Ultrasensitive ctDNA MRD Monitoring in early stage lung cancer with PhasED-Seq

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BACKGROUND

Circulating tumor DNA (ctDNA) minimal residual disease (MRD) detection is a promising approach for personalization of adjuvant therapy in non-small cell lung cancer (NSCLC). First generation ctDNA MRD assays that employ tumor-informed approaches to track single nucleotide variants (SNVs) have limits of detection (LOD95) of ~1E-4 and have high positive predictive values for recurrence. However, they have suboptimal clinical sensitivity, missing MRD at the completion of therapy in the majority of patients who will ultimately recur[1,2]. PhasED-Seq is a novel ctDNA MRD method that tracks multiple "phased" variants (PVs) within individual DNA fragments with an LOD95 ~100-fold better than first generation SNV based MRD assays[3]. Here we report PhasED-Seq ctDNA MRD results for the first prospective cohort of early stage NSCLC patients.

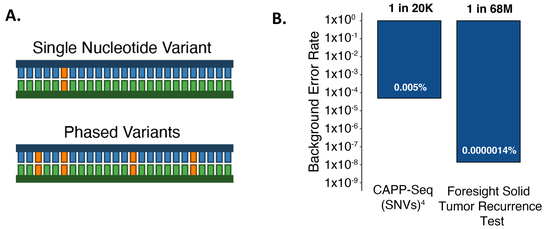

Fig. 1 - Genotyping phased variants on a single cfDNA molecule **(A)** decreases background error rate **(B)** and increases sensitivity ~100X.

METHODS

Tumor tissues (n=46), PBMCs (n=46) and plasma samples (n=169) from 46 Stage I-III NSCLC patients treated with curative intent were prospectively collected at Memorial Sloan Kettering Cancer Center. Patients were selected such that a sufficient number of recurrences were included. Foresight Diagnostics was blinded to clinical outcomes during the performance of testing. All patients underwent resection and received either neoadjuvant +/- adjuvant therapy (n=14), adjuvant therapy without neoadjuvant (n=17), or neither (n=15).

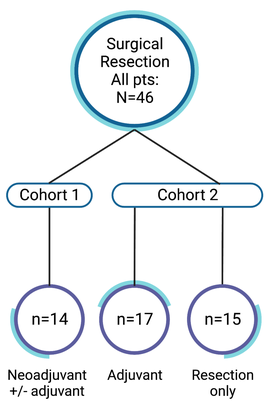

Fig. 2 – Breakdown of study cohorts

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METHODS

Samples were analyzed in Foresight Diagnostics' CLIA laboratory (Aurora, CO) using the Foresight Solid Tumor Recurrence Test which leverages PhasED-Seq. PhasED-Seq decreases background error rates and increases sensitivity by requiring the concordant detection of two or more distinct somatic mutation within a single DNA molecule. To enable comparisons, the same plasma samples were also analyzed using personalized CAPP-Seq tracking 16 truncal SNVs[4].

Step 1 - Identify patient specific phased variants

Whole genome sequencing (WGS) on tumor tissue

Input:
1. **Tumor tissue**
2. **Peripheral blood mononuclear cells (Germline DNA)**

Step 2 - Design patient-specific capture panel

Design custom hybrid capture oligonucleotide pool to capture patient-specific PVs identified using WGS (Step 1)

Step 3 - ctDNA assessment in plasma

ctDNA assessment at multiple timepoints including (as applicable)

1. **Pre-operatively**
2. **Post-operatively**
3. **Post treatment**
4. **During adjuvant/ neoadjuvant therapy**

RESULTS

Cohort 1 Neo-adjuvant therapy + Surgery (+/- adjuvant)	N	%
Total Patients	14	
Histology		
Adenocarcinoma	10	71%
Non-adeno	4	29%
Adjuvant therapy		
Yes	5	36%
No	9	64%
Outcome		
Relapse	6	43%
No Relapse	8	57%
Stage		
IA	1	7%
IB	0	0%
II	1	7%
III	12	86%

Cohort 2 Surgery (+/- adjuvant)	N	%
Total Patients	32	
Histology		
Adenocarcinoma	25	78%
Non-adeno	7	22%
Adjuvant therapy		
Yes	17	53%
No	15	47%
Outcome		
Relapse	12	38%
No Relapse	20	63%
Stage		
IA	7	22%
IB	6	19%
II	14	44%
III	5	16%

Table 1 – Study Cohort: Patients characteristics

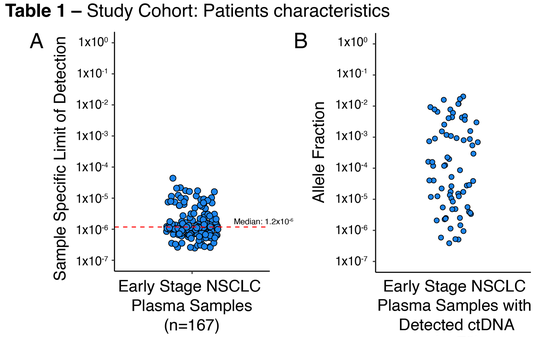


Fig. 3 – Dot plots depict **(A)** the sample specific limit of detection, defined as the ctDNA level for which the assay achieved 95% confidence for detection (LOD95), in all available plasma samples, and **(B)** mean ctDNA allele frequency in plasma samples with detectable ctDNA.

RESULTS

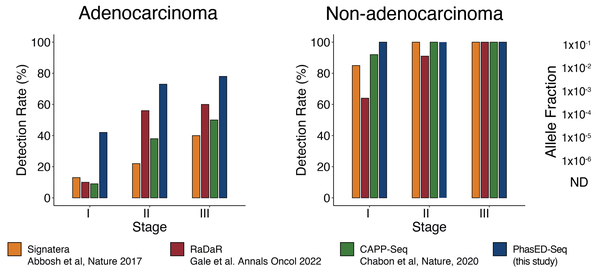


Fig. 4 – Bar plots depict rate of detection of ctDNA in pre-treatment plasma samples in patients with adenocarcinoma (left) and non-adenocarcinoma (right), in the indicated studies (color of bars).

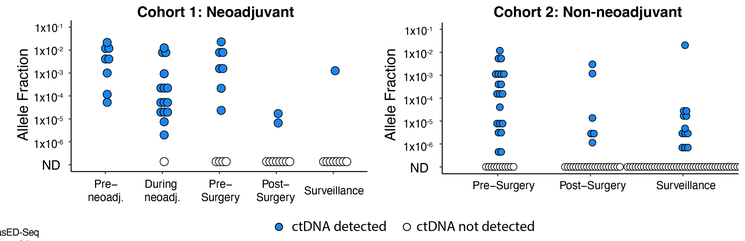
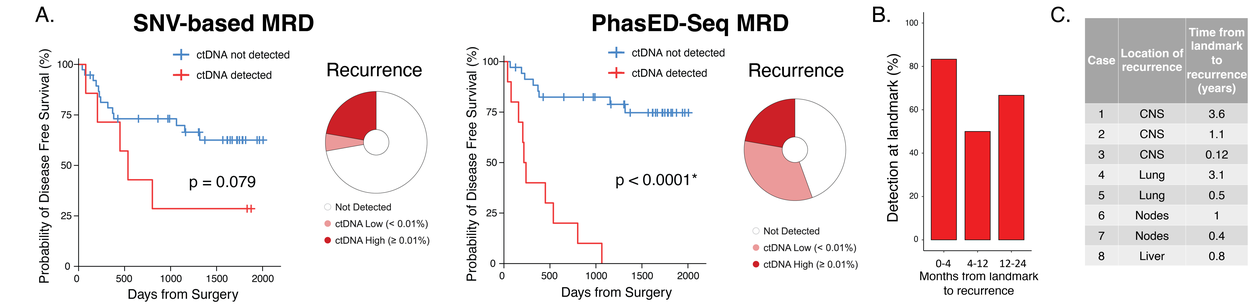


Fig. 5 – Dot plots depict the mean ctDNA allele fraction as measured using the Foresight Solid Tumor Recurrence Test in patient plasma samples collected at the indicated timepoints.



Case	Location of recurrence	Time from landmark to recurrence (years)
1	CNS	3.6
2	CNS	1.1
3	CNS	0.12
4	Lung	3.1
5	Lung	0.5
6	Nodes	1
7	Nodes	0.4
8	Liver	0.8

Fig. 6 – (A) Kaplan-Meier estimates of disease-free survival (DFS) in ctDNA-MRD negative (blue) versus ctDNA-MRD positive (red) patients at a post-treatment landmark, defined as the first post-therapy sample or, when not available, the last post-surgical sample taken during therapy. MRD detection with SNVs (left) or PhasED-Seq (right) are compared. Pie charts depict the relative proportion of undetected, ctDNA low (AF<0.01%) and ctDNA high (AF>0.01%) samples among patients that relapsed. **(B)** MRD detection rate at landmark in patients that recurred in the indicated timeframes relative to landmark sample. **(C)** Patients who recurred but had negative ctDNA-MRD at the landmark are enriched for CNS recurrences and solitary lung recurrences that may be second primaries. *p<0.05

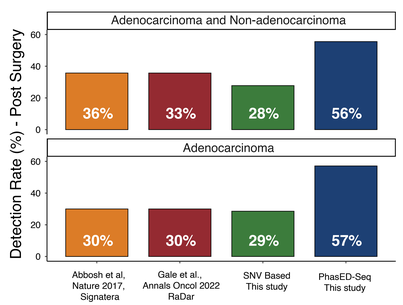


Fig. 7 – Bar plots depict rate of detection of ctDNA at post-treatment landmark in patients with both adenocarcinoma and non-adenocarcinoma (top) or adenocarcinoma only (bottom), using the indicated methods.

CONCLUSIONS

The Foresight Solid Tumor Recurrence Test achieved a LOD95 ≤ 1E-6 in the majority of plasma samples analyzed in this study and detected ctDNA at levels near or below 1E-6 in many samples. Furthermore, the assay demonstrated superior sensitivity compared to SNV-based ctDNA MRD approaches both pre-treatment and at the post-treatment landmark. Thus, PhasED-Seq is a promising technology for use in risk adapted trials in early-stage NSCLC.

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