Evaluation of the Fluorescence In Situ Hybridization Test to Predict Recurrence and/or Progression of Disease after bacillus Calmette-Guérin for Primary High Grade Nonmuscle Invasive Bladder Cancer: Results from a Prospective Multicenter Trial

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Abbreviations and Acronyms

BCG = bacillus Calmette-Guérin

FISH = fluorescence in situ hybridization

NMIBC = nonmuscle invasive bladder cancer

Purpose: Single center studies have shown that positive UroVysion® fluorescence in situ hybridization results were associated with recurrence of nonmuscle invasive bladder cancer treated with intravesical bacillus Calmette-Guérin. Our goal was to validate these findings.

Materials and Methods: We performed a prospective, multicenter diagnostic trial to determine whether the fluorescence in situ hybridization test could predict recurrence or progression in patients with primary high grade nonmuscle invasive bladder cancer who were scheduled to receive bacillus Calmette-Guérin. Fluorescence in situ hybridization testing was performed prior to the first bacillus Calmette-Guérin instillation, prior to the sixth instillation and at 3-month cystoscopy. The performance of fluorescence in situ hybridization was evaluated.

Results: A total of 150 patients were enrolled in analysis, including 68 with Ta disease, 41 with T1 disease, 26 with carcinoma in situ alone and 15 with papillary carcinoma plus carcinoma in situ. At 9 months of followup there were 46 events, including 37 recurrences and 9 progressions. For events with positive fluorescence in situ hybridization findings the HR was 2.59 (95% CI 1.42–4.73) for the baseline test, 1.94 (95% CI 1.04–3.59) for the 6-week test and 3.22 (95% CI 1.65–6.27) at 3 months. Patients with positive results at baseline, 6 weeks and 3 months had events 55% of the time and patients with negative results at each time point had no event 76% of the time.

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Conclusions: The study validated that a positive UroVysion fluorescence in situ hybridization test was associated with a 3.3-fold increased risk of recurrence. The test may be useful to risk stratify patients entering clinical trials in whom induction therapy fails. However, using the test to change management decisions is limited due to the discordance between results and outcomes as well as the variance of tests results with time.

Key Words: urinary bladder neoplasms; carcinoma in situ; in situ hybridization, fluorescence; BCG vaccine; neoplasm recurrence, local

Approximately 75% of the 79,000 newly diagnosed cases of bladder cancer are NMIBC at presentation.[1–3] Intravesical therapies are recommended in patients at intermediate and high risk to reduce recurrence.[4,5] In high risk cases intravesical BCG immunotherapy is the treatment of choice[4,5] but in 40% to 60% of cases the cancer recurs and 10% to 20% progress after BCG.[6] Pathological features help predict the probability of recurrence and progression after BCG but they are inadequate to predict the disease outcome.[7]

UroVysion® is a multitarget FISH assay developed to detect the presence of malignant urothelial cells in urine. Two previous studies showed that patients with a positive postinduction BCG UroVysion result had a higher rate of bladder cancer recurrence than patients with a negative result.[8,9] A subsequent prospective, single center study demonstrated that a positive UroVysion result was associated with a threefold to fivefold increased risk of bladder cancer recurrence and a fivefold to 13-fold increased risk of progression.[10] The studies were single center experiences and subject to several potential sources of error, including referral and other selection biases, spectrum bias,[11] diagnostic review bias (lack of blinding to UroVysion results)[12] and inadequate accounting for indeterminate test results.[13]

Therefore, we performed a prospective, multicenter, diagnostic trial to evaluate the ability of the UroVysion FISH assay to predict the early disease outcome (recurrence or progression within 9 months) in patients with NMIBC treated with induction BCG immunotherapy.

PATIENTS AND METHODS

Objective

The objective of the study was to determine whether the UroVysion FISH assay could predict bladder cancer recurrence or progression in patients with primary high grade NMIBC scheduled to receive BCG therapy.

Inclusion and Exclusion Criteria

Patients older than 18 years old with NMIBC who had high grade Ta or high grade T1 tumors, or CIS and who were starting the first course of induction BCG were eligible for study. Patients were excluded if they were unable to undergo routine surveillance cystoscopy at 3-month intervals or they failed to meet individual institutional criteria for starting or continuing induction intravesical BCG. Institutional Review Board ethical approval was required at each site and informed consent was obtained from each patient prior to study participation (IRB No. 062013-045).

Intravesical bacillus Calmette-Guérin and Bladder Surveillance

Patients were treated with an induction course of full dose intravesical BCG given weekly for a total of 6 doses with a target dwell time of 2 hours. To allow for generalizability of study results to real world settings investigators at each site were allowed to follow institutional standard operating procedures for intravesical BCG administration. Maintenance BCG was given at the discretion of each investigator and, when administered, they followed the SWOG (Southwest Oncology Group)/Lamm protocol.[4,5] Patients underwent surveillance cystoscopy and urine cytology 3, 6 and 9 months after completing the induction course of BCG.

Recurrence was defined as any histologically proven carcinoma in the bladder regardless of stage and grade. Stage progression was defined as the histological development of T2 or greater, or T1 cancer in patients who previously had stage Ta or CIS disease. The treatment of patients with negative cystoscopy but positive cytology findings was left to the discretion of each individual investigator.

Our goal was to assess recurrence and progression events 6 months after BCG. This time point defines BCG unresponsive bladder cancer.[14] If recurrence or progression develops within this early time window, treatment escalation to cystectomy is often recommended.[4,5] At the same time to allow for possible scheduling inefficiencies which could delay the 6-month disease ascertainment visit our study followup was extended to 9 months.

UroVysion Fluorescence In Situ Hybridization Assay

A voided urine specimen was obtained at 3 time points during the study, including 1) prior to initiating BCG therapy, 2) immediately prior to the sixth BCG instillation and 3) at a visit 3 months after BCG initiation. In patients unable to void a catheterized urine sample was accepted. Urine samples were sent to a single reference laboratory for FISH testing. To avoid diagnostic review bias in endoscopic disease ascertainment[12] clinicians were blinded to FISH assay results until study completion.

UroVysion is an in vitro diagnostic test designed to detect chromosomes 3, 7 and 17, aneuploidy and loss of the 9p21 locus in urine epithelial cells. The FISH assay was performed according to manufacturer labeling.[15] FISH assay results were reported as positive, negative or uninformative (ie inadequate cells for analysis or test failure).

Statistical Methods

To assess the association of the FISH assay with the 6-month post-BCG recurrence rate, the sample size was estimated to require at least 16 positive and 16 negative results. To achieve 80% power the proportion of the assay negative group with recurrence would need to be 0.169 and the proportion of the assay positive group with recurrence would need to be 0.692.

Baseline study patient characteristics were tabulated and analyzed as the mean ± SD for continuous variables, and the count and proportion for categorical variables. Since the FISH assay was repeated on 3 occasions per subject and status could change on each occasion, we visually summarized all patient state transitions using a Sankey (alluvial) flow diagram with the width of each ribbon band proportional to its sample size.[16]

Event-free survival was calculated with the Kaplan-Meier method and survival modeling was done using proportional hazards models for individual test time points. When modeling overall test performance across all testing time points, we used a multistate proportional hazards model since the standard Cox proportional hazards model does not allow for repeat testing in patients with time.[17] The proportional hazards assumption was assessed by testing the correlation between scaled Schoenfeld residuals and time. Statistical analyses were done with R 3.5.1 (https://cran.r-project.org/bin/windows/base/old/3.5.1/) with the lme4 and survival packages installed.

RESULTS

At 7 sites a total of 150 subjects were enrolled in the clinical trial. The supplementary table (https://www.jurology.com) shows study cohort characteristics overall and by the baseline UroVysion result. Mean patient age was 72 years, and 81% of the patients were male and 92% were Caucasian. FISH assay results were associated with patient age and disease stage. During the 9-month followup there were 46 bladder cancer related events, including 37 recurrences and 9 progressions. Four patients had low grade recurrence and 6 of the 9 with progression had T1, 2 had T2 and 1 had metastatic disease. The supplementary table (https://www.jurology.com) lists FISH assay results at each testing time point. Figure 1 shows the flow of individual patients through the time points.

The cumulative incidence of recurrence at 3, 6 and 9 months was 16% (95% CI 10–22), 23%

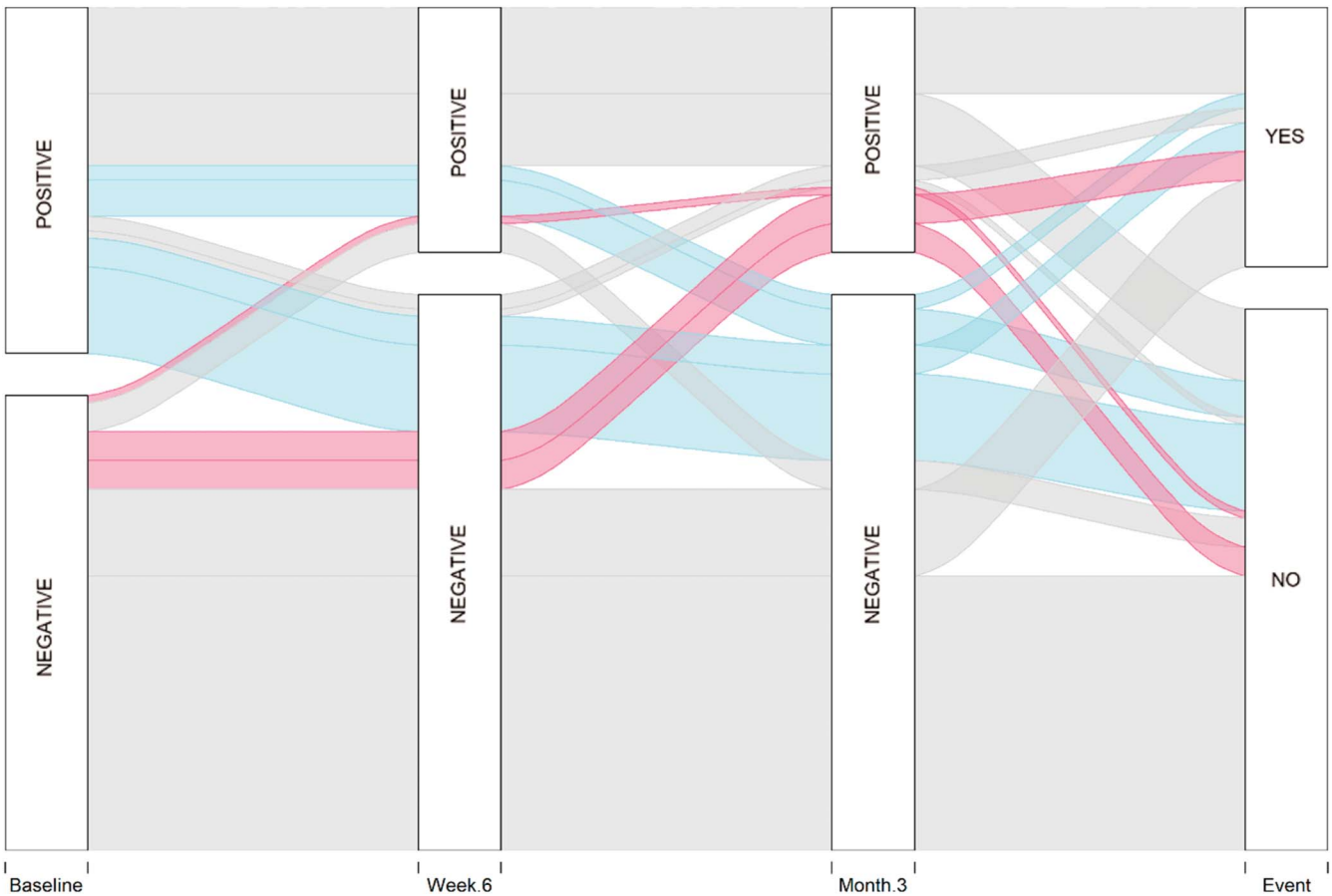

Figure 1. Sankey diagram shows FISH assay results at baseline, 6 weeks and 3 months, and whether there was event during 9-month followup.

(95% CI 16–30) and 30% (95% CI 22–37), respectively. Figure 2 shows the cumulative incidence of recurrence stratified by the FISH assay result at baseline before BCG, at the 6-week BCG instillation and at 3-month cystoscopy. At each time point a positive FISH assay result was associated with a significantly higher probability of subsequent tumor recurrence. The HR of recurrence with a positive FISH assay result was 2.59 (95% CI 1.42–4.73) at baseline, 1.93 (95% CI 1.04–3.59) at 6 weeks and

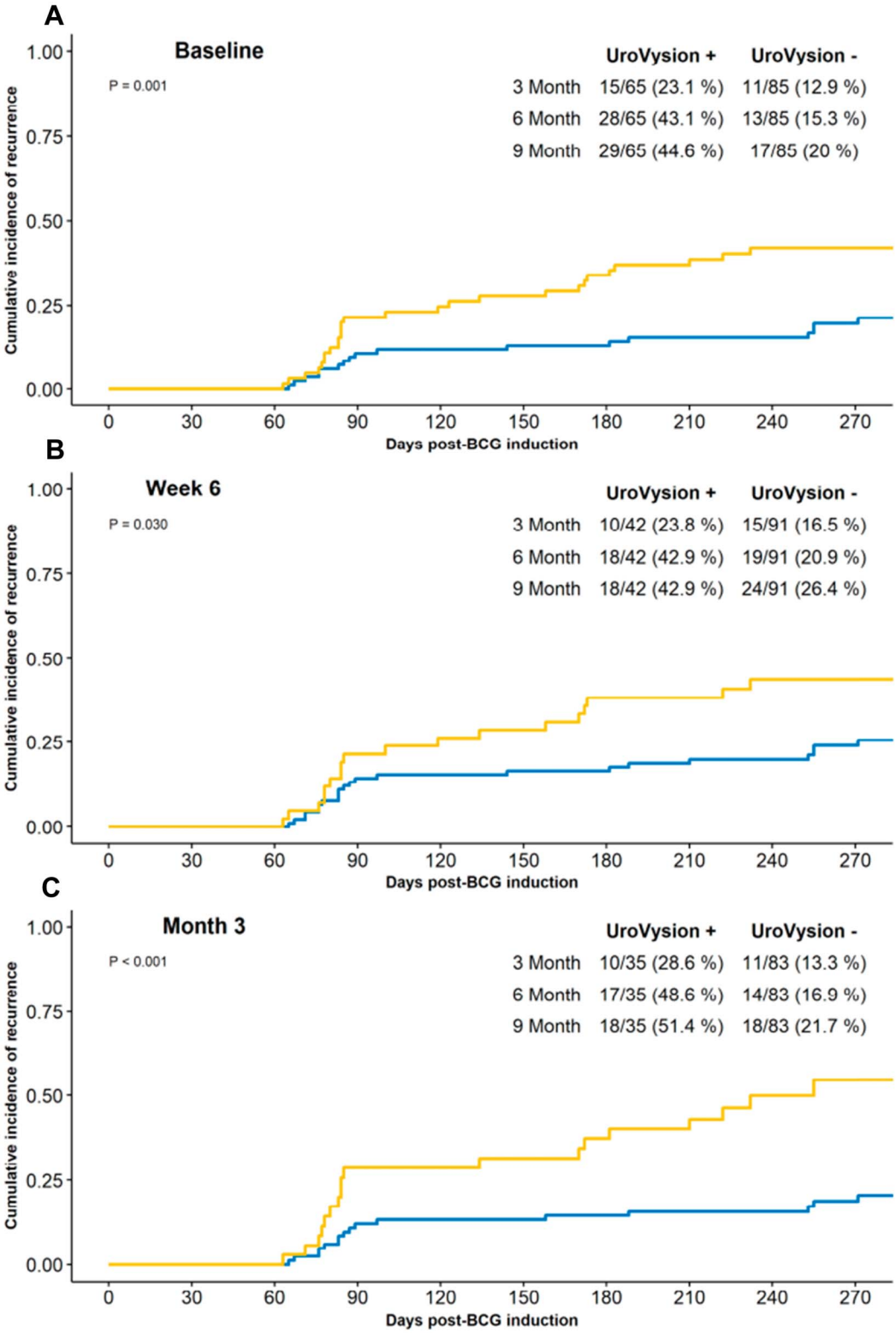

Figure 2. Event rate in FISH assay positive (yellow curve) and negative (blue curve) cases

Association of UroVysion results with events

Result				
Baseline	Wk 6	Mo 3	Event	% Pts
Neg	Neg	Neg	No	34.23
Neg	Neg	Neg	Yes	10.81
Pos	Pos	Pos	Yes	10.81
Pos	Pos	Pos	No	9.01
Pos	Neg	Neg	No	10.81
Pos	Neg	Neg	Yes	3.60
Pos	Pos	Neg	No	4.50
Pos	Pos	Neg	Yes	1.80
Pos	Neg	Pos	No	0.90
Pos	Neg	Pos	Yes	1.80
Neg	Neg	Pos	Yes	3.60
Neg	Neg	Pos	No	3.60
Neg	Pos	Neg	No	3.60
Neg	Pos	Neg	Yes	0
Neg	Pos	Pos	Yes	0
Neg	Pos	Pos	No	0.90

3.22 (95% CI 1.65–6.27) at 3 months. Using the multistate proportional hazards model the overall HR of recurrence given a positive FISH assay result regardless of the time point of testing was 3.35 (95% CI 1.59–7.05). There was no evidence of a departure from the proportional hazards assumption.

Notably 1% to 2% of the patients at each testing time point had an uninformative FISH assay result (supplementary table, https://www.jurology.com). For the mentioned analyses those tests were considered negative since most clinicians would not take clinical action based on an uninformative test result. However, as a sensitivity test to this assumption, we reanalyzed the data after reclassifying uninformative tests as positive. We found that the results were largely unchanged with an overall recurrence HR of 3.27 (95% CI 1.55–6.90).

Testing was repeated at baseline, 6 weeks and 3 months. The table lists FISH assay results with time along with how frequently they occurred and associations with an event.

In 45% of the patients all 3 repeated results were negative. Of these patients 76% did not have an event during the 9 months of followup. In 20% of patients the test was positive at each time point and 55% of this cohort had an event. Meanwhile, 14% of cases converted from a positive test at baseline to a negative test at 6 weeks and 3 months, including 75% without recurrence. Of the patients 35% had different FISH assay results (positive or negative) during the 3 time points, including 31% with events.

DISCUSSION

We report a multicenter, prospective clinical trial evaluating the usefulness of the UroVysion FISH assay to predict bladder cancer recurrence and progression in patients with primary high risk NMIBC undergoing induction BCG therapy. Within 6 and 9 months after BCG 27% and 31% of the patients, respectively, experienced an event. Overall a positive FISH assay test within the first 3 months of initiating BCG therapy was associated with a 3.3-fold higher risk of recurrence.

Our results largely validate prior single center studies showing the prognostic significance of UroVysion FISH assay status at BCG therapy initiation or conclusion. In a series of 126 patients with NMIBC Kamat et al found that those with a positive FISH assay result 6 weeks, or 3 or 6 months after initiating BCG therapy were 3 to 5 times more likely to experience recurrence and 5 to 13 times more likely to experience disease progression.[10] While our recurrence results align with these findings, the progression rate noted was far lower, likely reflecting differences in patient populations between our studies. Other potential etiologies for these varied findings include disparate performance of augmented endoscopy, second look transurethral resection and immediate postoperative chemotherapy.

Mengual et al evaluated the UroVysion FISH assay before and after BCG in 65 patients with high risk NMIBC.[8] They reported that patients with positive post-BCG FISH assay findings were at 2.7-fold increased risk for recurrence. Likewise, Whitson et al retrospectively evaluated 42 patients who underwent induction intravesical therapy with BCG, BCG plus interferon or mitomycin.[18] They noted that a positive FISH assay result after intravesical therapy was significantly associated with the risk of recurrence. Kipp et al were the first to report the FISH assay and the BCG response.[9] In a retrospective series of 37 patients receiving intravesical therapy 100% with a positive post-therapy FISH assay result experienced recurrence while recurrence was observed in only 52% of those with a negative result. Also, Savic et al prospectively evaluated 68 patients with NMIBC and determined that a positive post-BCG FISH assay result was associated with a 5.6-fold increased risk of recurrence.[19] While all of these studies agree that a positive UroVysion FISH assay is a negative prognostic factor, none characterized the entire diagnostic test performance as we did prospectively in this study. Furthermore, these prior studies were single institution experiences which lacked prospective validation.

When considering how to best apply the information in this study, on a population basis we noted that a positive FISH assay result translated to an increased recurrence risk in patients. However, in an individual patient changing the treatment based on the FISH assay result is challenging. Unlike many studies evaluating urine markers in which the marker was performed the same day as cystoscopy and determining performance characteristics

such as sensitivity was relatively straightforward, in this study we evaluated the prognostic role of the FISH assay. Thus, we correlated the results of a test performed at baseline (or 6 weeks or 3 months) with an event that occurred during a 9-month followup.

The impact of using a urine marker in this way is that the patient cancer status may change with time and marker status may also change. A patient with a positive marker at baseline may have cancer which later responds to BCG and the marker may convert to negative at 6 weeks or 3 months. Similarly a patient may have a negative marker at baseline if there is no cancer, and then cancer may develop 6 months later so that the marker did not predict future events but was correct on the day of the procedure. Furthermore, a patient may have a positive marker despite being free of cancer at baseline and then cancer may develop at 9 months. The marker will appear to have correctly predicted this event.

We observed that in a proportion of patients with an initially positive FISH assay the result remained positive and they did not experience an event by 9 months or convert to negative findings, which could occur due to a response to treatment (fig. 1). Changing management based on an initially positive result in these scenarios would not be to the benefit of the patient. Similarly some patients with a negative FISH assay result experienced an event. This suggests that the tumor developed after the biomarkers were evaluated or the biomarker result was false-negative. In each of those scenarios maintaining a similar surveillance schedule is necessary since patients have high grade disease.

These inconsistent performance characteristics were not affected by how uninformative test results were handled and they were consistent with the results of a recent series of 2,040 UroVysion tests done in a real world setting.[20] Additionally, we observed that the FISH assay was subject to some degree of spectrum bias as it performed differently in patients of different ages, again consistent with previous findings.[11]

While these factors are important to treat individuals, there is still a potential role for using the FISH assay to stratify patients for clinical trial inclusion. Kamat et al noted that patients with positive early FISH and negative 3-month cystoscopy results could be considered to have molecular BCG failure, which could be used to design clinical trials.[21] Since patients with a positive FISH assay are at higher risk for recurrence events, the test might help stratify patients for clinical trials based on the risk of recurrence.

We acknowledge that our study is limited by its 9-month followup. The rationale for that time frame was to identify the ability of the UroVysion FISH assay to predict early recurrence or progression following BCG and identify patients who might benefit from clinical trial enrollment or other changes in treatment strategy. We did not collect data on repeat transurethral bladder resection in patients with T1 disease. These data were not collected since the study was designed to be initiated when patients started BCG after a decision was made by the urologist that this was an appropriate course.

Also, the study was performed at well-known academic centers with experienced urological oncologists. Furthermore, only 2 patients progressed to muscle invasion during the study and in 1 metastatic disease developed, suggesting that it is unlikely that patients with muscle invasive disease were included in analysis. We also note that the current Medicare cost of the UroVysion FISH assay is $488 and in this study we did not assess cost-effectiveness.[22]

CONCLUSIONS

In a prospective multicenter trial we validated the association of a positive UroVysion FISH assay with a 3.3-fold increased risk of recurrence among patients with NMIBC who received BCG. The test may be useful to risk stratify patients entering clinical trials who have no response to respond to induction therapy. However, using the test to change treatment decisions in individual patients is limited due to the discordance between results and outcomes as well as the variance in test results with time.

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