Biopsy-Free Detection of Residual Urothelial Carcinoma via Cell-Free DNA Methylation in Pre-Operative Urine and Plasma

SUO
December 2-5, 3025
Phoenix, Arizona

Margaret F. Meagher,[*,1] Archana Shenoy,[*,2] Kelly McClintock,[2] Sarah M. Stuart,[2] Yinjie Gao,[2] Tanavi Shah,[1] Juan Javier-Deloges,[1] A. Karim Kader,[1] Aditya Bagrodia,[1] Rana R. McKay,[1] Amirali Salmasi,[1] Matthew H. Larson,[†,2] Tyler F. Stewart[†,1]

. [1]University of California San Diego, La Jolla, CA; [2]GRAIL, Inc., Menlo Park, CA *Joint lead authors. †Joint senior authors. Corresponding author: tstewart@health.ucsd.edu

Introduction

- Urine cell-free DNA (cfDNA) has emerged as an effective biomarker for bladder (BC) and upper tract urothelial carcinoma (UTUC), enabling non-invasive disease detection, treatment monitoring, and recurrence prediction.
- We previously demonstrated the potential of urine cfDNA methylation patterns to improve detection of non-muscle invasive bladder cancer (NMIBC) in patients with suspicious bladder lesions (by cystoscopy or imaging) compared to plasma cfDNA.[1]
- Here, we conducted an exploratory evaluation of the utility of pre-operative urine and plasma cfDNA to predict pathologic complete response (pCR), defined as no viable disease at time of surgery (pT0N0), for patients with localized BC or UTUC undergoing definitive surgery.

Objective

- To develop a non-invasive tool to predict pathologic complete response at time of surgery

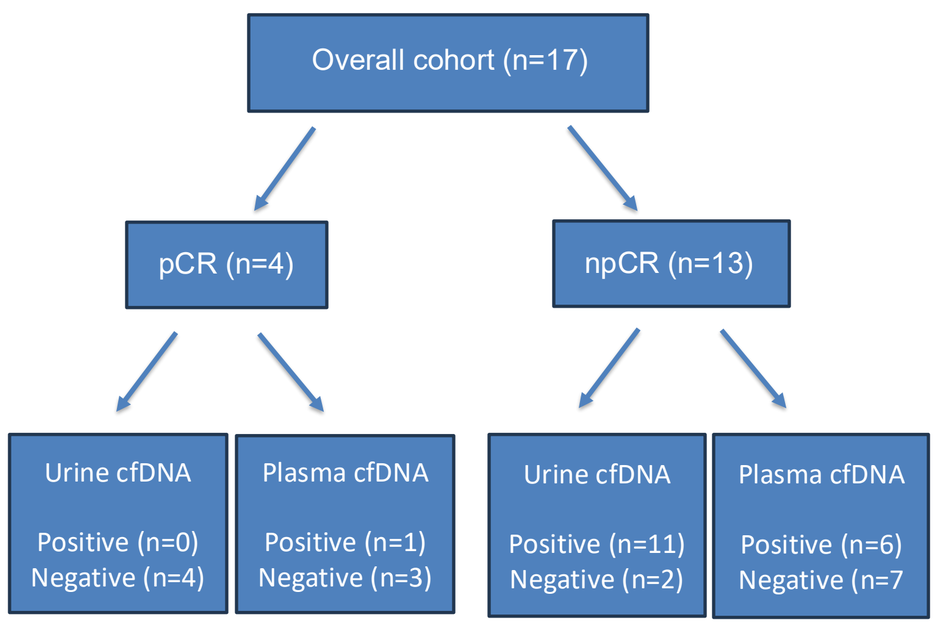
Methods

- Urine and blood were collected from patients with localized BC or UTUC (Ta-T4, N0/N1) prior to undergoing definitive surgery with either radical cystectomy, nephroureterectomy, or distal ureterectomy at a single institution.
- Patients with non-urothelial malignancies or evidence of distant metastatic disease were excluded.
- Urine cfDNA classifier predictions were generated using a previously trained classifier.[4]
- Plasma cfDNA was assessed using GRAIL's cancer research solution that detects circulating tumor DNA based on methylation signal. GRAIL team was blinded to clinical labels during results generation phase.[2-5]

Clinical Characteristics

Variable (n, %)	Total cohort (n=17)	BC cohort (n=11)	UTUC Cohort (n=6)
Age (median)	75	72	76
Male sex	14 (82)	10 (90.1)	4 (66.7)
Clinical Stage			
Ta/Tis	4 (23.5)	1 (9.1)	3 (50)
I	2 (11.8)	0 (0)	2 (33.3)
II	8 (47.1)	8 (72.7)	0 (0)
III	0 (0)	0 (0)	0 (0)
IV	3 (17.6)	2 (18.2)	1 (16.7)
Variant Histology	2 (11.8)	2 (18.2)	0 (0)
NAC Receipt	4 (23.5)	4 (23.5)	0 (0)
Pathologic Stage			
pCR	4 (23.5)	4 (36.4)	0 (0)
pTa/TisN0	4 (23.5)	2 (18.2)	2 (33.3)
pT1N0	3 (17.6)	2 (18.2)	1 (16.7))
pT2N0	1 (5.9)	0 (0)	1 (16.7)
pT3N0	2 (11.8)	0 (0)	2 (33.3)
pT4N0	0 (0)	0 (0)	0 (0)
pTxN1	3 (17.6)	3 (27.2)	0 (0)

Pre-operative cfDNA Status



pCR, pathologic complete response (pT0N0); npCR, non-pathologic complete response; cfDNA, cell-free DNA

KEY RESULT: SENSITIVITY AND SPECIFICITY OF METHYLATION PATTERNS IN CFDNA TO IDENTIFY RESIDUAL DISEASE AT TIME OF SURGERY

Pre-operative cfDNA detection	Specificity	Sensitivity
Urine cfDNA	100% (4/4)	84% (11/13)
Plasma cfDNA	75% (3/4)	46% (6/13)
Combined urine and plasma cfDNA	75% (3/4)	92% (12/13)

Conclusions

- In this study, analysis of methylation features in cfDNA from both urine and plasma detected residual disease prior to definitive surgery without the need for tissue biopsy.
- This pilot study suggests the combination of urine and plasma cfDNA has higher sensitivity to detect residual disease than either analyte alone.
- This warrants further investigation of the utility of urine and plasma cfDNA as biomarkers to predict neoadjuvant treatment response and could potentially be used for selective organ preservation.

References

1. Klein EA et al. *Ann Oncol.* 2021;32(9):1167-1177.
2. Bredno J et al. *J. Am J Pathol.* 2022;192(10):1368-1378.
3. Ward DG et al. *Transl Androl Urol.* 2017;6(2):331-335.
4. Stewart TF et al. *ASCO* 2025.
5. Liu MC et al. *Ann Oncol.* 2020;31(6):745-759.

Disclosures

Study funded by GRAIL, Inc., Menlo Park, CA. AS, KM, SS, YG, ML are current employees of GRAIL, Inc. with equity in the company. All financial relationships disclosed at abstract submission.