GALEAS™ Bladder

A non-invasive biomarker test for the detection and monitoring of bladder cancer

Accurate non-invasive detection of urothelial carcinoma in patients undergoing cystoscopy for either hematuria or surveillance for disease recurrence

Introduction

Bladder cancer is one of the most common cancers, ranking 10th in terms of prevalence and 8th in terms of survival [1]. Visual inspection of the bladder by cystoscopy is a key component for the investigation of both de novo bladder cancer in patients with hematuria and for the surveillance of local disease recurrence of non–muscle-invasive bladder cancer (NMIBC) [2,3]. However, bladder cancer is identified in only 10-20% of cystoscopies performed in either setting [4–6].

The necessity for visual inspection of the bladder via cystoscopy, for both the diagnosis of de novo bladder cancer in patients with hematuria and the regular surveillance of patients with NMIBC, means bladder cancer ranks as one of the most expensive cancers to manage [6,7]. There is a clear need to develop alternative, cost effective, approaches that can reduce the need for cystoscopy in all patients.

Cystoscopic inspection of the bladder

Despite being considered the diagnostic gold standard, cystoscopy is an approach not without issue; reported sensitivity and specificity lacks precision and accuracy for standard white light cystoscopy. Sensitivity ranges from 47- 100%, and specificity 93- 100% in the hematuria setting, to 68–100% and 57–97% respectfully in the surveillance setting [11,26,27].

Cystoscopy is expensive, highly invasive, uncomfortable and requires hospital attendance. It is also a cause of other morbidities, such as infection, which occur in greater than 3% of cases, significantly impacting patient quality of life [8]. Combined, these factors result in a reduction in cystoscopy compliance and delayed attendance, a significant issue in monitoring for NMIBC disease recurrence, resulting in potentially delayed diagnosis [7,9].

Increased demand on cystoscopy services, concerns around the overuse of cystoscopy, the increased economic costs and changes around patient behaviour foster the drive in development of non-invasive / remote testing approaches. Reducing the necessity for cystoscopy in both hematuria and surveillance settings will not only significantly reduce the economic burden of managing bladder cancer, but also improve patient quality of life [24]. However, for a non-invasive molecular biomarker to significantly impact the number of cystoscopies, it must provide both patient and physician alike with a high degree of certainty, equivalent to the perceived level of cystoscopy, that no tumor is present when the test result is negative [25].

A urine-based test that can be used for molecular triage, to improve the detection of both de novo and recurrent disease is essential for healthcare professionals, providers and patients alike [12,13]. Although several non-invasive urine-based molecular tests have been developed, and some are approved by the FDA, they have failed to be widely adopted, due to being unable to meet the necessary diagnostic performance [14–18]. This is in part due to their inability to consider inter-tumor or patient heterogeneity, as they focus on a single or low number of biomarkers [10–14]. They also generally suffer from a lack of sensitivity in the detection of low-grade tumors [14–18].

This combined with the historic scepticism around non-invasive biomarker tests, has resulted in low adoption rates. However, increased awareness and the acceptance of home self-sampling and non- or minimally-invasive remote monitoring post pandemic has changed both patient and physician perception of such tests, potentially increasing the likelihood of more rapid adoption, on the assumption they exhibit comparable clinical performance [19,20].

GALEAS Bladder

In this paper we discuss why we believe GALEAS Bladder [21] offers a viable alternative to cystoscopy, ensuring a better patient experience, which delivers on the clinical sensitivity and specificity expected. GALEAS Bladder identifies somatic mutations across 23 bladder cancer associated genes, to accurately identify patients with bladder cancer, from those who do not, from a simple urine sample [22,23].

Using GALEAS Bladder, as a non-invasive molecular triage in both the hematuria and surveillance pathways, has the potential to significantly reduce the number of cystoscopies.

Furthermore, given notable concerns around the overuse of cystoscopy, particularly in low-risk NMIBC surveillance or low-risk haematuria patients, the use of a molecular biomarker screen that can safely be used to triage patients to a diagnostic cystoscopy, could shift the paradigm of how we manage and treat patients with suspected bladder cancer [13]. The use of biomarker led molecular triage and remote monitoring would allow the creation of a long-term sustainable pathway for the delivery of bladder cancer care, improving the efficiency of hospital resources and increasing the delivery of a more personalised patient centric care.

Methodology

Sample cohort

This was a secondary analysis of a large cohort of samples from a single-visit trial, assessing the performance of GALEAS Bladder as a non-invasive urine test for the detection of bladder cancer in patients undergoing cystoscopic investigation for hematuria or surveillance for NMIBC [22,23]. All patients had previously given written informed consent for urine collection, processing, and analysis. Patient clinicopathological details are shown in Table 1. Voided urine was collected prior to cystoscopy.

	Bladder Cancer (n=354)	Negative by Cystoscopy (n=356)
Age, median (Min-Max), years	70.5 (26-90)	59.0 (19-93)
Gender:		
Male	284	174
Female	70	182
Tumour grade:		
G1	59	
G2	112	
G3+	183	
Tumour Stage:		
Ta	181	
T1	94	
T2+	79	

Table 1: Clinicopathological features of 710 patients tested via cystoscopy, utilised in GALEAS Bladder analysis.

GALEAS Bladder assay

DNA was obtained from urinary cell pellets; library preparation and enrichment was carried out using the GALEAS Bladder Next Generation Sequencing (NGS) kit [22]. FASTQ files were aligned to the GRCh38 human reference genome and analysed using the proprietary GALEAS Bladder software, to classify the samples as either likely positive or negative for bladder cancer.

To classify samples as likely positive or negative, a computational classifier was developed based on the presence of specific mutations associated with bladder cancer, as documented in existing literature [22,23], and their classifications according to the American College of Medical Genetics and Genomics (ACMG) and Association for Molecular Pathology (AMP) guidelines.

GALEAS Bladder technical performance

To demonstrate the analytical performance of GALEAS Bladder [21], two series of contrived reference standards were tested (cell line A and cell line B) and run in triplicate by two different operators, representing tumor cell fractions ranging from 100% to 0% in a wild-type normal DNA background. A total of 105 mutations were assessed across both cell lines and replicates.

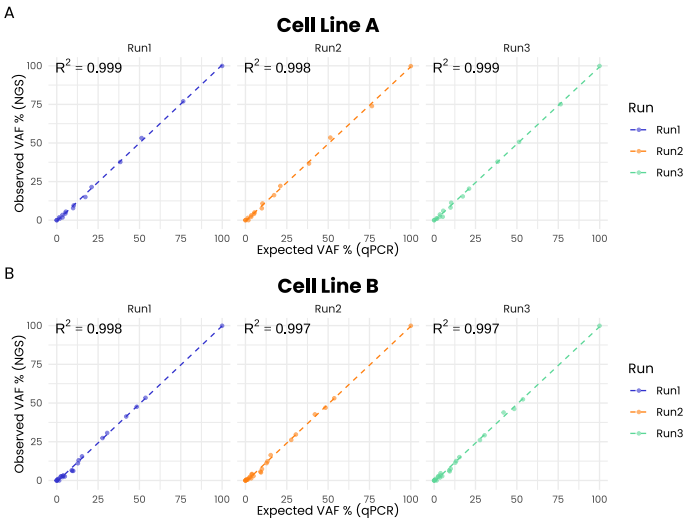
GALEAS Bladder demonstrated >99% sensitivity for the detection of variants. No false positives were identified, all wild type samples were defined as GALEAS Bladder negative (0/15).

Across the 105 mutations assessed there was high concordance (R2 >0.99) between GALEAS Bladder and qPCR derived VAFs, (Figure 1a and 1b).

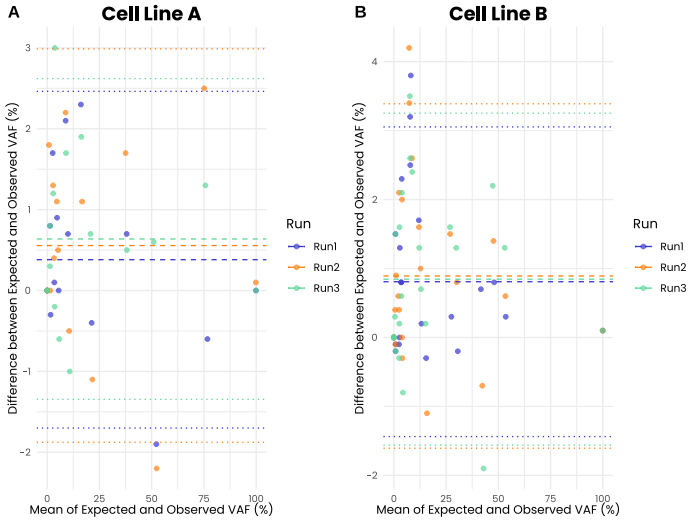
To assess inter-run variability a Bland-Altman analysis was performed across the two cell line triplicate runs (Figure 2a and 2b). Cell line A limits of agreement were -1.35% to +2.99% and the mean differences were 0.38% to 0.64%. Cell line B limits of agreement were 0.8% to 0.85% and the mean differences were -1.44 to + 3.34%.

The Bland-Altman analysis suggests that there is no significant systematic bias in any of the triplicate runs (run 1, run 2 and run 3, both cell lines), meaning that the observed VAF values are, on average, very close to the expected VAF values.

In summary, the small mean differences and narrow limits of agreement suggest that the experimental process is reliable and reproducible.



Figures 1A, 1B: GALEAS Bladder technical performance for cell line A and B. Comparing qPCR derived expected VAF with those derived from GALEAS Bladder next generation sequencing (NGS) across 42 and 63 variants respectively with an R2 >0.99



Figures 2A, 2B: Bland-Altman analysis to determine systematic bias in triplicate runs, includes horizontal lines for the mean difference and limits of agreement.

GALEAS Bladder hematuria clinical sensitivity

To validate GALEAS Bladder, urinary pellet DNA from a cohort of 710 hematuria patients was analysed. This included 335 samples confirmed cancer free hematuria patients and 375 confirmed cancer urines [22,23]. The clinicopathological features of the included cystoscopy patients are shown in Table 1.

The analysis yielded an overall sensitivity of 92% (CI 85-94%) and specificity of 86% (CI 82-90%) for the detection of any bladder cancer stage or grade (Table 2). The test exhibited high sensitivity across all grades of bladder cancer; when stratified by grade, the test sensitivity for the detection of Grade 3 disease was 96% (95% CI 92-98%), 93% (95% CI 86-97%) for Grade 2 disease and importantly the sensitivity for the detection of Grade 1 disease was 78% (95% CI 65-88%). When separated into non-muscle invasive (NMIBC) and muscle invasive (MIBC) disease, the sensitivity was 92% (CI 84-97) and 92% (CI 88-95%) respectfully (Table 2), whilst retaining a specificity of 86%. All cases of solitary carcinoma in situ (n=3) were GALEAS Bladder positive.

Overall, the prevalence corrected Negative Predictive Value (NPV) was 98.8%. When taking into account grade, the NPV remained >98% across all grades (Table 2).

In logistic regression analysis, a positive GALEAS Bladder result predicted the presence of bladder cancer with an odds ratio (OR) of 52.6 (95% CI 34.4 - 82.2, p<0.001). In multivariate analysis, adjusting for stage, grade, age and gender, GALEAS Bladder was an independent predictor of bladder cancer presence (OR 38.2, 95% CI 24.7 - 60.6, p<0.001).

	Sensitivity (%)	Specificity (%)	NPV (%)
pTa	89	86	99
T1	97	86	99
T2+	92	86	99
G1	78	86	99
G2	93	86	99
G3+	96	86	99
NMIBC	92	86	99
MIBC	92	86	99

Table 2: Sensitivity analyses for the performance of GALEAS Bladder in the detection of cancer in the 710 patients assessed

GALEAS Bladder surveillance clinical sensitivity

GALEAS Bladder was also validated using a cohort of urine samples collected from patients undergoing surveillance cystoscopy for NMIBC disease recurrence.

Of the 289 urine samples collected, 21 were confirmed to have cancer at the time of their surveillance cystoscopy. 20 passed GALEAS Bladder QC, of these, 19 were correctly identified as positive by GALEAS Bladder. This cohort of data indicates GALEAS Bladder has an overall sensitivity for the detection of bladder cancer recurrence of 95% (95% CI 76 – 100%), an NPV of 99.4% and an odds ratio of 3.63 (95% CI 1.71- 5.76, p<0.001).

Of the 268 patients negative on the day of their surveillance cystoscopy, 23 were subsequently diagnosed with bladder cancer recurrence at a future cystoscopy (2–24 months). Of these, 13 were identified as GALEAS Bladder positive at the time of their negative surveillance cystoscopy.

Viable molecular triage and surveillance of bladder cancer

Although many of the existing urine based molecular biomarkers for bladder cancer show comparable sensitivity for the detection of high-grade disease, with sensitivities approaching 90%[14–18], they invariably lack sensitivity for the detection of low-grade disease, one of the largest compounding factors in their low adoption[14–18].

GALEAS Bladder provides a significant advantage over other molecular tests; not only does it provide the essential >90% sensitivity for the detection of high-grade disease, it also exhibits a high sensitivity (nearing 80%) for the detection of low grade disease, a significant improvement on other existing molecular tests, and equivalent to the reported sensitivities of cystoscopy [11,26,27]. When combined with similarly high specificity (86%), GALEAS Bladder has the performance characteristics to safely act as a molecular triage for cystoscopy.

Furthermore, GALEAS Bladder has a negative predictive value of 99% in both the hematuria and surveillance settings, meaning, in effect, a false negative rate of only 1 in 100 results. This high NPV provides the assurance that, with a high degree of certainty, no tumor is present when the test result is negative.

GALEAS Bladder is a robust test, allowing the accurate non-invasive detection of urothelial carcinoma in patients undergoing cystoscopy for either hematuria or surveillance for disease recurrence. Given the performance of the assay, using GALEAS Bladder as a molecular biomarker test in the bladder cancer pathway could reduce the total number of cystoscopies necessary.

Summary

GALEAS Bladder offers a highly promising non-invasive urinary biomarker test for ruling out the presence of bladder cancer in patients undergoing investigation for both hematuria and disease recurrence.
The performance characteristics mean GALEAS Bladder has the potential to safely reduce the number of unnecessary cystoscopies across the entire bladder cancer pathway, reducing the cost of manging the disease and improving patients' quality of life.

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