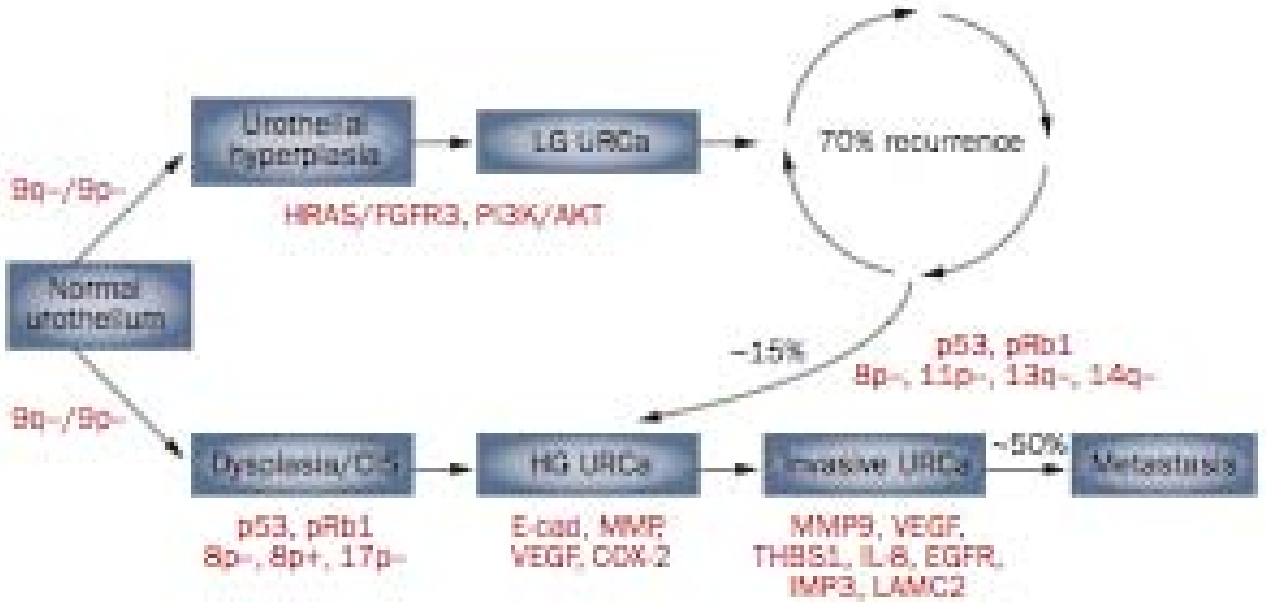
Cell cycle regulators – p16, p53, pRB

Hypermethylation and epigenetics

Tumour proliferation indices

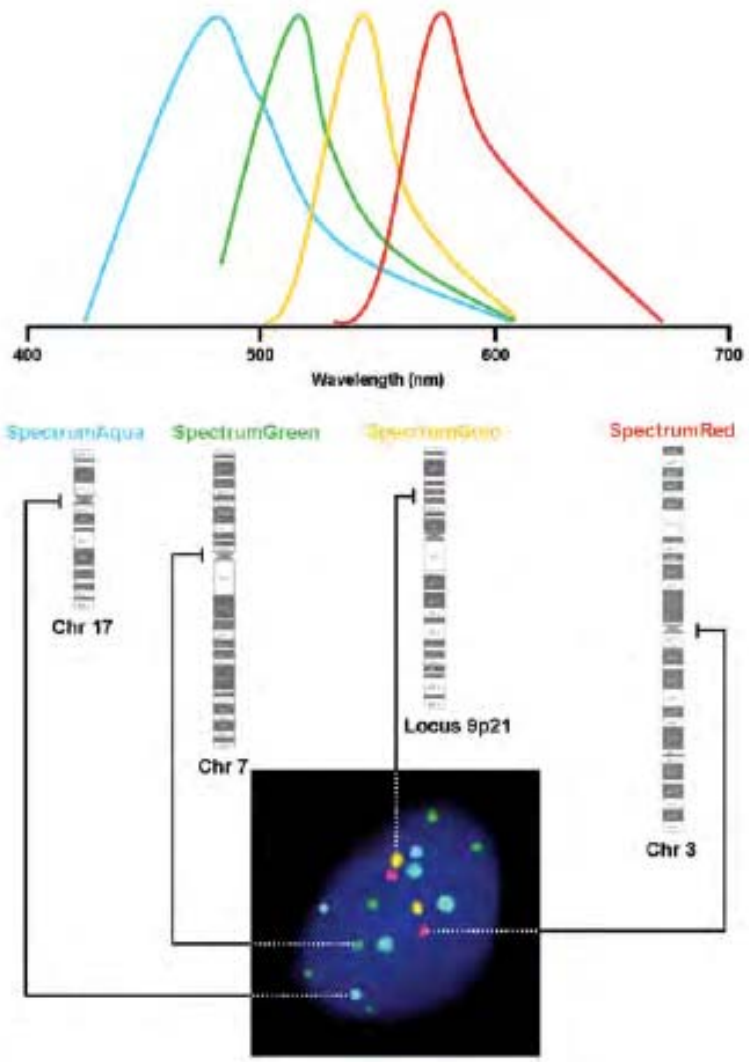
Proteomics

Invasive and non-invasive urothelial cancers are different diseases



Netto, Nat Rev Urol, 2011

FISH


400
500
600
700
Wavelength (nm)
SpectrumAqua
SpectrumGreen
SpectrumRed
Chr 17
Chr 7
Locus 9p21
Chr 3

Fluorescent In-Situ Hybridization

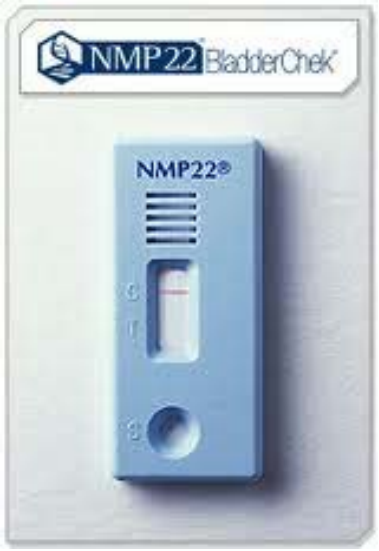
- FDA approved for
  - Surveillance of previously diagnosed UC
  - Haematuria investigation

- Sensitivity 69-87%
- Specificity 89-96%
  - (Lotan, Urology, 2003)

- Key advantages over cytology:
  - Not affected by inflammation
  - Anticipatory positive – (Yoder et al – 29m)
  - Predicts prognosis - BCG failure - 9.4x more likely to develop recurrence – (Kamat, J Urol, 2011)

NMP22

- Protein constituent of the nuclear mitotic apparatus
- 5x more abundant in urine of patients with UC



- Pooled data (n=10,019) (Mowatt et al, HTA, 2010)
  - Sensitivity 68%
  - Specificity 79%

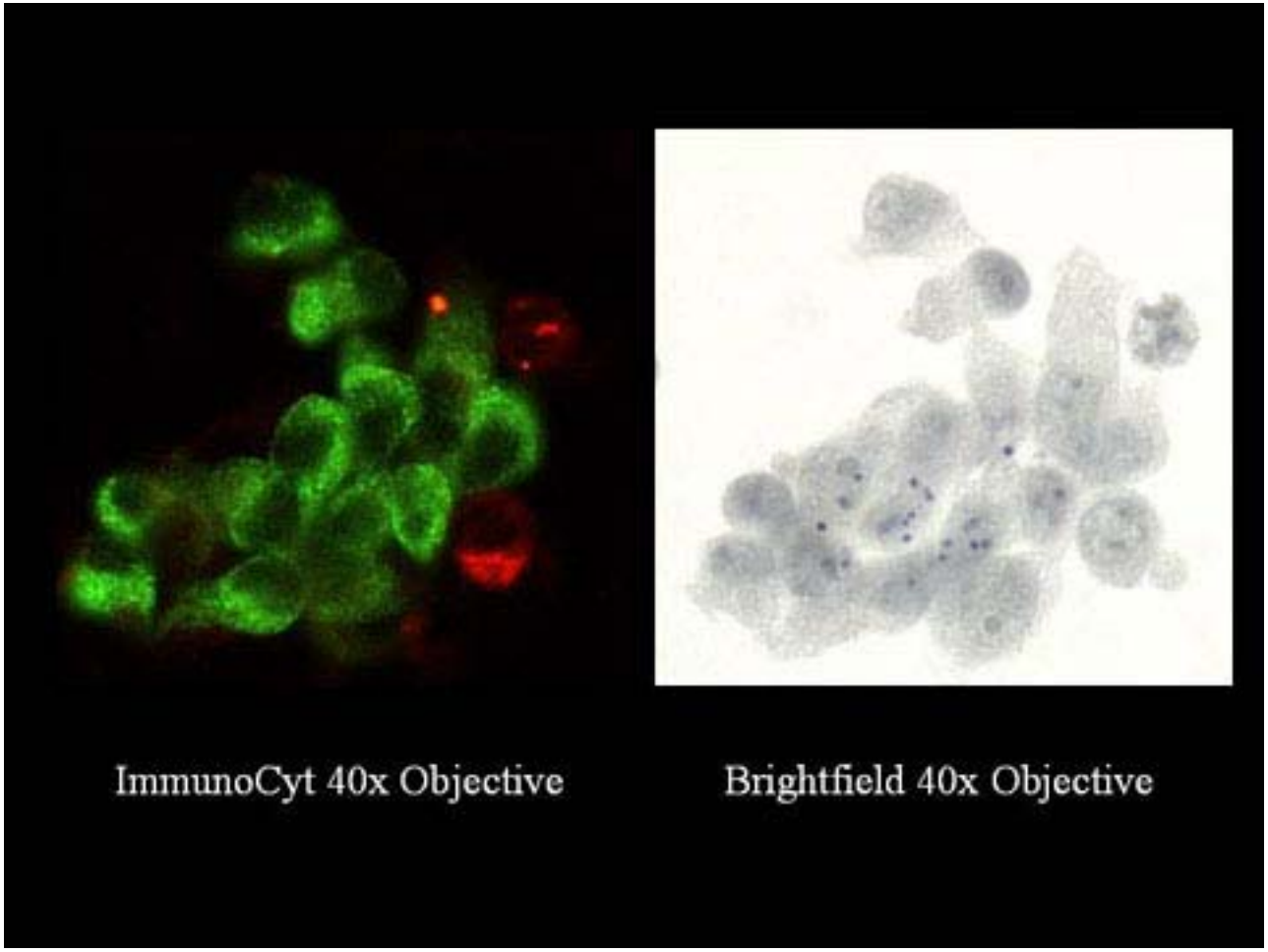
- Key advantages:
  - Point of care test – result in 30 mins
  - Better sensitivity for LG tumours v HG tumours
  - Better sensitivity for NMIBC v MIBC

Immunocyt

- Three fluorescent-labeled monoclonal antibodies target the M344, LDQ10, and 19A211 antigens, which are specific for bladder carcinoma

- Sensitivity of 84% (95% CI, 77–91%)
- Specificity of 75% (95% CI, 78–92%)
  - (Mowatt et al, HTA, 2010) n=2896

- Key advantages over cytology:
  - highly sensitive
  - increases the sensitivity of cytology without an appreciable loss of specificity when used together (Pfister, Jurol 2003, Tetu, Mod Pathol, 2005)
  - These findings have been demonstrated for both low- and high-grade, low-stage tumors.


ImmunoCyt 40x Objective
Brightfield 40x Objective

Bladder tumour antigen (BTA)

- Human complement factor H related protein
- Interrupts the complement cascade – conferring a growth advantage to cancer cells
- Point of care test - FDA approved for surveillance but not diagnosis

- Sens 65-70%
- Spec 75-78%

- False positives in infection, urolithiasis, previous BCG, bowel diversions

Urinary Marker	Sensitivity (%)	Specificity (%)	Clinical Status
Cytology	12.2–79	78.4–99.4	Laboratory
Quanticyt	42.1–69	67.9–87	Investigational
FISH	69–92.1	89–94.5	Laboratory
NMP22	49.5–92.1	66–87.3	Laboratory and point of care
BTA-Stat	50–70	67–78	Point of care
Immunocyt	66.7–84.9	62–84.7	Laboratory
FDP (Accu-Dx)	52–68.4	79.6–91	Point of care
Telomerase:			
TRAP	77.4–90	88–93.5	Investigational
hTERT	84.8–95	43.8–93.5	
Hyaluronic acid:			
HA	61–83.1	53.6–90.1	Investigational
HYAL-1	57.6–91	78–100	
HAase	81.5	83.8	
HA/HAase	88.1–94	63–84.4	
Lewis X	79.8–84	80–86.4	Investigational
Survivin	75	100	Investigational
LOH	60–97	93	Investigational
BLCA-4	89–96.4	95–100	Investigational
UPK3A	83	83	Investigational

Systematic review of the clinical effectiveness and cost-effectiveness of photodynamic diagnosis and urine biomarkers (FISH, ImmunoCyt, NMP22) and cytology for the detection and follow-up of bladder cancer

G Mowatt, S Zhu, M Kilonzo,
C Boachie, C Fraser, TRL Griffiths,
J N'Dow, G Nabi, J Cook and L Vale

Comparative Performance

- A total of 71 studies reported the performance of biomarkers (FISH, ImmunoCyt, NMP22) and cytology in detecting bladder cancer.

- Sensitivity
  - Immunocyt (84%) > FISH (76%) > NMP22 (68%) > cytology (44%)

- Specificity
  - Cytology(96%) > FISH (85%) > Immunocyt (75%) > NMP22 (79%) > Immunocyt (75%)

Cost

- PDD TURBT £2436
- WL TURBT £2002
- PDD cysto £1371
- WL cysto £937
- Flexi cysto £441
- cytology £92
- FISH £55
- Immunocyt £54
- NMP22 £39
- ImmunoCyt £54

Answers to the questions

- Sensitive for low grade disease
  - Immunocyt, NMP22
- Specific for high grade disease
  - Cytology
- Response to treatment
  - FISH
- Predicts prognosis
  - FISH

The next 20 years?

Proteomics

Epigenetics

Whole genome arrays

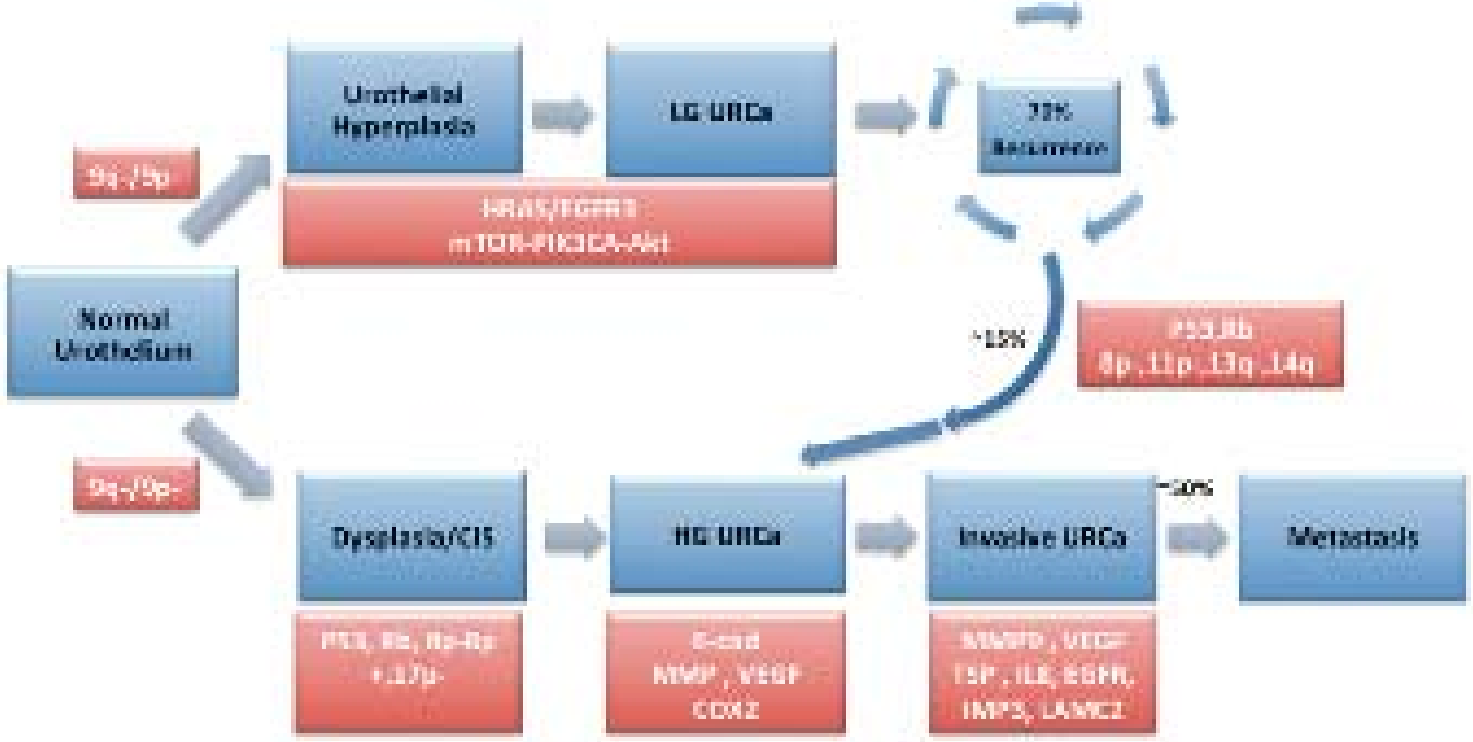
Theranostics

Will we ever dispense with cystoscopy?

- Trastuzumab (ERBB2)
- Cetuximab (EGFR)
- Lapatinib (EGFR and ERBB2)
- Sunitinib
- Bevacizumab (VEGF) – CALGB phase 3 trial with GC

FDP (Accu-Dx)

- The Accu-Dx test was developed as a qualitative point-of-care immunoassay utilizing murine monoclonal antibodies specific for FDP. However, since these antibodies have also been noted to react with intact fibrinogen typically found in human serum, the usefulness of the test in the presence of hematuria may be low[30].


Urothelial Hyperplasia
LG URCa
9q-/9p
HRAS/FGFR3
mTOR-PIK3CA-Akt
Normal Urothelium
9q-/9p-
Dysplasia/CIS
HG URCa
Invasive URCa
Metastasis
FGFR3, Ras, Ras-Rac
+17p-
E-cad
MMP , VEGF
CDX2
MMP9 , VEGF
TSP , IL8, EGFR,
IMP3, LAMC2

- Can we make cytology better?
- Commercially available urinary tests
- The future