Abstract LB058

Performance of a Cell-Free DNA-Based Multi-Cancer Detection Test as a Tool for Diagnostic Resolution of Symptomatic Cancers

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INTRODUCTION

- Delayed diagnostic resolution in individuals seeking care for signs and symptoms suspicious for cancer can affect outcome, patient experience, and the efficiency of care delivery.[1-6]
- A diagnostic triage tool that can detect the presence of cancer may inform the urgency and direction of diagnostic workup and shorten the time to correct diagnosis of cancer and non-cancer conditions.
- We developed a cell-free DNA (cfDNA)-based targeted methylation assay for a multi-cancer detection test that can detect the presence of cancer signal and predict cancer signal origin (ie, tissue of origin).[7]
- Here we report the performance of this multi-cancer detection test in participants with clinically presenting cancers. Additionally, we report performance in non-cancer participants and subgroups of non-cancer participants enrolled through a hematology clinic or with a significant non-malignant medical condition.

METHODS

Multi-Cancer Detection Test

- Detects cancer signal and predicts cancer signal origin through machine-learning analysis of methylation patterns in cfDNA circulating in the blood.[7]
  - Targeted methylation sequencing of 10^6 CpG sites in cfDNA isolated from a blood draw.
  - Machine-learning algorithm differentiates abnormal tumor cfDNA methylation patterns from normal cfDNA methylation patterns.
  - When cancer is detected, the machine-learning algorithm discerns cancer type-specific differences in methylation to predict cancer signal origin.

Study Design and Participants

- The Circulating Cell-free Genome Atlas (CCGA; NCT02889978) study is a prospective, longitudinal, multicenter, case-control study to develop and validate a multi-cancer detection test.
  - In the second CCGA substudy, multi-cancer detection test performance was assessed in a training set and an independent validation set.[7]
  - Here we report performance from the validation set from the second CCGA substudy of participants with clinically presenting cancers, non-cancer participants, and subgroups of non-cancer participants enrolled through a hematology clinic or with a significant non-malignant medical condition.
- Sensitivity and cancer signal origin prediction accuracy were assessed in participants with clinically presenting cancers.
- Specificity was assessed in non-cancer participants who had their non-cancer status confirmed at Year 1 follow-up, in the subgroup of non-cancer participants with significant non-malignant medical conditions at enrollment (eg, pancreatitis, liver diseases, inflammatory diseases), and in the subgroup of non-cancer participants enrolled through hematology clinics.

Measures

- Specificity: Estimated as the proportion of non-cancer participants with a "cancer signal not-detected" result.
- Overall sensitivity: Estimated as the proportion of cancer participants with a "cancer signal detected" result among all cancer participants.
- Sensitivity by clinical stage: Estimated as the proportion of cancer participants with a "cancer signal detected" result among cancer participants with a clinical stage or combined clinical stages across all cancer types.
- Sensitivity by cancer type: Estimated as the proportion of cancer participants with a "cancer signal detected" result among cancer participants with a cancer type across all clinical stages for that cancer type.
- Accuracy of cancer signal origin prediction: Estimated as the proportion of participants with a correctly predicted cancer signal origin label among true positives (cancer participants with a "cancer signal detected" result) with a trained target cancer signal origin label.
  - Multi-class confusion matrix comparing the target cancer signal origin label (x-axis) to the predicted cancer signal origin label (y-axis); Constructed among true positives with a single trained target cancer signal origin label.
  - Precision of cancer signal origin prediction: Estimated as the proportion of participants with a correctly predicted cancer signal origin label among true positives with that predicted cancer signal origin label.
- Survival: 503 of 518 participants with clinically presenting cancers had vital status available and were included in the survival analysis. The remaining 15 participants did not have vital status available because no electronic medical record was made or available and/or because the participants were not responsive to at least 2 contact efforts made by study coordinators.
  - Overall survival: Defined as the time from the date blood samples were collected to the date of death or the last date the participant was confirmed alive (censored). Survival curves were estimated using the Kaplan-Meier method, and differences were compared using the log rank test.[8,9]
  - Restricted mean survival time (RMST)[10,11]: Defined as the area under the survival curve; calculated using the survRM2 R package.

RESULTS

Study Population

- A total of 518 cancer participants who presented clinically and 398 non-cancer participants who had their non-cancer status confirmed at Year 1 follow-up were included in the analysis.
- In addition, 16 non-cancer participants with significant non-malignant medical conditions at enrollment and 137 non-cancer participants enrolled through a hematology clinic were also analyzed.

Specificity

- Overall, 99.5% of non-cancer participants who had their non-cancer status confirmed at Year 1 follow-up were called correctly as not having cancer (**Table 1**), corresponding to a false positive rate of <1%.

Table 1. Specificity

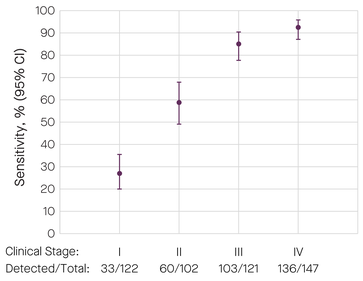
Group	Not Detected/Total	% (95% CI)
Non-Cancers With Non-Cancer Status Confirmed at Year 1 Follow-Up	396/398	99.5% (98.2-99.9%)
Non-Cancers With Significant Non-Malignant Medical Conditions at Enrollment	15/16	93.8% (71.7-99.7%)
Non-Cancers Enrolled Through a Hematology Clinic[a]	136/137	99.3% (96.0-100.0%)

CI, confidence interval.
[a]87 participants overlap between the non-cancers with non-cancer status confirmed at Year 1 follow-up and non-cancers enrolled through a hematology clinic.

Sensitivity

- Overall, 66.4% (344/518, 95% CI, 62.2-70.3%) of participants with clinically presenting cancers were called correctly as having cancer.
- Across all cancer types, sensitivity by clinical stage increased with increasing clinical stage (**Figure 1**).
  - 492 participants had stage I-IV cancers; 21 participants had cancers that were not expected to be staged; 4 participants had missing stage; and 1 participant had multiple primaries.
  - Sensitivity by clinical stage for stages I to IV together was 67.5% (332/492; 95% CI, 63.2-71.5%).
  - Sensitivity by clinical stage for stages I and II together was 41.5% (93/224; 95% CI, 35.3-48.1%).
  - Sensitivity by clinical stage for stages III and IV together was 89.2% (239/268; 95% CI, 84.9-92.4%).

Figure 1. Sensitivity by Clinical Stage Across All Cancer Types



- Sensitivity by cancer type was reported for multiple cancer types (**Table 2**).
  - Colon/rectum, liver/bile duct, and lymphoma cancers (among cancer types with >5 participants) had the highest sensitivity (all ≥90%).
  - Kidney, prostate, and uterus cancers (among cancer types with >5 participants) had the lowest sensitivity (all ≤30%).

Table 2. Sensitivity by Cancer Type

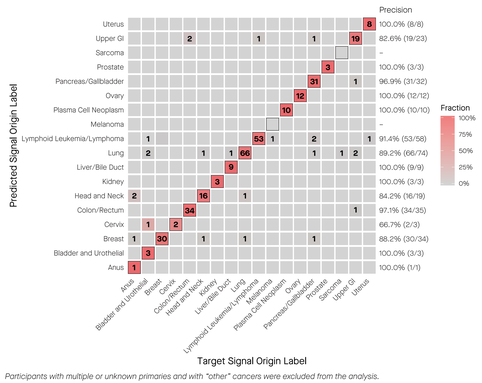
Cancer Type	Detected/Total	% (95% CI)
Overall	344/518	66.4 (62.2-70.3)
Bladder	5/5	100.0 (56.6-100.0)
Gallbladder	3/3	100.0 (43.9-100.0)
Melanoma	1/1	100.0 (5.1-100.0)
Urothelial	1/1	100.0 (5.1-100.0)
Colon/Rectum	36/39	92.3 (79.7-97.3)
Liver/Bile Duct	10/11	90.9 (62.3-99.5)
Lymphoma	45/50	90.0 (78.6-95.7)
Pancreas	33/39	84.6 (70.3-92.8)
Esophagus	18/22	81.8 (61.5-92.7)
Anus	4/5	80.0 (37.6-99.0)
Plasma Cell Neoplasm	10/13	76.9 (49.7-91.8)
Head and Neck	18/25	72.0 (52.4-85.7)
Ovary	12/17	70.6 (46.9-86.7)
Lung	68/99	68.7 (59.0-77.0)
Other[a]	16/25	64.0 (44.5-79.8)
Stomach	5/8	62.5 (30.6-86.3)
Breast	31/53	58.5 (45.1-70.7)
Cervix	2/4	50.0 (15.0-85.0)
Lymphoid Leukemia	9/19	47.4 (27.3-68.3)
Prostate	3/10	30.0 (10.8-60.3)
Uterus	9/36	25.0 (13.8-41.1)
Sarcoma	1/5	20.0 (1.0-62.4)
Kidney	3/25	12.0 (4.2-30.0)
Myeloid Neoplasm	0/1	0.0 (0.0-94.9)
Thyroid	0/1	0.0 (0.0-94.9)

CI, confidence interval.
[a]Adrenal (N=1), brain (N=1), unknown primary (N=12), orbit (N=1), penis (N=1), non-melanoma non-basal cell carcinoma/squamous cell carcinoma skin cancer (N=3), small intestine (N=1), testis seminoma (N=1), thymus (N=1), vagina (N=1), vulva (N=2).

Cancer Signal Origin Prediction

- Overall, cancer signal origin was predicted correctly in 91.7% (300/327, 95% CI, 88.3-94.3%) of true positives with a trained target cancer signal origin.
- Precisions of cancer signal origin prediction were >82% in all but one cancer signal origins with precisions calculated (**Figure 2**).

Figure 2. Precision of Cancer Signal Origin Prediction

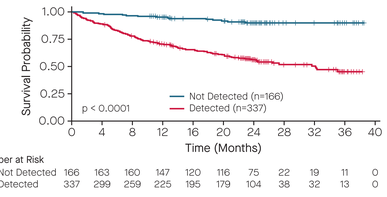


Participants with multiple or unknown primaries and with "other" cancers were excluded from the analysis.

Survival

- Participants with clinically presenting cancers in whom cancer signal was not detected with the multi-cancer detection test had better survival probability than those who had a cancer signal detected; the same trends were observed across stages I-IV (**Figure 3**).
  - At 36 months, participants with cancer signal not detected had a survival probability of 0.897 (95% CI, 0.849-0.949) compared to 0.453 (95% CI, 0.378-0.542) in participants with cancer signal detected.
  - At 36 months, participants with cancer signal not detected had an RMST of 33.6 months (95% CI, 32.4-34.8) compared to 23.6 months (95% CI, 22.1-25.1) in participants with cancer signal detected.

Figure 3. Kaplan-Meier Curve Depicting Overall Survival for Participants With Clinically Presenting Cancers That Had Cancer Signal Detected or Not Detected by the Multi-Cancer Detection Test



CONCLUSIONS

- This newly developed multi-cancer detection test had high specificity, including in non-cancer participants with significant non-malignant medical conditions or enrolled through hematology clinics.
  - Subgroups had limited sample size.
- For participants with clinically presenting cancers, this multi-cancer detection test detected cancer signal with high sensitivity across multiple cancer types and stages, and predicted cancer signal origin with high accuracy.
  - Cancer types with the highest sensitivities included colon/rectum, liver/bile duct, and lymphoma.
  - Cancers not detected by the multi-cancer detection test tended to be less aggressive.
- These findings support further development of this multi-cancer detection test and suggest that it has the potential to streamline the diagnostic workup of individuals presenting with signs and symptoms of cancer.

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Disclosures

AHB has consulted for Pfizer, Merck, Bayer, and Astellas Pharmaceuticals. **MCL** is an uncompensated advisory board member of GRAIL, Inc. **MVS** is a full-time employee of and has stock in McKesson Corporation and serves as a clinical advisor for GRAIL, Inc. **DDT**, **DR**, and **CB** have no conflicts of interest to declare. **KNK**, **TW**, **QZ**, and **AJ** are full-time employees of GRAIL, Inc., and have stock in GRAIL, Inc., and Illumina. **XC**, **JG**, **NZ**, **EH**, and **ETF** are full-time employees of and have stock in GRAIL, Inc. **EAK** is a consultant of GRAIL, Inc., Genomic Health, and GenomeDx.

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