A Targeted Methylation-Based MCED Blood Test Preferentially Detects High-Grade Prostate Cancer and Minimizes Overdiagnosis

Brandon Mahal[1]; Matthew Margolis[2]; Earl Hubbell[2]; Cheng Chen[2]; Jeffrey M. Venstrom[2]; John Abran[2]; Jordan J. Kartlitz[2]; Alexander W. Wyatt[3]; Eric A. Klein[2]

[1]*Sylvester Comprehensive Cancer Center, University of Miami Miller School of Medicine Miami, FL*

[2]*GRAIL, LLC, Menlo Park, CA*

[3]*Department of Urologic Sciences, Vancouver Prostate Centre, University of British Columbia, Vancouver, British Columbia, Canada*

Disclosure Information

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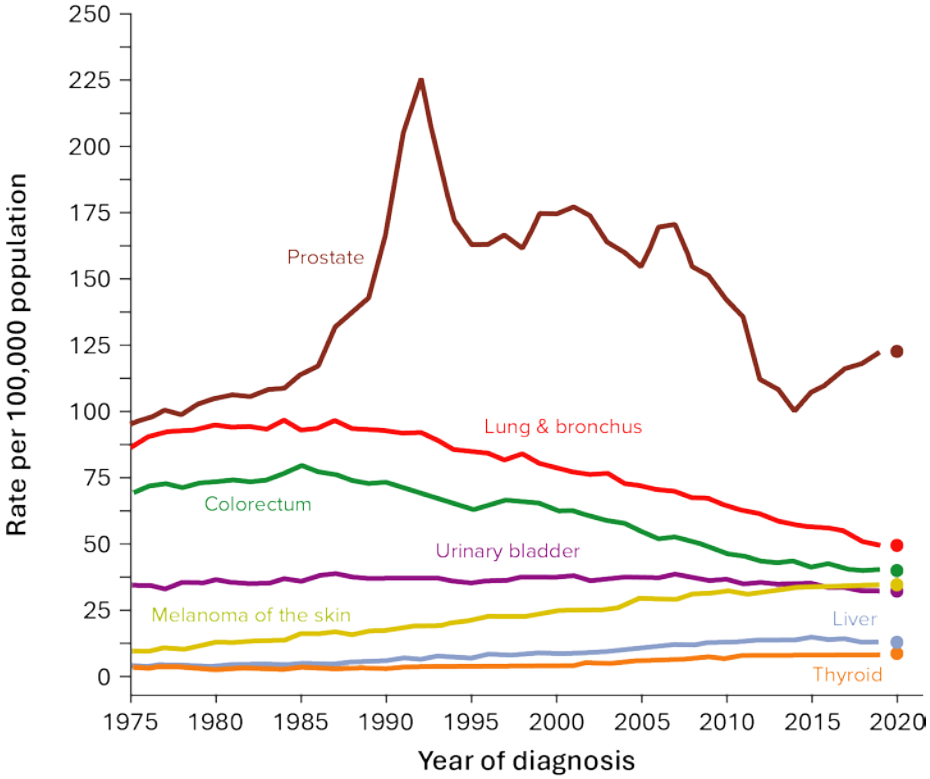
Eric A. Klein, MD

I have the following relevant financial relationships to disclose:

Employee of

GRAIL, LLC, Menlo Park, CA

Overdiagnosis of Prostate Cancer



Accounts for 23-42% of all screen-detected cases[1]

- Indolent disease typically defined as GG1-2, stage I-II

Associated harms

- Anxiety
- Overtreatment
- Unnecessary cost
- Overuse of healthcare resources

Imperative that new screening tests not exacerbate this problem

GG, Gleason grade group.
Figure reused from Siegel RL, et al. *CA Cancer J Clin*. 2024; 74(1):12-49.
[1]Loeb S, et al. *Eur Urol*. 2014;65(6):1046-1055.

Multi-Cancer Early Detection (MCED) is a New Screening Paradigm

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MCED test as a complement to existing standard of care screening, including PSA for prostate

Detect many lethal cancers, including unscreened cancers, using single blood sample collected at point-of-care

Identify signal origin to direct diagnostic workup

Large-scale clinical validation

High specificity (~99%) could limit false positives and unnecessary workups

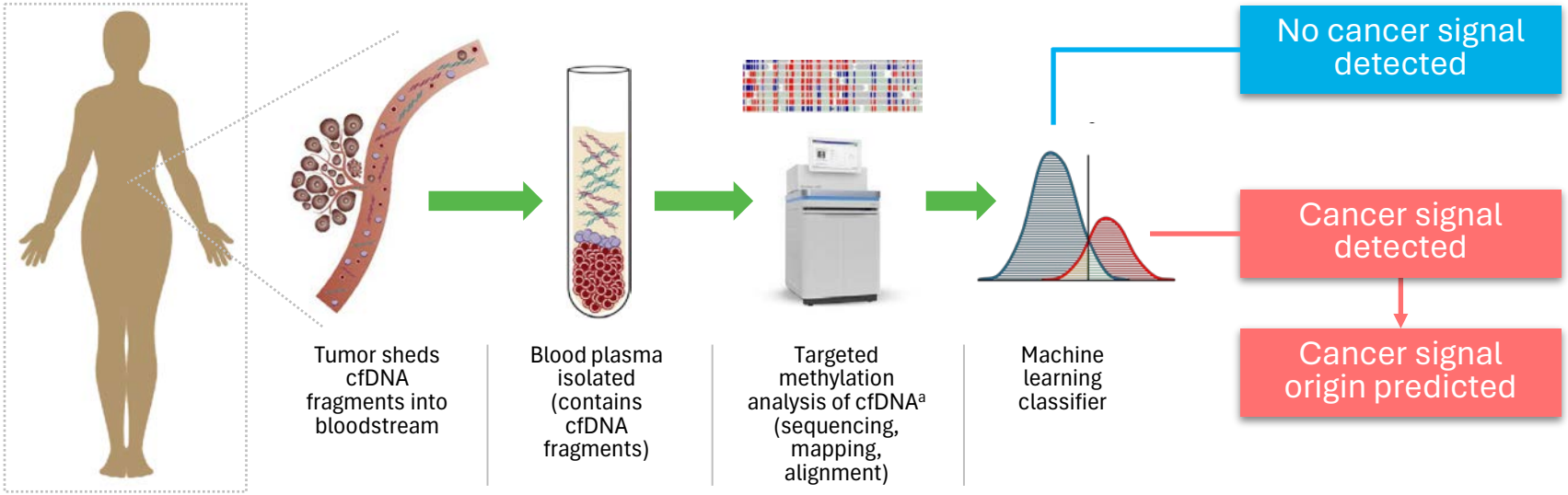
When used at population level, potential for:

- More cancer cases detected through screening
- Improved patient outcomes and survival
- Decreased burden of care

MCED, multi-cancer early detection; PSA, prostate-specific antigen.

Klein et al. *Cancer Epidemiol Biomarkers Prev*. 2023 Aug 1;32(8):1003-1010.

Blood-Based MCED Using Targeted Methylation



MCED tests are intended as a complement, not a replacement, to standard of care screening tests (including PSA)

cfDNA, cell-free DNA; PSA, prostate-specific antigen.
[a]Bisulfite treatment; targeted probes pull out fragments matching regions of interest.
Adapted from Liu MC, et al. *Ann Oncol*. 2020;31(6):745-759.

Age-Adjusted Prostate Cancer Incidence in the US Overlaps With the MCED Intended Use Population

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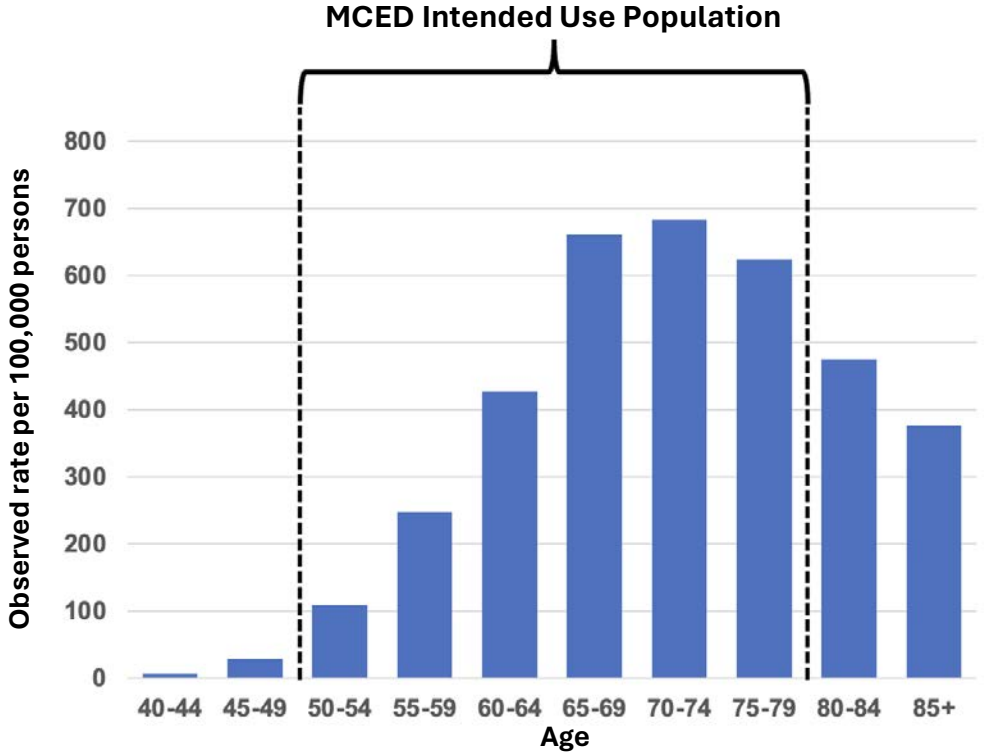

Observed SEER prostate cancer incidence rates by age at diagnosis, 2016-2020. Figure originally published by the National Cancer Institute and adapted from: www.seer.cancer.gov/statistics.

Study Objective

Assess MCED test performance for detecting prostate cancer in 2 prospective clinical studies

Substudy 3 of CCGA[1] NCT02889978	**PATHFINDER[2]** NCT04241796
▪ Case-control, observational ▪ Refine and validate a cell-free DNA-based MCED test	▪ Prospective, return of results ▪ Evaluate clinical implementation and perceptions of MCED test

Same MCED test with a locked classifier used in both studies

CCGA, Circulating Cell-Free Genome Atlas Study; MCED, multi-cancer early detection.
[1]Klein et al. *Ann Oncol*. 2021;32(9):1167-1177. [2]Schrag et al. *The Lancet*. 2023;402(10409):1251-1260.

MCED Test Performance Characteristics

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	Substudy 3 CCGA[1]	PATHFINDER[2]
Study design	Case-control	Prospective interventional
Number of participants	4077[a]	6621[b]
Specificity	99.5%	99.1%
Overall test sensitivity	51.5%[c]	–
Cancer signal origin (CSO) prediction accuracy	88.7%	97%
Positive predictive value (PPV)	44%	38%

[a]2823 cancer cases and 1254 non-cancer controls. [b]120 cancer cases. [c]Ranging from 16.8% in stage I to 90.1% in stage IV cancers.
CCGA, Circulating Cell-Free Genome Atlas Study; CSO, cancer signal origin; MCED, multi-cancer early detection; PPV, positive predictive value.
[1]Klein et al. *Ann Oncol*. 2021;32(9):1167-1177. [2]Schrag et al. *The Lancet*. 2023;402(10409):1251-1260.

Prostate Cancer Cohorts

	Substudy 3 of CCGA[1]	PATHFINDER[2]
Prostate cancer cases	420	18
Age (median, IQR)	65 (59, 70)	65 (58, 70)
White, non-Hispanic (%)	85%	89%
PSA (median, IQR)	6.53 (4.8-11.2)	Not collected

CCGA, Circulating Cell-Free Genome Atlas Study; IQR, interquartile range; PSA, prostate-specific antigen.
[1]Klein et al. *Ann Oncol*. 2021;32(9):1167-1177. [2]Schrag et al. *The Lancet*. 2023;402(10409):1251-1260.

Endpoints

- MCED test performance
  - Detectability of prostate cancer
    - Overall
    - By Gleason grade group (GG)[1]
    - By clinical stage[2]
  - CSO accuracy*
- Detectability as a function of Tumor Methylated Fraction (TMeF)* and PSA*
- Overall survival compared to SEER estimates adjusted for age, stage, and GG*

*Substudy 3 of CCGA only.
CCGA, Circulating Cell-Free Genome Atlas Study; CSO, cancer signal origin; GG, Gleason grade group; MCED, multi-cancer early detection; PSA, prostate-specific antigen; SEER, Surveillance, Epidemiology, and End Results; TMeF, tumor methylated fraction.
[1]Epstein et al. *Am J Surg Pathol*. 2016;40:244–252. [2]AJCC Cancer Staging Manual Seventh Edition.

Overall Prostate Cancer Detectability

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	Substudy 3 of CCGA[1] n (%)	PATHFINDER[2] n (%)
Sensitivity	47/420 (11.2)	1/18 (5.6)
CSO accuracy	43/47 (91.5)	Not calculated

CCGA, Circulating Cell-Free Genome Atlas Study; CSO, cancer signal origin.
[1]Klein et al. *Ann Oncol*. 2021;32(9):1167-1177. [2]Schrag et al. *The Lancet*. 2023;402(10409):1251-1260.

Sensitivity of Prostate Cancer Detection By Grade

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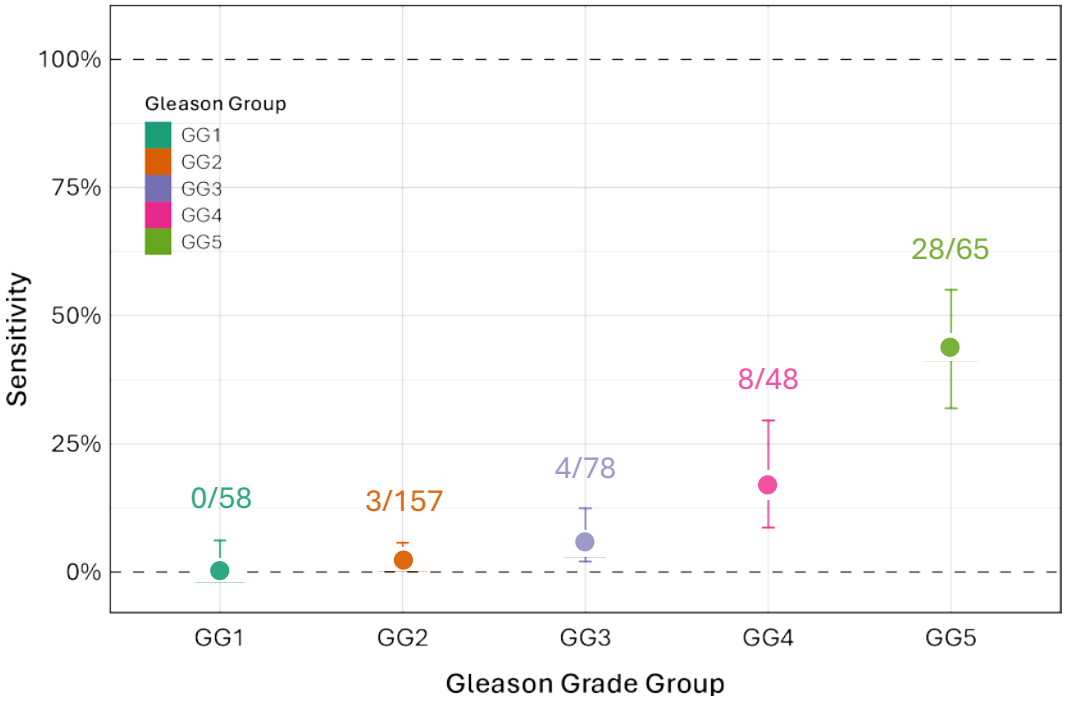

Substudy 3 of CCGA (N = 420)

Gleason Grade Group	Sensitivity (%)
GG1	0
GG2	1.9
GG3	5.1
GG4	16.7
GG5	43.1

CCGA, Circulating Cell-Free Genome Atlas Study; CSD, cancer signal detected; GG, Gleason grade group.

Sensitivity of Prostate Cancer Detection By Stage

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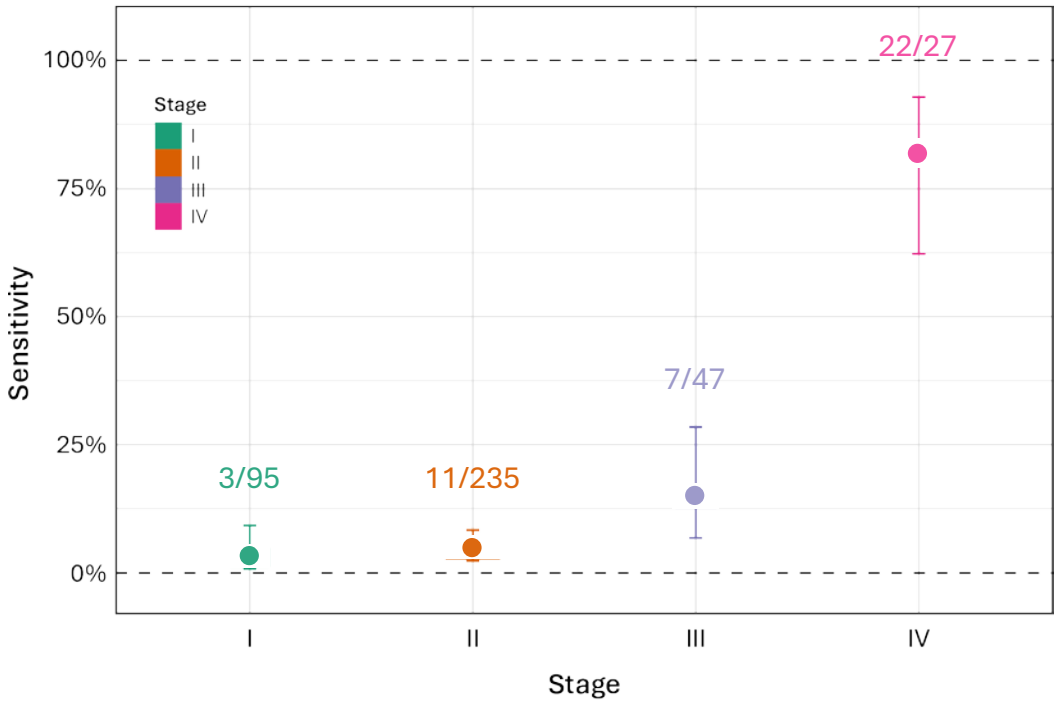

Substudy 3 of CCGA (N = 420)

Stage	Sensitivity (%)
I	3.2
II	4.7
III	14.9
IV	81.5

CCGA, Circulating Cell-Free Genome Atlas Study; CSD, cancer signal detected.

Relationship Between Cancer Detectability, TMeF,[1] and PSA

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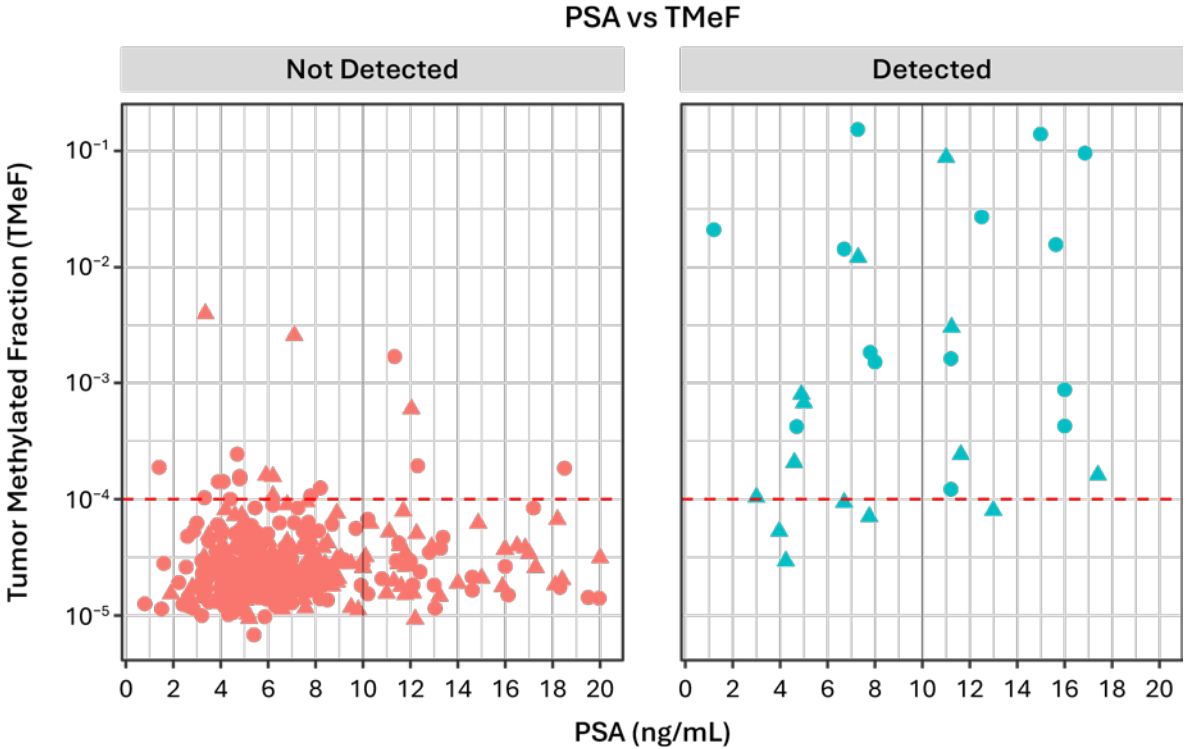

Substudy 3 of CCGA (N = 420)

Detected cases:
- 34% had PSA < 10 ng/mL
- 32% were ≥ GG3 & stage I-III

Approximate limit of detection

GG3–5 and Stages I–III
- False
- True

MCED Detection Status
- Not Detected
- Detected

CCGA, Circulating Cell-Free Genome Atlas Study; GG, Gleason grade group; PSA, prostate-specific antigen; TMeF, tumor methylated fraction.

[1]Melton et al. *Cancers*. 2023;16(1):82.

Observed Overall Survival of Prostate Cancer Cases Compared to SEER Expected Survival

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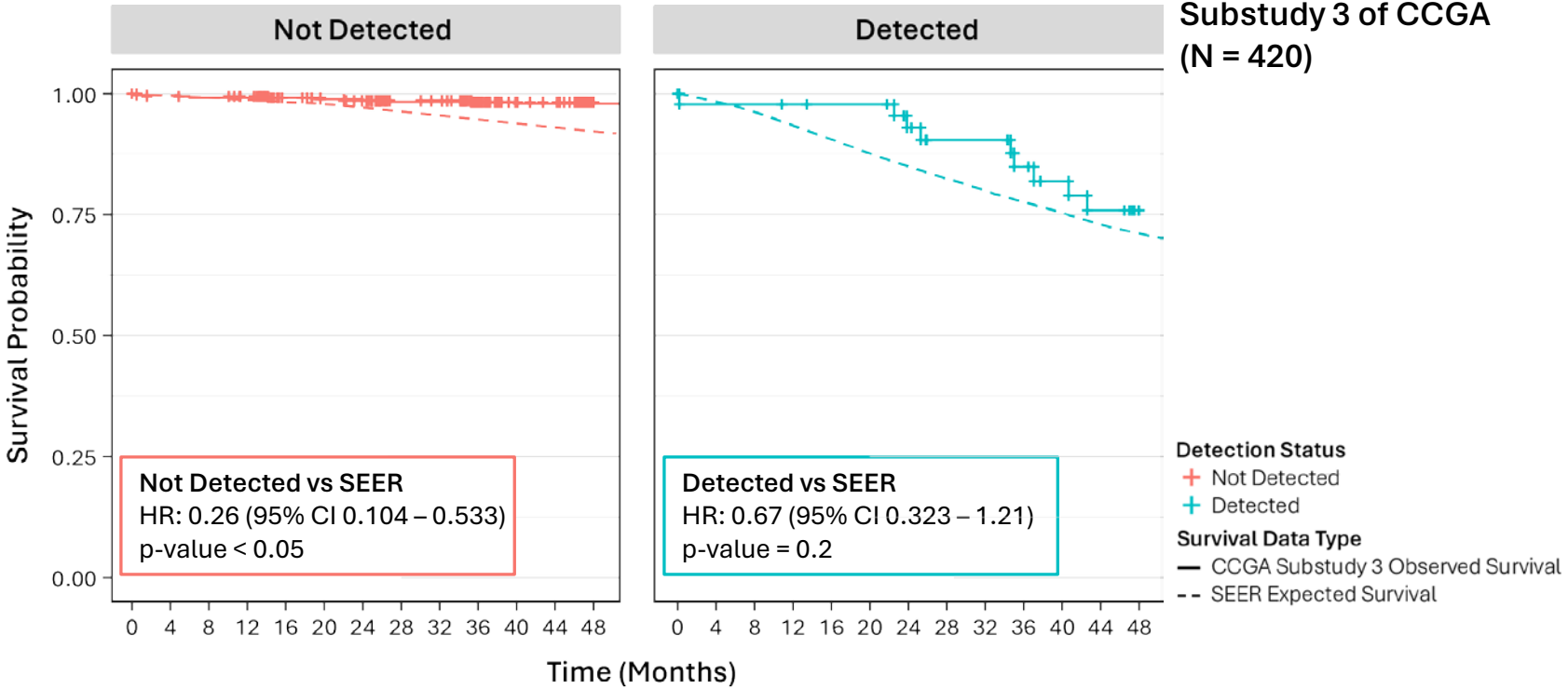
SEER expected survival adjusted for age, cancer stage, and grade



CCGA, Circulating Cell-Free Genome Atlas Study; CI, confidence interval; HR, hazard ratio; OS, overall survival; SEER, Surveillance, Epidemiology, and End Results.

Summary of Key Findings

- Overall detection of prostate cancer low
  - 11.2% in substudy 3 of CCGA
  - 5.6% in PATHFINDER
- CSO accuracy for prostate cancer high (> 90%)
- Preferential detection of high-grade and -stage disease
  - No GG1 cases detected
  - Few GG2 cases detected (2.1%)
  - 33% of cases potentially curable (PSA < 10 ng/mL and stage I-III)
- Prostate cancer cases with no cancer signal detected had better OS than comparable SEER estimates

CCGA, Circulating Cell-Free Genome Atlas; CSO, cancer signal origin; GG, Gleason grade group; OS, overall survival; PSA, prostate-specific antigen; SEER, Surveillance, Epidemiology, and End Results.

Strengths and Limitations

Strengths

- Data from 2 independent clinical studies using the same MCED test classifiers
- SEER reference data served as a synthetic control

Limitations

- Small number of prostate cancer cases and lack of PSA, TMeF, and OS data from PATHFINDER
- GG and stage assignment from multiple sites and not centrally reviewed

GG, Gleason grade group; OS, overall survival; PSA, prostate-specific antigen; SEER, Surveillance, Epidemiology, and End Results; TMeF, tumor methylated fraction.

Conclusions

- A clinically validated targeted methylation-based MCED test preferentially detects high-grade and high-stage clinically significant prostate cancer
- A cancer signal detected test result with a prostate CSO prediction strongly suggests the presence of aggressive disease and warrants a prompt diagnostic work-up
- Because this MCED test rarely detects low-grade disease, its use in population-based screening programs in addition to SOC screening is unlikely to exacerbate overdiagnosis of indolent prostate cancer

CSO, cancer signal origin; MCED, multi-cancer early detection; SOC, standard of care.

Acknowledgments

- Participants in CCGA and PATHFINDER
- Site investigators and study staff
- Co-authors for this analysis

CCGA, Circulating Cell-Free Genome Atlas.