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#NACLC20

Simultaneous Multi-Cancer Detection and Tissue of Origin Prediction Via Targeted Bisulfite Sequencing of Plasma Cell-Free DNA

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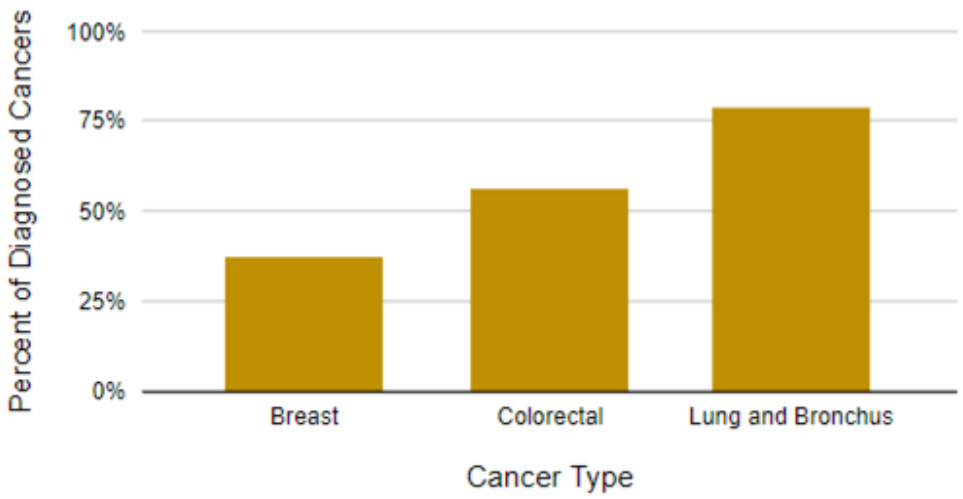
DISCLOSURES

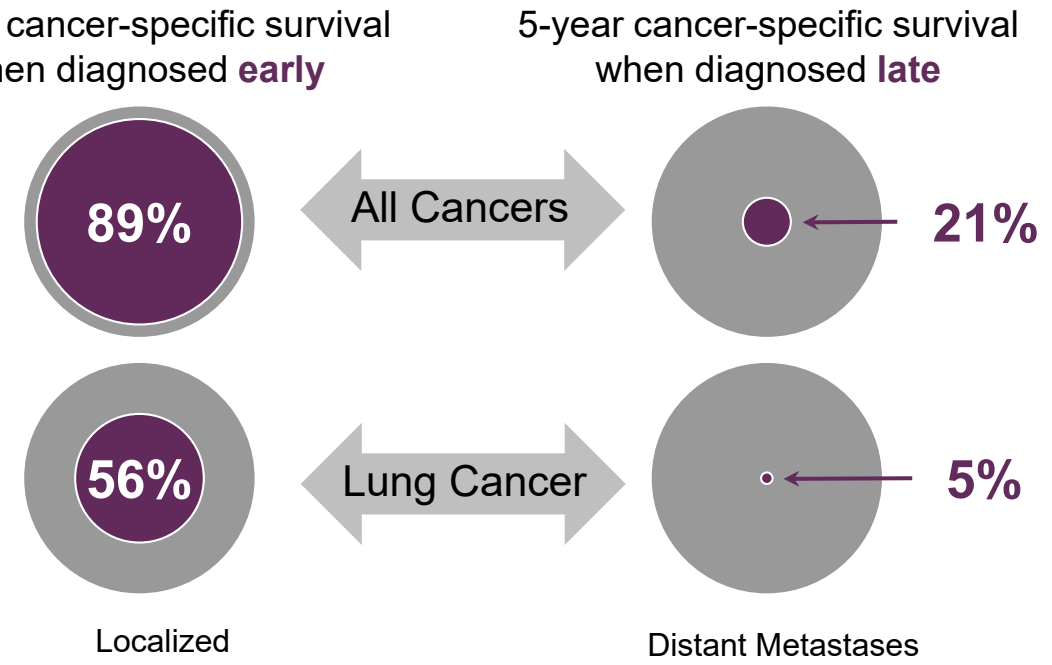
Commercial Interest	Relationship(s)
GRAIL, Inc.	Employee, equity holder

Many Cancers Are Detected Too Late

Cancers With Distant Metastases at Diagnosis[1]



Early Diagnosis Can Dramatically Improve Cancer Survival[2]



[1]Siegel R, et al. *CA Cancer J Clin.* 2018;68(1):7-30.
[2]Surveillance, Epidemiology, and End Results (SEER) Program (www.seer.cancer.gov) SEER*Stat Database: Incidence - SEER 18 Regs Research Data, Nov 2018 Sub. Includes persons aged 50-79 diagnosed 2006-2015 "Early/Localized" includes invasive localized tumors that have not spread beyond organ of origin, "Late/Metastasized" includes invasive cancers that have metastasized beyond the organ of origin to other parts of the body. Noone AM, Howlader N, Krapcho M, et al. (eds). SEER Cancer Statistics Review, 1975-2015, National Cancer Institute, Bethesda, MD, http://seer.cancer.gov/csr/1975_2015/, based on November 2017 SEER data submission, posted to the SEER website April 2018.

Circulating Cell-free Genome Atlas Study (NCT02889978)

Prospective, observational, longitudinal, case-control study for development of a cell-free DNA-based multi-cancer early detection test

15,254 participants
with and without cancer
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142 sites

Fully Enrolled

Blood samples
(from all participants)

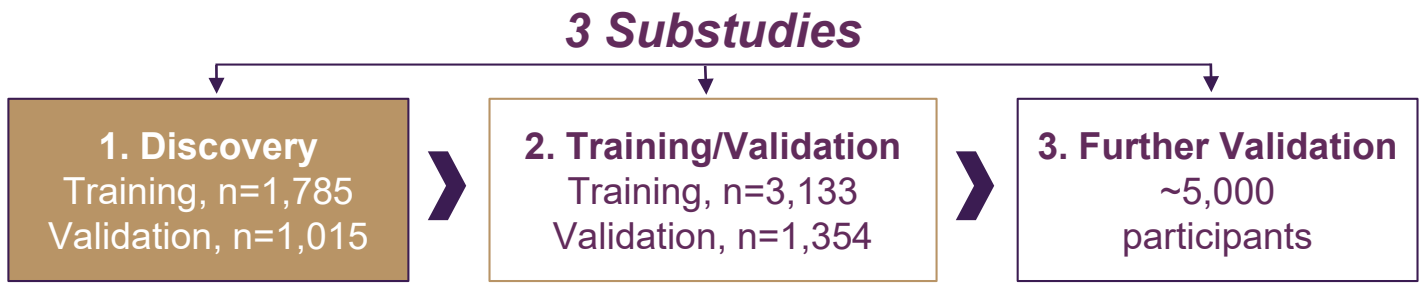
Tissue samples
(cancer only)

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

CCGA Divided Into 3 Pre-Specified Studies

Substudy 1: Comparison of assays to identify most suitable assay for further development

- **Discovery phase completed 2018[1]**
- Compared 3 sequencing approaches
  - Targeted sequencing (SNVs)
  - WGS (CNVs)
  - WGBS (methylation)
- Demonstrated feasibility of multi-cancer detection with low false positive rate (~2%)
- **Methylation-based assay selected for further development**



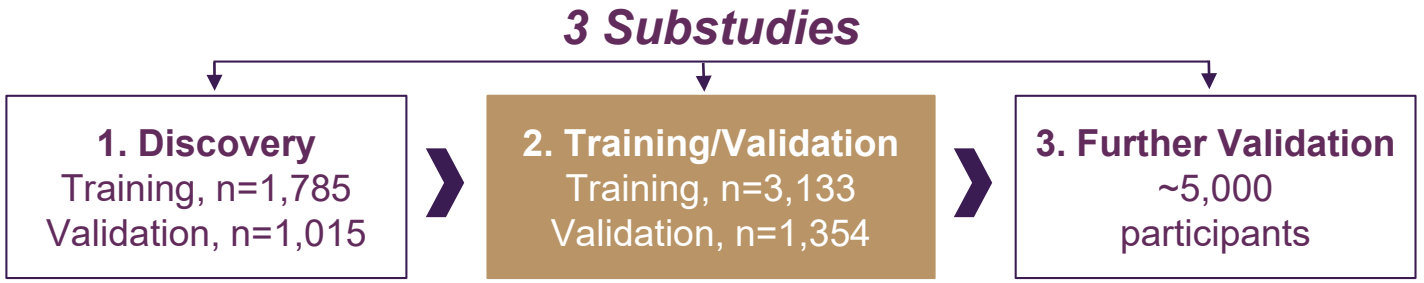
CCGA, Circulating Cell-free Genome Atlas study (NCT02889978); CNV, copy-number variation; SNV, single-nucleotide variant; WGBS, whole-genome bisulfite sequencing; WGS, whole-genome sequencing.

[1]Klein EA, *et al. J Clin Oncol* 2018;36(15_suppl):12021.

CCGA Divided Into 3 Pre-Specified Studies

Substudy 2: Training and Validation of a Targeted Methylation Assay

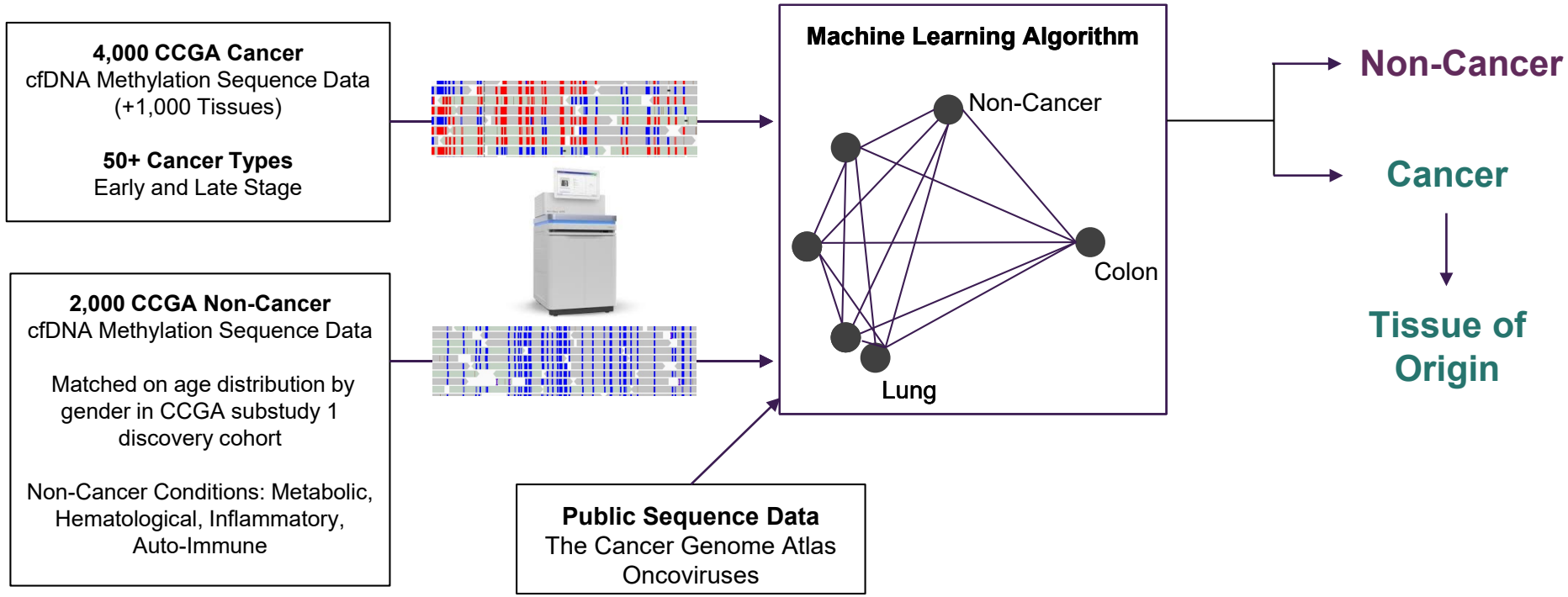
- **Training and initial validation completed 2020**[1]
- A **targeted methylation assay** was developed targeting key informative methylation regions
- Training and validation of a **targeted methylation classifier**



CCGA, Circulating Cell-free Genome Atlas study (NCT02889978).
[1]Liu MC, et al. *Ann Oncol*. 2020;31(6):745-759.

Target Selection Using a Machine Learning Algorithm

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



CCGA, Circulating Cell-free Genome Atlas study (NCT02889978); cfDNA, cell-free DNA; TOO, tissue of origin.

CCGA Divided Into 3 Pre-Specified Studies

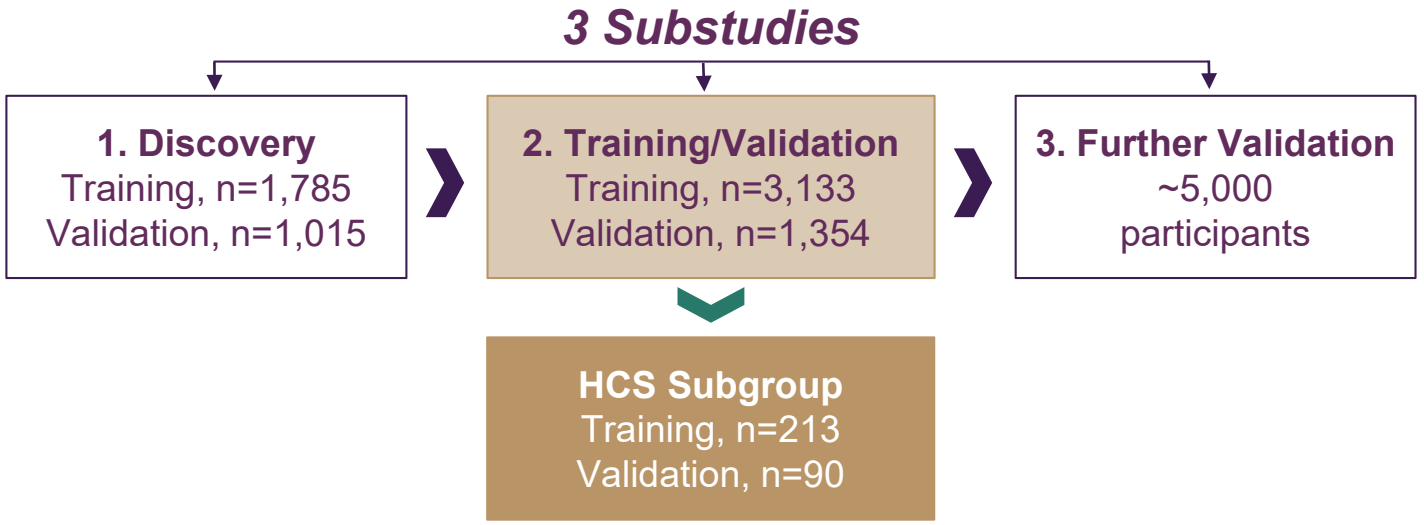
Substudy 2: High Clinical Suspicion (HCS)[1] of Cancer Subgroup Analysis

Participants

- Confirmed pathologic diagnosis of cancer, OR
- High suspicion of diagnosis of cancer

Objective

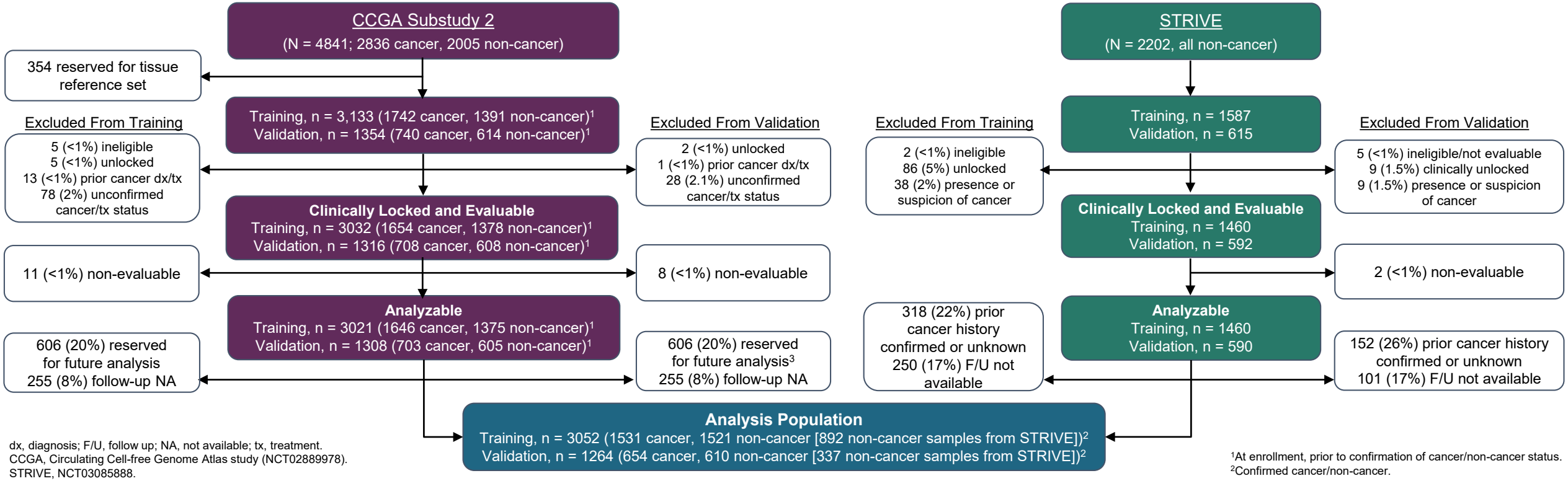
- Evaluate test performance in participants with HCS of cancer but without a confirmed pathologic diagnosis at the time of enrollment



CCGA, Circulating Cell-free Genome Atlas study (NCT02889978).
[1]High suspicion for a cancer diagnosis by clinical and/or radiological assessment, with planned biopsy or surgical resection to establish a definitive diagnosis within 6 weeks (42 days) after study blood draw.

Participant Disposition

To reach >99% specificity with >90% confidence, non-cancer samples were incorporated from an independent observational cohort study (STRIVE)



dx, diagnosis; F/U, follow up; NA, not available; tx, treatment.
CCGA, Circulating Cell-free Genome Atlas study (NCT02889978).
STRIVE, NCT03085888.

[1]At enrollment, prior to confirmation of cancer/non-cancer status.
[2]Confirmed cancer/non-cancer.

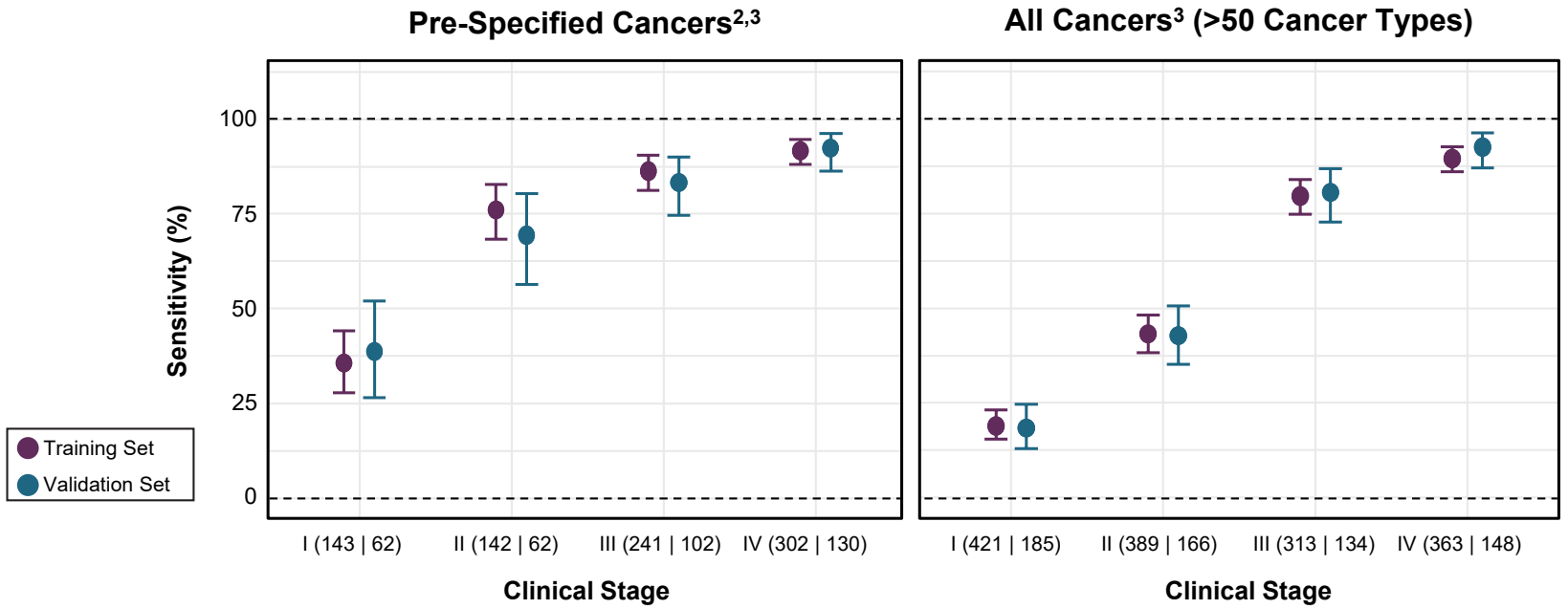
Comparable Demographics in Cancer and Non-Cancer Cohorts

	Training (N = 3052)		Validation (N = 1264)	
	Cancer[1] (n = 1531)	Non-Cancer (n = 1521)	Cancer[1] (n = 654)	Non-Cancer (n = 610)
Age, mean ± SD (years)	62 ± 12	59 ± 12	62 ± 12	59 ± 12
Female, n (%)	763 (50%)	1322 (87%)	321 (49%)	525 (86%)
White, Non-Hispanic	1264 (83%)	1315 (87%)	548 (84%)	533 (87%)
African American	105 (7%)	41 (3%)	47 (7%)	23 (4%)
Hispanic	97 (6%)	95 (6%)	37 (6%)	32 (5%)
Other[1]	65 (4%)	70 (5%)	22 (3%)	22 (4%)
Never-smoker, n (%)	679 (44%)	906 (60%)	292 (45%)	392 (64%)
BMI, Normal, n (%)	391 (26%)	525 (35%)	159 (24%)	185 (30%)

BMI, body-mass index; SD, standard deviation.
[1]Cancer status was derived per statistical analysis plan (ie, was confirmed). Cancers by training/validation: anus (13/5), bladder (11/4), breast (247/104), cervix (11/7), colon/rectum (122/53), esophagus (50/21), gallbladder (11/3), head and neck (62/25), kidney (56/25), liver/bile duct (29/11), lung (260/111), lymphoid leukemia (38/19), lymphoma (109/50), melanoma (7/1), myeloid neoplasm (4/1), ovary (37/17), pancreas (84/39), plasma cell neoplasm (34/12), prostate (188/84), sarcoma (17/5), stomach (17/8), thyroid (4/1), urothelial tract (5/0), uterus (84/36), and other (31/12); "other" in training included brain (3), Merkel cell carcinoma (1), mesothelioma (6), penis (1), pleura (1), small intestine (3), testis (1), thymus (2), unspecified (1), urethra (1), vagina (1), vulva (9); and in validation included brain (1), orbit (1), penis (1), skin cancer (not basal cell carcinoma, squamous cell carcinoma, or melanoma) (3), small intestine (1), testis seminoma (1), thymus (1), vagina (1), and vulva (2). [2]Includes Asian, Native Hawaiian, or Pacific Islander; American Indian, or Alaska Native; Other; Missing.

Cancers Detected at Early and Late Stages at 99.3% Specificity[1]

- 54.9% (95% CI, 51.0-58.8%) overall sensitivity (>50 cancers)
- 76.4% (95% CI, 71.6-80.7%) sensitivity in pre-specified[2] cancers
- Single, fixed false positive rate (99.3% specificity) across all cancers



CI, confidence interval.
[1]Liu MC, et al. *Ann Oncol*. 2020;31(6):745-759. DOI: 10.1016/j.annonc.2020.02.011.
[2]Includes anorectal, hormone receptor-negative breast, colorectal, esophagus, gallbladder, gastric, head and neck, hepatobiliary, lung, lymphoid neoplasm, lymphoma, multiple myeloma, ovary, and pancreas
[3]Plot excludes unstaged cancers.

Participant Disposition in HCS Subgroup

- Participants included in the HCS subgroup (N=303)
  - Clinically confirmed cancer
    - Training, n = 164
    - Validation, n = 75
  - Clinically confirmed non-cancer
    - Training, n = 49
    - Validation, n = 15
- The clinically confirmed cancer group in the HCS subgroup included participants with multiple cancers across stages I-IV

Cancer[1] and Stage for Participants in the HCS Subgroup with Subsequent Clinical Confirmation of Cancer

	Training				Validation			
	I	II	III	IV	I	II	III	IV
Bladder	4	-	1	-	-	-	-	-
Breast	3	1	-	1	1	-	-	-
Cervix	-	-	-	-	1	-	-	-
Colon/Rectum	1	-	1	2	-	-	1	3
Esophagus	-	-	-	-	-	-	-	1
Gallbladder	1	-	1	-	-	-	-	-
Head and Neck	-	-	1	1	-	-	-	-
Kidney	33	2	3	4	16	2	1	-
Liver/Bile Duct	-	1	-	1	-	-	-	-
Lung	18	4	4	10	7	2	3	7
Lymphoma	-	3	2	3	1	1	-	2
Melanoma	-	-	-	1	-	-	-	-
Ovary	4	1	11	4	1	1	7	2
Pancreas	-	-	-	5	2	1	-	-
Plasma Cell Neoplasm	2	4	1	-	-	1	2	-
Prostate	-	1	1	1	1	-	-	-
Sarcoma	-	2	-	1	1	-	-	-
Thyroid	-	-	-	1	1	-	-	-
Uterus	9	-	1	-	4	-	-	-
Lymphoid Leukemia	4 (no stage)				1 no stage			
Other[2]	1 stage I, 1 stage IV, 2 no stage				-	1	-	-
Total	**76**	**19**	**27**	**36**	**36**	**9**	**14**	**15**

HCS, high clinical suspicion.
[1]Cancers were grouped for reporting purposes.
[2]Brain, n=2; mesothelioma, n=1; thymus, n=1; vulva, n=1.

Comparable Specificity Between CCGA Substudy 2 and HCS Subgroup

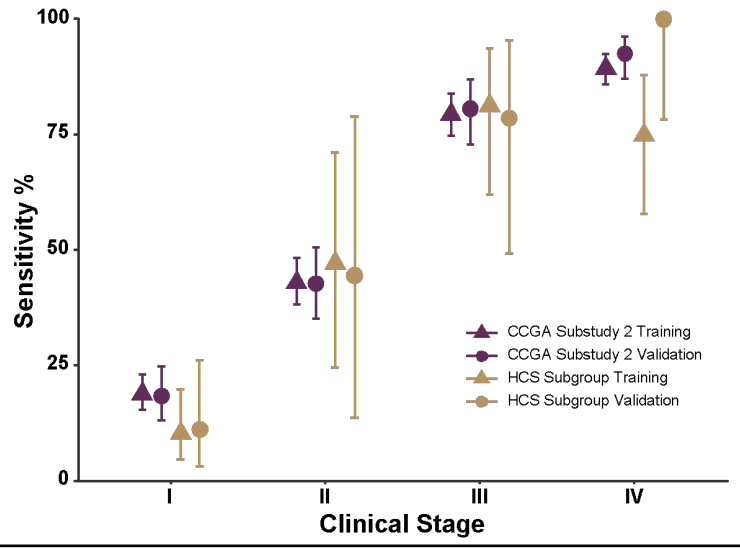
Suggests false positive rate was not elevated in individuals with HCS of cancer versus individuals with confirmed cancer diagnosis, although sample size was relatively small

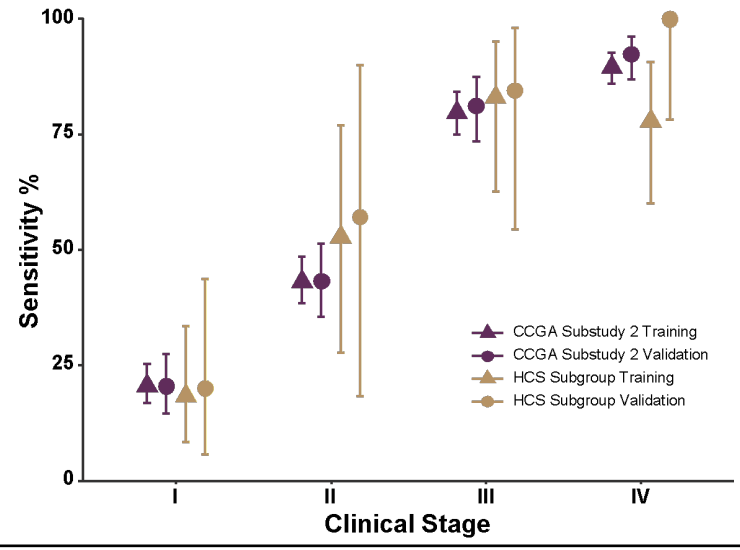
	CCGA Substudy 2		HCS Subgroup	
	N	Specificity	N	Specificity
Training	1521	99.8% (95% CI, 99.4–99.9%)	49	100% (95% CI, 92.7–100%)
Validation	610	99.3% (95% CI, 98.3–99.8%)	15	100% (95% CI, 78.2–100%)

CCGA, Circulating Cell-free Genome Atlas study (NCT02889978); CI, confidence interval; HCS, high clinical suspicion of cancer,

Comparable Sensitivity Between CCGA Substudy 2 and HCS Subgroup

	All Cancers		Excluding Renal Cancer	
	n/N	Sensitivity	n/N	Sensitivity
Training	66/164	40.2% (95% CI, 32.7-48.2%)	66/131	50.4% (95% CI, 41.5-59.2%)
Validation	35/75	46.7% (95% CI, 35.1-58.6%)	35/59	59.3% (95% CI, 45.7-71.9%)

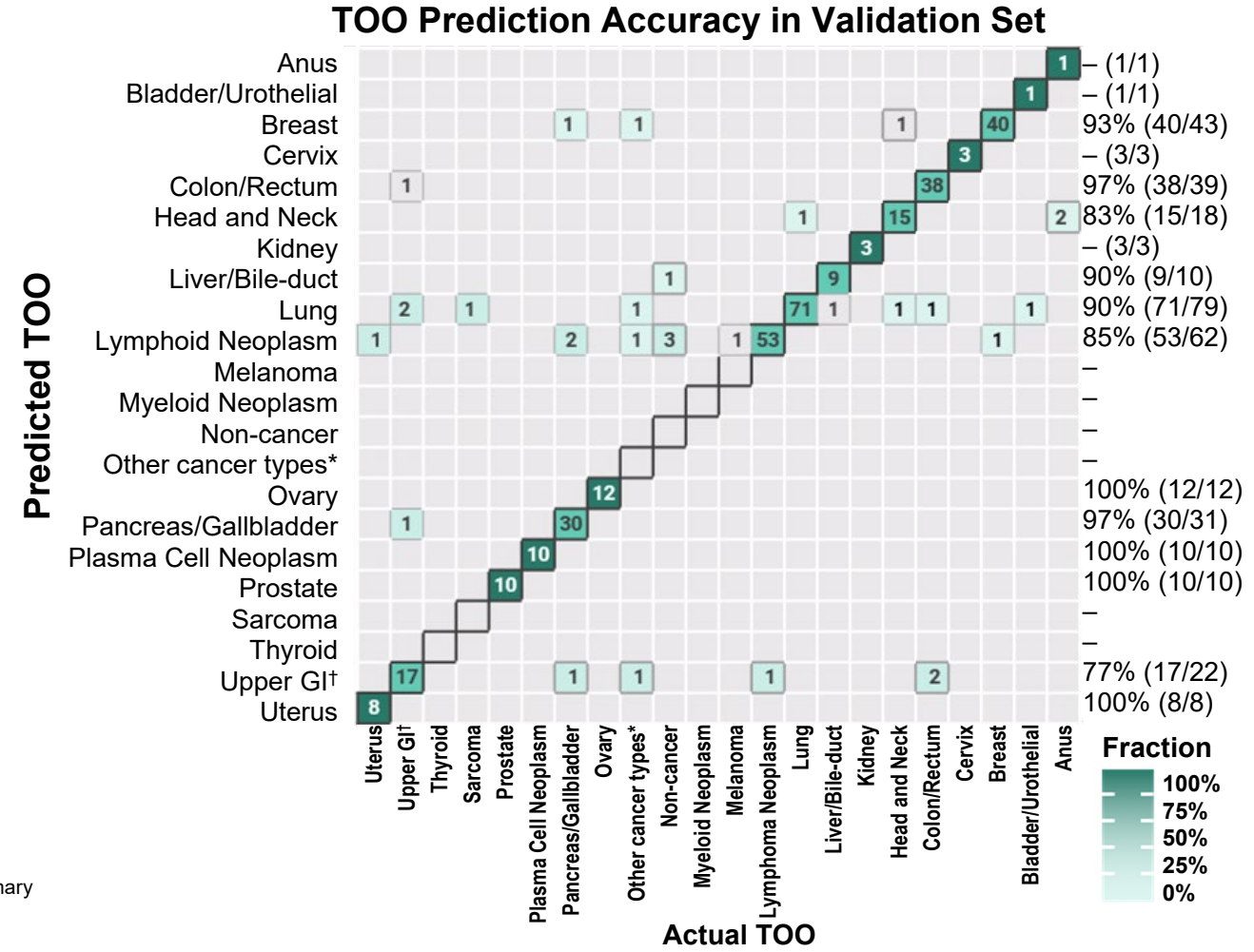




CCGA, Circulating Cell-free Genome Atlas study (NCT02889978); CI, confidence interval; HCS, high clinical suspicion of cancer,

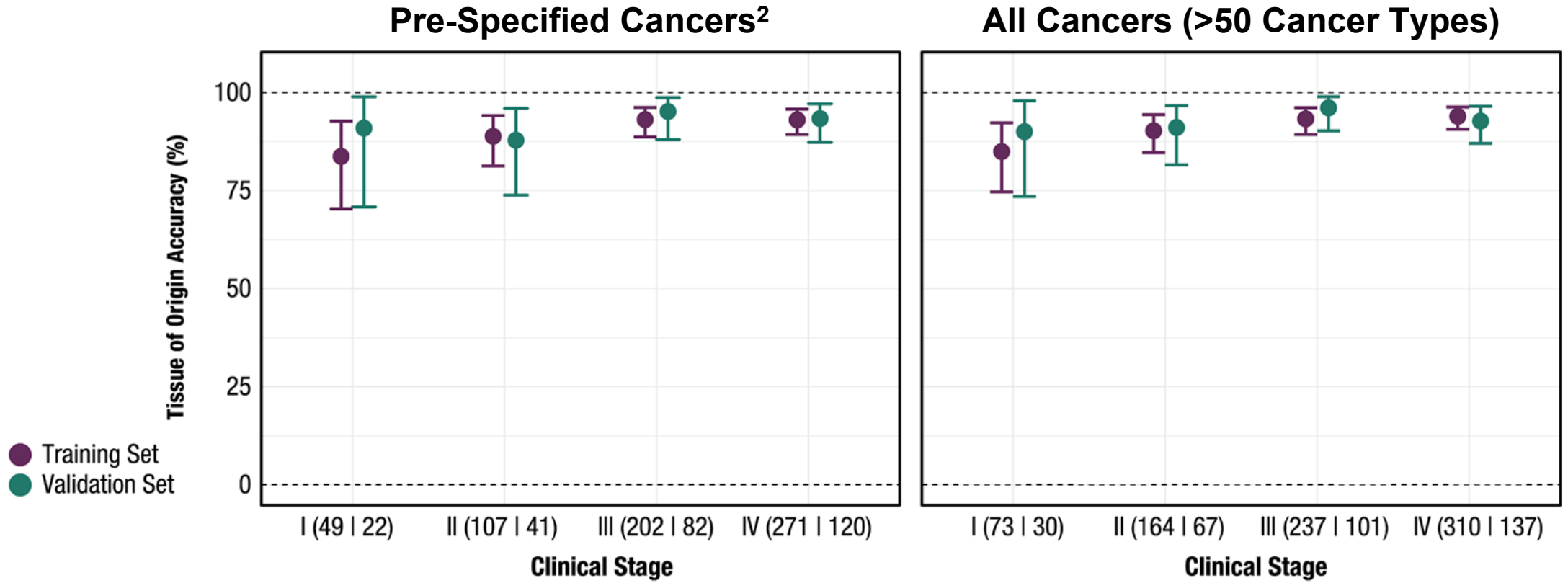
Highly Accurate Prediction of Tissue of Origin (TOO)[1]

- 96% of samples with assigned TOO (validation set)
- 93% of those calls were correct
- Highly accurate prediction of a single tissue site across a large number of tumor sites
- Consistent performance in the training set



GI, gastrointestinal.
[1]Liu MC, et al. *Ann Oncol*. 2020;31(6):745-759. DOI: 10.1016/j.annonc.2020.02.011.
*Other cancers, training: mesothelioma, penis, pleura, small intestine, testis, and vulva, as well as 1 sample missing primary cancer type information. Other cancers, validation: orbit, Merkel cell carcinoma of the scalp, penis, testis, vagina, vulva.
†Upper GI includes esophagus and stomach.

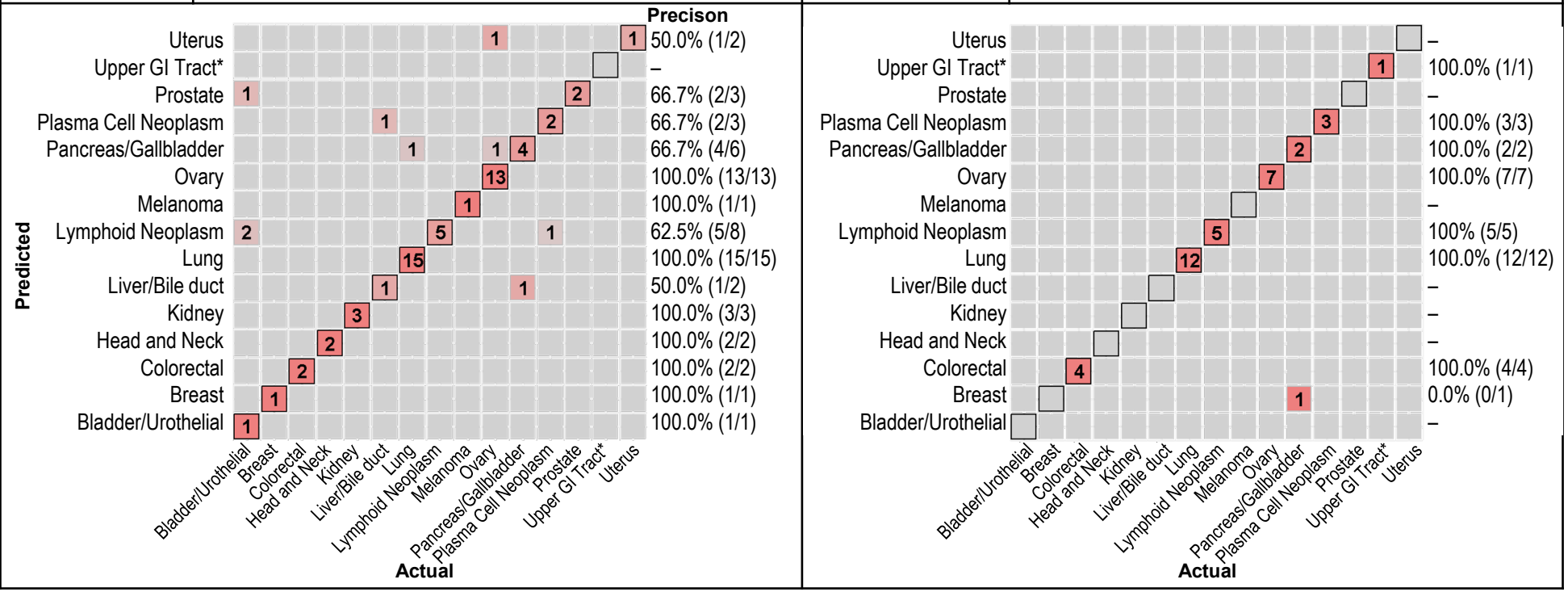
Tissue of Origin (TOO) Prediction Accuracy Is Consistently High Across Stages[1]



[1]Liu MC, et al. *Ann Oncol*. 2020;31(6):745-759. DOI: 10.1016/j.annonc.2020.02.011.
[2]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.

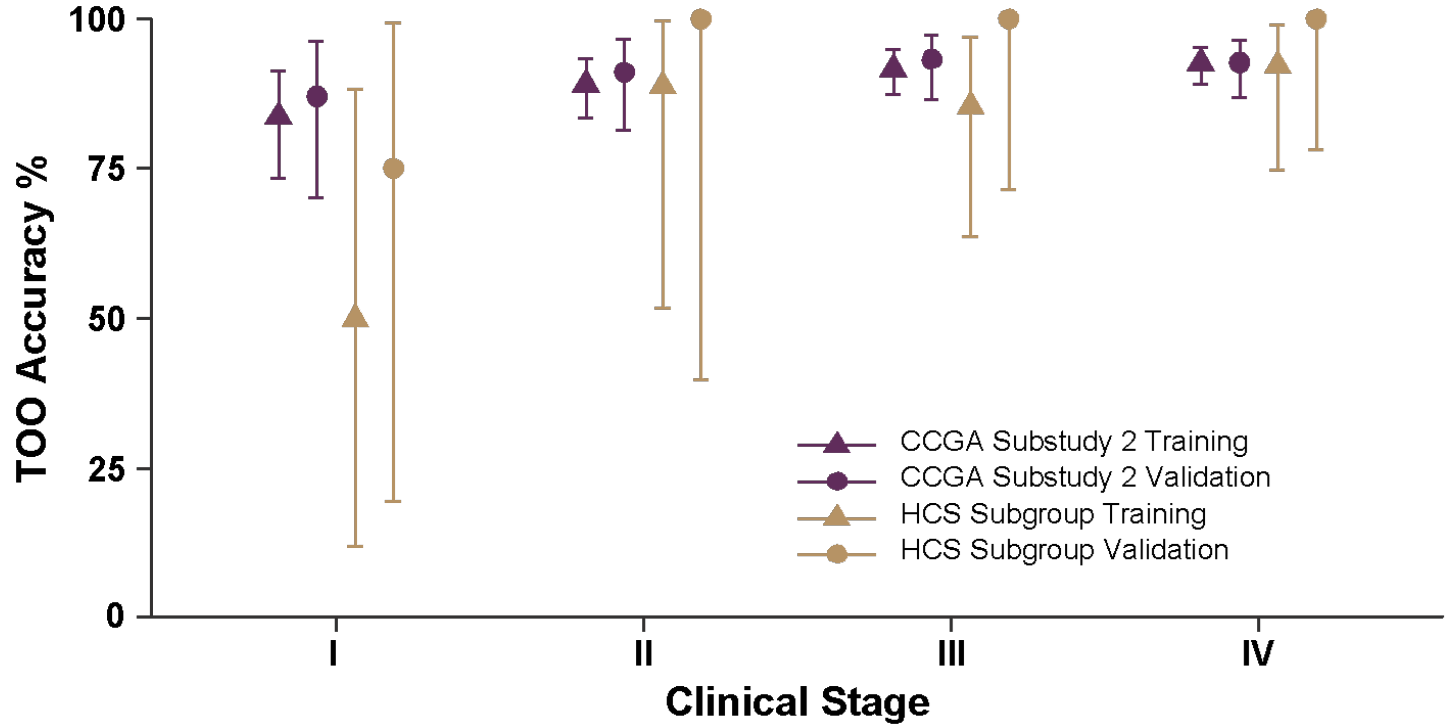
Tissue of Origin Prediction Accuracy in HCS Subgroup

	Training		Validation	
	n/N	Mean (95% CI)	n/N	Mean (95% CI)
Accuracy	53/62	85.5% (74.2-93.1%)	34/35	97.1% (85.1-99.9%)



*Upper GI includes esophagus and stomach. CI, confidence interval; GI, gastrointestinal; HCS, high clinical suspicion of cancer.

Comparable Tissue of Origin (TOO) Prediction Accuracy Between CCGA Substudy 2 and HCS Subgroup



CCGA, Circulating Cell-free Genome Atlas study (NCT02889978); HCS, high clinical suspicion of cancer.

Conclusions and Future Studies

- A targeted methylation cell-free DNA-based MCED test can simultaneously detect >50 cancers across all stages at **>99% specificity**
  - **Single, fixed, low false positive rate**
  - **Highly accurate tissue-of-origin prediction**
- MCED test performance in the HCS subgroup (although small *n*) was comparable to overall CCGA substudy 2 performance, suggesting that **this test could potentially be used to accelerate diagnosis of individuals with HCS of cancer**
- Together, **these findings support further clinical development of this MCED test**
- **Clinical implementation** (PATHFINDER [NCT04241796]) **and population-scale validation studies** (STRIVE [NCT03085888], SUMMIT [NCT03934866]) **are ongoing**

CCGA, Circulating Cell-free Genome Atlas study (NCT02889978); HCS, high clinical suspicion; MCED, multi-cancer early detection.

Acknowledgments

- Study participants who graciously donated their time, energy, and specimens
- Investigators and collaborators for advice, enrolling participants, and collecting data and specimens
- Advisors and Scientific Advisory Board members for their helpful feedback and advice
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