Poster 721

Analytical Validation of a Multi-Cancer Early Detection Test With Tissue Localization Using a Cell-Free DNA-Based Targeted Methylation Assay

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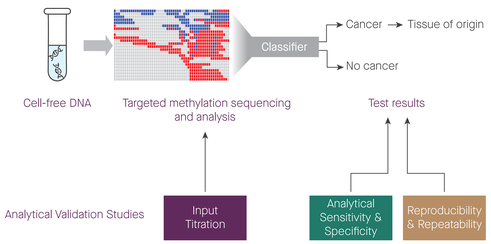
BACKGROUND

- Currently, population cancer screening is only performed for 5 cancers (breast, cervical, colon, lung, and on a per-patient basis, prostate cancer)[1-5]
- Although screening has reduced the mortality of these cancers, most cancers are diagnosed too late[6,7]
- A blood-based test using cell-free DNA (cfDNA) may address the unmet need for earlier detection of multiple cancers
- We have developed a cfDNA-based multi-cancer early detection (MCED) test that analyzes abnormally methylated regions in cell-free DNA fragments to detect cancer and predict the tissue of origin (TOO)
- In the Circulating Cell-free Genome Atlas study (NCT02889978), this test detected more than 50 cancers with a single, fixed false positive rate of 0.7% and predicted the TOO with 93% accuracy[8]
- Here, we describe analytical validation studies to characterize MCED test performance using known cancer and non-cancer samples from the CCGA study (Figure 1)
- Cancer detection was assessed by abnormal coverage, defined as the coverage of genomic regions likely to be abnormally methylated in cancer, and variant allele frequency (VAF; proportion of cfDNA fragments harboring tumor tissue-associated variants) or tumor fraction based on matched tissue samples

Figure 1. Analytical Validation of a Cell-Free DNA (cfDNA)-Based Multi-cancer Early Detection Test



Top, Plasma cfDNA samples were analyzed using targeted methylation sequencing and machine learning to classify samples as cancer or non-cancer, and if cancer was detected, to predict the tissue of origin.
Bottom, Test performance was characterized by 5 analytical validation studies. In addition to the 3 presented here, cross-contamination and potential interferents studies were performed.

INPUT TITRATION

Aim

- To characterize MCED test performance as a function of cfDNA input

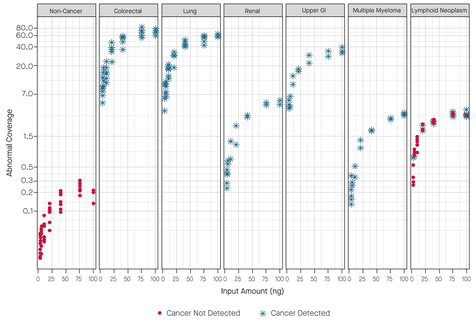
Methods

- Blood samples were obtained from 6 CCGA participants with cancer and 5 commercially-sourced samples from individuals without cancer (StemExpress [Folsom, CA], bioIVT [Hicksville, NY])
- 245 samples with 7 different cfDNA input amounts (3-100 ng) were analyzed

Results

- Abnormal coverage increased with cfDNA input amount and up to approximately 75 ng input cfDNA in cancer samples (Figure 2)
- Cancer was detected in 157/182 (86.3%) of cancer samples and was not detected in 62/62 (100%) of non-cancer samples (1 cancer sample failed quality control and was not analyzed)
- In all samples detected as cancer, TOO was correctly predicted at all input levels tested except lymphoid neoplasm, which exhibited a lower detection rate at all levels relative to the other cases

Figure 2. Correlation Between Cell-Free DNA Input Amount and Abnormal Coverage



Abnormal coverage increases with cfDNA input amount up to about 75 ng for both non-cancer samples (leftmost panel) and cancer samples (remaining panels). Samples detected as cancer are depicted by blue asterisks and those detected as non-cancer by red circles.
GI, gastrointestinal.

ANALYTICAL SENSITIVITY AND SPECIFICITY

Aim

- To determine analytical sensitivity and specificity of the MCED test

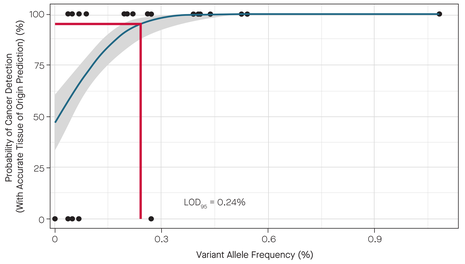
Methods

- Because the MCED test analyzes methylation patterns in more than 100,000 genomic regions, it was not possible to define a single traditional limit of detection
- Analytical sensitivity was characterized with respect to VAF and tumor fraction in samples produced by spiking cfDNA from cancer samples into cfDNA from non-cancer samples
- Analytical specificity was assessed by the false positive rate in 1,204 samples from 583 CCGA participants without cancer

Results

- In cancer samples, rate of cancer detection (with accurate TOO prediction) is positively correlated with VAF (Figure 3)
- Analytical sensitivity
  - 0.24% overall limit of detection with 95% probability (LOD_{95}) with respect to VAF
  - 0.06% LOD_{95} with respect to VAF excluding lymphoid neoplasm
  - 1.9% LOD_{95} with respect to tumor fraction
- 99.3% specificity (95% confidence interval, 98.6%-99.7%)

Figure 3. Correlation Between Variant Allele Frequency and Probability of Cancer Detection (With Accurate Tissue of Origin Prediction)



Probit fit (blue curve) of tissue of origin (TOO) accuracy versus variant allele frequency in samples detected as cancer (black circles). The gray shaded area represents the confidence region. The dotted red line indicates the overall limit of detection with 95% probability (LOD_{95}).

REPRODUCIBILITY AND REPEATABILITY

Aim

- To characterize reproducibility and repeatability of MCED test results

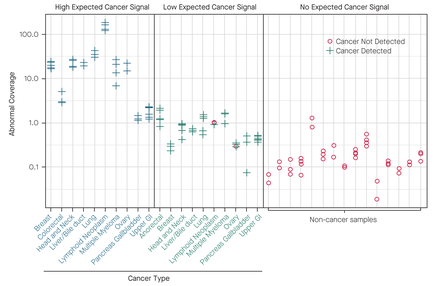
Methods

- Replicate blood samples from 20 CCGA participants with cancer (stage I to III) and 15 participants without cancer were analyzed in 4 independent test runs
- Two or more runs were performed on each set of samples to evaluate between-run variability
- Two replicates of all cancer samples and a subset of non-cancer samples were evaluated in a test run to determine within-run variability

Results

- Reproducibility
  - Across 4 independent runs
    - 77/81 (95.1%) replicates with cancer were called as cancer; of these, TOO was correct in 76/77 (98.7%) replicates
    - 45/45 (100%) replicates without cancer called as non-cancer
- Repeatability
  - In within-run tests
    - 31/34 (91.2%) concordant within-sample pairs with cancer were called as cancer; of these, TOO was correct in 30/31 (96.8%) concordant pairs
    - 17/17 (100%) concordant within-sample pairs without cancer called as non-cancer
- Consistent with these results, abnormal coverage was clustered among cancer and non-cancer sample replicates (Figure 4)

Figure 4. Abnormal Coverage in Cancer Samples and Non-cancer Samples



Clustering of abnormal coverage in cancer samples (left and center panels) and non-cancer samples (right panel). Cancer samples were grouped by their expected strength of the cancer signal (blue, high; green, low) from the multi-cancer early detection test as determined by computational titration tests. Samples detected as cancer are indicated by blue and green crosses and those detected as non-cancer by red circles.
GI, gastrointestinal.

CONCLUSIONS

- A targeted methylation-based cell-free DNA multi-cancer early detection test reproducibly and repeatedly detected cancer with high analytical sensitivity and specificity and highly accurate tissue-of-origin prediction
- These results support continued clinical development of this test
  - Clinical validation studies (STRIVE [NCT03085888] and SUMMIT [NCT03934866]) as well as a clinical implementation study (PATHFINDER [NCT04241796]) are ongoing

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Disclosures

All authors are employees and stockholders of GRAIL, Inc. AMA is an advisor to and holds equity in Foresite Labs, LLC.

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