Plasma Cell-free DNA (cfDNA) Assays for Early Multi-cancer Detection: the Circulating Cell-free Genome Atlas (CCGA) Study

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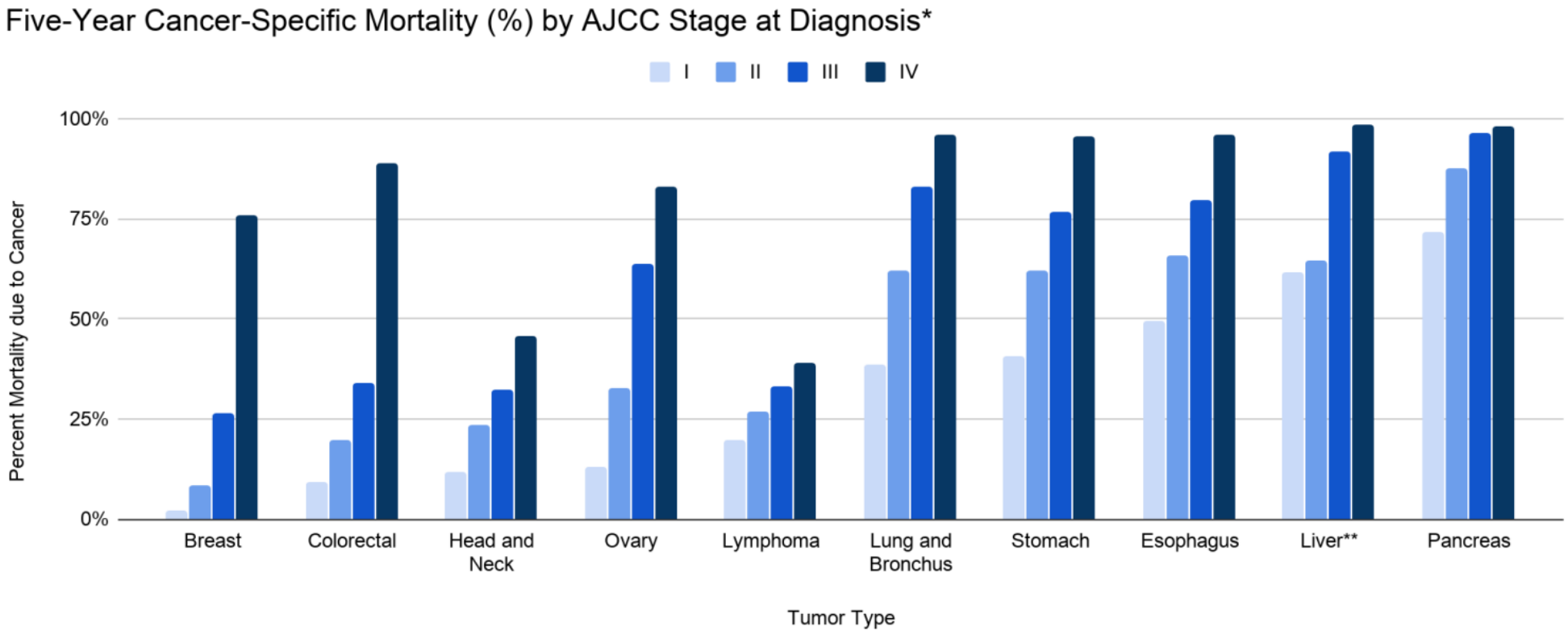
Cancer Mortality Increases with Later Stage



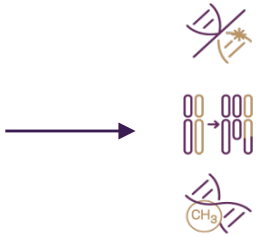
*Cancer specific survival data from SEER18 ages 50+ diagnosed 2006-2015. Surveillance, Epidemiology, and End Results (SEER) Program (www.seer.cancer.gov) SEER*Stat Database: Incidence - SEER 18 Regs Research Data, Nov 2017 Sub. **Includes intrahepatic bile duct.

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 cfDNA, WBCs

Targeted & whole-genome bisulfite sequencing cfDNA

Tissue samples
(cancer only)

Whole-genome sequencing of tumor tissue

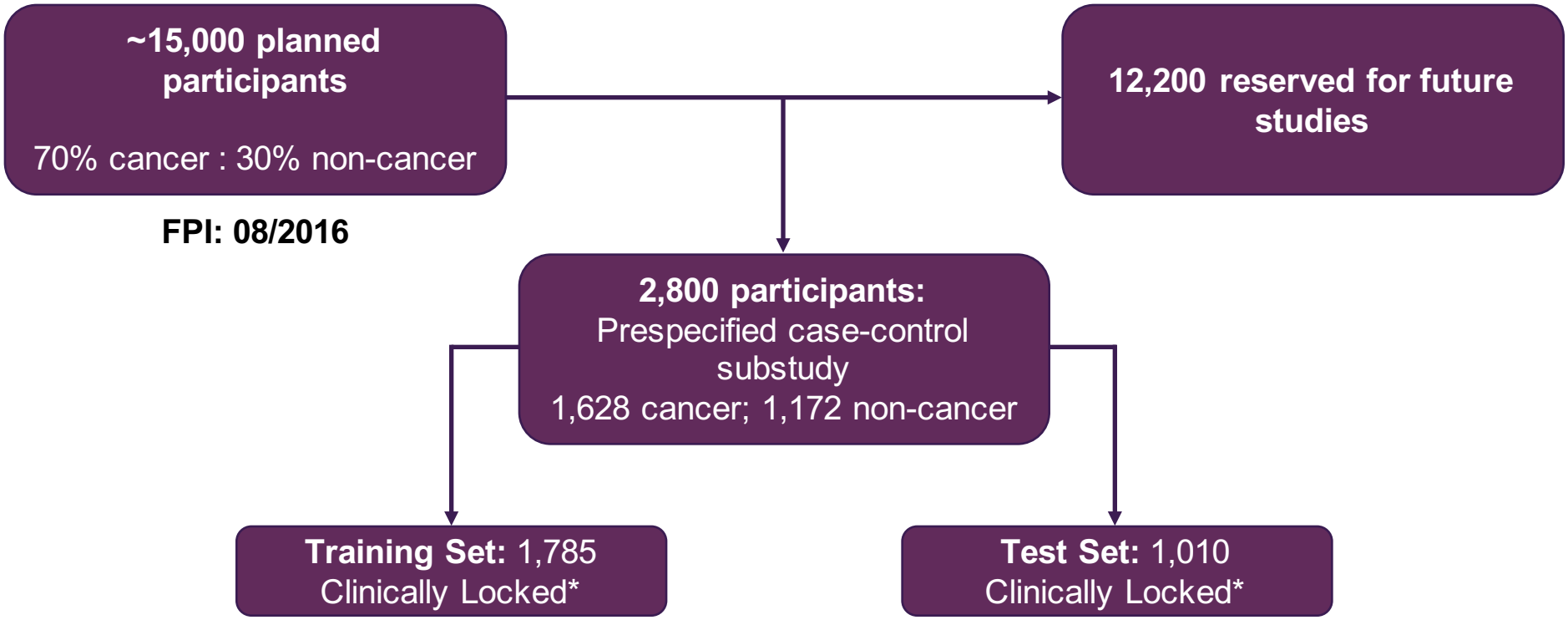
Follow-up for 5 yrs
Vital status & cancer status

People with cancer: Data on treatment, recurrence, mortality

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

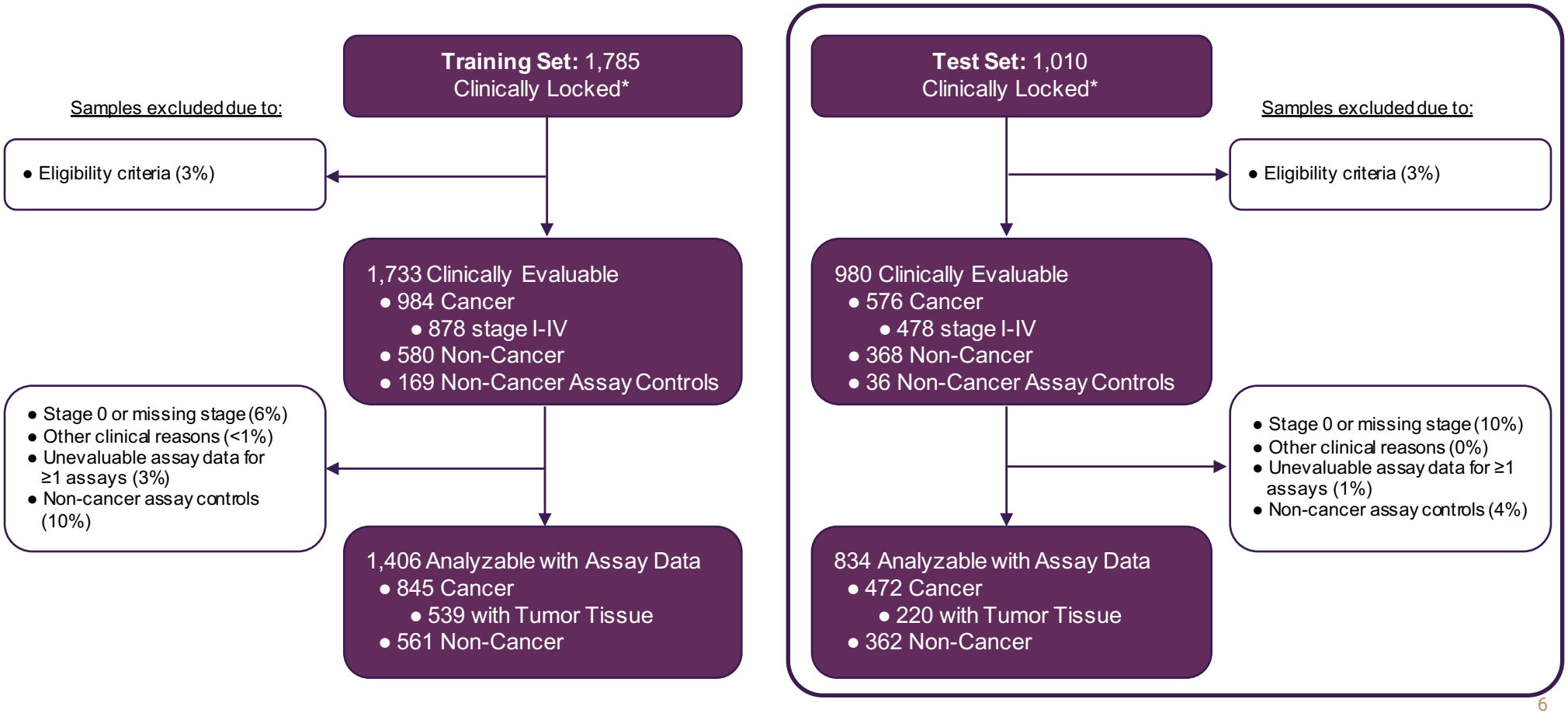
cfDNA, cell-free deoxyribonucleic acid; WBC, white blood cell.

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

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

*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).

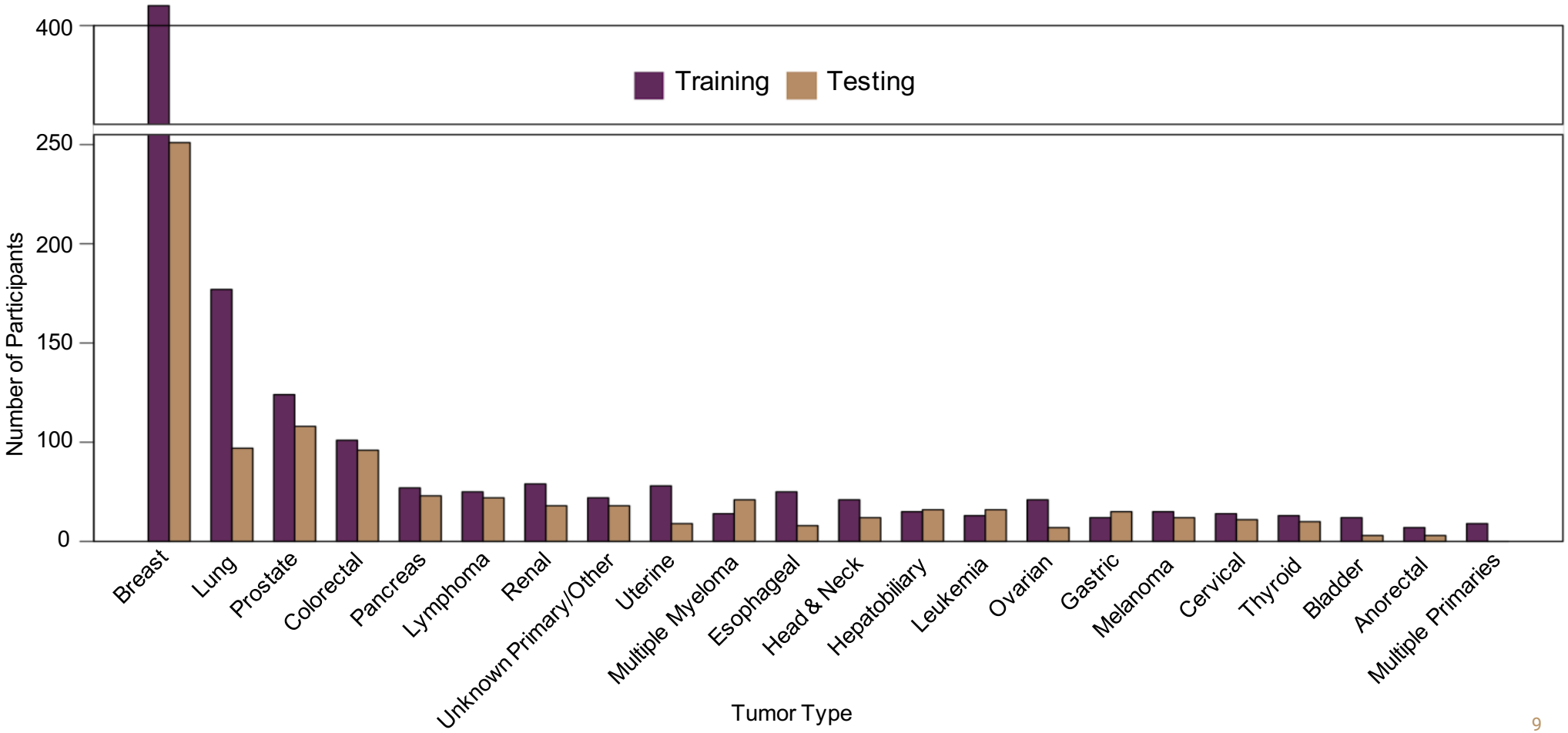
Stage Distribution and Method of Diagnosis were Consistent in Training and Test Sets

Broad distribution of stages in training and in test sets

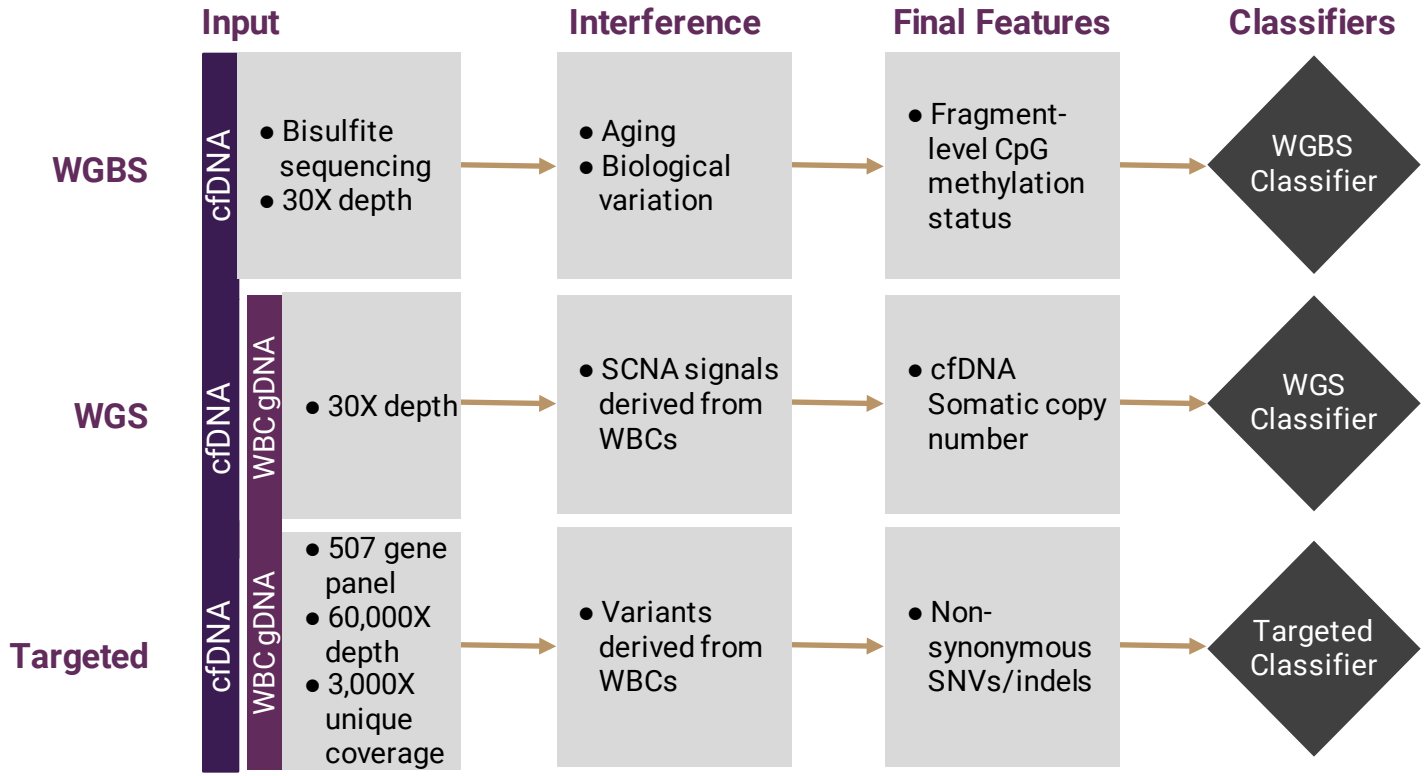
	Cancer, Training Set (n=984)	Cancer, Test Set (n=576)
Overall Clinical Stage (n, %)		
0*	56 (6%)	34 (6%)
I	300 (30%)	165 (29%)
II	249 (25%)	142 (25%)
III	165 (17%)	76 (13%)
IV	164 (17%)	95 (16%)
Non-Informative**	50 (5%)	64 (11%)
Method of Dx (n, %)		
Diagnosed by Screening§	354 (36%)	202 (35%)
Diagnosed by Clinical Presentation¶	630 (64%)	373 (65%)

*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).

Multiple Tumor Types Represented in the CCGA Cohort



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

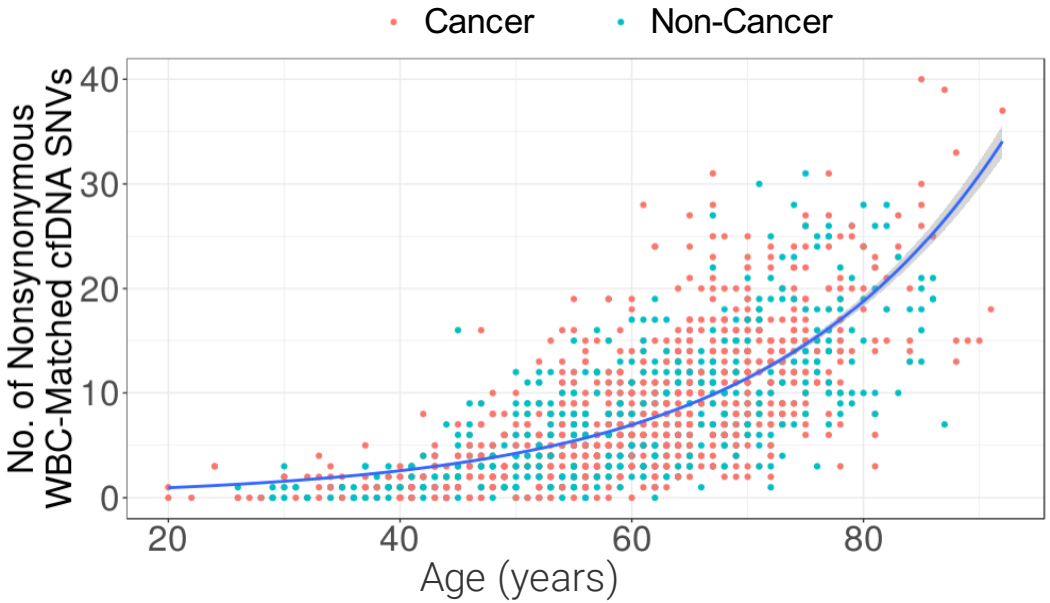


- **All major somatic and epigenetic cfDNA features characterized**

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

Majority of cfDNA Variants Are WBC-Matched Clonal Hematopoiesis

- Non-tumor WBC-matched cfDNA somatic variants (SNVs/indels) accounted for, on average:
  - **98% of all variants in non-cancer group**
  - **71% in cancer group**
- Number of WBC variants is positively associated with age in cancer and non-cancer groups

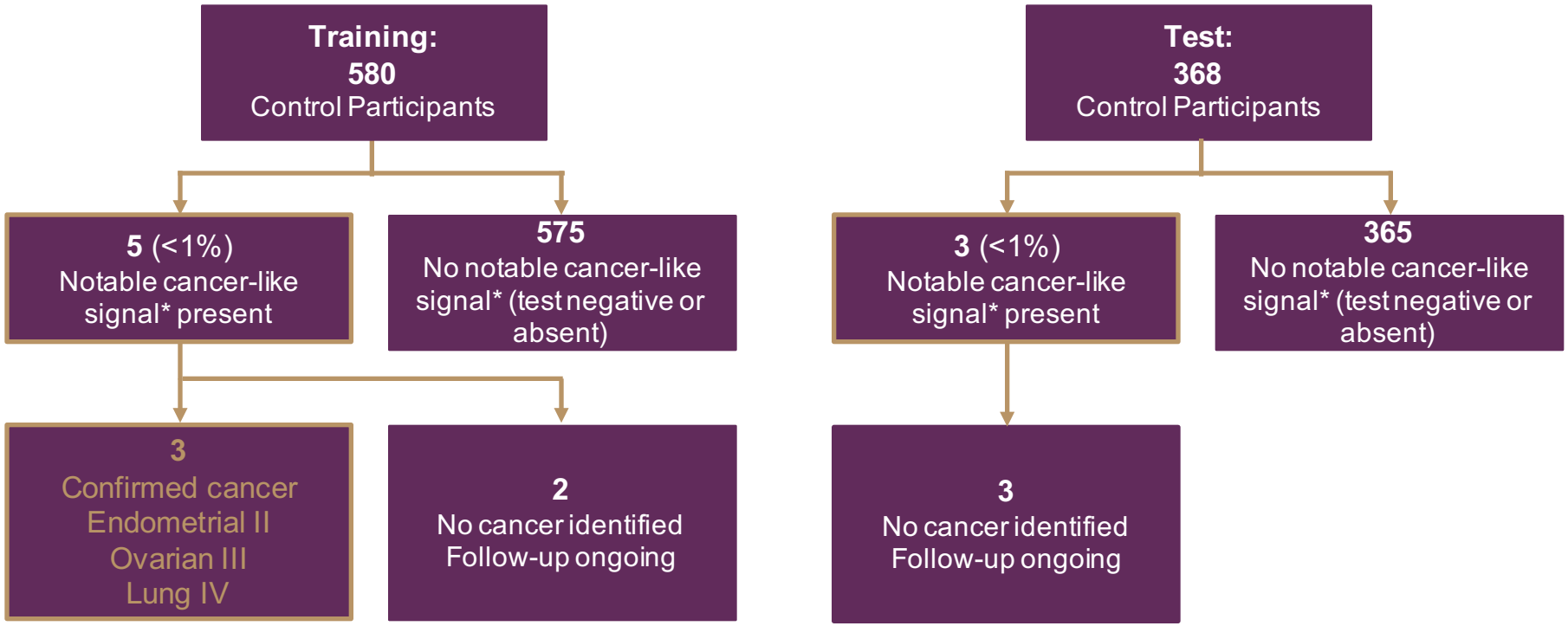


- Majority of CH variants were low variant allele frequency (<1.0%) and private[1]
- Accounting for CH using targeted methods is critical for high specificity

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

High Specificity (>99%) is Feasible

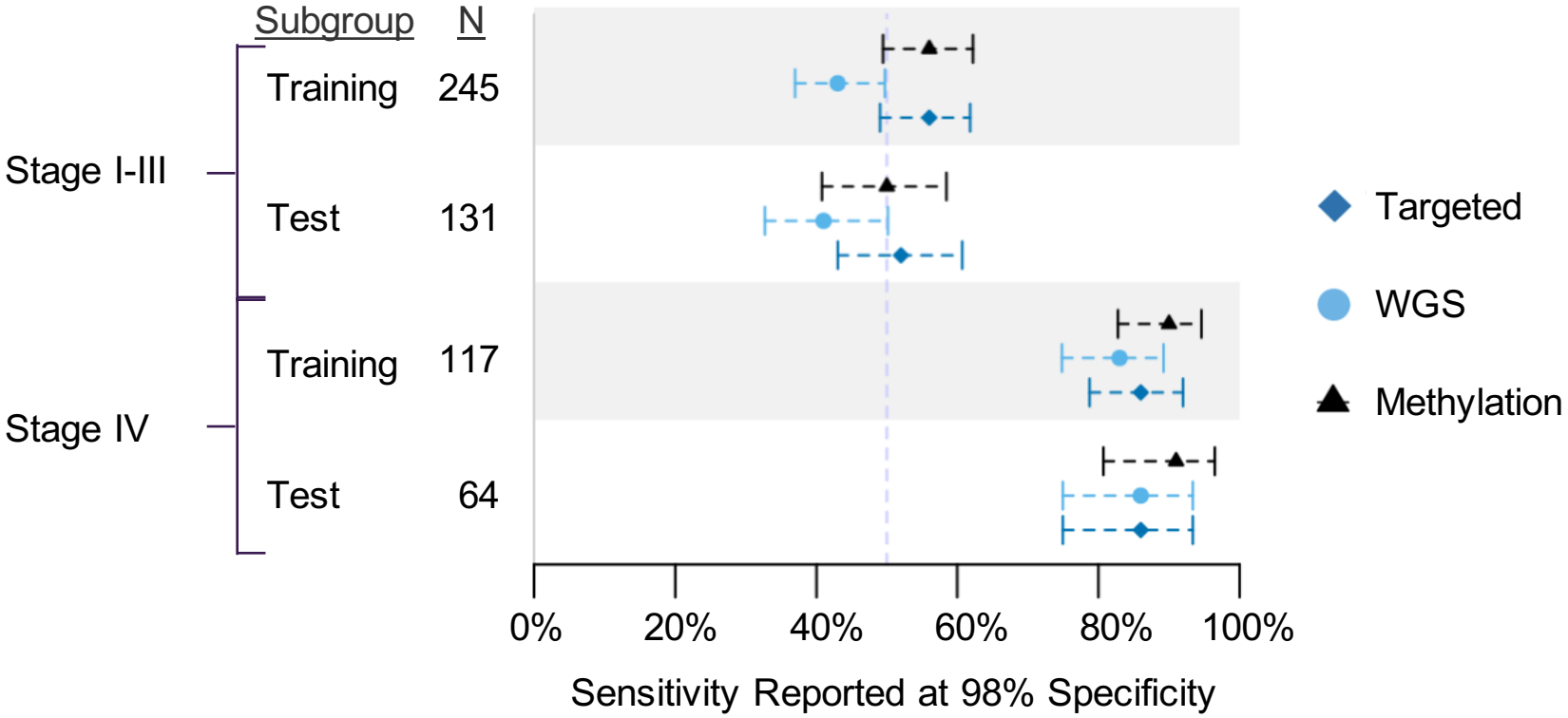
5-year follow-up will enable identification of participants who are subsequently diagnosed



*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.

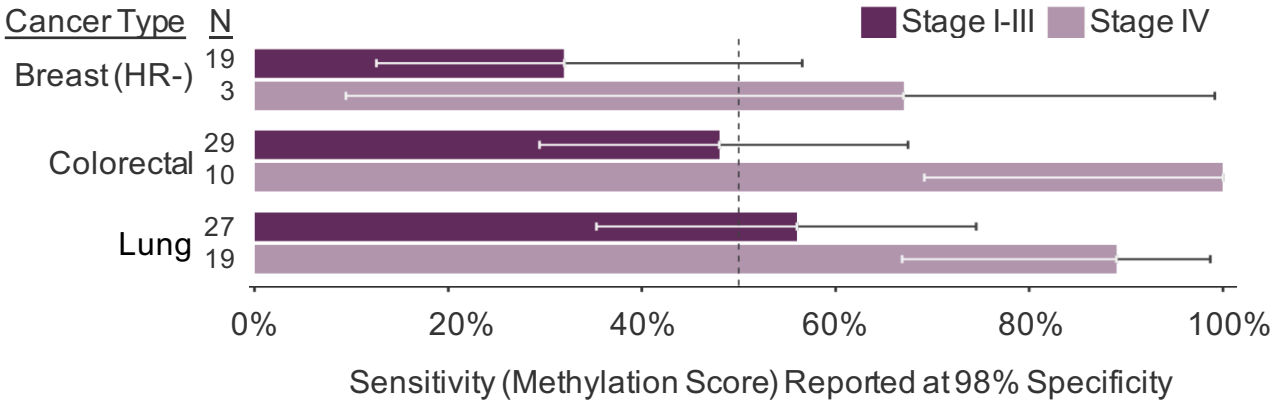
Consistent Results Across Assays and Between Training and Test Sets

- Depicted here: cancers with >50% signal in training: HR-negative breast, colorectal, esophageal, head & neck, hepatobiliary, lung, 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).
cfDNA, cell-free deoxyribonucleic acid; WBC, white blood cell; WGS, whole-genome sequencing; WGBS, whole-genome bisulfite sequencing.

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



- Assays detected later-stage cancers (Stage IV) in cancers with screening paradigms
- Signal also observed in participants with early-stage cancers (Stage I-III)

[1] Pham D et al. J Clin Oncol 36, 2018 (suppl; abstr 6504).

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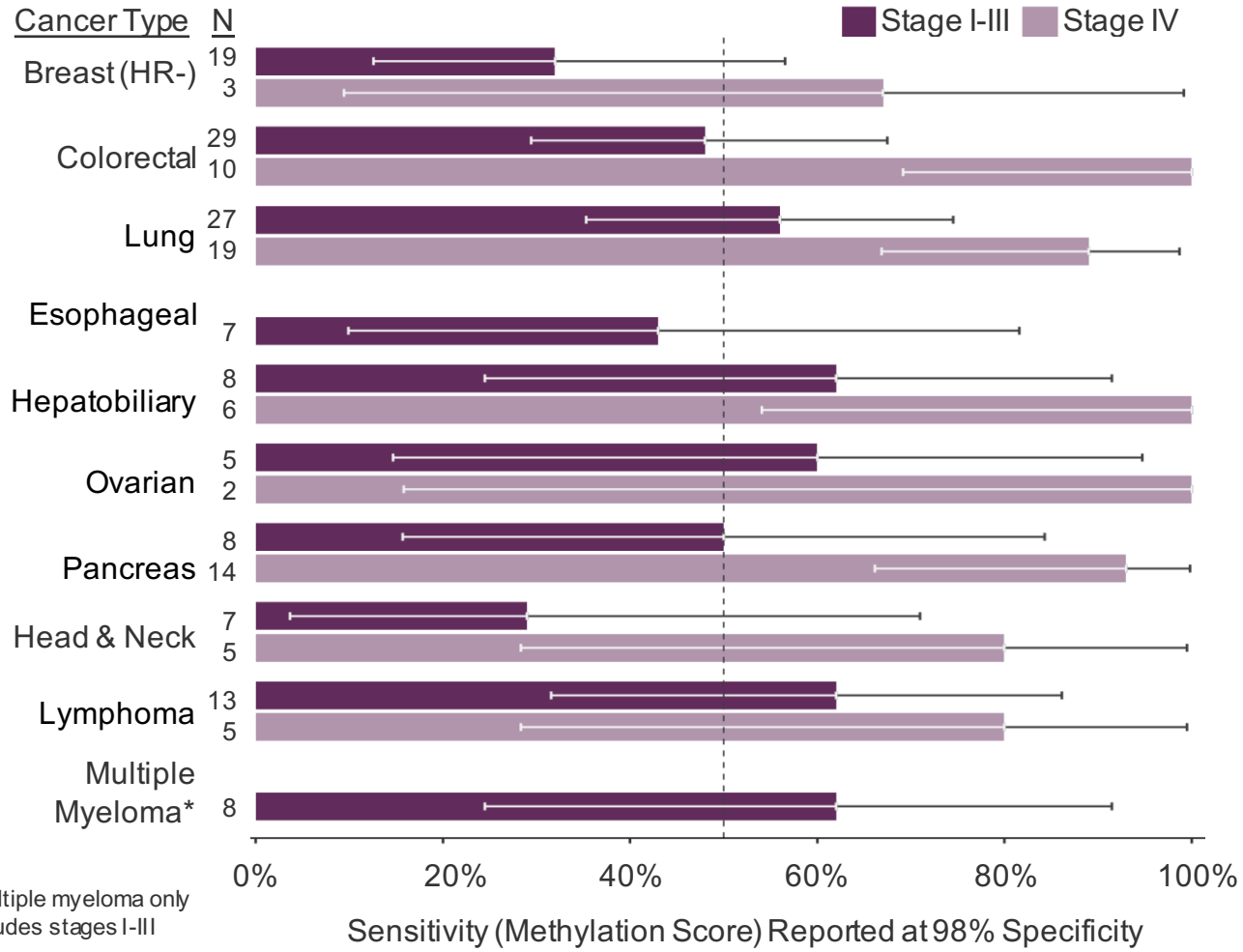

*Multiple myeloma only includes stages I-III

- Assays detected later-stage cancers (Stage IV) in cancers with screening paradigms
- Signal also observed in participants with early-stage cancers (Stage I-III)
  - **45% of cancers were stage I-II**
- Many of these cancers do not have a screening paradigm, or the screening paradigm is not well-adopted (eg, lung[1])

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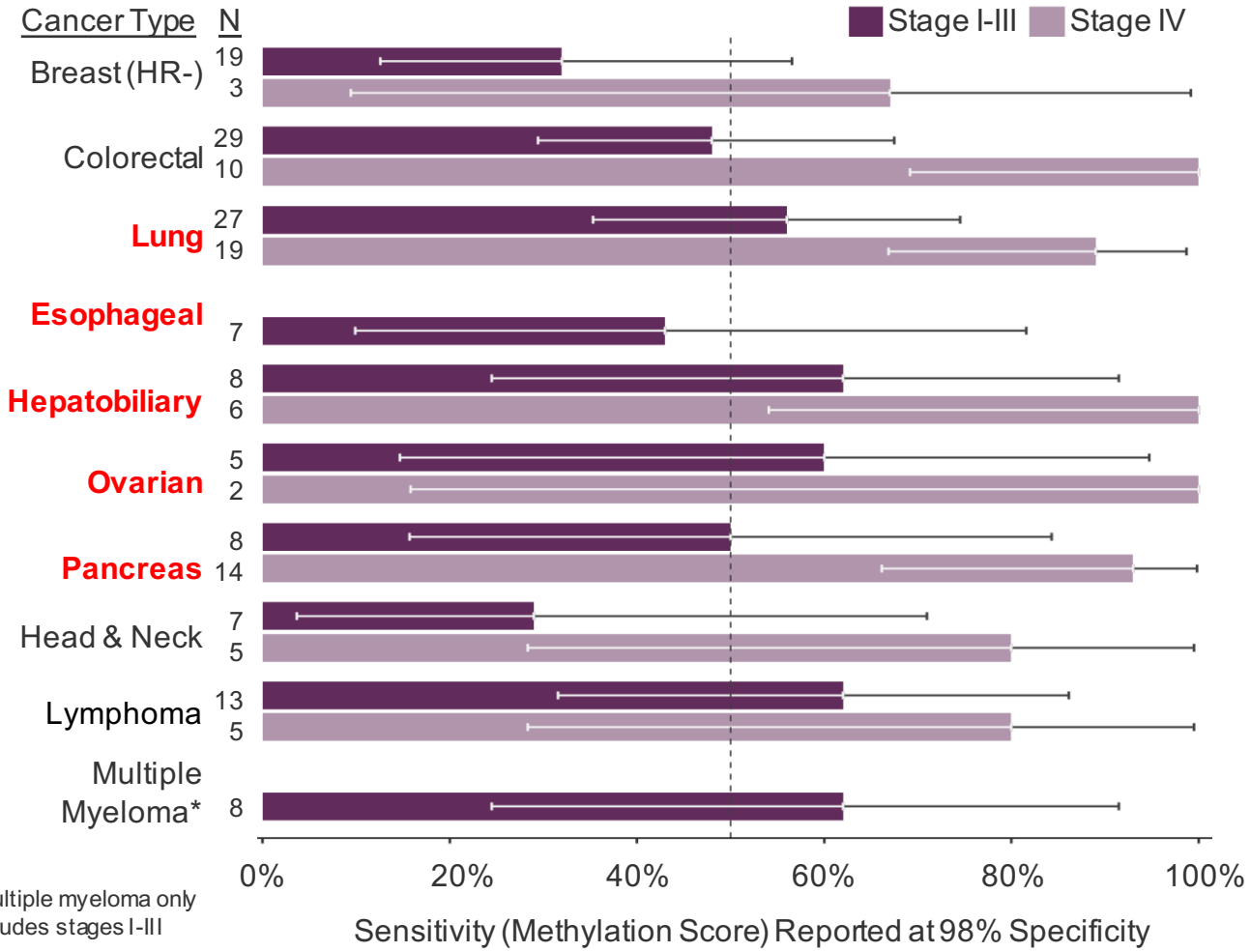

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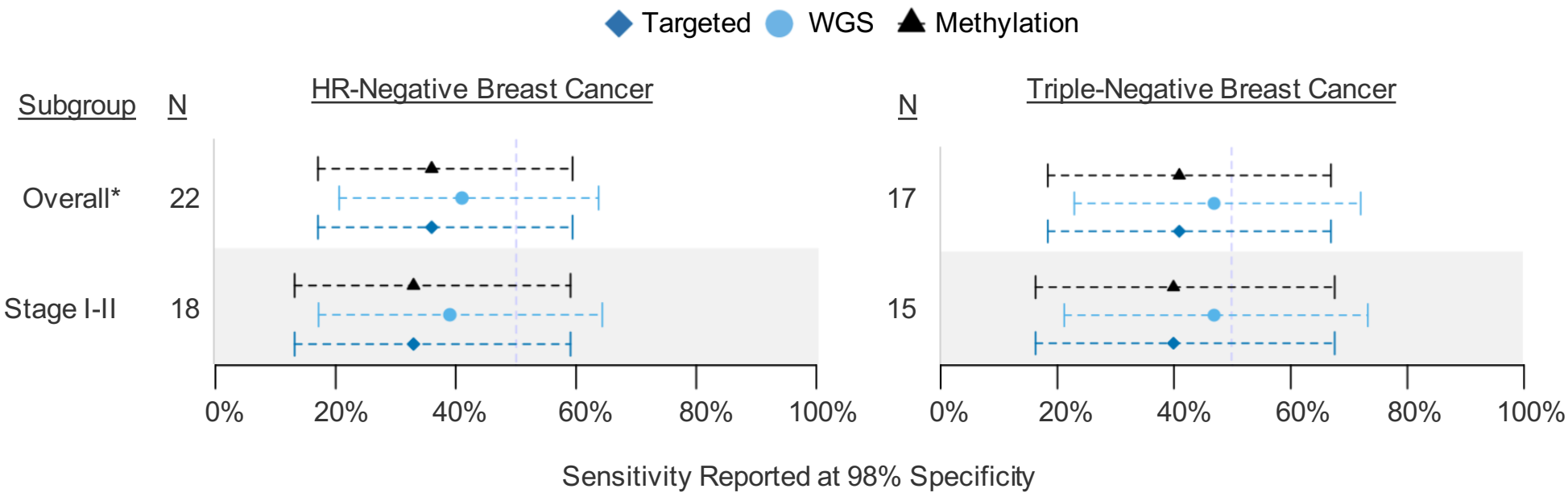
  - **45% of cancers were stage I-II**
- Many of these cancers do not have a screening paradigm, or the screening paradigm is not well-adopted (eg, lung[1])
- **Many of these cancers are associated with a >50% 5-year cancer-specific mortality[2]**

[1] Pham D et al. J Clin Oncol 36, 2018 (suppl; abstr 6504).
[2] 5-year cancer-specific mortality rates for persons aged 50-79 from SEER18, 2010-2014; https://seer.cancer.gov.

HR-Negative Breast Cancer Detection by Stage in the Test Set

- Signal was detected in early-stage (Stage I-II) as well as later-stage (Stage III-IV) HR-negative breast cancers, including TNBC
- 68% (15/22) of HR-negative breast cancers were Stage I or II TNBC

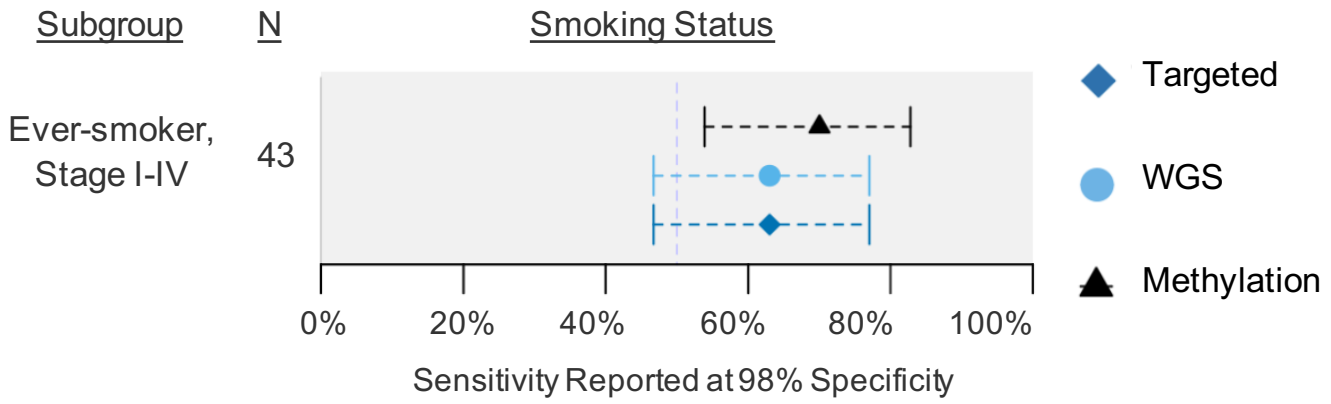


*Of the 4 stage III-IV HR- breast cancers, 2 were detected by each assay. Of the 2 stage III-IV TNBC breast cancers, 1 was detected by all 3 assays.

WGS, whole-genome sequencing; TNBC, triple-negative breast cancer.

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



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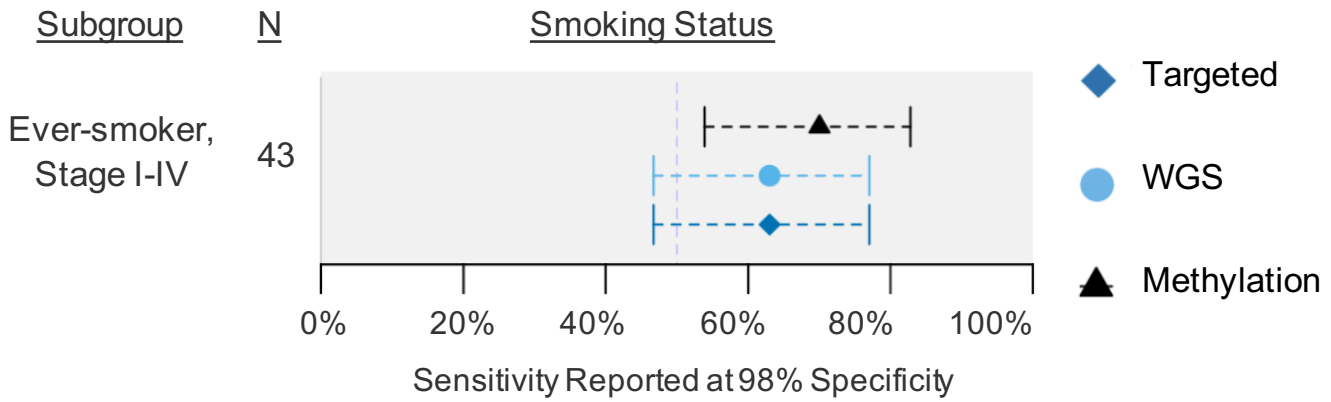
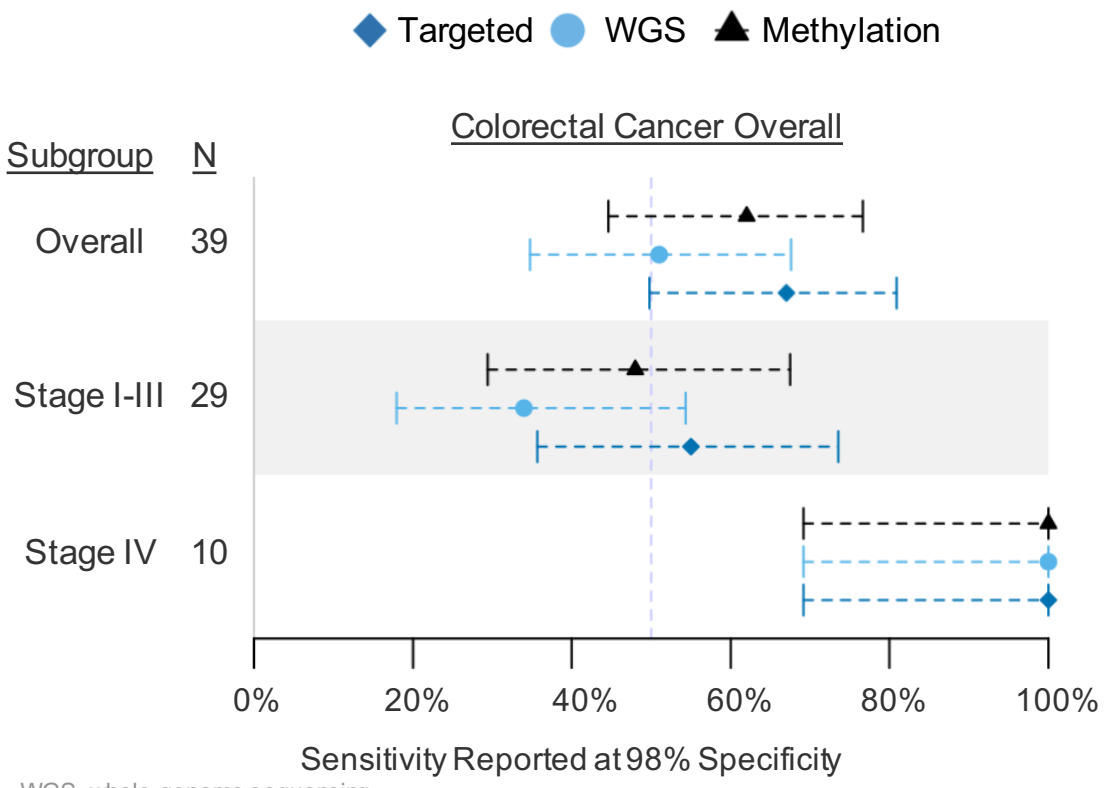


- Signal was also detected consistently across histologic subtypes (Stage I-IV methylation assay reported):
  - 100% (5/5) of SCLC cases were detected
  - 65% (11/17) of SCC cases were detected
  - 60% (12/20) of adenocarcinoma cases were detected

WGS, whole-genome sequencing.

Colorectal Cancer Detection by Stage and Location

- Signal was detected in early-stage (Stage I-III) as well as later-stage (Stage IV) colorectal cancers

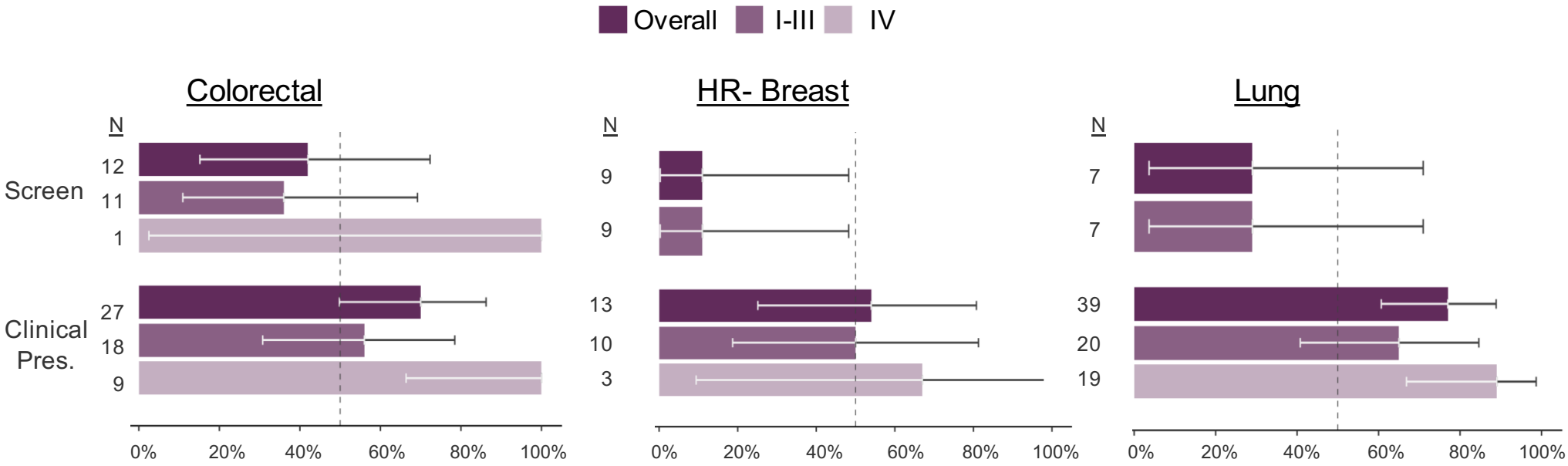


- Signal was also consistently detected across locations in colorectal cancer (methylation assay reported):
  - Rectum: 63% (10/16)
  - Left: 63% (5/8)
  - Right: 60% (6/10)
  - Other: 60% (3/5)

WGS, whole-genome sequencing.

Signal Observed in Participants with Screen-Detected Cancers and Cancers Detected by Clinical Presentation*

- Signal was generally stronger in cancers detected by clinical presentation



Sensitivity (Methylation Score) Reported at 98% Specificity

*Cancers detected by "clinical presentation" include all cancers not detected by screening (ie, detected symptomatically or as incidental findings).

Conclusions

- A cfDNA-based blood test detected multiple cancers across all stages, including at 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
- 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

cfDNA, cell-free DNA.

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- CCGA investigators and collaborators for advice, enrolling participants, and collecting data and specimens
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