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Non-Invasive Urinary Detection of Upper Tract Urothelial Carcinoma via Methylation Patterns in Cell-Free DNA

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Introduction

- Upper Tract Urothelial Carcinoma (UTUC) is an aggressive cancer associated with poor 5-year survival of 45–65%
- Accurate diagnosis and staging influences treatment choice, use of neoadjuvant therapy, and ultimately, long term outcomes.
- Ureteroscopy and biopsy is significantly limited by anatomical and technical factors, leading to unreliable results[1]:
  - Biopsy results have ~ 60% concordance with final path
  - ~ 40% are upstaged to >T2 disease after surgical resection
- This study evaluated whether methylation patterns in urine cell-free DNA (ucfDNA) could detect UTUC without the need for biopsy and whether estimates of of tumor burden (TMeF) correlate with pathologic tumor stage/grade.

Methods

- Prospective urine collection (2022–2025) at Stanford and Palo Alto VA.
- Blinded samples shared with study sponsor for analysis (Figure 1.)
- The urological cancer classifier was developed using the Galleri infrastructure[2]. The classifier was trained on both bio banked urine samples and prospectively collected samples from both cancer and non cancer paritipants.[3]
- Biopsy free TMeF estimates in urine cfDNA were generated as described previously[4]

	N=52	%
Age	Median: 72.8	Range: 47-86
Race		
White	36	69
Hispanic	7	13
Asian	7	13
Black	2	4
Gender		
Male	43	83
Female	9	17
Smoking Status		
Current	8	15
Former	22	42
Never	22	42
History of Upper Tract Urothelial C	25	48
History of Bladder Cancer	23	44
Cancer on Pathology	20	38
Variant Histology	3	6

Patient Category		
Control	9	17
UTUC Surveillance	11	21
Ureteroscopy and Biopsy	23	44
Definitive Surgery	9	17
Nephroureterectomy	8	15
Distal Ureterectomy	2	4
Path Grade (URS/bx)		
Benign or NED	10	43
HG	7	30
LG	6	26
Path T Stage (Definitive Surgery)		
Tis/Ta	4	44
T1	2	22
>T2	3	33

Figures

Figure 1. Urine sample processing workflow for classifier results and TMeF estimates

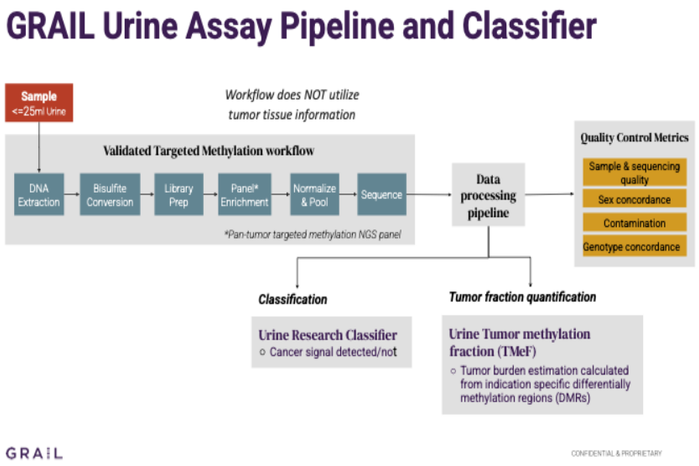


Figure 2. TMeF estimates and classifier prediction for UTUC pts compared to pts with negative endoscopic evaluation or benign path.

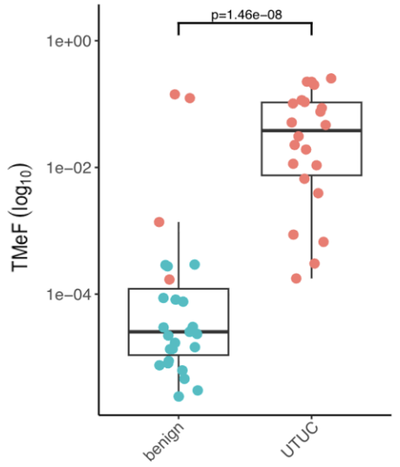
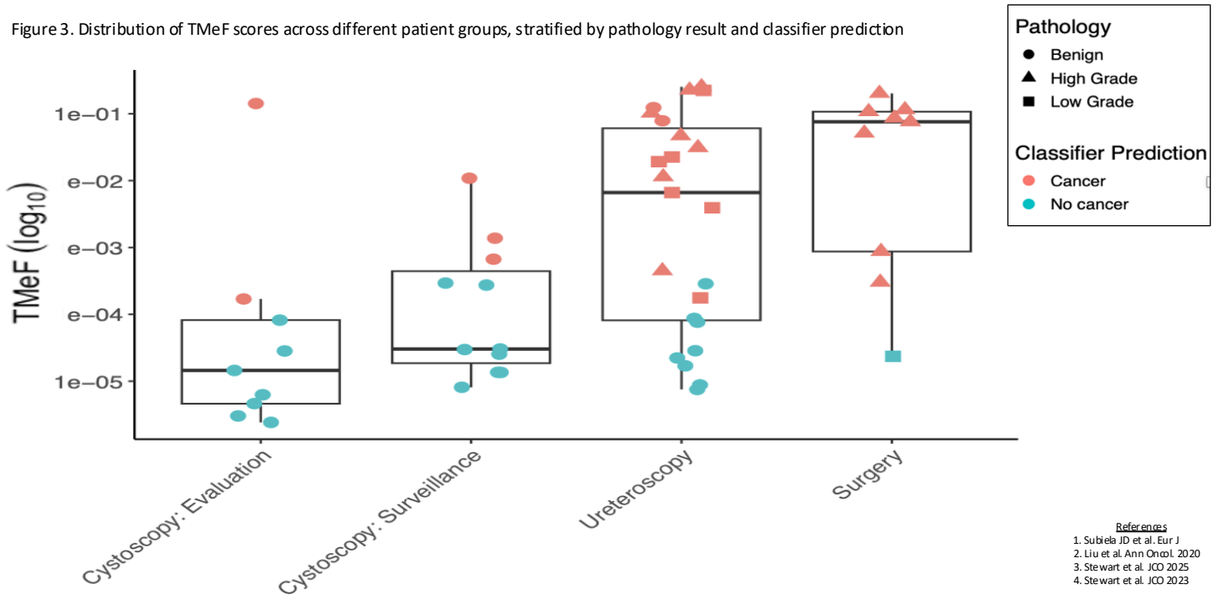


Figure 3. Distribution of TMeF scores across different patient groups, stratified by pathology result and classifier prediction



References
1. Subiela JD et al. Eur J
2. Liu et al. Ann Oncol. 2020
3. Stewart et al. JCO 2025
4. Stewart et al. JCO 2023

Results

- **UTUC can be detected using a urine cell free DNA methylation assay**
- **100% sensitivity (18/18)** and **72% specificity (21/29)** for detection of UTUC (Figure 2).
  - **Specificity improved to 81%** (21/26) if patients had **confirmed diagnosis of any urothelial cancer** within 6 months of sample collection.
- TMeF which estimates **overall tumor burden** can be a **valuable tool for clinical decision making**:
  - TMeF is **significantly higher in Bx confirmed UTUC patients** *(Figure 2. 0.0721 vs 0.0104, $p < 0.05$)*
  - Average TMeF is highest in patients undergoing definitive surgery *(Figure 3. Avg 0.071)*
  - TMeF **correlates with T stage** among patients who underwent definitive surgical resection (e.g., nephroureterectomy) (Figure 3).
  - TMeF does not correlate with stage or grade in patients undergoing ureteroscopy (Figure 3).

Conclusions

- **UTUC can be detected noninvasively** with excellent sensitivity and moderate specificity
- Limitations include **small sample size, cohort heterogeneity, and non-standardized collection**. Additionally, ureteroscopic biopsies can be inconclusive or suffer from sampling error, which makes it **challenging to define the "ground truth"** to assess assay performance.
- Future work requires prospective, multi-institutional studies with standardized collection protocols to further optimize the classifier.

Disclosure: This study received support from GRAIL, LLC, a subsidiary of Illumina, Inc, who provided the assay testing and results.