IASLC Asia Conference on Lung Cancer 2018
November 8-10, 2018 Guangzhou, China

Plasma-Based Molecular Testing for Early Detection of Lung Cancer

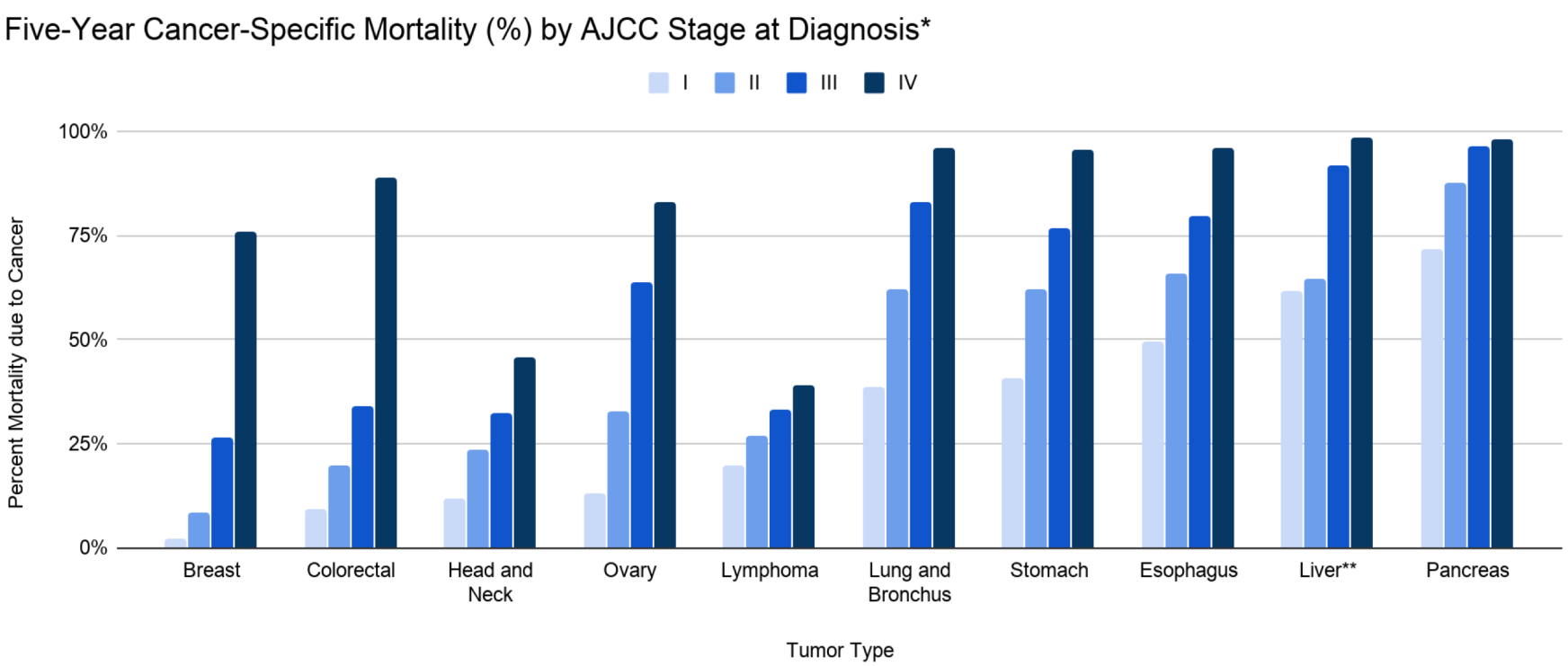
November 8, 2018

Eric Fung, MD, PhD
GRAIL, Inc.
Menlo Park, California, USA

Disclosures

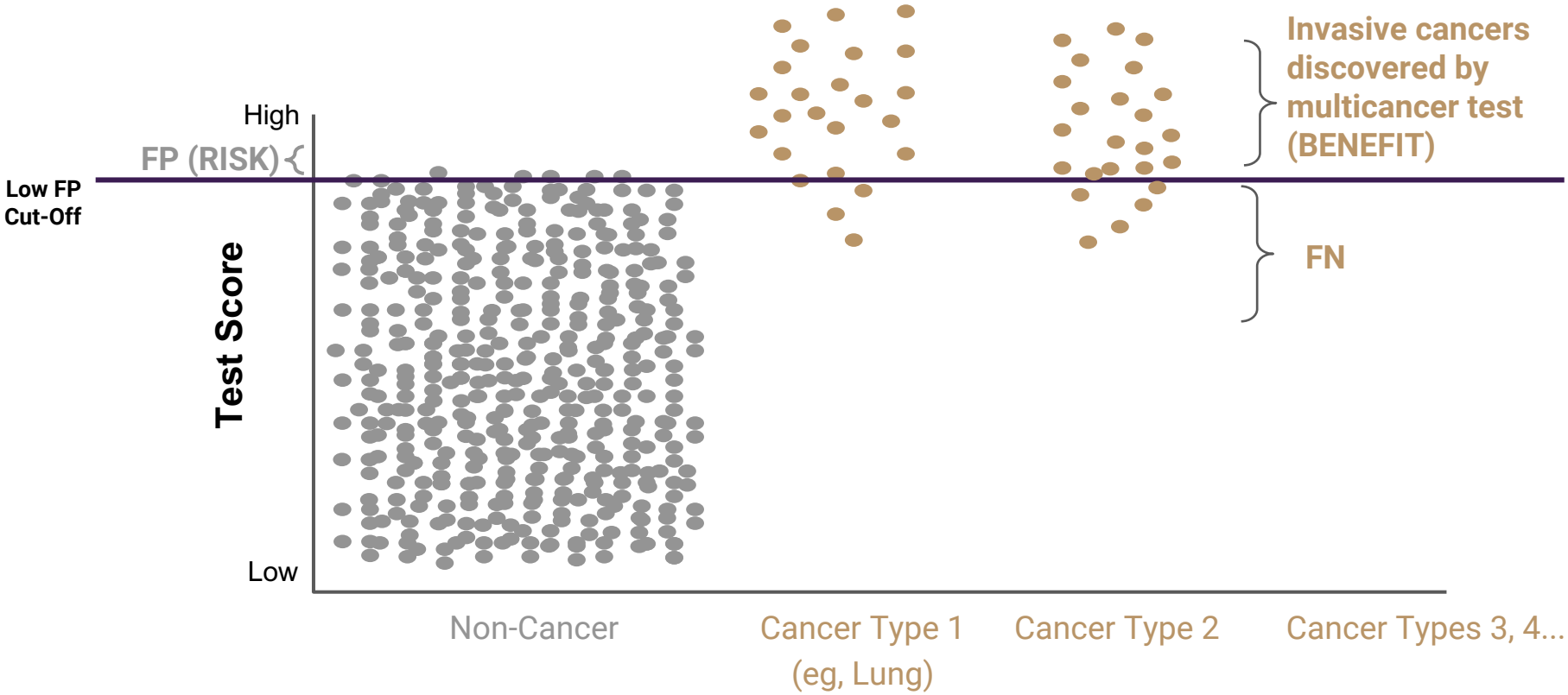
Eric Fung, MD, PhD is an employee of GRAIL and has stock options for GRAIL.

Cancer Mortality Increases with Later Detection



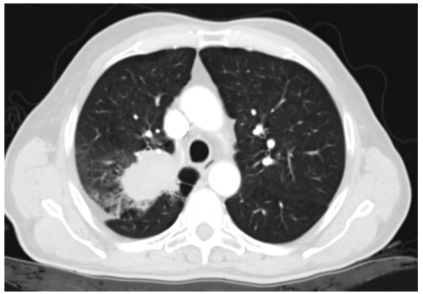
*Cancer specific survival data from SEER18 ages 50+ diagnosed 2006-2015. Surveillance, Epidemiology, and End Results (SEER) Program (www.seer.cancer.gov) SEER*Stat Database: Incidence - SEER 18 Regs Research Data, Nov 2017. **Includes intrahepatic bile duct.

Illustration of Benefit vs Risk of a Multi-Cancer Test



Limitations of Current Screening Paradigms Represent an Opportunity for Improved Cancer Detection

Example: Early Detection of Lung Cancer is a High Unmet Medical Need



- Low-dose computed tomography (LDCT) improves lung cancer mortality in high-risk individuals[1,2]
- Rate of clinical adoption remains low (1.9%)[3,4]
- Criticisms of LDCT include risk of false positives and logistical challenges[5]

How cfDNA-Based Tests can Improve Cancer Detection

- Cancer genotyping using plasma cfDNA
  - Adopted for detection of specific actionable mutations
  - Currently 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]de Koning H et al. IASLC World Conference on Lung Cancer. 2018(abstr PL02.05). [2]National Lung Screening Trial Research Team. *NEJM.* 2011;365:395-409. [3]Pham D et al. *J Clin Oncol.* 2018;36(suppl; abstr 6504). [4]Jemal A, Fedewa SA. *JAMA Oncol.* 2017;3:1278-1281. [5]McCunnet RJ et al. *Chest Journal.* 2014;145(3):618-24.

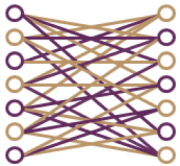
cfNA-Based Approaches to Cancer Detection

High-intensity sequencing to detect cfNAs

- Reliably detect cancer-defining cfNAs by looking broadly across the entire genome and at extraordinary depth to detect faint signals

Large-scale clinical studies

- Confirm clinical validity of tests through one of the largest clinical study programs ever conducted in genomic medicine



Powerful machine-learning approaches

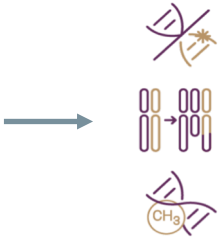
- Apply the latest tools of data science to classify participants according to the presence, type, and severity of cancer

The Circulating Cell-Free Genome Atlas (CCGA) Study

CCGA is a Prospective, Longitudinal Cohort Study Designed for Cancer Detection

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

Blood samples (all participants)



Targeted sequencing cfDNA, WBCs

Whole-genome sequencing (WGS) cfDNA, WBCs

Targeted & whole-genome bisulfite sequencing (WGBS) cfDNA

Tissue samples (cancer only)

Whole-genome sequencing of tumor tissue

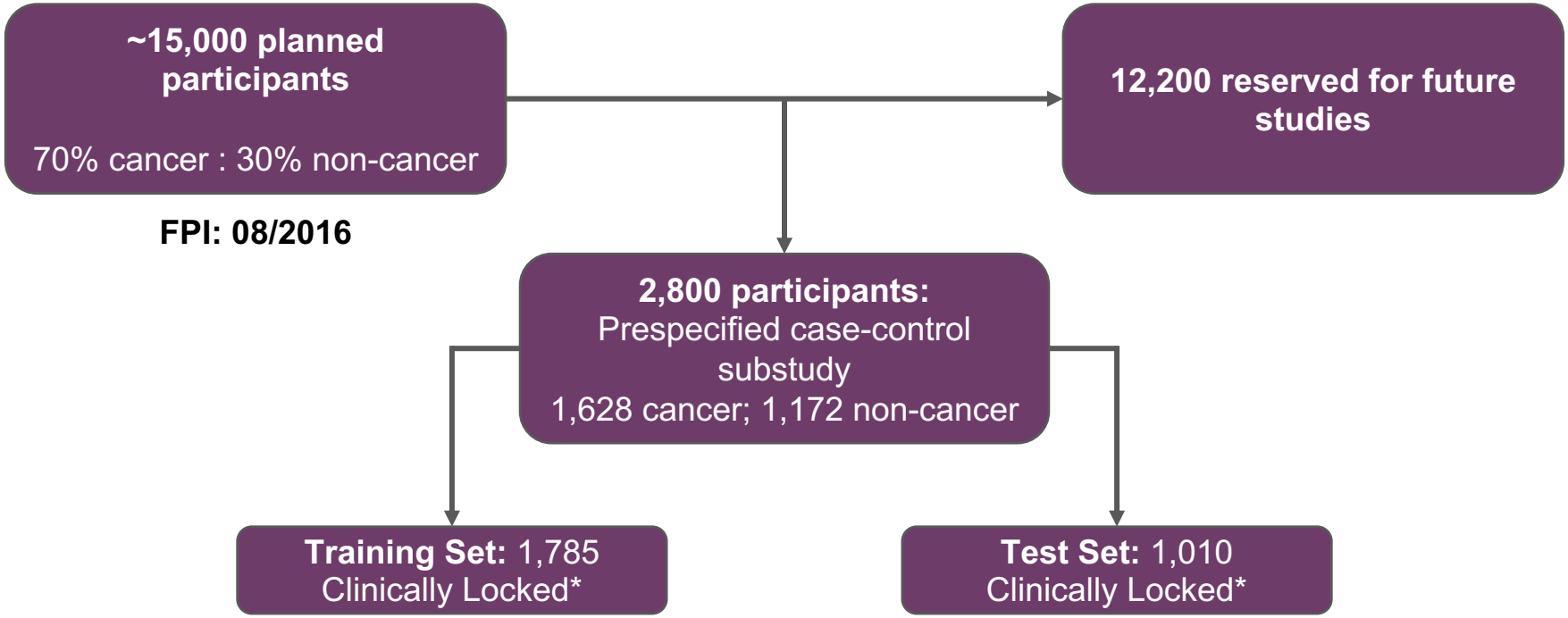
Follow-up for 5 yrs

Cancer Participants: Data on cancer status & treatment, new cancer diagnosis, mortality

Non-Cancer Participants: Remain cancer free or develop new cancer diagnosis, data on cancer status & treatment, mortality

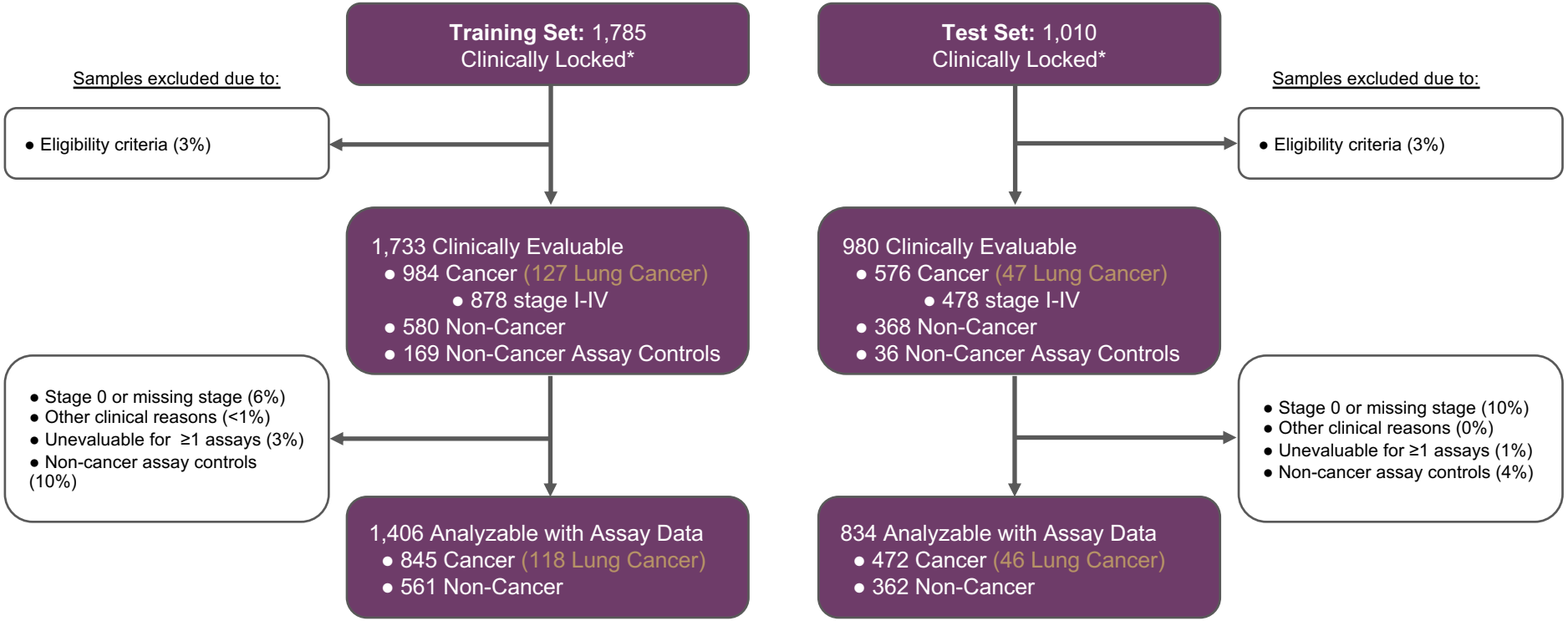
cfDNA, cell-free DNA; WBC, white blood cell; WGS, whole-genome sequencing; WGBS, whole-genome bisulfite sequencing.

CCGA: Discovery, Training, and Validation for a Multi-Cancer Test



*5 participants not clinically locked were excluded.

CCGA: Prespecified Case-Control Substudy of 2,800 Participants



*5 participants not clinically locked were excluded.

Comparable Cancer and Non-Cancer Groups

- Clinically evaluable cancer and non-cancer groups were comparable with respect to age, sex, race/ethnicity, and BMI
- A higher proportion of participants with lung cancer were male and were ever-smokers.

	Training			Test		
	Non-Cancer	**Cancer***	**Lung Cancer**	**Non-Cancer**	**Cancer***	**Lung Cancer**
Total, n (%)	580	984	127	368	576	47
Age, Mean ± SD (years)	60 ± 13	61 ± 12	67 ± 9	59 ± 14	62 ± 12	69 ± 8
Sex (%)						
Female	452 (78%)	697 (71%)	69 (54%)	238 (65%)	363 (63%)	25 (53%)
Race/Ethnicity (%)						
White, Non-Hispanic	489 (84%)	846 (86%)	112 (88%)	312 (85%)	475 (82%)	37 (79%)
African American	47 (8%)	67 (7%)	6 (5%)	25 (7%)	40 (7%)	5 (11%)
Hispanic, Asian, Other	44 (8%)	71 (7%)	9 (7%)	31 (8%)	61 (11%)	5 (11%)
Smoking Status (%)						
Never-smoker	330 (57%)	484 (49%)	19 (15%)	185 (50%)	290 (50%)	3 (6%)
BMI						
Normal/Underweight	156 (27%)	266 (27%)	41 (32%)	86 (23%)	162 (28%)	20 (43%)
Overweight**	184 (32%)	319 (32%)	48 (38%)	126 (34%)	190 (33%)	15 (32%)
Obese***	240 (41%)	398 (40%)	38 (30%)	155 (42%)	224 (39%)	12 (26%)

*Cancer types by training/test: Breast (410/201), lung (127/47), prostate (74/58), colorectal (51/46), renal (29/18), uterine (28/9), pancreas (27/23), esophageal (25/8), lymphoma (25/22), head & neck (21/12), ovarian (21/7), hepatobiliary (15/16), melanoma (15/12), cervical (14/11), multiple myeloma (14/21), leukemia (13/16), thyroid (13/10), bladder (12/3), gastric (12/15), anorectal (7/3), and unknown primary/other (22/18). **BMI≥25. ***BMI≥30.

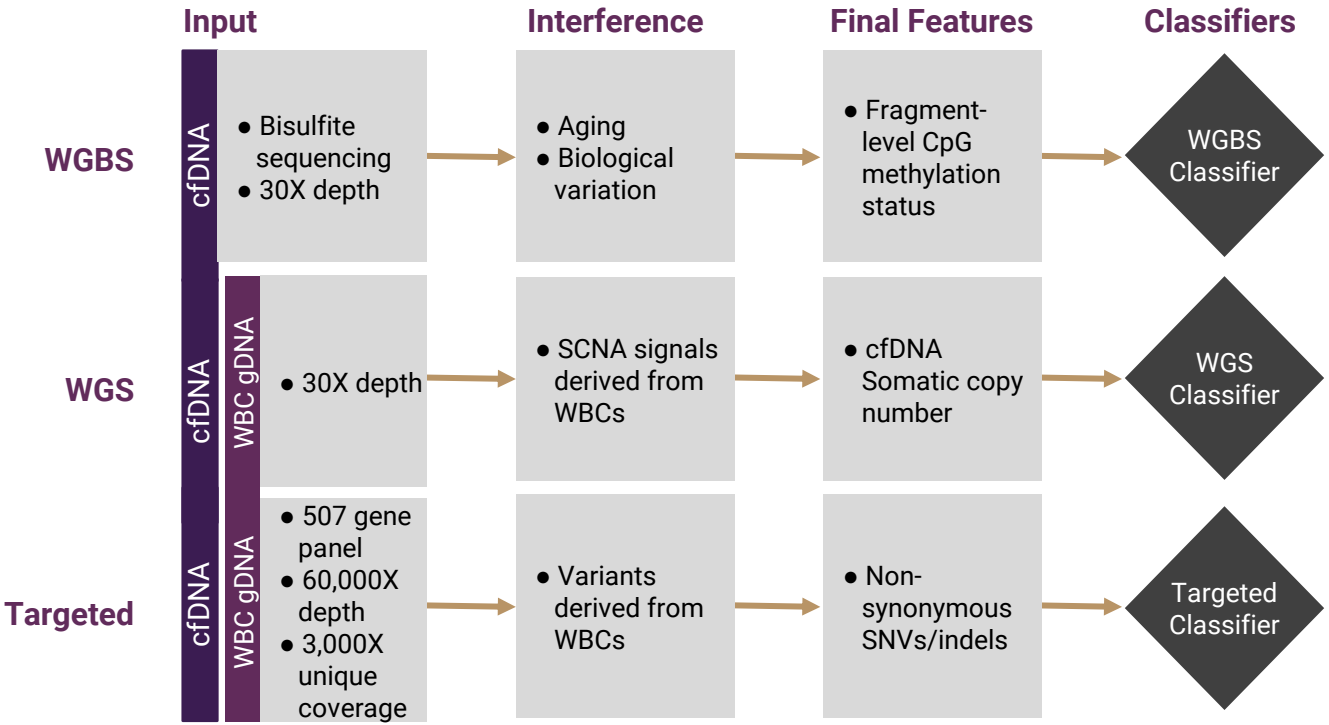
Stage Distribution and Method of Diagnosis were Consistent in Training and Test Sets

Broad distribution of stages in training and in test sets

	Training Set		Test Set	
	Cancer (n=984)	**Lung Cancer (n=127)**	**Cancer (n=576)**	**Lung Cancer (n=47)**
Overall Clinical Stage (n, %)				
0*	56 (6%)	1 (<1%)	34 (6%)	0 (0%)
I	300 (30%)	23 (18%)	165 (29%)	12 (26%)
II	249 (25%)	14 (11%)	142 (25%)	5 (11%)
III	165 (17%)	39 (31%)	76 (13%)	10 (21%)
IV	164 (17%)	47 (37%)	95 (16%)	19 (40%)
Non-Informative**	50 (5%)	3 (2%)	64 (11%)	1 (2%)
Method of Dx (n, %)				
Diagnosed by Screening§	354 (36%)	23 (18%)	202 (35%)	7 (15%)
Diagnosed by Clinical Presentation¶	630 (64%)	104 (82%)	373 (65%)	40 (85%)

*DCIS/CIS. **Staging information not available. §Percent screen-detected in training/test sets for breast cancer: 58%/58%, colorectal cancer: 29%/37%, lung cancer: 18%/15%, prostate cancer: 91%/90%, and other cancers 4%/4%. ¶Clinical presentation includes all cancers not detected by screening (ie, detected symptomatically or as incidental findings).

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

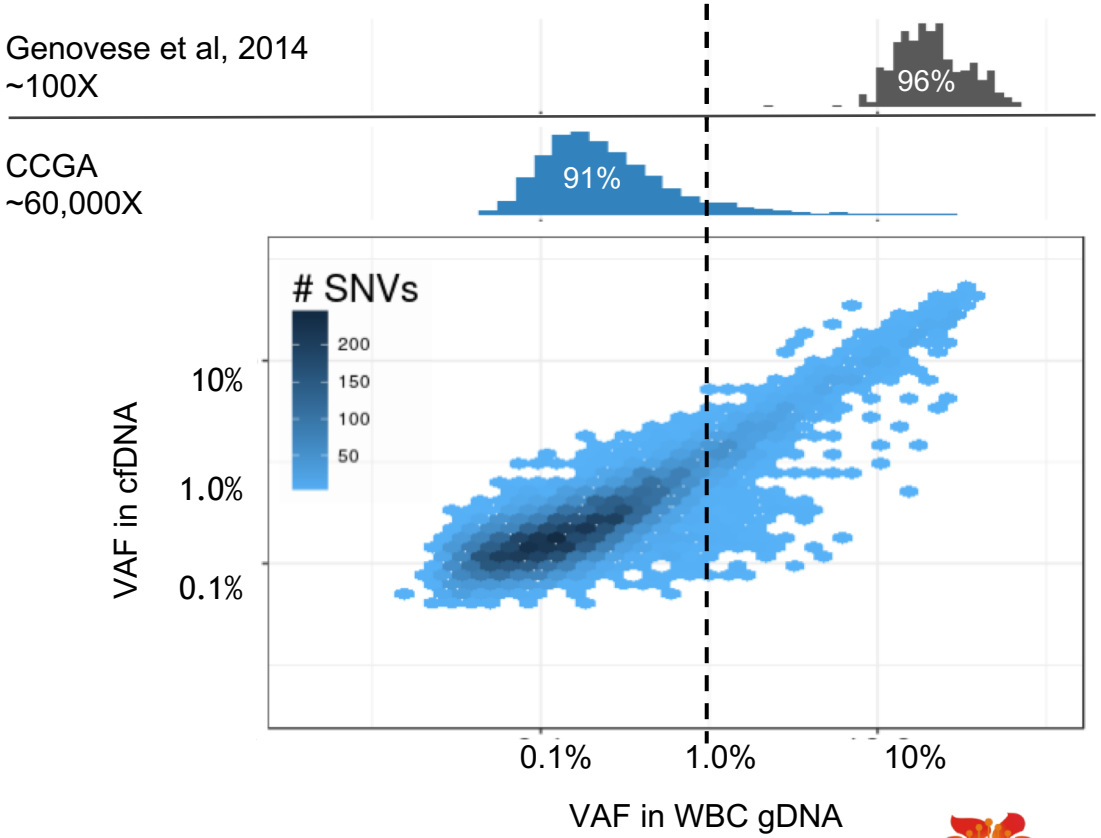


- **All major somatic and epigenetic cfDNA features characterized**

cfDNA, cell-free deoxyribonucleic acid; WBC, white blood cell; WGS, whole-genome sequencing; WGBS, whole-genome bisulfite sequencing.

Potential Source of False Positives: Clonal Hematopoiesis

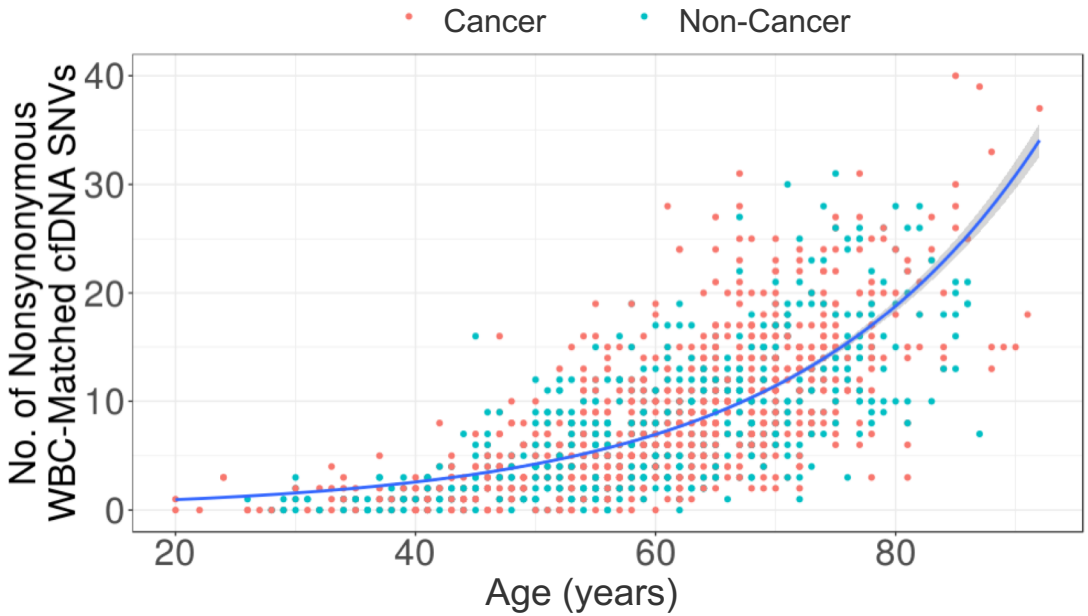
- Previous study sequencing at ~100X: 96% of variants at >1% variant allele frequencies (VAF)[1]
- Ultra-deep sequencing (this study [Training set]): <10% of WBC-matched variants at >1% VAF
- Variants at lower VAFs will require high-depth sequencing of WBCs to effectively exclude this confounding signal in cfDNA-based assays
- Early detection requires a low limit of detection, wherein low VAF CHIP is a confounding signal



[1]Genovese et al. *NEJM*. 2014;371:2477-2487.
CHIP, clonal hematopoiesis of indeterminate potential; cfDNA, cell-free DNA; WBC, white blood cell; gDNA, genomic DNA.

Majority of cfDNA Variants Are WBC-Matched Clonal Hematopoiesis

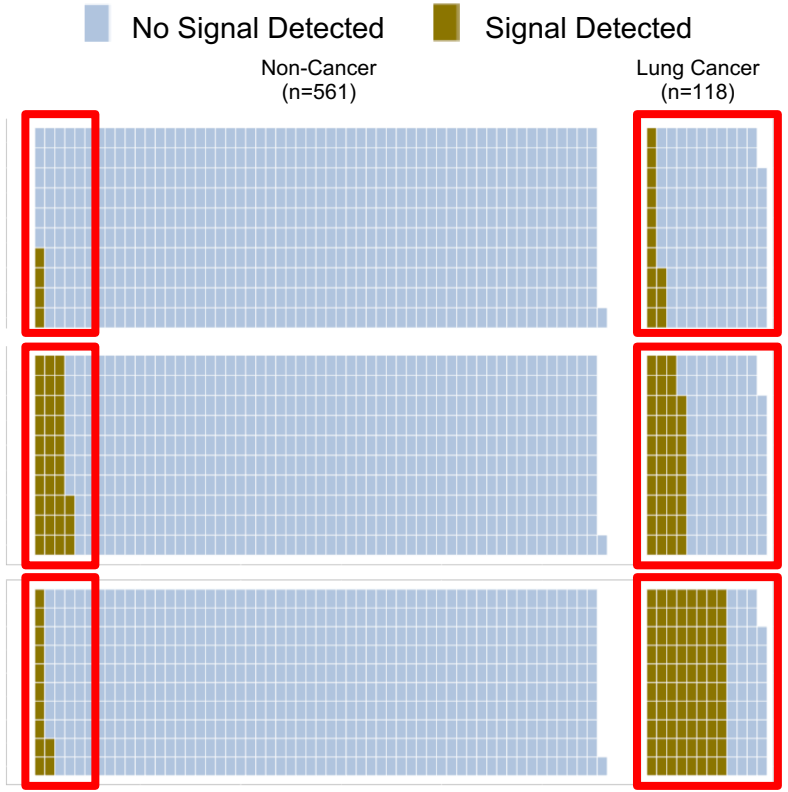
- In Training set, average non-tumor WBC-matched cfDNA somatic variants (SNVs/indels) were:
  - 98% of all variants in non-cancer group
  - 71% in total cancer group
  - 54% in lung cancer group
- Number of WBC variants is positively associated with age in cancer and non-cancer groups[1]



[1]Swanton C et al. *J Clin Oncol.* 2018;36(suppl; abstr 12003).
cfDNA, cell-free DNA; WBC, white blood cell; SNV, single-nucleotide variant; indel, insertion or deletion.

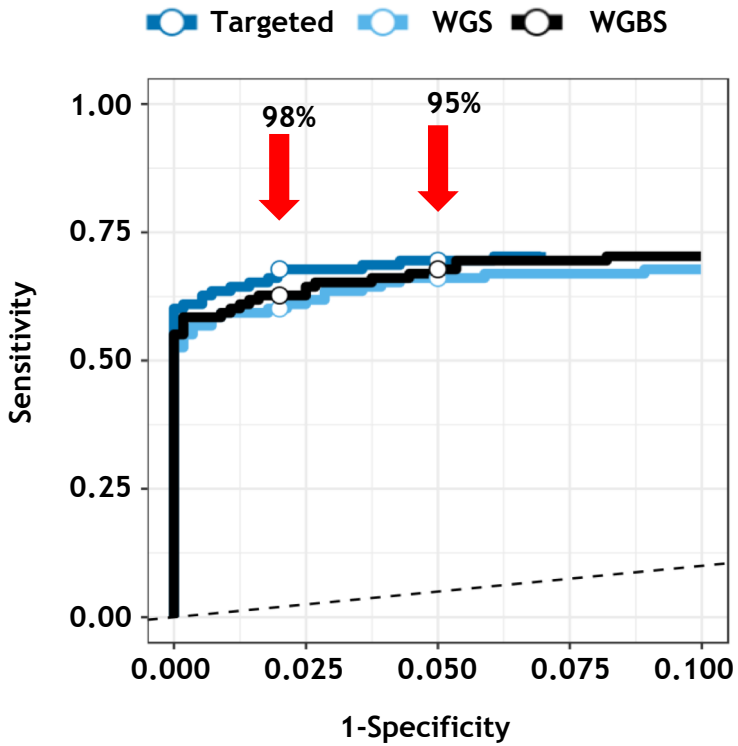
Simulating Existing Assays[1]: Not Optimized for Screening

- CCGA [Training set] used for simulation analysis:
  - 561 non-cancer; 118 participants with lung cancer
- Testing a single location (emulating ddPCR)
  - KRAS:p.G12X
  - Small number of cancer participants detected
  - Few non-cancer participants
- NGS panel reporting 813 clinically actionable variants from 30 genes[2]
  - More cancer participants detected
  - Many non-cancer participants detected due to WBC/CHIP variants
- CCGA targeted NGS assay with coverage of 507 genes and combined cfDNA and WBC sequencing
  - Joint cfDNA/WBC ML calling to remove WBC/CHIP variants
  - Increased detection of cancer participants
  - Reduced false-positives (specificity set at 98%)



[1]Oxnard GR et al. *J Clin Oncol*. 2018;36(suppl; abstr LBA8501). [2]Chakravarty D. *JCO Precis Oncol*. 2017;doi: 10.1200/PO.17.00011.
ddPCR, digital-droplet PCR; cfDNA, cell-free DNA; WBC, white blood cell; CHIP, clonal hematopoiesis of indeterminate potential; NGS, next generation sequencing; ML, machine learning.

Sensitivity Consistent Across Assays and at High Specificity—Training Set

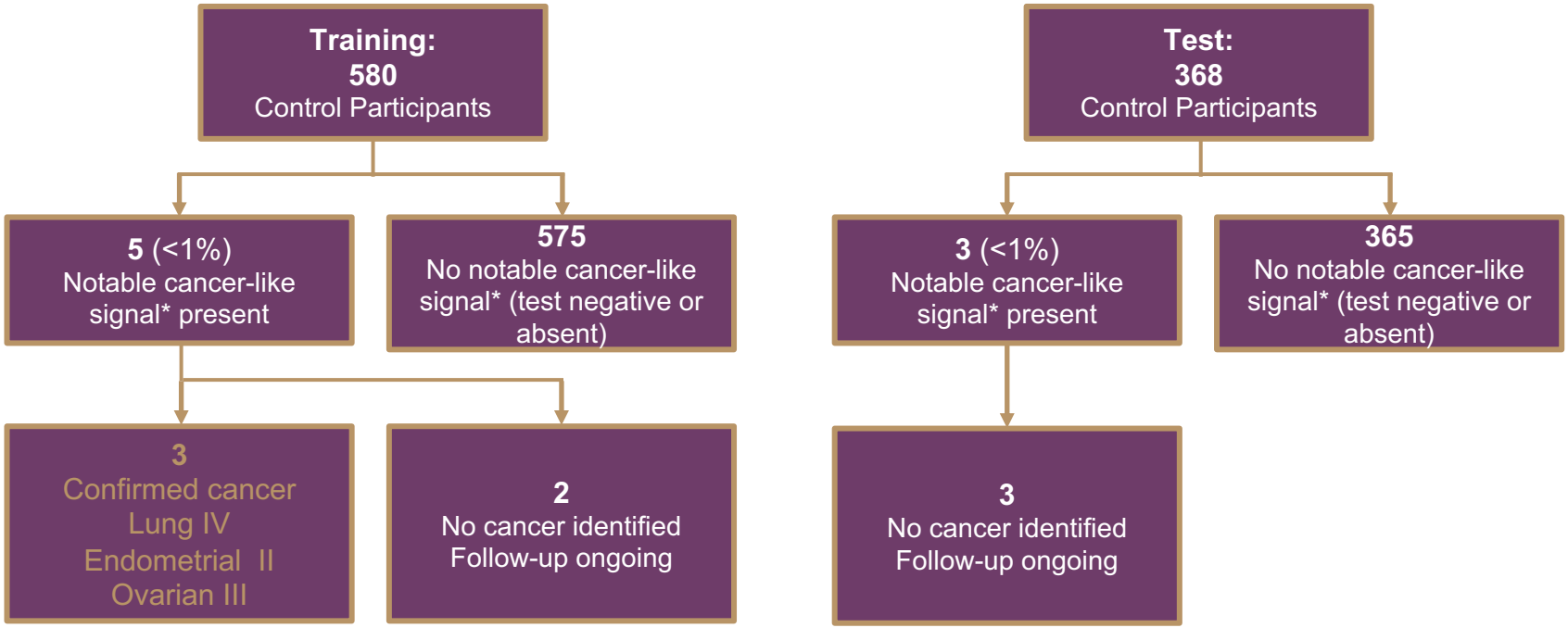


- In age-matched controls, there is expected to be a latent rate of 1% of undiagnosed cancer
  - Some of those cancers will be detectable by the prototype assays and classifiers
  - The longitudinal design of the study allows us to correctly assign cancer status to individuals post-enrollment once diagnoses 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

WGS, whole-genome sequencing; WGBS, whole-genome bisulfite sequencing.

High Specificity (>99%) is Feasible

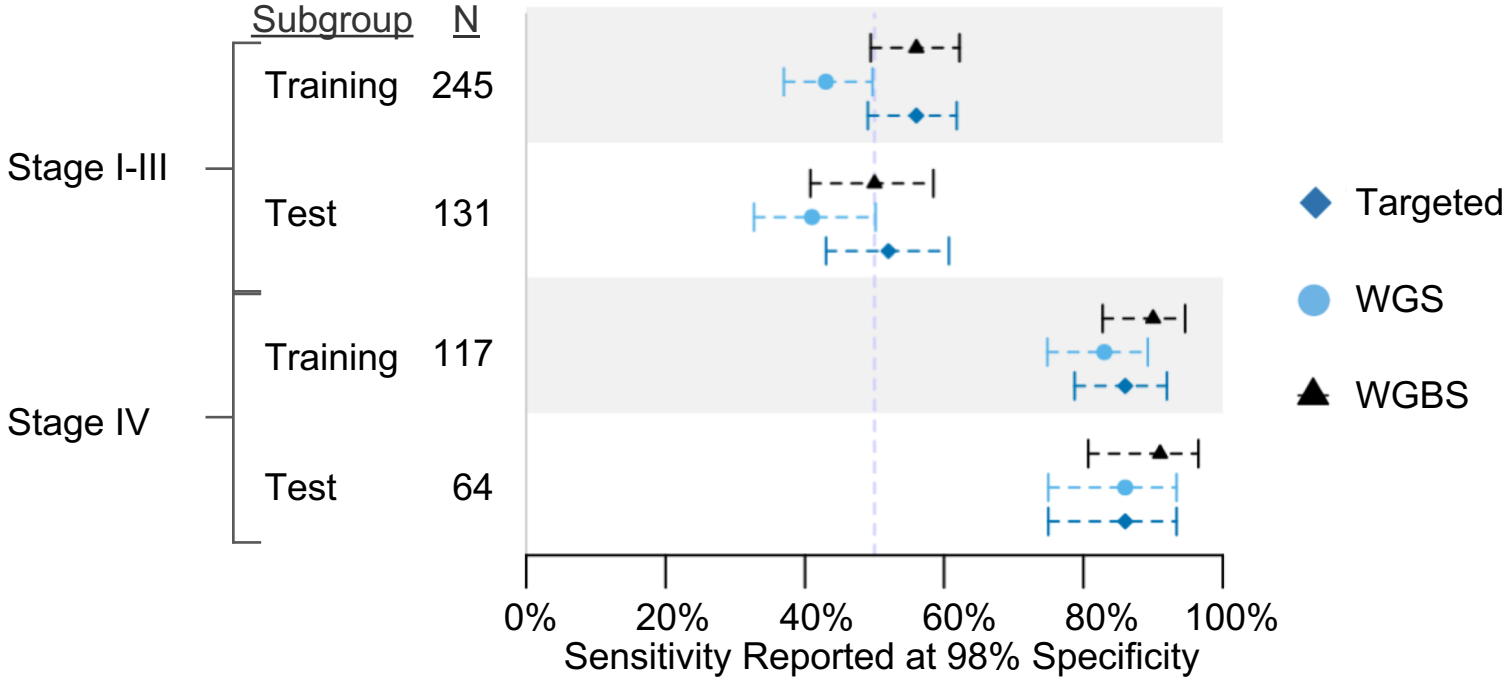
5-year follow-up will enable identification of participants who are subsequently diagnosed



*Notable cancer-like signal defined as ≥2 assays with significant abnormalities compared to the typical non-cancer population, or known cancer drivers present with ≥1 significant assay abnormality.

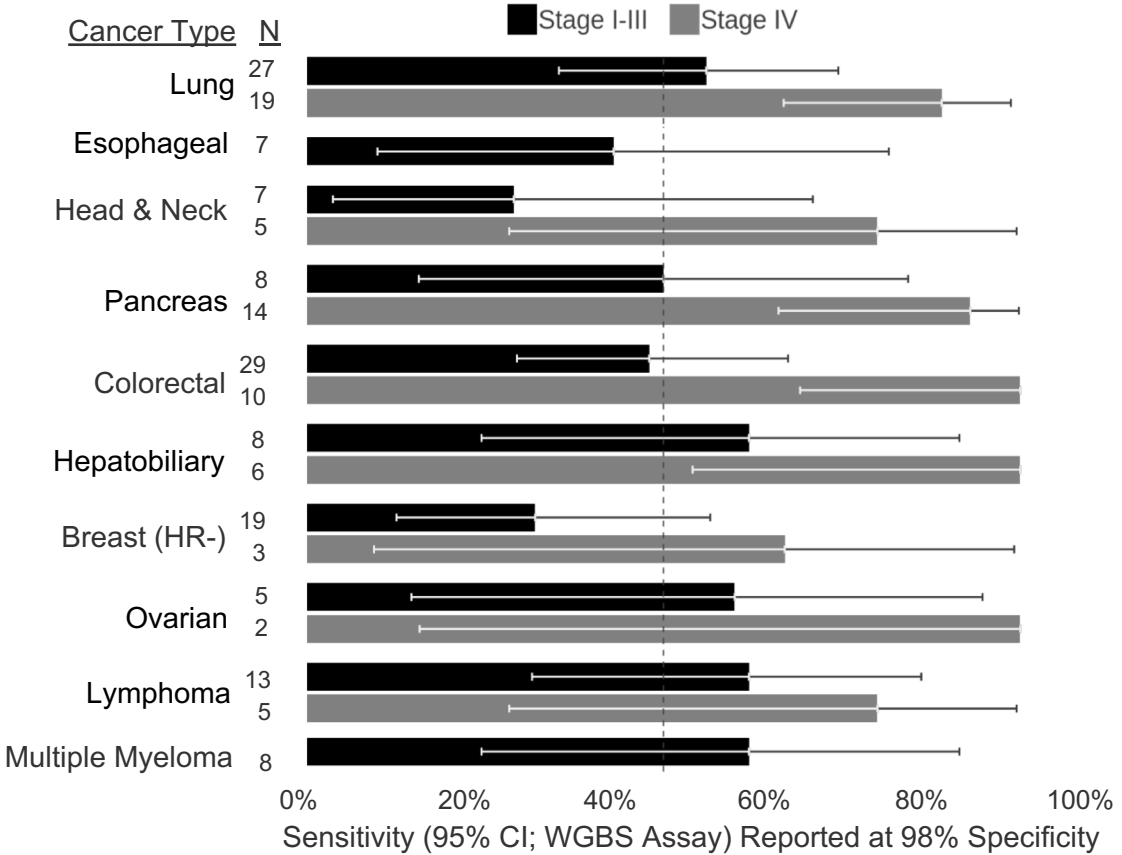
Consistent Results Across Assays and Between Training and Test Sets

- Cancers that had >40% detection in training included lung, HR-negative breast, colorectal, esophageal, head & neck, hepatobiliary, lymphoma, ovarian, and pancreatic cancers, and multiple myeloma



Signal was also consistent in low-signal cancers (<10% in training on any of the three assays: prostate, thyroid, gastric, melanoma).
WGS, whole-genome sequencing; WGBS, whole-genome bisulfite sequencing.

Early- and Late-Stage Cancers Detected in the Test Set

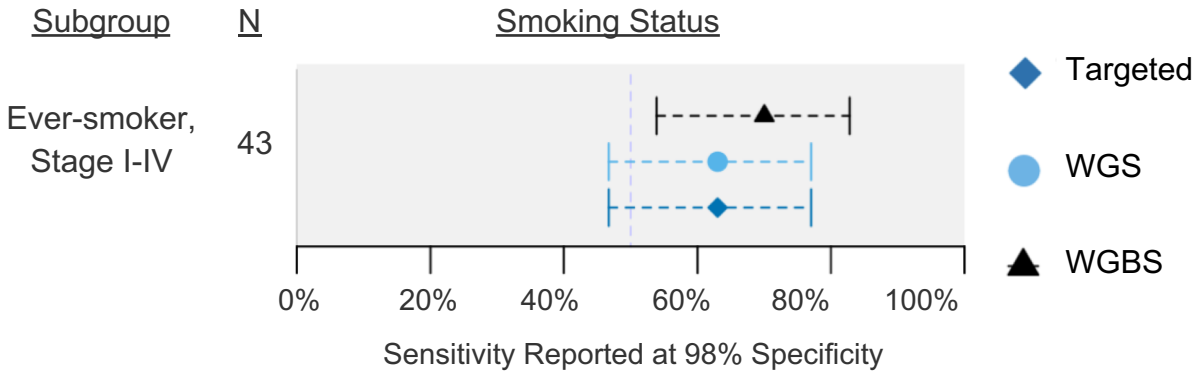


- Detection was observed in across all stages
  - Sensitivity was higher in Stage IV cancers
  - 45% cancers detected were Stages I-II
- Strong detection of cancers with high (>50%) cancer-specific mortality rates at five years[1]

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

Lung Cancer Detection by Smoking Status and Histologic Subtype in the Test Set

- 93% (43/46) of participants with lung cancer were ever-smokers
- Signal was detected in ever-smokers, as well as in never-smokers
  - Of 3 never-smokers, 2 were detected by the methylation assay, 1 by the WGS assay, and 3 by the targeted assay



- Signal was also detected consistently across histologic subtypes (Stage I-IV WGBS assay reported):
  - 100% (5/5) of SCLC cases were detected
  - 65% (11/17) of SCC cases were detected
  - 60% (12/20) of adenocarcinoma cases were detected

WGS, whole-genome sequencing.

Summary

- Preliminary cfDNA-based blood test results detected multiple cancers, including lung, and across all stages—even early stages when treatment may be more effective
  - Test set confirmed the signal observed in the training set
  - >99% specificity is feasible
    - Targeted methods require accounting for clonal hematopoiesis
  - High detection of cancers with high mortality and that lack screening paradigms or where screening is not well-adopted
- This approach is thus promising as a multi-cancer detection test, including for early-stage cancers
- Further assay and clinical development in large-scale clinical studies, including CCGA, is ongoing

WGS, whole-genome sequencing; WGBS, whole-genome bisulfite sequencing.

Acknowledgements

- Study participants who graciously donated their time, energy, and specimens
- CCGA investigators and collaborators for advice, enrolling participants, and collecting data and specimens
    - Principal Investigators from sites enrolling >35 participants in this preplanned substudy: Minetta C. Liu (Mayo Clinic, MN); David Thiel (Mayo Clinic, FL); Rosanna Lapham, MD (Spartanburg Regional Health Services, SC); Donald Richards, MD, PhD (TOPA Tyler, TX, US Oncology Network); Nicholas Lopez, MD (Baptist Health Paducah, KY); Daron G. Davis, MD (Baptist Health Lexington, KY); Mohan Tummala, MD (Mercy Springfield, MO); Peter Yu, MD (Hartford Hospital, CT); Wangjian Zhong, MD (Baptist Health, Louisville, KY); Alexander Parker, MD (Mayo Clinic Jacksonville, FL); Kristi McIntyre, MD (TOPA Dallas Presbyterian, TX, US Oncology Network); Fergus Couch (Mayo Clinic Rochester, MN); Robert Seigel (Bon Secours Greenville, SC); Allen L. Cohn, MD (Rocky Mountain Cancer Center Hale Parkway Denver, CO, US Oncology Network); Alan H. Bryce (Mayo Clinic Phoenix, AZ)
- Advisors and Scientific Advisory Board members for their helpful feedback and advice
- The many GRAIL teams who have worked and continue to work on this study