Abstract #8518: Leveraging phased variants for personalized minimal residual disease detection in localized non-small cell lung cancer

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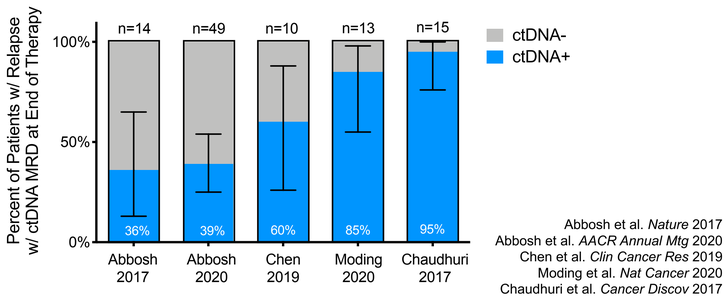
1. Background

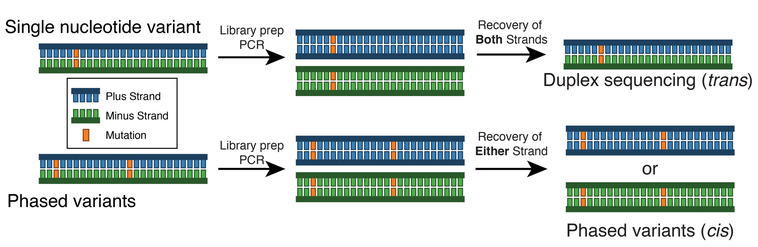
Detection of circulating tumor DNA (ctDNA) has prognostic value in lung cancer and could facilitate minimal residual disease (MRD) driven approaches. However, the sensitivity of ctDNA detection is suboptimal due to the background error rates of existing assays.

- Only 54% detection-rate for MRD at landmark time-point in prior studies (i.e., after completion of therapy)
- Concordant detection of mutations on both original strands of DNA, or "duplex sequencing", can lower error-rates but has poor efficiency (mutations in *trans*).
- Detection of multiple mutations seen on a single strand of cell-free DNA ("phased variants" or PVs) may also lower the background error-rate (mutations in *cis*).
- We developed and applied PhasED-Seq (Phased variant enrichment and detection by sequencing) to detect low-burden MRD in limited-stage lung cancer.



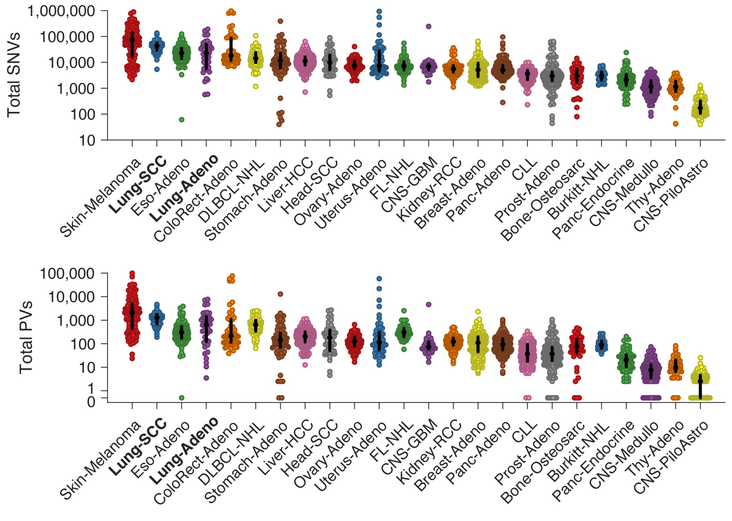
Abbosh et al. *Nature* 2017
Abbosh et al. *AACR Annual Mtg* 2020
Chen et al. *Clin Cancer Res* 2019
Moding et al. *Nat Cancer* 2020
Chaudhuri et al. *Cancer Discov* 2017

2. Phased variants are common genome-wide



- Concordant detection of a single nucleotide variant (SNV) in *trans* (i.e., duplex sequencing) has a low error-rate, but is inefficient as recovery of both strands is uncommon.
- Simultaneous detection of multiple variants (Phased Variants, "PVs") has a low error-rate & is efficient as only one DNA strand needs to be recovered.

Frequency of SNVs, PVs in Multiple Cancer Types

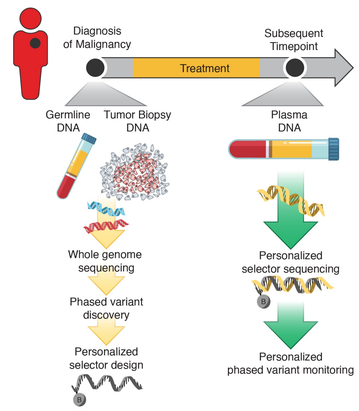


Pan-Cancer Analysis of Whole Genomes (PCAWG). *Nature* 2020.

3. PhasED-Seq improves the limit of detection for ctDNA

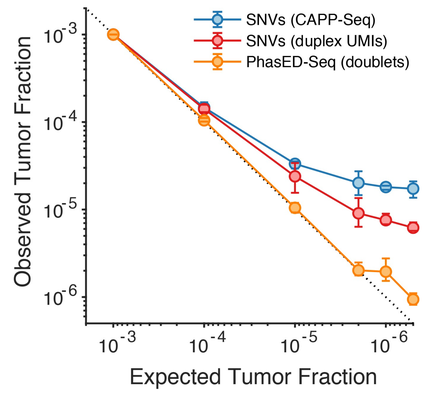
Personalized PhasED-Seq workflow

1. Whole genome sequencing (WGS) on tumor / germline DNA (~50x)
2. PV identification and hybrid capture panel synthesis
3. MRD assessment of plasma cell-free DNA



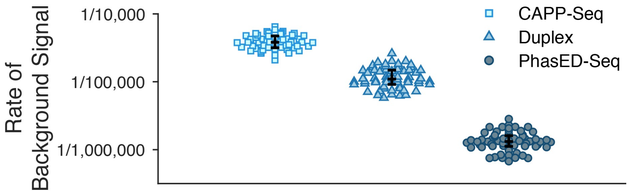
Analytical limit of detection below one part-per-million

- Detection assessed in 3 limiting dilution series of cfDNA from lymphoma patients diluted into healthy cfDNA
- PhasED-Seq demonstrates linearity down to ctDNA levels below one part-per-million



PhasED-Seq has a lower background error rate than SNV-base detection approaches

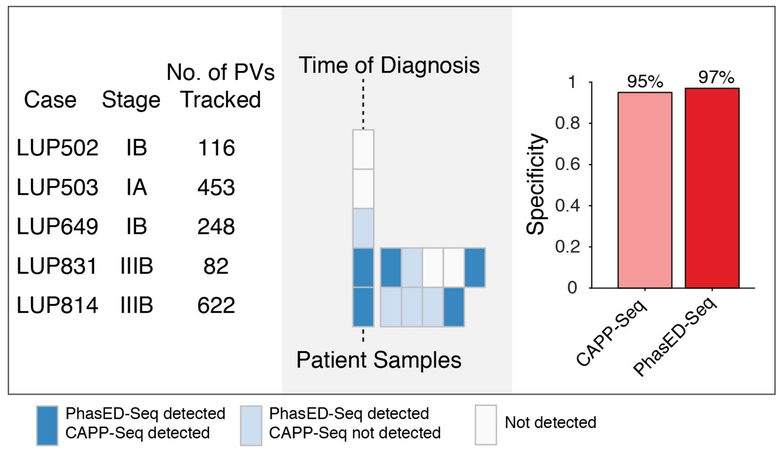
- The background error rate of PVs identified from WGS of 5 NSCLC patients was assessed in 12 healthy controls and compared to the background error rate of SNVs tracked by CAPP-Seq and duplex sequencing

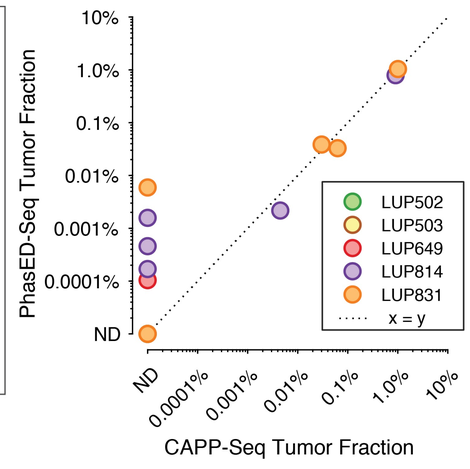


4. PhasED-Seq improves ctDNA detection in localized NSCLC

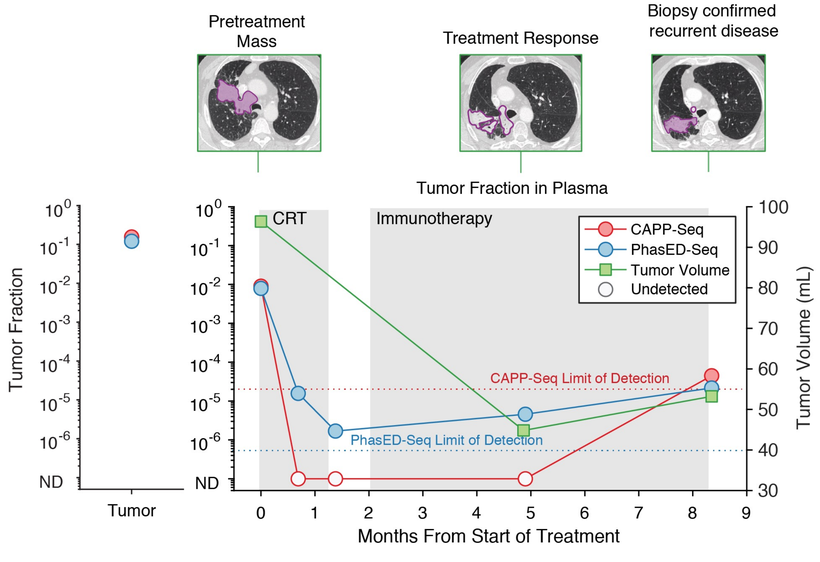
- Personalized PhasED-Seq was applied to 5 patients with limited-stage lung cancer
  - Tumor/germline WGS & personalized panel design
  - Median of 248 PVs were used to track ctDNA (range 82-622) in 14 plasma samples
- ctDNA detection results were compared to SNV-based detection with CAPP-Seq
  - Specificity = 97%
- CAPP-Seq detected ctDNA in 36% (5/14) of samples (0/3 for stage I cases)
- PhasED-Seq detected ctDNA in 71% (10/14) of samples, including 100% (5/5) of samples detected by CAPP-Seq, including:
  - ctDNA levels of 0.000094% in a stage I patient (LUP649)
  - MRD+ sample missed by CAPP-Seq after chemoradiotherapy at 0.00016% (LUP814)

Case	Stage	No. of PVs Tracked
LUP502	IB	116
LUP503	IA	453
LUP649	IB	248
LUP831	IIIB	82
LUP814	IIIB	622





5. PhasED-Seq detects MRD that is missed by SNVs



6. Conclusions

- ctDNA detection has potential to change the clinical management of cancer, however detection using SNV-based approaches is challenging for MRD after curative intent treatment
- PVs are common in most tumor types.
- By identifying and tracking PVs, PhasED-Seq can detect ctDNA at levels in the parts-per-million range.
- PhasED-Seq improves MRD detection rates compared to SNV-based approaches in low disease burden settings while maintaining high specificity.
  - Potential uses in MRD-adapted and personalized therapeutic approaches, including novel clinical trial designs

Related Abstract:
Kurtz DM, et al. Phased variants improve DLBCL minimal residual disease detection at the end of therapy. Abstract #7565.