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RAPID COMMUNICATION

Genotyping on circulating tumor DNA improves mutation detection rate in high-risk diffuse large B-cell lymphoma

Diffuse large B-cell lymphoma (DLBCL) is the most common lymphoma with heterogeneous clinical outcomes. Patients who are primarily refractory to the frontline therapy or relapse less than 12 months after diagnosis have an extremely dismal prognosis.[1] The heterogeneous clinical outcomes of DLBCL patients result from variable genetic profiles. However, the invasiveness of tissue biopsy often hampers the clinical application of genetic analysis. Circulating tumor DNA (ctDNA) is a non-invasive source of tumor genetic material. Rossi et al reported that pre-treatment plasma ctDNA from DLBCL patients have >90% sensitivity and ~100% specificity compared with mutations that were represented in >20% of the alleles of the tumor gDNA.[2] Moreover, in patients with multiregional diseases, ctDNA mutations offer a comprehensive representation of tumor genetics, regardless of anatomical biases.

In this study, we collected ctDNA and tumor gDNA from 104 DLBCLs, including 62 with paired ctDNA and gDNA samples. The samples were divided into four clinical groups according to the disease status at the time of sample collection: treatment-sensitive (TS), primary refractory (Pri-R), early-relapse/refractory (ERR), and late-relapse (LR) (Fig. 1A). Patients' characteristics and previous treatment were shown in Table S1. Peripheral blood samples and tissue biopsies were collected at enrollment. A targeted sequencing gene panel including 112 genes that are recurrently mutated in DLBCL was used (Burning Rock Biotech Co. Ltd, Guangzhou, China; Fig. S1). Germline variants were filtered out by analyzing gDNA extracted from paired white blood cells of each patient. The data have been deposited in the GSA-Human database with access number HRA004312. Detailed methods were available in the supplementary materials.

At least one variant was detected in 90.6% (154/170) samples. The median number of variants was 8 (range: 0–100) and 9 (range: 0–39) in ctDNA and gDNA, respectively. In the 102 ctDNA samples, the detection rate was 88.2% and the most frequently mutated genes were *PIM1*, *KMT2D*, *MYD88*, *TP53*, and *MYC* (Fig. S2). In the 68 gDNA samples, the detection rate was 94.1% and the most frequently mutated genes were *PIM1*, *MYD88*, *BCL2*, *KMT2D*, and *MYC* (Fig. S3).

In the 62 patients who had paired ctDNA and tumor gDNA samples, 83.9% (52/62) patients had at least one common mutation between the paired samples. A total of 1433 variants were detected in the paired samples, among which 1012 (506 pairs) were detected in both ctDNA and gDNA (Table S2). The sensitivity of biopsy-matched variants was 76.1% (506/665). When considering only the mutations present with >20% variant allelic frequency (VAF) in gDNA, we detected up to 85.0% (414/487) of these mutations in ctDNA.

In the 102 ctDNA samples, the mean VAF of mutations was 7.9% (range: 0–49.8%). The mean VAF of mutations showed a good correlation with elevated lactate dehydrogenase (LDH; $P = 0.0008$) and β2-microglobulin (β2-MG; $P = 0.039$), as well as the metabolism tumor volume (MTV, R 0.38; $P = 0.0024$) and the total lesion glycolysis (TLG, R 0.40; $P = 0.0012$) assessed by 18F-fluorodeoxyglucose-positron emission tomography with computed tomography (FDG-PET/CT; Fig. 1B–E). Besides, patients with a mean VAF of ≥1.5% (determined by X-tile) had significantly shorter progression-free survival and overall survival (Fig. 1F, G and Table S3).[3,4]

The comparison of mutational profiles among the four groups is shown in Figure S4–13 and Table S4. In the 102 ctDNA samples, genes of the NFκB, apoptosis/cell cycle, and BCR signaling pathways were more frequently mutated in ERR DLBCLs compared with the other three groups (Fig. S14). Also, mutations of these three pathways were

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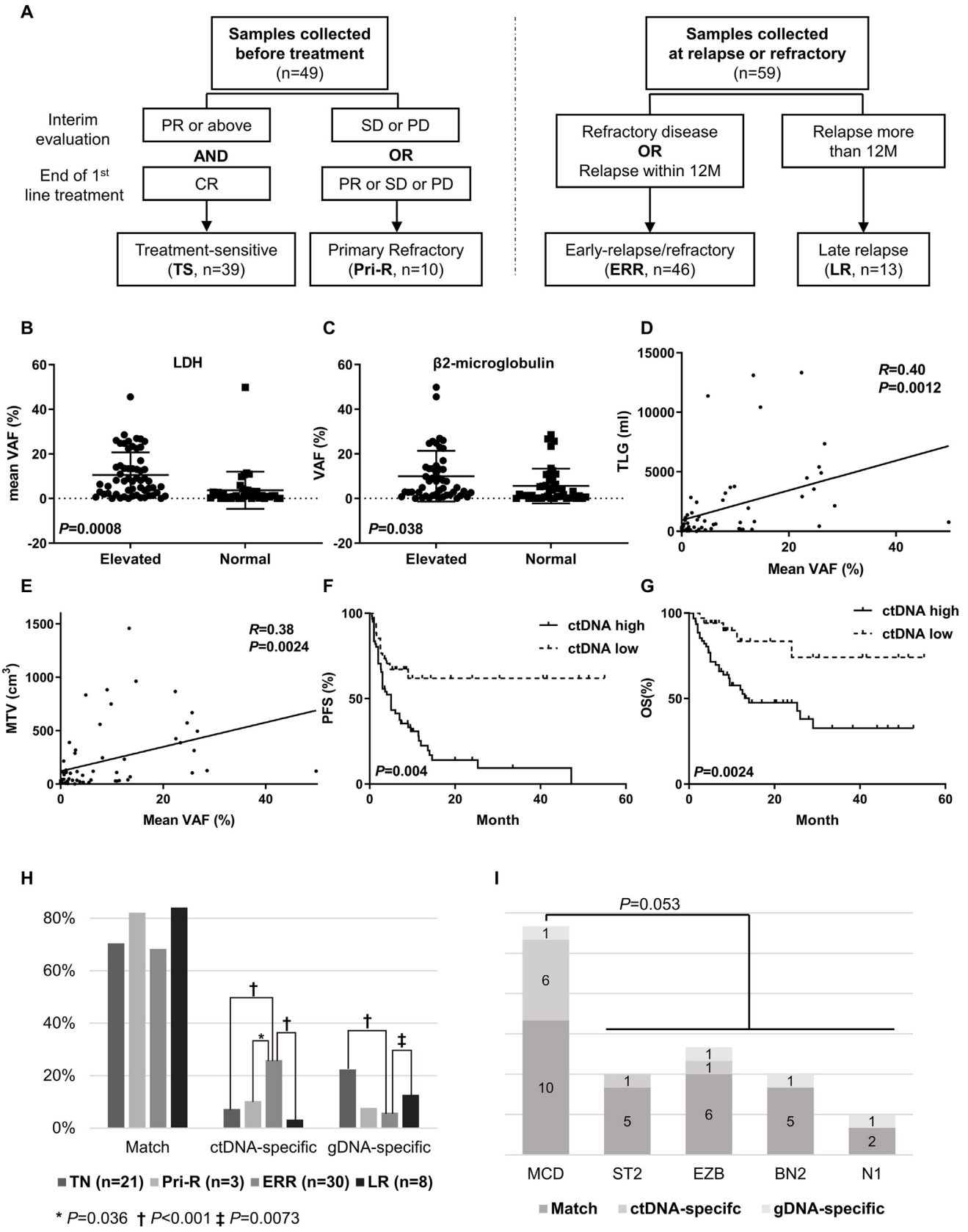

Figure 1 ctDNA genotyping in patients with high-risk DLBCL. **(A)** The graphic view of four groups of patients. For the TS and Pri-R groups, samples were collected before treatment. TS represents patients who responded well to frontline treatment and Pri-R represents patients who failed to achieve PR at the interim evaluation or CR at the end of frontline treatment. For ERR and LR patients, samples were collected after relapse. ERR represents patients who relapsed within 12 months after diagnosis and LR

associated with inferior prognosis (Fig. S15, 16). However, similar trends were not observed in the tissue gDNA samples, likely due to the low sample size ($n = 68$; Fig. S17, 18). The prognostic significance and clinical relevance of each mutation are shown in Figure S19−23.

In the 62 patients who had paired ctDNA and gDNA samples, we found 43.5% (27/62) had ctDNA-specific variants. Although the mean VAF of ctDNA-derived mutations among the four groups were similar (Fig. S24), the proportion of ctDNA-specific variants in ERR DLBCLs was higher than that of the other three groups (TS: 7.3%; Pri-R: 10.3%; ERR: 25.9%; LR: 3.2%; $P < 0.001$). In contrast, gDNA-specific variants were more common in TS and LR DLBCLs compared with ERR DLBCLs (TS: 22.4%; Pri-R: 7.7%; ERR: 5.9%; LR: 12.7%; $P < 0.001$; Fig. 1H; Fig. S25). Some subtype-characterizing mutations, *PRDM1* mutation (60%, 3/5) of MCD, *EP300* mutation (57.1%, 4/7) of EZB, and *TP53* mutation (30%, 3/10) of A53, were exclusively found in ctDNA, which prompt us to investigate the role of ctDNA genotyping in DLBCL genetic subtype identification.

According to the genetic subtypes proposed by Wright et al, 49.0% (50/102) of ctDNA samples and 52.9% (36/68) of gDNA samples could be classified (the A53 subtype was excluded due to lack of CNA information). The MCD subtype was more commonly seen in ERR patients compared with others (ctDNA: 34.9% *vs*. 18.6%, $P = 0.063$; gDNA: 21.2% *vs*. 14.3%, $P = 0.454$; Table S5, 6). In the 62 patients with paired ctDNA and gDNA samples, the detection rate of genetic subtypes was 51.6% (32/62) and 50% (31/62) in ctDNA and gDNA, respectively. Among these patients, 88.3% (53/62) had matched results, 6.5% (4/62) had gDNA-specific subtypes, 8.1% (5/62) had ctDNA-specific subtypes, and 3.2% (2/62) had inconsistent results (the ctDNA sample had 1 more subtype identified than the paired gDNA sample). When the two sample types were combined, the detection rate of genetic subtypes could reach 58.1% (36/62). Interestingly, it was found that 6 of 7 ctDNA-specific subtypes belonged to the MCD subtype, suggesting ctDNA-specific mutations were more common in the MCD subtype than other subtypes ($P = 0.053$). 35.3% (6/17) MCD DLBCLs were only identified by ctDNA but not by gDNA (Fig. 1I; Fig. S26). Clinically, patients with MCD DLBCL are more likely to be subject to extranodal disease and poor prognosis. In this respect, the ctDNA genotyping may be particularly advantageous to avoid selection bias resulting from spatial heterogeneity. Undoubtedly, the identification of the MCD subtype would encourage the early application of novel target therapies.[5]

However, there were limitations in this study. The reliability of noninvasive genotyping solely using plasma ctDNA still needs confirmation, as it can be influenced by factors like ctDNA concentration, germline variants, and clonal hematopoiesis of indeterminate potential. Simultaneous evaluation of tumor tissue and plasma is recommended, especially during initial diagnosis, for accuracy. Additionally, in this study, we assessed a cohort of patients who demonstrated high tumor burden and challenging treatment outcomes. While most of our analysis involved this specific subgroup, further research is needed to explore the broader application of ctDNA-based genotyping in the general population, particularly in cases of limited-stage DLBCL.

In conclusion, we conducted target gene sequencing of ctDNA and gDNA in 104 Chinese patients with DLBCL. Our findings confirmed a positive correlation between ctDNA levels and tumor burden indicators. Analysis of patients in different disease states revealed that those with ERR DLBCLs exhibited higher frequencies of ctDNA-specific mutations. Additionally, we observed an enrichment of mutations in the NFκB, BCR, and apoptosis/cell cycle pathways, which may contribute to chemo-resistance. Furthermore, ctDNA genotyping demonstrated potential for improving the detection of DLBCL genetic subtypes, particularly the MCD subtype. Consequently, we propose that ctDNA analysis serves as a valuable complementary tool to tissue biopsy-based gDNA genotyping, particularly in high-risk and R/R DLBCL cases.

Ethics declaration

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of Jiangsu Province Hospital (2022-SR-319).

Author contributions

WX and JYL designed the research. YX, LW, JHL, and HRS performed the research. HY, YL, and HYZ analyzed the data. YX and LW wrote the manuscript. JYL and WX revised the manuscript and finalized the last version of the manuscript. All authors checked and approved the submitted version.

represents patients who relapsed more than 12 months after diagnosis. The level of ctDNA positively correlated with **(B)** LDH, **(C)** β2-MG, **(D)** TLG, and **(E)** MTV assessed by PET/CT. A high ctDNA level correlated with shorter **(F)** PFS and **(G)** OS. **(H)** The proportion of ctDNA-specific mutations in ERR DLBCLs was significantly higher than that of the other three groups. **(I)** The proportion of ctDNA-specific mutations in MCD DLBCLs was significantly higher than that of the other molecular subtypes. β2-MG, β2-microglobulin; CR, complete remission; ctDNA, circulating tumor DNA; ERR, early-relapse/refractory; gDNA, genomic DNA; LDH, lactate dehydrogenase; LR, late-relapse; MTV, metabolism tumor volume; OS, overall survival; PFS, progression-free survival; PR, partial remission; Pri-R, primary refractory; TLG, total lesion glycolysis; TS, treatment-sensitive.

Conflict of interests

The authors declare no conflict of interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gendis.2023.101116.

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Yi Xia [a,b,c,1], Li Wang [a,b,c,1], Jinhua Liang [a,b,c], Haorui Shen [a,b,c], Jiazhu Wu [a,b,c], Hua Yin [a,b,c], Yue Li [a,b,c], Huayuan Zhu [a,b,c], Jianyong Li [a,b,c], Wei Xu [a,b,c,*]

[a] *Department of Hematology, The First Affiliated Hospital of Nanjing Medical University, Jiangsu Province Hospital, Nanjing, Jiangsu 210029, China*

[b] *Key Laboratory of Hematology of Nanjing Medical University, Nanjing, Jiangsu 210029, China*

[c] *Collaborative Innovation Center for Cancer Personalized Medicine, Nanjing, Jiangsu 210029, China*

*Corresponding author. Department of Hematology, The First Affiliated Hospital of Nanjing Medical University, Jiangsu Province Hospital, Nanjing, Jiangsu 210029, China.
E-mail address: xuwei10000@hotmail.com (W. Xu)

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[1] These authors shared the first authorship.