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Blood-based cancer detection in plasma cell-free DNA (cfDNA): evaluating clinical and pathologic tumor characteristics in participants with breast cancer

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BACKGROUND

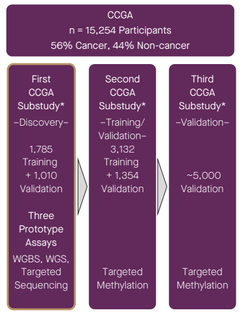
- The Circulating Cell-free Genome Atlas (CCGA; NCT02889978) study is a prospective, multicenter, observational, case-control study with longitudinal follow-up to support the development of a plasma cfDNA-based multi-cancer early detection test.
- We previously reported that a targeted methylation assay detected over 20 cancer types with a single, fixed false positive rate of <1%, and localized the cancer with >90% accuracy.[1-3]
  - Additionally, we noted that tumor fraction (TF) was associated with detection of multiple cancer types using an earlier prototype whole-genome bisulfite sequencing (WGBS) assay.[4]
- Differences in detection of hormone receptor (HR)+ and HR- breast cancer subtypes were noted,[1] which may be due to underlying biological differences.
- We thus used breast cancer as a model to understand the clinical and biological determinants of cancer detectability with our prototype WGBS assay.

METHODS

- In CCGA, blood samples were prospectively collected from participants with newly diagnosed, untreated cancer and from participants without a diagnosis of cancer (non-cancer); tumor tissue was also obtained from participants with cancer (Figure 1A).
  - As previously described, specimens were subjected to a prototype WGBS assay and were classified as cancer/non-cancer and localized to a tissue of origin.[1-3]
  - Samples from participants with breast cancer were used to model the determinant variables of cancer detectability with the WGBS assay.

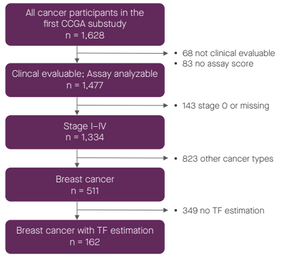
Figure 1. CCGA and Substudy 1 Participants

A. CCGA Divided into Three Substudies



*Includes participant samples used in evaluating the classifiers; approximately 2,700 participants not included in evaluating classifiers (eg, used as tissue references or in panel design) not represented. WGBS, whole-genome bisulfite sequencing; WGS, whole-genome sequencing.

B. Detail of Training and Test Cohorts from First Substudy



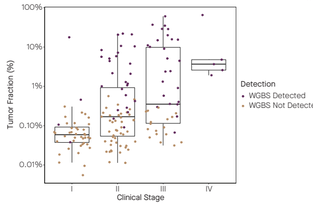
- In the training and test sets of the first prespecified substudy of CCGA, 511 of 1,628 (31.4%) participants had a clinical diagnosis of stage I-IV breast cancer and a WGBS result. TF was estimated for 162 participants as previously described (Figure 1B).[4]
  - The following prespecified biological and clinical factors were assessed for correlation with TF: clinical stage, clinical T stage (primary tumor size), clinical N stage (lymph node involvement), clinical M stage (distant metastasis), Bloom-Richardson histologic grade, proliferation fraction (Ki-67 by IHC), HR (estrogen receptor and progesterone receptor) status, HER2 status, and histologic subtype.
  - The relative importance of each variable was calculated using the LMG method[5]; metrics were normalized to sum 100%.

Table 1. CCGA1 Breast cancer patient cohort and tumor characteristics

Total	162
Age, Mean ± SD	56.9 ± 13.0
Age Group, ≥ 50 yrs, n (%)	117 (72.2)
Sex, Female, n (%)	162 (100)
Race/Ethnicity, n (%)	
White, Non-Hispanic	129 (79.6)
African American	18 (11.1)
Hispanic, Asian, Other	15 (9.3)
Never-smoker, n (%)	102 (63.0)
Body Mass Index, Normal/Underweight, n (%)	37 (22.8)
Dx by Screening, n (%)	61 (37.7)
Clinical Stage, n (%)	
I	44 (27.2)
II	71 (43.8)
III	42 (25.9)
IV	5 (3.1)
Histological Subtype, n (%)	
Ductal	134 (82.7)
Lobular	15 (9.3)
Mixed	10 (6.2)
Other	3 (1.8)

RESULTS

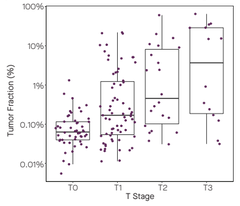
Figure 2. Correlation of TF with detection by WGBS across clinical stages I-IV



Of 162 breast cancer cases, 60 (37.0%; 68.9% in HR- and 25.9% in HR+) had a WGBS-detected cancer signal. Higher TF was significantly associated with cancer detection, and TF increased with higher clinical stage (Kruskal-Wallis rank sum test, p < 0.0001).

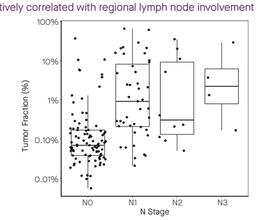
Figure 3. Tumor fraction correlates with tumor size, nodal involvement, and distant metastasis

A. TF positively correlated with primary tumor size (T stage)



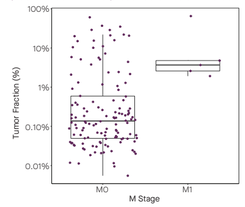
(A) Correlation of TF and primary tumor size (T stage). Tumor size was based on radiographic staging (Kruskal-Wallis rank sum test, p < 0.0001).*

B. TF positively correlated with regional lymph node involvement



*(B) Correlation of TF and lymph node involvement (N stage**). Lymph node status was based on clinical staging (Kruskal-Wallis rank sum test, p < 0.0001).*

C. TF positively correlated with the presence of distant metastatic disease

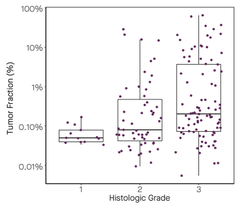


*(C) Correlation of TF and presence of distant metastasis (M stage***). Presence of metastasis was assessed clinically (Kruskal-Wallis rank sum test, p < 0.0001).*

*T1 ≤ 20 mm, 20 < T2 ≤ 50 mm, T3 > 50 mm. **N0 refers to no regional lymph node metastases. N1 disease refers to metastases to movable ipsilateral level I, II axillary lymph node(s). Clinical N2 disease refers to metastases in ipsilateral level I, II axillary lymph nodes that are clinically fixed or matted; or in clinically detected ipsilateral internal mammary nodes in the absence of clinically evident axillary lymph node metastases. Clinical N3 disease refers to metastases in ipsilateral infraclavicular (level III axillary) lymph node(s) with or without level I, II axillary lymph node involvement; or in clinically detected ipsilateral internal mammary lymph node(s) with clinically evident level I, II axillary lymph node metastases; or metastases in ipsilateral supraclavicular lymph node(s) with or without axillary or internal mammary lymph node involvement. ***M0 reflects absence of metastatic disease at the time of enrollment. M1 reflects presence of metastatic disease at the time of enrollment.

Figure 4. Histologic Bloom-Richardson grade 3 was associated with higher TF, specifically for stage II breast cancer

A



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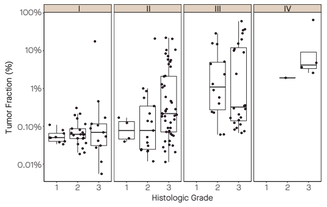
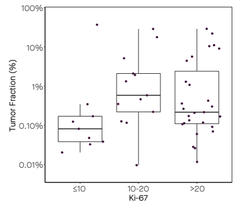


Correlation of TF and histologic grade. Bloom-Richardson histologic grade was obtained from local pathology reports and analyzed for the whole cohort (Kruskal-Wallis rank sum test, p<0.001) (A) and by clinical stage (B) (Kruskal-Wallis rank sum test, p=0.059 in stage II).

Figure 5. Relationship between TF and Ki-67 (proliferative rate) was observed

A



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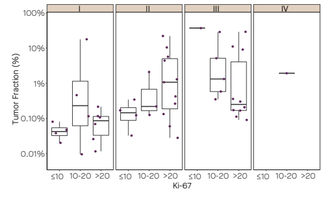
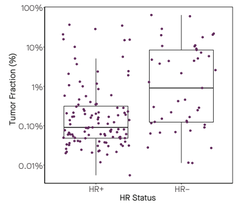


A positive trend between TF and proliferative rate (Ki-67) was observed. A. X-axis displays binned % Ki-67 positive tumor cells (Kruskal-Wallis rank sum test, p = 0.084). B. Distribution of Ki-67 positivity observed within each clinical stage. Limited sample size (n=49) may have contributed to the limited statistical significance.

Figure 6. HR-negative cases were more likely to have higher TF, particularly for stage II breast cancer

A



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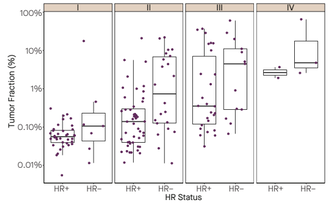
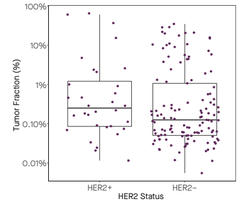


Correlation of TF and HR status. A. Correlation for the whole sample set (Kruskal-Wallis rank sum test, p < 0.0001). HR positivity is defined as positivity for either ER or PR based on the local pathology report. HR negativity is defined as negativity for both ER and PR based on the local pathology report. B. Higher TF specifically in stage II HR- cases (Kruskal-Wallis rank sum test p = 0.0051), possibly reflecting the greater contribution of biological factors to tumor cfDNA shedding (i.e., greater shedding in more aggressive HR- cancers) at earlier stages before significant nodal involvement and/or distant metastatic spread are clinically evident. The additional multivariate analysis below supports this hypothesis.

Figure 7. TF was not associated with HER2 status or histologic subtype

A) HER2 status had no significant association with TF



(A) Lack of correlation between TF and HER2 status. HER2 status was determined by local pathology reports as determined by immunohistochemical staining and/or FISH testing (Kruskal-Wallis rank sum test, p = 0.13). (B) Lack of correlation between TF and histologic subtypes (Kruskal-Wallis rank sum test, p = 0.13).

B) Histologic subtype had no significant association with TF

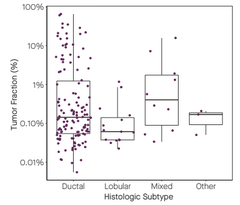


Table 2. Multivariate analysis

		p-value
T stage	T2 vs. T1	0.045
T stage	T3 vs. T1	0.055
T stage	T4 vs. T1	<0.001
N stage	N1/2/3 vs. N0	<0.001
HR status	HR- vs. HR+	<0.001
Histologic grade	2 vs. 1	0.98
Histologic grade	3 vs. 1	0.87
Distant metastasis	M1 vs. M0	0.17
Ki-67	10-20% vs. <10%	0.90
Ki-67	>20% vs. <10%	0.53
Histologic type	Lobular vs. ductal	0.60
Histologic type	Mixed vs. ductal	0.53
HER2 status	HER2- vs. HER2+	0.96

In a multivariate regression model, T stage, N stage, and HR status were statistically significantly associated with log-transformed TF in participants with breast cancer, while adjusting for histological grade, M stage, Ki-67, histological type, and HER2 status.

- The relative importance metric using the LMG method[5] shows that the relative proportion of R^2 explained by T stage, N stage, and HR status was 31.7%, 30.9%, and 16.6%, respectively (R^2 = 44.68%, metrics are normalized to sum to 100%)

CONCLUSIONS

- Higher cfDNA TF was significantly associated with WGBS-based cancer detection.
- cfDNA TF was found to be associated with varied clinical and pathologic features of breast cancer.
- Characteristics associated with disease burden (clinical stage, tumor size, and lymph node positivity) had high correlation with TF.
- Breast cancer pathologic features routinely used to assess clinical aggressiveness, such as HR status and histologic grade, were also highly correlated with TF. Ki-67 demonstrated a weak association with TF, but this may be due to small sample size.
- Stronger positive trends between TF and histologic grade, Ki-67, or HR were observed in stage II cancers, suggesting that these are strong prognostic factors in the absence of significant nodal involvement and distant metastases.
- There was no difference in TF between histologic subtypes and HER2 status.
- Multivariate analysis in all stages revealed that T stage, nodal status, and HR status were most predictive of TF.
- Together, these data suggest that higher tumor fractions may be observed in more aggressive breast cancers, which is likely generalizable across cancer types.
  - Therefore, this cfDNA-based assay may preferentially detect more aggressive cancers and may not be subject to overdiagnosis

References: 1. Liu, MC, *et al.* ASCO (2018). 2. Oxnard GR, Klein EA, Seiden MV, *et al.* ASCO Breakthrough Meeting; October 11, 2019; Bangkok, Thailand. Abstract 44. 3. Oxnard GR, Klein EA, Seiden MV, *et al.* ESMO Annual Meeting; Sep 27-Oct 1, 2019; Barcelona, Spain. Abstract 5639. 4. Venn, O. *et al.* Cold Spring Harbor Laboratory Meeting: The Biology of Genomes (2019). 5. Chevan, A. and Sutherland, M. The American Statistician 45, 90–96 (1991).

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