

Bladder Cancer Detection Using FISH (UroVysion Assay)

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Abstract: UroVysion is a fluorescence in situ hybridization assay that was developed for the detection of bladder cancer in urine specimens. It consists of fluorescently labeled DNA probes to the pericentromeric regions of chromosomes 3 (red), 7 (green), and 17 (aqua) and to the 9p21 band (gold) location of the *P16* tumor suppressor gene. The UroVysion assay works by detecting urinary cells that have chromosomal abnormalities consistent with a diagnosis of bladder cancer. Studies have shown that UroVysion is more sensitive than urine cytology for the detection of all stages and grades of bladder cancer. UroVysion is Food and Drug Administration-approved for the detection of recurrent bladder cancer in voided urine specimens from patients with a history of bladder cancer and for the detection of bladder cancer in voided urine specimens from patients with gross or microscopic hematuria, but no previous history of bladder cancer. Recent studies also suggest that UroVysion may be useful for assessing superficial bladder cancer patients' response to bacillus Calmette-Guerin therapy and in detecting upper tract urothelial carcinoma.

Key Words: bladder cancer, fluorescence in situ hybridization, urine cytology, UroVysion, BCG, urothelial carcinoma, hematuria
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BLADDER CANCER

There are approximately 60,000 new cases of bladder cancer in the United States each year and approximately 500,000 individuals in the United States living with a diagnosis of bladder cancer.¹ Owing to its high prevalence and continued monitoring for tumor recurrence that is required in patients who have a diagnosis, bladder cancer is one of the most expensive cancers to treat.²

Patients with bladder cancer are typically divided by urologists into 2 groups: those who have muscle invasive bladder cancer and those who do not. Patients with muscle invasive cancer generally undergo cystectomy as

there is a high risk of developing metastatic disease if the bladder is not removed. Cystectomy is generally not performed in patients with superficial bladder cancer as there is a reasonable chance that the tumor can be managed without cystectomy and because cystectomy is associated with morbidity.^{3,4}

“Nonmuscle invasive” tumors, often referred to as “superficial bladder cancer,” are a pathologically and genetically heterogeneous group of tumors that includes patients with noninvasive papillary tumors, carcinoma in situ (CIS), and tumors that invade into the lamina propria but no deeper (T1 tumors).⁵ Papillary tumors, especially low-grade papillary tumors, generally behave in a benign fashion and are often treated by tumor fulguration plus adjuvant intravesical chemotherapy/immunotherapy or excision alone. CIS and T1 tumors, on the other hand, often behave aggressively and are generally treated with intravesical therapies such as bacillus Calmette-Guerin (BCG) or mitomycin C.⁴

Patients with superficial bladder cancer have a 50% to 70% risk of having tumor recurrence. In addition, a subset of these patients is at risk of progressing to muscle invasive cancer. Reports suggest that 5%, 54%, and 46% of patients with Ta, Tis, and T1 tumors progress to muscle invasive disease. Tumor grade is also a significant predictor of progression to muscle invasive disease with an incidence of progression of 2% for grade 1, 11% for grade 2, and 45% for grade 3 cancers.⁶ Consequently, patients with superficial bladder cancer are regularly followed for tumor recurrence with cystoscopy and cytology. Typical surveillance intervals are every 3 months for the first 2 years after diagnosis with greater intervals if there is no tumor recurrence.

BLADDER CANCER DETECTION: URINE CYTOLOGY

Urine cytology has been the main laboratory method used to detect bladder cancer in urine specimens for nearly half a century. Studies have shown that urine cytology has excellent specificity (ie, a low false positive rate), but suboptimal sensitivity (ie, fairly high false negative rate).⁷ The sensitivity of cytology is fairly high for high-grade tumors, but even for these tumors has a suboptimal false negative rate. Because urine cytology suffers from less than perfect sensitivity, various investigators have been trying to develop bladder cancer detection assays that have higher sensitivity than urine cytology. Food and Drug Administration (FDA)-approved assays for the detection of bladder cancer include assays that assess for proteins in the urine (so called

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“proteomic” assays) such as BTA-Stat and NMP22, immunocytochemical assays such as ImmunoCyt, and fluorescence in situ hybridization (FISH) using the UroVysion probe set.⁸

FLUORESCENCE IN SITU HYBRIDIZATION

The focus of this review article will be on the use of FISH for the detection of bladder cancer. FISH is a technique that uses fluorescently labeled DNA probes to assess cells for genetic alterations. FISH takes advantage of the fact that cancer is a genetic disorder and that most cancers have chromosomal alterations. There are 2 general types of FISH probes: chromosome enumeration probes (CEPs) and locus-specific indicator (LSI) probes. CEP probes hybridize (ie, stick to) the pericentromeric regions of chromosomes and are used to enumerate the number of chromosomes in a cell. LSI probes are generally designed to hybridize to genes of interest, such as the *HER2*, *P53*, or other genes. FISH probes are labeled with fluorescent molecules such as fluorescein isothiocyanate that are called fluorophores. Using a fluorescence microscope, fluorophores allow one to determine how many copies of a given probe target are present in the nucleus of a cell.

BLADDER CANCER GENETICS AND DEVELOPMENT OF FLUORESCENCE IN SITU HYBRIDIZATION PROBE SET FOR BLADDER CANCER DETECTION

Karyotyping, comparative genomic hybridization, and DNA ploidy studies have shown that most bladder cancers possess chromosomal abnormalities and that the degree of aneuploidy and structural chromosomal abnormalities (deletions and gains) increase with increasing tumor grade.^{9–11} This suggested that the detection of chromosomally abnormal cells in the urine might be a good way to assess for bladder cancer. However, conventional cytogenetic analysis by karyotyping does not work well for detecting bladder cancer in urine specimens as urothelial cells must be in the mitotic phase of the cell cycle to obtain a karyotype. Few urothelial cells, even in patients with bladder cancer, are found to be undergoing mitosis. A number of investigators began to explore the possibility of using FISH to detect bladder cancer in urine specimens in the 1990s.^{12–16} The advantage of FISH over karyotyping is that it allows one to assess interphase cells (ie, cells in phases of the cell cycle other than the mitotic phase) for chromosomal abnormalities. Thus, FISH allows one to assess the chromosomes of all the cells in the urine, not just cells in the mitotic phase.

In August 2000, a report describing the development of a new FISH probe set for bladder cancer detection that is now referred to as UroVysion (Abbott Molecular Inc, Des Plaines, IL) was published.¹⁷ This 4-target, multicolor FISH probe set consists of directly labeled DNA probes to the pericentromeric regions of chromosomes 3 (CEP3), 7 (CEP7), and 17 (CEP17) and

to the 9p21 locus (LSI 9p21) location of the *P16* tumor suppressor gene. These FISH probes were chosen from 10 different candidate loci that were evaluated because they demonstrated the highest combined sensitivity for detecting urothelial carcinoma (UC). A 4-target probe set was selected because 4 probes provide a higher sensitivity than just 1 or 2 probes and because 4 is the maximum number that one can easily have in a single probe set owing to spectral overlap of the different fluorophores light emissions.

HOW IS UROVYSION TESTING CARRIED OUT?

UroVysion is performed by obtaining a urine specimen, harvesting the cells from the urine and placing them on a slide, preparing the cells on the slide for hybridization (“prehybridization”), hybridizing the FISH probes to the cells, washing the slide to remove any probe that is not specifically bound to its target, and then analyzing the cells on the slide for chromosomal alterations by fluorescence microscopy. The FDA-approved specimen for UroVysion is voided urine. However, numerous laboratories have performed this testing on a variety of other specimen types including bladder washings, urine obtained by catheterization, upper tract washings, and stomal specimens.^{18–20} The steps required to perform UroVysion on these other specimen types are the same as for voided urine. However, there is little data regarding the appropriate criteria for considering a case positive for other specimen types. If a laboratory uses a non-FDA-approved specimen, they should do appropriate validation studies before using clinically. For greater detail on the technical aspects of performing UroVysion testing, refer to the UroVysion package insert and paper on methodology by Bubendorf and Grilli.²¹

TYPES OF GENETIC ALTERATIONS OBSERVED WITH UROVYSION IN BLADDER CANCER

To truly understand the FISH assay, people performing the FISH test need to have a firm understanding of what is normal by FISH before one can confidently diagnose FISH abnormalities. Normal cells should in theory show 2 copies (“disomy”) of each of the 4 probes by FISH as there are 2 copies of each chromosome. However, studies carried out on urine specimens from normal patients reveal that about 5% to 10% of cells will show only 1 copy (“monosomy”) of a given probe, and a small percentage of cells (~1% to 3%) may show 3 (“trisomy”) or 4 (“tetrasomy”) copies of a probe.¹⁷ The finding of normal cells with monosomy is attributed to the fact that 2 signals may overlap and appear as 1 signal or that hybridization may not be 100% efficient. The most likely explanation for the finding of occasional normal cells with trisomy or tetrasomy is that these cells are in the S or G2 phase of the cell cycle. These findings indicate that the presence of small numbers of cells with monosomy, trisomy, or tetrasomy should not be construed as evidence of neoplasia. However, an

abundance of cells with trisomy, tetrasomy, and even monosomy may be indicative of neoplasia.

Cells with gains (ie, 3 or more copies) of 2 or more of the 4 probes in the same cell are referred to as “polysomic” cells. Polysomic cells, with the exception of occasional cells that are likely in the G2 or S phase of the cell cycle, are uncommon in urine specimens from normal individuals, but are common in the urine of patients with bladder cancer. Sokolova et al¹⁷ performed receiver operator curve analysis to assess the ability of the finding of polysomic cells to serve as a marker for the presence of bladder cancer. They found that a cutoff of 4 or more polysomic cells in the urine as evidence of bladder cancer was associated with a sensitivity and specificity of approximately 90% each for bladder cancer.

Four types of genetic abnormalities (polysomy, tetrasomy, trisomy, and homozygous 9p21 deletion) have been observed with UroVysion in patients with bladder cancer. Representative examples of each of these are shown in Figure 1. In our practice, over 90% of the cases

that are called positive by UroVysion demonstrate polysomic signal patterns. Polysomy generally correlates with presence of a high-grade tumor. Only a small fraction of cases are considered positive because of trisomy, tetrasomy, or homozygous 9p21 loss. Relatively little is known about the clinical correlates of cases showing trisomy or tetrasomy. However, homozygous 9p21 loss seems to generally correlate with the presence of a low-grade papillary tumor.

Studies have revealed that there is a strong correlation between the morphologic features of the nuclei and polysomy.^{17,22} Polysomic cells tend to have large and irregular nuclei and to have a mottled chromatin staining pattern. For this reason, the microscopic analysis portion of the UroVysion assay uses a scanning technique to assess the slides for cells that are most likely to have chromosomal abnormalities. This technique is described in the UroVysion package insert. Using this technique, the technologist starts at 1 end of the slide and scans the slide for cells that have atypical

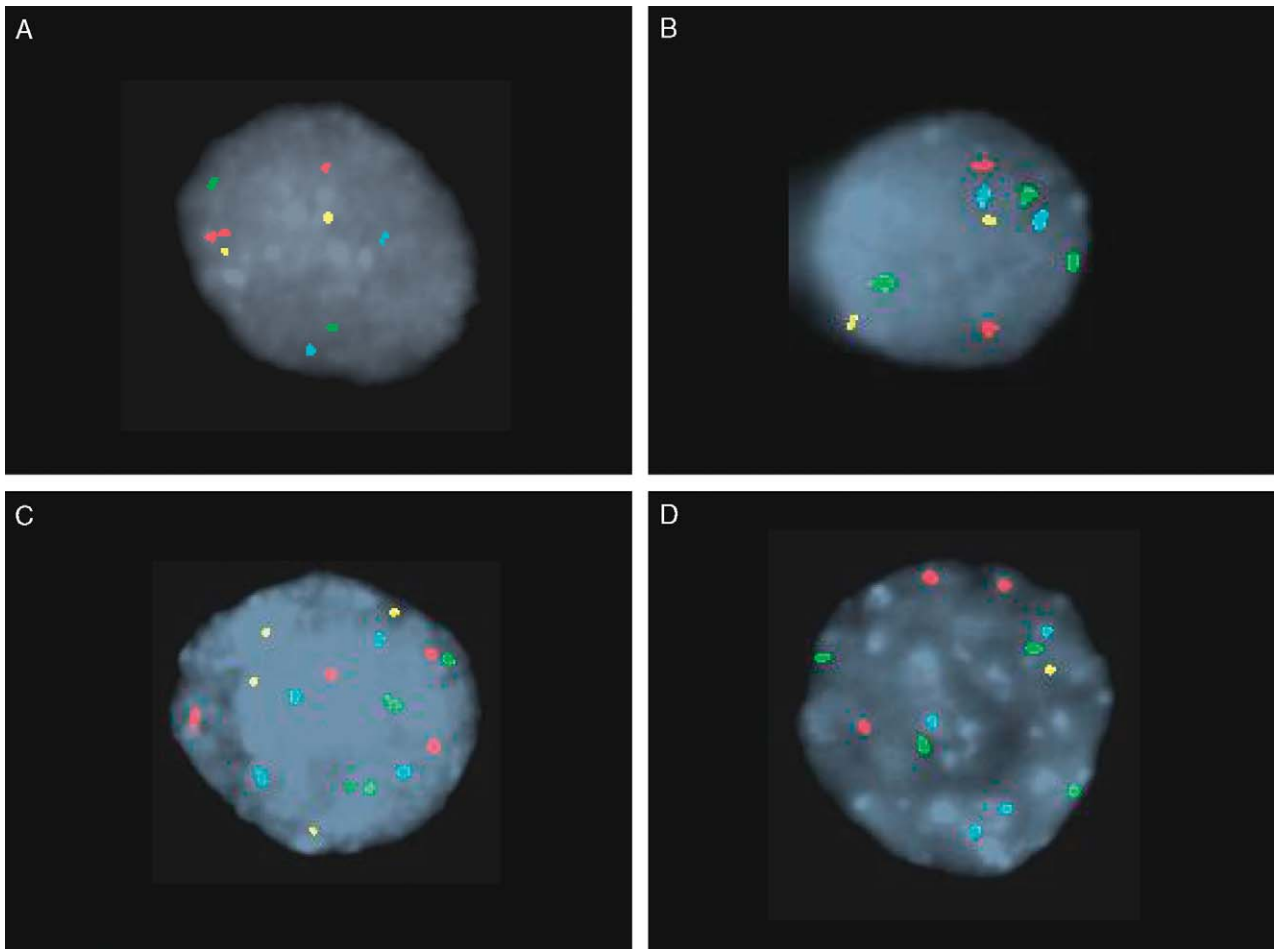


FIGURE 1. Representative examples of normal and abnormal cells with UroVysion FISH probe set: (A) normal (“disomic”) cell demonstrating 2 signals for all 4 probes, (B) trisomy 7 cells showing 3 copies of CEP7 but 2 copies of the other 3 probes, (C) tetrasomic cell showing 4 copies of all 4 probes, and (D) polysomic cell with gains (3 or more copies) for 2 or more of the 4 probes. UroVysion probe set: CEP3 (red), CEP7 (green), CEP17 (aqua), and 9p21 (gold). CEP indicates chromosome enumeration probe; FISH, fluorescence in situ hybridization.

nuclear features with the 4'-6-diamidino-2-phenylindole stain. The technologist then changes the filters to visualize the different probe signals in the atypical cell. The signal patterns of the first 25 abnormal cells are recorded on a worksheet. If 4 or more cells exhibit polysomy, the case is considered positive for tumor. If less than 4 of the 25 cells exhibit polysomy, scanning is continued till at least 4 or more cells exhibiting polysomy are observed or until the whole slide has been scanned. The slide analysis portion is relatively time consuming and on average takes approximately 15 to 20 min/case. At least 2 companies, BioView and Ikonysis, manufacture instruments that automate the microscopic analysis of UroVysion slides.²³

STUDIES EVALUATING THE SENSITIVITY AND SPECIFICITY OF FLUORESCENCE IN SITU HYBRIDIZATION (UROVYSION) FOR BLADDER CANCER DETECTION

A study at our institution published in the year 2000 was the first to evaluate the clinical utility of UroVysion. In that prospective study, 280 urine cytology specimens from 265 patients that were being assessed for UC were evaluated with FISH and urine cytology. The study found that with biopsy as the gold standard, the overall sensitivity of FISH was significantly higher than the sensitivity of routine cytology (81% vs. 59%, $P = 0.001$). Since then, subsequent studies have confirmed the utility of FISH in detecting bladder cancer in voided urines and bladder washing specimens. In a review of 12 studies comparing FISH and cytology, the weighted mean sensitivity of FISH was higher than cytology for the detection of all stages and grades of bladder cancer. The sensitivity of cytology and FISH was 28% and 67% for Ta tumors, 73% and 97% for Tis, 67% and 90% for T1, and 74% and 92% for T2-T4 tumors, respectively. The sensitivity of cytology and FISH by pathologic grade was 18% and 50% for grade 1 tumors, 45% and 75% for grade 2 tumors, and 69% and 90% for grade 3 tumors.²⁴ The specificity of cytology was slightly higher than FISH (93% vs. 85%). In a more recent meta-analysis, the pooled sensitivity and specificity of UroVysion was reported to be 72% (69% to 75%) and 83% (82% to 85%), respectively.²⁵ Some other studies suggest that FISH has a combined sensitivity and specificity superior to that of several other bladder cancer markers including BTA-Stat, hemoglobin dipstick, telomerase, NMP 22, and Lewis X antibody.²⁶⁻²⁸

FOOD AND DRUG ADMINISTRATION APPROVAL OF UROVYSION

Two FDA trials have been conducted with UroVysion. The first trial led to FDA approval of UroVysion for the detection of recurrent bladder cancer in voided urine specimens from patients with a history of bladder cancer in the year 2001.²⁹ The second trial led to FDA approval of UroVysion for the detection of bladder cancer in voided urine specimens from patients with gross or

microscopic hematuria, but no previous history of bladder cancer in the year 2005.³⁰

"ANTICIPATORY POSITIVE" FLUORESCENCE IN SITU HYBRIDIZATION RESULTS

The FISH assay is quite sensitive and it is not uncommon for FISH to be positive before there is evidence of recurrent tumor by cystoscopy. Patients with a positive FISH result but negative cystoscopy/cytology have been referred to as anticipatory positive cases.²⁹ In the FDA trial that led to approval of UroVysion, Sarosdy et al²⁹ reported that there were 36 patients with a negative cystoscopic examination but a positive FISH result. Fifteen of these patients (42%) were found to have biopsy-proven tumor recurrence on follow-up, with the time to tumor diagnosis ranging from 3 to 16 months (mean: 6.0 mo). Conversely, among 68 patients who had a negative cystoscopy and a negative FISH result, only 13 (19%) had a biopsy proven recurrence at 3 to 19 months (mean: 11.2 mo). In a study by Yoder et al,³¹ approximately 27% of patients with a negative or atypical cytology result had a positive FISH result, but no evidence of tumor by cystoscopy at the time that the cytology/FISH analysis specimen was collected. Clinical follow-up of these patients found that approximately 65% of these patients were found to have tumor recurrence within 29 months compared with only 5% of patients who had FISH-negative results. These findings provide strong evidence that FISH frequently detects tumor before it is clinically detectable by either cystoscopy or cytology.

REFLEX FLUORESCENCE IN SITU HYBRIDIZATION TESTING IN PATIENTS WITH EQUIVOCAL CYTOLOGY RESULTS

Equivocal urine cytology diagnoses are very common in clinical practice. Equivocal cytology diagnoses include cases that are diagnosed as atypical, suspicious, or cell clusters consistent with low-grade neoplasm, stones, or instrumentation. The decision of whether to further evaluate a patient with an equivocal cytology result can be difficult. Data suggest that fewer than 50% of patients with an equivocal cytology result have cancer on follow-up.³² Patients with an equivocal cytology result may undergo unnecessary additional testing and experience undue anxiety. A test that could help identify which patients with an equivocal cytology result truly have tumor would be beneficial.

Kipp et al³³ performed a study to determine if FISH can help identify which patients with an equivocal cytology diagnosis have cancer. FISH was performed on residual urine from 124 patients with an equivocal cytology diagnosis. Of the 124 equivocal cytology specimens, 58 (47%) were positive by FISH. Fifty-three of these 58 (91%) patients had subsequent evidence of carcinoma on the first follow-up biopsy. Sixty-six specimens were diagnosed as negative by FISH. Thirty-four (52%) of these patients were found not to have tumor and

32 (48%) were found to have tumor. The majority of the tumors (20 of 32) that FISH missed were papillary tumors and most of these papillary tumors were detected by cystoscopy. In conjunction with cystoscopy, FISH was significantly more sensitive than cystoscopy alone for detecting cancer (87% vs. 67%, $P < 0.001$) and muscle invasive cancer (94% vs. 56%, $P = 0.031$).

A more recent study by Lotan et al³⁴ also found that FISH was helpful for clarifying equivocal cytology results in patients with equivocal or negative cystoscopy results, but not necessary for patients with a positive cystoscopy result.

DETECTION OF BLADDER TUMORS OTHER THAN UROTHELIAL CARCINOMA

It is important to remember that a positive UroVysion result is not specific for UC. Other primary tumors of the bladder, prostatic cancer that invades into the urethra, and tumors metastatic to the bladder are occasionally the cause of a positive urine FISH result. Nonetheless, little is known about the sensitivity of UroVysion for detecting non-UC variants of bladder cancer. In an attempt to address whether these other tumor types would have genetic alterations that are detectable with the UroVysion probe set, Kipp et al³⁵ recently evaluated 22 paraffin embedded rarer histologic variants of bladder cancer [4 adenocarcinoma, 5 urachal adenocarcinoma, 6 small cell carcinomas, and 7 squamous cell carcinomas (SCCs)] and 9 typical UC and found that they all had genetic alterations that were detectable with the UroVysion probe set. Twenty-nine of the 31 tumors showed polysomy and 2 showed no evidence of polysomy but homozygous 9p21 deletion (1 SCC and 1 adenocarcinoma). The genetic alterations detected with UroVysion in the different tumor types were not markedly different except that the SCC tended to show much more homozygous 9p21 loss than the other tumors. The homozygous 9p21 loss in SCC cases was generally observed in cells that were polysomic for other signals. The results of this study suggest that UroVysion should have good sensitivity for the detection of rarer histologic variants of bladder cancer. However, the actual sensitivity for voided urine specimens might be lower than for paraffin embedded tumors if the tumor cells do not readily exfoliate.

UROVYSION FOR PATIENTS RECEIVING BACILLUS CALMETTE-GUERIN

FISH seems to be useful for assessing bladder cancer patients who are being treated with BCG for tumor recurrence. Patients with T1, Tis, or multiple high-grade papillary bladder tumors frequently receive BCG to treat their tumors.³⁶ BCG is a live, attenuated form of bovine tuberculosis bacillus that has been shown to have efficacy in bladder cancer treatment.³⁷ BCG leads to a self-limiting cystitis. It is thought that the immune response that BCG evokes is responsible for the antitumor effect of the agent. BCG treatment is generally performed by instilling a suspension of BCG into the

bladder through a catheter. The patient holds the instilled BCG for approximately 2 hours and then is allowed to void. Patients generally receive 6 courses of BCG over 6 weeks. The problem with BCG is that it induces marked inflammation of the bladder mucosa, and this makes it temporarily difficult to use cystoscopy and cytology to determine if the patient has had a tumor recurrence. This difficulty is owing to the fact that the mucosal erythema that results from BCG can mimic CIS on cystoscopic examination and in addition the inflammation induces cytologic atypia that makes cytologic interpretation difficult.³⁸ Thus, an assay that could be reliably used to detect tumor recurrence in patients who are undergoing BCG therapy would be quite useful.

In the year 2005, we published the results of a study carried out at our institution that was conducted to determine if FISH could be used to assess response to treatment in superficial bladder cancer patients receiving BCG or other intravesical therapies.³⁹ In that study, a sample for FISH analysis was collected just before the first and last BCG treatments from 37 patients undergoing BCG treatment for superficial bladder cancer. The patients were then followed for up to 2 years to determine if they developed recurrent tumor and if they did, whether the tumor was muscle invasive. These findings were correlated with the patients' FISH results. Twenty-five of the 37 patients had a negative FISH result at the end of therapy and 12 had a positive FISH at the end of therapy. All 12 patients with a positive FISH at the end of therapy were found to have tumor recurrence whereas tumor recurrence was observed in 13 of the 25 patients with a negative posttherapy FISH result [hazard ratio (HR): 4.6, 95% confidence interval (CI): 1.9 to 11.1, $P < 0.001$]. Seven of the 12 patients with a positive FISH at the end of their therapy developed muscle invasive bladder cancer compared with 2 of 25 patients with a negative FISH at the end of their therapy (HR: 9.4, 95% CI: 1.9 to 45.3, $P = 0.001$).

A similar study was recently published by Mengual et al.⁴⁰ The authors of that study found that patients with a positive FISH diagnosis after BCG therapy had a 2.7 times higher risk for tumor recurrence than patients with a negative FISH diagnosis ($P = 0.017$; 95% CI: 1.18 to 6.15).⁴⁰ In addition, patients who had a positive FISH result before and after BCG therapy had a risk for tumor recurrence 3.0 times higher than patients whose FISH result changed from positive to negative after BCG ($P = 0.02$; 95% CI: 1.17 to 7.54). However, in contrast to the first study, there was not a significant difference in the risk for tumor progression in patients with a positive versus a negative post-BCG FISH result ($P = 0.49$). In summary, the results of the 2 studies suggest that FISH is useful for assessing recurrent bladder cancer in patients who are being treated with BCG.

UROVYSION IN PATIENTS WITH BK POLYOMA VIRUS INFECTION

Kidney transplant patients receive immunosuppressive therapy to prevent rejection of the transplanted

kidney. Unfortunately, immunosuppression can lead to recrudescence of a latent BK polyoma viral infection that can then lead to rejection of the transplant. This is generally circumvented by reducing the immunosuppression. BK virus (BKV)-infected epithelial cells are often observed in the urine of such patients. These cells, often referred to as "decoy cells," have large atypical nuclei that can be confused with malignancy. Interestingly, DNA ploidy studies with image cytometry have shown that these cells are markedly aneuploid.⁴¹ As the BKV is a DNA virus, this apparent aneuploidy by DNA ploidy analysis is not for the most part owing to any abnormalities of the human chromosomes in the cell, but owing to the fact that nucleus is stuffed with viral particles. Nonetheless, there has been some concern that BKV infection might cause false positive FISH results with UroVysion. To address this, we performed FISH (UroVysion) and DNA ploidy analysis by image cytometry on 32 urine cytology specimens with evidence of BKV infection. Thirty of the 32 (94%) cases were aneuploid by DNA ploidy analysis whereas only 4 of the 32 (12.5%) cases were abnormal by FISH (1 polysomic case, 2 homozygous 9p21 deletion cases, and 1 trisomy 7 case). None of these patients were found to have cancer. These results suggest that BKV infection can be a rare cause of false positive FISH results, but that most patients with BKV infection (and decoy cells) do not have a positive FISH result.

UROVYSION FOR DETECTING BLADDER CANCER IN PATIENTS WITH HEMATURIA

It has been reported that approximately 90% of patients who are diagnosed with muscle invasive bladder cancer have that diagnosis at their initial presentation.⁴²⁻⁴⁴ Most bladder cancer mortality comes from this group of patients. Consequently, it has been argued that if significant reductions in bladder cancer mortality are to take place, then we should be screening for bladder cancer in an attempt to identify tumors at an early, more treatable stage. Hematuria is generally the first presenting sign for bladder cancer, and assessing individuals for hematuria could be used as a way to screen for bladder cancer. Hematuria can be inexpensively assessed for with urinalysis or a hemoglobin dipstick. Some studies have suggested that screening at-risk populations for bladder cancer by assessing regularly (once a year for instance) for hematuria may be a cost effective way of screening for bladder cancer.⁴⁵ The main problem with this strategy is that hematuria lacks specificity for bladder cancer. Other disorders, such as kidney stones, cystitis, etc., can cause hematuria. In a bladder cancer screening study by Messing et al,⁴⁶ approximately 10% of the screened patients were found to have hematuria by home dipstick testing, but only about 10% of the patients that had hematuria were found to have bladder cancer by cystoscopy. This demonstrates that most of the patients who undergo cystoscopy for hematuria are not found to have bladder cancer. Tests with higher specificity could

potentially be used to further assess the risk that a patient with hematuria may have bladder cancer before undergoing cystoscopy. UroVysion has been shown to have higher specificity than hemoglobin dipstick analysis for the detection of bladder cancer.²⁶ Thus, it is possible that UroVysion could be used to assess patients with hematuria to further assess their risk of having bladder cancer before undergoing cystoscopy.

On the basis of this concept, an FDA trial was carried out to address the utility of UroVysion testing for diagnosing bladder cancer in patients with hematuria.³⁰ In that study, patients with microscopic or gross hematuria, but no previous history of bladder cancer, were assessed for bladder cancer with urine cytology, UroVysion, and cystoscopy. Suspicious lesions on cystoscopy were biopsied and biopsy results were used as the gold standard for the study. Bladder cancer was histologically diagnosed in 50 of 497 (10.2%) of the patients enrolled into the study and ureteral cancer was diagnosed in 1. FISH detected 69% (95% CI: 54 to 81) of the bladder cancer cases and cytology detected 38% (95% CI: 25 to 52). When TaG1 tumors were excluded, FISH detected 25 of 30 cancers (83%) (95% CI: 65 to 94) whereas cytology detected 15 (50%) (95% CI: 31 to 69). It was concluded that the UroVysion was significantly more sensitive than voided urine cytology for detecting bladder cancer in patients evaluated for gross or microscopic hematuria for all grades and stages. On the basis of these data, UroVysion was approved by FDA for use in patients with hematuria in the year 2005.

Although UroVysion shows potential for assessing patients with hematuria for bladder cancer, it needs to be used wisely. The reason for this is that even a test with reasonably good specificity such as UroVysion can have a low positive predictive value (PPV) if used in a low prevalence population. In the Sarosdy et al³⁰ study that led to FDA approval, the PPV of UroVysion for bladder cancer was 65% in patients with a 40+ pack year history of smoking compared with only 20% in nonsmokers. This illustrates the importance of using the FISH assay in the right group of patients. For the most part, UroVysion testing of patients with hematuria should be restricted to patients that have other risk factors for bladder cancer. The most important risk factors are previous or current smoking history and age of at least 45 years or older.

DETECTION OF UPPER TRACT UROTHELIAL CARCINOMA WITH UROVYSION

Approximately 10% of all UC arise in the upper urinary tract. UroVysion sometimes detects tumors of the upper genitourinary tract (ie, ureters, renal pelvis) in voided urine specimens. Patients with a positive cytology result but a negative cystoscopic examination are generally evaluated for upper tract UC with radiology (excretory urograms or intravenous pyelography), ureteroscopy, and upper tract washings for cytologic examination. Biopsies can be difficult to obtain because of the narrow caliber of the ureters. A filling defect by

radiology could be consistent with either a tumor or stones. CIS does not lead to a filling defect and would be missed by radiology and possibly by ureteroscopy. Techniques that increase the ability to accurately diagnose upper tract UC, especially CIS, are needed.

Only 2 studies have been published on the use of FISH for the detection of upper tract UC.^{47,48} Marin-Aguilera et al⁴⁸ assessed urine specimens from 30 consecutive patients with upper tract UC and 19 healthy controls with FISH and cytology. They found that the sensitivity of FISH was higher than cytology (76.7% vs. 36%, $P = 0.00056$), but that the specificities of FISH and cytology were not significantly different (94.7% vs. 100%). In a smaller study, Akkad et al⁴⁶ examined 16 patients with suspected upper tract UC and found the sensitivity of FISH and cytology to be 87.5% and 60%, respectively, and the specificity of FISH and cytology to be 80% each.

Our own personal experience (unpublished results) also suggests that FISH is more sensitive than cytology for upper tract UC. However, we have found that tetrasomic and near-tetrasomic cells are much more abundant in upper tract washing specimens than voided urine specimens and that the presence of these cells can lead to false positive results. The high number of tetrasomic and near-tetrasomic cells could be owing to the abundance of umbrella cells in upper tract washing specimens or could reflect a higher proliferative rate of upper tract urothelial cells as dividing cells that are in the S or G2 phase of the cell cycle would have near-tetrasomic or tetrasomic signal patterns. We have found that if we restrict our positive diagnoses to cases that show 4 or more hypertetrasomic cells, that the assay has excellent specificity for upper tract UC.

Together, these studies suggest that FISH may be a promising way to assess patients for upper tract UC. However, additional larger studies are needed to clearly define the criteria for positivity for upper tract UC.

CONCLUSIONS

Studies to date provide strong evidence that UroVysion has higher sensitivity and similar specificity to urine cytology. However, additional studies are needed to help answer a number of other important questions regarding the use of UroVysion for bladder cancer detection. These include studies that address (1) Does earlier detection of bladder cancer with UroVysion translate into decreased bladder cancer mortality?, (2) Can the relatively high negative predictive value of the assay for high-grade tumors be used to decrease the frequency of cystoscopy in patients with negative UroVysion results?, (3) What are the performance characteristics (sensitivity, specificity, and positive and negative predictive value) of UroVysion for the detection of upper tract UC?, and (4) Can quantitative UroVysion analysis in which percentages of FISH-positive urothelial cells are reported provide additional information that would be useful in patient management but which is not

provided with the current qualitative (ie, positive or negative) mode of analysis?

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