ctDNA detection in early-stage non-small cell lung cancer

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Poster Number: 736, Circulating Markers I
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INTRODUCTION

- In non-small-cell lung cancer (NSCLC) , overall survival rates are poor as patients are frequently diagnosed at late-stage when treatment options are limited.
- Circulating tumor DNA (ctDNA) can be used as a non-invasive liquid biopsy for the detection of cancer. However, ctDNA levels have been shown to be low in patients with early-stage disease.

OBJECTIVE

- The primary objective of this study was to assess the distribution of ctDNA in baseline plasma samples from early-stage NSCLC patients prior to treatment with curative intent. ctDNA levels were assessed using either patient-specific hybrid capture or amplicon sequencing.

METHODS

- 100 stage IA-IIIB treatment-naïve NSCLC patients were recruited to the LUCID (LUng cancer - CIrculating tumour DNA) study. The cohort predominantly consisted of stage IA/IB patients (n=60). 55% of patients had adenocarcinoma **(Figure A, Figure B).**

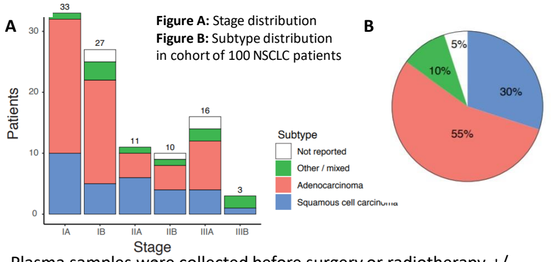


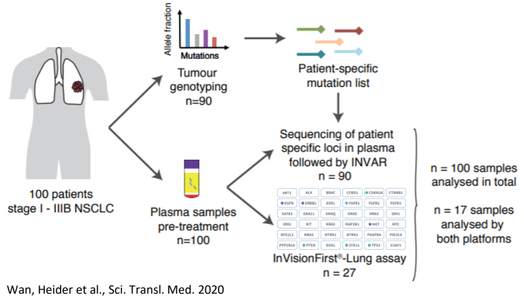
Figure A: Stage distribution
Figure B: Subtype distribution in cohort of 100 NSCLC patients

- Plasma samples were collected before surgery or radiotherapy +/- chemotherapy.
- Tumor tissue was available from 90 patients. Exome sequencing was performed to identify tumor-specific mutations, and patient-specific capture panels were developed to assess ctDNA levels in plasma.
- Data was analyzed using INtegration of VAriant Reads (INVAR), a method we developed which uses sequencing data across 100s-1000s of tumor patient-specific mutated loci combined with error suppression and signal enrichment methods to detect ctDNA at high sensitivity. Assays targeting a greater number of mutations can further improve sensitivity.
- To assess ctDNA in patients without available tumor, plasma samples were analyzed using InVisionFirst®-Lung assay (not including gene fusion analysis) **(Figure C).**

STUDY DESIGN

- **Figure C** shows the study design. Baseline plasma from 100 NSCLC patients were analyzed using either patient-specific hybrid capture and INVAR analysis (n=90) or InVisionFirst-Lung (n=27). 17 were analyzed by both platforms.

C



Wan, Heider et al., Sci. Transl. Med. 2020

RESULTS

Patient-specific hybrid capture & INVAR analysis

- Exome sequencing identified a median of 328 mutations/patient (IQR: 205 - 491). 99.8% were private mutations.
- Patient and healthy control plasma were analyzed using hybrid capture.
- ctDNA was detected in 60/90 (66.7%) patients (mean specificity: 95.63%).
- A median of 87,523 informative reads were generated at patient-specific loci (IQR 44,149 – 156,436).

D

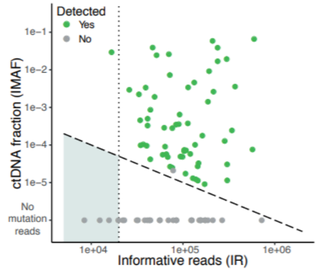


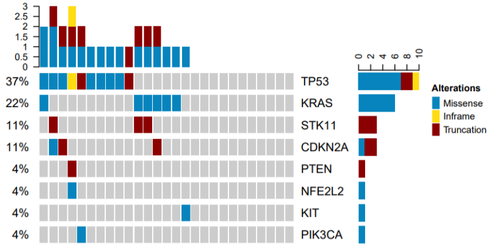
Figure D: Number of Informative Reads (IR) plotted against Integrated Mutant Allele Fraction (IMAF), showing samples where ctDNA was detected by INVAR. ctDNA was detected down to an IMAF of 9.1×10^{-6}.

Informative reads are the product of the number of targeted patient-specific loci and the number of haploid genomes analysed at these loci.
Dark grey dashed line: estimation of the limit of sensitivity (~ 1/IR).
Shaded region: samples with IR<20,000 that are below the sensitivity threshold.

Amplicon sequencing using InVisionFirst®-Lung

- For 27 patients, plasma was analyzed using InVisionFirst®-Lung to assess SNVs in genomic regions of 36 cancer-related genes.
- ctDNA was detected in 16/27 patients (59%) with a mean 1.67 alterations identified per patient. ctDNA was detected down to an allele fraction (AF) of 6×10^{-4}.
- **Figure E** shows the genes where mutations were identified and the mutation types.

E



Concordance between ctDNA analysis platforms

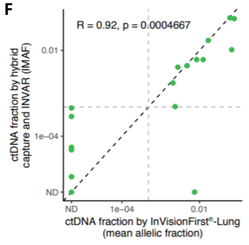
- Plasma from 17 patients was analyzed using both InVisionFirst®-Lung and INVAR.
- Concordance in ctDNA detection was seen in 11/17 patients (64.7%; **Table 1**), with a significant correlation (Spearman's r= 0.92, p = 4.667 x 10-4) in observed AFs **(Figure F)**.
- Of the discordant samples, 5 were detected by INVAR alone, and one by InVisionFirst®-Lung alone.

Table 1

INVAR analysis

InVisionFirst®-Lung	Detected	Not detected	Not evaluated	Total
Detected	10	1	5	16
Not detected	5	1	5	11
Not evaluated	45	28	0	73
Total	60	30	10	100

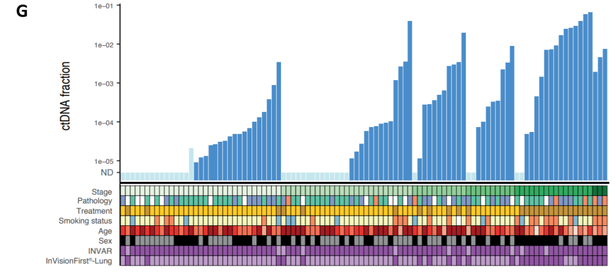
F



Overall ctDNA results

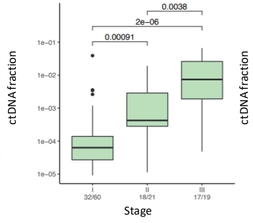
- Overall using both platforms, ctDNA was detected in 66/100 patients at baseline in treatment-naïve NSCLC patients, with detection of ctDNA in 51.7% stage I, 85.7% stage II and 89.5% stage III patients **(Figure G, Figure H).**
- Analyzing different histological subtypes, overall ctDNA was detected in 79% of squamous cell carcinomas and 60% of adenocarcinomas **(Figure G)**.

G

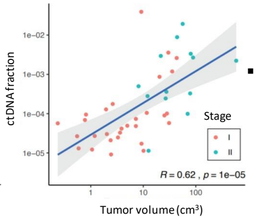


	Adenocarcinoma	Squamous cell carcinoma	Other	Not reported
I	18/39 (46.2%)	9/15 (60%)	3/4 (75%)	1/2 (50%)
II	7/8 (87.5%)	9/10 (90%)	1/2 (50%)	1/1 (100%)
III	7/8 (87.5%)	5/5 (100%)	4/4 (100%)	1/2 (50%)

H



I



- Tumor volumetric data was available for 43 patients where ctDNA was detected.
- **Figure I** shows a significant correlation between ctDNA fraction and tumor volume in patients with stage I-II disease (Pearson's r= 0.62, p = 1x10-5) where ctDNA was detected.

CONCLUSIONS

- Analysis of plasma from 100 stage IA-IIIB treatment-naïve NSCLC patients using both a patient-specific and amplicon sequencing approach identified ctDNA in 66% of all patients, with a median allele fraction across all stages of 3.2×10^{-4} AF, and ctDNA detection down to 9.1×10^{-6} AF. Our findings suggest that an assay sensitivity below 10 parts per million may enable ctDNA detection in two-thirds of patients with early-stage NSCLC prior to treatment, including the majority of adenocarcinoma cases.