Detection and Quantification of Triple-Negative Breast Cancer Across Ethnicities Through Analysis of Cell-Free DNA Methylation

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INTRODUCTION

- Triple-negative breast cancer (TNBC) is an aggressive subtype that contributes to race-related disparities in breast cancer outcomes[1]
- These disparities are partly explained by the higher burden of TNBC in Black women and unequal access to new mammography technologies; disparities are further compounded by limited efficacy of screening mammography for early detection of TNBC[2]
- Blood-based multi-cancer early detection (MCED) tests are an emerging technology that may help address race-related cancer disparities; the Galleri® MCED test leverages methylation patterns in circulating tumor DNA (ctDNA) to help detect and diagnose cancers earlier
- Because cancer aggressiveness correlates with higher ctDNA shedding,[3] it is necessary to estimate the relative abundance of ctDNA to ensure consistent cancer detection performance across clinically and ethnically diverse patient populations

OBJECTIVE

- To determine whether the Galleri MCED test and the tumor methylated fraction (TMeF) quantification algorithm can detect cancer signals across cancer stages and self-reported race/ethnicity (SRE) groups in TNBC patients from the Circulating Cell-free Genome Atlas (CCGA) study[4]

KEY RESULTS: A CANCER SIGNAL FROM STAGE II, III, AND IV TNBC WAS DETECTED BY THE MCED TEST ACROSS SELF-REPORTED RACES/ETHNICITIES

- For the 84 patients with TNBC in the third CCGA substudy (CCGA3), baseline characteristics of age, smoking status, or cancer stage distribution were not statistically different between SRE groups (**Figure S1, Table S1**)
- In CCGA3, 49% (19/39) of Black women diagnosed with breast cancers had TNBC, which was a higher percentage than for White women (15% [58/396]) and women of Other/Unreported ethnicities (13% [7/52])
- In patients with stage II-IV TNBC, the MCED test successfully detected a cancer signal in 94% (16/17) of Black women, 72% (31/43) of White women, and 100% (6/6) women of Other/Unreported ethnicities (**Table 1**)
  - The MCED test did not detect any stage I TNBC cases, potentially because early-stage breast cancers tend to shed less ctDNA than later-stage cancers[3]

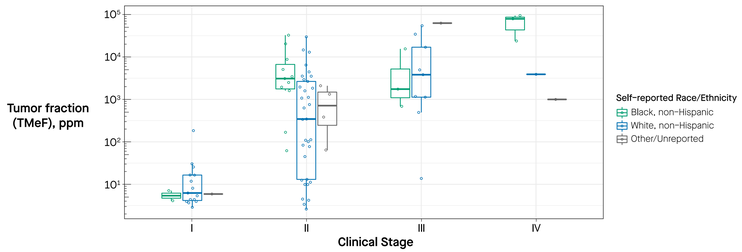
Table 1. MCED cancer signal detection rate by cancer stage and SRE among TNBC patients.

	Black, non-Hispanic (n = 19)		White, non-Hispanic (n = 58)		Other/Unreported (n = 7)	
Stage	Detected/ Total	% (95% CI)	Detected/ Total	% (95% CI)	Detected/ Total	% (95% CI)
I	0/2	0% (0-80)	0/15	0% (0-25)	0/1	0% (0-95)
II	10/11	91% (57-100)	22/33	67% (48-81)	4/4	100% (40-100)
III	3/3	100% (31-100)	8/9	89% (51-99)	1/1	100% (5.5-100)
IV	3/3	100% (31-100)	1/1	100% (5.5-100)	1/1	100% (5.5-100)

CI, confidence interval; SRE, self-reported race/ethnicity; TNBC, triple-negative breast cancer.

- TMeF significantly varied between cancer stages ($p < 0.001$), and there was no significant difference in mean TMeF between SRE groups ($p = 0.09$; **Figure 1 and Table 2**)
  - Cancer stage and SRE also displayed a significant interaction ($p < 0.001$), which could represent an impact of stage on TMeF that varies by SRE or differences in cancer stage distribution between SRE groups

Figure 1. Box plot of estimated TMeF across cancer stages and SRE groups among TNBC patients.



Median TMeF and IQR reported in ppm.

IQR, interquartile range; ppm, parts per million; SRE, self-reported race/ethnicity; TMeF, tumor methylated fraction; TNBC, triple-negative breast cancer.

Table 2. Estimated median TMeF across cancer stages and SRE groups among TNBC patients.

Stage	Black, non-Hispanic (n = 19)	White, non-Hispanic (n = 58)	Other/Unreported (n = 7)
I	5.6 (4.8-6.3)	6.2 (4.2-17)	5.9 (5.9-5.9)
II	3100 (1800-6900)	340 (13-2700)	850 (300-1500)
III	1700 (1200-8600)	3800 (1100-17000)	63,000 (63,000-63,000)
IV	79,000 (51,000-87,000)	3900 (3900-3900)	1000 (1000-1000)

Median TMeF (IQR) reported in ppm.

IQR, interquartile range; ppm, parts per million; SRE, self-reported race/ethnicity; TMeF, tumor methylated fraction; TNBC, triple-negative breast cancer.

CONCLUSIONS

- The MCED test detected a cancer signal across SRE groups for stage II-IV TNBC
- The higher detection rate for TNBC in Black women, compared to White women, may be due to differences in biological and/or clinical characteristics beyond stage and will need to be evaluated with a larger sample size before conclusions can be drawn
- The ctDNA abundance estimate, TMeF, significantly varied by cancer stage but not by SRE group
- This pilot study provides hypothesis-generating evidence that analysis of methylation signals across the genome can provide meaningful information for screening and detection of TNBC, unbiased by SRE

METHODS

Cancer Detection and TMeF Estimation

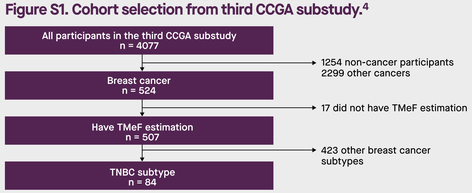
- MCED testing was performed as part of the CCGA3 case-control study[4]
  - The MCED test uses a methylation-based assay and machine-learning classifier to detect a common cancer signal shared across multiple cancer types and predict a cancer signal origin
  - The test assesses alterations in methylation patterns associated with ctDNA within the total cell-free DNA (cfDNA) in a peripheral blood sample
- TMeF is a methylation-based estimate of ctDNA abundance
  - TMeF estimates are based on cancer-indicative methylation patterns of cfDNA that were identified by machine learning models trained on whole-genome bisulfite sequencing of tumor biopsy tissue and non-cancer cfDNA plasma samples

Data Analysis

- TMeF was estimated in 507 women with breast cancer participating in CCGA3, 84 of whom had TNBC (**Figure S1, Table S1**)
- Cancer detection by MCED test and ctDNA abundance estimation by TMeF were performed across three SRE groups: non-Hispanic Black, non-Hispanic White, and Other (Hispanic, Asian, Pacific Islander) or Unreported ethnicities
- Differences in TMeF were evaluated using two-way ANOVA

SUPPORTING INFORMATION

Figure S1. Cohort selection from third CCGA substudy.[4]



CCGA, Circulating Cell-free Genome Atlas; TMeF, tumor methylated fraction; TNBC, triple-negative breast cancer.

Table S1. Demographic characteristics of pilot TNBC cohort.

		Black, non-Hispanic (n = 19)	White, non-Hispanic (n = 58)	Other/Unreported (n = 7)	*p*-value[a]
Age, median years (IQR)		52 (43-62)	56 (44-66)	60 (48-70)	0.7
Smoking Status, n (%)	Never-smoker	13 (68)	37 (64)	5 (71)	>0.9
	Ever-smoker	6 (32)	21 (36)	2 (29)	
	Other/Missing	0 (0)	0 (0)	0 (0)	
Clinical Stage, n (%)	I	2 (11)	15 (26)	1 (14)	0.2
	II	11 (58)	33 (57)	4 (57)	
	III	3 (16)	9 (16)	1 (14)	
	IV	3 (16)	1 (2)	1 (14)	

[a]Kruskal-Wallis rank sum test; Fisher's exact test.
IQR, interquartile range.

References

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2. Mitchell E, et al. *J Natl Med Assoc.* 2022 Jun;114(3):236-250.
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4. Klein EA, et al. *Ann Oncol.* 2021 Sep;32(9):1167-1177.

Disclosures

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