Residual ctDNA after treatment predicts early relapse in patients with early-stage NSCLC

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INTRODUCTION

- Identification of minimal residual disease (MRD) in patients with localized non-small cell lung cancer (NSCLC) following treatment with curative intent holds promise for identifying patients who are at higher risk of relapse who may benefit from adjuvant therapy.
- Liquid biopsies based on circulating tumor DNA (ctDNA) analysis are being investigated for detection of residual disease and recurrence.
- Due to low ctDNA levels in early-stage disease or post-treatment, effective methods require high analytical sensitivity to detect variant allele fractions (VAF) below 0.01%.
- Here, we have evaluated detection of ctDNA in serial plasma samples collected from the LUCID (LUng cancer - CIrculating tumor DNA) study using a personalized sequencing assay.

STUDY OBJECTIVE

- The objective of this study was to test the feasibility and prognostic value of detecting ctDNA at or before relapse in 88 stage IA - IIIB NSCLC patients following treatment with curative intent, either surgery (n=69) or radiotherapy (RT) ± chemotherapy (n=19) **[Table 1].**

Characteristics	Patients (n=88)
Age, Median (range)	
Stage I	73 (52-88)
Stage II	74 (57-83)
Stage III	67 (44-78)
Sex	
Male	45 (51.1%)
Female	43 (48.9%)
Smoking status	
Never	8 (9.1%)
Ex-smoker	63 (71.6%)
Smoker	16 (18.2%)
Cancer history	29 (33%)

Characteristics	Patients (n=88)
Histology	
Adenocarcinoma	55 (62.5%)
Squamous cell carcinoma	27 (30.7%)
Other	6 (6.8%)
Pathology	
Stage I	43 (48.9%)
Stage II	25 (28.4%)
Stage III	20 (22.7%)
Treatment	
Surgery	69 (78.4%)
ChemoRadiation	19 (21.6%)
Time points	
Baseline	78
Follow-up	285

Table 1: Patient demographics of NSCLC patients enrolled in the study who had available tumor tissue for exome sequencing & an available ctDNA assay (n=88).

- Plasma samples (n=363) were collected before and after treatment, and at 3, 6 and 9 months. For 17 patients, additional plasma was collected at disease relapse. Patients were followed for a median of 3 years (range: 9 months to 5 years) and disease outcomes recorded.
- Plasma ctDNA was analysed using RaDaR^{TM} assays that target up to 48 tumor-identified variants per patient. Detection of residual ctDNA after end of treatment was compared to outcomes including Relapse Free Survival (RFS) and Overall Survival (OS).

Analysis of patient samples using patient-specific ctDNA assays

Figure 1: Tumor exome sequencing was performed to identify somatic mutations, and a personalized ctDNA assay developed for each patient.

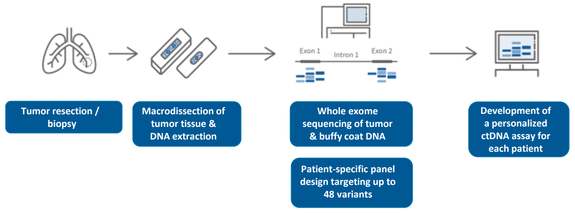
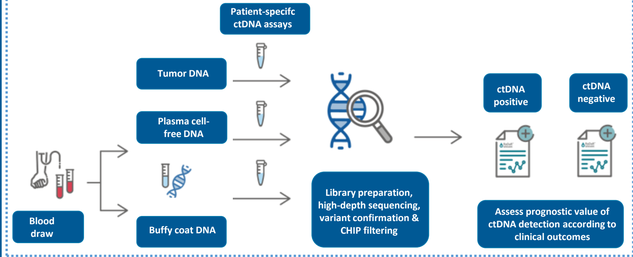


Figure 2: Tumor and buffy coat DNA were analysed using personalized assays to confirm somatic mutations and exclude clonal hematopoiesis (CHIP). Plasma samples were analysed and ctDNA detection compared to outcomes.



RESULTS

ctDNA detection prior to treatment

- ctDNA was detected prior to treatment in 87%, 77% and 24% of patients with stage III, II and I disease respectively. Overall, ctDNA was detected in 26% of all samples collected at baseline and follow-up at a median variant allele fraction (VAF) of 0.047% (range: 0.0007% to >2%).

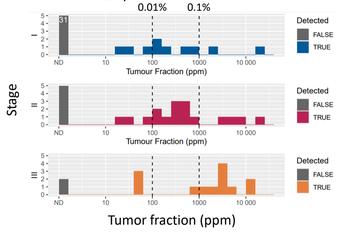


Figure 3: ctDNA levels (VAFs), measured in parts per million (ppm), detected in plasma samples prior to treatment.

Dotted lines indicate variant allele fractions of 0.01% and 0.1%.

Longitudinal monitoring for residual disease and recurrence

Figure 4: Examples of longitudinal monitoring of ctDNA

- **Patient 1:** ctDNA detected before surgery, but not at 20 days after surgery, and detected at day 174, 222 days prior to clinical progression.
- **Patient 2:** ctDNA detected at all timepoints, lead time 203 days
- **Patient 3:** ctDNA detected before and after surgery, but not after adjuvant chemotherapy. No progression in 3 years of further follow-up.
- **Patient 4:** ctDNA detected before and after surgery, and at all follow-up time points, many months prior to clinical progression.

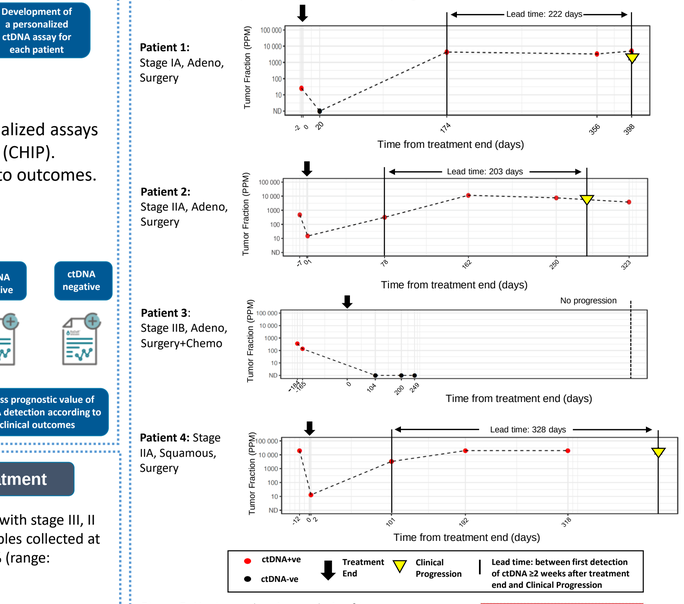


Figure 5: Heatmap showing analysis of tumor, buffy coat and plasma samples from Patient 4 using a patient-specific assay. Each row represents a different sample type and each column is a different variant. Variants shaded in grey were excluded from analysis due to absence in tumor DNA or presence in buffy coat DNA

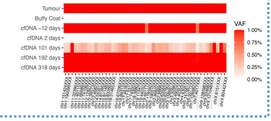
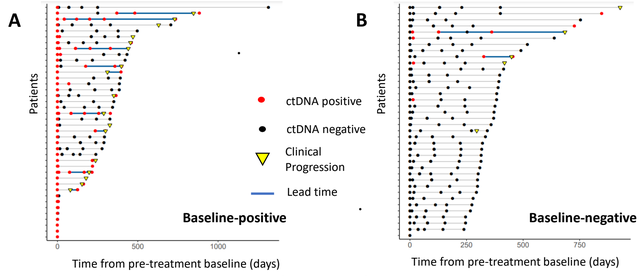


Figure 6: Longitudinal monitoring of plasma from patients with (A) ctDNA detected (n=40) and (B) ctDNA not detected prior to treatment (n=38).



- In analysis of samples collected ≥2 weeks after the end of treatment, ctDNA was detected at any timepoint in 20 cases. Of these, 17 had clinical progression (85%), 2 patients were diagnosed with a second primary cancer (genomic investigation is underway), and one patient died of other causes. 5 patients had no samples available in the 200 days prior to progression. In the remaining 12 patients, ctDNA detection preceded clinical progression by a median of 212.5 days. In 8 cases who progressed >200 days after end of treatment, median ctDNA detection lead time was 402.5 days.

ctDNA detection after treatment is associated with shorter relapse free survival

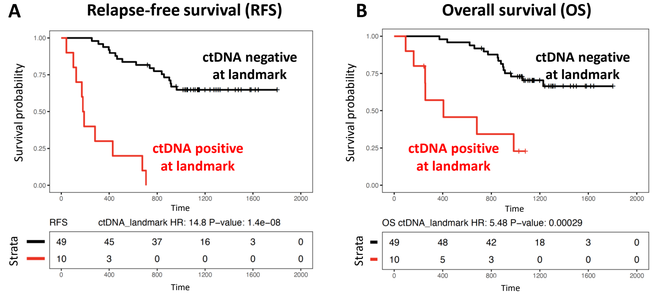


Figure 7: In survival analysis of 59 patients, plasma was available within a landmark timepoint, between 2 weeks and 4 months after end of initial treatment. ctDNA detection at the landmark timepoint was strongly predictive of clinical disease relapse Kaplan-Meier curve showing (A) Relapse Free Survival, Hazard Ratio: 14.8, p-value<10^{-5}. (B) Overall Survival, Hazard ratio 5.48 (p-value<0.0003). All 10 patients with ctDNA detected at landmark had clinical progression within the study period.

CONCLUSIONS

Our results demonstrate the ability to monitor ctDNA in NSCLC patients at or prior to relapse using a sensitive patient-specific plasma sequencing assay. ctDNA was detected in plasma samples at levels as low as 7 ppm. ctDNA detection within the landmark timepoint (2 weeks to 4 months after treatment end), was associated with shorter Relapse Free Survival (HR 14.8, p-value <10^{-5}) and Overall Survival (HR 5.48, p-value<0.0003). In patients who progressed, detection of ctDNA preceded clinical progression by a median lead time of 212.5 days. Our results support emerging evidence that ctDNA monitoring can reliably detect residual disease in patients treated with curative intent, many months before clinical progression, and offers an opportunity to identify ctDNA-positive patients who may benefit from adjuvant therapy.