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ORAL ABSTRACTS

621.LYMPHOMAS: TRANSLATIONAL-MOLECULAR AND GENETIC

Longitudinal Noninvasive Surveillance & Fragmentomic Characterization of Follicular Lymphoma

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Introduction

While cell-free DNA (cfDNA) plays an increasingly defined role in aggressive lymphomas, its characteristics in indolent lymphomas are less established. Many follicular lymphoma (FL) patients experience durable remissions and late relapses (**Fig A**). We therefore explored circulating tumor DNA (ctDNA) kinetics during long-term blood-based surveillance in patients with FL. In addition to noninvasive detection using somatic mutations, we also evaluated gene expression inference from cfDNA utilizing a novel fragmentomic method to detect histological transformation (tFL).

Methods

We studied 121 serial samples in a cohort of 17 FL patients (median 7 per patient) with long term clinical and blood-based MRD monitoring (median 5.6 years, max 10.8). We considered serial ctDNA status at 4 milestones: baseline/pre-treatment, post-treatment, in radiographic remission (MRD), and at relapse. We also profiled pre-treatment cfDNA for 9 tFL cases. Cases were genotyped to identify somatic mutations from diagnostic tumor or baseline plasma, with matched PBMCs used to censor germline variants and clonal hematopoiesis. ctDNA status in subsequent samples was evaluated via CAPP-Seq (SNVs) and PhasED-Seq (PVs). The cfDNA fragmentation profiles of pre-treatment FL and control samples were evaluated for inferred gene expression via EPIC-seq (Esfahani Nat Biotech 2022) using a novel 1676-gene panel focused on lymphoid neoplasms, including classic and transformed FL.

Results

Treatment & Relapse Kinetics

Most patients had advanced disease (88% stage III-IV) and Low to Intermediate risk FLIPI (71% 0-2). At first cfDNA evaluation, 9/17 (53%) were previously untreated. Median pre-treatment ctDNA levels were 73.4 hGE/mL in FL, as compared to 236 hGE/mL in DLBCL (Kurtz *JCO* 2018). Post-treatment, median ctDNA levels dropped to 1.2 hGE/mL. Depth of response was similar between rituximab monotherapy and cytotoxic regimens, with median -2.2 log10 fold change from baseline.

MRD Detection

PVs were observed in all tumors, consistent with expected aberrant somatic hypermutation in FL. To evaluate MRD detection via PhasED-Seq, we considered plasma timepoints with undetectable ctDNA by CAPP-Seq (n=26) from patients (n=9) with subsequent clinical relapse. MRD was detected in 11 additional specimens (42%) by PhasED-Seq. Among FL patients tested for MRD while in radiographic remission, low-level ctDNA was detectable in 62% of plasma specimens (8/13) obtained within 1 month of negative PET/CT, suggesting potential additional utility for surveillance.

Long-term Genomic Stability

Sequential biopsies of FL tumors have shown genomic heterogeneity between diagnosis and relapse. We therefore evaluated ctDNA concordance across variant classes & regions. Relative AF was calculated pre-treatment and at relapse (median 2 years between samples, range 1-8 years), normalized by total ctDNA levels. The most stable lesions included missense mutations

in *CREBBP* and *TP53*, nonsense *KMT2D* mutations, and *BCL2* 5′-UTR variants. *BCL6* intronic mutations and chr2/chr14 SHM varied between diagnosis/relapse.

Plasma versus Cellular Concentrations

In contrast to DLBCL, circulating FL cells are frequently detectable at low levels in blood. To compare relative FL concentrations in the cellular versus plasma compartments, we profiled 10 paired specimens from patients with radiographic disease. While tumor-confirmed variants were detected in 7 of 10 cellular specimens, mean allelic frequency (AF) was 5.3x higher in plasma.

Fragmentomic Assessment of Transformation

We compared gene expression profiles inferred from cfDNA from pre-treatment lymphoma (FL=15, tFL=9) versus control (n=20) samples (**Fig B**). Considering genes associated with FL (Huet *Lancet Onc* 2018), FL patients had significantly higher inferred expression than healthy adults (p=0.036). Likewise, patients with tFL showed higher inferred expression of genes associated with histologic transformation (Gentles *Blood* 2009, p=0.025).

Conclusions

Sensitive methods are required for effective MRD assessment in FL given lower ctDNA shedding as compared with DLBCL. Frequent detectable MRD in radiographic remission suggests that integrating molecular surveillance may augment serial imaging in the long-term management of FL. Simultaneous inferred gene expression from FL cfDNA appears to hold promise, including for noninvasive detection of transformation.

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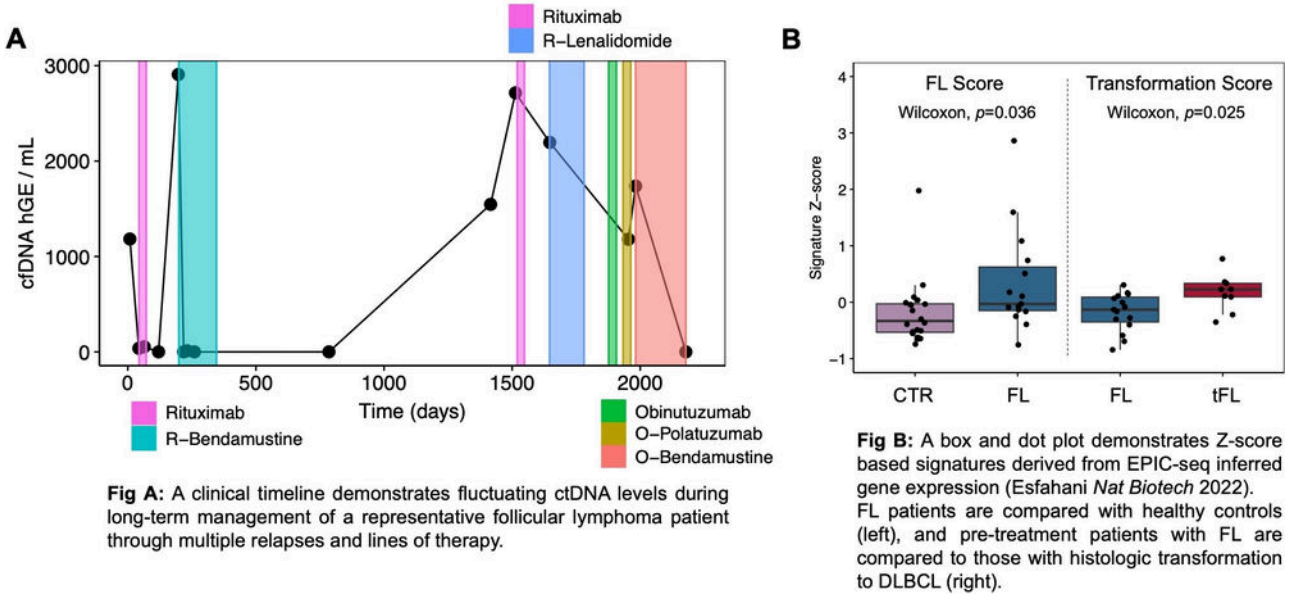

Fig A: A clinical timeline demonstrates fluctuating ctDNA levels during long-term management of a representative follicular lymphoma patient through multiple relapses and lines of therapy.

Fig B: A box and dot plot demonstrates Z-score based signatures derived from EPIC-seq inferred gene expression (Esfahani *Nat Biotech* 2022). FL patients are compared with healthy controls (left), and pre-treatment patients with FL are compared to those with histologic transformation to DLBCL (right).

Figure 1

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