Abstract #52 | Poster

Circulating Tumor DNA Reveals Lymphoma-Associated Mutations Long Before Clinical Diagnosis

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Background: The timing between early lymphoma-driving events and clinical presentation remains poorly defined. Diffuse large B-cell lymphoma (DLBCL) and Classic Hodgkin lymphoma (cHL) arise from germinal-center B cells and harbor recurrent genetic alterations. Aberrant somatic hypermutation generates multi-nucleotide variants (MNVs), often targeting super-enhancers and key B-cell regulatory loci early in lymphomagenesis. These MNVs may serve as a sensitive mutational fingerprint in cfDNA, enabling early tumor detection and mapping of lymphoma development.

Aim: By integrating single nucleotide variant (SNV) and MNV calling, we aim to identify early molecular aberrations preceding clinical diagnosis of DLBCL and cHL.

Methods: We analyzed pre-diagnostic plasma from the Dutch Lifelines biobank in a retrospective case-cohort of 33 DLBCL and 13 cHL patients, including matched diagnostic tumor tissue samples and white blood cells for 41 cases. Targeted sequencing of 115 genes (~391 kbp), including SEs of 14 genes was performed. SNV analysis was restricted to DLBCL, as tumor cell content in cHL tissue is too low to reliably call SNVs. MNVs were called using our in-house developed MNVista tool.

Results: Overall, pre-diagnostic ctDNA was detected in 31/46 (67%) cases. The median pre-diagnostic plasma sample was drawn 21 months prior to diagnosis (DLBCL range = 1–106 months, cHL range = 3–55 months). MNVs were detected in pre-diagnostic ctDNA in 20/33 DLBCL and 9/13 cHL patients. Both MNVs and SNVs were identified in 11/33 DLBCL cases, while only MNVs were identified in 9/33 and only SNVs in 2/33 cases. The earliest pre-diagnostic timepoints with detectable ctDNA were 106 and 55 months before diagnosis of DLBCL and cHL, respectively (Figure 1A). In cHL, pre-diagnostic ctDNA levels correlated strongly with sTARC ($r = 0.86$, $p<0.001$). CtDNA-positive plasma samples were collected significantly closer to diagnosis (median 17, range 1–106 months) than ctDNA-negative samples (median 33, range 50–3 months) (Figure 1B). CtDNA levels significantly correlated with sampling timepoint (Figure 1C). DLBCL and cHL seem to expand at a similar rate.

Summary/Conclusion: ctDNA can be detected years prior to B-cell lymphoma diagnosis. Our data show that MNV-approaches are sensitive and can be used for pre-diagnostic ctDNA detection in a tumor-informed setting. They provide novel insight in early molecular aberrations in DLBCL and cHL and the latent interval of these diseases.

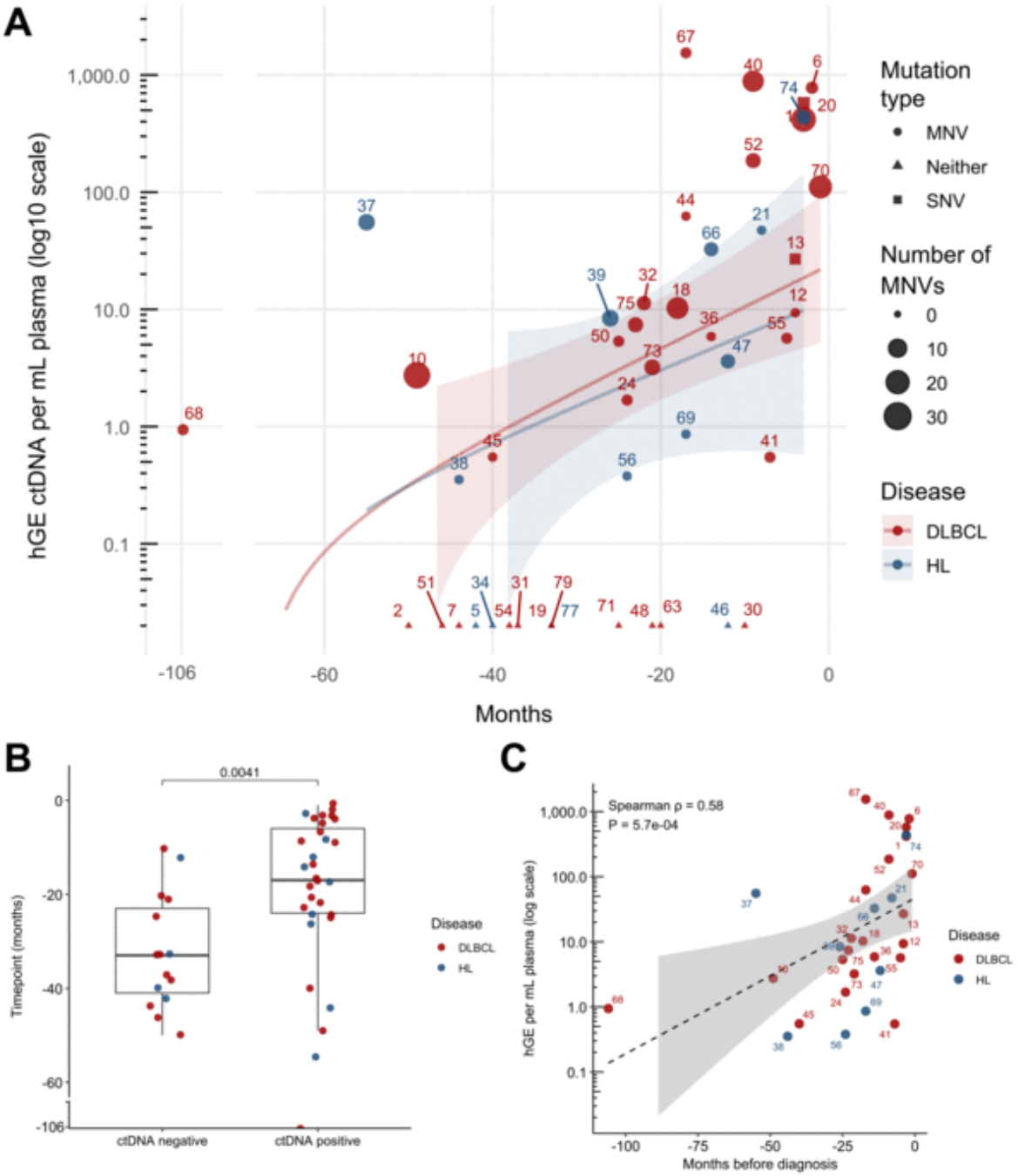

Figure 1: Pre-diagnostic circulating tumor DNA (ctDNA) detection in DLBCL and cHL
A) Haploid genome equivalents (hGE) of circulating tumor DNA (ctDNA) per mL plasma by analyzing multi-nucleotide variants (MNVs) over time per disease in both DLBCL and cHL patients. The point size reflects MNV counts. **B)** Comparison of pre-treatment plasma sample timepoints in ctDNA-positive (+) versus ctDNA-negative (–) patients (overall ctDNA+ vs ctDNA– p = 0.0041). **C)** Scatterplot correlating (Spearman) plasma sampling timepoint and ctDNA level in hGE/mL plasma. In all panels, color depicts disease (DLBCL in red, cHL in blue) and each dot represents an individual patient sample.