Ultrasensitive circulating tumor DNA (ctDNA) minimal residual disease (MRD) detection in early stage non-small cell lung cancer (NSCLC).

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BACKGROUND

- Early studies of ctDNA-based MRD show promise in early stage NSCLC.[1-4]
- Translation of ctDNA MRD in NSCLC has been hampered by suboptimal sensitivity of 1st-generation assays.
- Phased variant enrichment and detection sequencing (PhasED-Seq) is a method which uses multiple somatic mutations in individual DNA fragments to improve the sensitivity of ctDNA detection.[5]
- Here, we explore how improvements in analytical sensitivity can drive improved clinical sensitivity for MRD after surgery in NSCLC.
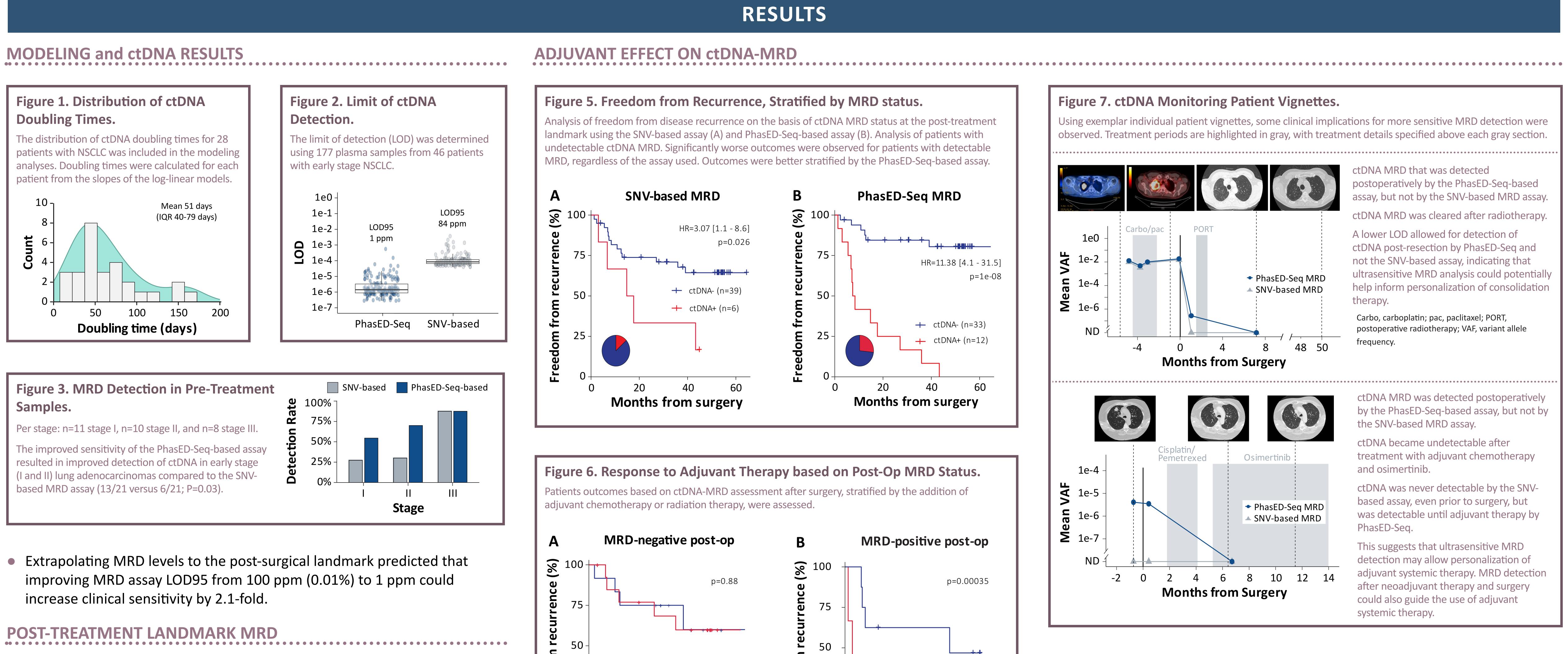
METHODS

- To understand MRD kinetics, we analyzed longitudinal ctDNA in early-stage NSCLC using data from the TRAcking non-small cell lung Cancer Evolution through therapy (NCT01888601; TRACERx) study.
  - ctDNA MRD dynamics were assessed in 23 patients from TRACERx with ≥3 consecutive samples with detectable ctDNA without intervening therapy.
- Patient-specific mathematical models were generated to predict MRD levels at the post-surgical landmark and estimate the impact of an assay's 95% limit of detection (LOD95) on clinical sensitivity.
- To test these predictions, tumor-informed ctDNA testing was performed using a first-generation SNV-based assay (personalized CAPP-Seq)[6] and Foresight CLARITY™, a MRD assay utilizing PhasED-Seq technology, on 269 samples from 46 patients with NSCLC (Table 1).
- Performance between the CAPP-Seq and PhasED-Seq-based MRD assays was compared.
- We also assessed MRD performance for predicting patient outcomes at the landmark timepoint and the effect of adjuvant treatment.
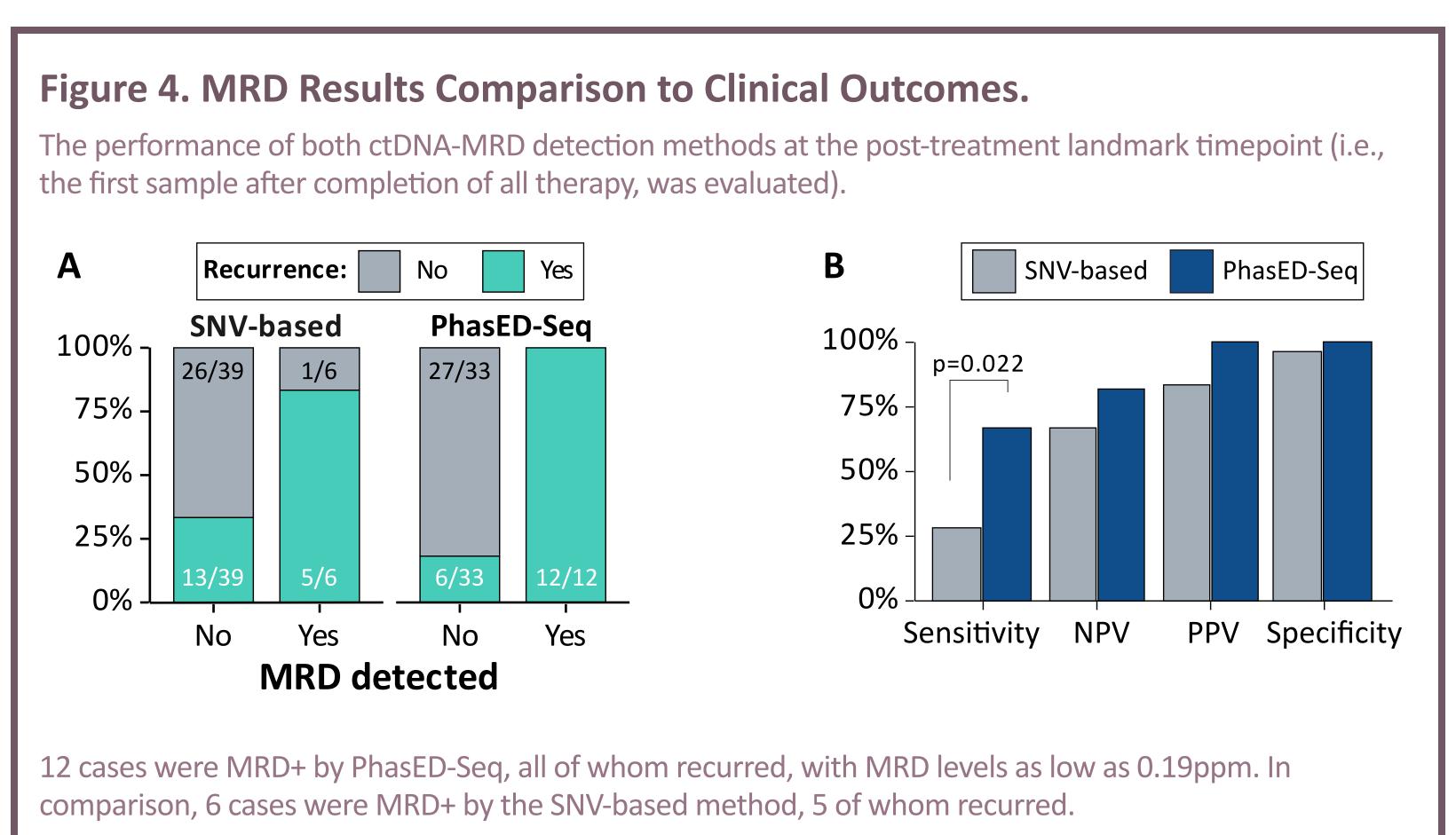
Table 1. Description of NSCLC Cohort (N=46)

		n (%)
Histology	Adeno.	35 (76%)
	Non-Adeno.	11 (24%)
Stage*	IA	7 (15%)
	IB	7 (15%)
	II	14 (30%)
	III	18 (39%)
Neoadjuvant Therapy	No	32 (70%)
	Yes	14 (30%)
Adjuvant Systemic Therapy	No	25 (54%)
	Yes	21 (46%)
Postoperative Radiotherapy	No	34 (74%)
	Yes	12 (26%)
Relapse	No	27 (59%)
	Yes	19 (41%)

*Stage: clinical stage determined before neoadjuvant therapy or, for patients without neoadjuvant therapy, pathological stage determined after surgery; Adeno, adenocarcinoma.

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RESULTS

MODELING and ctDNA RESULTS

Figure 1. Distribution of ctDNA Doubling Times.

The distribution of ctDNA doubling times for 28 patients with NSCLC was included in the modeling analyses. Doubling times were calculated for each patient from the slopes of the log-linear models.

Figure 2. Limit of ctDNA Detection.

The limit of detection (LOD) was determined using 177 plasma samples from 46 patients with early stage NSCLC.

Figure 3. MRD Detection in Pre-Treatment Samples.

Per stage: n=11 stage I, n=10 stage II, and n=8 stage III.

The improved sensitivity of the PhasED-Seq-based assay resulted in improved detection of ctDNA in early stage (I and II) lung adenocarcinomas compared to the SNV-based MRD assay (13/21 versus 6/21; P=0.03).

- Extrapolating MRD levels to the post-surgical landmark predicted that improving MRD assay LOD95 from 100 ppm (0.01%) to 1 ppm could increase clinical sensitivity by 2.1-fold.

POST-TREATMENT LANDMARK MRD

Figure 4. MRD Results Comparison to Clinical Outcomes.

The performance of both ctDNA-MRD detection methods at the post-treatment landmark timepoint (i.e., the first sample after completion of all therapy, was evaluated).

12 cases were MRD+ by PhasED-Seq, all of whom recurred, with MRD levels as low as 0.19ppm. In comparison, 6 cases were MRD+ by the SNV-based method, 5 of whom recurred.

As a result, PhasED-Seq had a higher clinical sensitivity and specificity than the SNV-based method.

ADJUVANT EFFECT ON ctDNA-MRD

Figure 5. Freedom from Recurrence, Stratified by MRD status.

Analysis of freedom from disease recurrence on the basis of ctDNA MRD status at the post-treatment landmark using the SNV-based assay (A) and PhasED-Seq-based assay (B). Analysis of patients with undetectable ctDNA MRD. Significantly worse outcomes were observed for patients with detectable MRD, regardless of the assay used. Outcomes were better stratified by the PhasED-Seq-based assay.

Figure 6. Response to Adjuvant Therapy based on Post-Op MRD Status.

Patients outcomes based on ctDNA-MRD assessment after surgery, stratified by the addition of adjuvant chemotherapy or radiation therapy, were assessed.
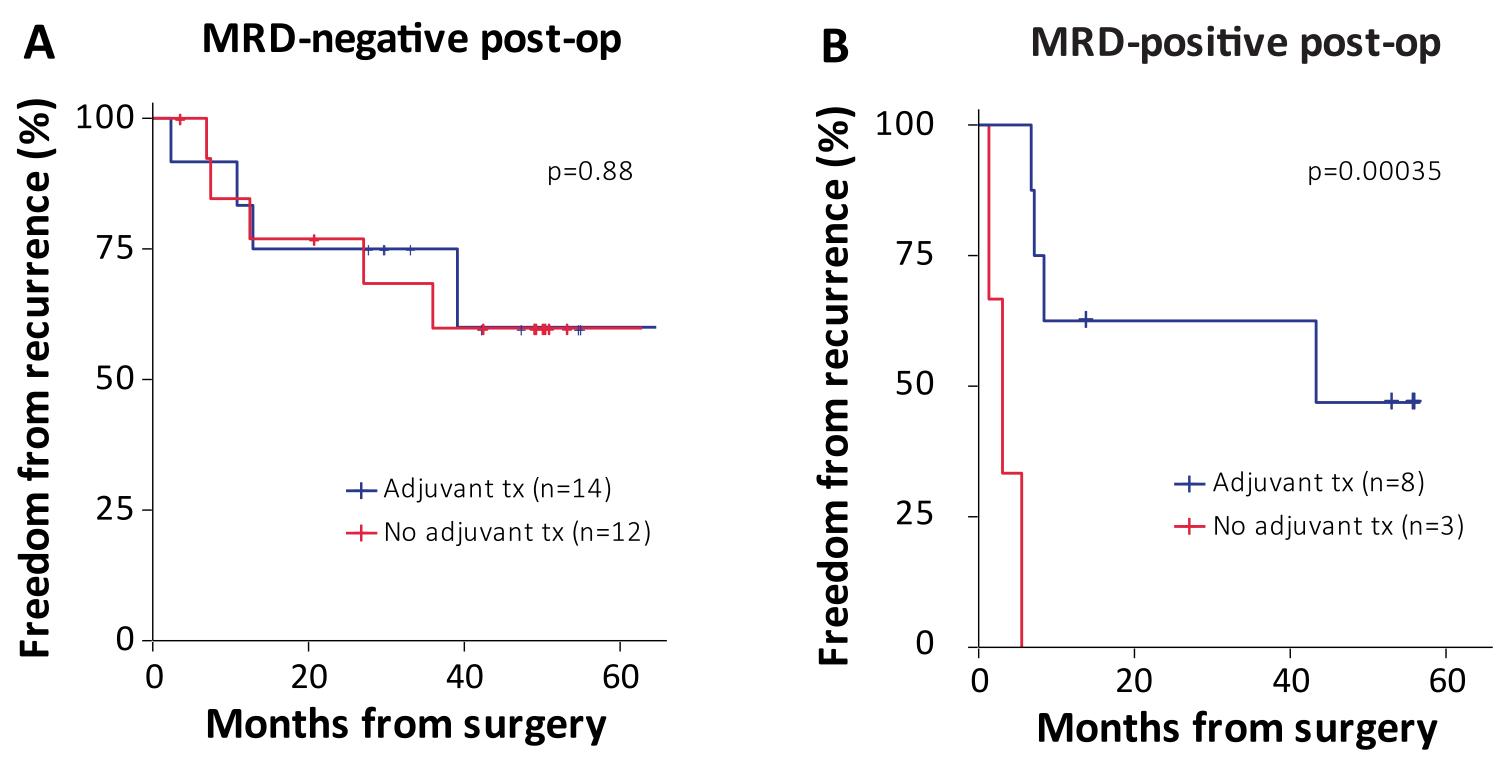

Figure 6A shows that among patients who were MRD- by PhasED-Seq after surgery, similar outcomes were observed for regardless of whether adjuvant therapy (chemotherapy and/or radiotherapy) as received.

Figure 6B shows that among patients who were MRD+ by PhasED-Seq after surgery, those who received adjuvant therapy had significantly better outcomes than those who did not (HR 8.2, P=0.00035).

A similar benefit for adjuvant therapy was not observed with the SNV-based assay.

Figure 7. ctDNA Monitoring Patient Vignettes.

Using exemplar individual patient vignettes, some clinical implications for more sensitive MRD detection were observed. Treatment periods are highlighted in gray, with treatment details specified above each gray section.

ctDNA MRD that was detected postoperatively by the PhasED-Seq-based assay, but not by the SNV-based MRD assay.

ctDNA MRD was cleared after radiotherapy.

A lower LOD allowed for detection of ctDNA post-resection by PhasED-Seq and not the SNV-based assay, indicating that ultrasensitive MRD analysis could potentially help inform personalization of consolidation therapy.

Carbo, carboplatin; pac, paclitaxel; PORT, postoperative radiotherapy; VAF, variant allele frequency.

ctDNA MRD was detected postoperatively by the PhasED-Seq-based assay, but not by the SNV-based MRD assay.

ctDNA became undetectable after treatment with adjuvant chemotherapy and osimertinib.

ctDNA was never detectable by the SNV-based assay, even prior to surgery, but was detectable until adjuvant therapy by PhasED-Seq.

This suggests that ultrasensitive MRD detection may allow personalization of adjuvant systemic therapy. MRD detection after neoadjuvant therapy and surgery could also guide the use of adjuvant systemic therapy.

CONCLUSIONS

- Ultrasensitive detection of ctDNA was achieved using PhasED-Seq technology, with MRD detected at levels below 1 ppm.
  - This translated to improved clinical sensitivity of MRD at key landmarks in early-stage NSCLC compared to current SNV-based detection assays.
- PhasED-Seq predicted benefit to adjuvant therapy, such that there was no observed therapeutic benefit in the MRD- population, but there was a benefit observed in the MRD+ population.
- Together, these data suggest that ultrasensitive MRD detection is promising for use in risk-adapted trials in early-stage NSCLC.