Genome-Wide Sequencing for Early Stage Lung Cancer Detection from Plasma Cell-Free DNA (cfDNA): The Circulating Cell-free Genome Atlas (CCGA) Study

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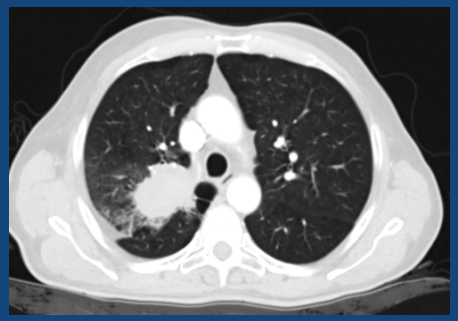
Disclosures

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. Tara Maddala, Earl Hubbell, Alexander M. Aravanis, Nan Zhang, Oliver Venn, Anton Valouev, Ling Shen, Shilpen Patel, Arash Jamshidi, Samuel Gross, Darya Filippova, John F. Beausang, Richard T. Williams, and Anne-Renee Hartman are current or former GRAIL employees with options to hold stock in the company. Richard T. Williams is an employee of WuXi NextCODE with options to hold stock in the company. Arash Jamshidi is a shareholder in Illumina. Charles Swanton is a grant recipient from Pfizer and AstraZeneca; receives honoraria or consultant fees from Roche Ventana, Celgene, Pfizer, Novartis, Genentech, and BMS; holds stock in GRAIL, Epic Biosciences, and Apogen Biotech; and is a co-founder of Achilles Therapeutics. The remaining authors have no conflicts to declare.

Current Lung Cancer Screening Paradigm is Not Widely Adopted

Early Detection of Lung Cancer is a High Unmet Medical Need



- Low-dose computed tomography (LDCT) improves lung cancer mortality in high-risk individuals
- Rate of clinical adoption remains low (1.9%)[2,3]
- Criticisms of LDCT include risk of false positives and logistical challenges[4]

cfDNA-Based Tests Represent an Untapped Opportunity for Cancer Detection

- Cancer **genotyping** using plasma cfDNA
  - Adopted for detection of specific actionable mutations
  - Only validated for advanced cancer
  - Uses smaller targeted gene panels
- Cancer **detection** using plasma cfDNA
  - Aims to identify a broader cancer "signature" rather than specific individual mutations
  - Genome-wide approaches offer additional information that allow early detection
  - Could address the unmet medical need

[1]National Lung Screening Trial Research Team. N Engl J Med 2011; 365:395-409. [2]Pham D et al. *J Clin Oncol* 36, 2018 (suppl; abstr 6504). [3]Jemal A, Fedewa SA. *JAMA Oncol* 2017;3:1278-1281. [4]McCunnet RJ et al. *Chest Journal* 2014;145(3):618-24.

CCGA is a Prospective Longitudinal Cohort Study Designed for Early Cancer Detection (NCT02889978)

15,000+ participants:

70% with cancer

- Previously untreated
- Any malignancy

30% without cancer

- Benign comorbid conditions were not excluded

Enrollment

Sample Collection

Blood samples (from all participants)

Tissue samples (cancer only)

Clinical data All pts

Sequencing and Follow-Up for 5 Years

Targeted sequencing: cfDNA, WBCs

Whole-genome sequencing: cfDNA, WBCs

Targeted & whole-genome bisulfite sequencing: cfDNA

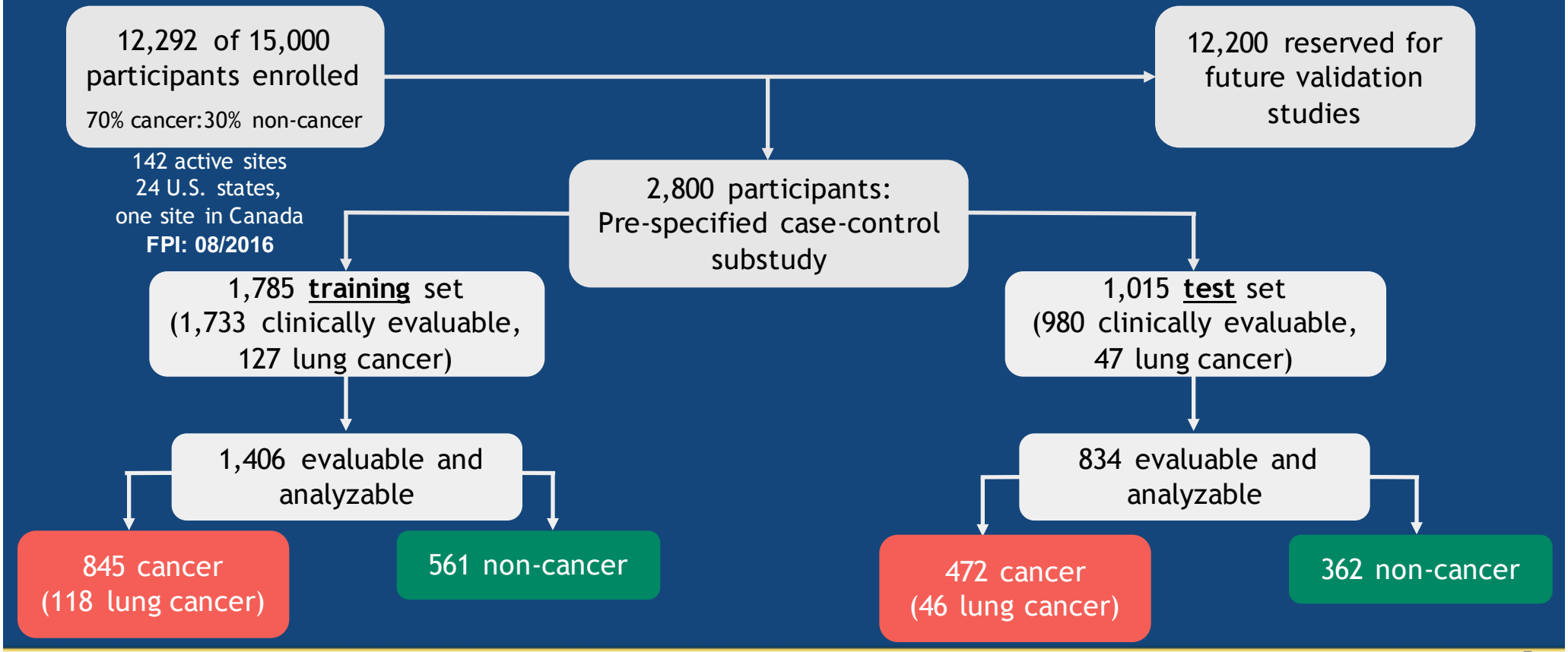
Whole transcriptome sequencing: cfRNA

Whole genome sequencing: tumor tissue

Pts with cancer: Treatment, recurrence, mortality

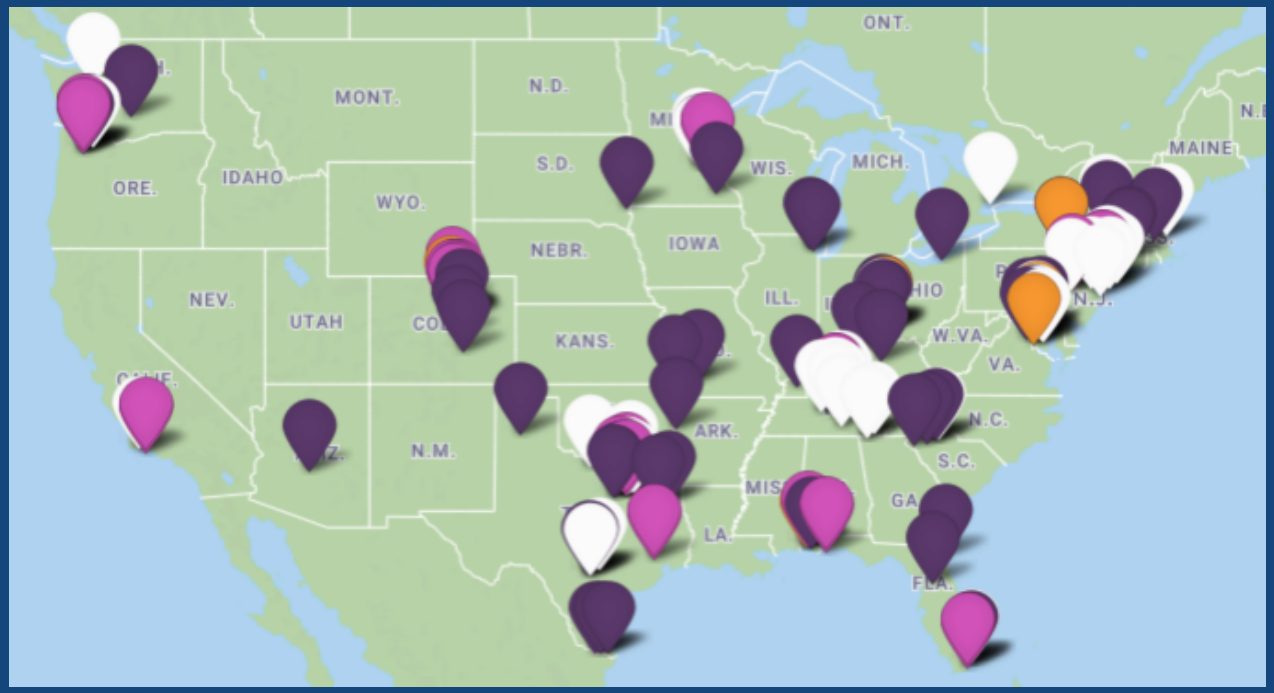
Pts without cancer: Remain cancer free or new cancer diagnosis, data on cancer status & treatment, mortality

CCGA is a Prospective Longitudinal Cohort Study Designed for Early Cancer Detection: First Training and Test Set Analyses



CCGA is Geographically Diverse with Enrollment Representative of United States Population

Non-cancer and cancer participants are enrolled from the same institutions to control for preanalytical variability



Participant Demographics

- To identify markers of cancer versus non-cancer, a comparable non-cancer group is important.
- Lung cancer and non-cancer participants were comparable with respect to age, race, and BMI.
- A higher proportion of participants with lung cancer were male and were ever-smokers.

	Training		Test	
	Lung Cancer	Non-Cancer	Lung Cancer	Non-Cancer
Total, n (%)	127	580	47	368
Age, Mean ± SD	67 ± 9	60 ± 13	69 ± 8	59 ± 14
Sex (%)				
Female	69 (54%)	452 (78%)	25 (53%)	238 (65%)
Race/Ethnicity (%)				
White, Non-Hispanic	112 (88%)	489 (84%)	37 (79%)	312 (85%)
African American	6 (5%)	47 (8%)	5 (11%)	25 (7%)
Hispanic, Asian, Other	9 (7%)	44 (8%)	5 (11%)	31 (8%)
Smoking Status (%)				
Never-smoker	19 (15%)	330 (57%)	3 (6%)	185 (50%)
BMI				
Normal/Underweight	41 (32%)	156 (27%)	20 (43%)	86 (23%)
Overweight/Obese	86 (68%)	423 (73%)	27 (57%)	281 (77%)

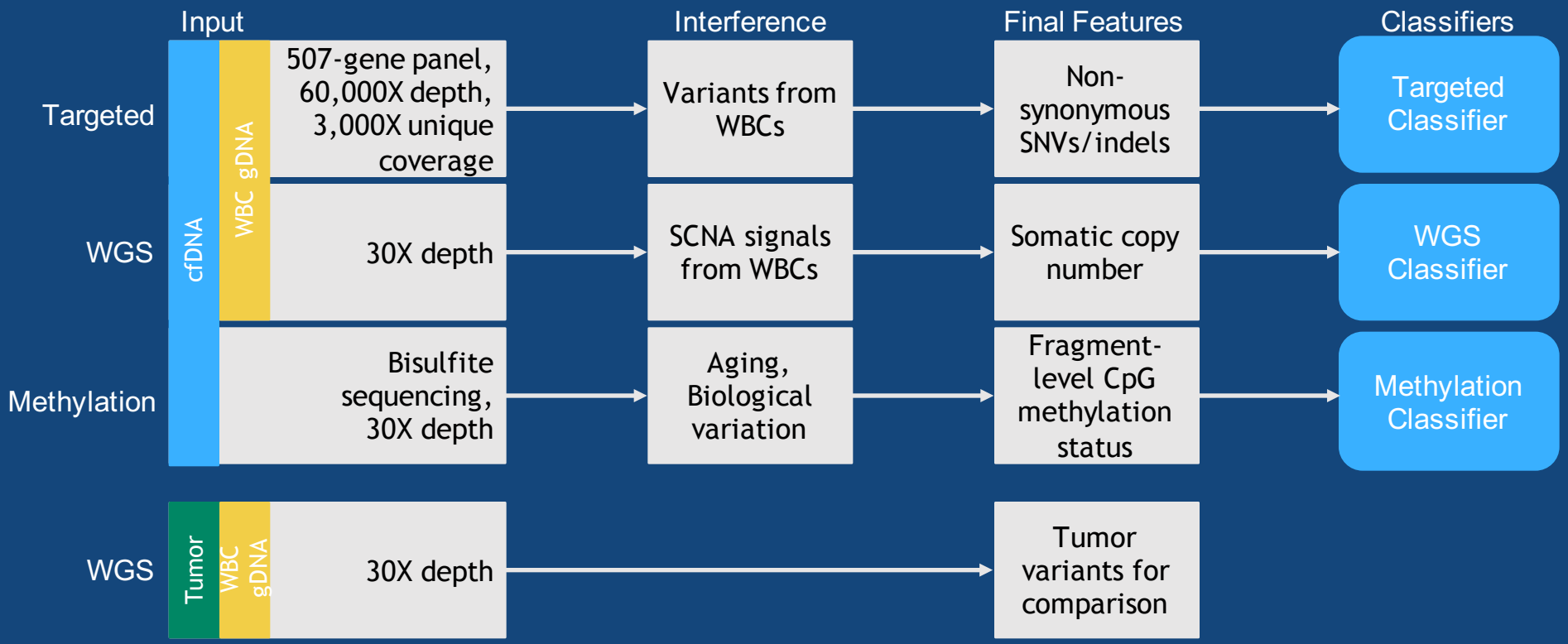
Participant Demographics

- Stage distribution consistent with United States distribution[1]
- A subset of participants with lung cancer were diagnosed by screening

	Lung Cancer, Training Set (n=127)	Lung Cancer, Test Set (n=47)
Overall Clinical Stage (n, %)		
0*	1 (<1%)	0 (0%)
I	23 (18%)	12 (26%)
II	14 (11%)	5 (11%)
III	39 (31%)	10 (21%)
IV	47 (37%)	19 (40%)
Non-Informative**	3 (2%)	1 (2%)
Method of Dx (n, %)		
Dx by Screening	23 (18%)	7 (15%)
Dx by Clinical Presentation	104 (82%)	40 (85%)

*Carcinoma *in situ*. **Staging information not available.
[1]Staging by the Surveillance, Epidemiology, and End Results (SEER) program 2010-2014 involves the higher of pathological or clinical stage. https://seer.cancer.gov.

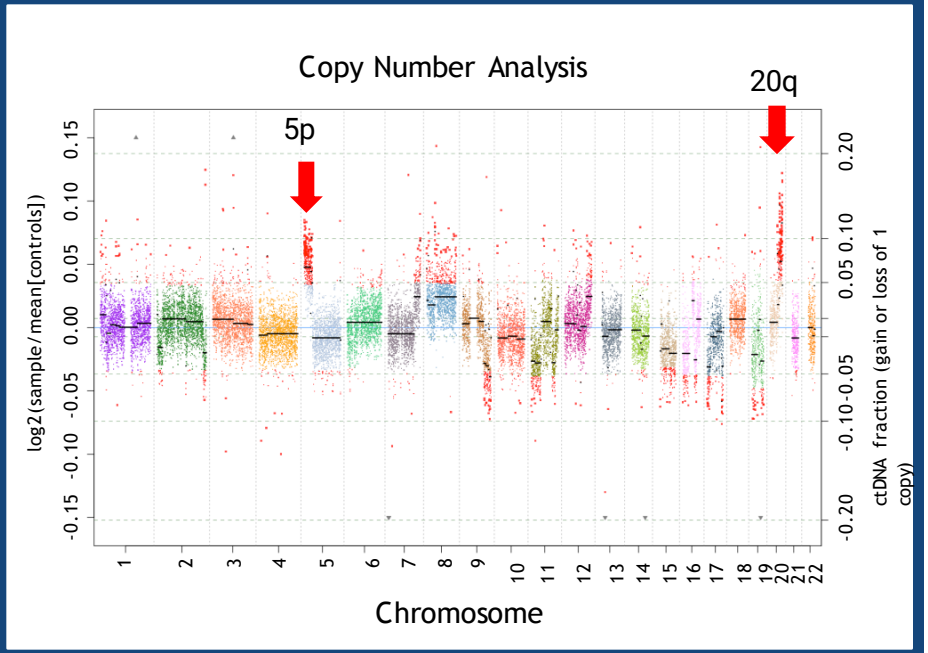
Prototype Sequencing Assays Used to Comprehensively Characterize Cancer-Specific cfDNA Signals



- All major somatic and epigenetic cfDNA features characterized

Correcting for WBC Variants (Clonal Hematopoiesis) Allows for High Specificity

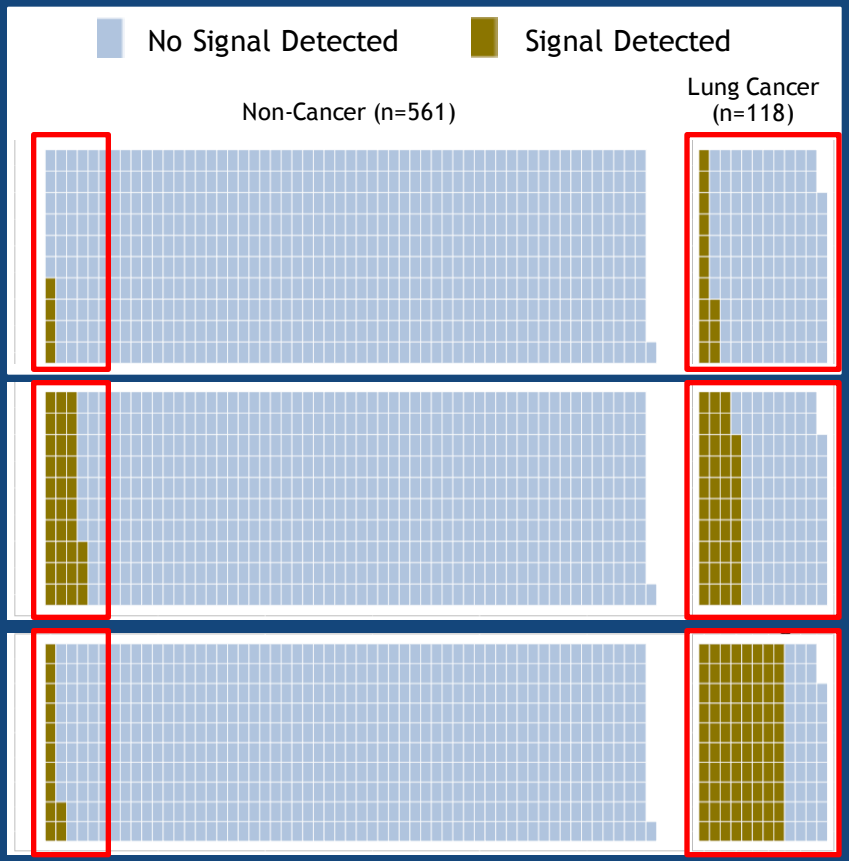
- The majority of cfDNA single-nucleotide somatic variants (SNVs) were WBC-matched and accounted for, on average:
  - 3,562 of 3,648 (98%) of all variants in non-cancer group
  - 1,154 of 2,156 (54%) in cancer group
  - Number of variants positively associated with age[1] (**ASCO Abstract 12001**)
- After correcting for WBC-matched variants:
  - Only 5 of 580 (<1%) non-cancer samples had a cancer-like signal across multiple assays
  - Two pts subsequently diagnosed with cancer (ovarian and endometrial)
- Potential for highly specific tests (>99%) when controlling for CHIP



1. Aravanis et al. Development of plasma cell-free DNA (cfDNA) assays for early cancer detection: first insights from the Circulating Cell-Free Genome Atlas Study (CCGA). Oral presentation at the 2018 American Association for Cancer Research; April 14-18, 2018; Chicago, IL.

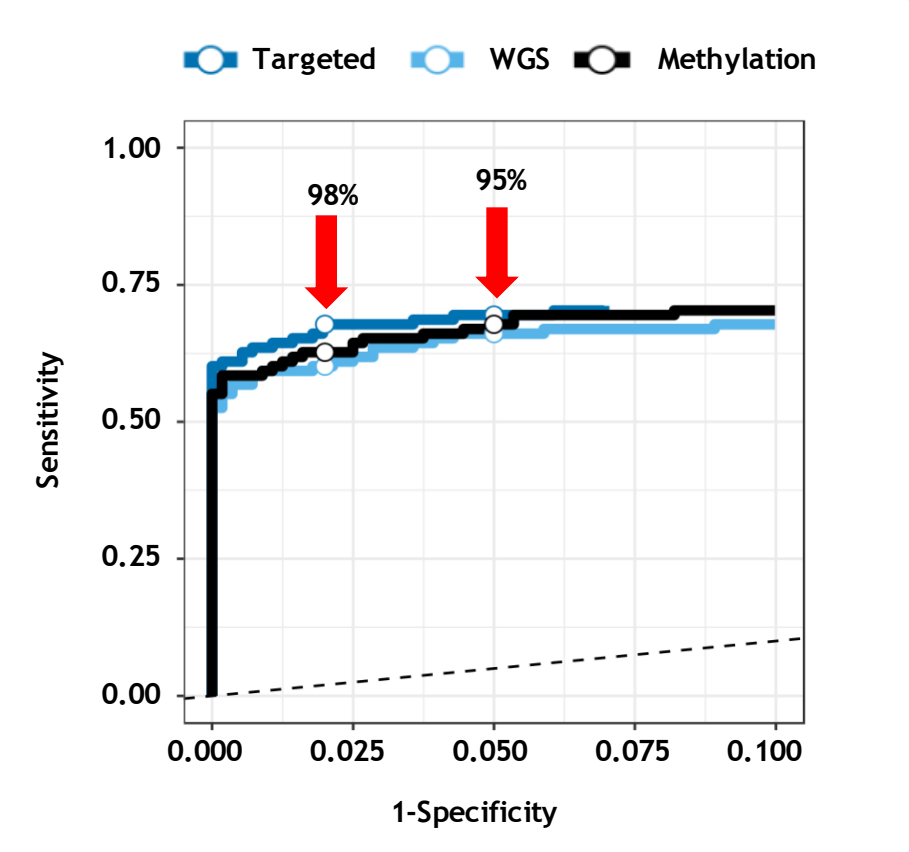
Simulating Existing Assays: Not Optimized for Screening

- 561 non-cancer and 118 pts with lung cancer from CCGA analyzed
- Testing a single location (emulating ddPCR) **without WBC filtering**
  - KRAS:p.G12X
  - Excellent specificity
  - Small number of cancer cases detected
- NGS panel reporting 813 clinically actionable variants (OncoKB levels 1-4)* **without WBC filtering**
  - Many cancer cases with variants
  - Many non-cancer cases with variants
- CCGA Targeted Assay **with WBC filtering**
  - Increased detection
  - Reduced false-positives (set at 98% here)
  - Continuous statistical score allows for tradeoffs in sensitivity/specificity



*Chakravarty D et al. *JCO Precis Oncol* 2017;doi: 10.1200/PO.17.00011. Epub 2017 May 16

Sensitivity Consistent Across Assays and at High Specificity

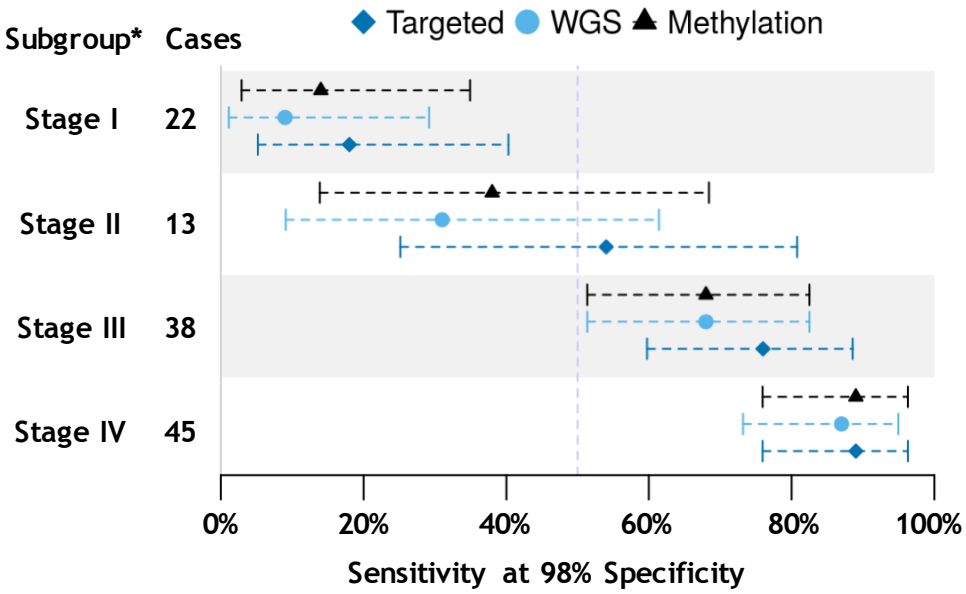


- In age-matched controls, there is a latent rate of 1% of undiagnosed cancer
  - Some of those cancers will be detectable by the prototype assays and classifiers
  - The longitudinal design of the study allows us to correctly assign cancer status to individuals post-enrollment once diagnoses are reported from normal clinical practice
- We conservatively look at 98% specificity to account for these latent cases
  - Specificity will continue to evolve as follow-up is completed

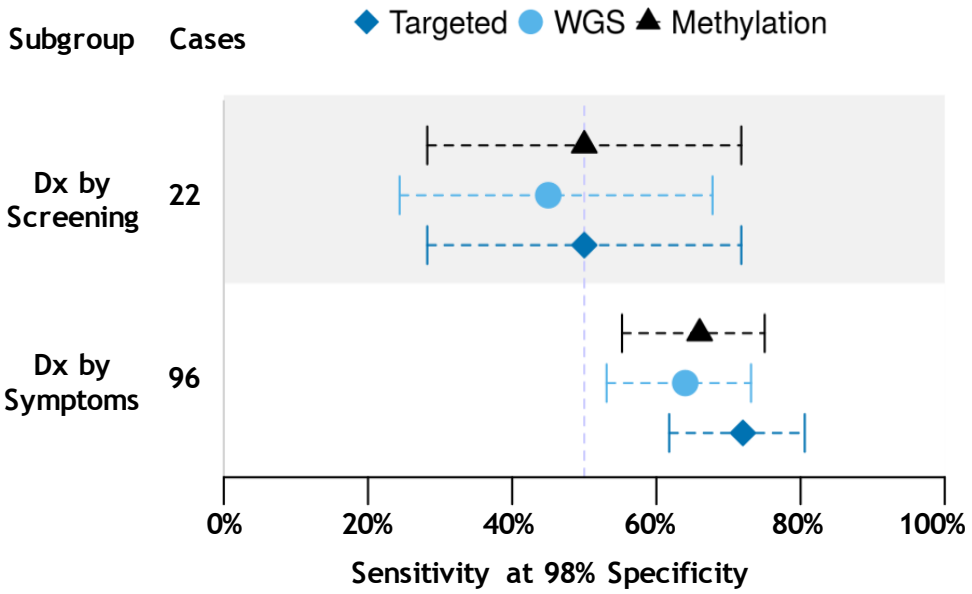
Consistent Assay Performance Across Lung Cancer Stages and by Diagnosis Method

Sensitivity by Stage (Training)

- Signal increased with stage



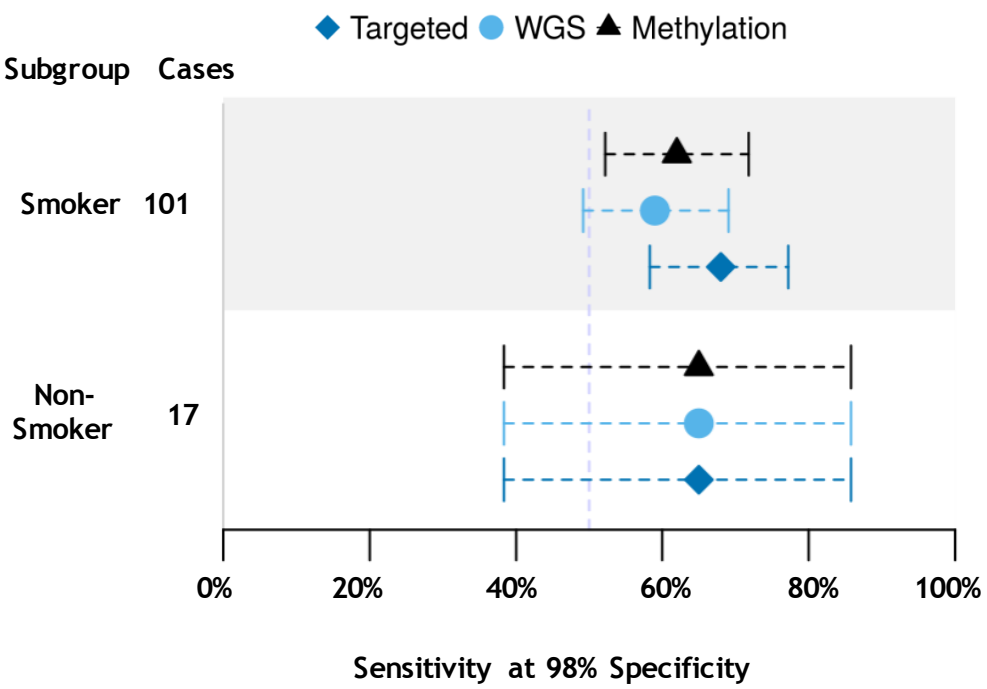
Sensitivity by Diagnosis Method (Training)



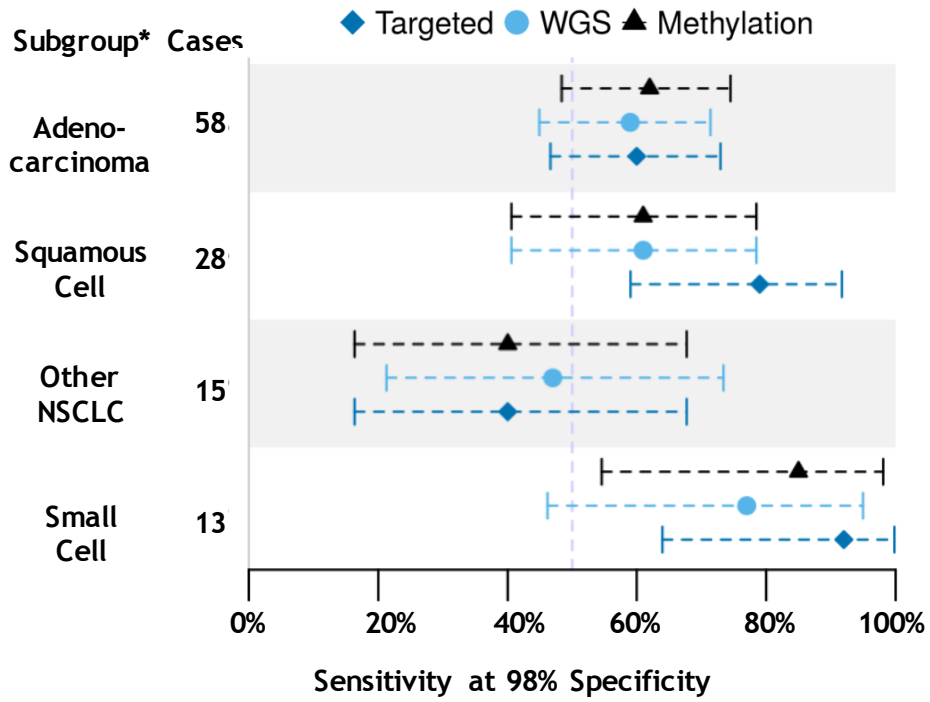
*Excludes 3 participants with non-informative stage

Consistent Biological Signal In Smokers and Non-Smokers and Across Histologies

Sensitivity by Smoking Status (Training)



Sensitivity by Histological Subtype (Training)

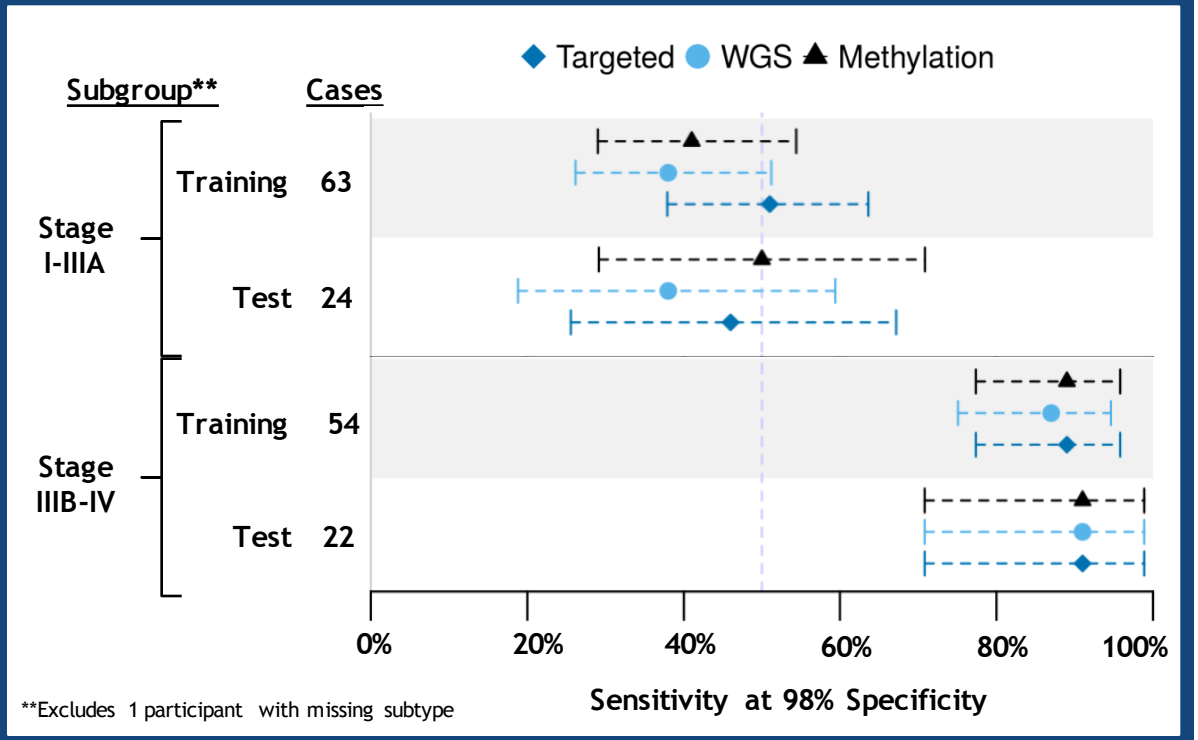


*Excludes 3 participants with non-informative stage, 7 participants with missing subtype

Consistent Biological Signal Across Lung Cancer Stages in Training and Test Sets

Methylation Score*
(Sensitivity [95% CI])

- Early stage lung cancer (stage I-IIIA)
  - Training: 41% (29-54%)
  - Test: 50% (29-71%)
- Advanced lung cancer (stage IIIB-IV)
  - Training: 89% (77-96%)
  - Test: 91% (71-99%)



*Results comparable across assays

Conclusions

- This first interim analysis of the CCGA study (2,800 participants, 174 with lung cancer) shows:
  - Comprehensive sequencing of plasma cfDNA generates high-quality data across the spectrum of genomic features that permits non-invasive cancer detection
  - These assays detect lung cancer across stages, histologies, and populations, and results replicate in an independent test set
  - WBC-derived mutations and copy number variations are a major source of potential false positives that must be accounted for to achieve high specificity
- Together, these early results support the promise of using cfDNA-based sequencing to develop an early cancer detection test with high specificity
  - Further assay and clinical development in large-scale clinical studies is ongoing
    - CCGA (NCT02889978): remaining participants for further training and clinical validation
    - STRIVE (NCT03085888): clinical validation in an intended use population

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