Urine cell-free DNA improves detection of bladder cancer compared to blood-based screening

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INTRODUCTION

- Plasma cell-free DNA (cfDNA) has shown great promise as a non-invasive approach for multi-cancer early detection (MCED), but early-stage urological cancers have low tumor fraction in plasma, making them more difficult to detect.[1,2]
- Urine cfDNA has the potential to improve detection and monitoring of early-stage urinary tract cancers due to its proximity to the affected organs and ease of collection.[3]
- Prior studies have shown bladder cancer is detectable in cfDNA from urine by evaluating different features (eg, mutations, copy number aberrations, methylation patterns).[4,5]
- Targeted analysis of methylation patterns in cfDNA can improve the limit of detection in plasma by an order of magnitude compared to other cfDNA features.[6]
- We conducted an exploratory study to assess the utility of targeted analysis of methylation patterns to detect bladder cancer in urine cfDNA compared to matched plasma cfDNA from patients with suspicion for bladder cancer.

OBJECTIVES

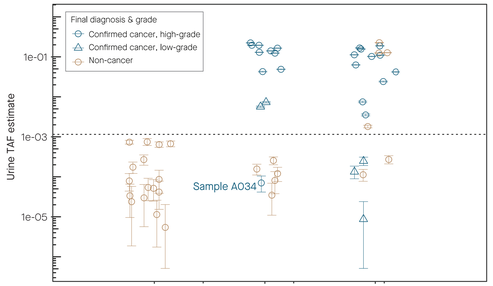
- Assess the feasibility of detecting early-stage bladder cancer in urine cfDNA using biopsy-free tumor allele fraction (TAF) estimates.
- Assess concordance of biopsy-free and biopsy-informed TAF estimates in urine cfDNA.
- Compare TAF estimates in urine vs blood in patients with bladder cancer.

KEY RESULTS: TARGETED ANALYSIS OF METHYLATION PATTERNS IN URINE cfDNA DETECTS LOCALIZED BLADDER CANCER

Biopsy-free TAF estimates in urine cfDNA can distinguish non-cancer and bladder cancer patients.

- Among patients with confirmed bladder cancer, urine cfDNA sensitivity was 91.7% overall (11/12; 95% CI 61.5%–99.8%) and 90% for high grade (9/10) (**Figure 3**).
- Sensitivity of urine cfDNA for recurrence detection was 78.6% overall (11/14; 95% CI 49.2%–95.3%), and 100% for high grade (11/11).
- TAF in urine from non-cancer patients (N=16) and patients with suspicion of new localized bladder cancer found to be benign by TURBT (N=5) were all below the detection threshold.
- Among patients with suspicion of recurrent localized bladder cancer but not found to have bladder cancer by TURBT, TAF estimates for 4/6 (66.7%) were above the detection threshold. Clinical follow-up is needed to determine if these increased TAF estimates are predictive of future disease recurrence.

Figure 3. Biopsy-free TAF Estimates in Urine cfDNA

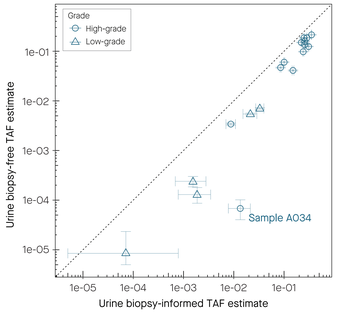


Each point represents a study sample. The error bars represent the 95% credible interval of the posterior distribution. The dotted line is the detection threshold used to calculate detection rates in each cohort. cfDNA, cell-free DNA; TAF, tumor allele fraction.

Biopsy-free and biopsy-informed TAF estimates in urine cfDNA are concordant.

- We observed concordance between biopsy-free and biopsy-informed approaches suggesting that biopsy-free TAF estimates are quantitative down to 10^{-4} (**Figure 4**).
- Further calibration is needed to address potential biases in TAF estimates.
- Sample A034 was undetectable in urine using biopsy-free estimate, but had a higher biopsy-informed TAF estimate, suggesting that tissue-derived methylation features were detected in the patient's urine, but that they were distinct from features typically found in bladder cancer tissue.

Figure 4. Biopsy-free vs Biopsy-informed TAF Estimates in Urine cfDNA of Confirmed Cancer Patients

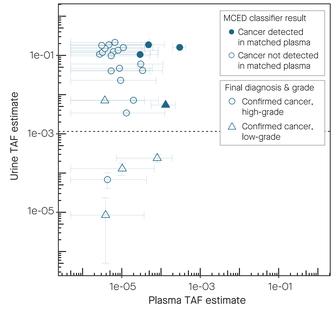


A scatterplot depicts the TAF estimates derived independently using the biopsy-free or biopsy-informed method. Each point represents a single cancer patient sample (N=18). Error bars represent the 95% credible interval of the posterior distribution.
TAF, tumor allele fraction.

Plasma cfDNA TAF estimates were lower than urine cfDNA TAF estimates and consistent with low detection rates using an MCED classifier.

- Using an MCED classifier trained at 99% specificity (**Figure 5**):
  - Among patients with confirmed new bladder cancer, plasma cfDNA sensitivity was 16.7% overall (2/12) and 10.0% for high grade (1/10).
  - For recurrence detection, plasma cfDNA sensitivity was 14.3% (2/14) overall and 18.2% (2/11) for high grade.
- Samples detected using the MCED classifier had higher plasma TAF estimates relative to undetected samples but lower TAF estimates than matched urine cfDNA from the same patient (**Figure 5**).
- All non-cancer samples (data not shown; includes non-cancer cohort [N=16] and those suspected of cancer without confirmed cancer [N=11]) were undetectable with the MCED classifier.

Figure 5. Urine vs Plasma cfDNA TAF Estimates for Confirmed Cancer Patients



A scatterplot depicts matched biopsy-free urine and plasma TAF estimates derived from each confirmed cancer patient in the new/recurrent cohorts. Each point is a single patient and error bars represent the 95% credible interval of the posterior distribution.
cfDNA, cell-free DNA; MCED, multi-cancer early detection; TAF, tumor allele fraction.

CONCLUSIONS

- We observed high sensitivity in urine cfDNA for detection of diagnosed localized bladder cancer using biopsy-free TAF estimates. The observed non-cancer data are consistent with the ability to achieve high specificity.
- Biopsy-free TAF estimates in urine cfDNA were concordant with those derived from matched tumor biopsies although with some systematic bias, suggesting that biopsy-free estimates provide a quantitative estimate of tumor burden.
- Analysis of matched plasma showed lower tumor fraction and detection rate using an MCED classifier in plasma cfDNA. A urine-based cfDNA assay with high sensitivity at high specificity, combined with non-invasive sampling, may be an ideal tool to use alongside the standard of care (eg, cystoscopy) for clinical diagnosis and monitoring of bladder cancer.
- Further studies are needed to validate these findings and determine the clinical utility of urine cfDNA in the diagnosis and surveillance of bladder cancer.

References

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Disclosures

Study funded by GRAIL, LLC , a subsidiary of Illumina, Inc., currently held separate from Illumina Inc. under the terms of the Interim Measures Order of the European Commission dated 29 October 2021, Menlo Park, CA. AS, SS, KM, SB, and ML are current or former employees of GRAIL, LLC. with equity in the company. All financial relationships disclosed at abstract submission.

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METHODS

Patient Recruitment

- Urine and blood were collected from patients with suspicion of new (N=17) or recurrent (N=20) localized bladder cancer, and from non-cancer patients with urological conditions (N=16) (**Table 1**).
- Patients with suspicion of localized bladder cancer underwent transurethral resection of bladder tumor (TURBT) and conventional imaging when clinically indicated.
- Tissue was collected and processed for patients with a confirmed bladder cancer diagnosis when available.

Table 1. Patient Cohort

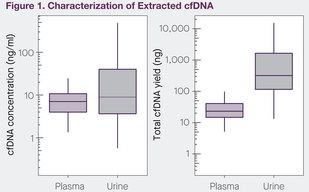
	Non-cancer (N=16)*	Confirmed Cancer (N=26)*		Suspected of Cancer, Without Confirmed Cancer (N=11)
		New Cancer (N=12)	Recurrent Cancer (N=14)	
Age (median)	69.5 years	70.5 years		59 years
Sex (%, [n/N])	81% male (13/16)	69% male (18/26)		55% male (6/11)
Stage (%, [n/N])				
Oa	—	50% (6/12)	57% (8/14)	—
Ois	—	0% (0/12)	14% (2/14)	—
I	—	42% (5/12)	14% (2/14)	—
II	—	8% (1/12)	14% (2/14)	—
Grade (%, [n/N])				
High grade	—	83% (10/12)	79% (11/14)	—
Low grade	—	17% (2/12)	21% (3/14)	—

**The non-cancer and confirmed cancer cohorts were comparable with respect to age and gender.*

Sample Processing

- Whole blood (up to 20 mL from each patient) was collected in Streck Cell-Free DNA Blood Collection Tubes and processed to plasma as previously described.[7]
- Urine samples (up to 100 mL from each patient) were collected in a specimen collection cup and preserved by the addition of Streck Urine Preserve within 1 hour of collection. Up to 50 mL was spun to remove cell sediment and concentrated prior to extraction of cfDNA.
- cfDNA was extracted from plasma and concentrated urine (up to 4.2 mL) using a modified version of the MagMax Cell-Free DNA Isolation Kit (ThermoFisher Scientific).
- The yield of extracted cfDNA from blood and urine was quantified prior to preparation of preparation of targeted methylation libraries (**Figure 1**).
- Up to 75 ng of cfDNA was subjected to bisulfite conversion, converted to dual indexed sequencing libraries, and enriched with a custom panel.
- Libraries were sequenced to a target depth of 75 to 100 million reads.

Figure 1. Characterization of Extracted cfDNA



cfDNA, cell-free DNA.

Data Analysis

- Biopsy-informed TAF estimates (defined as TAF estimates generated with the inclusion of matched tumor tissue biopsy data) in urine cfDNA were generated using a method previously developed for plasma[7] (**Figure 2**). Modifications to generate urine cfDNA estimates included the use of an external reference dataset of urine cfDNA from individuals without cancer (N=176) (instead of plasma cfDNA) and additional filtering of methylation variants using healthy urological tissue WGBS data.[8]
- The biopsy-informed TAF estimation method was modified to generate biopsy-free estimates (defined as TAF estimates generated without matched tumor tissue biopsy data) by (1) utilizing methylation data from a GRAIL bladder cancer tissue biobank (N=49) in training (instead of matched biopsy), and (2) updating the statistical model to include a recurrence term for each variant. The recurrence estimate is defined as the rate at which a particular variant is observed in the tissue biobank (**Equation 1**).
- We set a detection threshold, using a maximum TAF value (1×10^{-3}) from an independent set of non-cancer urine samples (N=50), to assess detection rates in our study cohorts.
- Sensitivity in plasma cfDNA was determined using a validated MCED test classifier at 99% specificity.[1]

Equation 1. Biopsy-free Tumor Fraction Model

$$Prob(tf \mid X) \sim \prod_{i=1}^{I} \left[r \cdot Pois(x_i, \lambda_i) + (1-r) \cdot Pois(x_i, \lambda_{ni}) \right] * Prob(tf)$$

r = recurrence rate

x_i = abnormal counts of site i in cfDNA

tf = tumor fraction

λ_i = Poisson lambda for site i given tumor contains variant $i = [tf * vaf_i \cdot (1-tf) * noise_i] * depth_i$

λ_{ni} = Poisson lambda for site i given background noise process $= noise_i * depth_i$

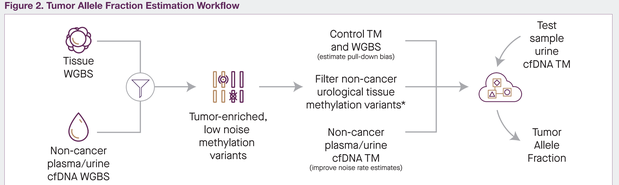
vaf_i = variant allele fraction for site i in the biopsy

$depth_i$ = estimated depth of site i in the cfDNA

$noise_i$ = estimated noise of site i in the cfDNA

cfDNA, cell-free DNA.

Figure 2. Tumor Allele Fraction Estimation Workflow



**This step occurs only in the TAF estimation workflow for urine samples.*
cfDNA, cell-free DNA; TM, targeted methylation; WGBS, whole-genome bisulfite sequencing.