Abstract 536
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Breast Cancer Cell-free DNA (cfDNA) Profiles Reflect Underlying Tumor Biology: The Circulating Cell-free Genome Atlas (CCGA) Study

2018 ASCO Annual Meeting
June 1–5, 2018
Chicago, Illinois, USA

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BACKGROUND

- Breast cancer is the second leading cause of death among women in the United States
  - Approximately 40,000 women die each year from breast cancer in the U.S.[1]
- Patients diagnosed with early-stage breast cancer have a significantly better prognosis than patients with advanced disease at presentation[2]
  - Mammography is the main screening paradigm/standard-of-care
  - Mammography does not consistently detect clinically aggressive subtypes
  - Mammography is problematic in women with dense breasts, has a high rate of false positives, and can result in overdiagnosis[2]
- Recent updated guidelines recommended altering the screening paradigm from mammography once a year starting at age 40 to once every two years starting at age 50, but this is not widely adopted and remains somewhat controversial[2]
- New breast cancer screening approaches, complementary to mammography, are needed to detect clinically aggressive subtypes, as well as cancers that present at later stages in unscreened populations
  - Stage shift due to earlier detection may improve outcomes
- Development of blood tests to detect typically unscreened cancers that affect women is highly desirable
- The Circulating Cell-free Genome Atlas (CCGA) study (NCT02889978) is a prospective, multi-center, longitudinal, observational study to support development of a noninvasive assay for multi-cancer detection (Figure 1)
  - >12,000 of 15,000 planned participants enrolled as of May 2018 (70% cancer, 30% non-cancer)
- A preplanned substudy of a WomenOnly Cohort is reported here.

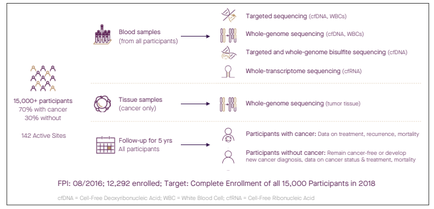


Figure 1. CCGA study design

METHODS

- The primary objective was to develop classifiers of invasive breast cancer versus non-cancer among clinically eligible participants with all assays evaluable (339 pts with invasive breast cancer [IBC] and 437 female control participants).
- Blood was prospectively collected (N=1,733) for plasma cfDNA extraction from 984 participants (pts, 878 with stage information) with newly diagnosed, untreated cancer (20 tumor types, all stages) and 749 participants with no cancer diagnosis (580 controls and 169 assay controls) (Figure 2).
- Three prototype sequencing assays were performed: paired cfDNA and white blood cell (WBC) targeted sequencing (507 genes, 60,000X) for single nucleotide variants/indels, paired cfDNA and WBC whole-genome sequencing (WGS, 30X) for copy number variation, and cfDNA whole-genome bisulfite sequencing (WGBS, 30X) for methylation. WBC sequencing identified the contribution of clonal hematopoiesis (Figure 3).
- Clonal hematopoiesis is described in more detail in oral presentation 12003.
- Sensitivity was estimated at 98% specificity after accounting for clonal hematopoiesis.

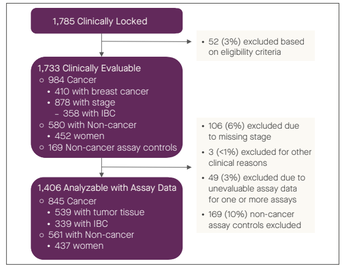


Figure 2. 2,800 participants sampled for first case-control sub-study. The training set (N=1,785) was used to develop classifiers of cancer versus non-cancer. The training portion of this first sub-study included 339 participants with invasive breast cancer (IBC) and 437 female control participants. Analysis followed a pre-specified statistical analysis plan, with clinical and assay data locked and blinded to each other.

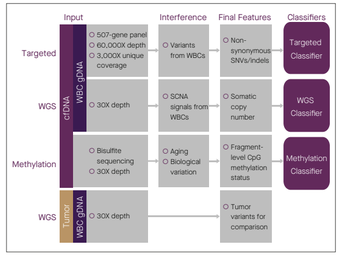


Figure 3. Ultra-Deep Prototype Sequencing Assays Used in CCGA to Comprehensively Characterize the Cancer-Specific cfDNA Signals. For each assay, a classification model using 10-fold cross-validation was developed to discriminate IBC from controls.

RESULTS

Participant demographics

- Participants with breast cancer and control participants were comparable
- The majority (82%) of samples from participants with cancer were stage I/II
- Similarly, the majority (67%) of samples were from participants with HR+ cancer, which was more likely to be screen-detected than in participants with HR- cancer (60% vs 30%)

Table 1. Participant demographics.

	Breast Cancer (n=410)					Non-Cancer
	HR+/HER2-	HR+/HER2+	HR-/HER2+	TNBC	Other/Missing	
Total	232	41	21	53	63	452
Age, Mean ± SD	59 ± 12	54 ± 15	55 ± 13	53 ± 13	60 ± 11	59 ± 12
Race/Ethnicity (%)						
White, Non-Hispanic	209 (90%)	36 (88%)	17 (81%)	38 (72%)	51 (81%)	385 (85%)
African American	10 (4%)	2 (5%)	2 (10%)	10 (19%)	8 (13%)	34 (8%)
Hispanic, Asian, Other	13 (6%)	3 (7%)	2 (10%)	5 (9%)	4 (6%)	33 (7%)
Smoking Status (%)						
Never-smoker	127 (55%)	27 (66%)	15 (71%)	34 (64%)	42 (67%)	269 (60%)
Diagnostic Method, n (%)						
Screening	147 (63%)	16 (39%)	8 (38%)	14 (26%)	52 (83%)	-
Clinical Presentation	85 (37%)	25 (61%)	13 (62%)	39 (74%)	11 (17%)	-
Clinical N Staging, n (%)						
Stage 0	1 (<1%)	0 (0%)	0 (0%)	0 (0%)	48 (76%)	
Stage I	134 (58%)	11 (27%)	5 (24%)	13 (25%)	5 (8%)	-
Stage II	75 (32%)	17 (42%)	7 (33%)	24 (45%)	4 (6%)	-
Stage III	16 (7%)	12 (29%)	5 (24%)	13 (25%)	3 (5%)	-
Stage IV	4 (2%)	0 (0%)	2 (10%)	0 (0%)	0 (0%)	-
Noninformative/ Missing	2 (1%)	1 (2%)	2 (10%)	3 (6%)	3 (5%)	-

Specificity

- Majority of cfDNA variants were WBC-matched (clonal hematopoiesis)
  - In women with invasive breast cancer (357 with targeted panel results), 2,397 nonsynonymous SNVs were called, of which 2,060 (86%) matched WBC
  - In women enrolled in the non-cancer cohort (448 with targeted panel results), 2,871 nonsynonymous SNVs were called, of which 2,799 (98%) matched WBC
  - These represent potential false-positive signal that must be accounted for in cfDNA-based assays
- After accounting for clonal hematopoiesis, 4 of 452 (<1%) women enrolled in the non-cancer cohort had a cancer-like signal

Sensitivity

- Sensitivity was higher in clinically aggressive breast cancers (e.g., triple-negative breast cancer [TNBC], HR-/HER2+ breast cancer, Figure 4) versus hormone-receptor (HR)-positive cancers,
- Sensitivity was also higher in breast cancers detected via clinical presentation vs detected via screening (Figure 5)

Figure 4A. Sensitivity was higher for TNBC vs HR-/HER2+ vs HR+/HER2+ vs HR+/HER2- breast cancer. Includes participants returning a result across all three assays.

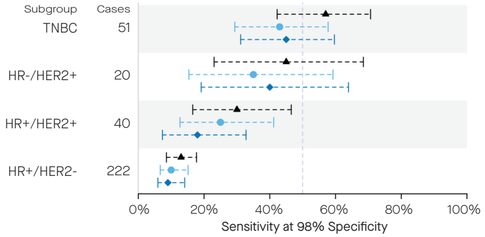


Figure 4B. HR-/HER2+ breast cancer was detected at early stages. Includes participants returning a result across all three assays.

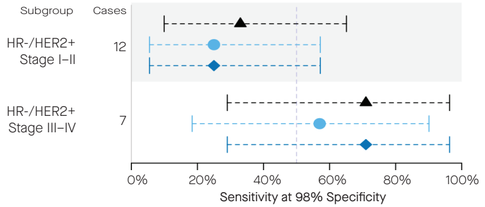


Figure 4C. TNBC was detected at early stages. Includes participants returning a result across all three assays.

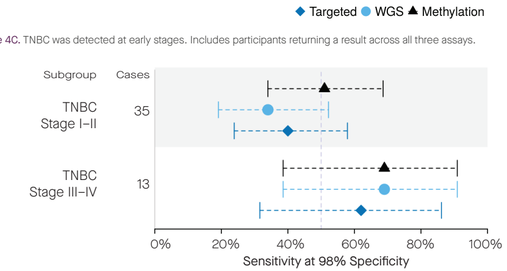
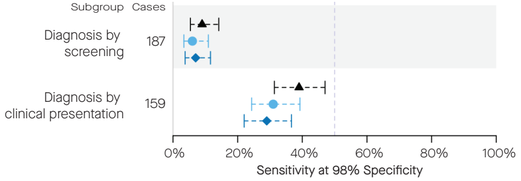


Figure 5. Sensitivity was higher for breast cancers detected via clinical presentation vs screen-detected breast cancers. Includes participants returning a result across all three assays.



CONCLUSIONS

- Breast cancers with detectable cfDNA signals at time of diagnosis included clinically aggressive subtypes (e.g., TNBC) and breast cancers detected by clinical presentation.
  - Earlier identification of clinically aggressive subtypes that are more likely to be missed by screening mammography could improve outcomes
- A comprehensive set of prototype sequencing assays (copy number, methylation, SNVs/indels) generated large-scale, high-quality datasets that allowed discovery of breast cancer-specific features.
- Data and analysis from this first women-only pre-planned substudy demonstrated the promise of using cfDNA-based assays to develop an early cancer detection test.
  - Suggests that high specificity is possible achieve when accounting for clonal hematopoiesis
- Further assay and clinical development in CCGA (NCT02889978) and in an intended-use population is ongoing (NCT03085888).

References

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2. Pace, L. E. & Keating, N. L. A systematic assessment of benefits and risks to guide breast cancer screening decisions. *JAMA* 311, 1327-1335, doi:10.1001/jama.2014.1398 (2014).

Acknowledgements: Medical writing assistance provided by Megan P. Hall, PhD (GRAIL, Inc. Menlo Park, CA). Formatting assistance provided by PosterDocs (Oakland, CA).

Disclosures: T.M., A.A., E.H., J.F.B., D.F., S.G., A.J., K.K., L.S., A.V., O.V., N.Z., R.T.W., and A.-R.H., are or were employees of GRAIL, Inc. with options to hold stock in the company. RTW is an employee of WuXi NextCODE with options to hold stock in the company. E.H. holds stock in ThermoFisher. A.J. and K.K. hold stock in Illumina. C.C. is an advisory board member for GRAIL, Inc. E.A.K. is a consultant for GRAIL, Inc., Genomic Health, and GenomeDx. J.B. is an advisory board member for Juno Therapeutics, Northern Biologics, Varian Medical Systems, Foghorn Therapeutics, Tango Therapeutics, PMV Pharmaceuticals, and GRAIL; is a former board member for GRAIL; is a consultant for Genentech and Novartis; holds stock in Aura Biosciences and GRAIL; and has research funding from Puma Biotechnologies.