Abstract #7565: Phased variants improve DLBCL minimal residual disease detection at the end of therapy

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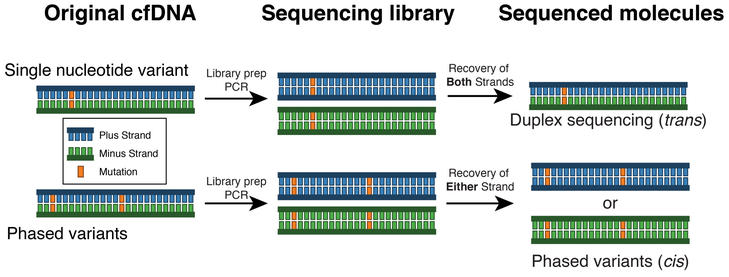
1. Background

Detection of circulating tumor DNA (ctDNA) has prognostic value in DLBCL 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.

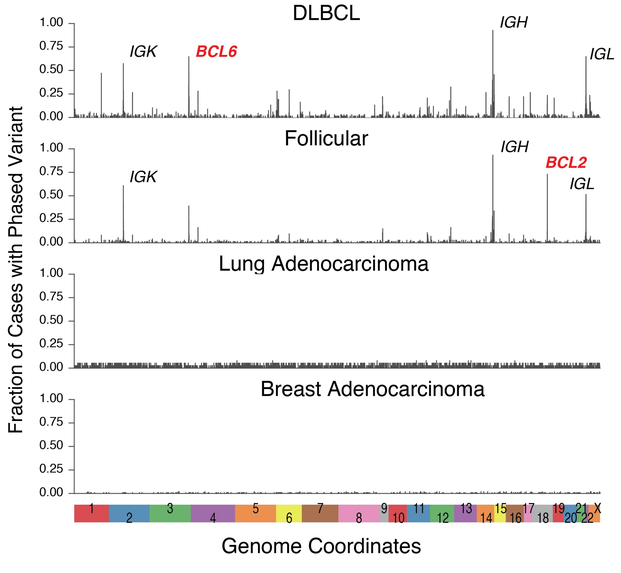
- Concordant detection of mutations on both original strands of DNA, or "duplex sequencing", can lower error-rates but has poor efficiency (mutations in *trans*).
- Detection of multiple mutations seen on a single strand of cell-free DNA ("phased variants" or PVs) may also lower the background error-rate (mutations in *cis*).
- We developed PhasED-Seq, a method for ctDNA detection and disease monitoring leverage PVs, and compared this to prior ctDNA methods.

2. PVs are stereotyped in lymphoma



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

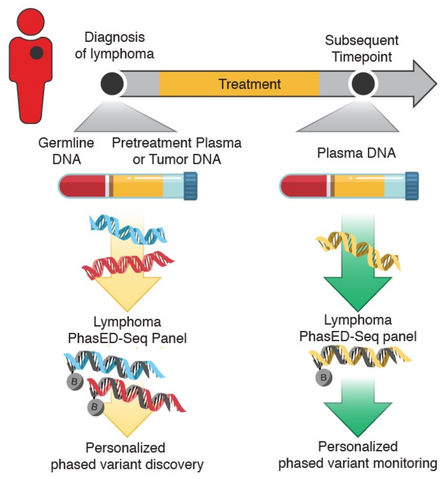
PVs occur in stereotyped genomic locations in B-NHLs



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

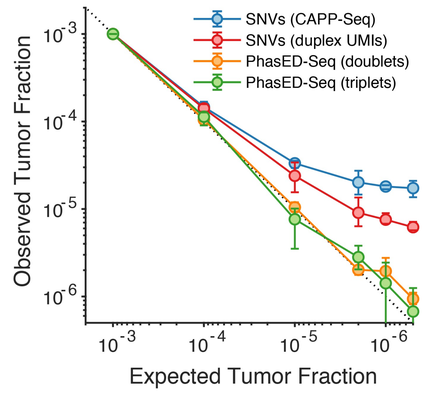
3. PhasED-Seq improves the limit of detection for ctDNA

Phased Variant Enrichment and Detection by Sequencing (PhasED-Seq)



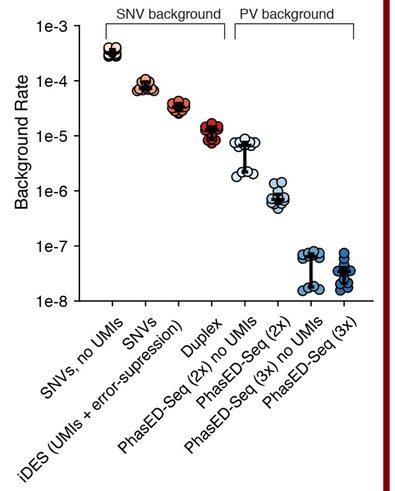
Lymphoma PhasED-Seq workflow

- WGS from 79 DLBCL/FL patients analyzed to select recurrent regions with PVs
- ~315kb panel to capture PVs designed
- PVs identified from tumor or pretreatment plasma samples, tracked in subsequent plasma samples



- Detection assessed in 3 limiting dilution series of cfDNA from lymphoma patients diluted into healthy cfDNA
- PhasED-Seq demonstrates linearity down to parts-per-million
- Background signal in 12 healthy controls assessed (*right*)
  - Compared to background of CAPP-Seq and duplex
- PhasED-Seq demonstrated lowest background signal
- PhasED-Seq improves background signal even without unique molecular identifiers

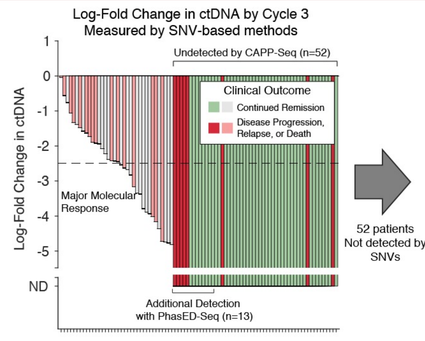
Background Profile of different types of error-suppression

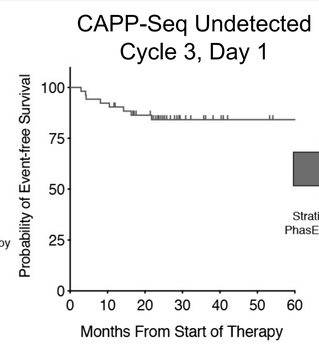


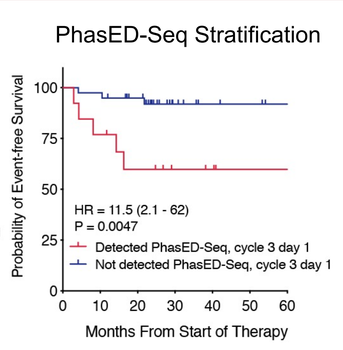
4. PhasED-Seq improves ctDNA detection in localized NSCLC

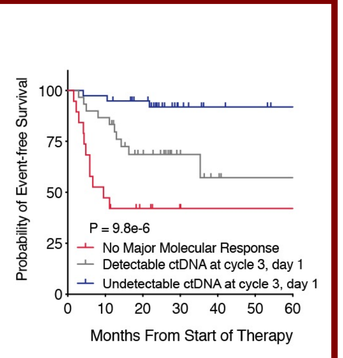
Study Population

	Interim	EOT
Total Patients	107	19
Age	56	57
Stage		
1 - 2	34%	37%
3 - 4	66%	63%
International Prognostic Index		
0 - 1	39%	37%
2	26%	26%
3	21%	15%
4 - 5	14%	16%



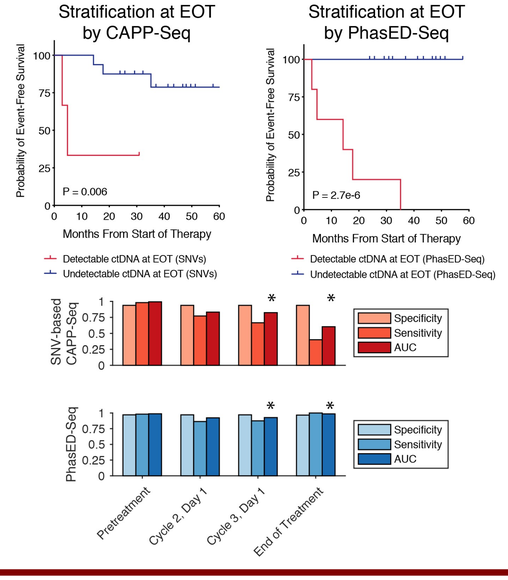




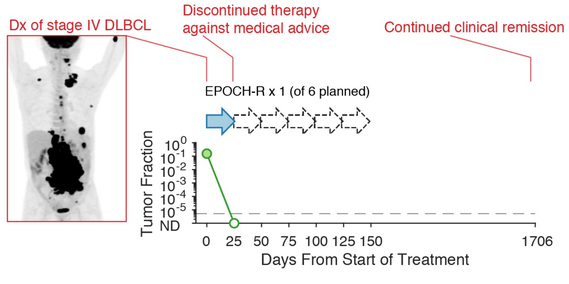


- Performance of PhasED-Seq assessed for stratifying outcomes (EFS) in patients with large B-cell lymphoma undergoing first-line therapy
- Cycle 3, Day 1 (C3D1) and End of Therapy (EOT) time-points assessed
- CAPP-Seq previously described to stratify patient outcomes at cycle 3, day 1 – Major Molecular Response
- However, 52/88 patients undetectable at this timepoint
- PhasED-Seq detected 13/52 patients not detected by CAPP-Seq
- PhasED-Seq detected vs undetected further stratifies CAPP-Seq undetectable patients
- Similar results seen at cycle 2, day 1

5. PhasED-Seq improves stratification at end of therapy



- We explored performance of CAPP-Seq and PhasED-Seq at the time-point of lowest disease burden (i.e., end of therapy)
- 19 patients, 5 with events
- CAPP-Seq detection at EOT stratified outcomes, but only detects 2/5 patients experiencing relapse
- PhasED-Seq detection at EOT detects 5/5 relapsers
- PhasED-Seq correctly identifies undetectable disease in a subject who discontinued therapy after 1 cycle
  - Remains in remission at ~5 years after 1 treatment cycle



6. Conclusions

- ctDNA detection has potential to change management of DLBCL in the clinic, however detection is still challenging for MRD after curative intent treatment
- PVs are common in B-NHLs, occur in stereotyped locations
- PhasED-Seq can identify, track PVs for improved ctDNA MRD detection to parts-per-million
- PhasED-Seq improves MRD detection compared to SNV-based methods at interim and end-of-treatment time-points
  - Potential uses in MRD-adapted and personalized therapeutic approaches, including novel clinical trial designs

Related Abstract:
Kurtz DM, et al. Leveraging phased variants for personalized minimal residual disease detection in localized non-small cell lung cancer. Abstract #8518.