Utilizing Cell-Free Urinary and Plasma Tumor DNA to Predict Pathologic Stage at Radical Cystectomy


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Abstract

Objective To assess the ability of cell-free urinary and plasma tumor DNA (cfDNA) to predict pathologic stage at radical cystectomy for patients with clinical muscle-invasive bladder cancer.

Methods A total of 25 patients with clinical muscle-invasive bladder cancer were enrolled before undergoing radical cystectomy. Blood and urine were collected before surgery. The 600-gene PredicineATLAS panel was used to sequence blood buffy-coat germline DNA, plasma cfDNA, and urine cfDNA samples. Low-pass whole genome sequencing was performed on plasma- and urine-derived cfDNA. CfDNA tumor fraction (TF), genome-wide copy number burden (CNB), and estimated tumor mutational burden (TMB) were measured in both plasma and urine samples and their correlation with pathologic T-stage was examined.

Results Three of 25 plasma samples had insufficient cfDNA. In 22 of 22 plasma samples and 24 of 25 urine samples, at least one nonsynonymous somatic variant was detected. Across the cohort, 44% of plasma variants were concordant with paired urine variants. The mean number of variants did not differ between noninvasive (< pT1/pN0) and invasive disease (≥ pT1 or N+) for both plasma (8 vs. 9.5 variants; P = 0.85) and urine (33.7 vs. 30 variants; P = 0.45). A strong correlation was observed between urine TF and urine CNB score within patients (rv = 0.92). Plasma TF (r = 0.38), urine TF (r = 0.21), and urine CNB score (r = 0.16) exhibited positive correlations with pT stage. Patients with carcinoma in situ (CIS) had higher mean urine TF and CNB scores (P = 0.07 and P = 0.05, respectively). Plasma TF and CNB score did not correlate with the presence of CIS.

Conclusions Combining plasma- and urine-based cfDNA analysis may help identify patients with residual disease at radical, although we were unable to predict pathologic T-stage based on these metrics. The presence of CIS may contribute to greater urinary CNB and TF levels. Considering CIS in the analysis may improve the ability to correlate tumor metrics with pathologic stage. Low-pass whole genome sequencing–derived urinary CNB correlates strongly with urinary TF and may provide a less resource-intensive method for future longitudinal disease monitoring.
Survival following radical cystectomy (RC) for urothelial carcinoma of the bladder is strongly influenced by the pathologic T-stage and the presence of lymph node metastases. Most patients with non–organ-confined disease or lymph node involvement have nearly 50% lower survival rates at 5 years. The ability to predict minimal residual disease has proven feasible for patient management, especially considering that more than 60% of patients who are pT0 on transurethral resection before radical cystectomy harbor residual disease. These patients have significantly decreased survival rates at 5 years. The administration of adjuvant treatment, Board-certified genitourinary pathway specialists reviewed the pathologic specimens. Postsurgical surveillance consisted of cross-sectional imaging, urine cytology, and laboratory assessment every 3 to 6 months. Pathologists blinded to the patients’ clinical data interpreted the slides. The pathologic T-stage prior to RC.

Materials and Methods

After obtaining institutional review board (IRB) approval (MCC 21616), we prospectively enrolled 25 patients diagnosed with muscle-invasive bladder cancer (MIBC) between November 2021 and August 2022, prior to their radical cystectomy. Patients with prior history of upper tract urothelial carcinoma or non–organ-confined bladder were not eligible for enrollment. A previous history of nonmuscle-invasive bladder cancer, with or without history of intravesical treatment, was not an exclusion criterion. All patients provided written informed consent and the study was approved by the institutional review board. Treatment and surveillance followed national guidelines. The multidisciplinary treatment team made recommendations regarding the omission of neoadjuvant chemotherapy, the performance of template-based lymphadenectomy, and the administration of adjuvant treatment. Board-certified genitourinary pathway specialists reviewed the pathologic specimens. Postsurgical surveillance consisted of cross-sectional imaging, urine cytology, and laboratory assessment every 3 to 6 months. Pathologists blinded to the patients’ clinical data interpreted the slides. The pathologic T-stage prior to RC.

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Cell-free circulating tumor DNA in the plasma (ctDNA) and urine (uDNA) are emerging as promising biomarkers for identifying the presence of bladder cancer, predicting pathologic complete response (pCR), detecting disease recurrence following RC, and assessing response to adjuvant immunotherapy.4–9. Though significant inroads have been made to maximize clinical utility, neither ctDNA nor uDNA has been shown to predict pathologic T-stage following RC. Instead, binary outcomes related to the presence or absence of ct/ctDNA have been associated with survival following radical cystectomy.[8,9] In this study, we employed ultra-low-pass whole genome sequencing and ultra-deep–targeted sequencing of both ctDNA and uDNA to investigate the potential of these biomarkers for predicting pathologic stage prior to RC.

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Gene fusions
DNA rearrangement was detected by identifying the alignment break points based on the BAM files before consensus filtering. Suspicious alignments were filtered based on repeat regions, low entropy calculation, and similarity between reference and alternative alignments. To report a DNA fusion, larger than 3 unique alignments (at least one of them double stranded) were required.

Tumor fraction
The ctDNA fraction was estimated based on the mutant allele fraction of autosomal somatic mutations, as described previously[17]. Briefly, under the conservative assumption that each SNV may have loss of heterozygosity, the mutant allele fraction (MAF) and ctDNA fraction are related as MAF = (ctDNA * 1) / [(1 – ctDNA) * 2 + ctDNA *4], and so ctDNA = 2 / (1 / MAF + 1). Some somatic mutations in genes with a detectable copy number gain were omitted from ctDNA fraction estimation, thus only a subset of samples could have the ctDNA fraction accurately estimated from mutation data.

TMB score estimation
Blood- and urine-based tumor mutational burden (TMB) was defined as the number of somatic coding SNVs, including synonymous and nonsynonymous variants, within panel target regions. Because TMB estimation considers all variants (including synonymous), higher variant call specificity is required. More stringent cutoffs were used for variant calls, and only variants with allele frequency ≥ 0.35% were used in score calculation. The plasma tumor fraction of each sample was estimated by the total effective targeted panel size within the coding region. Samples with the maximum somatic allelic frequency (MSAF) < 0.7% were excluded from TMB estimation.

Outcomes and statistical analyses
The primary objective was to investigate the ability of preoperative plasma and urine to predict pathologic disease stage at cystectomy. All differences between patient group means were tested using a Wilcoxon rank sum test. Correlations between tumor burden metrics and pathologic T-stage rank were measured using the Spearman correlation coefficient. Multivariate models to predict CIS were fit to the entire dataset using binomial logistic regression with pathologic tumor stage to predict CIS were fitted to the entire dataset using binomial logistic regression with pathologic tumor stage to predict CIS were fitted to the entire dataset using binomial logistic regression with pathologic tumor stage and presence of CIS.

Across the cohort, we detected 656 non-synonymous single nucleotide variants (SNVs) and indels, 377 gene level copy number variants (CNVs), and 5 fusion mutations (Online Supplementary Table 2). Plasma samples exhibited a median of 5 SNVs/indels (range, 1–36) and 1 CNV (range, 0–7). In contrast, urine samples showed a median of 17 SNVs/indels (range, 0–77) and a median of < 1 CNV (range, 0–54). For concordant variants, the mutant allele frequency was always higher in urine than in plasma (Figure 1A). Overall, 44% of plasma variants were concordant with paired urinary variants (Figure 1B). Seven nonconcordant plasma variants and 13 urinary variants were observed. The plasma and urine fraction of each sample was estimated by the total effective targeted panel size within the coding region. Samples with the maximum somatic allelic frequency (MSAF) < 0.7% were excluded from TMB estimation.

Using these metrics, we assessed the correlation between preoperative plasma sample quality and pathologic stage and the presence of CIS.

Online Supplementary Table 3). Similarly, neither urine- or plasma-derived CMB score nor tumor fraction could distinguish between noninvasive and invasive disease in this small group. Despite the study lacking statistical power to evaluate the accuracy of combining metrics for predicting the presence of invasive vs. noninvasive disease in this cohort of patients, we noted that several patients with fewer than 5 variants detected in plasma still had a high urinary CMB score (Online Supplementary Figure 2). Thus, a combined approach using both plasma variant detection and urinary low-pass sequencing to determine CMB score might be useful for early detection of MIBC.

It is important to note that utDNA metrics were strongly influenced by the presence of CIS. Both urine mean TF and CMB score were higher in patients with pure or concomitant CIS (Figures 4 A and B, P = 0.07 and P = 0.05, respectively). The correlations remained statistically significant or marginally significant while controlling for pathologic T-stage within CIS-only/CIS groups (multivariate regression coefficient = 0.63, P = 0.04, and coefficient = 0.48, P = 0.08, respectively). In contrast, plasma TF and CMB score did not exhibit significant correlations with the presence of CIS.

Using these metrics, we assessed the correlation between preoperative ut/cDNA burden and pathologic T-stage identified in the RC specimen. Plasma and urine TF showed weak correlations with T-stage (Figure 3A, r = 0.38 and 0.21, respectively). Urine CMB score also showed a positive correlation with T-stage (Figure S3B, r = 0.16). The number of variants observed in plasma and subsequently the estimated plasma TMB showed the greatest difference between the group of 3 patients with noninvasive disease and the 22 patients with invasive disease, although the difference did not reach statistical significance (pTMB, 0.7 vs. 3.4, P = 0.12, Online Supplementary Table 3).
NAC followed by RC[8]. The researchers employed a tumor-informed approach to select 50 somatic variants per patient from transurethral resection specimens to create a custom sequencing panel. cfDNA positivity was established by comparing patient-specific variants to all nonpatient-specific variants, and cfDNA levels were monitored throughout the NAC course using mean variant allele frequency. While the investigators were able to predict treatment response to NAC based on utDNA and ctDNA clearance, they did not identify a significant correlation between mean variant allele frequency of the samples collected after NAC but prior to RC and pathologic stage at RC. In our study, 6 of 9 patients treated with NAC showed a decrease in urinary tumor fraction post-treatment (median change in TF, –1.3%, data not shown). Yet, neither urinary tumor fraction nor CNB levels could distinguish between noninvasive and invasive disease. Future studies should focus on the specific alterations more frequently found in noninvasive versus invasive bladder cancer to improve disease staging.

Consistent with previous studies, urine-derived cfDNA exhibited a larger spectrum of unique tumor-derived alterations and higher mean mutant allele frequency compared to plasma-derived cfDNA[19]. Furthermore, we observed a positive correlation between tumor fraction and CNB score derived from both utDNA and ctDNA with increasing pathologic T-stage. However, in this pilot study, we were unable to accurately predict the presence of invasive versus noninvasive residual disease before cystectomy.

To examine the role of cfDNA in predicting response to NAC, Christensen et al. used a custom next-generation sequencing panel to longitudinally assess urine and plasma samples on a cohort of 92 patients undergoing various bladder cancer settings[5,6,8,9,19]. Significant advances in next-generation sequencing technologies and the integration of genome-wide tumor DNA metrics have improved the ability to predict pathologic complete response to neoadjuvant chemotherapy and estimate survival after RC[6]. However, the accurate prediction of pathologic T-stage using quantitative cfDNA metrics has not yet been demonstrated.

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cohort received neoadjuvant chemotherapy, which may have an unknown impact on sample variant makeup. In this study, we lacked the power to test multivariate models, but we did find several non-concordant mutations across plasma and urine in white-blood-cell-associated genes, highlighting the utility of mutational profiling using both urine and plasma. Future work incorporating larger datasets is needed to reassign the value of combining cfDNA and clinical features to predict the presence of invasive versus noninvasive disease. Additionally, further studies should explore incorporating the sequencing of personalized markers derived from initial tumor tissue biopsy with the panel genes used in this study.

Conclusions

We observed a positive correlation between both utDNA and ctDNA metrics with progressive disease in patients who underwent radical cystectomy. Specifically, patients with CIS on final pathology exhibited higher urinary copy number burden and tumor fraction. Future assessments that control for the presence of CIS may improve the ability to correlate cfDNA metrics with pathologic staging. The observed changes in urinary tumor burden in response to treatment, along with optimized plasma- and urine-based cfDNA metrics, may help to identify patients who are candidates for bladder preservation. The use of combined plasma- and urine-based liquid biopsy techniques holds promise in the early detection of MBIC and the measurement of minimal residual disease during treatment.

References


