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Phased Variants Allow Robust Profiling of Circulating Tumor DNA in Untreated Follicular Lymphomas

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BACKGROUND

- In the absence of tumor tissue, several studies have shown the utility of plasma cell-free DNA (cfDNA) for blood-based noninvasive genotyping of diverse human tumors, including aggressive lymphomas.
- However, it remains unclear whether these same cfDNA advantages also apply to follicular lymphoma (FL), and if they could inform noninvasive minimal residual disease (MRD) monitoring in the context of modern regimens inducing deep FL remissions.
- Here, we address these questions in previously untreated patients with FL receiving standard first-line (1L) therapy, using Phased Variant Enrichment and Detection Sequencing (PhasED-Seq)[1] to monitor ctDNA and MRD.

METHODS

COHORT

- We profiled 298 samples from 61 patients with FL, including:
  - Archival FFPE tumor tissues
  - Serial blood samples before, during, and following 1L therapy.
- All patients were treatment-naive, with most (75%) presenting with advanced stage FL.
- At a median follow-up of 23 months from start of 1L therapy, 80% of patients were alive and progression free, while 11% experienced progression within 24 months (POD24).

ctDNA MRD TESTING

- MRD was analyzed by PhasED-Seq (Foresight Diagnostics).
  - Phased variants (PVs) were genotyped using baseline blood plasma, or FFPE tumor tissue.
  - Matched leukocytes were used as a source of constitutional DNA to censor germline variants and clonal hematopoiesis of indeterminate potential (CHIP).
- Baseline PV genotypes from each baseline source were then used to longitudinally assess MRD serially during 1L therapy.
  - Blood timepoints included baseline (pre-treatment), Cycle 2 Day 1 (C2D1), C3D1, C4D1, C5D1, C6D1, at End of Induction (EOI), and serially thereafter.
- Levels of ctDNA were compared to known FL prognostic factors, including radiographic responses, POD24, and progression-free survival (PFS).

RESULTS

- PVs were successfully identified from tumor FFPE samples in all patients.
- The median number of PVs identified was 530 (IQR 285-1152).
- The median tumor fraction in baseline plasma was 0.28%, significantly lower than pre-treatment DLBCL.[2]

Figure 1. LOD for Phased Variants and SNVs

ctDNA detection using phased variants has a substantially lower limit of detection (LOD) in comparison to SNVs.[1,3]

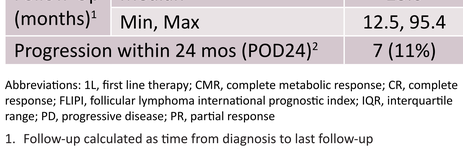


Table 1. Cohort Description

Characteristic		N (%)
Age	Median (IQR)	56 (51, 65)
Sex	Female	28 (46%)
	Male	33 (54%)
Stage	I/II	14 (23%)
	III/IV	46 (75%)
	Unknown	1 (2%)
FLIPI Score	Low (0-1)	15 (25%)
	Intermediate (2)	18 (30%)
	Poor (>3)	24 (39%)
	Unknown	4 (7%)
1L Therapy	Bendamustine/Rituximab	39 (66%)
	R-CHOP	16 (26%)
	Radiotherapy	3 (5%)
	Watch and Wait	3 (5%)
Best Response after 1L	CR/CMR	47 (77%)
	PR	8 (13%)
	PD	2 (3%)
	Unknown or N/A	4 (7%)
Follow-Up (months)[1]	Median	23.6
	Min, Max	12.5, 95.4
Progression within 24 mos (POD24)[2]		7 (11%)

Abbreviations: 1L, first line therapy; CMR, complete metabolic response; CR, complete response; FLIPI, follicular lymphoma international prognostic index; IQR, interquartile range; PD, progressive disease; PR, partial response

1. Follow-up calculated as time from diagnosis to last follow-up
2. Progression of disease <24 months after completion of 1L therapy

Figure 2. Phased Variants in DLBCL vs FL

WGS data from the PCAWG cohort[1] was reanalyzed. PVs were identified and placed into 1,000bp genomic bins. PVs are common and occur in stereotyped regions of the genome in FL.

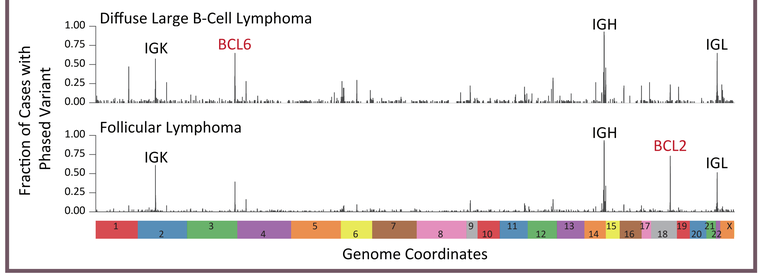


Figure 3. ctDNA Levels in FL Correlate with Stage

Median pre-treatment ctDNA levels were significantly higher in advanced stage disease (stage I/II, 0.04%, stage III/IV, 0.53%, P=0.006), and correlated with FLIPI risk scores (P=0.04).

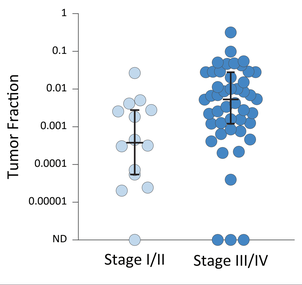


Figure 4. Similar ctDNA Levels using Tumor or Plasma-Derived PVs

Prior to therapy, ctDNA was detectable in 94% (57/61) cases using tumor-derived PVs and in 75% of cases using plasma-derived PVs.

ctDNA levels were highly correlated, regardless of the baseline sample used to identify PVs.

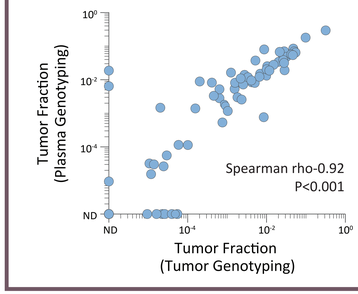


Figure 5. Mutational Genotyping is Highly Concordant Between Tumor and Plasma

Despite high genotypic concordance between tumor and plasma, significant tumor heterogeneity was observed across compartments with variable rates of concordance between specific mutated genes

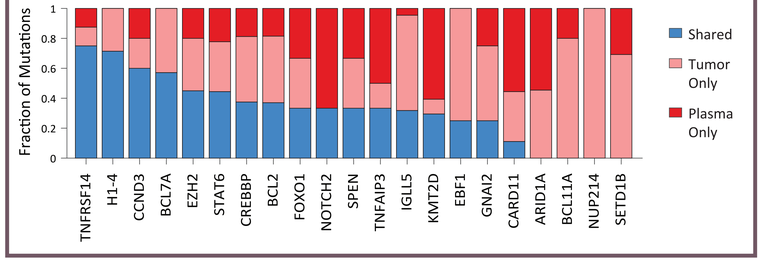


Figure 6. ctDNA Levels at C3D1 Predict Outcomes

ctDNA levels prior to treatment were not significantly prognostic for time to progression.

In contrast, in patients with ctDNA MRD assessed after two cycles of therapy (i.e., at C3D1), detection of residual ctDNA was significantly associated with inferior time to progression (log-rank P=0.03, HR = 8.9).

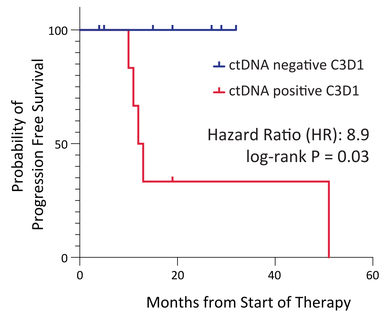
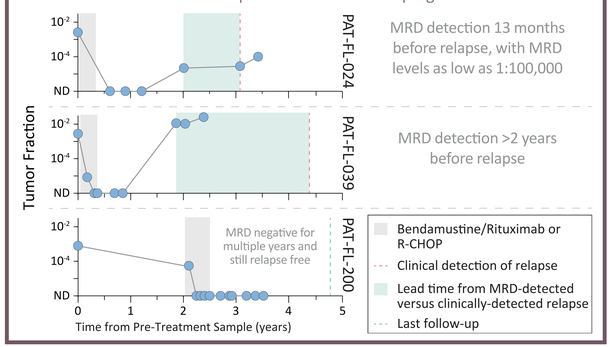


Figure 7. ctDNA Monitoring Patient Vignettes

In patients with subsequent clinical relapse of disease, reemergence of detectable MRD was observed prior to clinical disease progression.



CONCLUSIONS

- Circulating tumor DNA analysis with PhasED-Seq is feasible in FL, with baseline ctDNA levels significantly lower than those observed in DLBCL.
- In cases without tissue samples available, plasma DNA can frequently be used as a surrogate for tissue samples.
- ctDNA detection during treatment is strongly associated with eventual treatment responses, and serial disease surveillance with ctDNA can anticipate clinical relapse.

ACKNOWLEDGMENTS

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