Poster 2308

Classifier Performance of a cfDNA-Based Multi-Cancer Detection Test on Uncommon Cancer Types

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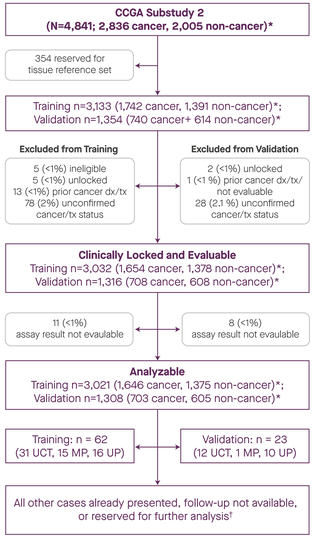
INTRODUCTION

- A noninvasive cell-free DNA (cfDNA) blood test with the potential to detect multiple cancer types at pre-metastatic stages (eg, stages I–III) could decrease cancer mortality.[1]
- To be effective at population scale, such a multi-cancer test should:
  - Detect as many cancer types as possible, to maximize the total number of cancer cases detected and intercept as many cancers as possible before they reach metastatic stages,
  - Have a low, fixed false positive rate (FPR; ie, high specificity) to minimize potential harms,[2,3] with compelling sensitivity, and
  - Accurately predict the tissue of origin (TOO) of a cancer signal to direct further evaluation and streamline the diagnostic workup.
- We previously reported multi-cancer detection[4] in plasma cfDNA samples across ~50 cancers[5] with >99% specificity, and accurate TOO prediction in >90% of cases, in the second substudy from the Circulating Cell-free Genome Atlas study (CCGA; NCT02889978).
- When transitioning from such a case-control study to application in a screening population, a multi-cancer early detection (MCED) test will be confronted with cancers with rare histologic types that were not present during test development or for which no TOO class was assigned (ie, untrained cancer types [UCT]).
- The ability of a multi-cancer test to detect UCT and uncommon clinical scenarios (eg, multiple primaries [MP] or metastatic cancers with uncertain primary [UP; metastatic cancers with unknown clinical primary tumor site, or unknown due to insufficient primary source data]) as cancer in this case-control study, may indicate MCED test behavior in similarly difficult scenarios in a general screening population.

METHODS

- CCGA plasma cfDNA samples from a pre-specified substudy[4] (Figure 1) were subjected to a targeted methylation (TM) assay.
  - Methylation states per DNA fragment across ~100,000 targeted genomic regions were input to create a classifier that detects cancer signal and predicts TOO for 20 classes defined in training (breast, ovary, uterus, cervix, anus, prostate, bladder & urothelial, kidney, colon & rectum, liver & intrahepatic bile duct, pancreas & gallbladder, upper GI, lung, head & neck, thyroid, sarcoma, and melanoma, as well as plasma cell-, myeloid-, and lymphoid neoplasms).
- Figure 1 depicts the CONSORT diagram for participants in the second substudy of CCGA and provides a breakdown of participants reported here.
- Test results were obtained for 43 participants with UCT (Figure 2 and Table 1), for 16 participants with MPs (Table 2), and 26 with UP (Table 3).
- For the 31 UCT cases in the training cohort, classifier results are obtained in 6-fold cross-validation of the training set. For all cases in the hold-out cohort and all cases with MP and UP, classifier results are obtained from the locked classifier created using the training cohort.

Figure 1. Study Design and CONSORT Diagram of the Second Substudy of CCGA



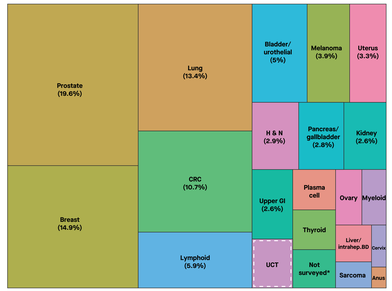
*At enrollment, prior to confirmation of cancer versus non-cancer status.
†Samples reserved for future analysis include, for example, a cohort of participants recruited from hematology clinics meant to understand ctDNA signal in premalignant or other hematologic conditions.
CCGA, Circulating Cell-Free Genome Atlas; MP, multiple primary; UCT, untrained cancer type; UP, uncertain primary.

- Two blood tubes were processed separately in different batches for each participant, and detection and TOO predictions were obtained independently for each tube.
- Stage-specific sensitivity estimates for cases with an assigned TOO class were obtained from published performance data using a cfDNA-based MCED test[4]; these detection rates were broken down to compare stage-specific detection of cases with assigned TOO class to stage-specific detection of cases with UCT, MP, and UP.

RESULTS

- Based on SEER data,[6] the UCT reported here are included in the approximately 1.85% (70,871 out of a total of 3,827,540) of cancer cases listed in SEER18 (for patients aged 50+ between 2000-2014) with a single, known primary that are mapped to the CCGA cohort but for which no TOO class is assigned (Figure 2).
- Multiple primaries and cancers of unknown primary site account for approximately 2-17%[7] and 3-5%[8], respectively, of all malignant epithelial tumors.

Figure 2. Depiction of Cancer Incidence for Prespecified TOO Classes and UCT



SEER incidence for trained and untrained cancer types with single, known TOO class. Untrained cancer types (UCT, dashed box) account for approximately 1.85% of all cases.
**Rare cancers in SEER that are not mapped to the CCGA cohort.*
CCGA, Circulating Cell-Free Genome Atlas; TOO, tissue of origin; UCT, untrained cancer type.

- Table 1 shows the classifier detection and TOO predictions for UCTs. Cancers of the vulva, vagina, and penis have mostly TOO prediction for other HPV-driven cancers (eg, head & neck) (see poster #2114[9]). The most frequent TOO prediction for mesothelioma of pleura is lung.
  - In general, TOO predictions for detected UCTs were consistent with biologic features of trained classes.

Table 1. Detection and TOO Results for Untrained Cancer Types

Cancers Without TOO Class	Cases (n)	Detected in 2/1/0 Tubes	ICD-O-3 Morphology Code and Site for Detected Cases	TOO Prediction (n)
Vulva/Vagina/ Penis	15	8/2/5	8070/3 Vulva	Head & neck (4)
				Cervix (2)
				Lymphoid neoplasm (1)
			8070/3 Vagina	Indeterminate localization (1)
			8070/3 Penis	Head & neck (1)
			8010/3 Penis	Head & neck (1)
Mesothelioma	7	4/0/3	9050/3 Pleura	Lung (3)
				Head & neck (1)
Small intestine	5	1/0/4	8140/3 Duodenum	Pancreas/gallbladder (1)
Merkel cell	4	1/0/3	8247/3 Skin	Lung (consistent with other neuroendocrine cancers) (1)
Brain	4	0/0/4		
All other	8	5/0/3	9061/3 Mediastinum	Upper GI tract (1)
			8200/3 Orbit	Breast (1)
			9064/3 Testis	Breast (1)
			8070/3 Other site	Cervix (1)
			8070/3 Urethra	Head & neck (1)

GI, gastrointestinal; TOO, tissue of origin.

- Table 2 shows the classifier detection and TOO predictions for MP. In addition to the TOO prediction, the TOO class with second highest score is also listed. TOO prediction was consistent with the cancer class with the stronger signal in 11 out of 12 cases with detected cancer signal.

Table 2. Detection and TOO Results for Participants with Multiple Primary Cancers

First Primary Cancer		Second Primary Cancer			
Type	Stage	Type	Stage	Detection	Highest and Second-Highest TOO Prediction
Bladder	III	Prostate	II	2/2	Bladder and urothelial, lung
Anus	III	Essential thrombocythemia	/†	2/2	Head and neck, cervical
Uterus	I	Lymphoid leukemia	/†	0/2	
Lung	II	Polycythemia vera	/†	2/2	Lung, breast (1 tube) Lung, ovary (1 tube)
Lung	I	Lymphoid leukemia	/†	2/2	Lymphoid neoplasm, myeloid neoplasm (1 tube) Lymphoid neoplasm, breast (1 tube)
Lung	I	Other	II	2/2	
Kidney	I	Prostate	/‡	2/2	
Prostate	III	Colorectal	II	1/2	Colorectal, prostate
Uterus	I	Breast	II	2/2	Breast, uterus
Uterus	I	Ovary	II	2/2	Uterus, ovary
Prostate	II	Essential thrombocythemia	/†	0/2	
Colorectal	I	Kidney	II	2/2	Colorectal, upper GI
Lung	IV	Pancreas	IV	2/2	Pancreas/gallbladder, lung (1 tube) Pancreas/gallbladder, upper GI (1 tube)
Pancreas	III	Breast	I	2/2	Pancreas/gallbladder, upper GI
Breast	III	Lung	I	2/2	Breast, Head and neck
Lung	I	Lymphoid leukemia	/†	2/2	Lymphoid neoplasm, prostate

† *Not expected to be staged.*
‡ *Non-informative stage.*
GI, gastrointestinal; TOO, tissue of origin.

- Table 3 shows the classifier detection and TOO results for UP. While TOO prediction cannot be assessed as correct or incorrect in this situation, some TOO classes obtained sufficiently low scores from the classifier to be excluded as likely possible origin.

Table 3. Detection and TOO Results for Participants with Metastatic Cancer of Uncertain Primary

ICD-O-3 Morphology Code	Detection	TOO Prediction	Supported† TOO Classes (N)
8010/3 Carcinoma NOS (5 cases)	2/2	Lung	5
	2/2	Lung (1 tube), indeterminate localization (1 tube)	13
	2/2	Pancreas/gallbladder	3
	2/2	Upper GI	2
	2/2	Upper GI (1 tube), lung (1 tube)	6
8033/3 Pseudosarcomatous carcinoma	2/2	Liver/intrahepatic bile duct	4
8041/3 Small cell carcinoma, NOS	2/2	Lung	6
8070/3 Squamous cell carcinoma, NOS (2 cases)	2/2	Cervical	3
	0/1	Not detected	/
8140/3 Adenocarcinoma, NOS (9 cases)	2/2	Liver/intrahepatic bile duct (4 cases)	1, 1, 7, 9
	2/2	Lung (4 cases)	1, 1, 1, 2
	1/1	Lung	4
8240/3 Carcinoid tumor, NOS (2 cases)	0/2	Not detected (2 cases)	/
8246/3 Neuroendocrine carcinoma, NOS (4 cases)	2/2	Breast (1 tube), pancreas/gallbladder (1 tube)	16
	2/2	Indeterminate localization	16
	2/2	Lung	14
	2/2	Pancreas/gallbladder	4
8480/3 Mucinous adenocarcinoma (2 cases)	2/2	Breast	17
	2/2	Upper GI	1

† *Number of TOO classes with sufficiently high scores per case, see text.*
GI, gastrointestinal; NOS, not otherwise specified; TOO, tissue of origin.

- Table 3 lists the single most supported TOO prediction for each UP case. By excluding unsupported TOO classes (summing to less than 0.5% of all TOO scores for each sample), 50% of samples had 4 or fewer TOO predictions with the number of remaining supported TOO predictions also shown in Table 3.
- Table 4 shows a breakdown of the sensitivity for cancer detection in cfDNA by clinical stage for cases in the second CCGA substudy training cohort assigned to one of the 20 TOO classes as well as UCT, MP, and UP cases.

Table 4. Sensitivity for Detection in cfDNA at >99% Specificity

Stage	Cancers with Assigned TOO Class	UCT	MP	UP
I	18.2%	36.4%	50.0%	/
II	43.5%	35.7%	66.7%	/
III	78.3%	65.4%	90.0%	/
IV	90.0%	62.5%	100%	88.5%

cfDNA, cell-free DNA; MP, multiple primary; TOO, tissue of origin; UCT, untrained cancer type; UP, uncertain primary.

CONCLUSIONS

- This targeted methylation MCED test detected cancers with rare histologic types and from uncommon clinical scenarios; detection was similar to that for cases with a TOO assigned in training.
  - These data suggest that there are biologic features in the methylome of circulating tumor cfDNA that are associated with cancer, and that are consistent between trained and untrained classes and detectable by this MCED test.
- The TOO capability of an MCED test can have direct implications for the clinic as the TOO readout can direct the clinical workup
- Given that further classifier development is ongoing (see poster #2114[9]), it is possible that the number of reportable, prespecified TOO classes and/or the TOO accuracy, may increase over time.
- Detection and TOO predictions for MPs further suggest that the test handles superposition of signals from existing TOO categories, and could lead to at least one of the primaries in most cases, allowing confirmation of cancer status.
- Prediction of a TOO for UP could potentially lead to altered treatment, and thus potentially improve outcomes in such cases.
- Overall, these data suggest that the MCED test classifier exploits general features associated with malignancy, and supports its generalizability to population-scale multi-cancer early detection.

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Disclosures

All authors were employees of GRAIL, Inc. at the time of the study and have equity in the company. **AJ** and **KNK** hold equity in Illumina. **JB** holds equity in Roche. **AMA** is an advisor to, and equity holder in, Foresite Labs.

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