Lung Cancer Subtyping Using Cell-free DNA and Protein Biomarkers

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BACKGROUND

Lung cancer is the leading cause of cancer-related mortality worldwide. Accurate histological subtyping of lung adenocarcinoma (LUAD), squamous cell carcinoma (LUSC), and small cell lung cancer (SCLC) is critical for guiding optimal therapeutic strategies; however, up to 20% of patients lack sufficient tissue for conventional histopathological classification. Liquid biopsies using cell-free DNA (cfDNA) offer a promising non-invasive alternative for cancer characterization when tissue is not available

METHODS

We examined 745 patients with newly diagnosed, treatment-naive lung cancer of all stages, including LUAD (n=459), LUSC (n=152), SCLC (n=42) and other subtypes (n=92) from the prospective Lung Cancer Early Molecular Assessment trial. We performed low-coverage whole-genome sequencing of plasma cfDNA to derive fragmentation features; we applied a machine-learning classifier trained on tissue-based copy-number signatures from the Clinical Lung Cancer Genome Project to these data for lung cancer subtyping, and we estimated ctDNA burden using the DELFI-TF method.

RESULTS

The subtyping classifier, trained on tissue CLCGP samples and validated in LEMA plasma samples achieved an AUC of 0.98 (95% CI = 0.96-1.00) for distinguishing NSCLC from SCLC, and an AUC of 0.87 (95% CI = 0.83-0.91) for differentiating LUAD from LUSC. The model correctly classified 93% of SCLC, 77% of LUAD, and 76% of LUSC cases. The analysis included 551 plasma samples collected from different stages of lung cancers (I=91, II=48, III=138 and IV=274) meeting the minimum ctDNA fraction threshold of 0.15%.

Among a subset of 361 NSCLC patients, integration of five serum protein biomarkers resulted in a multimodal model that differentiated LUAD from LUSC across all tumor fractions with high performance (AUC=0.85, CI = 0.80-0.90), an improvement over cfDNA (p<0.01; AUC=0.78, CI = 0.74-0.82) or protein-only classifiers (p<0.001; AUC=0.70, CI = 0.62-0.78).

CONCLUSIONS

These findings establish cfDNA fragmentation and protein biomarkers as a viable non-invasive approach for lung cancer subtyping across all lung cancer stages when tissue is unavailable, with potential to expedite subtype-specific treatment selection and improve clinical outcomes.

Table 1.

Patient characteristics

Participants/samples, n	
745	
Median Age	71 (39-94)
Male	398 (53%)
Female	347 (47%)

Stage, n (%)

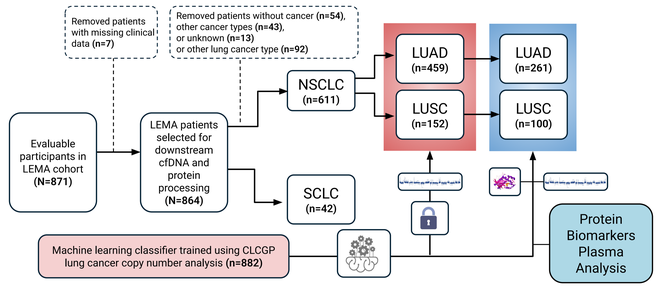
Stage I	188 (25.2%)
Stage II	69 (9.2%)
Stage III	186 (24.9%)
Stage IV	301 (40.4%)

Histology, n (%)

Adenocarcinoma (ADC)	459 (61.6%)
Squamous cell carcinoma (SCC)	152 (20.4%)
Large cell carcinoma (LCC)	15 (2%)
Mixed phenotype (ASC)	13 (1.7%)
Small Cell Lung cancer (SCLC)	42 (5.5%)
Unknown histology (Other)	64 (8.6%)

Figure 1:

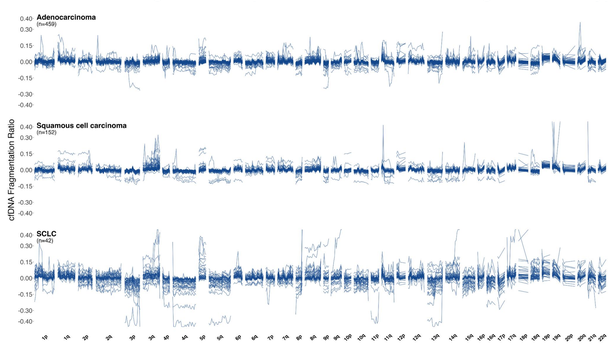
Approach for developing a lung cancer subtype classifier using cell-free DNA and protein biomarkers



- The flow diagram illustrates the processing and analysis of the DELFI pWGFrag-Lung assay for the 653 patients selected for subtyping analysis (NSCLC n=611, SCLC n=42)
- A machine learning classifier was trained using copy number data from CLCGP lung cancer tissue samples
- This classifier was then used to analyze cfDNA WGS data to categorize patients to SCLC, LUAD and LUSC subtypes
- The model performance was further improved by combining fragmentation with protein biomarkers into a multimodal model

Figure 2:

Genome-wide fragmentation profiles across different lung cancer subtypes



- The ratio of short to long genome-wide cfDNA fragmentation profiles in 5Mb bins across LUAD, LUSC, and SCLC lung cancer subtypes in the LEMA cohort
- Consistent aberrations in fragmentation patterns are observed in the subtypes, with SCLC showing the most divergent profile, characterized by widespread genomic alterations

Figure 7:

Implementation of cfDNA-fragmentome-based approaches for lung cancer subtyping in clinical practice

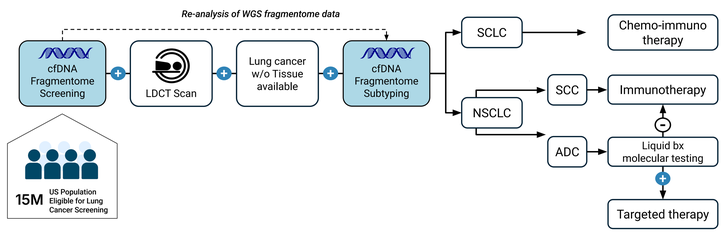
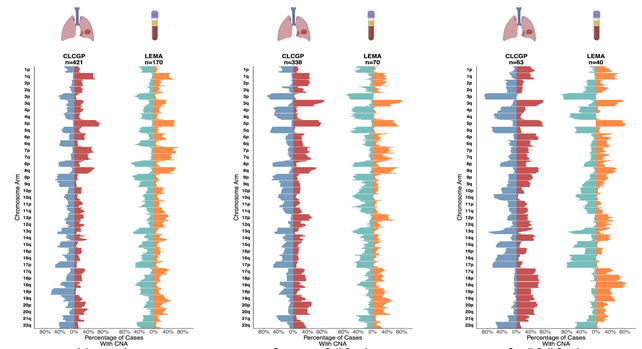


Figure 3:

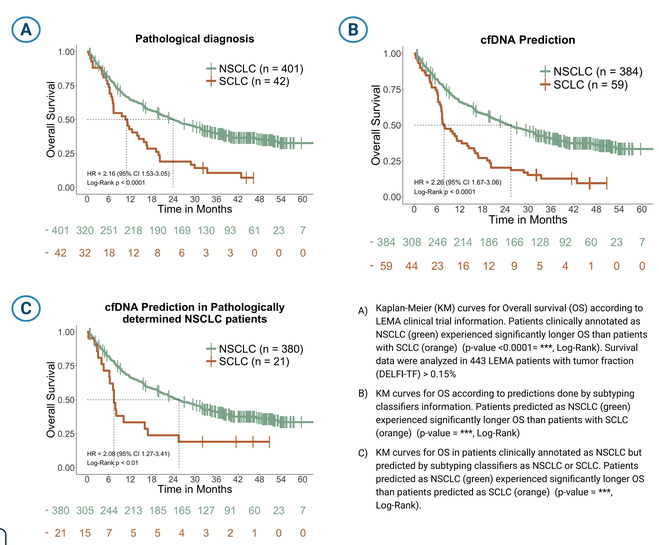
Copy number signatures distinguish subtypes in tissue and plasma



- Copy number analysis using WGS data generated from lung cancer tissue samples obtained from the CLCGP database and cfDNA collected from patients from the LEMA cohort with a DELFI-Tumor Fraction ≥0.15%
- Segmentation scores were derived for each sample for adjacent non-overlapping 1 Mb windows
- The right-hand bars indicate proportion of samples with amplifications and left-hand bars indicate proportion of samples with deletions

Figure 5:

Clinical outcome of clinically and molecularly identified NSCLC and SCLC



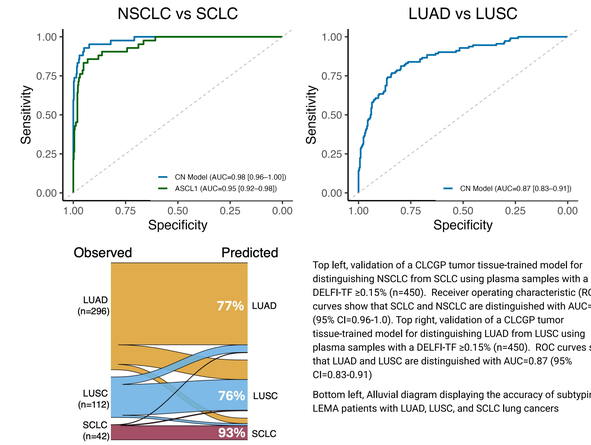
A) Kaplan-Meier (KM) curves for Overall survival (OS) according to LEMA clinical trial information. Patients clinically annotated as NSCLC (green) experienced significantly longer OS than patients with SCLC (orange) (p-value <0.0001= ***, Log-Rank). Survival data were analyzed in 443 LEMA patients with tumor fraction (DELFI-TF) > 0.15%

B) KM curves for OS according to predictions done by subtyping classifiers information. Patients predicted as NSCLC (green) experienced significantly longer OS than patients with SCLC (orange) (p-value = ***, Log-Rank)

C) KM curves for OS in patients clinically annotated as NSCLC but predicted by subtyping classifiers as NSCLC or SCLC. Patients predicted as NSCLC (green) experienced significantly longer OS than patients predicted as SCLC (orange) (p-value = ***, Log-Rank).

Figure 4:

Tumor tissue-trained model applied to cfDNA fragmentomes with tumor fraction ≥0.15%

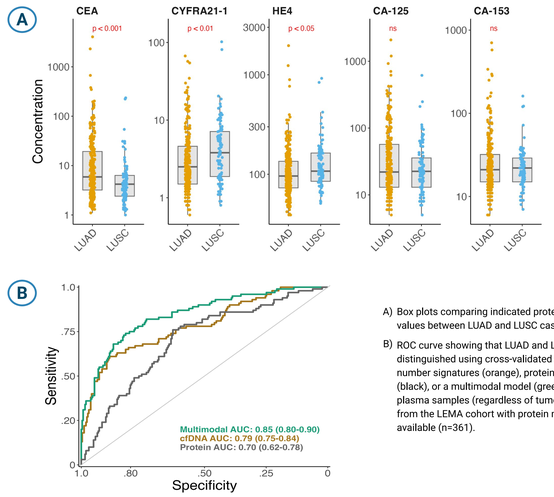


Top left, validation of a CLCGP tumor tissue-trained model for distinguishing NSCLC from SCLC using plasma samples with a DELFI-TF ≥0.15% (n=450). Receiver operating characteristic (ROC) curves show that SCLC and NSCLC are distinguished with AUC=0.98 (95% CI=0.96-1.0). Top right, validation of a CLCGP tumor tissue-trained model for distinguishing LUAD from LUSC using plasma samples with a DELFI-TF ≥0.15% (n=450). ROC curves show that LUAD and LUSC are distinguished with AUC=0.87 (95% CI=0.83-0.91)

Bottom left, Alluvial diagram displaying the accuracy of subtyping of LEMA patients with LUAD, LUSC, and SCLC lung cancers

Figure 6:

Multimodal fragmentome-protein model distinguishes LUAD from LUSC in all LEMA samples with both biomarkers available



A) Box plots comparing indicated protein biomarker values between LUAD and LUSC cases

B) ROC curve showing that LUAD and LUSC are distinguished using cross-validated cfDNA copy number signatures (orange), protein biomarkers (black), or a multimodal model (green) for all plasma samples (regardless of tumor fraction) from the LEMA cohort with protein measurements available (n=361).

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References:
[1] Mazzone et al. Cancer Discov. 2024 Nov 1;14(11):2224-2242. [2] Mathios et al. Nat Commun. 2021 Aug 20;12(1):5060. [3] Cristiano S, et al. Nature. 2019;570:385-9. [4] Schouten et al PLoS One. 2024 Jul 31;19(7) [5] van't Erve et al. Nat Commun. 2024 Oct 21;15(1):8801

- **Tumor subtyping is necessary for therapy selection in cancer patients, but not feasible in some individuals.**
- **Cell-free DNA fragmentomes and protein measurements can distinguish lung cancer subtypes with high performance.**
- **This approach provides an avenue for non-invasive lung cancer subtyping to guide treatment selection that could lead to improve clinical outcomes.**