Development of Plasma Cell-Free DNA Assays for Early Cancer Detection: First Insights from the Circulating Cell-Free Genome Atlas (CCGA) Study

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Disclosures

Alexander M. Aravanis, Tara Maddala, Earl Hubbell Oliver Venn, Arash Jamshidi, Ling Shen, Hamed Amini, John F. Beausang, Craig Betts, Daniel Civello, Konstantin Davydov, Saniya Fayzullina, Darya Filippova, Sante Gnerre, Samuel Gross, Chenlu Hou, Roger Jiang, Byoungsok Jung, Kathryn Kurtzman, Collin Melton, Shivani Nautiyal, Jonathan Newman, Joshua Newman, Cosmos Nicolaou, Richard Rava, Onur Sakarya, Ravi Vijaya Satya, Seyedmehdi Shojaee, Kristan Steffen, Anton Valouev, Hui Xu, Jeanne Yue, Nan Zhang, Richard Williams, and Anne-Renee Hartman are current or former GRAIL employees with options to hold stock in the company. Hamed Amini, Chenlu Hou, Arash Jamshidi, Byoungsok Jung, Kathryn Kurtzman, Shivani Nautiyal, Onur Sakarya, and Hui Xi are shareholders in Illumina. Geoffrey R. Oxnard is an advisory board member and consultant for Inivata, an honorarium recipient from Guardant Health, Sysmex, and BioRad, and a consultant for DropWorks, AstraZeneca, and GRAIL. Jose Baselga 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; receives research support from Puma Biotechnology; is a consultant for Genentech and Novartis; and is shareholder in GRAIL and Aura Biosciences. Michael V. Seiden is an employee of and shareholder in McKesson Corporation. Christina Curtis is an advisory board member for GRAIL. Eric Klein is a consultant for GRAIL, Genomic Health, and GenomeDx. The remaining authors have no conflicts to declare.

CCGA Is A Prospective Longitudinal Cohort Study

15,000+ participants
70% with cancer
30% without

141 Active Sites

Blood samples
(from all participants)

- **Targeted sequencing** cfDNA, WBCs
- **Whole-genome sequencing** cfDNA, WBCs
- **Targeted & whole-genome bisulfite sequencing** cfDNA
- **Whole transcriptome sequencing** cfRNA

Tissue samples
(cancer only)

- **Whole Genome Sequencing** of tumor tissue

Follow-up for 5 yrs
Vital status & cancer status

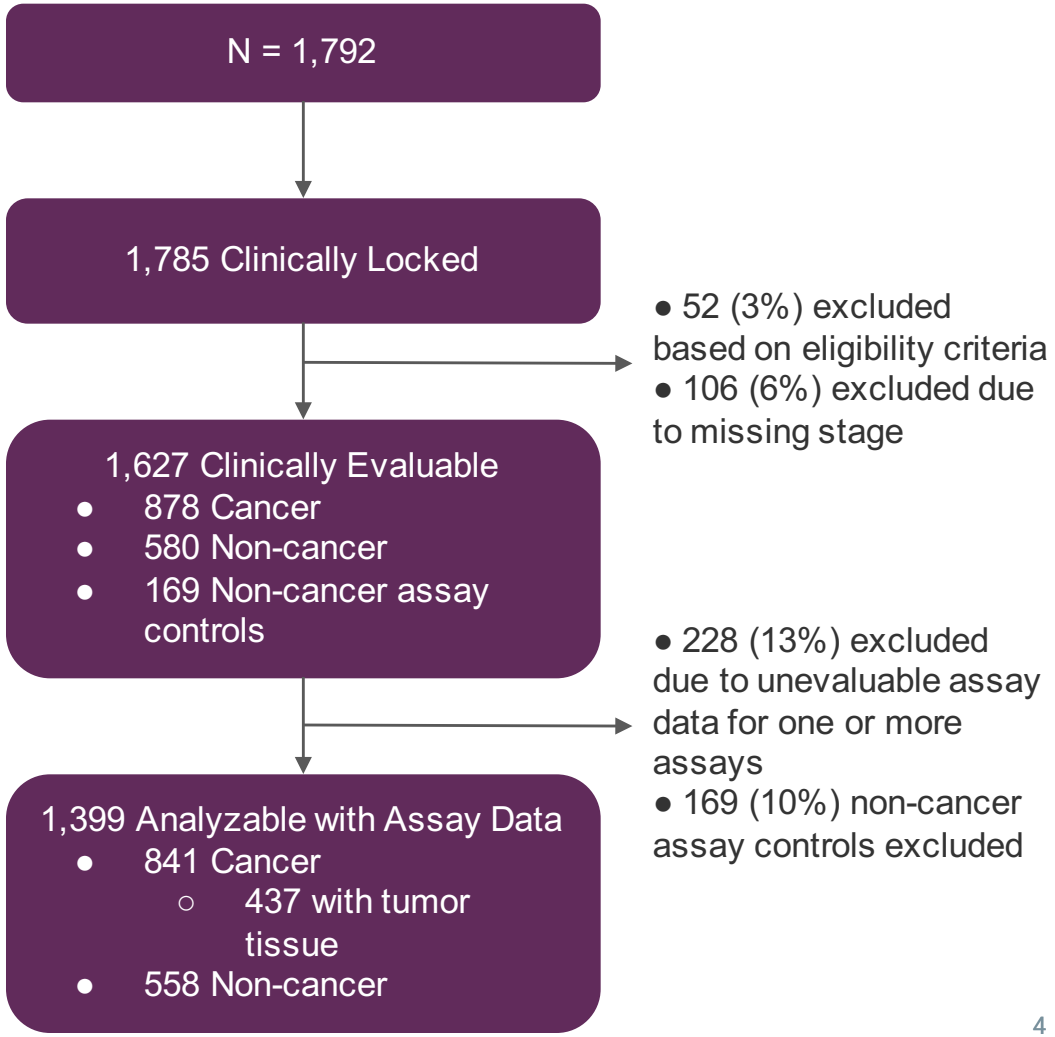
- **Participants with cancer:** Data on treatment, recurrence, mortality
- **Participants without cancer:** Data on cancer diagnosis + treatment, recurrence, mortality; or remain cancer-free

FPI: 08/2016; 11,648 enrolled; Target: Complete Enrollment of all 15,000 Participants in 2018

cfDNA = Cell-Free Deoxyribonucleic Acid; WBC = White Blood Cell; cfRNA = Cell-Free Ribonucleic Acid

First CCGA Training Set

- 2,800 participants sampled for first case-control sub-study
- Training set (N=1,792) to **develop classifiers of cancer vs. non-cancer** using rigorous cross-validation
- Test set (N=1,008) to test the classifiers
- Analysis followed a **pre-specified statistical analysis plan** with clinical and assay data locked and blinded to each other



Comparable Cancer and Non-Cancer Groups

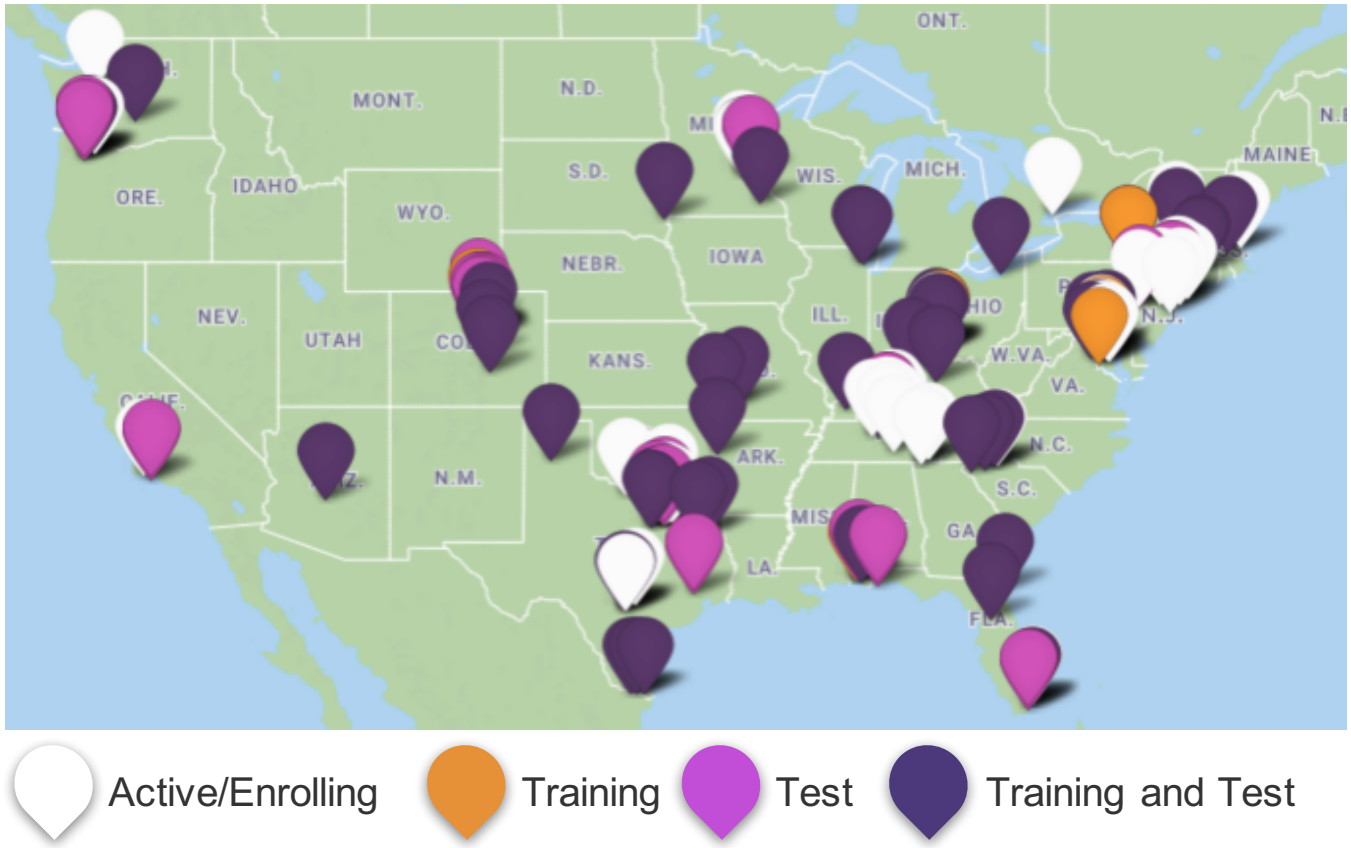
- **Cancer and non-cancer groups were comparable with respect to age, race, sex, and body mass index (not shown).**
- Participants with lung cancer tended to be older and a higher proportion were ever-smokers.

	Cancer					Non-Cancer
	Breast	**Lung**	**Prostate**	**Colorectal**	**Other***	
Total	410	127	74	52	321	580
Age, Mean ± SD	58 ± 13	67 ± 9	64 ± 8	60 ± 11	62 ± 12	60 ± 13
Sex (%)						
Female	100%	54%	0%	54%	9%	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%	62%	47%	57%

*Other includes renal, uterine, pancreas, esophageal, lymphoma, head & neck, ovarian, hepatobiliary, melanoma, cervical, multiple myeloma, leukemia, thyroid, bladder, gastric, anorectal, unknown primary/other.

CCGA Has Geographically Diverse Enrollment Representative of United States Population

141 active sites representing 24 states in the U.S. and one site in Canada

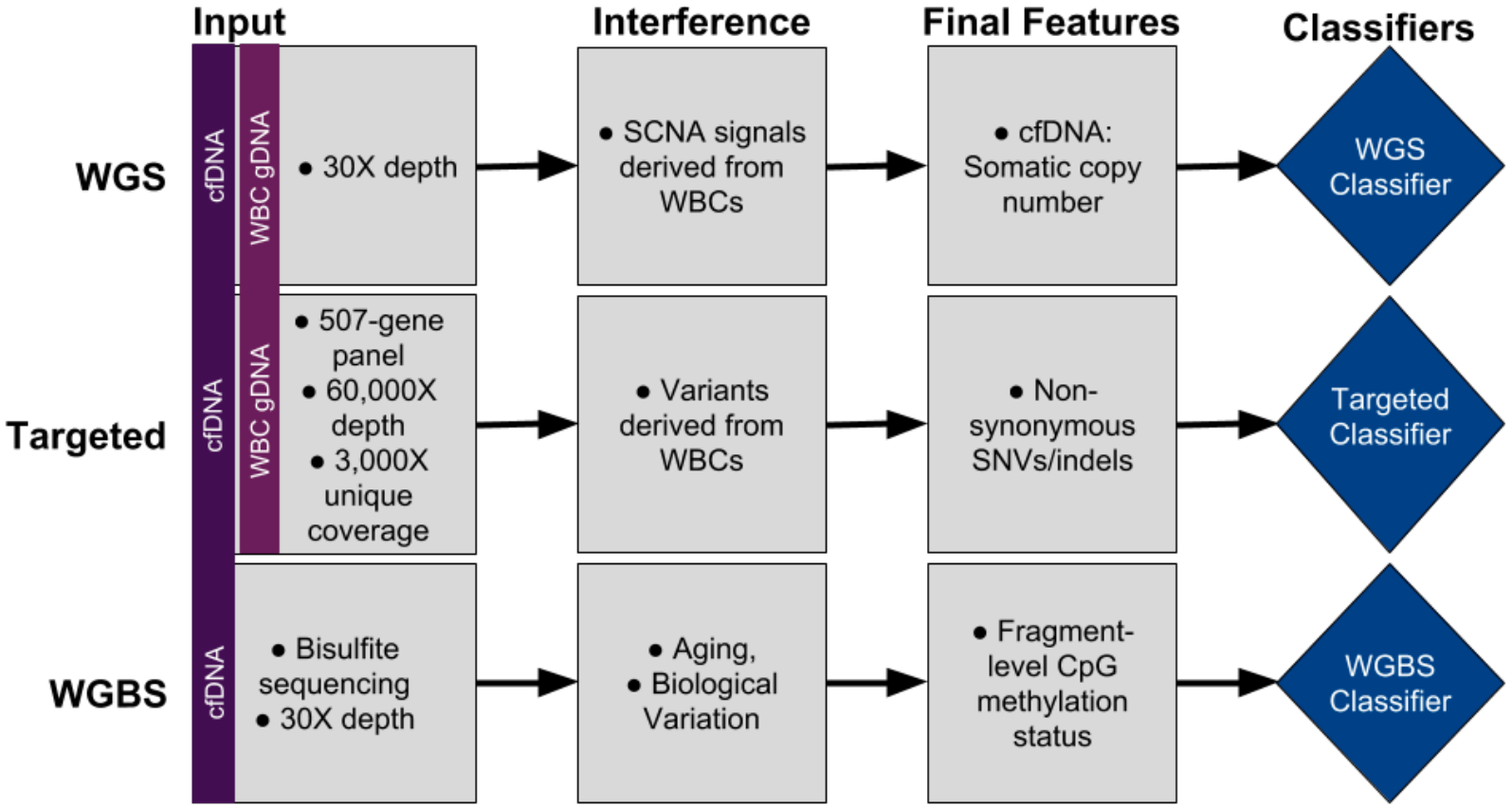


Stage Distribution Consistent with United States Cancer Incidence[1]

	Breast	Lung	Prostate	Colorectal	Other*
Total (n)	410	127	74	52	321
Method of Dx (%)					
Dx by Screening	58%	18%	91%	31%	3%
Overall Clinical Stage (%)					
0**	12%	<1%	<1%	2%	2%
I	41%	18%	23%	10%	27%
II	31%	10%	66%	15%	16%
III	12%	32%	4%	31%	18%
IV	2%	37%	5%	36%	27%
Non-Informative***	2%	2%	1%	6%	10%

*Other includes renal, uterine, pancreas, esophageal, lymphoma, head & neck, ovarian, hepatobiliary, melanoma, cervical, multiple myeloma, leukemia, thyroid, bladder, gastric, anorectal, unknown primary/other. **DCIS ***Staging information not available.
[1]https://seer.cancer.gov

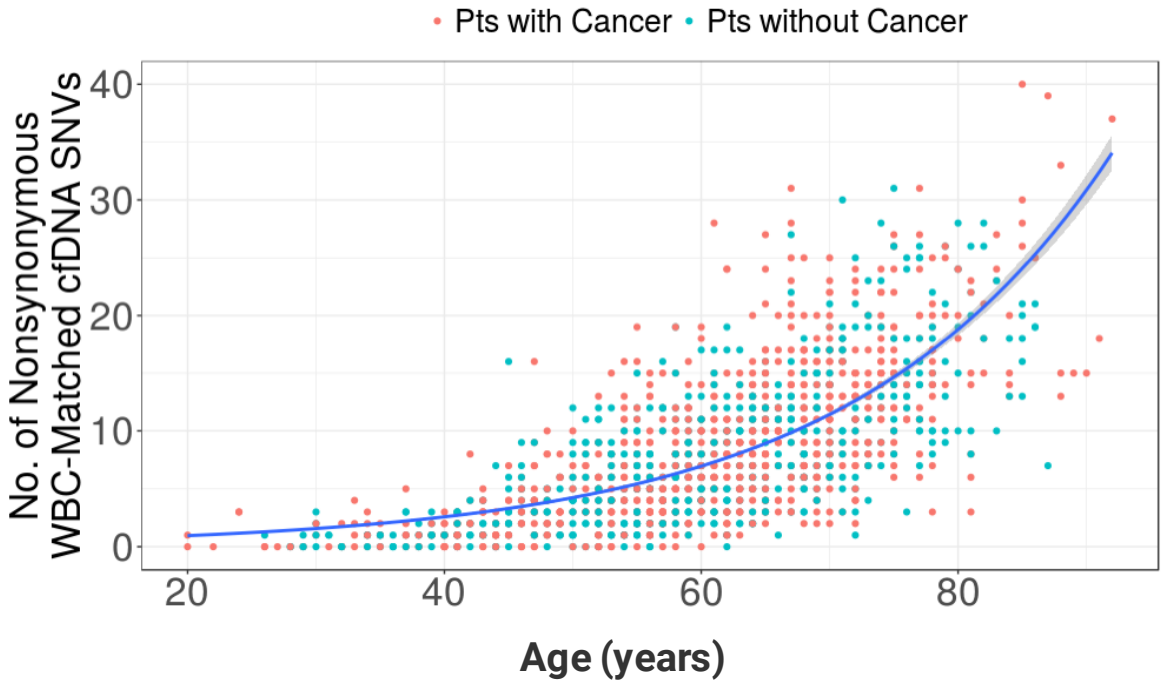
Prototype Sequencing Assays Generate Signals Used by Classifiers for Cancer vs Non-Cancer



- **All major somatic and epigenetic cfDNA features characterized**

Majority of cfDNA Variants Are WBC-Matched Clonal Hematopoiesis

- Non-tumor WBC-matched cfDNA somatic variants (SNVs/indels) accounted for, on average:
  - **78% (SD: 10%) of all variants in non-cancer group**
  - **66% (SD: 14%) in cancer group**
- Number of WBC variants is positively associated with age in cancer and non-cancer groups

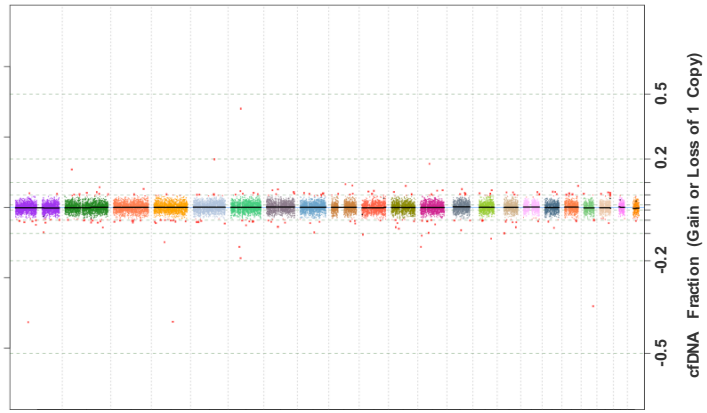


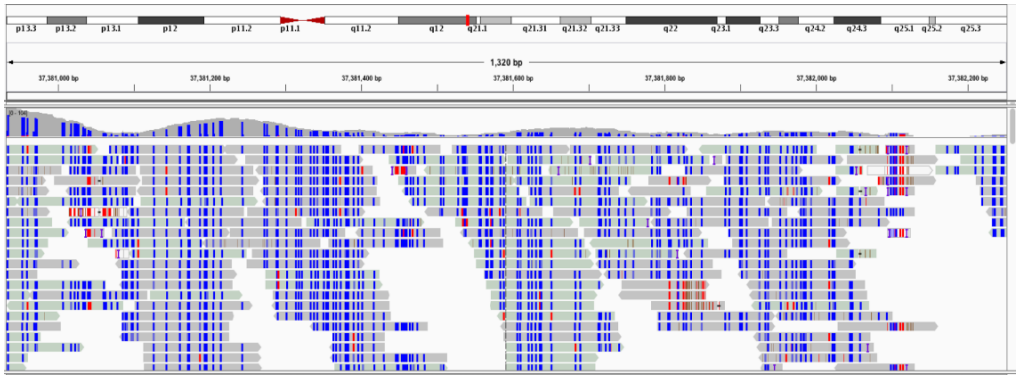
Whole Genome and Whole Genome Methylation Assays Detect Cancer-Specific Features

Copy Number

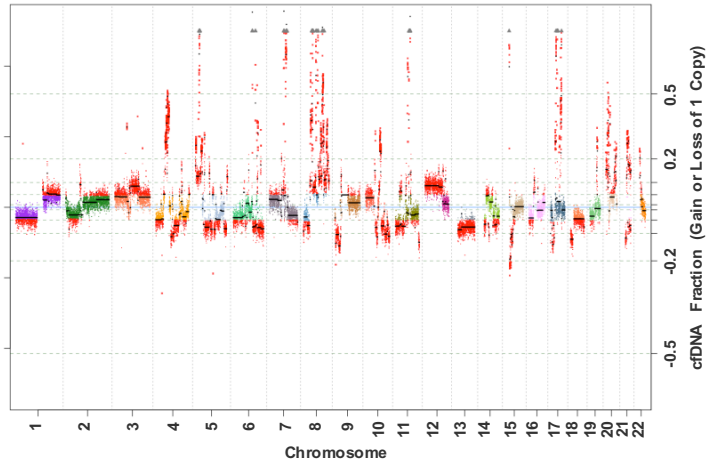
Methylation

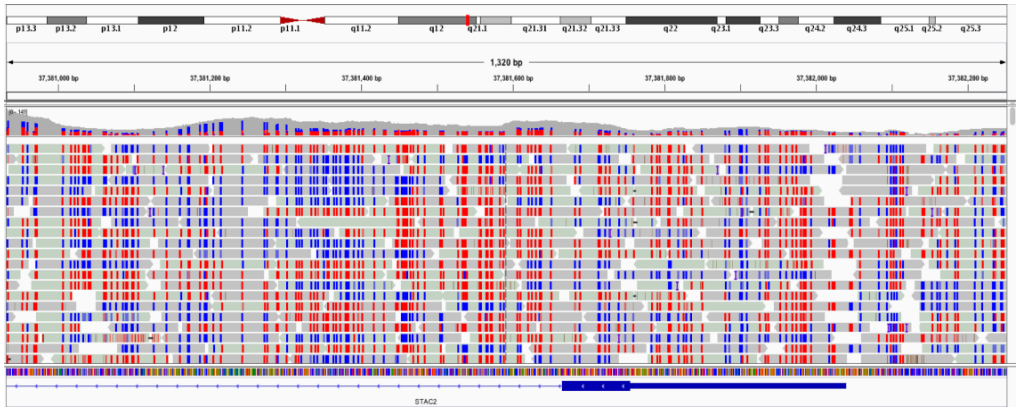
Participant w/o Cancer (Female Age Matched)





Participant w/ Invasive Breast Cancer - Stage II



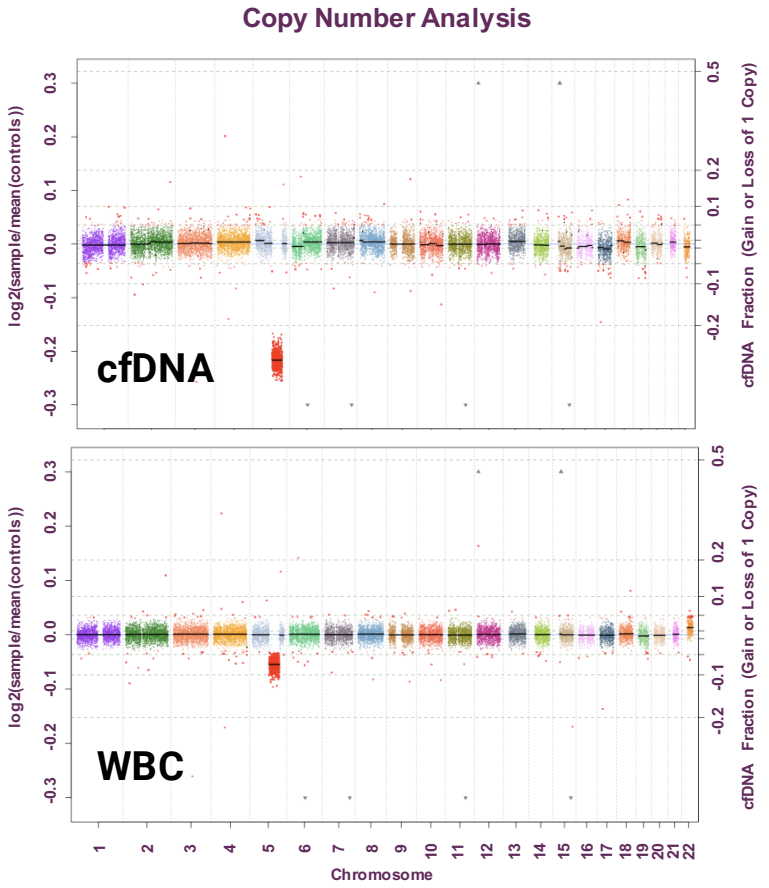


unmethylated CpG

methylated CpG

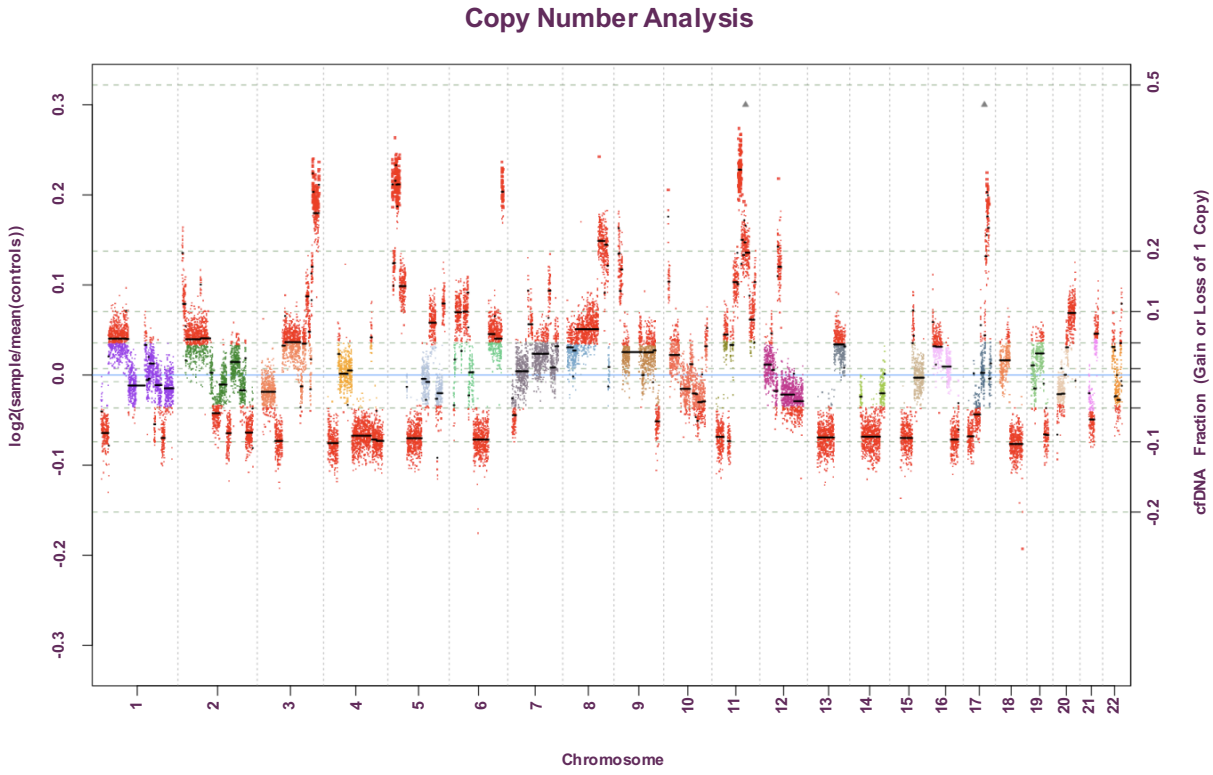
Cancer-Like Signal Found in Less Than 1% of Non-Cancer Group

- Only 5 of 580 **(<1%)** non-cancer samples had a cancer-like signal **across all three assays**
  - **With WGS**, 8 of 575 non-cancer samples had somatic copy number alterations in cfDNA
    - 4 were WBC-matched, due to clonal hematopoiesis
    - 4 were not WBC-matched **(<1% of all non-cancer samples)**
- **Potential for highly specific tests (>99%) when controlling for CHIP**

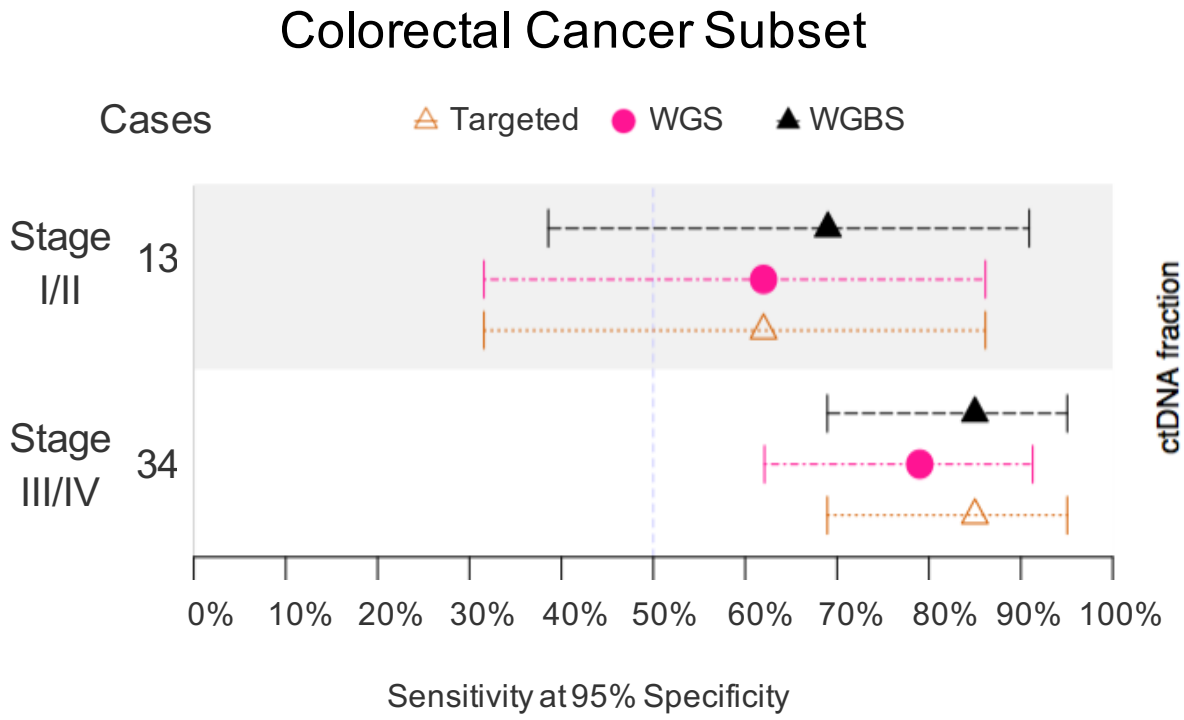


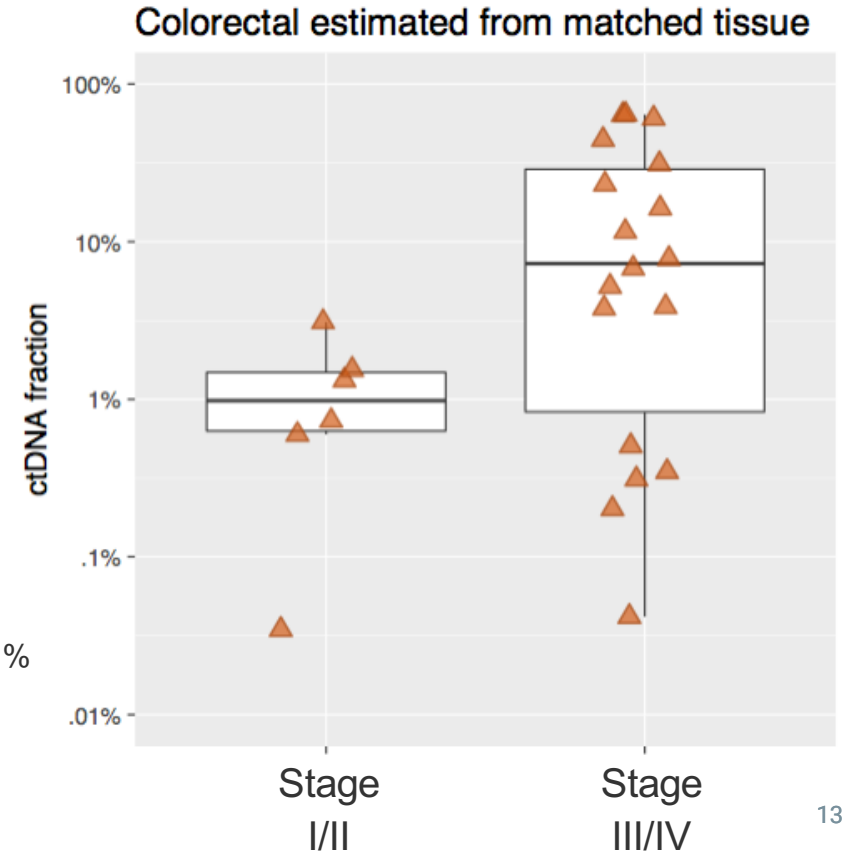
Subsequent Cancer Diagnoses In Participants From Non-Cancer Group With Cancer Signals

- Cancer-like signal detected:
  - SCNAs (shown here)
  - TP53 variant, not WBC matched
  - Abnormally methylated fragments
- Subsequently diagnosed with ovarian cancer
- Second participant diagnosed with endometrial cancer
- Cancer signals anticipated cancer diagnosis



Classification Scores Across Assays Are Highly Correlated and Proportional to Circulating Tumor DNA Fraction (ctDNA)





High Biological Signal in Typically Unscreened Cancers

- Subset analysis of 196 CCGA cases with cancer diagnosis associated with 5-year cancer-specific mortality of **>50%**[1]
  - Includes lung (118), ovarian (16), pancreatic (25), hepatobiliary (13), and esophageal (24) cancers

[1]5-year cancer-specific mortality rates for persons aged 50-79 from SEER18, 2010-2014; https://seer.cancer.gov.

Summary

- The CCGA study is a large, prospective, longitudinal, cohort study with a representative control population that will support development of early cancer detection tests
- Strong performance of genome-wide approaches like WGS and WGBS
- High biological signal in unscreened cancers that typically present at late stages
- Less than 1% of controls had a cancer signal, indicating feasibility of highly specific tests (>99%)
- Assay optimization is ongoing:
  - Deeper sequencing in informative regions
  - With increased sample sizes, machine learning approaches are expected to improve performance
- Next steps are to analyze the test set, and perform validation studies using the larger CCGA cohort with more early-stage participants
- **Together, these findings lay the groundwork for the development of a blood-based tests for early cancer detection**

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