

Fluorescence *In Situ* Hybridization for the Detection of Bladder Cancer

a report by

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Fluorescence *in situ* hybridization (FISH) is a technique that utilizes fluorescently labeled DNA probes to detect chromosomal abnormalities in cells. FISH is widely used to aid in the diagnosis and management of patients with cancer. Most solid tumors, including urothelial carcinoma (UC), are characterized by numerical and structural chromosomal abnormalities. Since urothelial carcinoma cells readily exfoliate into the urine, FISH can be used to detect cells in the urine that have chromosomal abnormalities consistent with a diagnosis of UC.

UroVysion™ is a set of FISH probes that were specifically developed for the detection of urothelial carcinoma. This probe set contains chromosome enumeration probes (CEP) for chromosomes 3, 7, and 17, and an locus specific indicator (LSI) probe to the 9p21 band that are labeled with red, green, aqua, and gold fluorophores, respectively. UroVysion received US Food and Drug Administration (FDA) approval for the detection of recurrent tumor in patients with a history of bladder cancer in 2001 and for the detection of bladder cancer in patients with microhematuria but no previous history of bladder cancer in 2005.

Performing FISH for Urothelial Carcinoma Detection

FISH, with UroVysion, can be performed on any type of urine specimen including voided urine, urine obtained by catheterization, bladder and ureteral washings, and stomal urine specimens. However, voided urine is the specimen type that is approved by the FDA. If specimens cannot be processed the same day of collection, it is recommended that a fixative be added or that the specimen be refrigerated. Cytec (Marlborough, MA) offers a collection kit for this assay which is known as UroCyte™. An explanation of how to perform the UroVysion FISH assay is provided with the UroVysion test packet insert. For further information on the technical aspects of performing the assay, refer to Bubendorf et al.

Criteria for FISH Abnormality

When analyzing urinary cells with the UroVysion

probe set, non-neoplastic cells should show two copies for each of the four probes. Urothelial carcinoma cells, on the other hand, will show one of several types of chromosomal abnormality which include polysomy, tetrasomy/near-tetrasomy, trisomy, and 9p21 loss. In the authors' laboratory, the frequency of the different types of chromosomal abnormalities among our positive cases is: polysomy—90%, tetrasomy—7%, trisomy 7 or 3—2%, homozygous 9p21 loss—1%.

The criteria for considering a case positive for these abnormalities vary. For polysomy, the finding of four or more polysomic cells on the slide, regardless of the total number of cells on the slide, is considered positive for tumor. The finding of polysomy generally correlates with the presence of a higher grade tumor. A case can be considered positive for 9p21 loss if 12 or more of 25 morphologically abnormal cells demonstrate homozygous 9p21 loss. Patients whose samples demonstrate homozygous 9p21 loss alone are almost invariably found to have a low-grade papillary tumor.

The criteria that should be used for considering a case positive for tetrasomy or trisomy are not well established. In the authors' laboratory, a case is diagnosed as positive for tetrasomy or trisomy if 10 or more cells, regardless of the total number of cells on the slide, show this abnormality. However, the authors' group and other groups have found a higher number of tetrasomic cells in specimens collected during bladder washing procedures or in specimens from the upper tract. Concern has been expressed by some that tetrasomic cells may be umbrella cells, dividing cells, or overlapping cells. However, tumors are frequently tetraploid or near tetraploid, and consequently tetrasomic cells cannot always be ignored. A recent article by Zellweger and colleagues (2006) found that by increasing the threshold of tetrasomic cells from four to more than 10 cells, the performance of the FISH assay significantly improved for predicting bladder tumor recurrence.

FISH Versus Conventional Urine Cytology

A review of 12 studies that compared the performance

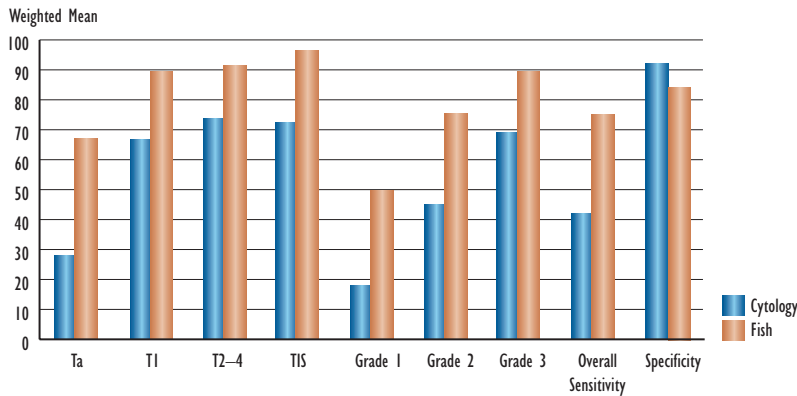


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Figure 1: Sensitivity and Specificity of Conventional Urine Cytology and FISH (UroVysion) for the Detection of Urothelial Carcinoma



This is a weighted analysis of 12 studies which shows that the sensitivity of UroVysion was higher than cytology for every stage and grade of tumor.

of FISH with conventional cytology revealed that FISH is more sensitive than cytology for all grades (1, 2, and 3) and stages (Ta, Tis, T1, T2–T4) of urothelial carcinoma (see *Figure 1*). In these same studies, the specificity of FISH is slightly lower than the specificity of urine cytology. The lower sensitivity of FISH for low-grade tumors is explained by the fact that some of these tumors are diploid or near-diploid tumors with relatively few chromosomal abnormalities. However, failing to detect some low-grade tumors is less of a concern than missing high-grade tumors, since low-grade tumors behave less aggressively.

Quantitative Molecular Cytology?

The UroVysion assay, as currently performed by most labs, is simply reported qualitatively as positive or negative for abnormality. However, one patient might have a positive result because five cells out of 1,000 on the slide demonstrate polysomy while a second patient might have a positive result because 500 out of 1,000 cells on the slide show polysomy. Both patients exceed the cut-off required to call the case positive for tumor, but the second patient is likely to have a greater tumor burden than the first patient. The first patient might have a small high-grade papillary tumor while the second patient might have extensive carcinoma *in situ* and/or invasive tumor. Since the authors feel that the percentage of urothelial cells showing FISH abnormality roughly reflects the percentage of the urothelial mucosa that is involved by tumor, the authors have been determining a percentage abnormal value for all positive cases. Among cases that are called positive, the percentage of abnormal cells can range from as little as 1% up to 100%. A retrospective analysis of our cases revealed that of our positive cases, about 25% fall into the 1–4%, 5–10%, 11–30%, and 31–100% categories each. Further studies

are needed to determine if the percentage of abnormal cells correlates with prognosis and/or response to treatment.

FISH for Assessing Response to Intravesical Therapy

Bacillus Calmette–Guerin (BCG) is commonly used to treat superficial bladder cancer. Unfortunately, the intense inflammatory reaction induced by BCG makes the interpretation of cystoscopic findings difficult since areas of erythema can represent either carcinoma *in situ* or inflammation. In addition, cytology interpretation is complicated by reactive changes in the cells that make it difficult to distinguish malignant from reactive non-neoplastic cells.

Our group conducted a study to determine if FISH could be used to assess response to treatment in superficial bladder cancer patients receiving BCG or other therapy. This study suggested that BCG does not interfere with the interpretation of FISH, i.e. BCG did not appear to cause false positive FISH results. Among patients who had a positive FISH result at the beginning of therapy, nearly all showed a significant reduction in the percentage of abnormal cells by the end of their treatment. The study also showed that patients with a positive FISH result at the end of their treatment were 4.6 times more likely to develop recurrent tumor and 9.4 times more likely to develop muscle-invasive tumor than patients with a negative FISH result.

Early Detection of Recurrent Tumor with UroVysion—‘Anticipatory Positives’

The FISH assay is sensitive, and it is not uncommon for the assay to be positive for a patient in whom tumor cannot be identified. A number of studies have now demonstrated that FISH can detect recurrent UC before it is clinically evident by cystoscopy. In the initial study at the Mayo Clinic, the authors found that 11 patients with a history of superficial UC had a positive FISH result but negative biopsy. It was uncertain whether these cases represented false positive FISH results or an ability of the UroVysion probe set to detect recurrent tumor before it could be detected clinically.

Seven of the 11 patients subsequently developed biopsy proven recurrent UC 3–12 months later. The seven tumors included three pTa tumors, a pTis tumor, and three pT3 tumors. In the trial that led to FDA approval of UroVysion, Sarosdy et al. reported that there were 36 patients with a negative cystoscopic examination but a positive FISH result. With continued longitudinal follow-up, 15 (41.6%) of these cases were found to

biopsy proven tumor recurrence with time to tumor diagnosis of 3–16 months (mean 6). Conversely, among 68 patients who had a negative cystoscopy and a negative FISH result, only 13 (19.1%) had a biopsy proven recurrence at 3–19 months (mean 11.2, chi-square $p=0.014$). The patients with a positive FISH result but negative cystoscopy were referred to as ‘anticipatory positive’ cases. A Kaplan-Meier curve showed that the time to tumor recurrence was significantly less ($p=0.014$) for patients with ‘anticipatory positive’ FISH results compared with those with negative FISH results. Based on these findings, the FDA approved the claim that UroVysion can be used to detect tumor recurrence before it is easily detectable by cystoscopy.

FISH and Other Tumor Markers

There is a plethora of other markers that can be utilized for the detection of UC. These include ‘proteomic assays’ such as BTA-Stat® and NMP-22®, which are designed to detect proteins that are associated with UC, immunocytochemical assays such as ImmunoCyt™, and genetic assays such as microsatellite analysis. Most published studies have compared the sensitivity and specificity of FISH with urine cytology alone, and not to other bladder tumor markers. Our group assessed the relative sensitivity of FISH, hemoglobin dipstick, telomerase, and BTA-stat. The sensitivity of these assays was 81%, 74%, 46%, and 78%, and the specificities were 96%, 51%, 91%, and 74%, respectively. In our study, the sensitivity of BTA-Stat, a point of care assay, was similar to FISH (78% versus 81%, respectively); but the specificity of BTA-Stat was significantly lower than FISH (74% versus 96%, respectively). The FDA trial that led to UroVysion approval also compared the sensitivity of FISH with BTA-stat and found sensitivities of 71% and 50%, respectively. These findings suggest that BTA-stat may be useful for screening patients for recurrent UC (though FDA trial suggests that there will be more false negatives than with FISH) but that positive results should be confirmed with a test with higher specificity, but similar or better sensitivity such as FISH.

FISH for the Detection of Upper Tract UC

The UroVysion assay is not FDA-approved for the detection of upper tract UC. The main difficulty with assessing the utility of FISH for upper tract UC detection is that it is often difficult to determine if the patient does or does not have tumor since it is hard to obtain biopsies of the upper tract. Consequently, long-term follow up is often required to determine if a positive FISH result in the absence of obvious tumor is a false positive FISH result or an ‘anticipatory positive’ result. In our experience, a low percentage (approximately 1–4%) of

tetrasomic cells is a fairly common finding in upper tract washing specimens, even in patients who do not appear to have tumor. For this reason, the authors are conservative about interpreting the finding of tetrasomic cells as evidence of tumor except when such cells both exceed the 10-cell cut-off and are present in high percentages. In contrast, the authors have found that the finding of four or more cells with hyper-tetrasomic signal patterns (i.e. cells in which at least one of the four probes shows five or more copies) has high specificity for the presence of UC and interpret such findings as consistent with a diagnosis of UC or other tumor involving the upper tract.

FISH and Polyomavirus Infection

Patients who have had renal transplants often develop a recrudescence of latent BK polyomavirus infections. The polyomavirus infected cells that are shed into the urine are referred to as ‘decoy cells.’ To the inexperienced cytologist, decoy cells can mimic UC cells. Interestingly, recent studies have shown that decoy cells are markedly aneuploid when assessed for ploidy status by digital image analysis. This brought up the question as to whether such cells might cause false positive FISH results. To evaluate this, the authors assessed 38 patients with decoy cells in their urine for DNA ploidy status using digital image analysis (DIA) and for chromosomal abnormalities with FISH (UroVysion probe set). The authors found that 84% of the cases showed aneuploidy by DIA but only 13% showed chromosomal abnormalities by FISH. The patients with the chromosomal abnormalities were the patients with the highest viral titers. None of the patients are known to have UC. These findings suggest that polyomavirus infection may occasionally cause false positive FISH results but that appears to be only in patients with high titers of BK polyomavirus.

Conclusions

Studies to date reveal that FISH is a useful assay for detecting UC. However, additional studies are needed to determine if:

- the frequency of cystoscopy can be reduced in patients with a negative UroVysion result;
- patients with a positive UroVysion result but negative cystoscopic and/or upper tract evaluation should be evaluated more aggressively (e.g. by obtaining random biopsies or performing fluorescence cystoscopy); and
- the percentage of cells with abnormality correlates with recurrence and/or progression. ■

A version of this article containing references and additional graphics can be found in the Reference Section on the website supporting this briefing (www.touchgenitourinarydisease.com).