Performance of A Blood-based Test for the Detection of Multiple Cancer Types

Brian M. Wolpin, MD, MPH[1]

Donald A. Richards, MD, PhD[2,3]; Allen L. Cohn, MD[3,4]; Xiaoji Chen, PhD[5]; Joerg Bredno, PhD[5]; Kathryn N. Kurtzman, MD[5]; Jessica Yecies, PhD[5]; Rita Shaknovich, MD, PhD[5]; Nan Zhang, PhD[5]; Mohan K. Tummala, MD[6]; David R. Spigel, MD[7]; David Cosgrove, MD[8]; Sharon T. Wilks, M.D., FACP[9]; Robert D. Siegel MD, FACP[10]; Eric T. Fung, MD, PhD[5]; Arash Jamshidi, PhD[5]; Alexander M. Aravanis, MD, PhD[5]; Anne-Renee Hartman, MD[5]; Minetta C. Liu, MD[11]; Eric A. Klein, MD[12]

1. Dana-Farber Cancer Institute, Boston, MA; 2. Texas Oncology, Tyler, TX; 3. US Oncology Research, The Woodlands, TX; 4. Rocky Mountain Cancer Center, Denver, CO; 5. GRAIL, Inc, Menlo Park, CA; 6. Mercy Clinic, Springfield, MO; 7. Tennessee Oncology, Nashville, TN; 8. Vancouver Cancer Center, Vancouver, WA; 9. Texas Oncology, San Antonio, TX; 10. Bon Secours, Greenville, SC; 11. Mayo Clinic, Rochester, MN; 12. Cleveland Clinic, Cleveland, OH.

Disclosures

Study funded by GRAIL, Inc. BMW: Grant funding from Celgene and Eli Lilly; Consulting for BioLineRx, Celgene, G1 Therapeutics, and GRAIL; Institutional support for clinical trials: AbGenomics, ALLIANCE, Astra-Zeneca, Aveo, BioLineRx, BMS, Celgene, Curegenix, Five Prime, Genentech, Eli Lilly, Lustgarten Foundation, Merck, Pancreatic Cancer Action Network, Parker Institute, Revolution Medicines, Seattle Genetics, Stand Up to Cancer, Tesaro. AC [up-to-date on ASCO website]. XC, JB, JY, RS, NZ, ETF, AMA, and A-RH are employees of GRAIL, Inc. with equity in the company. KNK and AJ are employees of GRAIL, Inc. with equity in the company, and shareholders in Illumina, Inc. AMA is an advisor to and equity holder in Foresite Labs. EAK is a consultant for GRAIL, Inc. and Cellanyx, LLC.

Multi-cancer Test for Early Detection at Population Scale

Early-stage cancer is more likely to be treated successfully

Realize benefits of early detection while minimizing harms and cost:

- **Low false positive rate:** achieved through high specificity
- **High detection rate:** achieved through sensitivity for >20 cancer types
- **Localizing ability:** identify anatomic location to direct appropriate diagnostic evaluation
- **Limited over-diagnosis:** preferential detection of clinically significant cancers

Demonstrate test performance, reproducibility, and applicability to target populations

The Circulating Cell-free Genome Atlas (CCGA) Study

Prospective, longitudinal, case-control study for development of a multi-cancer test

15,254 participants
with or without cancer

---

142 sites

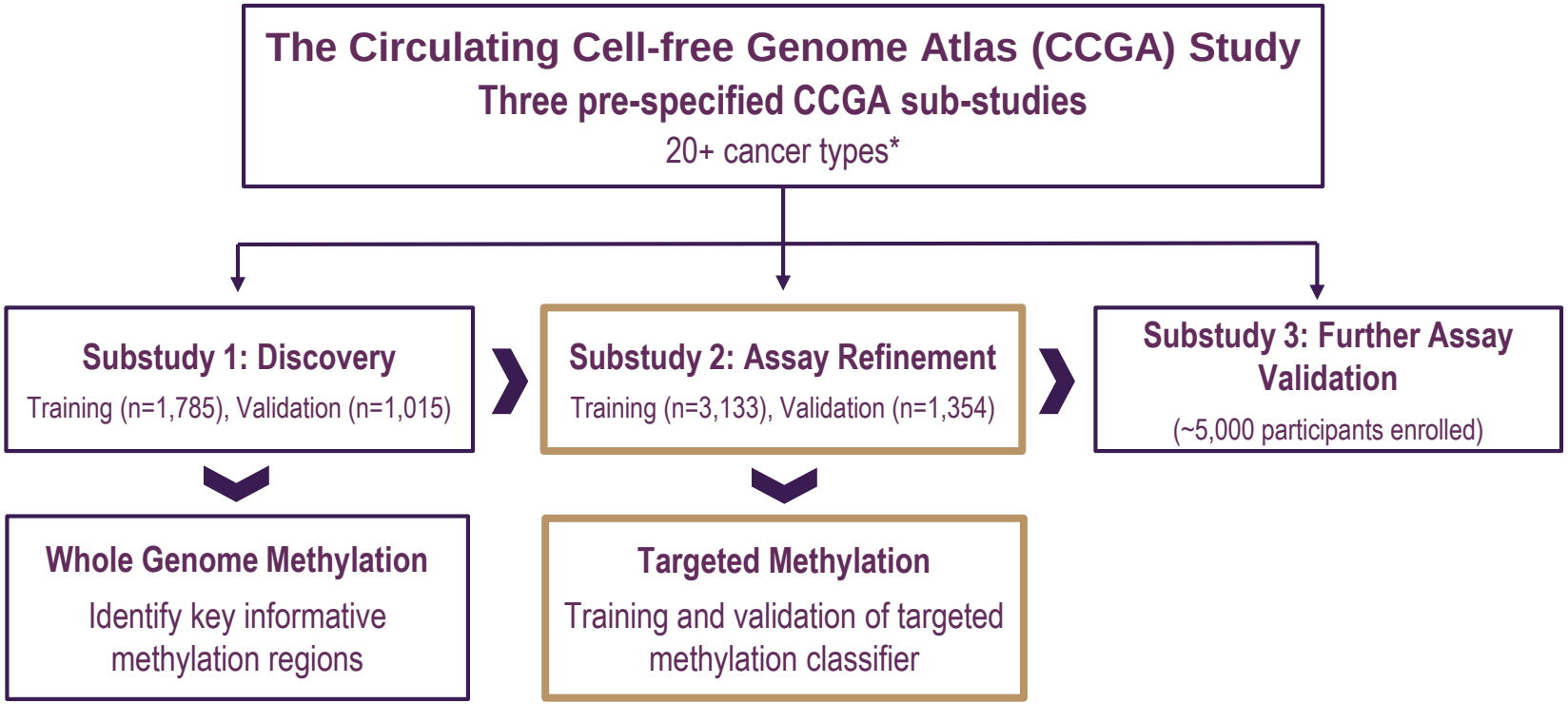
Fully Enrolled

Blood samples
(from all participants)

Tissue samples
(cancer only)

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

The CCGA: Overview of Substudies



**Anus, Bladder, Breast, Cervix, Colon/Rectum, Esophagus, Gallbladder, Head and Neck, Kidney, Liver/bile duct, Lung, Lymphoid leukemia, Lymphoma, Melanoma, Myeloma, Ovary, Pancreas, Prostate, Sarcoma, Stomach, Thyroid, Urothelial tract, Uterus, Other (including Brain, Mesothelioma, Orbit, Penis, Pleura, Skin Cancer [not basal cell carcinoma, squamous cell carcinoma, or melanoma], Small Intestine, Testis, Thymus, Urethra, Vagina, Vulva)*

Gastrointestinal Cancers (N=447) in CCGA Substudy 2

A subset of the total 2,185 cancer participants across >20 cancer types in CCGA substudy 2

Liver / Bile Duct

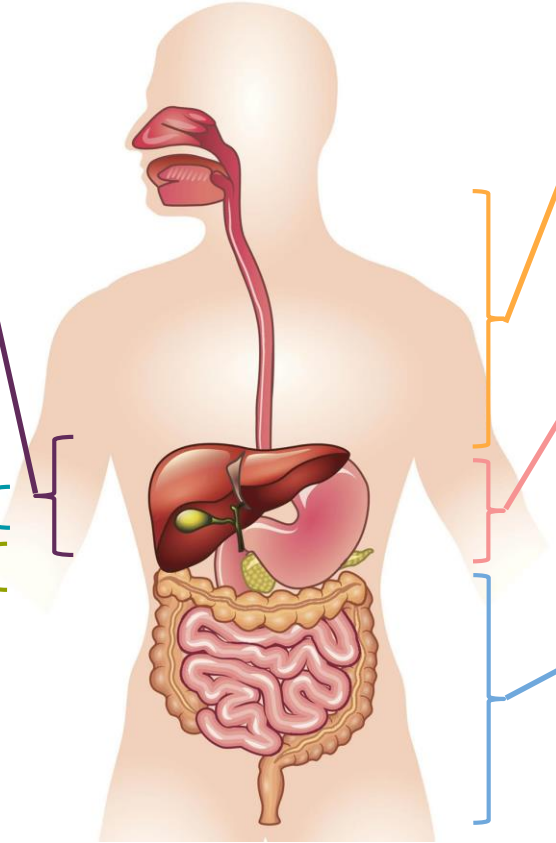
Stage	I-III	IV	All
Training	19	10	29
Validation	7	4	11
All	**26**	**14**	**40**

Gallbladder

Stage	I-III	IV	All
Training	7	4	11
Validation	3	0	3
All	**10**	**4**	**14**

Pancreas

Stage	I-III	IV	All
Training	42	42	84
Validation	22	17	39
All	**64**	**59**	**123**



Esophagus

Stage	I-III	IV	All
Training	31	19	50
Validation	13	8	21
All	**44**	**27**	**71**

Stomach

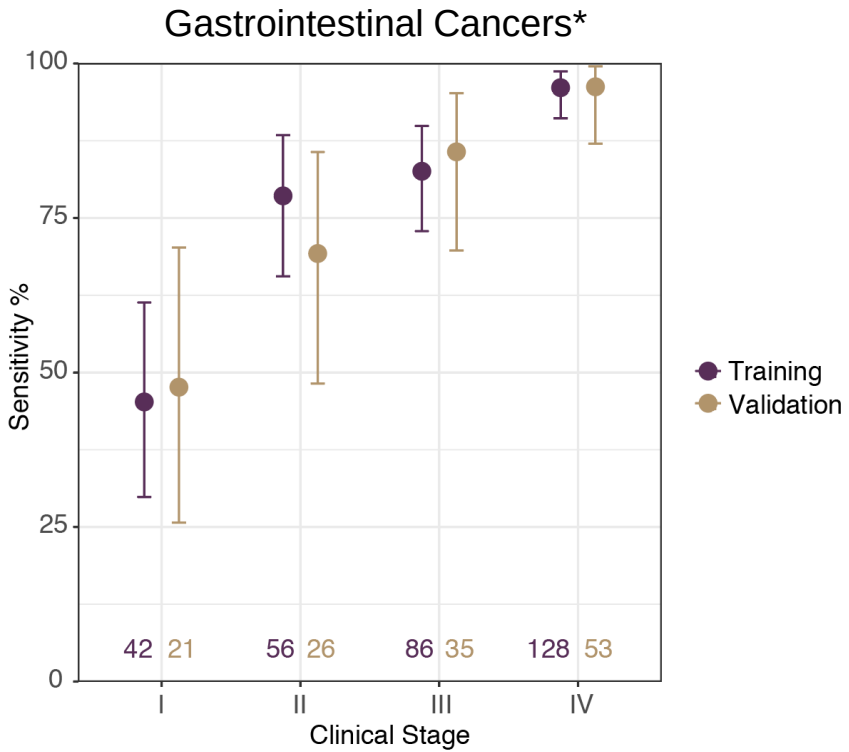
Stage	I-III	IV	All
Training	9	8	17
Validation	5	3	8
All	**14**	**11**	**25**

Colon / Rectum

Stage	I-III	IV	All
Training	76	45	121
Validation	32	21	53
All	**108**	**66**	**174**

Sensitivity for GI Cancer Detection at >99% Specificity

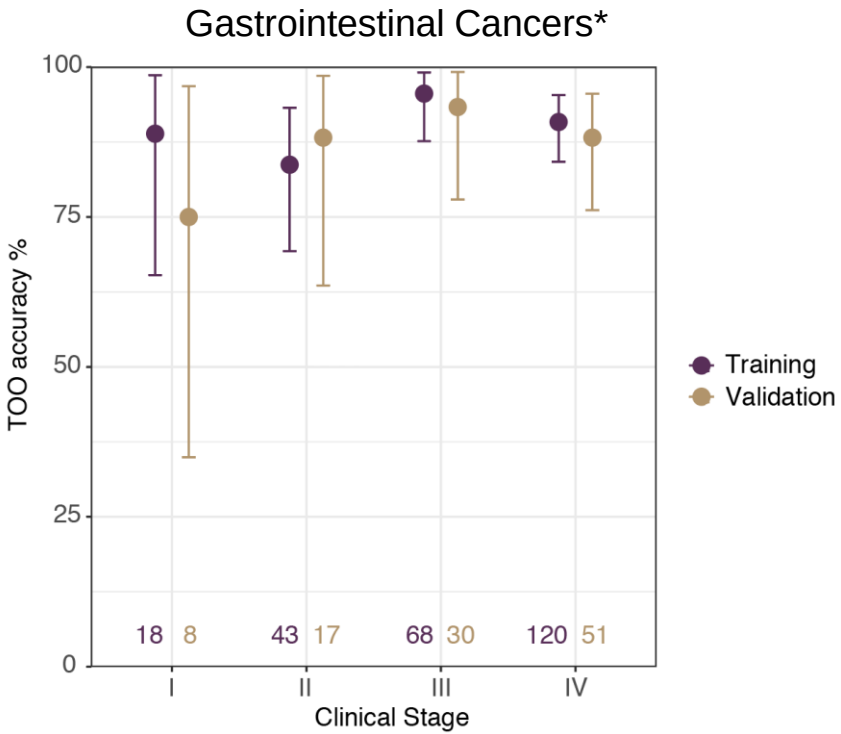
- Classifier achieved specificity of **99.8%** in the cross-validated training set and **99.3%** in the independent validation set
- False positives were **3/1,521 (0.2%)** in training and **4/610 (0.7%)** in validation
- Training and validation sets had similar sensitivity
- Stage I-III sensitivity was **73%** (95% CI, 66%-79%; training) and **71%** (CI, 60-80, validation)
- Stage I-IV sensitivity was **82%** (95% CI, 78%-86%, training) and **81%** (CI, 73%-87%, validation)



* Cancers of esophagus, stomach, liver/bile duct, pancreas, gallbladder, and colon/rectum

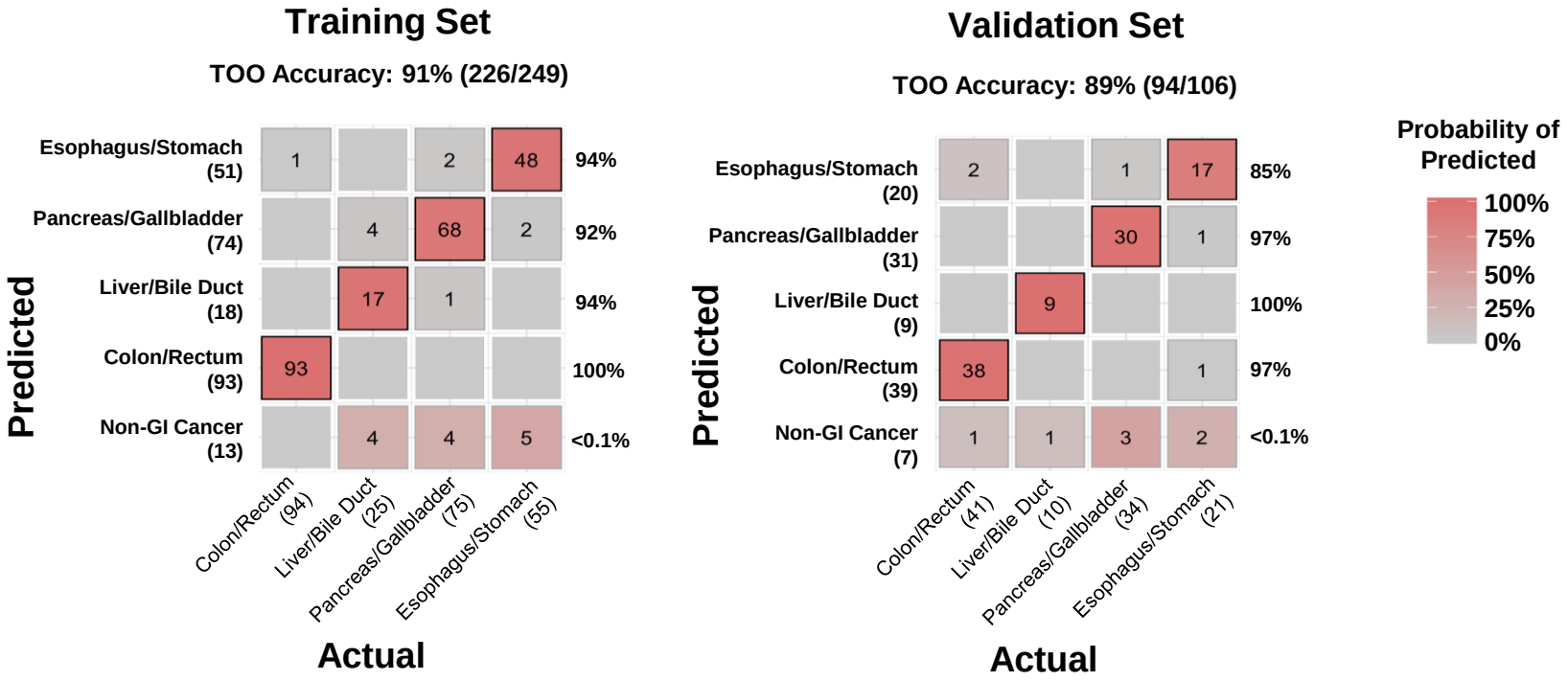
Accuracy of TOO Localization for GI Cancers

- 20+ trained cancer TOO classes enables localization to single tissue site
- **97%** of detected GI cancers were assigned a TOO in both training and validation sets
- Training and validation sets had consistent TOO accuracy across all stages
- Overall, the **predicted TOO accuracy** for patients with GI cancers was **91%** (95% CI, 86%-94%) in the training and **89%** (95% CI, 81%-94%) in the validation set



* Cancers of esophagus, stomach, liver/bile duct, pancreas, gallbladder, and colon/rectum

Tissue of Origin (TOO) Classification for GI Cancers



Localization to a single tissue site was accurate as demonstrated by a high level of agreement between the true (x axis) and predicted (y axis) TOO per sample in training and validation sets.

See poster for Abstract 283 to view data for the full (>20 cancer type) dataset.

Conclusions

- Targeted methylation analysis of plasma cfDNA simultaneously detected multiple GI cancers at high sensitivity with pre-specified high specificity
  - >99% specificity was maintained in the independent validation set.

- 97% of detected GI cancers were assigned a TOO in training and validation sets.

- Highly accurate TOO localization was achieved in GI cancers.

- This investigational multi-cancer early detection test evaluating cfDNA methylation may be a practical method for detecting and localizing GI and numerous other cancers, thus directing the downstream diagnostic evaluation.

Acknowledgements

- 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
- The many GRAIL teams who have worked and continue to work on this study