Sensitive detection of ctDNA in early-stage non-small cell lung cancer patients with a personalized sequencing assay

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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.
- Current routine clinical practice involves serial radiographic imaging following surgery to detect macroscopic disease.
- Recent research has shown that liquid biopsies can identify patients who have MRD without macroscopic disease.
- However, many currently available assays have identified circulating tumor DNA (ctDNA) in only a limited number of cases with early-stage NSCLC. More sensitive methods are needed to accurately identify the majority of patients who will subsequently relapse.
- Here, we have evaluated detection of ctDNA in serial plasma samples collected from the LUCID (LUng cancer - CIrculating tumor DNA) study using the RaDaR™ assay.

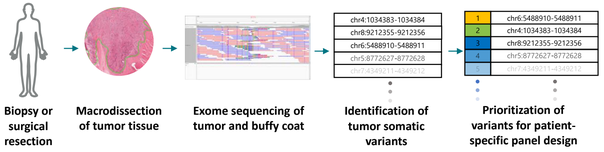
OBJECTIVE

- The primary objective of this study was to test the feasibility and prognostic value of detecting ctDNA at or before relapse using the RaDaR™ assay in stage IA - IIIB NSCLC patients following treatment with curative intent.
- RaDaR™ is a highly sensitive personalised sequencing ctDNA assay. Tumor-specific variants are first identified by exome sequencing of tumor tissue, followed by multiplex PCR and high-depth next-generation sequencing to track low levels of ctDNA in patient plasma.

METHODS

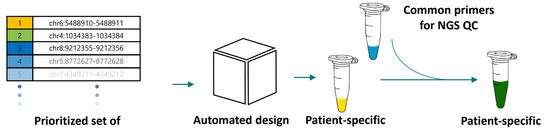
- Stage IA-IIIB NSCLC patients were recruited to the LUCID study. 90 patients undergoing radical treatment with curative intent, either surgery (n=70) or radiotherapy (RT) ± chemotherapy (n=20) had tumor tissue available for analysis (**Table 1**).
- Plasma samples (n=366) were taken at recruitment and at follow-up visits (~every 3 months for 9 months). Patients undergoing surgery also had a sample taken within 72 hours of surgery. Patients were followed for a minimum of 9 months and up to five years.
- Tumor exome sequencing was performed to identify mutations, and a RaDaR assay developed for each patient.
- Detection of residual disease was correlated with progression-free survival data.

Step 1: Creation of a patient-specific list of tumor mutations



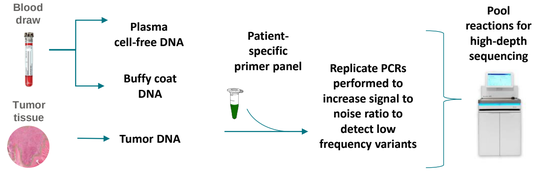
Biopsy or surgical resection | Macrodissection of tumor tissue | Exome sequencing of tumor and buffy coat | Identification of tumor somatic variants | Prioritization of variants for patient-specific panel design

Step 2: Creation of a RaDaR™ patient-specific ctDNA panel



Prioritized set of somatic variants (median 48 variants) | Automated design of patient-specific primers | Patient-specific primers | Patient-specific primer-pool covering 48 variants

Step 3: NGS testing of patient samples and analysis



Patient demographics

Table 1: Patient demographics of NSCLC patients enrolled in the study who had available tumor tissue for exome sequencing (n=90).

Characteristics	Patients (n=90)
Age, Median (range)	
Stage I	73 (52-86)
Stage II	74 (57-88)
Stage III	63 (44-78)
Sex	
Male	45 (50.0%)
Female	45 (50.0%)
Smoking status	
Never	8 (9.0%)
Ex-smoker	63 (70.8%)
Smoker	18 (20.2%)
Cancer history	31 (34.4%)

Histology	
Adenocarcinoma	48 (53.3%)
Squamous cell carcinoma	26 (28.9%)
Other	16 (17.8%)
Stage at diagnosis	
I	55 (61%)
II	18 (20%)
III	17 (19%)
Treatment	
Surgery	70 (77.8%)
ChemoRadiation	20 (22.2%)
Time points	
Baseline	79
Follow-up	287

RESULTS

- 89/90 RaDaR assays passed QC (median of 48 patient-specific mutations designed per panel).
- A median of 43 amplicons passed QC (minimal target read depth of 40,000 per sample per variant).

ctDNA detection at baseline

- In analysis of 79 baseline samples, ctDNA was detected in a higher proportion of patients with lung squamous cell carcinoma (84%) compared to adenocarcinoma (34%) (**Figure A**).

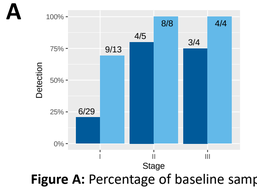


Figure A: Percentage of baseline samples with ctDNA detected according to cancer subtype and disease stage.

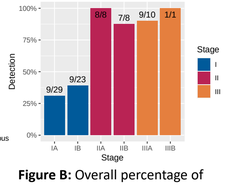


Figure B: Overall percentage of baseline samples with ctDNA detected according to disease stage.

- ctDNA was detected in 35% of baseline samples from stage I patients, and >90% samples from stage II-III patients, with detection of ctDNA in 54% of baseline samples overall (**Figure B**).
- Tumor fractions (TF) detected ranged from 6 parts per million (ppm, 0.0006% AF) to 20,000 ppm (2%) in baseline samples. Median TF across all 124 tumor-positive samples was 136 ppm (**Figure C**).

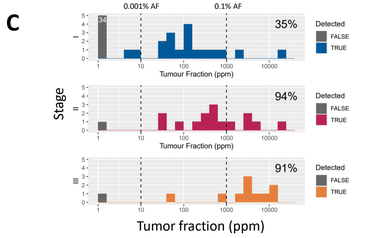
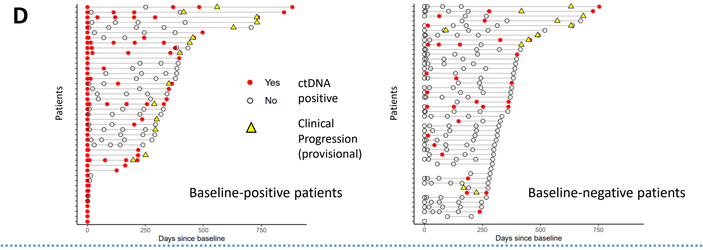


Figure C: ctDNA tumor fractions in parts per million (ppm) detected in baseline plasma samples according to disease stage. Percentages indicate overall detection per stage.

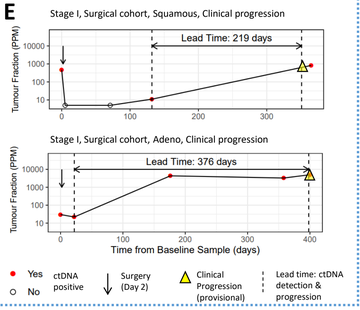
Dotted lines indicate allele fractions of 0.001% AF and 0.1% AF

Longitudinal monitoring for residual disease and recurrence

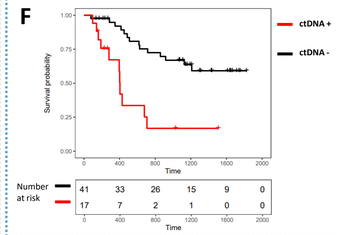
- Preliminary results, including provisional clinical data, indicate that overall ctDNA was detected at baseline or follow-up in 71.9% of patients. ctDNA was detected between 6 - 12 months before clinical progression in 60% of patients where samples were available within this time period.
- **Figure D** shows longitudinal monitoring of serial plasma samples from 89 patients, indicating when ctDNA was detected and whether the patient subsequently relapsed. Provisional clinical progression data is indicated with a yellow triangle.



- **Figure E** shows examples of longitudinal monitoring of ctDNA in plasma taken from 2 patients. Vertical lines indicate the lead time between the earliest detection of ctDNA post-treatment and when clinical progression was first recorded.



- For patients with a sample available within the landmark timepoint (2 weeks to 4 months, n=58), ctDNA detection in those samples was associated with lower progression-free survival among ctDNA+ group (Hazard Ratio 4.6, CI: 2.04-10.6; p-value 0.00023) compared to ctDNA- group; **Figure F).**



CONCLUSIONS

- Results demonstrate the ability to detect and monitor ctDNA in NSCLC patients at or prior to relapse using patient-specific plasma sequencing assays. ctDNA was detected at baseline or during follow-up in 71.9% of patients, at levels as low as 6 ppm. ctDNA detection post-treatment (2 weeks - 4 months) was associated with lower progression-free survival among the ctDNA+ group (Hazard Ratio 4.6, CI: 2.04-10.6; p-value 0.00023) compared to the ctDNA- group. In patients who progressed, ctDNA was detected between 6 - 12 months ahead of progression in 60% of patients where samples were available within this time period.