Abstract 12021
#134

Development of a comprehensive cell-free DNA (cfDNA) assay for early detection of multiple tumor types: The Circulating Cell-free Genome Atlas (CCGA) study

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INTRODUCTION

- The Circulating Cell-free Genome Atlas (CCGA) study (NCT02889978) is a prospective, multicenter, longitudinal, observational study for the development of a noninvasive assay for cancer detection (Figure 1)
  - >12,000 of 15,000 planned participants enrolled (~70% cancer, 30% non-cancer)
- A preplanned case-control substudy is reported here

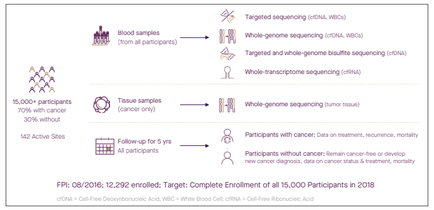


Figure 1. CCGA study design

BACKGROUND

- The majority of cancers are detected at advanced stages when treatment burden is high and cure rates are low[1], thus early diagnosis is likely to improve survival and improve quality of life[2]
- The use of circulating cell-free DNA (cfDNA) for early cancer detection would require very high specificity in a screening population to avoid false positives and thus unnecessary workups and follow-up testing
- Available cfDNA-based tests for cancer are almost exclusively focused on detecting later-stage tumors, when ctDNA levels are high
- Additionally, there are few studies of people without cancer to define specificity in the intended use population[3,4]
- Tumor fraction in ctDNA is lower in early stage cancers versus later-stage cancers, and can be low even in some metastatic disease[5,6]
- High levels of technical and biological specificity near the molecular limit of detection will be required to detect low-prevalence cfDNA shed from cancer across large genomic regions with confidence
  - This is especially true for a test aimed at detecting multiple cancers from a single blood draw

METHODS

- Blood was prospectively collected (N=1,785) 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 (controls) for plasma cfDNA extraction (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 (CH) (Figure 3).
- Informative features were extracted for each assay: support for somatic variants in genes, variation in read depth, and unusually methylated fragments, respectively. ML classifiers were independently trained on each feature type to estimate probability of cancer. For each assay, a classification model using 10-fold cross-validation was developed to avoid over-fitting to the training data and to discriminate cancer from controls.
- Sensitivity was estimated at 98% specificity after accounting for clonal hematopoiesis.

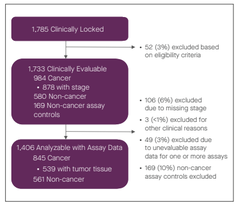


Figure 2. 2,800 participants sampled for first case-control sub-study. The training set (N=1,785, depicted here) was used to develop classifiers of cancer versus non-cancer. Analysis followed a pre-specified statistical analysis plan, with clinical and assay data blinded to each other prior to lock.

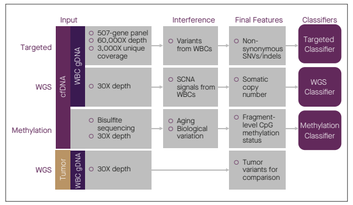


Figure 3. Ultra-Deep Prototype Sequencing Assays Used in CCGA to Comprehensively Characterize the Cancer-Specific cfDNA Signals.

RESULTS

Participant demographics

- Overall, the cancer and non-cancer groups were comparable with respect to age, sex, race/ethnicity, and BMI (not shown) (Table 1)
  - Participants with lung cancer tended to be older, and more were ever-smokers
- Stage distribution was consistent with the United States cancer incidence (SEER reference) (Table 2)

Table 1. Participant demographics.

	Cancer					Non-Cancer
	Breast	Lung	Prostate	Colorectal	Other*	
Total	410	127	74	51	322	580
Age, Mean ± SD	58 ± 13	67 ± 9	64 ± 8	60 ± 11	62 ± 12	60 ± 13
Sex (%)						
Female	100%	54%	0%	53%	59%	78%
Race/Ethnicity (%)						
White, Non-Hispanic	86%	88%	82%	92%	85%	84%
African American	8%	5%	12%	<1%	6%	8%
Hispanic, Asian, Other	6%	7%	6%	7%	9%	8%
Smoking Status (%)						
Never-smoker	60%	15%	50%	63%	47%	57%

Table 2. Stage distribution was consistent with SEER-observed United States distribution (https://seer.cancer.gov)

	Breast	Lung	Prostate	Colorectal	Other*
Total (n)	410	127	74	51	322
Method of Dx (%)					
Dx by Screening	58%	18%	91%	29%	4%
Overall Clinical Stage (%)					
0**	12%	<1%	<1%	0%	2%
I	41%	18%	23%	8%	27%
II	31%	11%	66%	16%	16%
III	12%	31%	4%	31%	18%
IV	2%	37%	5%	37%	27%
Non-Informative***	2%	2%	1%	8%	10%

*Other includes anorectal, bladder, cervical, esophageal, gastric, head & neck, hepatobiliary, lymphoma, melanoma, multiple myeloma, ovarian, pancreas, renal, thyroid, uterine, unknown primary/other. **DCIS/CIS. ***Staging information not available.

Specificity

- Non-tumor WBC-matched cfDNA non-synonymous somatic variants (SNVs/indels) accounted for, on average:
  - 3,633 of 3,724 (98%) of all variants in non-cancer group
  - 5,996 of 8,501 (71%) in cancer group
    - Clonal hematopoiesis of indeterminate potential (CHIP) is being presented in more detail in oral presentation 12003
- Only 5 of 580 (<1%) samples from participants enrolled as non-cancer had a cancer-like signal across multiple assays
  - With WGS, 8 of 575 non-cancer samples had somatic copy number alterations in cfDNA
    - 4 were WBC-matched
    - 4 were non-WBC-matched (<1% of all non-cancer samples)
  - Two non-cancer participants with a cancer-like signal were subsequently diagnosed with cancer (ovarian, diagnosed two months after blood draw; endometrial, diagnosed three months after blood draw)
    - This suggests that the few other cancer-like signals may also be due to undiagnosed cancer

Sensitivity

- The assays were able to detect cancers with high mortality at early stages
- Cancers with the highest signal (>50% sensitivity overall) included anorectal, triple-negative breast cancer (see poster 536), colorectal, esophageal, head & neck, hepatobiliary, lung, lymphoma, ovarian, and pancreas (Figure 5)
- There were high biological signal (>50%) in cancers with high mortality (>50%, SEER reference) for which there are no standard-of-care screening paradigms or for which screening uptake is low[7] (Figure 6)
  - These include esophageal, hepatobiliary, lung, ovarian, and pancreatic cancers
  - Lung cancer and breast cancer are reported in more detail in late-breaking oral presentation LBA8501 and poster 536, respectively

Figure 5. Sensitivity was reported at 98% specificity for stage I-III and stage IV, separately, across tumor types.

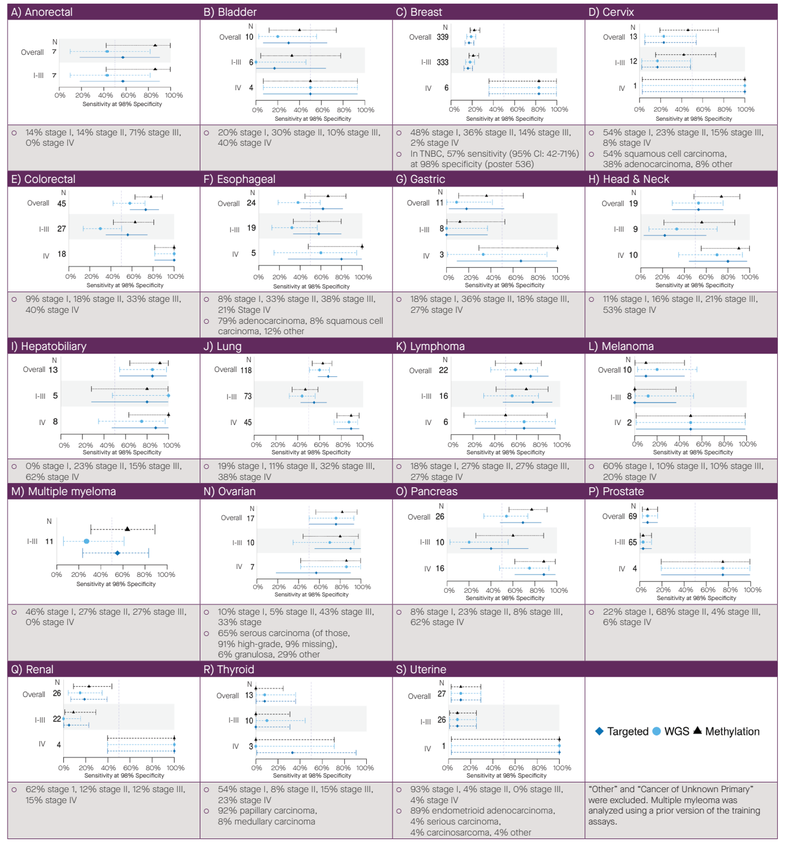
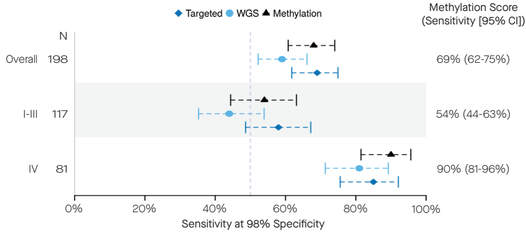


Figure 6. High biological signal (>50% sensitivity overall on any assay) in typically unscreened cancers with high mortality (>50%, SEER). Sensitivity for Stage I-III and Stage IV at 98% specificity is reported in aggregate for esophageal, hepatobiliary, lung, ovarian, and pancreatic cancers.



CONCLUSIONS

- The CCGA study is a prospective, longitudinal cohort study of a representative and generalizable cohort with >12,000 of a planned 15,000 cancer and non-cancer participants enrolled
- A comprehensive set of sequencing assays (copy number, methylation, SNVs/indels) performed on peripheral blood generated large-scale, high-quality datasets that allowed discovery of cancer-specific features, and facilitated the development of prototype classifiers
- Data and analysis from the first training set (~1700 individuals) demonstrate:
  - WBC derived copy number and SNVs are a significant source of signal that must be accounted for to obtain high specificity
  - Some cancer-like signal in non-cancer participants was due to undiagnosed cancer
  - Signals across assay features are highly correlated
  - Strong biological signal in unscreened cancers with high mortality
  - Signal observed in early-stage cancer across several tumor types
- This prototype cfDNA-based blood test detected multiple cancers at various stages with high specificity
  - Supports that this approach is promising as a multi-cancer screening test, including for lethal unscreened cancers where stage shift can impact mortality
- Further assay and clinical development of a multi-cancer cfDNA test in an asymptomatic population is ongoing (NCT03085888)
- Due to limitations in assessing accuracy using the same dataset in which the classifiers are developed, classification models will be evaluated in the independent test set from this substudy

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