1947

Assessment of Cancer Subtypes Across Multiple Cancer Types Using a Circulating Tumor DNA (ctDNA)-Based Targeted Methylation Assay

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INTRODUCTION

- Accurate identification of cancer subtypes is essential for effective diagnosis, prognosis, and treatment selection[1-3]
- Additionally, as subtype switching becomes more recognized as a major resistance mechanism to targeted therapies,[4] serial subtype assessment is expected to become more widely adopted
- Histologic type, cell of origin, and underlying pathogenesis have traditionally been determined using tissue histology, requiring invasive biopsies; this approach presents several challenges, including inaccessibility of some tumor tissue samples, limited interpretation of some tissue-based results due to tumor heterogeneity, and impracticality of longitudinal assessments due to the invasive nature of biopsies[5]
- GRAIL's ctDNA-based targeted methylation platform is a robust, tissue-free, scalable assay that has been shown to distinguish methylation patterns between cancers of different lineages and anatomical origins[6]
- We have previously described a set of classification algorithms that accurately predict subtypes of three common cancer types (lung, breast, and head & neck) using GRAIL's ctDNA-based targeted methylation assay from a plasma sample[7]
- The GRAIL-sponsored Circulating Cell-free Genome Atlas (CCGA)[6,8] study provides a rich database of plasma samples from patients with cancer in which to explore differences in ctDNA methylation among cancer subtypes

OBJECTIVE

- This study evaluates the use of GRAIL's ctDNA-based targeted methylation sequencing assay to accurately identify cancer subtypes across multiple cancer types through a tissue-free blood test

KEY RESULTS: A ctDNA-BASED TARGETED METHYLATION ASSAY ACCURATELY PREDICTS CANCER SUBTYPES ACROSS MULTIPLE CANCER TYPES FROM A PLASMA SAMPLE

PARTICIPANT CHARACTERISTICS

- 1,404 cases with 54 different staging cancer types as defined by the American Joint Committee on Cancer (AJCC)[9] were detected in a held-out test set with 2,669 cancer participants by GRAIL's Cancer Research Solution, a tissue-free, Research Use Only (RUO) multi-cancer detection test (**Table 1**)

Table 1: Characteristics of Participants in Held-Out Test Set

Demographics	Held-Out Test Set (n = 1,404)
Clinical Stage	
I	165
II	293
III	416
IV	493
Missing	37
Self-Reported Race and Ethnicity	
American Indian or Alaska Native	4
Asian, Native Hawaiian, or Pacific Islander	21
Black, non-Hispanic	102
Hispanic	119
White, non-Hispanic	1,143
Other/missing race	15
Smoking Status	
Ever-smoker	801
Never-smoker	582
Other/missing smoking status	21
Sex	
Female	665
Male	739
Age, years	
<40	77
40–49	137
50–59	295
60–69	460
70–79	334
≥80	101

SUBTYPING ALGORITHM RESULTS IN THE HELD-OUT TEST SET

- Performance assessment based on the 1,404 RUO-detected, ctDNA-positive cancer samples indicated that the classifier prediction accuracy was 92.7% (95% confidence interval [CI]: 91.3–94.0; 1,302/1,404) across all 14 subtypes (**Figure 1**)
- For subtypes with >50 samples in the data, high accuracy was observed in histologic types (adenocarcinoma), lineage of the cell of origin (lymphoid, Müllerian), and oncogenic process (human papillomavirus [HPV]-mediated) (**Table 2**)

Figure 1. Confusion Matrix of Predicted Cancer Subtypes vs Clinical Cancer Subtypes

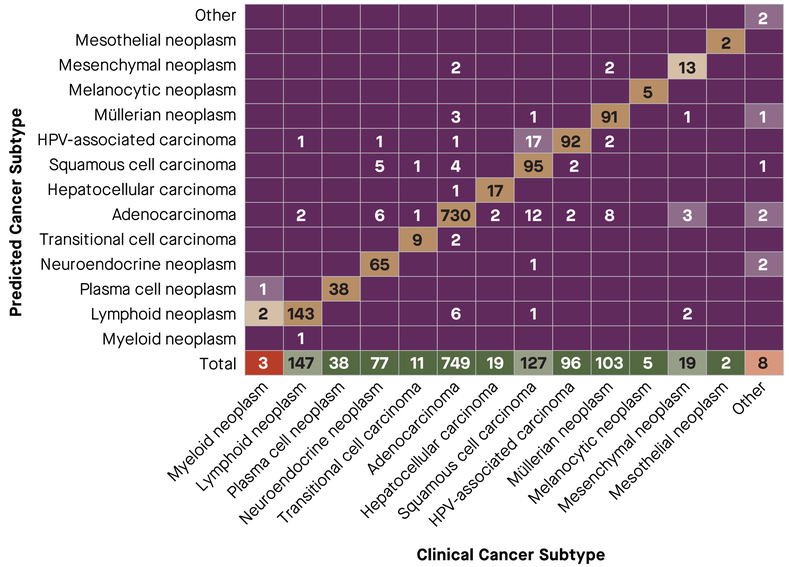


Table 2. Accuracy Assessment of Subtypes With >50 Samples in the Held-Out Test Set

Subtype	Correctly Classified	Misclassified	Total	Accuracy	95% CI
Lymphoid neoplasm	143	4	147	97.3%	92.7–99.1
Neuroendocrine neoplasm	65	12	77	84.4%	74.0–91.3
Adenocarcinoma	730	19	749	97.5%	96.0–98.4
Squamous cell carcinoma	95	32	127	74.8%	66.2–81.9
HPV-associated carcinoma	92	4	96	95.8%	89.1–98.7
Müllerian neoplasm	91	12	103	88.3%	80.2–93.6

- For squamous cell carcinoma, classifier prediction accuracy was 74.8% (95% CI: 66.2–81.9; 95/127)
  - Among the 32 misclassified samples, 17 were classified as HPV-associated carcinoma (14/17 were head & neck cancers), and 12 were classified as adenocarcinoma (9/12 were lung cancers)
    - Most of the HPV-associated carcinomas were squamous cell carcinomas
      - To accurately reflect the cell type origin of the cancer samples, we defined squamous cell carcinoma as HPV-negative squamous cell carcinoma so that it could be clearly distinguished from HPV-associated carcinoma
      - HPV infection has been established as a risk factor for developing head & neck squamous cell carcinoma[10]
      - The 14 head & neck cancers had no information on a positive HPV test in this multi-site study's electronic data capture and were therefore labeled as "squamous cell carcinoma"
      - However, it is possible that the multi-cancer–focused electronic data capture did not catch the HPV status of all head & neck cancers
    - For the 9 lung cancers classified as adenocarcinoma, it is possible both adenocarcinoma and squamous cell carcinoma cell types coexisted in the same tumor; this mixed histology is typically referred to as "adenosquamous carcinoma," and typically happens in the lung[11]
- For neuroendocrine neoplasm, classifier prediction accuracy was 84.4% (95% CI: 74.0–91.3%, 65/77)
  - Among the 12 misclassified samples, 5 were classified as squamous cell carcinoma, and 6 were classified as adenocarcinoma
  - Neuroendocrine neoplasm is not usually adenocarcinoma or squamous; but in rare cases, a tumor can contain both neuroendocrine and adenocarcinoma or squamous components.[12] It is possible that these misclassifications were due to mixed histology in the cancer samples

CONCLUSIONS

- This study demonstrated that GRAIL's ctDNA-based targeted methylation platform accurately predicts cancer subtypes in a wide range of cancers using a single blood draw
- This powerful multi-cancer subtyping classifier offers a potential tool to assist clinical decision-making, especially when tissue biopsies are unavailable, by supporting early cancer detection, identifying cancer types with unknown primaries, informing treatment selection, and monitoring resistance mechanisms
- Further validation and investigation of this subtyping classifier in real-world clinical settings is needed to fully decipher its utility in guiding diagnostic and treatment decisions and predicting patient outcomes

METHODS

Sample Selection and Processing

- Within the CCGA (NCT02889978) study, clinical data were recorded and plasma samples were collected, accessioned, and stored from 3,239 cancer patients prior to any treatment and 3,340 participants without cancer
- Plasma samples were processed on GRAIL's targeted methylation platform, which determines methylation status at over a million CpG sites and was optimized for cancer detection and localization of cancer signal (**Figure S1**)

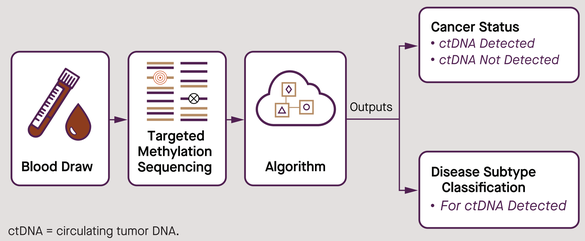
Clinical Subtype Determination

- We defined a set of 14 cancer subtypes spanning histologic types (adenocarcinoma, squamous cell carcinoma, neuroendocrine neoplasm, urothelial carcinoma), lineages of the cell of origin (lymphoid, myeloid, plasma cell, hepatocellular, mesenchymal, melanocytic, mesothelial, Müllerian), or an oncogenic process (HPV-mediated) across 54 AJCC-defined cancer types. **Figure S2** illustrates how these cancer subtypes are categorized in relation to their associated cancer types, with the shades of the circles indicating the frequency of the cancer types in the cancer subtype category in the training and intended-use populations. Pathology reports were used to assign a subtype to each sample. An "other" category was used for less frequent subtypes not captured by these subtypes

Algorithm and Performance Evaluation

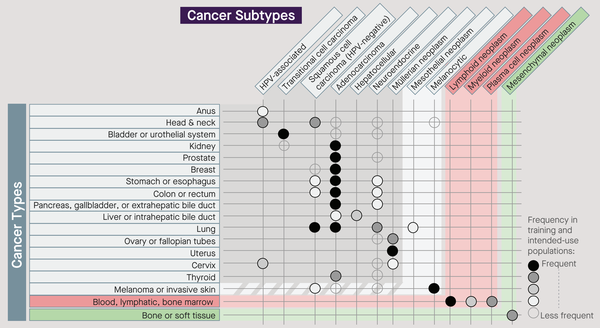
- A machine-learning classifier was trained using ctDNA methylation data from 3,340 non-cancer and 3,239 cancer participants with informative pathology to predict subtype. Performance was then assessed by using a held-out test set with 2,669 cancer participants
- GRAIL's Cancer Research Solution is a tissue-free, RUO multi-cancer detection test. To ensure a reliable assessment of the subtypes, 1,404 cancer participants in the held-out test set found positive by the RUO test and who were shedding sufficient cell-free DNA were reported for performance evaluation
- Wilson score interval with continuity correction[13] was used to calculate the confidence interval of the prediction accuracy

Figure S1. GRAIL's ctDNA-Based Targeted Methylation Platform



ctDNA = circulating tumor DNA.

Figure S2. 13 Defined Cancer Subtypes Mapping to Common Cancer Types



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Disclosures

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