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The Circulating Cell-free Genome Atlas (CCGA) Study: Follow-up (F/U) on Non-cancer Participants with Cancer-like Cell-free DNA Signals

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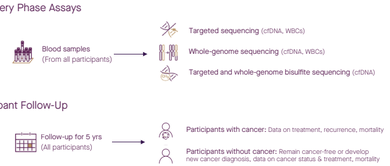
BACKGROUND

- A noninvasive cell-free DNA (cfDNA)-based cancer detection assay offers the hope of a blood test that might enable early detection, which remains an unmet need.
  - Early cancer detection has the potential for better outcomes by reducing cancer morbidity and mortality, particularly for those without recommended screening tests (eg, some gynecologic cancers).
- Circulating Cell-free Genome Atlas (CCGA) (NCT02889978) is a prospective, multi-center, longitudinal, case-control study evaluating models for discriminating cancer versus non-cancer.
  - Previous results from a prespecified case-control substudy[1] reported multi-cancer detection across stages at high specificity.
- To further understand the nature of assay signals, this *post hoc* analysis examined non-cancer participants with outlier signals beyond the non-cancer classification distribution.

METHODS

- The CCGA study enrolled participants without a cancer diagnosis (non-cancer) and those with a newly diagnosed treatment-naive cancer (cancer) from 142 research sites.
- In a prespecified case-control substudy from the CCGA study, clinically evaluable samples (N=2,508) were divided into training (n=1,564: 580 non-cancer, 984 cancer) and test (n=944: 368 non-cancer, 576 cancer) sets.
- Classification performance (cancer/non-cancer) was assessed via 3 prototype assays: whole-genome bisulfite (WGBS), whole-genome (WGS), and targeted (507 gene) sequencing (Figure 1A).
  - Features that resulted in a cancer classification call included (A) WGS: presence of chromosomal amplifications and deletions; (B) WGBS: multiple unusually methylated or unmethylated locations within molecules across the genome; and (C) targeted: sufficient presence of somatic non-synonymous mutations (eg, TP53).
- Follow-up in accordance with study protocol is being conducted for all participants (to date: 85% with 10 months and 25% with >22 months follow-up) (Figure 1B).

Figure 1. Schematic of CCGA Study Discovery Phase and Participant Follow-up



CCGA is a prospective, multi-center, longitudinal, observational study comprised of a discovery phase that includes training and validation for development of a multi-cancer detection test (A). All participants have five-year follow-up wherein outcomes are collected (no additional blood draws) (B).
cfDNA, cell-free deoxyribonucleic acid; WBC, white blood cell.

- In this *post hoc* analysis, participants that were enrolled in the CCGA non-cancer arm were defined as outliers if they had a classification result suggesting cancer via either (a) ≥2 assay classification results, or (b) ≥1 assay classification result in addition to known cancer drivers.
- During follow-up, some participants in the non-cancer group were diagnosed with cancer via standard of care; these events are referred to as occult cancers.

RESULTS

- In this *post hoc* analysis, 8 non-cancer participants were identified with an outlier cancer-defining feature (non-cancer outlier [NCO]) among training and test sets.
- During follow-up, 3 of these NCO participants were subsequently diagnosed with cancer; participant characteristics and prototype assay classification features are summarized in Table 1.

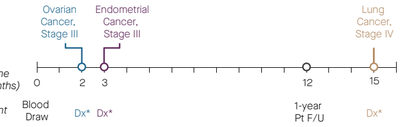
Table 1. Outlier Non-cancer Participant Characteristics

Non-cancer Participant	Participant Characteristics			Classification Features			Occult Diagnoses			Clinical Follow-Up
	Age	Sex	Race	WGBS	WGS	Targeted	Cancer Type	Stage	Time from Enrollment to Dx	
1	55	F	White/ Non-Hispanic	High	High	High (TP53 R282W[a])	Ovarian	III	2 mo	Alive at 9 months after enrollment
2	87	F	White/ Non-Hispanic	NR[b]	High	High (TP53 L319X[a]; TP53 R175H[a])	Endometrial Clear Cell Carcinoma	III	3 mo	Alive at 11 months after enrollment
3	63	M	White/ Non-Hispanic	High	Not High	High (TP53 G154W[a])	Lung	IV	15 mo	Alive at 15 months after enrollment

[a]Non-white blood cell (WBC)-matched; WBC sequencing was used to remove non-cancer feature noise (eg, a source of TP53 mutations).[2] [b]No result. TP53, tumor protein 53; mo, months.

- Two of those three NCO participants have been diagnosed with a gynecologic malignancy during the follow-up period to-date:
  - 1 stage III clear cell endometrial carcinoma 3 months post-enrollment;
  - 1 stage III ovarian cancer 2 months post-enrollment (Figure 2).
  - The third NCO participant was diagnosed with stage IV lung cancer 15 months post-enrollment (Figure 2).
- The sensitivity (reported at 98% specificity; WGBS) for detecting these cancer types in the training and test sets of a prespecified analysis was: uterine/endometrial: 11% (n=27) and 22% (n=9); ovarian: 82% (n=17) and 71% (n=7); lung: 63% (n=118) and 70% (n=46), respectively.

Figure 2. Diagnosis of Occult Cancer in Non-cancer Participants Post-enrollment



*Post enrollment; diagnosed by standard of care.

Schematic depicting the time after enrollment that participants enrolled in the cancer cohort were diagnosed with cancer via standard of care. Dx, diagnosis; Pt, participant; F/U, follow-up.

References: 1.Liu MC, Klein EA, Hubbell E, et al. Plasma cell-free DNA (cfDNA) assays for early multi-cancer detection: the Circulating Cell-Free Genome Atlas (CCGA) study. *Annals of Oncology.* 29(suppl_8):viii14-viii57. doi:10.1093/annonc/mdy269; 2. Yuebi Hu, Bryan C. Ulrich, Julianna Supplee, et al. False-Positive Plasma Genotyping Due to Clonal Hematopoiesis. *Clin Cancer Res.* 2018 Sep 15;24(18):4437-4443; 3. James T. Robinson, Helga Thorvaldsdóttir, Wendy Winckler, Mitchell Guttman, Eric S. Lander, Gad Getz, Jill P. Mesirov. Integrative Genomics Viewer. *Nature Biotechnology.* 29, 24–26 (2011); 4. Helga Thorvaldsdóttir, James T. Robinson, Jill P. Mesirov. Integrative Genomics Viewer (IGV): high-performance genomics data visualization and exploration. *Briefings in Bioinformatics.* 14, 178-192 (2013); 5. James T. Robinson, Helga Thorvaldsdóttir, Aaron M. Wenger, Ahmet Zehir, Jill P. Mesirov. Variant Review with the Integrative Genomics Viewer (IGV). *Cancer Research.* 77(21) 31-34 (2017).

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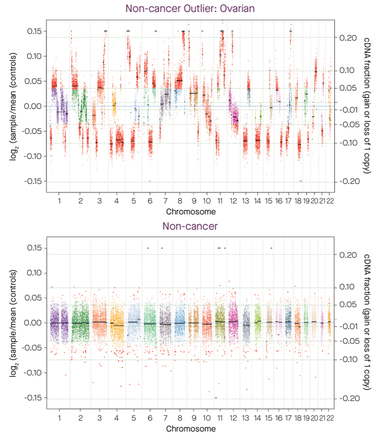
Ovarian Cancer

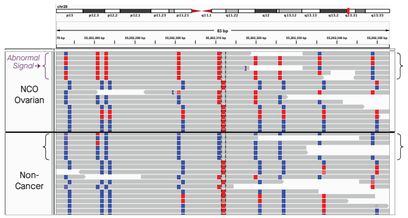
- WGS assay results for the NCO-ovarian cancer participant were compared to an age- and sex-matched non-cancer participant in Figure 3A; chromosomal aberrations were observed for the NCO-ovarian participant, but not for the non-cancer participant.
- A cancer-defining, unusual hypermethylated signature classified by the WGBS assay was also detected in this individual (Figure 3B).

Figure 3. Classification Features Comparing Non-cancer Outlier (Ovarian) vs Non-cancer

A. Genome-wide Somatic Copy Number Alterations (WGS)



B. Local Fragment-level Methylation (WGBS)

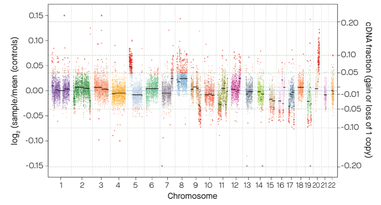


(A) Copy number analyses (WGS assay) for the NCO-ovarian participant (top) and an age- and sex-matched non-cancer participant (bottom). Shown here are normalized log-ratios of amplification and deletions beyond expected depth at each 100kb bin along the genome. Each color reflects an individual chromosome; bins with log-ratios outside 3 SD are colored red. (B) Integrative Genomics Viewer (IGV)3-5 plots depicting hyper- and hypomethylated locations on chromosome 20 (WGBS assay) in the same participants. Blue depicts unmethylated CpG locations within a cfDNA molecule, red depicts methylated CpG locations within a cfDNA molecule; each continuous horizontal stripe is one read. Arrow points to sequencing reads coming from an unusually hypermethylated molecule.

Endometrial Cancer

- Aberrant copy numbers were observed across multiple chromosomes in the NCO- endometrial participant who was diagnosed 3 months post-enrollment (Figure 4); this cancer-defining feature was somewhat less pronounced than the feature from the NCO-ovarian cancer participant who was diagnosed with cancer 2 months post-enrollment (Figure 3A).
- This participant did not return a result on the WGBS assay (Table 1). As such, no WGBS result is shown for this participant.

Figure 4. CNA Plot for Non-cancer Outlier: Endometrial



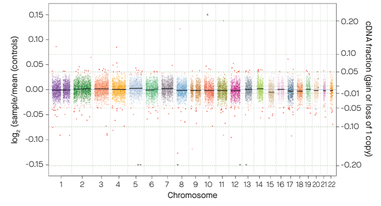
NCO-endometrial participant copy number analysis (WGS) classification features. Shown here are normalized log-ratios from expected depth at each 100kb bin along the genome. Each color reflects an individual chromosome; bins with log-ratios outside 3 SD are colored red.

Lung Cancer

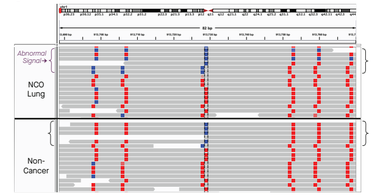
- For the NCO-lung participant, no obvious aberrant chromosome copy numbers were observed (Figure 5A).
- A cancer-defining, unusual hypomethylated signature was detected in this individual (Figure 5B).

Figure 5. Classification Features Comparing NCO-Lung vs Non-cancer

A. Genome-wide Somatic Copy Number Alterations (WGS)



B. Local Fragment-level Methylation (WGBS)

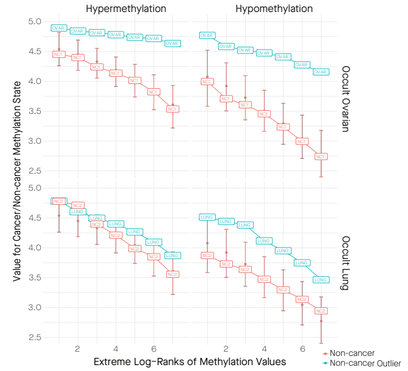


(A) Copy number analysis (WGS assay) of NCO-lung participant. Shown here are normalized log-ratios of amplification and deletions beyond expected depth at each 100kb bin along the genome. Each color reflects an individual chromosome; bins with log-ratios outside 3 SD are colored red. (B) IGV plots depicting hyper- and hypomethylated locations on chromosome 1 (WGBS assay) in the same NCO-lung participant (top) compared to features in an age- and sex-matched non-cancer participant (bottom). Blue depicts unmethylated CpG locations within a cfDNA molecule, red depicts methylated CpG locations within a cfDNA molecule; each continuous horizontal stripe is one read. Arrow points to sequencing reads coming from an unusually hypomethylated molecule.

WGBS Cancer-defining Features

- WGBS outperformed targeted sequencing and WGS prototype assays for cancer detection (see poster 3049), therefore, the reasons for the cancer-defining call among the NCO-ovarian and -lung participants – both of whom returned an outlier result on WGBS – were closely examined.
- Informative methylation values for NCO-ovarian and -lung participants were clearly separated from those for the age- and sex-matched non-cancer individuals (and the population of non-cancer individuals in general) (Figure 6), providing evidence that these NCO participants had detectable cancer features at the time of the blood draw.
- The most informative cancer-defining hypermethylated molecules for NCO-ovarian and NCO-lung are depicted in the top-left boxes in Figure 6A and 6B, respectively (which also represent the molecules for which local fragment-length methylation is depicted in Figures 3B and 5B, respectively).

Figure 6. WGBS Features for Non-cancer Outlier (Ovarian and Lung) vs Non-cancer Participants



WGBS features for the NCO ovarian (top), NCO lung cancer (bottom), and age- and sex-matched non-cancer (NC1 and NC2, respectively) participants. Boxes represent the set of molecules with the most informative cancer/non-cancer values for both hyper- and hypomethylation; each box represents one observed molecule. Red depicts the distribution of non-cancer values; blue depicts the cancer-defining values for the NCO-ovarian and -lung participants. Higher values indicate greater cancer-defining methylation state for a molecule (Y-axis). Higher ranked molecules indicate more accumulation of cancer-like behavior across the genome in a person (X-axis). The combination of these two factors produced the classification prediction for each participant.

CONCLUSIONS

- In this *post hoc* analysis, outlier cancer-defining features anticipated a clinical diagnosis of cancer in some participants enrolled as non-cancer (without a diagnosis of cancer) as early as 15 months prior to the actual diagnosis.
  - These cases provide evidence that tumor-derived cfDNA signals anticipated a cancer diagnosis, which is what will be required of early detection tests (ie, to find cancers earlier than they would otherwise present) in order to improve outcomes.
- Given that apparent false positive signals in clinical trials may in fact be occult cancers highlights the importance of having long-term follow-up to confirm non-cancer status for the purposes of determining false positives.
- Furthermore, the detection of occult cancers in the CCGA non-cancer group during follow-up suggests that even higher specificity levels can be achieved.
  - Poster 3049 reports performance at 99% specificity using an optimized targeted methylation approach in a cohort of ~3,100 additional samples from the CCGA study.
- Taken together, these data suggest that a methylation-based approach for detecting cancer using cfDNA may have high performance detecting a variety of gynecological and other cancers.