Value of UroVysion FISH in non-muscle invasive bladder cancer surveillance: first UK experience

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Background

Patients with intermediate and high-grade **non-muscle invasive bladder cancer (NMIBC)** are often treated with transurethral resection and intravesical Bacillus Calmette-Guérin immunotherapy in combination with electromotive Mitomycin C (Di-Stasi regimen). It is estimated that up to 70% of these patients will have a reoccurrence of their disease, and presently their treatment response cannot be predicted.

According to the European Association of Urology Guidelines, the gold standard for post-transurethral resection bladder tumour (TURB) surveillance of high-risk NMIBC involves three-monthly flexible cystoscopies (FC) under local anaesthetic for two years and equivocal results require further white and blue light cystoscopy (LC) under general anaesthetic. Both procedures are quite invasive. Furthermore LC interpretation can be compromised by the level of inflammation resulting in unnecessary biopsies of inflamed but non-malignant tissue.

Improvement in diagnostic and follow up testing would be significantly beneficial to the management of these patients.

Studies have demonstrated how gains and losses of specific chromosomes can be used to detect bladder cancer cells. We report the validation, implementation, challenges and preliminary findings of a single-centre prospective study aimed to assess the value of Urovysion-FISH performed in combination with FC at different time-points during the follow-up of patients undergoing Di-Stasi.

In 2015, new NICE guidelines for the diagnosis and monitoring of bladder cancer have introduced the use of urinary biomarkers, such as FISH, in association with cystoscopy.

Study: establishment of a new patient pathway for the follow up of high grade NMIBC patients undergoing Di-Stasi therapy, combining routine FISH analysis of urine samples with conventional flexible white light cystoscopy both performed every three months (see workflow in **Figure 1**).

Proposed introduction of Bladder cancer FISH testing in high risk non-muscle invasive bladder cancer to replace GA blue light cytoscopy in main theatre with LA cystoscopy in day surgery.

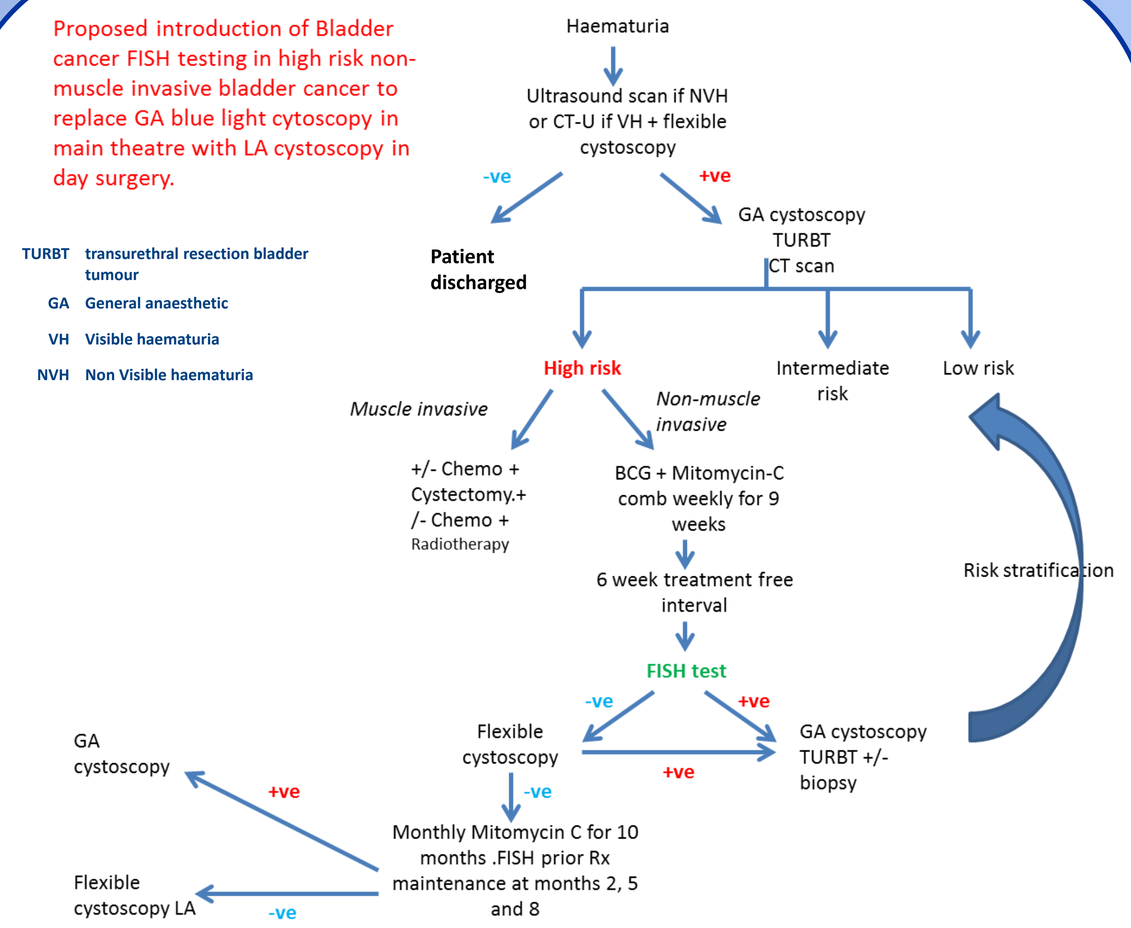

Figure 1 Proposed workflow for incorporating Bladder FISH testing for the management of high risk NMIBC patients

Methodology

We have used the Abbott UroVysion fluorescence *in situ* hybridisation (FISH) probe-combination to identify bladder cancer cells in the urine through the detection of cells carrying polysomies of chromosomes 3, 7 and 17 and/or loss of 9p21.

The UroVysion kit consists of a 4-color, 4-probe mixture of DNA probe sequences homologous to specific chromosomal regions: 9p21 (Spectrum Gold), chromosome 3 centromere (CEP3, Spectrum Red), chromosome 7 centromere (CEP7, Spectrum Green), chromosome 17 centromere (CEP17, Spectrum Aqua) (**Figure 2**).

Fresh urine samples (<12 hours from collection) are collected in a universal without additives. Cell pellets from the urine containing epithelial bladder cells are fixed onto slides. The slides are treated to denature the DNA in cells to its single stranded form to allow the UroVysion probes to hybridize. A number of washes follow to remove unbound probe, the nuclei are counterstained with DAPI (4,6 diamidino-2-phenylindole) and the slides are viewed using a fluorescence microscope.

Enumeration of CEP 3, 7, and 17, and LSI 9p21 signals is conducted in 25 **morphologically abnormal** cells by microscopic examination. The abnormal cells are identified by their characteristic irregular cell morphology and uneven DAPI staining in the nuclei (**Figure 3 A**). A sample is considered positive if there are ≥ 4 cells with gain of 2 or more chromosomes or ≥ 12 cells loss of both 9p21.

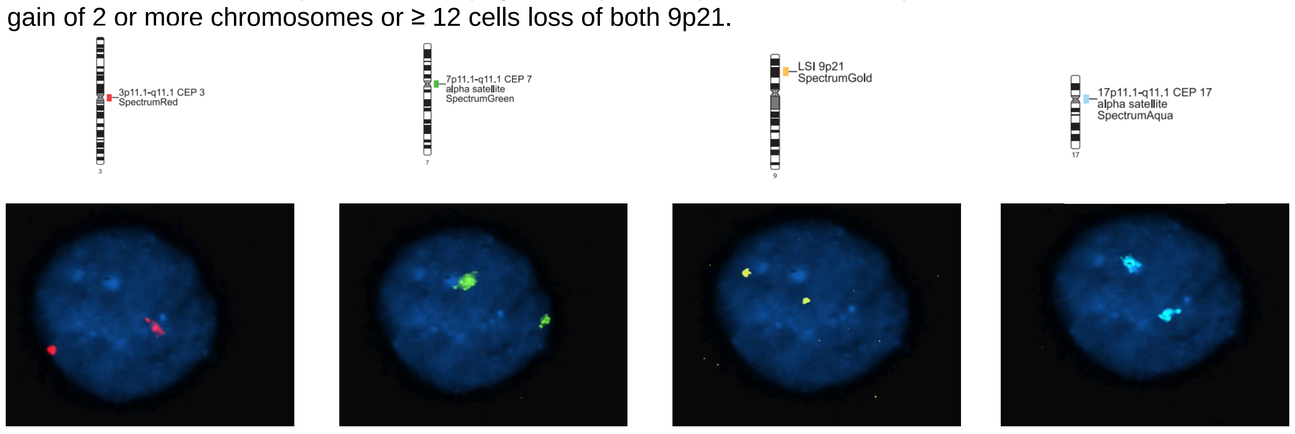

Figure 2 Loci and fluorophore colours of the Urovysion probes

Validation

- Urine samples from ten local Salisbury District Hospital patients with prior history of high risk NMIBC and positive by cytoscopy were examined
- Cytology and FISH was performed on all samples
- A normal and abnormal control were provided by Abbott and analysed blindly. The normal sample showed no evidence of an abnormal signal pattern, while the tumour sample showed all cells with multiple numerical abnormalities for the tested *loci*.
- Nine out of ten samples provided an informative result
- Eight of these nine cases showed an abnormal FISH signal pattern, consistent with the presence of a malignant population (**Figure 3 B**)
- The only case with a false negative result showed more than 70% of cells being represented by neutrophils, consistent with an ongoing infection

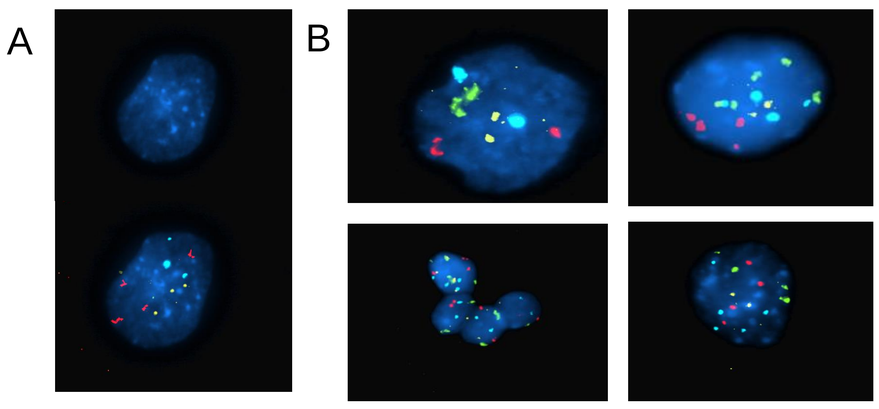

Figure 3 A) Enumeration is carried out on abnormal cells which have distinct morphology and uneven DAPI staining which correlates with polysomy. **B)** Examples of abnormal cases from the validation cohort

Limitations

- Uninformative results due to ongoing infections
- Sparse samples
- Sensitivity reliant on presence of tumor cells shed in the urine

Conclusions

- Out of the patients tested by FISH, ten completed FC
- FISH showed no evidence of residual tumour cells in any of these ten samples
- FC showed no recurrence in seven out of ten patients
- Three patients had a suspicious patch on FC; however, biopsy examination from this patch showed non-malignant bladder tissue
- All ten patients remain recurrence-free to date (range 1-28 months, mean 8 months)

These preliminary results suggest that UroVysion-FISH has the potential to reliably assess absence of disease post Di-Stasi and is likely to reduce the number of unnecessary biopsies of inflamed bladder tissue. Introduction of genetic testing significantly improves the treatment experience of individuals affected by bladder cancer by reducing the number of invasive procedures under general anaesthetic.

References

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