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Identification of Cancer Subtypes With a ctDNA-Based Targeted Methylation Assay

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INTRODUCTION

- Identifying cancer histological and molecular subtypes is necessary for cancer diagnosis, prognosis determination, and treatment selection[1–3]
- Furthermore, as transformation between subtypes is increasingly recognized as a key resistance mechanism to targeted therapies,[4] serial subtype reassessment will likely gain adoption
- Cancer subtypes have traditionally been determined histopathologically by tissue architecture and immunohistochemistry (IHC); more recently, molecular subtyping is being performed using RNA sequencing (RNAseq) or assays that detect genetic alterations[5]
- However, existing molecular subtyping methods have significant limitations, including tissue biopsy requirements (IHC and RNAseq), inability to resolve complex epigenetically-driven subtypes (IHC and mutation detection), and poor reproducibility and feasibility (RNAseq)
- The GRAIL plasma-only, circulating tumor DNA (ctDNA)-based targeted methylation platform is a robust, biopsy-free, scalable assay that has previously been shown to distinguish methylation patterns between cancers of different lineages and anatomical origins[6]
- The GRAIL-sponsored Circulating Cell-free Genome Atlas (CCGA)[6,7] study provides a rich database of plasma samples from patients with cancer in which to explore differences in ctDNA methylation among cancer subtypes

OBJECTIVE

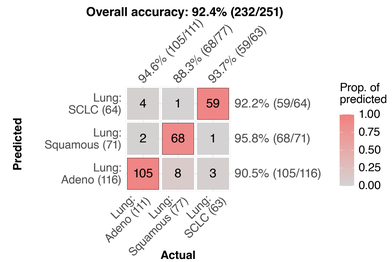
- To demonstrate the GRAIL platform's potential to detect fine-scale differences in cancer biology by developing classification algorithms to identify subtypes of three common cancer types using only a plasma sample

KEY RESULTS: A ctDNA-BASED TARGETED METHYLATION ASSAY ACCURATELY PREDICTS LUNG, BREAST, AND HEAD & NECK CANCER SUBTYPES FROM A PLASMA SAMPLE

Lung Cancer

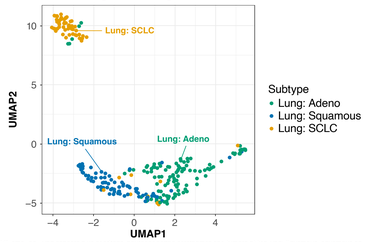
- The lung histology algorithm correctly classifies 95% (105/111) of adenocarcinomas, 88% (68/77) of squamous cell carcinomas, and 94% (59/63) of small cell lung cancers, plus additional neuroendocrine carcinomas and tumors of the lung, in a held-out test set (**Figure 1**)
- A reduced-dimension representation of targeted methylation data for held-out samples shows distinct clustering of lung cancer histological subtypes (**Figure 2**)

Figure 1. Lung Histology ctDNA Algorithm Classifies Subtypes With >90% Accuracy.



Adeno, adenocarcinoma; Prop, proportion; SCLC, small cell lung cancers plus additional neuroendocrine carcinomas and tumors of the lung; Squamous, squamous cell carcinoma.

Figure 2. Dimensionality Reduction of ctDNA Targeted Methylation Data Shows Separation of Lung Histological Subtypes.

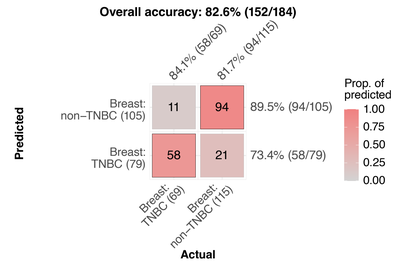


Adeno, adenocarcinoma; SCLC, small cell lung cancers plus additional neuroendocrine carcinomas and tumors of the lung; Squamous, squamous cell carcinoma; UMAP, Uniform Manifold Approximation and Projection.

Breast Cancer

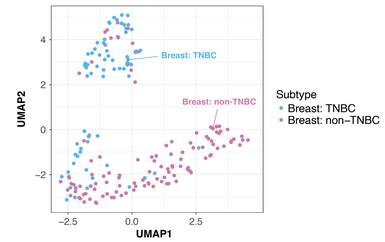
- The TNBC algorithm correctly classifies 84% (58/69) of TNBCs and 82% (94/115) of non-TNBCs in a held-out test set (**Figure 3**)
- A reduced-dimension representation of methylation data for held-out samples shows distinct clustering of TNBC and non-TNBC breast cancer subtypes (**Figure 4**)

Figure 3. TNBC ctDNA Algorithm Classifies Subtypes With >80% Accuracy.



Prop, proportion; TNBC, triple negative breast cancer.

Figure 4. Dimensionality Reduction of ctDNA Targeted Methylation Data Shows Separation Between TNBC and non-TNBC.

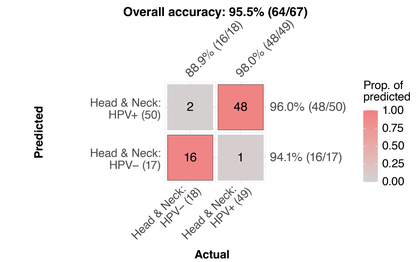


TNBC, triple negative breast cancer; UMAP, Uniform Manifold Approximation and Projection.

Head & Neck Cancer

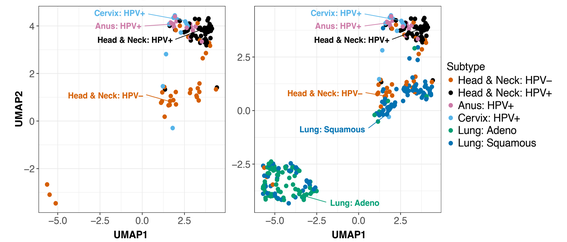
- The HPV algorithm correctly classifies 98% (48/49) of HPV-positive head & neck cancers and 89% (16/18) of HPV-negative head & neck cancers (**Figure 5**)
- A reduced-dimension representation of methylation data for training samples shows distinct clustering of HPV-positive and HPV-negative cancers (**Figure 6**)
- Training samples are shown in the dimensionality reduction to illustrate that HPV-positive head & neck cancers cluster with other HPV-driven cancers (e.g., anal, cervical, and that HPV-negative head & neck cancers cluster with lung squamous cell carcinomas (**Figure 6**)

Figure 5. HPV ctDNA Algorithm Classifies Subtypes With >95% Accuracy.



HPV, human papillomavirus; HPV+, HPV positive; HPV-, HPV negative; Prop, proportion.

Figure 6. Dimensionality Reduction of ctDNA Targeted Methylation Data Shows Separation Between HPV-Positive and HPV-Negative Squamous Cell Cancers, Including Head & Neck Cancers.



Adeno, adenocarcinoma; HPV, human papillomavirus; HPV+, HPV positive; HPV-, HPV negative; Squamous, squamous cell carcinoma; UMAP, Uniform Manifold Approximation and Projection.

CONCLUSIONS

- GRAIL's proprietary ctDNA-based targeted methylation platform accurately predicts subtypes of lung, breast, and head & neck cancers using only a plasma sample
- This work demonstrates the feasibility of cancer subtyping without the considerable safety risks and practical obstacles of tumor biopsy
- Future efforts will be directed towards generalizing the GRAIL subtyping method to additional cohorts and cancer subtypes
- The disease subtyping capabilities of GRAIL's technology may inform standard-of-care patient management and be used to discover and assess biomarkers in clinical research

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DISCLOSURES

Study funded by GRAIL, LLC. TN, TS, YZ, MA, YG, JH, RC, JH, OV, JB, JB, and CZ are current or former employees of GRAIL, LLC, and may have equity in the company. CS is an AstraZeneca advisory board member and Chief Investigator for two AZ clinical trials. CS is a paid member of GRAIL's Scientific Advisory Board (SAB) and receives consultant fees from Achilles Therapeutics (SAB member), Genentech, Medixci, Roche Innovation Centre Shanghai, Bicycle Therapeutics (SAB member), and the Sarah Cannon Research Institute. CS currently has stock options in Epic Bioscience, Bicycle Therapeutics, and Achilles Therapeutics and is co-founder of Achilles Therapeutics. LN declares no conflicts of interest.

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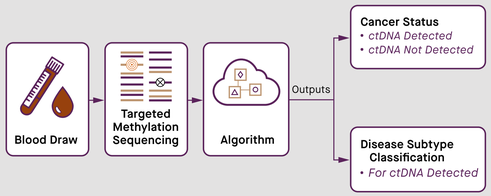
METHODS

Sample Selection and Processing

- As part of the CCGA (NCT02889978) and STRIVE (NCT03085888) studies, clinical data were recorded and plasma samples were collected, accessioned, and stored from 3989 patients with cancer prior to any treatment, and 6013 patients without cancer (no patients with cancer were used from the STRIVE study)
- 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**)
- Algorithms were developed to predict ctDNA status (ctDNA-detected or ctDNA-not-detected) and subtype for:
  - **Lung cancer:** lung histology algorithm (334 patients with lung cancer used in training; **Table S1**)
  - **Breast cancer:** TNBC algorithm (459 patients with breast cancer used in training; **Table S1**)
  - **Head & neck cancer:** HPV algorithm (157 patients with head & neck cancer, 30 with anal cancer, 71 with cervical cancer, and 2832 patients with other cancers used in training; **Table S1**)
  - Samples from patients without cancer were also used to train ctDNA status for each algorithm
- Performance of these three algorithms was assessed on a held-out cohort of ctDNA-detected cancer samples (184 breast, 241 lung, 67 head & neck)
- For each sample in the reduced-dimension plots, feature activation values derived from methylation states were retrieved from the corresponding classifier. Principal components analysis and then UMAP dimensionality reduction[8] were performed on these data to produce 2-dimensional plots. Samples shown are ctDNA-detected. Lung and breast plots show held-out test data, and the HPV plot shows training data in order to show relationships among non-head & neck cancers
- Because non-linear projections of high-dimensional data into the Euclidean plane like UMAP have known limitations in reproducibility,[9] they were used here simply to provide a visual representation of the concept and feasibility of classification, which is quantified more rigorously by classifier performance in the held-out test set

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



ctDNA = circulating tumor DNA.

Table S1. Patient Characteristics.

Training data	TNBC Algorithm		Lung Histology Algorithm			HPV Algorithm		
	Breast:TNBC (n=111)	Breast: non-TNBC (n=348)	Lung: SCLC (n=73)	Lung: Squamous (n=84)	Lung: Adeno (n=177)	HPV+ (n=102)	HPV- (n=85)	Other Cancer (n=2903)
Clinical stage								
I	30 (27%)	148 (43%)	12 (16%)	17 (20%)	54 (31%)	17 (17%)	9 (11%)	904 (31%)
II	55 (50%)	141 (41%)	8 (11%)	16 (19%)	15 (8%)	29 (28%)	14 (16%)	727 (25%)
III	22 (20%)	40 (11%)	21 (29%)	33 (39%)	41 (23%)	22 (22%)	28 (33%)	542 (19%)
IV	3 (3%)	17 (5%)	32 (44%)	17 (20%)	66 (37%)	34 (33%)	30 (35%)	549 (19%)
Missing	1 (1%)	2 (1%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (2%)	181 (6%)
Self-reported race and ethnicity								
American Indian or Alaska Native	0 (0%)	0 (0%)	1 (1%)	0 (0%)	0 (0%)	1 (1%)	1 (1%)	10 (0%)
Asian, Native Hawaiian or Pacific Islander	2 (2%)	11 (3%)	2 (3%)	0 (0%)	3 (2%)	1 (1%)	1 (1%)	56 (2%)
Black, non-Hispanic	24 (22%)	28 (8%)	1 (1%)	6 (7%)	20 (11%)	5 (5%)	5 (6%)	223 (8%)
Hispanic	8 (7%)	25 (7%)	2 (3%)	2 (2%)	4 (2%)	8 (8%)	6 (7%)	164 (6%)
Other/missing	0 (0%)	0 (0%)	1 (1%)	0 (0%)	0 (0%)	1 (1%)	3 (4%)	58 (2%)
White, non-Hispanic	77 (69%)	279 (80%)	66 (90%)	76 (90%)	150 (85%)	86 (84%)	69 (81%)	2392 (82%)
Smoking status								
Ever-smoker	32 (29%)	141 (41%)	61 (84%)	82 (98%)	146 (82%)	59 (58%)	63 (74%)	1498 (52%)
Never-smoker	79 (71%)	206 (59%)	11 (15%)	1 (1%)	30 (17%)	33 (32%)	14 (16%)	1284 (44%)
Other/missing	0 (0%)	0 (0%)	1 (1%)	1 (1%)	1 (1%)	10 (10%)	8 (9%)	121 (4%)
Sex								
Female	111 (100%)	348 (100%)	38 (52%)	18 (21%)	92 (52%)	37 (36%)	34 (40%)	1561 (54%)
Male	0 (0%)	0 (0%)	35 (48%)	66 (79%)	85 (48%)	65 (64%)	51 (60%)	1342 (46%)

Adeno, adenocarcinoma; HPV, human papillomavirus; SCLC, small cell lung cancers, plus additional neuroendocrine carcinomas and tumors of the lung; Squamous, squamous cell carcinoma; TNBC, triple negative breast cancer.