Ultrasensitive Detection of Circulating Tumor DNA In Untreated Diffuse Large B-cell Lymphoma

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Background

- DLBCL response criteria rely on imaging scans which cannot detect disease at the molecular level
- Circulating tumor DNA (ctDNA) is a prognostic biomarker in DLBCL before and after therapy
- Detection of ctDNA at the end-of-therapy (EoT) has low sensitivity with approaches that have a limit of detection (LOD) of 1 x 10^{-4}
- PhasED-Seq is an ultrasensitive ctDNA assay that reduces background error rate by 100x and has a LOD of 1 x 10^{-6}

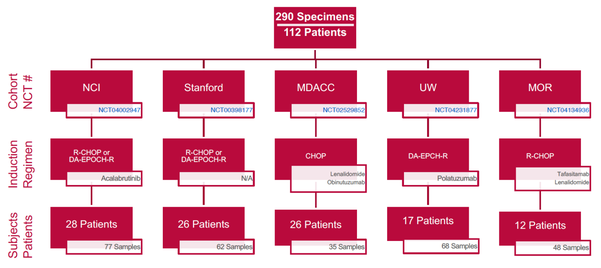
Hypothesis

- The improved analytic sensitivity of PhasED-Seq could improve disease detection at EoT in untreated DLBCL

Methods

Subjects and Trials

- Pooled analysis of 5 prospective frontline trials for DLBCL
- Integrated PhasED-Seq data during therapy and EoT
- First, we assessed the prognostic value of ctDNA as MRD at landmark timepoints during therapy and at EoT
- Next, we compared the prognostic value of ctDNA as MRD at EoT to conventional response criteria



Specimen Collection and Analysis

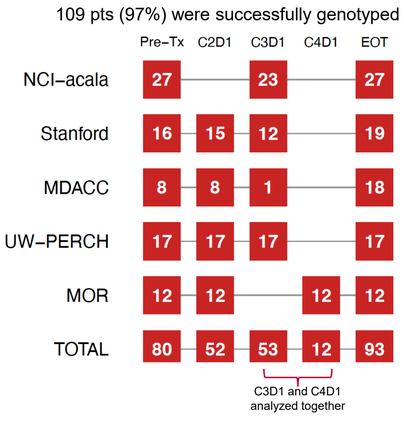
- Pre-treatment tumor or plasma & PBMC were used to identify Phased Variants (PVs) for tracking
- PVs were tracked as MRD in plasma that had been prospectively collected at baseline, C2D1, C3D1, C4D1, and EoT timepoints
- Cell-free DNA was profiled by PhasED-Seq blinded to clinical outcomes at 2 labs:
  - Foresight Diagnostics (Aurora, CO)
  - Stanford University (Palo Alto, CA)
- Plasma was reported as MRD positive when ctDNA levels exceeded an analytical detection threshold of 1 x 10^{-6}

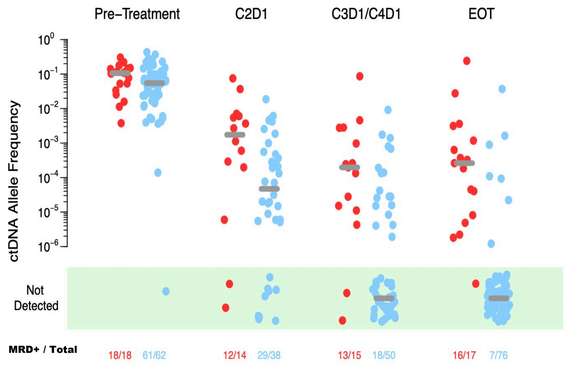
Results

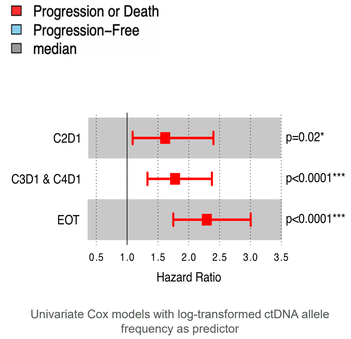
Table 1. Characteristics of the Patients

Characteristic	N (%)
Number of patients	112
Female sex	44 (39%)
Median age (range) - yr	61 (20-85)
DLBCL, subtype	
DLBCL, GCB	60 (54%)
DLBCL, non-GCB	28 (25%)
High grade B-cell lymphoma with *MYC* and *BCL2*	4 (4%)
High grade B-cell lymphoma with *MYC* and *BCL6*	0 (0%)
Primary mediastinal B-cell lymphoma	4 (4%)
T-cell histocyte rich DLBCL	0 (0%)
Unknown	16 (14%)
Stage	
I	0 (0%)
II	29 (26%)
III	83 (74%)
IV	0 (0%)
International Prognostic Index	
0 to 1	31 (28%)
2	29 (26%)
3	33 (29%)
4 to 5	19 (17%)
Cell-free DNA samples available	
Prior to therapy	80
End of cycle 1	52
End of cycle 2	53
End of cycle 3	12
End of therapy	93

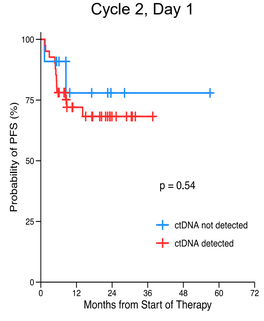
Abbreviations: DLBCL, diffuse large B-cell lymphoma, GCB, germinal center B-cell, non-GCB, non-germinal center B-cell,

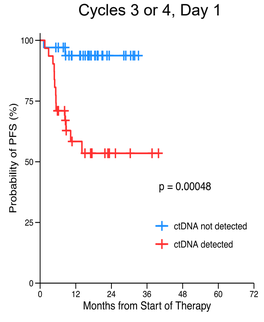


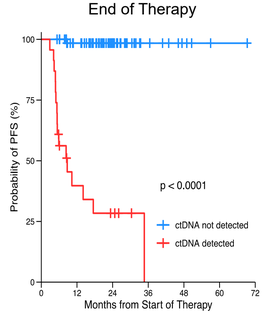




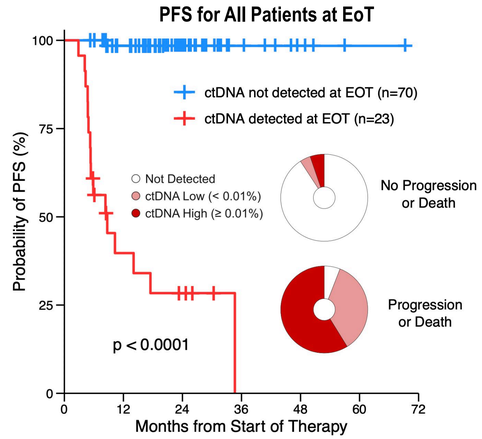
Univariate Cox models with log-transformed ctDNA allele frequency as predictor

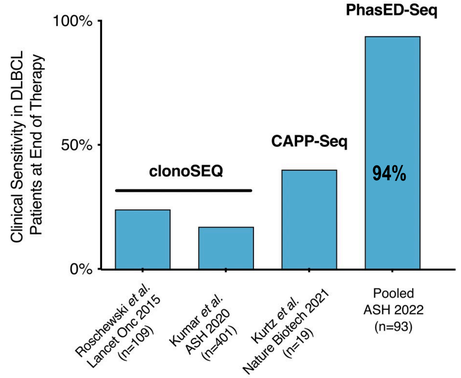


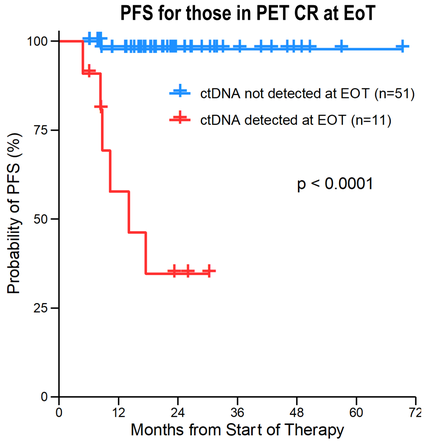


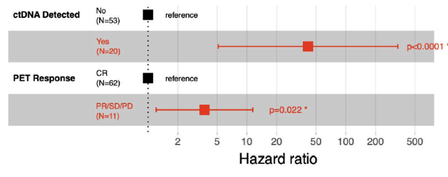


Results









Conclusions

- PhasED-Seq ctDNA a highly prognostic biomarker for DLBCL; MRD status at EoT has the strongest prognostic value
- Limit of detection of 1 x 10^{-6} is essential to detect ctDNA at EoT
- MRD status at EoT may enhance current response criteria and has promise as a surrogate endpoint for clinical trials
- All DLBCL trials should prospectively collect plasma at baseline, during therapy, and at EoT for MRD