Abstract 283

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

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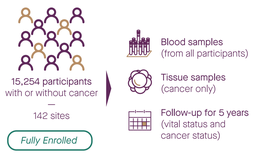
BACKGROUND

- Cancers of the esophagus, stomach, pancreas, gallbladder, liver, bile duct, colon, and rectum accounted for an estimated 17% of incident cancer diagnoses and 26% of cancer-related deaths in the United States in 2019.[1]
- A noninvasive cell-free DNA (cfDNA) blood test capable of detecting multiple cancers, including the gastrointestinal (GI) cancers, at pre-metastatic stages (stages I–III) could lead to earlier cancer detection and decreased cancer mortality.
- For a multi-cancer test to be effective at population scale, it should:
  - Detect clinically significant cancers with a low false positive rate (ie, very high specificity [>99%]) to limit overdiagnosis and unnecessary diagnostic workups;
  - Identify a specific tissue of origin (TOO) to direct appropriate diagnostic work-up for detected cancers.[2,3]
- The Circulating Cell-free Genome Atlas (CCGA; NCT02889978) study is a prospective, multicenter, observational, case-control study with longitudinal follow-up to support the development of a plasma cfDNA-based multi-cancer early detection test (Figure 1A,B).
- We previously reported that a targeted methylation-based cfDNA test using a machine-learning classifier detected over 20 cancer types, including GI cancers, with a single, fixed false positive rate of <1%, and predicted TOO with >90% accuracy.[4,5]
- Here we report the performance of this targeted methylation assay for GI cancers (esophagus, stomach, pancreas, gallbladder, liver, bile duct, colon, and rectum) in the second pre-specified substudy of CCGA (Figure 1C).

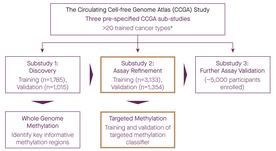
Figure 1. The CCGA Study

A. CCGA Study Design

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

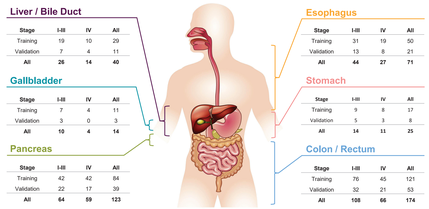


B. CCGA Divided Into Three Pre-specified Substudies



C. 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

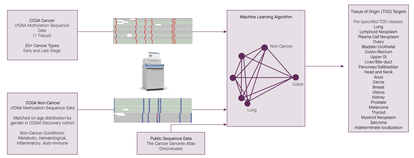


Anus, Bladder, Breast, Cervix, Colon/Rectum, Esophagus, Gallbladder, Head and Neck, Kidney, Liver/bile duct, Lung, Lymphoid leukemia, Lymphoma, Melanoma, Myeloma, Ovary, Pancreas, Prostate, Sarcoma, Stomach, Thyroid, Urothelial tract, Uterus, Other (including Brain, Mesothelioma, Orbit, Penis, Pleura,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

METHODS

- 15,254 participants (44% non-cancer, 56% cancer) were enrolled in the CCGA study.
- Blood samples were prospectively collected from participants with newly diagnosed, untreated cancer and from participants without a diagnosis of cancer (hereafter referred to as cancer and non-cancer cohorts). Tumor biopsy tissue was collected from participants with cancer when available.
- Plasma cfDNA from blood samples was subjected to a targeted methylation approach that included high-efficiency methylation chemistry to enrich for methylation targets and a cross-validated machine-learning classifier to predict cancer status and TOO (Figure 2).
  - Observed methylation fragments characteristic of cancer and TOO were combined across targeted regions and assigned a relative probability of cancer and specific TOO; accuracy was defined as the fraction of correct calls.
  - The machine-learning classifier was trained and locked, including decision thresholds, targeting >99% specificity with >90% confidence.
  - Assay and classifier performance was validated in an independent validation set.

Figure 2. Target Selection and Machine Learning Algorithm



A large database of DNA methylation patterns from individuals with and without cancer (including healthy individuals and those with other medical conditions) was generated to enable target selection for a single test that is able to classify the presence or absence of cancer for multiple cancer types at high specificity and identify TOO with high accuracy.

RESULTS

Specificity

- The classifier achieved 99.8% specificity in the cross-validated training set and 99.3% specificity in the independent validation set.
- In non-cancer controls, there were 3/1521 (0.2%) false positives in training and 4/610 (0.7%) in validation.
- Two false positives were identified as GI cancer—1 was called colorectal cancer in training, and 1 was called liver/bile duct cancer in validation.

Sensitivity

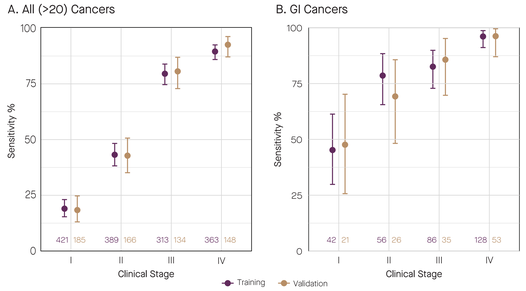
- Assay sensitivity was consistent between the cross-validated training and independent validation sets across stages (Figure 3).
- Stage I-III sensitivity (95% confidence interval [CI]) for cancer detection was 73% (66-79%) in the training set and 71% (60-80%) in the validation set for patients with GI cancers.
- Stage I-IV sensitivity (95%CI) was 82% (78-86%) in the training set and 81% (73-87%) in the validation set for patients with GI cancers.
- Sensitivity by tumor stage for GI cancers is shown in Table 1.

Table 1. Test Sensitivity by Tumor Stage for Patients With Gastrointestinal Cancers in Training and Validation

	Test Sensitivity (95% CI) (%)	
Tumor Stage	Training Set	Validation Set
I	45 (30-61)	48 (26-70)
II	79 (66-88)	69 (48-86)
III	83 (73-90)	86 (70-95)
IV	96 (91-99)	96 (87-100)

CI, confidence interval.

Figure 3. Stage-specific Performance Reported at >99% Specificity



A. Cancer detection sensitivity in GI Cancers (esophagus, stomach, liver/bile duct, pancreas, gallbladder, and colorectal).
B. Sensitivity in >20 cancer types.

Tissue of Origin

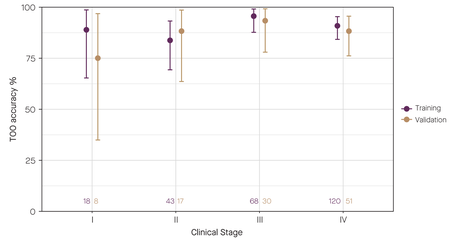
- 97% (both training and validation) of detected GI cancers were assigned a TOO.
- TOO accuracy in GI cancers was consistent across all stages in training and validation (Figure 4).
- Overall, predicted TOO accuracy for patients with GI cancers was 91% (95% CI, 86%-94%) in the training set and 89% (95% CI, 81%-94%) in the validation set among the samples for which TOO was assigned.
- TOO accuracy for GI cancers by tumor stage is shown in Table 2.

Table 2. Accuracy of Tissue of Origin Predictions by Tumor Stage for Patients With Gastrointestinal Cancers in Training and Validation

	TOO Accuracy (95% CI) (%)	
Tumor Stage	Training Set	Validation Set
I	89 (65-99)	75 (35-97)
II	84 (69-93)	88 (64-99)
III	96 (88-99)	93 (78-99)
IV	91 (84-95)	88 (76-96)

CI, confidence interval; TOO, tissue of origin.

Figure 4. Tissue of Origin Accuracy Across Stages in GI Cancers

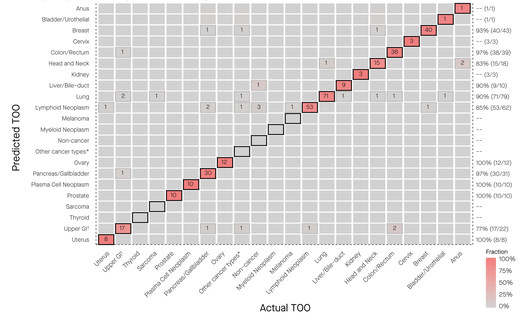


TOO accuracy in all GI cancers (esophagus, stomach, liver/bile duct, pancreas, gallbladder, colorectal).

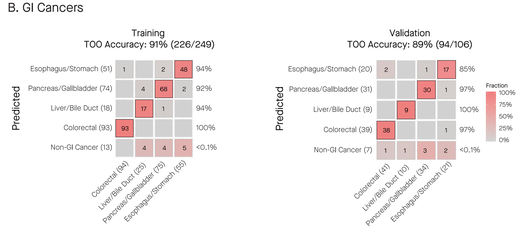
- TOO localization was highly accurate as demonstrated by a high level of agreement between the actual (*x* axis) and predicted (*y* axis) TOO per sample (Figure 5).

Figure 5. Tissue of Origin Localization

A. All (>20) Cancers (Validation Set)



B. GI Cancers



Numbers in each box represent the total number of predicted TOO. Color corresponds to the proportion of predicted TOO, as indicated to the right of the plot. Percent correct predictions from the total predictions for each cancer type is indicated to the right of the plot. A. All cancer types. B. GI cancers (esophagus, stomach, liver/bile duct, pancreas, gallbladder, and colorectal).
**Other cancer types, training: mesothelioma, penis, pleura, small intestine, testis, and vulva, as well as one sample missing primary cancer type information. Other cancer types, validation: orbit, merkel cell carcinoma of the scalp, penis, testis, vagina, vulva.*

CONCLUSIONS

- Targeted methylation analysis of plasma cfDNA simultaneously detected multiple GI cancers at high sensitivity with pre-specified high specificity (>99%).
  - >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 tissue of origin 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 other cancers, to help guide downstream clinical evaluation.

References

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[1]Upper GI: Esophagus and stomach.