Evaluating lung cancer clinical characteristics and tumor subtypes using cell-free DNA fragmentomes

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BACKGROUND

- Lung cancer is a heterogeneous disease classified into two major types based on its biology, therapy, and prognosis: Non-Small Cell Lung Cancer (NSCLC) and Small Cell Lung Cancer (SCLC)
- Although molecular profiling of NSCLC is essential for optimising treatment decisions, it is often incomplete. Tumour biopsies are complicated and invasive.
- Circulating tumour DNA (ctDNA) analyses have the potential to overcome some of the issues encountered with tissue-based tests.
- Here we evaluate the ability of a liquid biopsy approach to predict the clinical and tumor subtype characteristics of lung cancer cases using a cohort of 578 individuals from a prospective clinical trial (LEMA, NCT02894853)

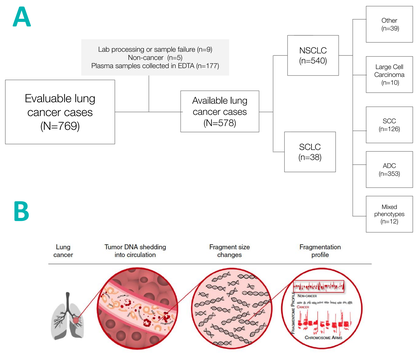
METHODS

- Pre-treatment plasma samples were processed through the DELFI assay, a validated cell-free DNA (cfDNA) test based on a locked machine learning analysis of genome-wide fragmentation patterns [**1**]
- Clinical data were collected from all patients (N=578) and included tumor stage (I=165, II=60, III=147, IV=206), histologic subtypes, lymph node invasion, comorbidities, medications, smoking history, treatment type and overall-survival
- Tissue molecular profiling was performed to identify actionable alterations in driver oncogenes [**4**]
- A machine learning model utilizing copy-number signatures was trained on TCGA NSCLC data. This model was then applied to cfDNA fragmentation data from the LEMA cohort

RESULTS

- Lung adenocarcinoma (ADC) cases displayed lower DELFI scores compared to squamous cell carcinomas (SCC) (p<0.01, Wilcoxon rank-sum), while small-cell lung cancer cases had the highest scores among all subtypes (p<0.0001, Wilcoxon rank-sum)
- cfDNA fragmentome changes in patients with ADC and SCC reflected chromosomal alterations observed in TCGA cohorts (ADC n=518; SCC n=501)
- A machine learning model trained on TCGA copy-number changes was able to predict lung cancer subtypes with high accuracy in a non-invasive manner (AUC for stages I-IV=0.91)
- DELFI scores obtained before treatment and surgery showed predictive capability for both overall survival and cancer relapse in lung cancer patients across early and late stages.

Figure 1. LEMA study design



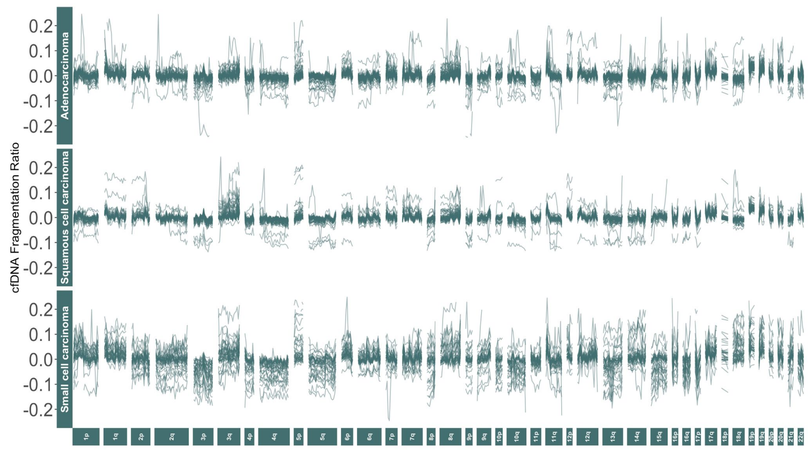
A) Study design of the prospective observational trial of 769 individuals examined consecutively at the Lung cancer Early Molecular Assessment (LEMA) trial (NCT02894853)

B) Illustration representing the DELFI approach for lung cancer through noninvasive assessment of cell-free DNA fragmentation profiles [**1**]

Table 1. Patient characteristics

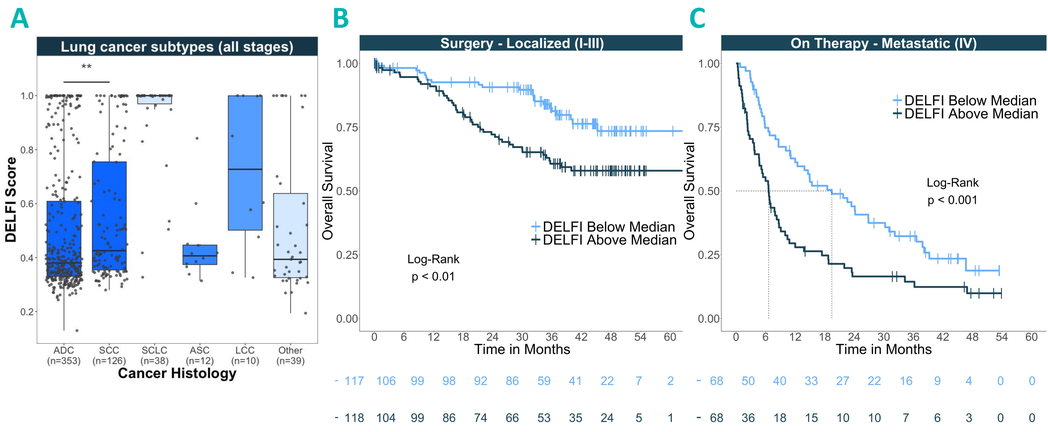
Participants/samples, n	578
Median Age	71 (39-94)
Sex, n (%)	
Male	319 (55%)
Female	259 (45%)
Stage, n (%)	
Stage I	165 (28.5%)
Stage II	60 (10.3%)
Stage III	147 (25.4%)
Stage IV	206 (35.6%)
Histology, n (%)	
Adenocarcinoma (ADC)	353 (61%)
Squamous cell carcinoma (SCC)	126 (21.7%)
Large cell carcinoma (LCC)	10 (1.7%)
Mixed phenotype (ASC)	12 (2%)
Small Cell Lung cancer (SCLC)	38 (6.5%)
Unknown histology (Other)	38 (6.7%)
Baseline pre-treatment, n (%)	
Yes	578 (100%)
Smoking Status, n (%)	
Current	242 (41.8%)
Former	293 (50%)
Never	43 (7.4%)
Tissue molecular profile, n (%)	
No mutation identified	188 (43.8%)
KRAS	134 (31.2%)
EGFR	53 (12.3%)
ALK	5 (1.1%)
ERRB	11 (2.5%)
ROS1	6 (1.3%)
MET	15 (3.4%)
BRAF	17 (3.9%)
Number of Comorbidities, n (%)	
0	252 (43.5%)
1	218 (37.7%)
2	99 (17%)
3	9 (1.5%)

Figure 2. Genome-wide fragmentation profiles across different lung cancer subtypes



Fragmentation profiles of lung cancer revealed genome-wide fragmentation alterations, and reflect the underlying tissue histology of lung cancer subtypes in adenocarcinoma (ADC), squamous cell carcinoma (SCC), and Small cell Lung Cancer (SCLC)

Figure 3. DELFI scores among lung cancer subtypes and their prognostic potential across lung cancer stages

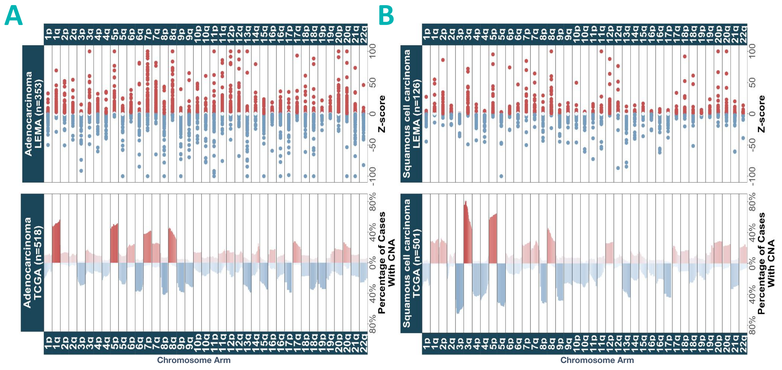


A) DELFI classifier derived from recently published locked machine learning model [1] showed differences across lung cancer subtypes, suggesting genome-wide fragmentation features have the potential to capture tumor-specific histologies.

B) Overall survival Kaplan-Meier curves for stage I-III lung cancer patients, stratified by pre-surgery DELFI score (above/below median)

C) Overall survival in stage IV lung cancer patients, stratified by pre-treatment DELFI score (above or below median), visualized with Kaplan Meier curves.

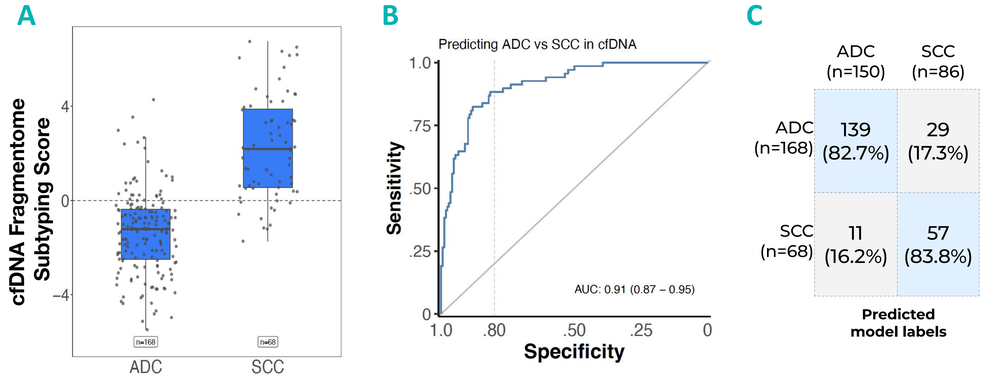
Figure 4. cfDNA fragmentome changes in the circulation of patients with lung cancer reflect chromosomal alterations observed in lung cancer subtypes



A) Genome-wide cell-free DNA fragmentomes of the LEMA patients had similar chromosomal gains and losses to those of lung cancer specimens analyzed in TCGA (ADC, n = 518). LEMA and TCGA subjects show increased cfDNA representation of 1q, 2p, 3q, 5p, 8q and decreased levels of 3p, 4q, 5q, 10q, and 13q, all known to be gained or lost in lung cancer

B) We observed a distinct aneuploidy profile in LEMA and TCGA SCC (n=501) compared to ADC, suggesting a potential opportunity to differentiate these cancer types via a non-invasive approach based on cfDNA features

Figure 5. Machine learning model using TCGA copy-number signatures predicts lung cancer subtype with high accuracy in the LEMA cohort

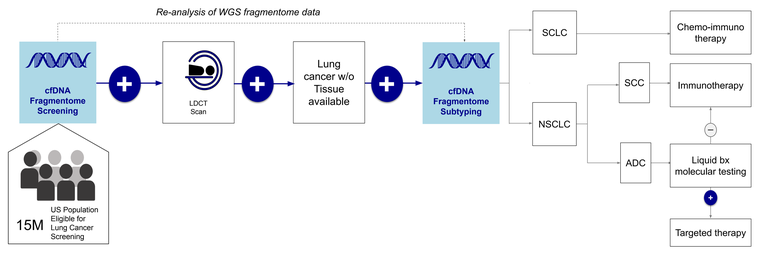


A) Subtyping score derived from a machine learning model using copy-number signatures from TCGA NSCLC and applied to cfDNA fragmentation data from the LEMA cohort (limited to samples with ctDNA >0.3% as measured by DELFI-TF [**5**]: ADC=168, SCC=68)

B) ROC curve of the cfDNA fragmentome classifier distinguishing ADC from SCC in stage I-IV NSCLC cases (LEMA cohort).
A machine learning model, trained on copy-number signatures from TCGA NSCLC and applied to cfDNA fragmentation data from the LEMA cohort (ctDNA >0.3% as measured by DELFI-TF [5]), yielded a subtyping score. The analysis included 168 ADC and 68 SCC samples.

C) Confusion matrix of the cfDNA fragmentome classifier prediction in the LEMA cohort

Figure 6. Implementation of cfDNA-fragmentome-based approaches for lung cancer subtyping in clinical practice



cfDNA fragmentomes show promise as prognostic biomarkers in both US and European lung cancer patients. DELFI scores correlate with cancer stage and predict survival, independent of mutational status or comorbidities. cfDNA fragmentome analysis could differentiate lung cancer subtypes and guide personalized treatment.

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