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Multi-cancer detection of early-stage cancers with simultaneous tissue localization using a plasma cfDNA-based targeted methylation assay

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DISCLOSURE INFORMATION

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- Consulting fees from AstraZeneca; Sysmex; Takeda; Boehringer-Ingelheim; Inivata; Genentech; Loxo; DropWorks; GRAIL, Inc.; Janssen; Illumina.
- Honoraria from Chugai, BioRad, Guardant, Foundation Medicine.
- Licensing fees from MolecularMD paid to my institution.
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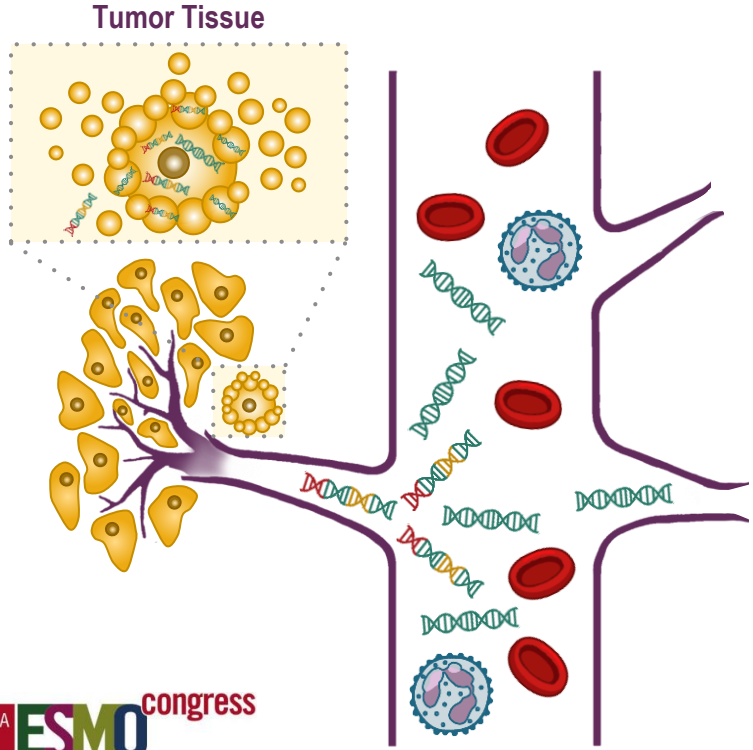
- Chair, Scientific Leadership Board, GO2 Lung Cancer Foundation.

Cancer is a Disease of the Genome

Tumors shed cell-free DNA into the blood, carrying signals specific to cancer



Hallmarks of cancer DNA in the blood:

- Mutations (Single Base Changes)
- Chromosome Alterations (Copy Number)
- DNA Methylation Patterns (Chemical Modification)

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Adapted from Crowley E, Di Nicolantonio F, Loupakis F, Bardelli A. Liquid biopsy: monitoring cancer-genetics in the blood. *Nature reviews Clinical oncology*. 2013; **10**(8):472-84.

Requirements for Multi-cancer Test for Use at Population Scale

Benefits of early detection while minimizing harms:

- **Low false positive rate:** achieved through high specificity
- **Localizing ability:** ability to identify anatomic location to direct appropriate diagnostic work-up
- **Limited over-diagnosis:** preferential detection of clinically significant cancers

Demonstrate test performance, reproducibility, and generalizability to population:

Pre-specified statistical analyses to reduce bias

Assessment of performance in an independent test set

Inclusion of potentially confounding conditions to ensure specificity

Multiple study sites for demographic diversity

Evaluation of performance in population scale studies with people with no known diagnosis

The Circulating Cell-free Genome Atlas (CCGA) Study: Supporting Development of a Multi-Cancer Test

Prospective, observational, longitudinal, case-control study for discovery, training, and validation of multi-cancer test

15,254 participants
with & without cancer

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142 sites

Fully Enrolled

Blood samples
(from all participants)

Tissue samples
(cancer only)

Follow-up for 5 years
(vital status & cancer status)

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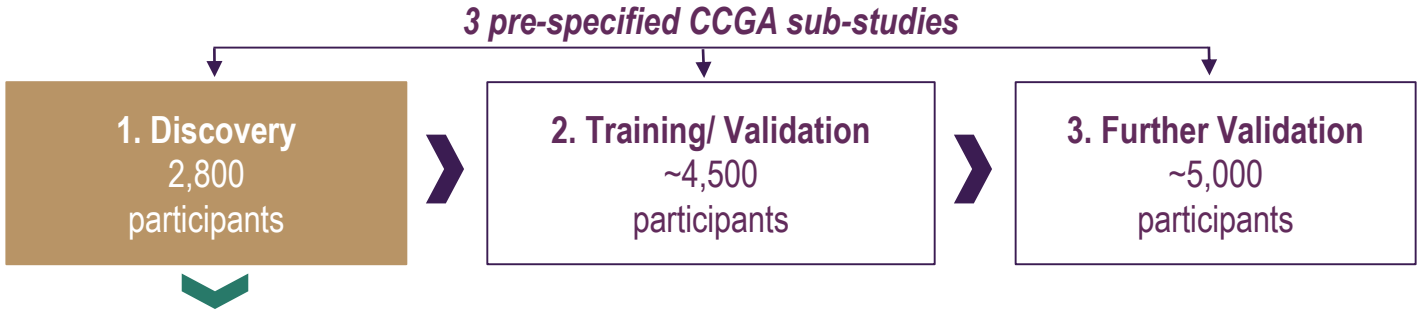
The CCGA Study: Targeted Methylation Assay for Further Development

Pre-specified studies for discovery and validation



Discovery phase completed 2018[1,2]

3 prototype assays detecting mutations, CNVs, DNA methylation

Demonstrated feasibility of multi-cancer detection with low false positive rate (~2%)

Methylation assay selected for further development

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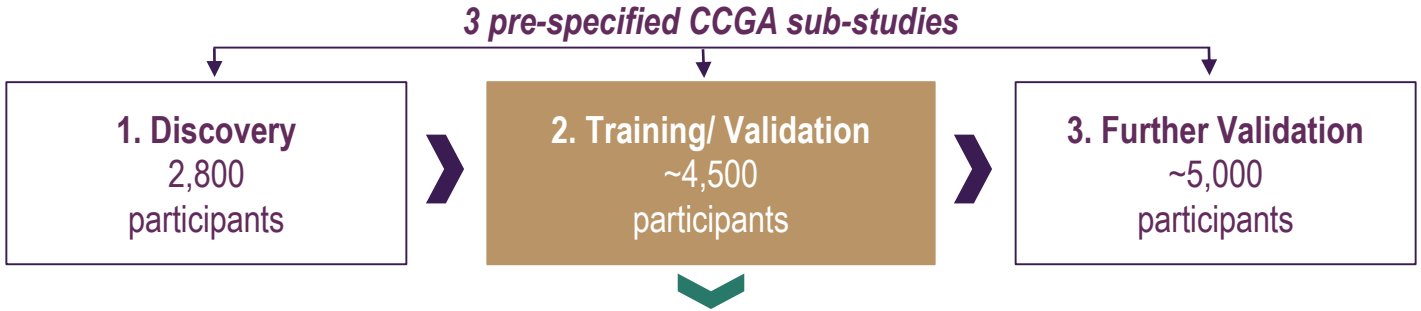
1. Klein EA, *et al. J Clin Oncol* 2018;36(15_suppl):12021. 2. Liu MC, *et al. J Clin Oncol* 2018;36(15_suppl):536.

The CCGA Study: Targeted Methylation Assay for Further Development

Pre-specified studies for discovery and validation



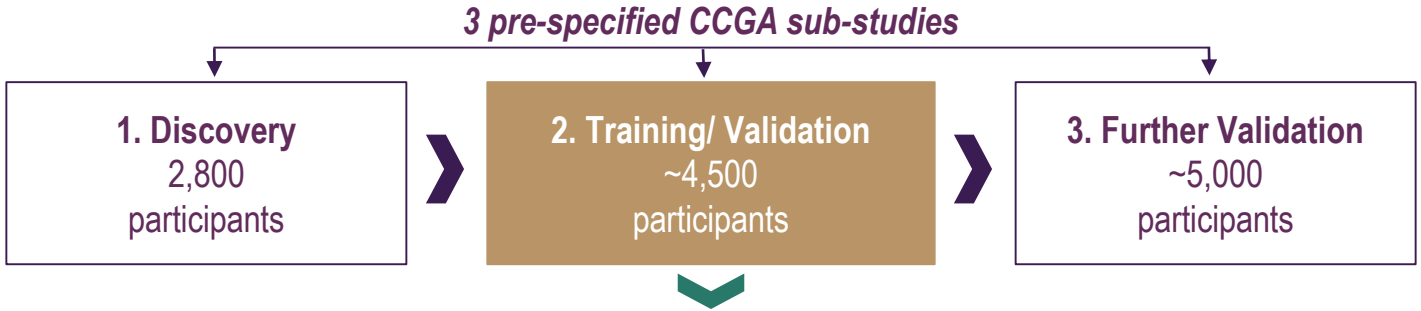
A **targeted methylation assay** was developed targeting key informative genomic regions

Here, we report training and internal cross-validation

Assessment of an independent validation set is ongoing

The CCGA Study: Targeted Methylation Assay for Further Development

Pre-specified studies for discovery and validation



14 pre-specified cancers types:

Anorectal, hormone receptor-negative breast, colorectal, esophagus, gallbladder, gastric, head and neck, hepatobiliary, lung, lymphoid neoplasm, lymphoma, multiple myeloma, ovary, pancreas

These cancer types account for ~63% of US cancer deaths[1]

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[1]Surveillance, Epidemiology, and End Results (SEER) Program SEER*Stat Database: Incidence - SEER 18 year 2016 data based on the November 2018 submission.

Target Selection using Machine Learning Algorithm

Targeted methylation panel developed through generation and analysis of an extensive database of plasma and tissue methylation patterns

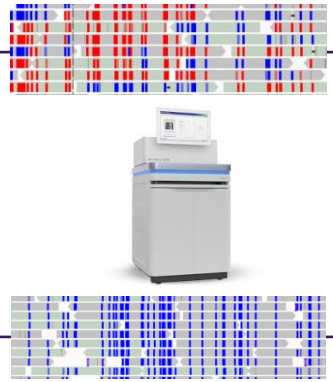
4000 CCGA Cancer
cfDNA Methylation Sequence Data
(+1000 Tissue)

20+ Cancer Types
Early and Late Stage

2000 CCGA Non-Cancer
cfDNA Methylation Sequence Data

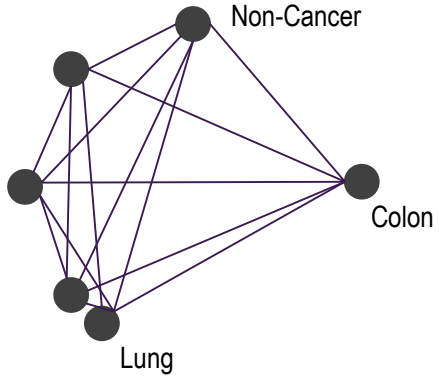
Matched on age distribution by gender in CCGA1 Discovery cohort

Non-Cancer Conditions: Metabolic, Hematological, Inflammatory, Auto-Immune



Public Sequence Data
The Cancer Genome Atlas
Oncoviruses

Machine Learning Algorithm



Multi-cancer and Tissue of Origin (TOO) Targets

Pre-specified TOO classes:

1	Head and neck
2	Upper gastrointestinal
3	Colorectum
4	Anorectum
5	Hepatobiliary
6	Pancreas
7	Lung
9	Melanoma
10	Breast
11	Cervix
12	Uterus
13	Ovary
14	Prostate
15	Kidney
16	Bladder and urothelium
18	Thyroid
19	Myeloid neoplasms
20	Lymphoid neoplasms
21	Multiple myeloma
22	Indeterminate localization

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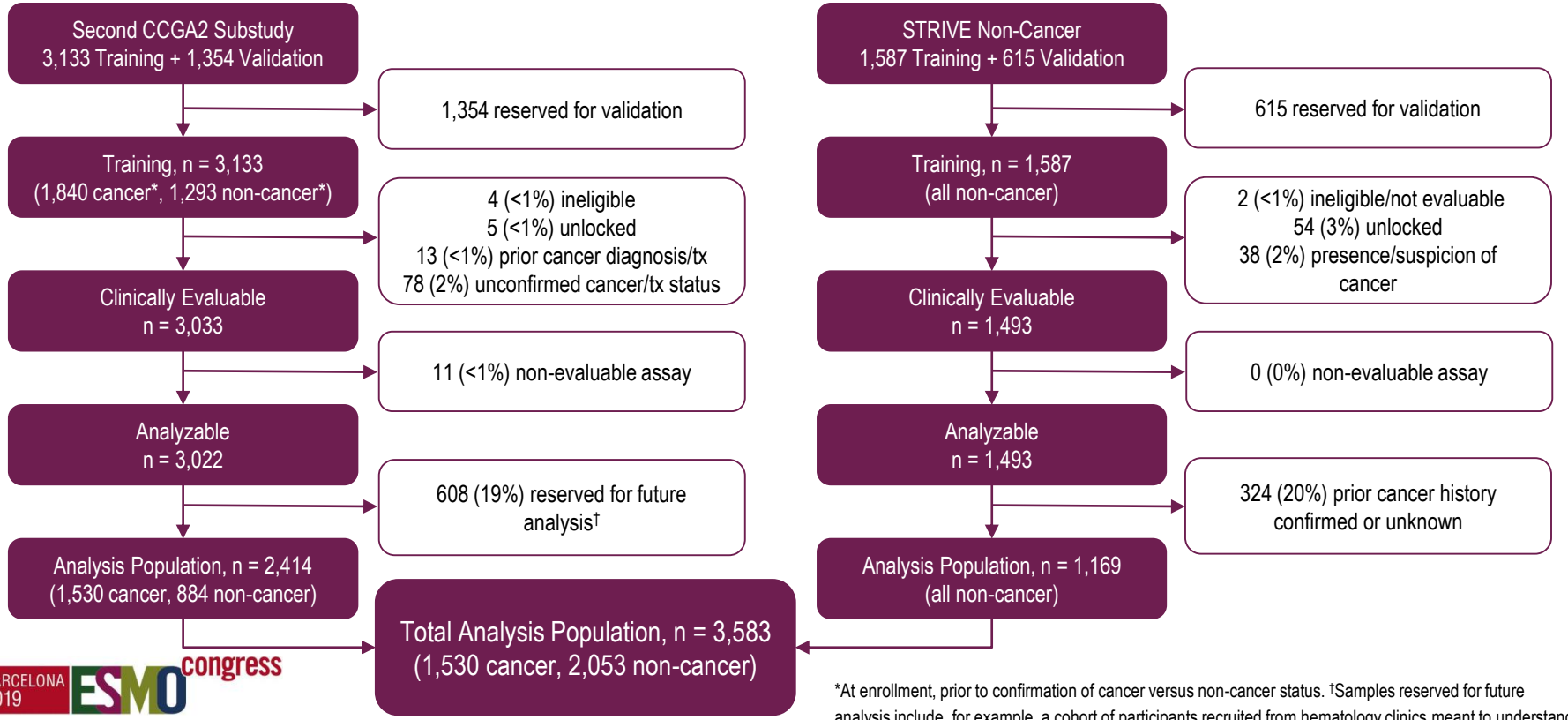
Cancer and Non-Cancer Participant Disposition

To reach >99% specificity with >90% confidence, additional non-cancer samples were incorporated from an independant observational cohort study



*At enrollment, prior to confirmation of cancer versus non-cancer status. †Samples reserved for future analysis include, for example, a cohort of participants recruited from hematology clinics meant to understand cfDNA signal in premalignant or other hematologic conditions.

Comparable Cancer and Non-Cancer Groups

Total (N=3,133)	Second CCGA Substudy		STRIVE
	Cancer N = 1,530	Non-Cancer N = 884	Non-Cancer N = 1,160
Age, Mean ± SD	62.1 ± 12.0	54.3 ± 13.6	60.6 ± 9.6
Female, N (%)	763 (49.9%)	585 (66.2%)	1,169 (100%)
Race/Ethnicity, N (%)			
White, Non-Hispanic	1,263 (83.1%)	719 (81.4%)	1,017 (87.7%)
Black, Non-Hispanic	105 (6.9%)	66 (7.5%)	7 (<1%)
Hispanic, Asian, Other	152 (10.0%)	98 (11.1%)	136 (11.7%)
Never-smoker, N (%)	679 (45.2%)	500 (57.3)	716 (62.5%)
BMI, Normal/Underweight, N (%)	415 (27.1%)	218 (24.7%)	493 (42.2%)

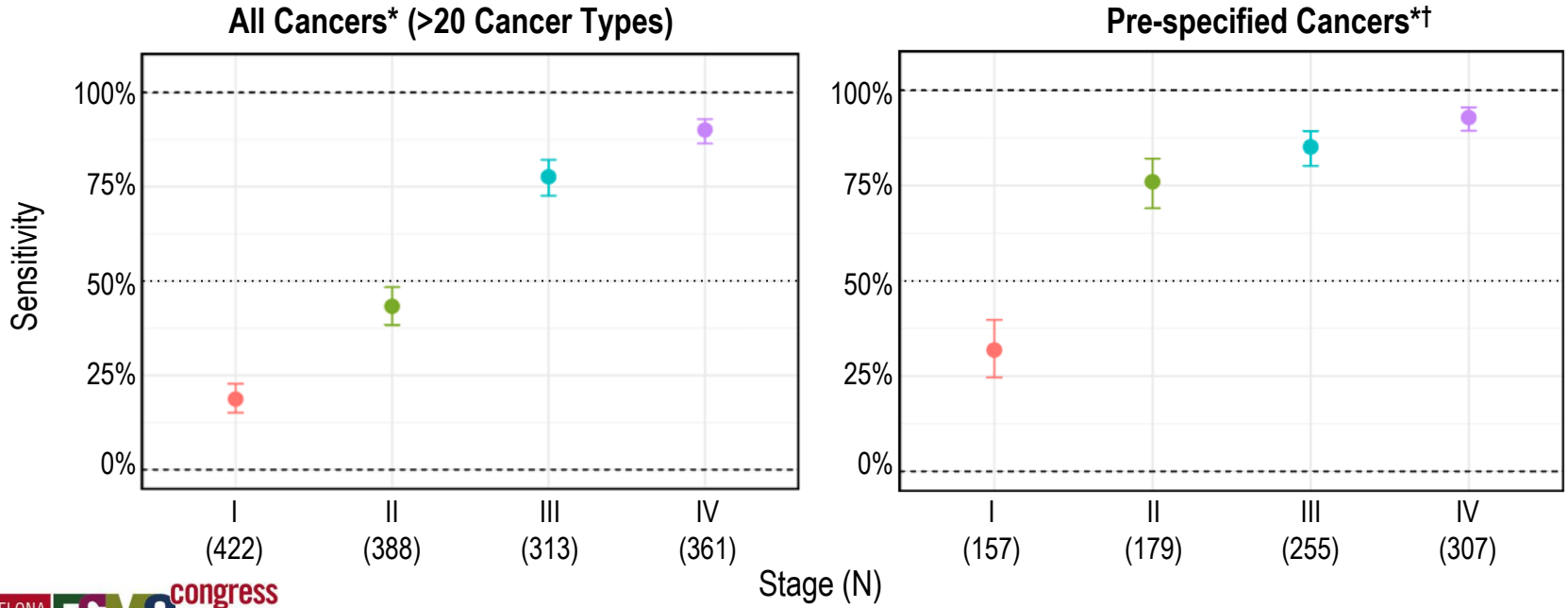
Broad Cancer Stage Distribution

Total (N=3,133)	Second CCGA Substudy		STRIVE
	Cancer N = 1,530	Non-Cancer N = 884	Non-Cancer N = 1,160
Clinical Staging, N (%)			
Stage I	422 (27.6%)	--	--
Stage II	388 (25.4%)	--	--
Stage III	313 (20.5%)	--	--
Stage IV	361 (23.6%)	--	--
Unstaged*	46 (3.0%)	--	--
Method of Diagnosis, N (%)			
Screening	367 (24.0%)	--	--

*Includes leukemia and brain cancer.

Cancers Detected at Early and Late Stages at 99.4% Specificity

- 54.7% (95% CI: 52.2-57.2%) overall sensitivity (>20 cancer types)
- 75.8% (72.9-78.5%) sensitivity in pre-specified† cancer types
- Single fixed false-positive rate (99.4% specificity) across >20 cancer types



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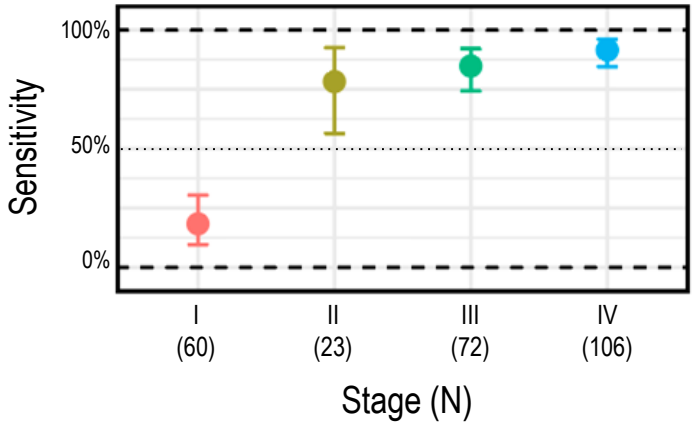
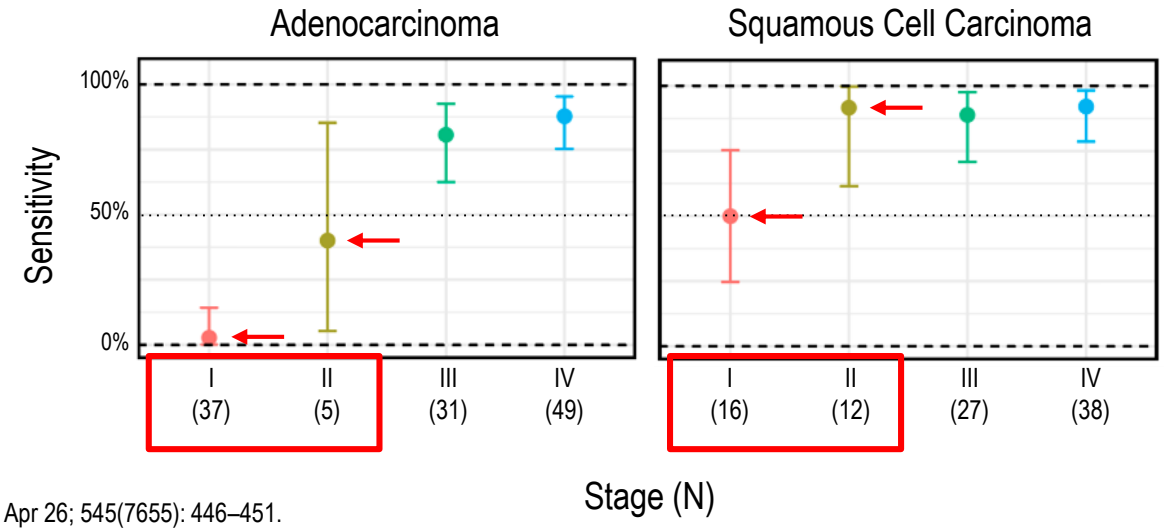
*Plot excludes unstaged cancers. †Includes anorectal, hormone receptor-negative breast, colorectal, esophagus, gallbladder, gastric, head and neck, hepatobiliary, lung, lymphoid neoplasm, lymphoma, multiple myeloma, ovary, and pancreas.

Lung Cancer Detection Varies by Subtype at 99.4% Specificity



- Overall lung cancer sensitivity: 71.6% (95% CI: 65.8-77.0%)

- Detection rate affected by early-stage adenocarcinomas
  - Detection higher in squamous cell carcinoma
- Consistent with prior report showing ctDNA detection was higher in squamous cell carcinoma than adenocarcinoma[1]



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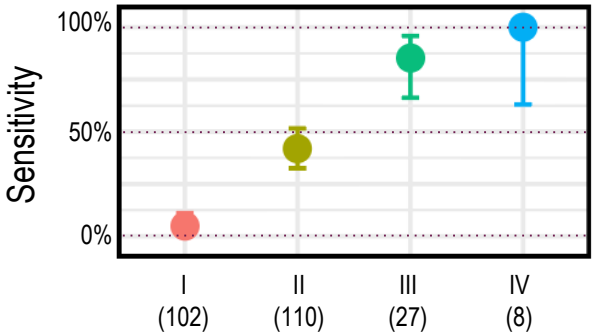
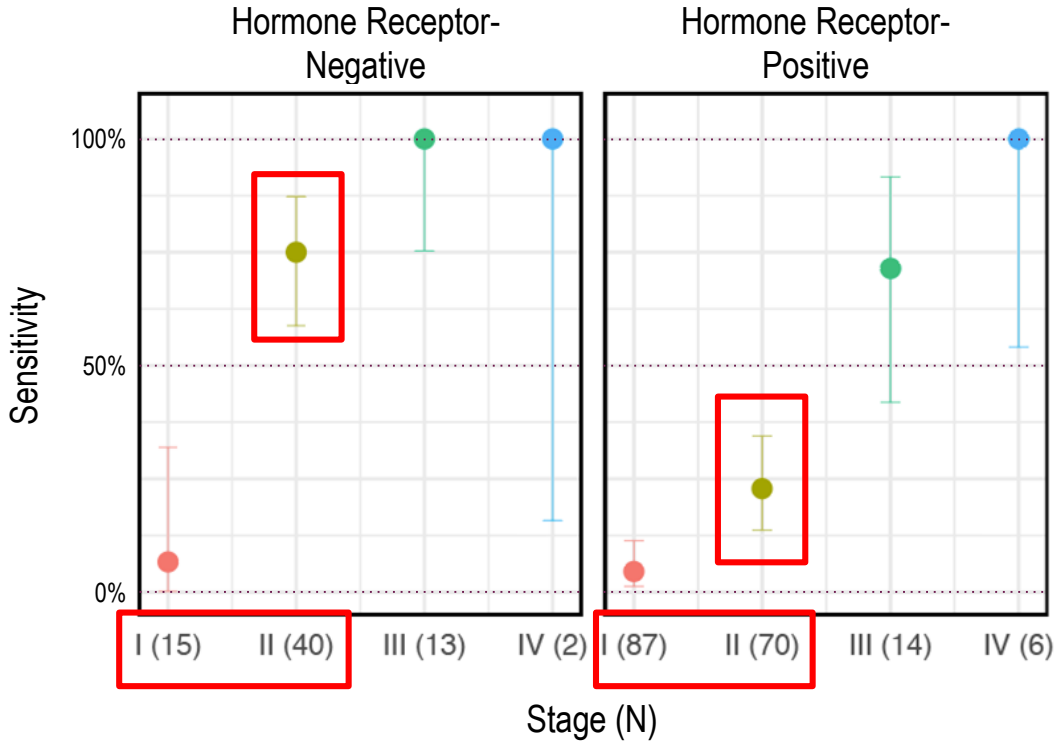
[1]Abbosh C, *et al. Nature.* 2017 Apr 26; 545(7655): 446–451.

Breast Cancer Detection Varies by Subtype at 99.4% Specificity



- Overall breast cancer sensitivity: 33.2% (95% CI: 27.4-39.4%)
- Detection rate affected by preponderance of participants with early-stage hormone receptor-positive breast cancer



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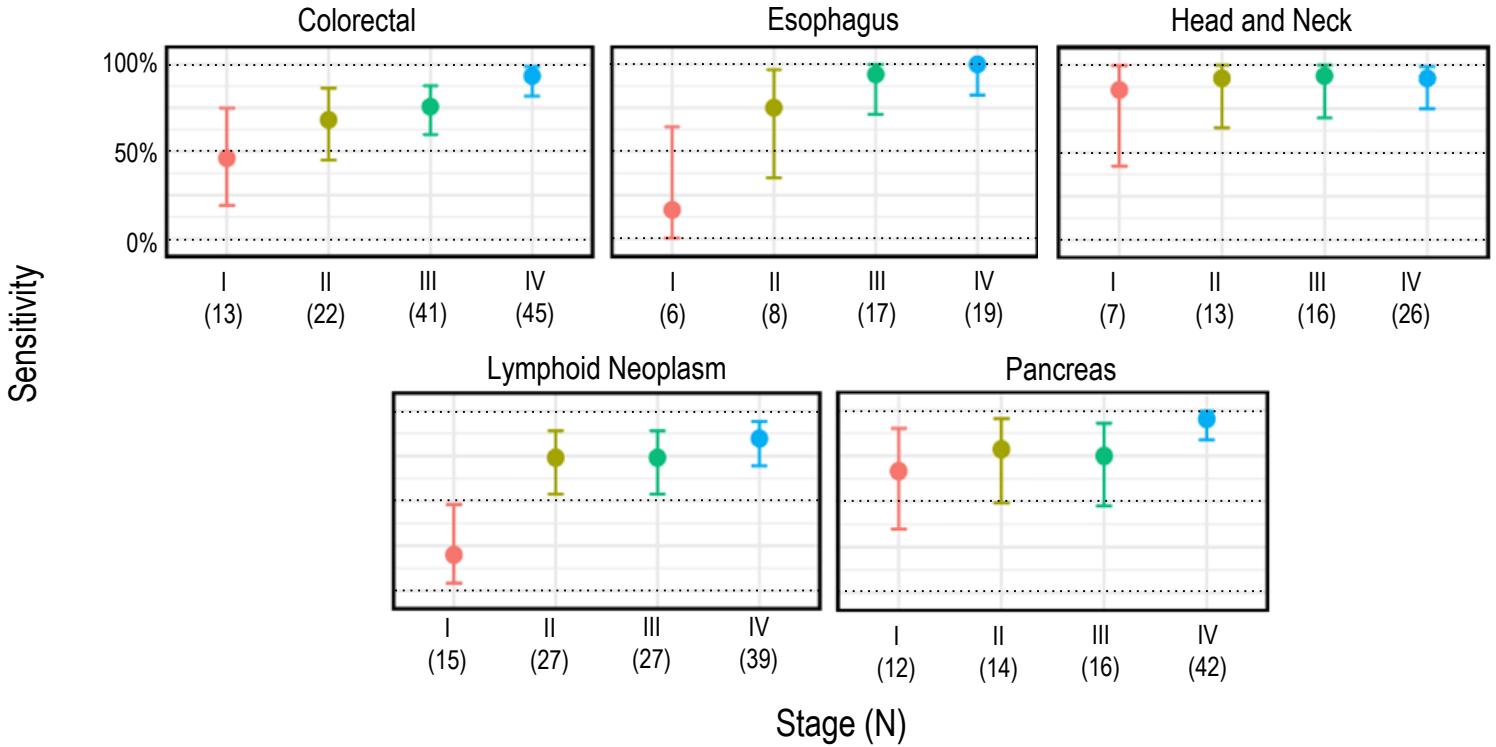
Additional Pre-Specified Cancer Detection* at 99.4% Specificity

Many cancer types lack screening paradigms



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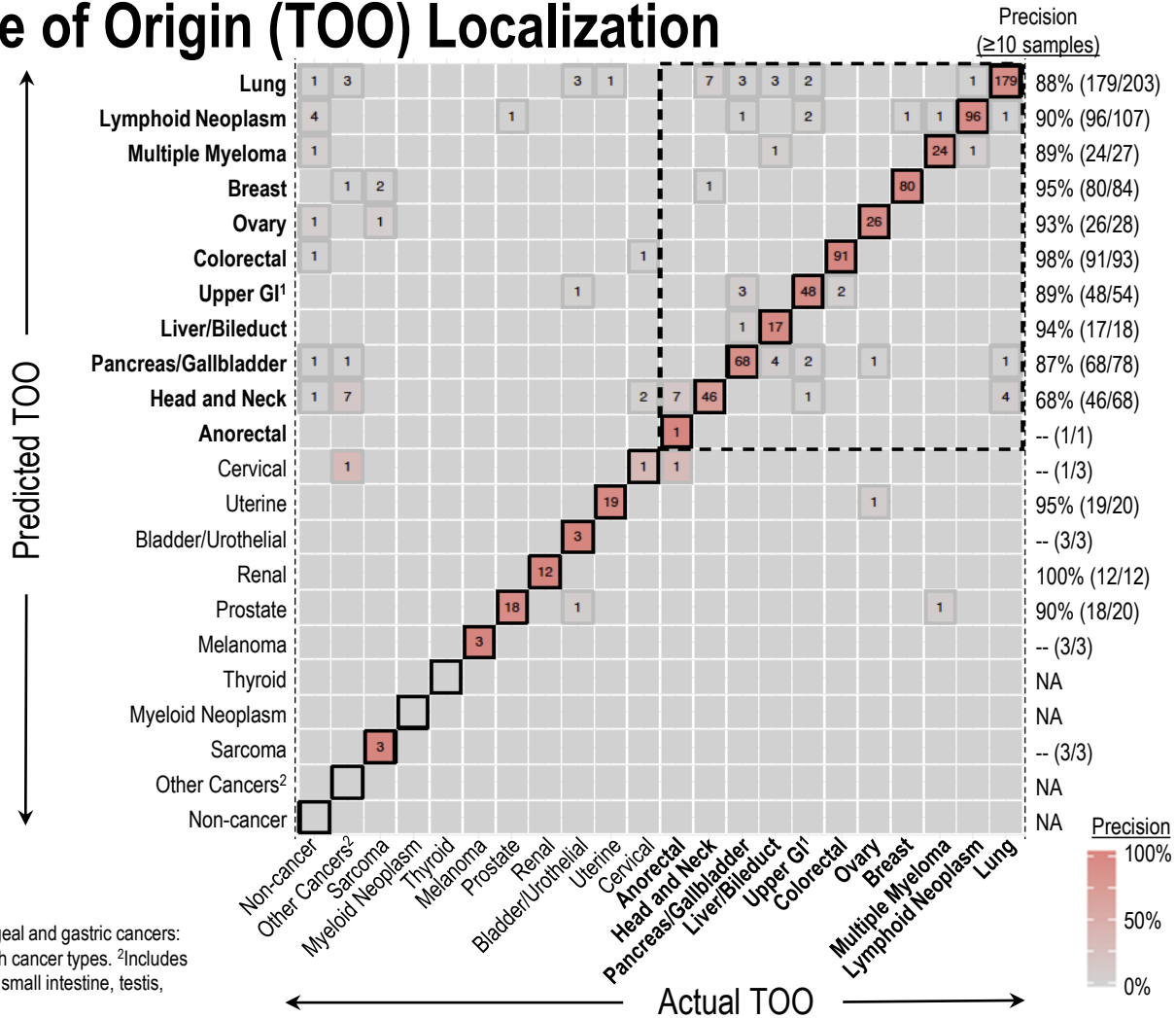
*Cancer types with >50 samples displayed (not displayed: anorectal, hormone receptor-negative breast, gallbladder, gastric, hepatobiliary, leukemia, lung, multiple myeloma, ovary).

Highly Accurate Tissue of Origin (TOO) Localization

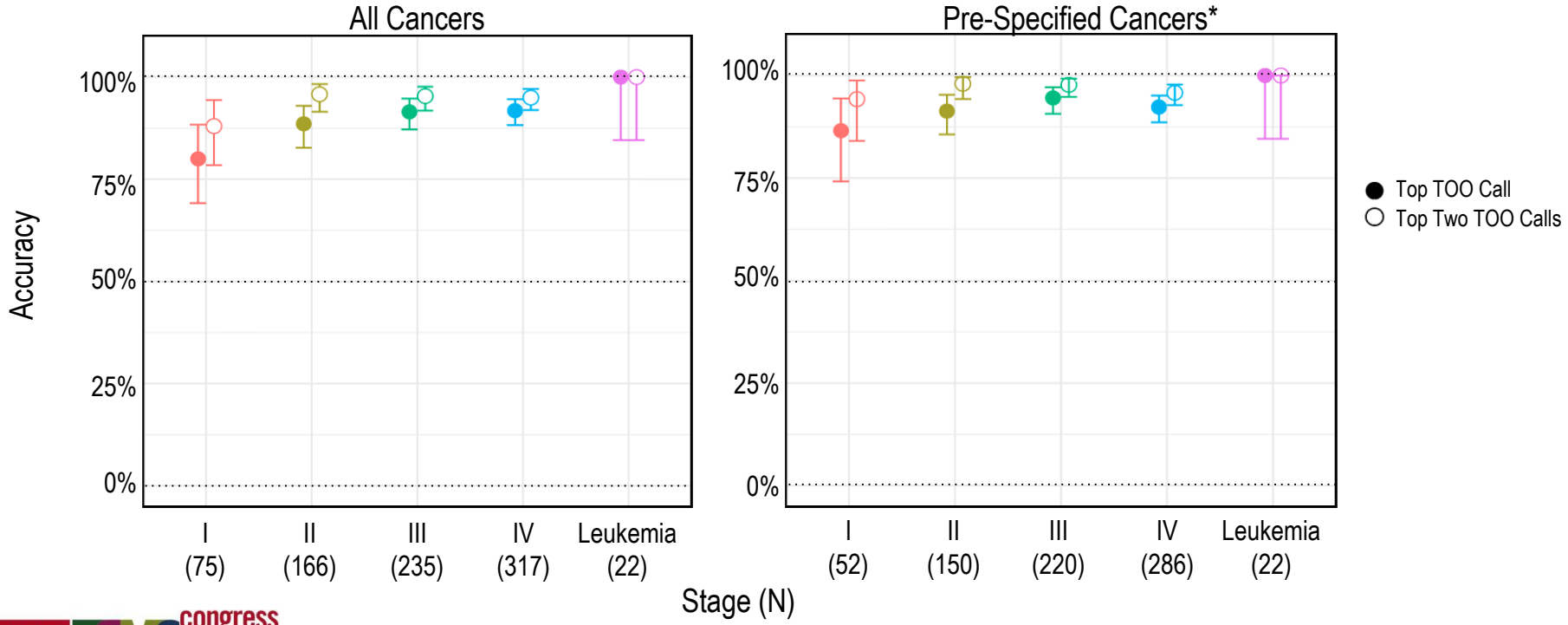
- 97% of samples with assigned TOO
- 89% of those calls were correct
- Highly precise localization **to a single tissue site** across >20 distinct tumor types



[1]Upper GI combines esophageal and gastric cancers: diagnostic workup covers both cancer types. [2]Includes mesothelioma, penis, pleura, small intestine, testis, unspecified, vulva.

Tissue of Origin (TOO) Accuracy is Consistently High Across Stages

- Single tissue localization: **89% (~9 of 10) of TOO calls were correct**
- Localization to the top two TOO calls: **94% (~19 of 20) of TOO calls were correct**



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*Includes hormone receptor-negative breast, ovary, head and neck, colorectal, anorectal, lung, esophagus, gastric, hepatobiliary, pancreas, gallbladder, lymphoid neoplasm (lymphoma and lymphoid leukemias), and multiple myeloma.

Conclusions

- Targeted methylation analysis of cfDNA simultaneously detected multiple cancer types, at early stages, at a specificity (>99%) appropriate for population screening
    - Detection of >20 cancer types was achieved with a **single, fixed, low false positive rate**
    - This approach also **accurately localized the TOO**, which will streamline subsequent diagnostic work-up
    - Both should be requirements for a blood-based multi-cancer test
- Results from an independent validation set will be presented at a future meeting
- Together, **these findings support the further clinical development of this targeted methylation approach as a multi-cancer detection test for numerous clinically significant cancer types**

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