


Original Article

Longitudinal follow-up and performance validation of an mRNA-based urine test (Xpert® Bladder Cancer Monitor) for surveillance in patients with non-muscle-invasive bladder cancer

Barrett Cowan¹, Eric Klein², Ken Jansz³, Karl Westenfelder⁴, Timothy Bradford⁵, Chad Peterson⁶, Douglas Scherr⁷, Lawrence I. Karsh⁸, Blair Egerdie⁹, Alfred Witjes¹⁰, Andrew Trainer¹¹, Richard Harris¹², Bernard Goldfarb¹³, Stanley Flax¹⁴, Robert Kroeger¹⁵, Buffi Boyd¹⁶, Joseph Liao¹⁷, Sanjay Patel¹⁸, Julia Bridge¹⁹, Victor Reuter²⁰, Neil Quigley²¹, Sarah Brown²², Suling Zhao²², Malini Satya²², Michael Bates²², Iris M. Simon²², Scott Campbell²² and Yair Lotan²³ 

¹Urology Associates, Englewood, CO, ²Idaho Urologic Institute, Meridian, ID, USA, ³Jansz Medicine Professional Corp, Burlington, ON, Canada, ⁴Five Valleys Urology, Missoula, MT, ⁵Virginia Urology, Richmond, VA, ⁶North Idaho Urology, Coeur d Alene, ID, ⁷Weill Cornell Medicine, New York, NY, ⁸The Urology Centre of Colorado, Denver, CO, USA, ⁹Urology Associates/Urology Medical Research, Kitchener, ON, Canada, ¹⁰Radboud University Medical Centre, Nijmegen, The Netherlands, ¹¹Adult and Paediatric Urology, Omaha, NE, ¹²UroPartners, Melrose Park, IL, USA, ¹³Medical Offices, North Bay, ON, ¹⁴Urology Clinic, North York, ON, Canada, ¹⁵Urology Centre, St. Omaha, NE, ¹⁶Urology Associates of Savannah, Savannah, GA, ¹⁷VA Palo Alto Health Care System, Palo Alto, CA, ¹⁸Stephenson Cancer Centre, Oklahoma City, OK, ¹⁹University of Nebraska Medical Centre, Omaha, NE, ²⁰MSKCC, New York, NY, ²¹Genevity Clinical Research Services, Maryville, TN, ²²Cepheid, Sunnyvale, CA, and ²³UT Southwestern, Dallas, TX, USA

Objective

To evaluate the performance of the Xpert Bladder Cancer Monitor (Xpert; Cepheid, Sunnyvale, CA, USA) test as a predictor of tumour recurrence in patients with non-muscle-invasive bladder cancer (NMIBC).

Patients and Methods

Patients ($n = 429$) undergoing surveillance for NMIBC underwent Xpert, cytology, and UroVysion testing. Patients with a positive Xpert and a negative cystoscopy result (positive-negative [PN] group, $n = 66$) and a control group of double negative patients (negative Xpert and cystoscopy results [NN] group) were followed for 12 months (± 90 days).

Results

Histology-confirmed recurrences were detected in 58 patients (13.5%). Xpert had an overall sensitivity of 60.3% and a specificity of 76.5%. The sensitivity for high-grade (HG) cancer was 87% with a negative predictive value (NPV) of 99%. Urine cytology showed an overall sensitivity of 23.2% (47.6% sensitivity for HG tumours) and a specificity of 88.3%. In the PN group, 32% ($n = 21$) developed a recurrence within 12 months, 11 of which were HG tumours. In the NN control group, 14% ($n = 9$) developed a recurrence and only two were HG tumours. The hazard ratio for developing recurrence in the PN group was 2.68 for all tumours and 6.84 for HG cancer.

Conclusions

The Xpert test has a high sensitivity for detecting the recurrence of cancer and a high NPV for excluding HG cancer. In addition, the data suggest that patients with a positive Xpert assay in the setting of negative cystoscopy are at high risk for recurrence and need close surveillance.

Keywords

non-muscle-invasive bladder cancer, molecular diagnostic, liquid biopsy, surveillance, anticipatory positive, #blcsm, #BladderCancer, #uroonc

Introduction

Frequent recurrences and the potential for progression of non-muscle-invasive bladder cancer (NMIBC) demand close and repeated follow-up to identify recurrences as early as possible. The frequency and length of follow-up of patients depend on their individual risk and might involve the use of cystoscopy, cytology, urinary biomarkers, ultrasonography and other methods [1,2]. However, all follow-up methods are associated with limitations, either in sensitivity, specificity, ease-of-use, cost and/or patient's comfort. Therefore, a combination of methods might improve and personalize the surveillance strategy for patients with NMIBC [3].

The Xpert Bladder Cancer Monitor (Xpert; CE-IVD [Conformite Europeene *In-vitro* Medical Device]; Cepheid, Sunnyvale, CA, USA) measures the expression of five mRNA targets that are frequently overexpressed in bladder cancer and can be detected in voided urine [4]. The Xpert assay is run in a self-contained cartridge using the GeneXpert Instrument (Cepheid) with hands-on time of <2 min and a turnaround time of 90 min. Previously, the assay has been validated in 239 patients with a history of NMIBC and demonstrated a sensitivity of 74% for all tumours and a sensitivity of 83% for high-grade (HG) tumours with a specificity of 80%. The negative predictive value (NPV) was 93% overall and 98% for HG tumours [5]. In addition, independent single-centre studies have confirmed the overall sensitivity and specificity and high NPV for HG tumours in clinical routine [6,7].

In this prospective, multicentre study, the performance of the Xpert assay was validated as a predictor of tumour recurrence using pathological verification. The performance of the Xpert assay was compared to cytology and the UroVysion fluorescence *in situ* hybridization (FISH) assay [8]. We also assessed the significance of a positive or negative marker result in the setting of a negative cystoscopy. Since it is known that standard white-light cystoscopy can miss disease, especially carcinoma *in situ* [9], we also studied whether patients with a positive Xpert result were more likely to have recurrence than patients with a negative Xpert result in patients with negative cystoscopy.

Materials and Methods

Objectives

The primary objective of this prospective, multicentre study was to establish the clinical performance characteristics of Xpert on the GeneXpert Instrument Systems to detect the recurrence of bladder cancer in patients previously diagnosed with bladder cancer. In addition, the performance of Xpert was compared with the performance of UroVysion and cytology as compared to clinical truth. The secondary

objective of the study was to assess the positive predictive value (PPV) and NPV of Xpert for the likelihood of detecting bladder cancer at an earlier time point than current standard of care testing in patients who have recurrence with a positive Xpert result and who present as disease-negative at the initial visit.

Patients

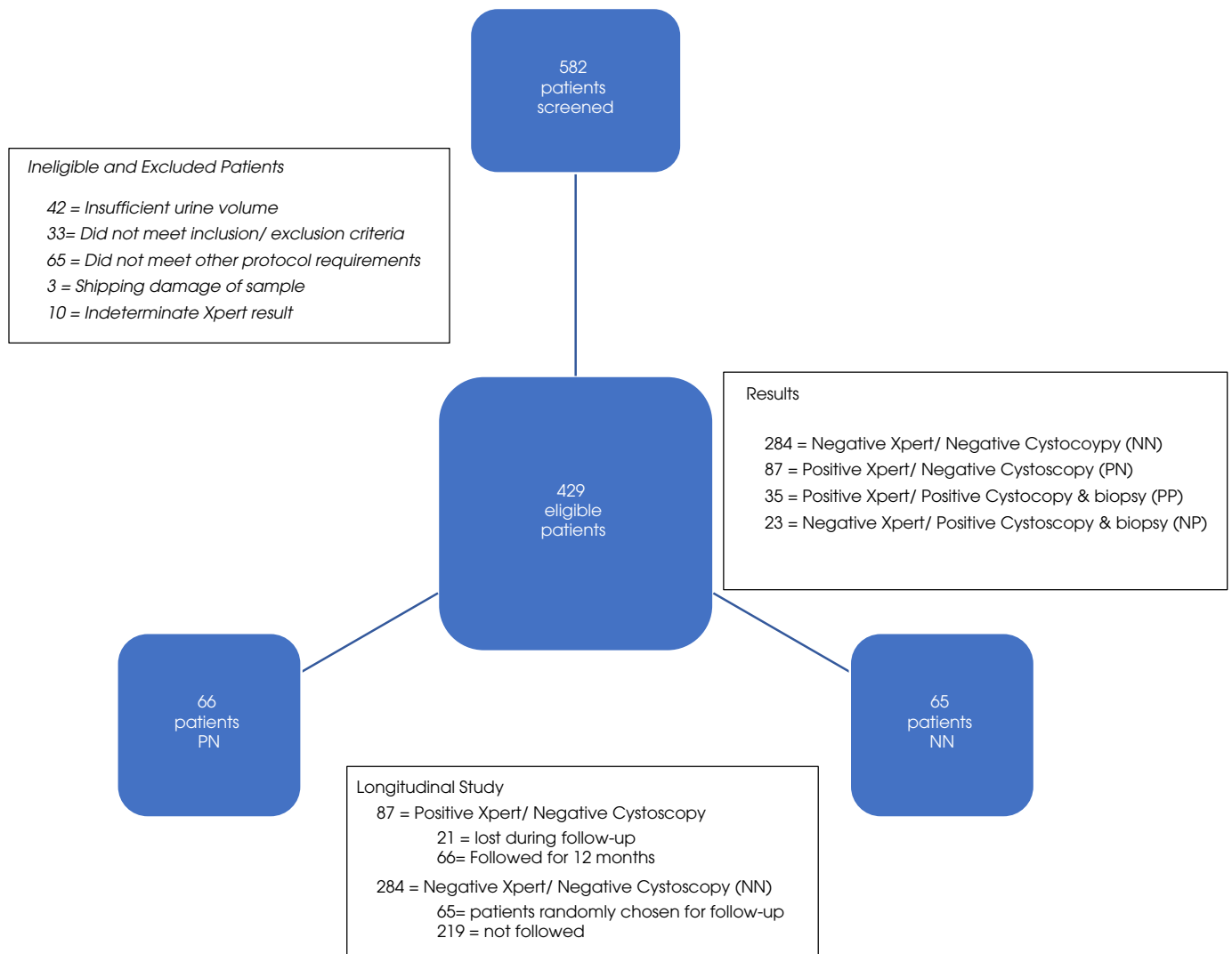
The study was conducted from December 2016 to May 2019 at medical clinics and reference laboratories in the USA, Canada and the Netherlands. The appropriate institutional review boards or ethics committee approved the study at all sites. All patients gave informed consent as required and, for California only, signed the Experimental Subjects Bill of Rights. Inclusion criteria were initial diagnosis or recurrence of NMIBC within 12 months of enrolment, with scheduling for routine white-light cystoscopy for surveillance of disease. Patients above the age of 40 years from 19 centres were enrolled in the study. Exclusion criteria were first morning void urine or a volume of <60 mL, and transurethral resection of bladder tumour (TURBT) or other bladder excision procedure within 42 days of cystoscopy. At baseline, the same voided urine was aliquoted into the transport tubes for Xpert, UroVysion and cytology assessment.

In total, 582 patients were screened at 19 clinical sites over 29 months. Of these, there were a number of screen failures who were not enrolled for the following reasons: inability to provide sufficient volume of urine ($n = 42$); not meeting one or more of the inclusion criteria ($n = 33$); and not meeting other protocol requirements (no biopsy of positive/suspicious cystoscopy, TURBT >6 weeks after study visit, cystoscopy >72 h after specimen collection, haematuria not evaluated, Xpert testing >7 days after specimen collection; $n = 65$). Three samples were excluded because of shipping damage and 10 samples (1.7%) had an indeterminate Xpert result, resulting in the enrolment of 429 patients for the baseline analysis (Fig. 1).

Results from Xpert and UroVysion and cytology performed at the reference laboratory were not used for patient management. The operator(s) performing Xpert and UroVysion and cytology performed at the reference laboratory were blinded to patient status, cystoscopy, UroVysion, cytology, and central histology results. The local pathology review and the initial central pathology review were independent and blinded to each other.

Xpert Bladder Cancer Monitor

Xpert detects five mRNA targets (ABL1, ANXA10, UPK1B, CRH, IGF2) in a self-contained cartridge. The marker expression levels combined in a linear discriminant analysis are used to classify samples as negative or positive [4]. In

Fig. 1 Subject accountability in the baseline and longitudinal Xpert Monitor Study.

In addition, the cartridge contains multiple internal controls to assure RT-PCR. The GeneXpert System automates and integrates cell lysis, nucleic acid amplification, and detection of the target sequences using real-time RT-PCR. Voided urine samples (4.5 mL) were mixed with the Xpert urine transport reagent within 1 h of urine collection, making the sample stable for 1 week at 2–28°C, and then run on the GeneXpert System. External positive and negative controls (Maine Molecular Quality Controls, Inc., Saco, ME, USA) were run on each day study specimens were tested.

Pathology, Cytology and UroVysion Procedures

For all positive or suspicious cystoscopies, tissue was obtained through a cystoscopic biopsy or TURBT within 6 weeks of the original cystoscopy. All tissue specimens were assessed by the local pathology laboratory. Two central

pathologists (V.R., D.G.) independently evaluated all biopsy specimens using the same or an adjacent slide. If the central pathologists did not agree, a concordance review was scheduled with both pathologists to agree on tumour grade and stage. Only if central pathology confirmed the presence of tumour were patients classified as having recurrence. All voided urine specimens were analysed by cytology and UroVysion (Abbott Molecular, Des Plaines, IL, USA) at the reference laboratory (J.B., N.Q.). Cytology categorization was performed according to the Paris system within the reference laboratory's standard procedures. The Paris system was developed to rule in/rule out HG urothelial carcinoma, which is consistent with the goal of this study [10]. Positive and suspicious results were considered positive; atypical and negative results were considered negative. UroVysion testing and reporting was performed according to the manufacturer's instructions.

Longitudinal Study

The secondary objective for this study was to determine if patients with a negative cystoscopy and a positive Xpert assay were more likely to experience recurrence than patients with a negative cystoscopy and negative Xpert result. All patients with a positive Xpert and a negative cystoscopy result (positive-negative [PN] group) were followed for 12 months (± 90 days). As a control, a random subset of patients with a negative Xpert and negative cystoscopy result at the baseline visit (negative-negative [NN] group) was also enrolled in the longitudinal cohort. Since this was a random sample we did not match for prior stage and grade.

Statistical Analysis

Sensitivity, specificity, PPV and NPV, including 95% CIs, were calculated for all and HG recurrences to assess clinical test performance. Bladder cancer recurrence status was determined by cystoscopy alone (if negative) or histology (if cystoscopy was suspicious or positive) as determined by central pathology. The longitudinal cohort was analysed with a Kaplan–Meier analysis. The Kaplan–Meier method, or product limit estimator, is a non-parametric statistic used to estimate time-to-event, and can take censored data into account such as patient withdrawal, loss to follow-up, or alive without event occurrence at last follow-up. In our Kaplan–Meier analysis, the two groups were analysed separately (PN vs NN at baseline). The two groups were compared using a log-rank test in SAS.

Sample Size Calculation

We chose the superiority design to prove that Xpert would perform equally, or better than the UroVysion test in sensitivity, while being non-inferior in specificity. The minimum overall sample size for primary and secondary objectives would be $N = 40$ disease-positive cases (minimum 40 patients with low-grade (LG) or HG tumours) for our sensitivity claims and $N = 210$ disease-negative cases for our specificity claims. Assuming that the difference in the sensitivity of Xpert and UroVysion is 24%, the power to detect the difference would be 80%. Given historical prevalence rates in similar sampling populations, we predicted accrual of a total sample size of between 250 and 530 patients in the study. The specificity requirement would be that the lower two-sided 95% CI be $>65\%$. However, because of the low prevalence, the number of reference disease-negative cases was expected to be much greater than needed to power the test.

Results

Patient Characteristics

Table 1 shows the patient characteristics. The average patient age was 71 years and most were men (80%) and White

(95%). Almost 68% of patients were current or former smokers. Before enrolment, 178 patients (41.5%) had been diagnosed with HG tumours and 230 patients (53.6%) with LG tumours (4.9% had missing grade information). Most patients had a stage Ta tumour ($n = 324$). Microscopic haematuria was detected in 31% of patients at enrolment. Half of the patients had no BCG or intravesical chemotherapy treatment before enrolment ($n = 213$), 63 patients received only intravesical chemotherapy and 149 patients received BCG before enrolment (four patients had missing information).

Xpert Bladder Cancer Monitor Performance

In the eligible patients, 58 patients (13.5%) had recurrence, of whom 35 had LG and 23 had HG recurrence of cancer. The sensitivity of Xpert to detect recurrences was 60.3% with a specificity of 76.5%. The NPV was 92.5%. In the analysis of

Table 1 Patient information of patients at baseline ($n = 429$).

Category	Type	
Sex, n (%)	F	85 (19.8)
	M	344 (80.2)
Race, n (%)	Asian	4 (0.9)
	Black/African American	8 (1.9)
	Other	3 (0.7)
	Two or more races	6 (1.4)
	White	408 (95.1)
Smoking history, n (%)	Current	52 (12.1)
	Former	239 (55.7)
	Never a smoker	138 (32.2)
Haematuria at baseline, n (%)	Missing data	1 (0.2)
	Macroscopic haematuria	3 (0.7)
	Microscopic haematuria	131 (30.5)
Age, years	None	294 (68.5)
	Overall	71.4 \pm 9.5 (40–93)
	F	69.8 \pm 9.6 (45–91)
Treatment before enrolment, n (%)	M	71.8 \pm 9.4 (40–93)
	BCG	149 (34.7)
	Intravesical chemotherapy	63 (14.7)
Tumour grade before enrolment, n (%)	No treatment	213 (49.7)
	Missing data	4 (0.9)
	HG	178 (41.5)
Tumour stage before enrolment, n (%)	LG	230 (53.6)
	Missing/other	21 (4.9)
	Ta (HG)	94 (21.9)
	Ta (LG)*	228 (53.1)
	Ta (other)	2 (0.5)
	T1/T2 (HG)	46 (10.7)
	T1S (HG)	37 (8.6)
	Tx (HG)	1 (0.2)
	Tx (LG)	2 (0.5)
	Missing	19 (4.4)
Median time from last tumour to enrolment		5 months

HG, high grade; LG, low grade. Data are mean \pm SD (range) unless otherwise stated. *PUNLMP (Papillary Urinary Neoplasm of Low Malignant Potential) is classified as LG.

only HG tumours, the sensitivity to detect recurrences was 87%, the specificity was 76.5% and the NPV was 99% (Table 2a,b). The sensitivity to detect LG tumours was 43%. The sensitivity to detect T1, T2 and TIS tumours was 100%, 100% and 80%, respectively (Table 3).

The sensitivity to detect recurrences in patients who received BCG within the last 3 months before enrolment ($n = 90$ with nine recurrences) was 78%, with a specificity of 69%. The sensitivity to detect recurrences in patients who received BCG at any time before enrolment (13 recurrences) was 69%, with a specificity of 73% (NPV 96%). The performance of Xpert in patients who had received no treatment before enrolment (37 recurrences) was reflected in a 57% sensitivity with a specificity of 81%.

Performance of UroVysion and Cytology

Of the 429 patients enrolled at baseline, eight had no valid cytology results and UroVysion results were not available or indeterminate for 17 patients. To best compare results among the biomarkers, a performance analysis was conducted in only those patients with all biomarker results ($n = 405$). In this study cohort, 35 patients had an LG recurrence and 21 an HG recurrence. The overall sensitivity of Xpert, UroVysion and cytology to detect any recurrence was 59%, 45% and 23%, with a specificity of 76%, 73% and 88%, respectively (Table 4). The Xpert assay had a higher

Table 2 (A) Performance of the Xpert Bladder Cancer Monitor to detect all tumours and (B) to detect high-grade tumours with using cystoscopy/histology as the 'gold standard'.

	Cystoscopy/histology		
	Positive	Negative	Total
(A)			
Xpert			
Positive	35	87	122
Negative	23	284	307
Total	58	371	429
Sensitivity, %		60.3 (95% CI 47.5–71.9)	
Specificity, %		76.5 (95% CI 72.0–80.6)	
PPV, %		28.7 (95% CI 21.4–37.3)	
NPV, %		92.5 (95% CI 89.0–95.0)	
Accuracy, %		74.4 (95% CI 70.0–78.3)	
Prevalence, %		13.5 (95% CI 10.6–17.1)	
(B)			
Xpert			
Positive	20	87	107
Negative	3	284	287
Total	23	371	394
Sensitivity, %		87.0 (95% CI 67.9–95.5)	
Specificity, %		76.5 (95% CI 72.0–80.6)	
PPV, %		18.7 (95% CI 12.4–27.1)	
NPV, %		99.0 (95% CI 97.0–99.6)	
Accuracy, %		77.2 (95% CI 72.8–81.0)	
Prevalence, %		5.8 (95% CI 3.9–8.6)	

NPV, negative predictive value; PPV, positive predictive value; Xpert, Xpert Bladder Cancer Monitor.

Table 3 Sensitivity of the Xpert Bladder Cancer Monitor to detect tumour subgroups.

Stage	Sensitivity	
	n/N (%)	95% CI
ALL	35/58 (60)	47.5–71.9
Ta, HG	7/9 (78)	45.3–93.7
Ta, LG	15/35 (43)	28.0–59.1
T1	5/5 (100)	56.6–100.0
T2	4/4 (100)	51.0–100.0
TIS	4/5 (80)	37.6–96.4
Grade		
HG	20/23 (87)	67.9–95.5
LG	15/35 (43)	28.0–59.1

HG, high grade; LG, low grade; NPV, negative predictive value; PPV, positive predictive value.

sensitivity for LG tumours (43%) compared to 29% and 8.6% for UroVysion and cytology. The Xpert assay also had a higher sensitivity for HG tumours (86%) compared to 71% and 48% for UroVysion and cytology in this study cohort.

Combining Xpert with cytology did not increase the sensitivity of detecting tumours (data not shown). Combining Xpert with UroVysion would have identified one additional HG/TIS tumour.

Longitudinal Study

To investigate whether patients with a positive Xpert result but a negative cystoscopy (PN group) had a higher risk of recurrence than patients with a negative cystoscopy and negative Xpert assay (NN), 131 patients (66 PN and 65 NN patients) with at least 12 months (± 90 days) of follow-up were observed. At baseline, there were 284 NN patients, 65 of whom were randomly chosen to be followed for 12 months. A comparison of patient characteristics between the 65 randomly chosen NN patients and the 219 not-chosen NN patients showed that the two groups did not differ in any variable analysed (age, smoking status, previous stage or grade of tumour). This indicates that the 65 NN patients represented the total of all NN patients in the study (Table S1).

The patient characteristics of the 65 NN patients and the 66 PN patients are shown in Table S2. Patients with a positive Xpert result and negative cystoscopy were more likely to have had an HG tumour before enrolment ($P = 0.004$) and prior intravesical therapy. Of the 131 specimens in patients who completed the longitudinal study, 129 specimens had valid cytology test results. Patients in the PN group were more likely to have positive cytology than those in the NN group, with 22.7% (15/66) vs 6.3% (4/63), respectively.

The median time since last tumour, age and smoking status were similar in the two groups. In the NN group, nine patients (14%) experienced a recurrence of their tumour

Table 4 Performance of the Xpert Bladder Cancer Monitor, UroVysion and cytology in the subset of patients with results for all three biomarkers ($N = 405$).

	Xpert	UroVysion	Cytology
Sensitivity, %	59 (95% CI 46–71)	45 (95% CI 32–58)	23 (95% CI 14–36)
Sensitivity LG, %	43 (95% CI 28–59)	29 (95% CI 16–45)	8.6 (95% CI 3.0–22)
Sensitivity HG, %	86 (95% CI 65–95)	71 (95% CI 50–86)	48 (95% CI 28–68)
Specificity, %	76 (95% CI 72–80)	73 (95% CI 68–78)	88 (95% CI 85–91)
PPV, %	28 (95% CI 21–37)	21 (95% CI 15–29)	24 (95% CI 15–37)
NPV, %	92 (95% CI 88–95)	89 (95% CI 85–92)	88 (95% CI 84–91)
Accuracy, %	74 (95% CI 69–78)	69 (95% CI 65–73)	79 (95% CI 75–83)
Prevalence, %	14 (95% CI 11–18)	14 (95% CI 11–18)	14 (95% CI 11–18)

HG, high grade; LG, low grade; NPV, negative predictive value; PPV, positive predictive value; Xpert, Xpert Bladder Cancer Monitor.

within 1 year of follow-up: seven patients had an LG tumour and two patients had an HG Ta recurrence. In the PN group, 21 patients (32%) had recurrence, 10 of whom had an LG and 11 an HG recurrence Ta ($n = 2$), T1 ($n = 4$) and TIS ($n = 5$).

The hazard ratio (HR) for experiencing a recurrence in the PN group was 2.7 (95% CI 1.2–5.9) with a P value of 0.0098 (Fig. 2a). The HR for experiencing an HG recurrence in the PN group was 6.8 (95% CI 1.5–30.9) with a P value of 0.0037 (Fig. 2b). The results indicate that patients with a positive Xpert result are more likely to experience an HG recurrence, even if cystoscopy is negative. There was no difference in recurrence rates for patients who had LG tumours in the PN and NN groups (Fig. 2c).

We compared UroVysion-positive patients with negative cystoscopy ($N = 43$) and UroVysion-negative patients with negative cystoscopy ($N = 82$) and found a higher recurrence rate in those with a positive UroVysion result (Fig. S1).

Of the 429 patients in the study, 80 patients had a suspicious cystoscopy, which was followed by TURBT or a cystoscopic biopsy. Eight additional patients with a suspicious cystoscopy did not have a follow-up TURBT or biopsy for reasons related to their particular clinical situation. Of these 80 patients, 25 were classified as negative by central pathology (31.2%). When evaluating the results by Xpert in those 25 patients who were false-positive by cystoscopy, Xpert had a true-negative rate of 64%, i.e. 16 of those 25 patients were negative by Xpert.

Discussion

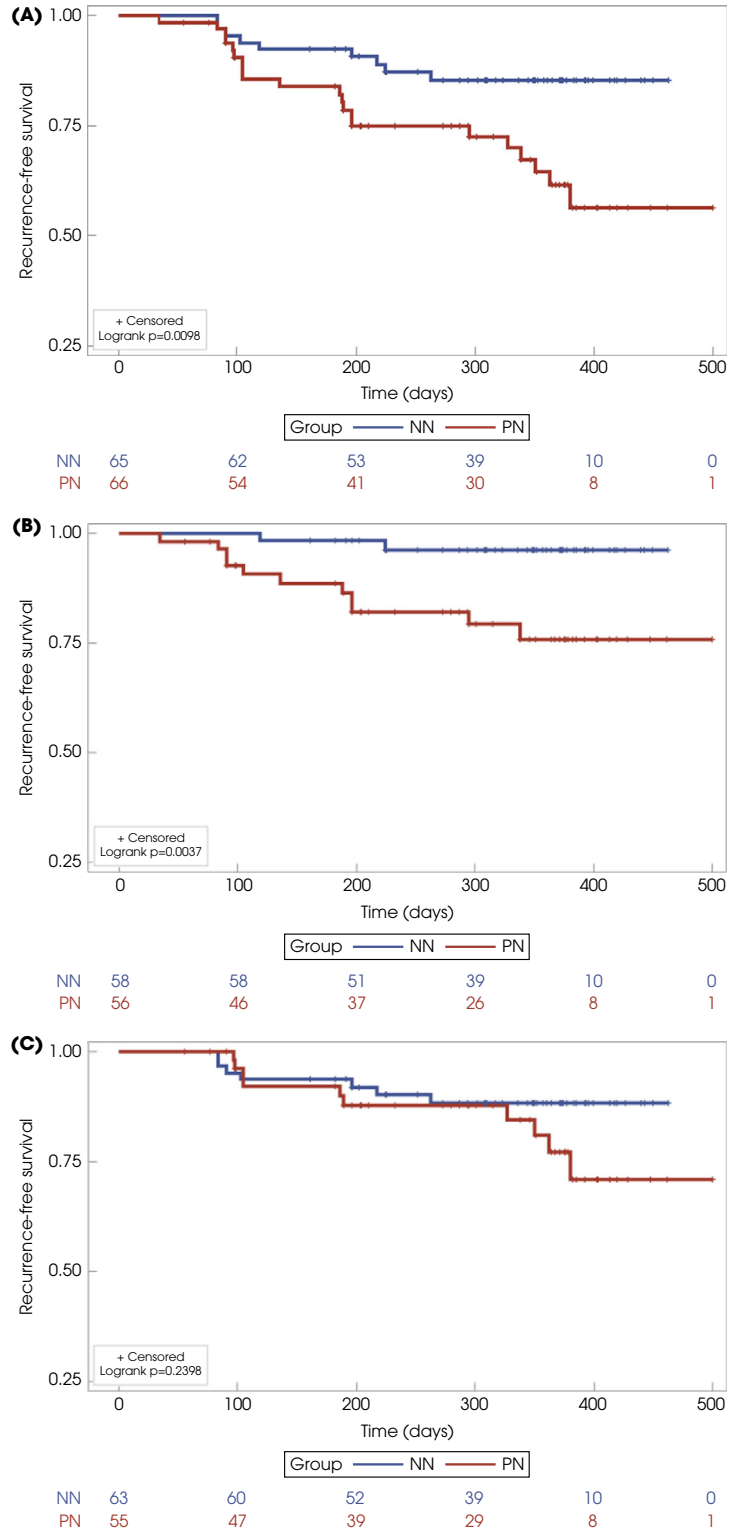
This multicentre trial found that Xpert had a high sensitivity for detecting HG recurrences of 87%, with an NPV of 99%. These results were not impacted by use of intravesical therapies such as BCG. The sensitivity was also superior to the UroVysion FISH assay and cytology for both LG and HG disease. Overall the specificity was 76.5% and was not impacted significantly by use of intravesical BCG, where overall specificity was 73%. This multicentre cohort validates prior studies of the Xpert test and demonstrate that it can be

effective at detecting bladder cancer recurrence during NMIBC surveillance [5–7].

One of the goals of the present study was to evaluate the significance of a positive Xpert assay in the setting of a negative cystoscopy. The use of enhanced cystoscopy has highlighted the fact that white-light cystoscopy can miss up to 10–30% of cancers, especially carcinoma *in situ*, but also lower-grade disease [11–13]. There is also recognition that urine-based tumour markers may be able to identify cancer early prior to visual detection. This has led to the term ‘anticipatory positive’ which implies that a positive urine marker in the setting of ‘normal’ cystoscopy is really a true-positive marker that has yet to be detected cystoscopically [8,14]. In this study, patients with a positive Xpert assay and negative cystoscopy were 2.7 times more likely to have recurrence than patients with a negative Xpert and negative cystoscopy result. More importantly, the HR for experiencing an HG recurrence in the PN group was 6.8. This means that PN patients were much more likely to have a missed HG tumour than a missed LG tumour. While, this trial was not designed to assess how to evaluate patients with a positive Xpert assay, it would seem reasonable to consider enhanced cystoscopy at the time of the next evaluation or to have a closer surveillance if not already scheduled at 3-month intervals. One limitation of this longitudinal analysis was that a higher proportion in the PN group had a positive cytology (22.7% vs 6.3%) which may have contributed to higher recurrence. The study was underpowered to study PN vs NN when excluding these patients.

Prior studies evaluating anticipatory positives for UroVysion found that patients with a positive UroVysion result in the setting of a normal cystoscopy had a shorter time to recurrence but similar recurrence rates when compared to patients with a negative UroVysion result [8]. Two studies in patients who underwent BCG therapy found that UroVysion-positive patients were more likely to have recurrence than patients with a negative UroVysion result in the setting of normal cystoscopy [15,16]. In the present study, we also found that UroVysion-positive patients with negative cystoscopy were more likely to have recurrence compared

Fig. 2 Kaplan–Meier curves for recurrence in patients in the negative Xpert and negative cystoscopy (NN) group and those in the positive Xpert and a negative cystoscopy (PN) group for recurrences of (A) all tumours, (B) only high-grade tumours and (C) only low-grade tumours.



with UroVysion-negative patients with negative cystoscopy. Another study of 114 patients undergoing surveillance for bladder cancer who had a negative cystoscopy evaluated UroVysion, immunocytology and nuclear matrix protein 22 (NMP22) ELISA. Recurrence rates in patients with positive vs negative cytology, FISH, uCyt+ and NMP22 were all more likely to have recurrence [17].

Of the 582 patients, 88 had a positive or suspicious cystoscopy and 80 underwent TURBT. Of these 80 patients, 25 were classified as negative by central pathology (31.2%). Xpert was negative in 16 of these 25 negative cases. This study did not collect data on what was deemed positive or suspicious and relied on the judgement of the urologist as to which patients to biopsy. It is known that many suspicious lesions are found to be benign and there is a risk of biopsy for many patients who are older and chronic smokers [11].

Previous studies have shown that markers may be able to adjudicate indeterminate bladder lesions to try to avoid unnecessary biopsies [18,19]. The fact that 64% of the patients with negative biopsies and suspicious lesions had a negative Xpert test suggests the possibility that this marker could also be helpful in determining need for a biopsy if a lesion is indeterminate. The NPV for patients with HG disease was 99% and 96% overall in patients with prior BCG, which supports this potential use.

Similarly to recent contemporary studies, the sensitivity of cytology in the present study was poor overall and only 48% for HG disease. A recent study using three prospective cohorts including 1487 urine samples from 1375 patients found pooled sensitivity for cytology of 40.8%, and 54.3% for HG/WHO grade 3 disease [20]. The reliance on cytology is based on a high PPV, but a marker that misses half of HG cancers is flawed. In this study, the Xpert assay had a significantly higher sensitivity for HG tumours of 87%. Further evaluation will be necessary to determine the PPV of the Xpert test since there would need to be biopsies for patients with a positive marker to avoid verification bias. However, as noted above, with blue-light flexible cystoscopy it would be possible to evaluate these patients further to determine if there are suspicious lesions such as carcinoma *in situ* that were missed by white-light cystoscopy.

The present study has some limitations. A total of 153 patients were excluded, mostly due to lack of biopsy of patients with suspicious cystoscopy. This was necessary since cancer presence was only based on pathological confirmation. There were 42 patients with insufficient urine required for the three biomarker tests. Only 10 patients had an indeterminate Xpert result, which is <2% of the entire cohort. The other issue was a difference between the PN and NN groups resulting from the randomized nature of selecting NN patients. Furthermore, patients with a positive Xpert result

were at higher risk because they were more likely to have cancer. The marker positivity is selecting patients who are more likely to have a false-negative cystoscopy. In fact, a comparison of patient characteristics between the 65 randomly chosen NN patients and the 219 not-chosen NN patients shows that the two groups did not differ in any analysed parameter. This suggests that the difference between the PN and NN groups is that the marker selects higher-risk patients, not the study design itself. Finally, upper tract evaluation was not included as part of this trial.

In conclusion, the Xpert test has a high sensitivity for detecting the recurrence of tumours and a high NPV for excluding HG tumours. In addition, the data suggest that patients with a positive Xpert assay in the setting of a negative cystoscopy are at high risk for recurrence and need closer surveillance.

Acknowledgement

We would especially like to thank the late Dr David Grignon for his contribution and support in this study.

Disclosure of Interest

Dr Lotan reports personal fees from fergene, Ferring Research, Merck, C2I genomics, photocure, Astra Zeneca, AbbVie, Cleveland Diagnostics, BMS, Nucleix, Ambu, Seattle Genetics and Hitachi, outside the submitted work and grants from Abbott, Cepheid, Pacific Edge, and GenomeDx Biosciences, Inc., outside the submitted work. Dr Bridge reports grants from Cepheid, during the conduct of the study, and other from Merck and Bayer, outside the submitted work. Dr Quigley works for Genuity. Genuity received payment from Cepheid for performing testing on this study. Ms Brown reports personal fees from Cepheid, during the conduct of the study, personal fees from Cepheid, outside the submitted work, and is an employee of a company who sponsored the study. Mrs Zhao reports other from Cepheid (study sponsor and funding source), outside the submitted work. Ms Satya reports other from Cepheid, outside the submitted work. Dr Bates reports personal fees from Cepheid and Danaher, during the conduct of the study, personal fees from Cepheid and Danaher, outside the submitted work. Dr Campbell reports other from Cepheid (study sponsor and funding source), outside the submitted work. All other authors have nothing to declare.

References

- 1 Chang SS, Boorjian SA, Chou R *et al.* Diagnosis and treatment of non-muscle invasive bladder cancer: AUA/SUO guideline. *J Urol* 2016; 196: 1021
- 2 Babjuk M, Burger M, Comp erat EM *et al.* European association of urology guidelines on non-muscle-invasive bladder cancer (TaT1 and carcinoma *in situ*) – 2019 update. *Eur Urol* 2019; 76: 639

- 3 Witjes JA. Developments in the follow-up of nonmuscle invasive bladder cancer: what did we learn in the last 24 months: a critical review. *Curr Opin Urol* 2020; 30: 387
- 4 Wallace E, Higuchi R, Satya M et al. Development of a 90-minute integrated noninvasive urinary assay for bladder cancer detection. *J Urol* 2018; 199: 655
- 5 Valenberg FJPV, Hiar AM, Wallace E et al. Prospective validation of an mRNA-based urine test for surveillance of patients with bladder cancer. *Eur Urol* 2019; 75: 853
- 6 Pichler R, Fritz J, Tulchiner G et al. Increased accuracy of a novel mRNA-based urine test for bladder cancer surveillance. *BJU Int* 2018; 121: 29
- 7 D'Elia C, Pycha A, Folchini DM et al. Diagnostic predictive value of Xpert Bladder Cancer Monitor in the follow-up of patients affected by non-muscle invasive bladder cancer. *J Clin Pathol* 2019; 72: 140–4
- 8 Seideman C, Canter D, Kim P et al. Multicenter evaluation of the role of UroVysion FISH assay in surveillance of patients with bladder cancer: does FISH positivity anticipate recurrence? *World J Urol* 2015; 33: 1309
- 9 Daneshmand S, Patel S, Lotan Y et al. Efficacy and safety of blue light flexible cystoscopy with hexaminolevulinatate in the surveillance of bladder cancer: a phase III, comparative, multicenter study. *J Urol* 2018; 199: 1158
- 10 Rosenthal D, Wojcik E, Kurtycz D eds. *The Paris System for Reporting Urinary Cytology*. New York, NY: Springer International Publishing, 2016
- 11 Svatek RS, Lee D, Lotan Y. Correlation of office-based cystoscopy and cytology with histologic diagnosis: how good is the reference standard? *Urology* 2005; 66: 65
- 12 Kang W, Cui Z, Chen Q, Zhang D, Zhang H, Jin X. Narrow band imaging-assisted transurethral resection reduces the recurrence risk of non-muscle invasive bladder cancer: a systematic review and meta-analysis. *Oncotarget* 2017; 8: 23880
- 13 Burger M, Grossman HB, Droller M et al. Photodynamic diagnosis of non-muscle-invasive bladder cancer with hexaminolevulinatate cystoscopy: a meta-analysis of detection and recurrence based on raw data. *Eur Urol* 2013; 64: 846
- 14 Gopalakrishna A, Fantony JJ, Longo TA et al. Anticipatory positive urine tests for bladder cancer. *Ann Surg Oncol* 2017; 24: 1747
- 15 Kamat AM, Dickstein RJ, Messetti F et al. Use of fluorescence in situ hybridization to predict response to bacillus Calmette-Guérin therapy for bladder cancer: results of a prospective trial. *J Urol* 2012; 187: 862–7
- 16 Lotan Y, Inman BA, Davis LG et al. 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. *J Urol* 2019; 202: 920–6
- 17 Todenhöfer T, Hennenlotter J, Guttenberg P et al. Prognostic relevance of positive urine markers in patients with negative cystoscopy during surveillance of bladder cancer. *BMC Cancer* 2015; 15: 155
- 18 Konety B, Shore N, Kader AK et al. Evaluation of cxbladder and adjudication of atypical cytology and equivocal cystoscopy. *Eur Urol* 2019; 76: 238
- 19 Schlomer BJ, Ho R, Sagalowsky A, Ashfaq R, Lotan Y. Prospective validation of the clinical usefulness of reflex fluorescence in situ hybridization assay in patients with atypical cytology for the detection of urothelial carcinoma of the bladder. *J Urol* 2010; 183: 62
- 20 Freifeld Y, Lotan Y. Effect of blue-light cystoscopy on contemporary performance of urine cytology. *BJU Int* 2019; 124: 251

Correspondence: Yair Lotan, Department of Urology, 2001 Inwood Road, West Campus Building 3, Floor 4, Dallas, TX 75390-9110, USA.

e-mail: yair.lotan@utsouthwestern.edu

Abbreviations: FISH, fluorescence in situ hybridization; HG, high grade; HR, hazard ratio; NMIBC, non-muscle-invasive bladder cancer; NMP22, nuclear matrix protein 22; NPV, negative predictive value; PPV, positive predictive value; TURBT, transurethral resection of bladder tumour; Xpert, Xpert Bladder Cancer Monitor.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Comparison of NN patients randomly selected for the longitudinal study ($n = 65$) with NN patients not selected for follow-up ($n = 219$).

Table S2. Patient characteristics of NN and PN patients in the longitudinal study.

Fig. S1. Kaplan--Meier curves for recurrence in the urovysion NN and PN group for recurrences of all tumours.