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Targeted methylation analysis of circulating cell-free DNA to predict outcomes in clinical stage I NSCLC patients

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ABSTRACT

Introduction: A cell-free DNA (cfDNA) targeted methylation assay developed at GRAIL for multi-cancer early detection (MCED) and to predict cancer signal origin (CSO) has demonstrated prognostic potential in a pan-cancer analysis. In this MCED, plasma cfDNA undergoes bisulfite sequencing targeting a panel of >100,000 methylation regions to detect the presence of circulating tumor DNA (ctDNA) and assess CSO.
Methods: We tested pre-operative plasma samples from clinically diagnosed stage I lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC) patients using the MCED assay to evaluate prognostic utility in early-stage lung cancer. This retrospective cohort consisted of 180 patients with LUAD and 80 with LUSC. Patients were selected in a 2:1 ratio within each histology based on relapse (high-risk group) or non-relapse (low-risk group) within 5 years of tumor resection. Outcomes of interest were ctDNA detection, CSO, relapse after surgery, and clinical to pathological upstaging.
Results: Utilizing the classifier employed for MCED, ctDNA was detected in 86 of 260 (33%) patients with a detection rate of 63 of 174 (36%) in the high-risk group and 23 of 86 (27%) in the low-risk group. Detection rate was lower in LUAD (40 of 180, 22%) than LUSC (46 of 80, 58%). Top CSO prediction was accurate in 83% of ctDNA positive patients (71 of 86). The association between ctDNA detection and relapse within 5 years did not reach statistical significance (p=0.13). ctDNA detection was associated with clinical to pathological upstaging in all patients (p=0.0001), particularly in LUSC (p=0.0002).
Conclusions: Pre-operative ctDNA detection using MCED did not significantly associate with recurrence within 5 years of surgery in clinical stage I LUAD and LUSC, however it did predict clinical to pathological upstaging. Customization of MCED algorithms may be required to improve prognostic utility in stage I NSCLC.

BACKGROUND

- Surgery remains the first line of treatment for resectable early-stage non-small cell lung cancer (NSCLC).
- There is an urgent need for prognostic differentiation of patients following early-stage NSCLC resection.
- Clinical TNM understaging prevents patients from being able to access emerging neoadjuvant regimens.
- GRAIL has recently developed a plasma cell-free DNA (cfDNA) methylation assay for multi-cancer early detection (MCED, Klein et al. Ann Oncol 2021, PMID 34176681) and a previous version of the MCED assay has been associated with disease prognosis beyond clinical stage across a range of tumor types (Chen et al. Clin Cancer Res 2021, PMID 34088722).

OBJECTIVES

Evaluate the performance of the MCED assay in
1) detecting lung cancer pre-operatively,
2) predicting relapse within 5 years after surgery, and
3) refining clinical staging to match pathological staging.

MATERIALS AND METHODS

CLINICAL DATA AND SELECTION OF PATIENTS

- Patients were selected from a large biobank of resected lung cancer available at the *Institut universitaire de cardiologie et de pneumologie de Québec – Université Laval* (IUCPQ-UL, Quebec City, Canada).
- Lung cancer diagnosis was confirmed by surgical resection and pathologic evaluation was updated to the 8th edition of the TNM classification.
- A total of 260 patients were selected based on pre-operative plasma samples availability, clinical stage, relapse status within 5 years of surgery, and histology (**Table 1** and **Figure 1**).

GRAIL ASSAY

Plasma samples were shipped to GRAIL (Menlo Park, CA) where bisulfite conversion, targeted enrichment and sequencing were performed. In-house machine learning classifiers were used to derive the cancer status (cancer or non-cancer), the cancer score and the cancer signal origin as previously reported (Klein et al. Ann Oncol 2021, PMID 34176681).

STATISTICAL ANALYSIS

- Outcomes of interest: cancer detection, relapse within 5 years after surgery, and clinical and pathological stage agreement.
- Sensitivity and specificity were calculated from a 2x2 contingency table.
- Analyses were performed for all patients and also stratified by histology, risk groups (low and high), and clinical stage (**Figure 1**), as applicable.
- Receiver operating characteristic (ROC) curves were used to evaluate the performance of the MCED cancer score to identify relapse and pathological upstaging within each histology.

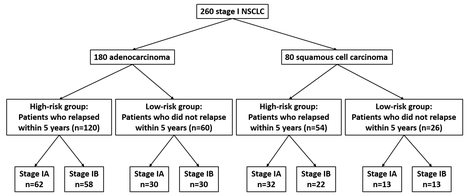

Figure 1. Study design and sample sizes. Staging is based on clinical stage.

Table 1. Patient's demographic and clinical information by risk group, histology and clinical stage

	Group							
	High-risk				Low-risk			
	LUAD		LUSC		LUAD		LUSC	
Characteristics	Stage IA	Stage IB	Stage IA	Stage IB	Stage IA	Stage IB	Stage IA	Stage IB
n	62	58	32	22	30	30	13	13
Age (years)	64.6 ± 8.6	65.2 ± 10.5	67.4 ± 8.4	69 ± 7.6	62.4 ± 9.0	63.3 ± 9.7	69.3 ± 8.1	65.4 ± 8.2
Sex								
Male	19	32	23	12	12	12	8	10
Female	43	26	9	10	18	18	5	3
Ethnicity								
White	57	56	31	21	30	30	13	13
Others	5	2	1	1	0	0	0	0
Body mass index (kg/m²)	26.3 ± 5.6	26.4 ± 4.7	26.9 ± 4.5	28.5 ± 6.0	26.2 ± 4.4	26.1 ± 4.8	27.7 ± 5.2	27 ± 4.9
Smoking status								
Never	4	1	0	2	1	0	0	0
Current	14	8	8	2	10	5	2	2
Former	44	49	24	18	19	25	11	11
Type of operation								
Lobectomy	44	49	17	17	20	27	11	10
Bilobectomy	1	3	1	0	1	3	0	1
Pneumonectomy	1	2	2	2	0	0	0	2
Wedge resection	7	1	4	1	2	0	1	0
Segmentectomy	9	2	6	0	7	0	1	0
Others	0	1	2	2	0	0	0	0
Pathological stage								
IA	32	9	16	0	18	9	11	1
IB	10	20	5	8	9	15	1	8
IIA	2	5	3	5	0	0	1	0
IIB	14	14	0	4	1	4	0	3
IIIA	3	9	6	2	1	2	0	1
IIIB	0	0	0	1	0	0	0	0
IV	0	0	1	0	0	0	0	0
Patho stage missing	1	1	1	2	1	0	0	0
Alive status (dead/alive)	43/19	45/13	22/10	19/3	4/26	3/27	5/8	4/9
PFS (median)	594.5	445	606	416	2268.5	2442.5	2543	2241

RESULTS

CANCER DETECTION AND CANCER SIGNAL ORIGIN

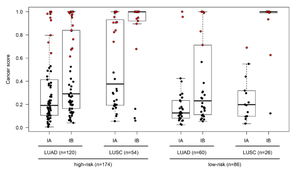

Figure 2. Boxplots showing the cancer score by group defined by relapse status at 5 years, histology and clinical stage. Cancer signal detected and cancer signal not detected by the MCED assay are depicted by red and black dots, respectively.

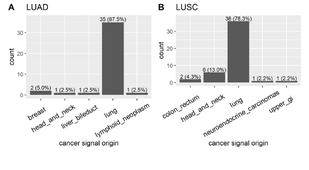

Figure 3. Cancer signal origin for detected cancer (n=86).

Table 2. Sensitivity of the MCED assay to detect cancer by histology, risk group and clinical stage

	Test results		
Subgroup	positive	negative	Sensitivity (95% CI)
All	86	174	0.33 (0.27, 0.39)
Adenocarcinoma	40	140	0.22 (0.16, 0.29)
Squamous cell carcinoma	46	34	0.57 (0.46, 0.68)
High-risk group	63	111	0.36 (0.29, 0.44)
Low-risk group	23	63	0.27 (0.18, 0.37)
LUAD high-risk stage IA	10	52	0.16 (0.08, 0.28)
LUAD high-risk stage IB	20	38	0.34 (0.22, 0.48)
LUAD low-risk stage IA	2	28	0.07 (0.01, 0.22)
LUAD low-risk stage IB	8	22	0.27 (0.12, 0.46)
LUSC high-risk stage IA	14	18	0.44 (0.26, 0.62)
LUSC high-risk stage IB	19	3	0.86 (0.65, 0.97)
LUSC low-risk stage IA	1	12	0.08 (0.00, 0.36)
LUSC low-risk stage IB	12	1	0.92 (0.64, 1.00)

PREDICTION OF RELAPSE

Table 3. Performance metrics of the MCED assay by histology to predict relapse within 5 years

		Relapse			
Subgroup	Test	True	False	Sensitivity (95% CI)	Specificity (95% CI)
All	+	63	23	0.36 (0.29, 0.44)	0.73 (0.63, 0.82)
	-	111	63		
Adenocarcinoma	+	30	10	0.25 (0.18, 0.34)	0.83 (0.71, 0.92)
	-	90	50		
Squamous cell carcinoma	+	33	13	0.61 (0.47, 0.74)	0.50 (0.30, 0.70)
	-	21	13		

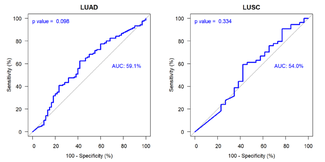

Figure 4. ROC curves by histology showing the value of the MCED cancer score at predicting relapse within 5 years after surgery.

CLINICAL AND PATHOLOGICAL STAGE AGREEMENT

Table 4. Performance metrics of the MCED assay to identify patients that will be up-staged by pathology

		Up-staged			
Subgroup	Test	True	False	Sensitivity (95% CI)	Specificity (95% CI)
All	+	40	42	0.49 (0.38, 0.60)	0.76 (0.69, 0.82)
	-	42	132		
Adenocarcinoma	+	16	23	0.29 (0.18, 0.43)	0.81 (0.73, 0.88)
	-	39	101		
Squamous cell carcinoma	+	24	19	0.89 (0.71, 0.98)	0.62 (0.47, 0.75)
	-	3	31		

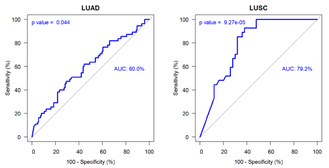

Figure 5. ROC curves by histology showing the value of the MCED cancer score at predicting subjects that are upstaged following pathological review.

CONCLUSION

- This study demonstrates that targeted methylation analysis of cfDNA using the recently developed MCED assay from GRAIL can detect early-stage lung cancer with modest sensitivity (33%), but with better performance in squamous cell carcinoma with clinical stage IB (**Table 2**). The assay may thus be useful for cancer detection in a more specific lung cancer niche.
- For cancer signal origin, 71 out of the 86 positive cancer calls were assigned to lung, which gives an accuracy of 83% (95%CI: 73%-90%) (**Figure 3**).
- Pre-operative cancer detection by the MCED assay was not significantly associated with relapse within 5 years after lung cancer resection (**Table 3** and **Figure 4**). Further development of the assay may facilitate predicting relapse following curative intent resection.
- The MCED assay was a predictor of pathological upstaging in both adenocarcinoma and squamous cell carcinoma (**Table 4** and **Figure 5**). This represents approximately 30% of patients who may benefit from treatment decisions that match pathological stage.

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