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ORIGINAL PAPER

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Dynamics of circulating tumour DNA in relapsed/refractory diffuse large B-cell lymphoma patients

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Summary

The response to salvage chemotherapy in relapsed/refractory (R/R) diffuse large B-cell lymphoma (DLBCL) is poor, and data on circulating tumour deoxyribonucleic acid (ctDNA) in this setting are limited. We evaluated ctDNA dynamics in 29 patients with relapsed or refractory DLBCL who received platinum-based salvage chemotherapy at the University Medical Center Groningen. In total, 124 plasma samples were analysed using low-coverage whole-genome sequencing to detect copy number alterations (CNAs) and targeted sequencing with a 115-gene panel for single- and multi-nucleotide variants (SNVs/MNVs). The complete response rate at the end of treatment was 55%, with a 1-year progression-free survival of 31%. At the R/R baseline, defined as the time after confirmation of relapse or refractory status and prior to initiation of salvage chemotherapy, CNAs were detected in 15 patients, SNVs in 27 and MNVs in 21. Patients with treatment failure had higher fraction of genome altered (FGA) and ctDNA levels at baseline. Low FGA combined with low metabolic tumour volume (MTV) at baseline was associated with favourable outcome. Clearance of all baseline SNVs at interim evaluation correlated with improved response, while persistence predicted failure ($p < 0.05$). Two of three patients with complete metabolic response at the end of treatment and detectable ctDNA relapsed, indicating ctDNA as a sensitive marker of minimal residual disease. These findings indicate the value of ctDNA profiling as a prognostic biomarker and as a tool for response-adapted treatment strategies in R/R DLBCL.

KEYWORDS

ctDNA, disease dynamics, relapsed/refractory DLBCL, salvage chemotherapy

INTRODUCTION

Diffuse large B-cell lymphoma (DLBCL) is the most prevalent B-cell lymphoma, accounting for approximately 30% of all lymphomas.[1] While 60%–80% of patients respond favourably to first-line treatment with rituximab in combination with cyclophosphamide, doxorubicin, vincristine and prednisone (R-CHOP), 20%–40% develop relapsed/refractory (R/R) disease.[2]

In general, most DLBCL patients present with R/R disease within 12 months post first-line treatment, whereas late relapses (>12 months) constitute approximately 30%–40% of cases.[3] The prognosis of R/R DLBCL is poor, with an overall response rate (ORR) to salvage chemotherapy of 26%–46% and a median overall survival (OS) of around 6 months in patients that show R/R disease within 12 months.[4,5] In contrast, those with late relapse have a relatively better, yet still adverse outcome, with an ORR of 80%–88% and a long-term

survival rate of 45%–66% when treated with salvage chemotherapy,[4,5] underscoring the need for novel biomarkers to identify poor responders that might benefit from chimeric antigen receptor T-cell (CAR-T) therapy[6,7] or bispecific monoclonal antibody therapy.[8] While durable responses are achieved in only 40%–50% of R/R DLBCL patients following CAR-T therapy,[7] the role of salvage chemotherapy following CAR-T failure remains unclear.

Circulating tumour DNA (ctDNA) has emerged as a promising biomarker, providing non-invasive assessment of tumour burden, molecular features and early treatment response. In first-line treatment, baseline ctDNA level, early ctDNA dynamics and molecular minimal residual disease (MRD) are associated with outcome.[9–12] Moreover, cell free DNA (cfDNA)-based mutational profiling, fragmentation analysis and molecular clustering further refine prognostic stratification beyond ctDNA levels alone.[13,14] In the context of CAR-T therapy, several studies have reported the prognostic significance of ctDNA. Higher pretreatment ctDNA concentration and increased number of focal copy number alteration (CNA) were associated with worse outcomes after CAR-T therapy, whereas early ctDNA clearance post-infusion correlates with better treatment outcomes.[15,16] In contrast, data on the utility of ctDNA as a biomarker in the setting of salvage chemotherapy remain limited. Detection of clonal immunoglobulin heavy chain rearrangements in ctDNA by high-throughput sequencing after second-line chemotherapy was negatively associated with outcome in DLBCL.[17] One study showed that ctDNA levels and molecular features can predict response and guide treatment in R/R mantle cell lymphoma.[18] However, to the best of our knowledge, there are no publications evaluating the predictive value of ctDNA to salvage chemotherapy in R/R DLBCL. This highlights a knowledge gap in current understanding of the potential value of ctDNA as a biomarker for treatment outcome for R/R DLBCL. Moreover, according to the National Comprehensive Cancer Network (NCCN) Guidelines, only ctDNA MRD assays with a detection limit of less than 1 ppm are recommended for first-line treatment.[19] To reach this sensitivity, it will be important to incorporate multi-nucleotide variant analysis ctDNA workflows.

To address the knowledge gap in R/R DLBCL, this study aims to investigate the utility of ctDNA in predicting patient response to salvage chemotherapy. In addition, we aimed to evaluate ctDNA dynamics in R/R DLBCL during salvage chemotherapy as a potential tool for disease monitoring.

METHODS

Patient population

Patients included in this study were enrolled in OncoLifes,[11] a prospective biobank for cancer patients at the University Medical Center Groningen (UMCG, The Netherlands). In total, we identified 35 patients diagnosed with R/R DLBCL between 2019 and 2023. Patients with central nervous system only involvement ($n = 3$) and those who achieved complete response (CR) but lacked end-of-treatment (EOT) plasma samples ($n = 3$) were excluded from the analysis. Ultimately, 29 patients were included, all with plasma samples available prior to salvage therapy. Immunohistochemical subtyping was performed using the Hans algorithm to classify cases as germinal centre B-cell–like (GCB) or non-GCB. Relapse was confirmed by biopsies for all patients. Pathology was reviewed by an experienced haematopathologist (A.D.). This study was conducted in accordance with the ethical principles of the Declaration of Helsinki and with the approval of the Medical Ethics Review Board of the University Medical Center Groningen. Written informed consent was obtained from all participants prior to sample collection.

Patient characteristics and sample process

Baseline characteristics, treatment and response assessment were retrieved from the medical records. Refractory or early relapse (<12 months) DLBCL was defined as stable disease (SD) or progressive disease (PD) during or by EOT, following first-line treatment; partial response (PR) at EOT; or relapse within 12 months after achieving CR at EOT. Late relapse DLBCL was defined as a relapse developing after >12 months achieving CR at EOT of front-line treatment.[20] Response evaluation was done using the 2014 Lugano criteria. Plasma samples were collected at R/R DLBCL diagnosis (baseline), after one to two cycles of salvage therapy (interim), at EOT and during follow-up. Blood samples were collected in DNA-STRECK tubes (STRECK, Nebraska, USA) and processed within 96 h (for details, see Supplementary Methods: Data S1).

^{18}F-FDG PET/CT acquisition and analysis

^{18}F-FDG positron emission tomography/computed tomography (PET/CT) scans were performed using a Siemens Biograph mCT scanner (Siemens Healthineers, Erlangen, Germany) following the European Association of Nuclear Medicine (EANM) procedure guidelines for tumour imaging.[21] Detailed information was provided in Data S1.

DNA library preparation and next-generation sequencing

DNA library preparation and target enrichment was conducted using Twist Kits (Twist Bioscience, San Francisco, USA). CNAs were called using QDNAseq (version = 1.36.0).[22] The fraction of genome altered (FGA) was calculated as the total length of CNA segments divided by the total size of the sequenced genome. The estimated tumour fraction (ETF, fraction of ctDNA in cfDNA) was calculated using ichorCNA (version = 0.2.0),[23] using merged data of 14 normal samples as controls. The maxCN setting was set to 4, ploidy = 2. A detailed description of the workflow is provided

in the Supplementary Methods: Data S1 and Table S1. Single nucleotide variants (SNVs) and multi-nucleotide variants (MNVs) were analysed using an in-house bioinformatics pipeline, as outlined in the Supplementary Methods: Data S1 and Tables S2 and S3.

Statistical analyses

The treatment success group was defined as patients who achieved CR at the EOT of salvage therapy and had no events during follow-up. The treatment failure group included patients with SD or PD during salvage therapy or at EOT, those with PR at EOT and those who achieved CR at EOT but relapsed during follow-up. Statistical differences between the success and failure groups in baseline lactate dehydrogenase (LDH), metabolic tumour volume (MTV), FGA, ETF, SNV load and ctDNA level ($\log_{10}$ haploid genomic equivalent per millilitre [hGE/mL]) were assessed using the Mann–Whitney *U*-test. Kaplan–Meier estimates were used to analyse progression-free survival (PFS) and overall survival (OS). Fisher's exact test was used to test the association between SNV clearance at interim and treatment response. Spearman's correlation coefficient was calculated to determine the correlation between baseline ETF and MTV, ETF and elevated LDH, MTV and ctDNA level ($\log_{10}$ hGE/mL), as well as elevated LDH and ctDNA level ($\log_{10}$ hGE/mL). Multi-variable analyses were not performed due to the relatively small sample size.

RESULTS

Clinical characteristics and sample overview

A total of 29 R/R DLBCL patients were included in this study. Among them, 22 patients (75.9%) had refractory/early relapse disease, while seven patients (24.1%) had a late relapse. The cohort consisted of 17 males (58.6%) and 12 females (41.4%), with a median age of 60 years (range: 28–69). Histological examination revealed DLBCL for 20 patients (69.0%) and transformed follicular lymphoma (tFL) for nine patients (31.0%). Immunohistochemistry-based cell-of-origin classification indicated a germinal centre B-cell-like (GCB) subtype for 19 cases (65.5%), a non-GCB type for eight cases (27.6%), while two cases were not classified. Elevated LDH levels were observed in 17 patients (58.6%) (Table S4). Of the 16 patients (55.2%) who achieved a CR at the end of salvage treatment (EOT), 12 underwent autologous stem cell transplantation (ASCT); second relapse occurred in five patients overall, including four who had received ASCT. The remaining 13 patients showed either PR or PD. The estimated 1-year PFS and OS rates were 31% and 69% respectively (Figure S1). A schematic summary of clinical characteristics and the timing of 124 cfDNA samples is provided in Figure 1. Treatment information for each individual is available in Table S5.

Pre-salvage therapy genomic landscape

At the time of relapse prior to salvage treatment, CNAs were detected in 15 of 29 cases (52%). The CNA profile was characterized by a widespread pattern of gains and losses, with gains observed on chromosomes 1, 7 and 18 and losses on 6, 9 and 17. Notably, gains and losses were detected more frequently in patients with PR or PD (10/13) as compared to those who achieved CR (3/11) following salvage chemotherapy (Figure 2A). The ETF of the 15 cases with CNAs ranged from 0.06 to 0.81 (median 0.36). The ETF value was not correlated with MTV ($\rho = 0.49$, $p = 0.07$; Figure 2B) or with LDH levels ($\rho = 0.03$, $p = 0.92$, Figure 2C). Interestingly, nine patients with ETF >0 and elevated LDH at baseline (dashed box) all experienced treatment failure (Figure 2C).

SNVs were detected in 27 of 29 patients. Non-synonymous SNVs were present in 26 patients (89%) (Figure 3A). LymphGen clustering identified three cases as A53 (*TP53*-altered subtype) (10.3%), three as EZB (*EZH2/BCL2* subtype) (10.3%), one as EZB/A53 (3.4%) and two as MCD (*MYD88/CD79B* subtype) (6.9%), while 20 cases (69.0%) as other. The DLB class algorithm assigned three cases to C1 (10.3%), 13 to C3 (45%), 2 to C4 (6.9%) and 6 to C5 (20.7%), whereas five cases remained unclassified. None of the cases were assigned to the C2 subtype, likely owing to the lack of structural variants and copy number alterations (low- or high-level gain/loss) data (Figure 3A). The most frequently mutated genes were *IRF2BP2* (34%), *ARID1B* (21%), *IGLL5* (21%), *KMT2D* (21%) and *PIM1* (21%). The median ctDNA concentration was 272 hGE/mL (range 10–28 421 hGE/mL). We observed a significant correlation between ctDNA level (expressed as $\log_{10}$ hGE/mL) and MTV ($\rho = 0.64$, $p < 0.001$) (Figure 3B), while there was no significant correlation between ctDNA levels and LDH ($\rho = -0.1$, $p = 0.68$) (Figure 3C). No significant differences were observed in non-synonymous SNV load, ctDNA level or MTV between refractory/early response cases and late relapses, DLBCL and tFL, nor between GCB and non-GCB (Figure S2). MNVs were detected in 21 cases with a median number of 12 (range: 1–61). The most frequently affected super-enhancer (SE) hypermutation regions included *BCL6* (55%), *BCL2* (40%) and *CXCR4* (24%) (Figure 3D).

Predictive value of ctDNA sampled before salvage chemotherapy

Prior to salvage chemotherapy, LDH and MTV were higher in the failure group, but did not reach statistical significance (Figure 4A,B). In contrast, FGA was significantly elevated in failures compared with CR group (0.05 [0–0.71] vs. 0.00 [0–0.53]), as was ETF (0.22 [0–0.82] vs. 0.00 [0–0.44]) (Figure 4C,D). Stratification by median FGA (0 vs. >0) and MTV (100 mL) separated patients based on outcomes: 7/10 patients with low FGA and low MTV achieved long-term CR, whereas 8/10 with high FGA and high MTV experienced PR/PD or relapse. The combined parameters outperformed single predictors in forecasting response to salvage therapy

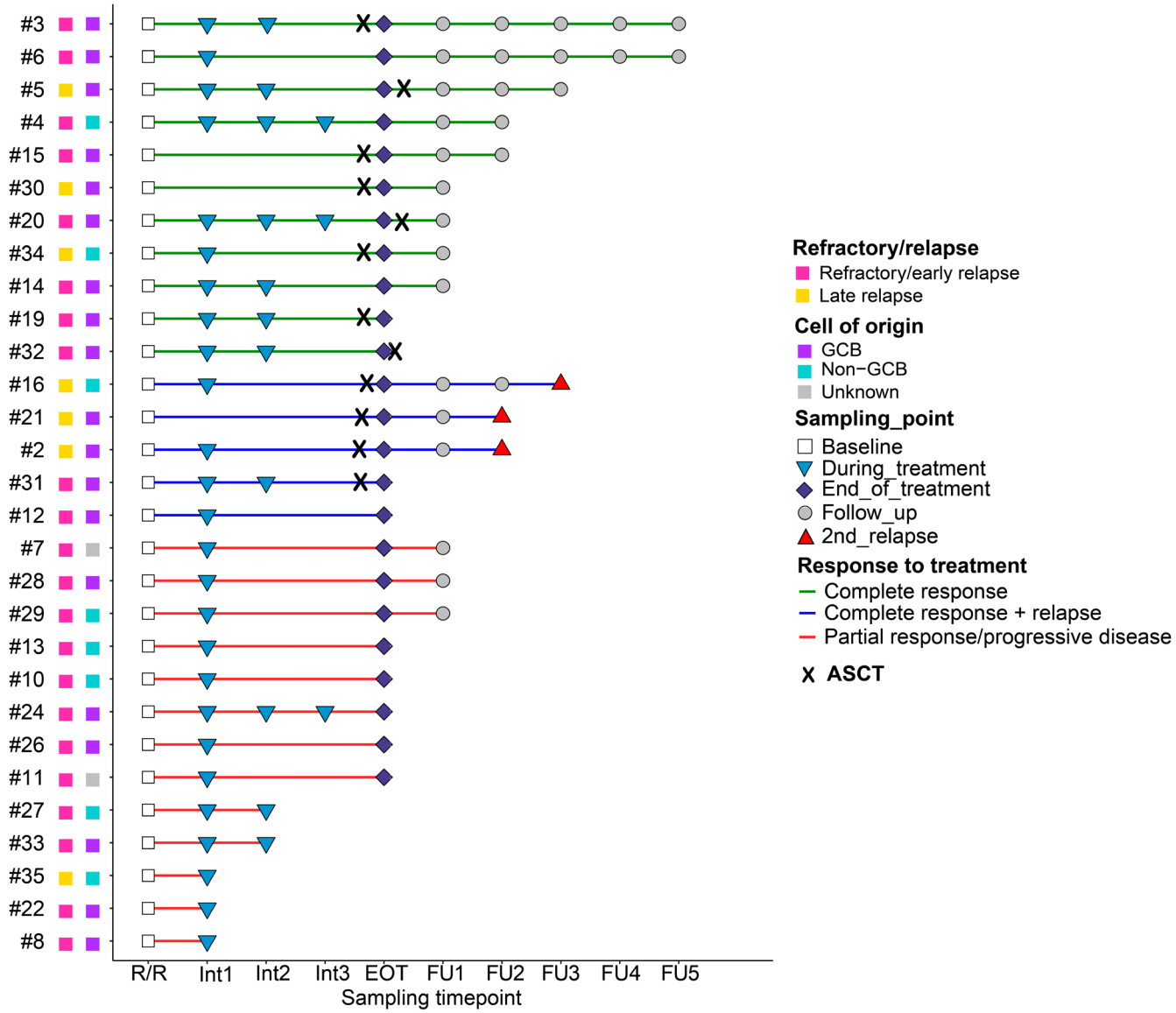

FIGURE 1 Sampling timeline and treatment response in relapsed/refractory (R/R) diffuse large B-cell lymphoma (DLBCL) patients undergoing salvage chemotherapy. Each horizontal line represents a single patient, with sampling points indicated at different time points. Patients with available plasma at a second relapse are marked with red triangles.

(Figure 4G). Baseline non-synonymous SNV-load was higher in the failure group (8.5 [1–27]) compared to long-term remission (3.0 [0–23]), as was ctDNA level (2.77 [1.12–4.45] vs. 1.51 [0–3.06]) (Figure 4E,F). When stratified by median ctDNA level into low (<median, $n = 14$) and high (≥median, $n = 15$) groups, the low-ctDNA group showed longer PFS ($p = 0.12$) and significantly longer OS ($p = 0.048$) compared with the high-ctDNA group (Figure S3).

ctDNA tracks disease dynamics

ETF dynamics were assessed in 15 patients with detectable CNAs at baseline. Interim samples were available for 14 patients and EOT samples for 13 (an overview of all results is provided in Table S6). Patients with PR or PD ($n = 10$) showed variable ETF changes, with ΔETF ranging from −77% to +9% at interim and −77% to +70% at EOT. Among the three CR cases, two had no detectable CNA at interim (ΔETF −100%). No CNAs were observed at interim or EOT in the two cases who achieved CR but later relapsed. Overall, the presence of CNA and a lack of ΔETF reduction correlated with poor clinical response (Figure S4).

Next, we performed a longitudinal analysis of ctDNA level by tracking recurrent non-synonymous and synonymous SNVs in 27 cases with SNVs at R/R baseline. Among nine patients achieving a durable CR, there were seven patients with interim samples. Six of seven patients exhibited ctDNA clearance—defined as loss of all baseline SNVs—after the first three treatment cycles and remained negative at EOT (Figure 5A). One patient remained ctDNA-positive yet clinically disease-free for 48 months, highlighting the

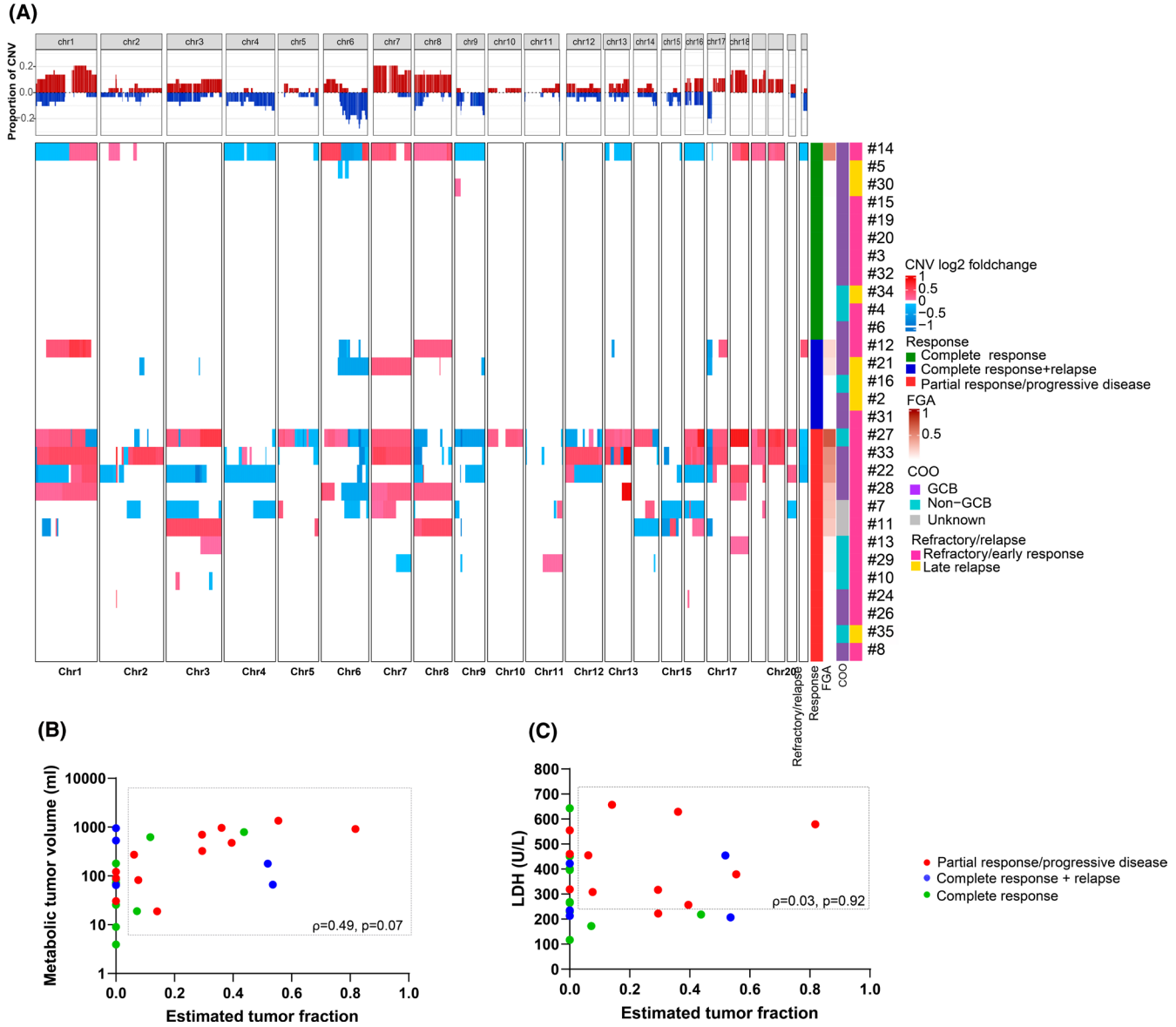

FIGURE 2 Overview of baseline copy number alterations. (A) Copy number alterations (CNAs) analysis across the genome in relapsed/refractory (R/R) diffuse large B-cell lymphoma (DLBCL) patients. (B) Correlation between estimated tumour fraction (ETF) and metabolic tumour volume (MTV). Patients within the dashed box (ETF > 0) were included in the correlation analysis. A positive correlation was observed, but it did not reach statistical significance ($r = 0.49$, $p = 0.07$). (C) Correlation between detectable ETF and elevated lactate dehydrogenase (LDH) levels. Patients within the dashed box (ETF > 0 and elevated LDH) were included in the analysis. No significant correlation was observed ($\rho = 0.03$, $p = 0.92$).

potential presence of biologically inactive residual mutations (Figure 5A; Figure S5). In patients who achieved CR but subsequently relapsed ($n = 5$), persistent or re-emerging ctDNA at interim and EOT was observed in four cases. In three cases, ctDNA reappearance preceded radiologic or clinical relapse, supporting its role as an early marker of molecular recurrence (Figure 5A; Figure S5). In the PR/PD group ($n = 13$), ctDNA generally remained detectable throughout treatment, indicating resistance to therapy, though one patient showed transient clearance followed by reappearance (Figure 5A; Figure S5). Across all evaluable patients, clearance of baseline SNVs at interim assessment was significantly associated with improved treatment response ($p < 0.05$), underscoring the prognostic value of early ctDNA dynamics.

In the context of ASCT, among eight CR patients who received ASCT, five had detectable SNVs at baseline and evaluable interim samples; four of five patients cleared ctDNA prior to ASCT (Figure 5B). In contrast, among four CR patients who later relapsed, three cases with interim samples remained ctDNA-positive (Figure 5B).

Monitoring of MNVs in patients with detectable baseline events ($n = 21$) revealed dynamics that closely mirrored SNV results in 17 cases (Figure S6). At the interim time point, three patients showed no detectable SNVs, whereas MNV tracking still revealed positivity. In contrast, one patient had detectable SNVs, but no MNVs at interim. At EOT, results were concordant between SNVs and MNVs in 14 of 15 evaluable cases (Figure S6). Among

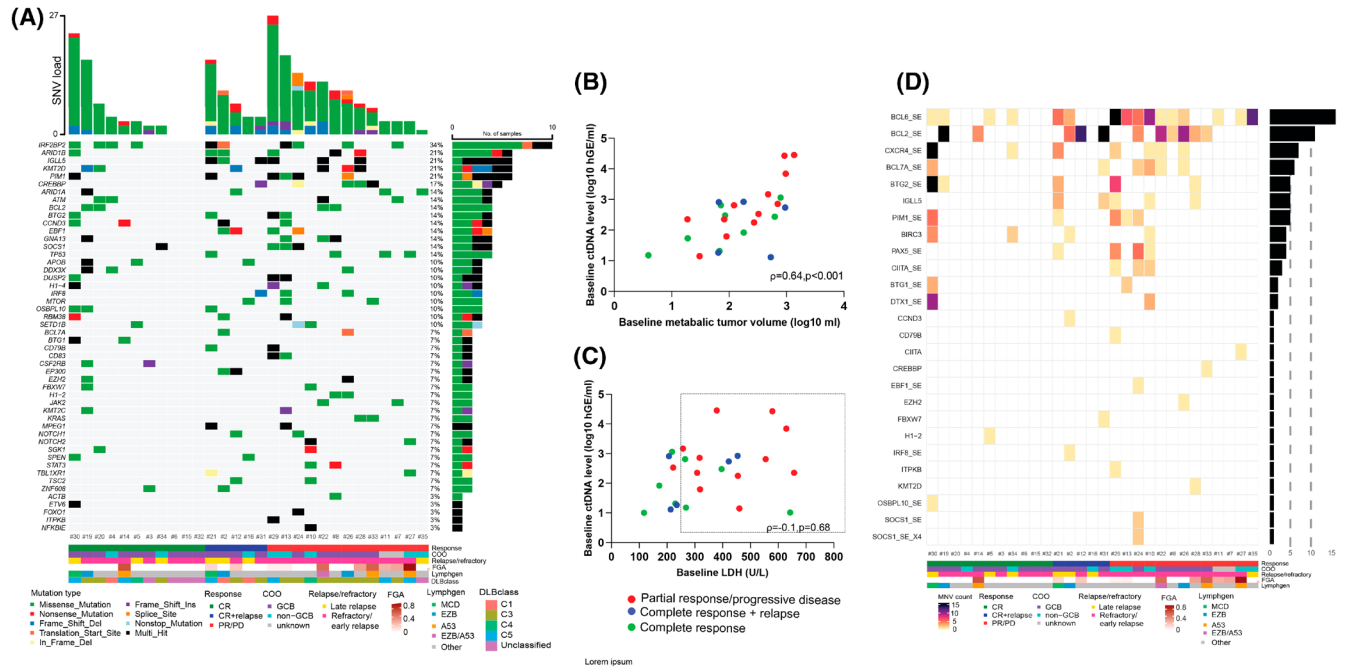

FIGURE 3 Baseline mutational landscape with SNV load in relapsed/refractory (R/R) diffuse large B-cell lymphoma (DLBCL). (A) Oncoplot showing the baseline mutational landscape of 29 R/R DLBCL samples. Non-synonymous variants were detected in 26 (90%) cases. (B) Correlation between ctDNA level ($\log_{10}$ haploid genomic equivalent per millilitre [hGE/mL]) and metabolic tumour volume (MTV). A significant positive correlation ($\rho = 0.64$, $p < 0.001$) was observed for ctDNA concentration and MTV. (C) Patients within the dashed box (elevated LDH) were included in the analysis. No correlation ($r = -0.1$, $p = 0.68$, Spearman coefficient test) was observed between ctDNA level ($\log_{10}$ hGE/mL) and LDH (U/L). (D) Oncoplot showing the baseline MNV of 29 R/R DLBCL samples. MNV were detected in 21 (72%) cases. ctDNA, circulating tumour deoxyribonucleic acid; LDH, lactate dehydrogenase; SNV, single-nucleotide variant.

MNV-based MRD-negative patients at EOT, two of six experienced relapse, compared with two of three patients with a detectable MRD signal, although all nine patients were imaging-negative at EOT.

DISCUSSION

In this study, we aimed to investigate the predictive value of ctDNA in R/R DLBCL patients undergoing salvage chemotherapy and evaluated its potential for disease monitoring. Our findings demonstrate that baseline ctDNA alterations in R/R patients, as well as their dynamics during treatment, predict treatment response and track MRD and second relapse, reinforcing the role of ctDNA as a biomarker in this setting.

RF2BP2 was the most frequently mutated gene in our R/R DLBCL cohort (34%), which was higher than in newly diagnosed cases (~15%).[24] Loss-of-function mutations in *IRF2BP2*, most common in the *MYD88/CD79B* and *NOTCH1* subtypes (20%–40%),[24] may enhance NF-κB (nuclear factor-κB) signalling and contribute to chemotherapy resistance.[25] Based on LymphGen classification, the majority of cases were categorized as other, whereas the most dominant subtype using DLB class was the C3 cluster.

MNVs were most frequently identified in SE hypermutation regions of *BCL6*, *BCL2* and *CXCR4*, consistent with findings in the literature.[26] Hypermutations within these SE regions disrupt the binding of transcriptional repressors, such as BLIMP1 and NR3C1, thereby preventing the downregulation of their target genes, including *BCL6*, *BCL2* and CXCR4.[26]

We detected CNAs at baseline in half of the R/R cases by low-coverage whole genome sequencing (lcWGS), with higher FGA and ETF being associated with worse outcomes. Targeted sequencing revealed SNVs in almost all cases, indicating a higher sensitivity for detection of ctDNA than lcWGS. The prognostic value of SNV load in DLBCL has been inconsistent across studies,[27] whereas increased large-scale CNAs were more consistently correlated with poor prognosis in the literature.[16,28] MTV is a known prognostic marker in DLBCL, with the MTV-age-stage model outperforming international prognostic index (IPI) in predicting progression-free and overall survival.[29] Moreover, combining ctDNA levels with cell-of-origin and IPI further improved prognostic accuracy.[30] In our cohort, the concurrent presence of high MTV and detectable CNA at baseline was associated with poorer outcomes (8/10 failures), whereas the combination of low MTV and undetectable CNA predicted better responses (7/9 CR). Notably, all nine patients with both detectable CNA and elevated LDH at baseline experienced treatment failure. These findings highlight the potential utility of integrating metabolic and molecular tumour burden assessment for risk stratification, though validation in larger cohorts is warranted.

Results of the ctDNA dynamics during salvage chemotherapy further highlight its value in detecting disease activity. Patients who achieved ctDNA clearance at interim exhibited

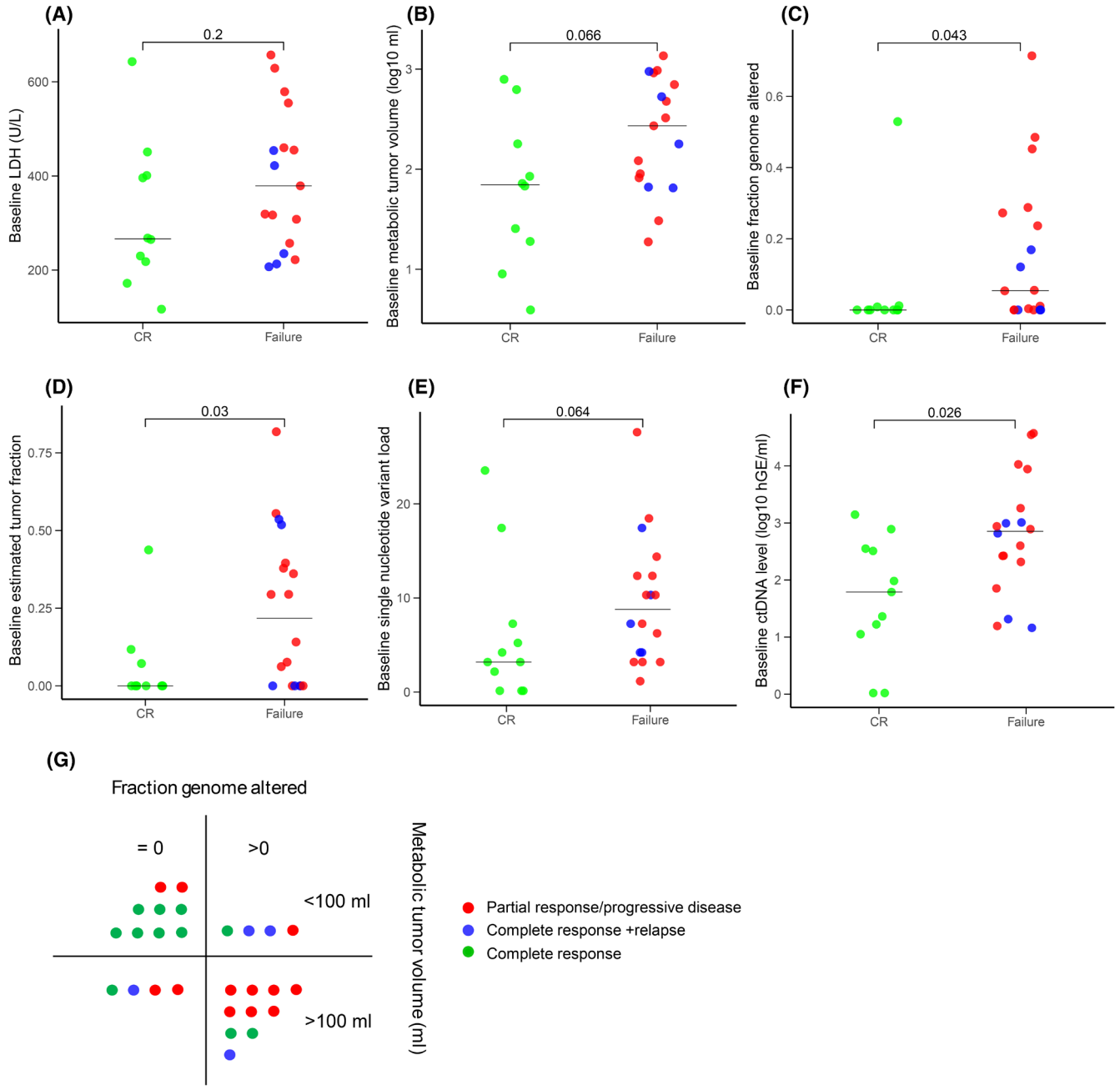

FIGURE 4 Baseline ctDNA and MTV with treatment outcomes in relapsed/refractory (R/R) diffuse large B-cell lymphoma (DLBCL). (A) Baseline LDH was not different between CR and failure group ($p = 0.2$). (B) Baseline MTV was not statistically higher in patients with treatment failure ($p = 0.066$). (C) Fraction of genome altered (FGA) was significantly higher in patients with treatment failure compared to CR group ($p = 0.04$). (D) Estimated tumour fraction (ETF) was elevated in the failure group compared with CR patients ($p = 0.03$). (E) Baseline non-synonymous single nucleotide variant (SNV) load was higher in the failure group than in the CR group ($p = 0.064$). (F) SNV-based ctDNA concentration was significantly higher in the failure group ($p = 0.026$). (G) Scatter plot showing the relationship between FGA and MTV. Patients with partial response/progressive disease (red) exhibit higher FGA and MTV, whereas those achieving CR (green) have lower values. Patients with CR followed by relapse (blue) show intermediate values. The dotted lines indicate the median value of MTV. CR, complete response; ctDNA, circulating tumour deoxyribonucleic acid; LDH, lactate dehydrogenase; MTV, metabolic tumour volume.

better clinical outcomes, whereas persistent ctDNA positivity was associated with treatment failure. These findings align with data from CAR-T therapy, where early ctDNA clearance correlates with long-term remission.[15,16] Additionally, the presence of ctDNA identified MRD in patients where PET/CT suggested a complete remission. This underscores the potential of ctDNA as a tool for MRD detection.

Considering that CAR-T therapy has become a second-line treatment option for R/R DLBCL, particularly for patients with refractory disease or early relapse, salvage chemotherapy remains prevalent in patients experiencing late relapse or in regions where access to CAR-T is limited.[31] Our data indicate that patients with detectable CNAs or high SNV load or high ctDNA level at the R/R baseline are more likely to exhibit resistance to

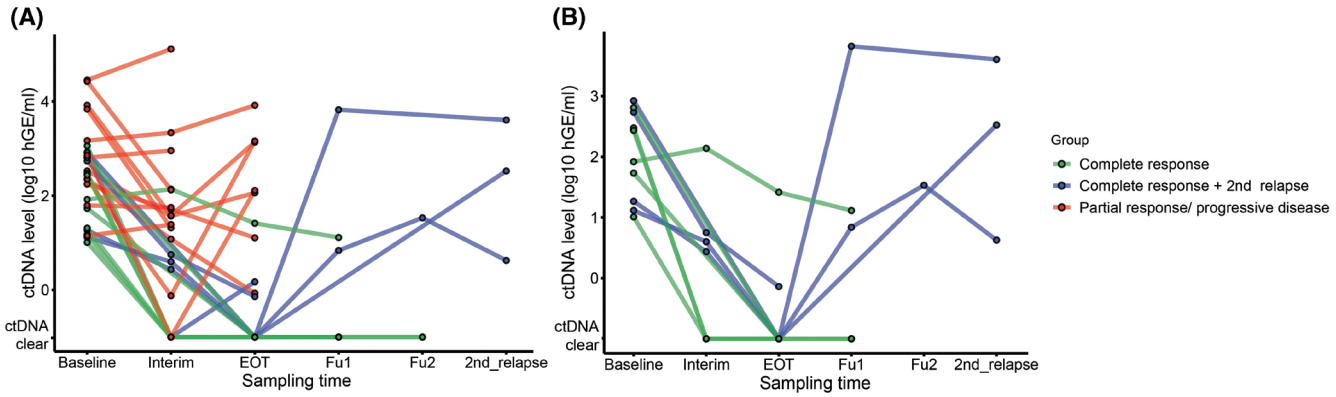

FIGURE 5 Dynamics of changes in ctDNA concentrations during salvage chemotherapy in relapsed/refractory (R/R) diffuse large B-cell lymphoma (DLBCL) patients. Longitudinal ctDNA levels (hGE/mL) across different response groups: (A) Patients with CR ($n = 9$, green): ctDNA levels declined significantly during treatment and remained undetectable at follow-up; patients with CR but presenting with a second relapse group ($n = 5$, blue): ctDNA decreased initially but re-emerged at second relapse; patients with PR/PD group ($n = 13$, red): ctDNA levels fluctuated but remained detectable, indicating poor treatment response. (B) ctDNA dynamics in patients achieving long-term remission after autologous stem cell transplantation (ASCT) ($n = 6$, green), patients with a second relapse after ASCT ($n = 4$, blue). CR, complete response; ctDNA, circulating tumour deoxyribonucleic acid; hGE/mL, haploid genomic equivalent per millilitre; PD, progressive disease; PR, partial response.

salvage chemotherapy. Furthermore, we observed that a lack of ctDNA clearance after the first three cycles of salvage chemotherapy strongly predicts poor outcome. These findings indicate that patients with higher FGA and MTV at the R/R baseline, or those with detectable ctDNA at interim assessments, should be considered for earlier CAR-T therapy to improve their prognosis. Future studies should explore how ctDNA monitoring might further optimize patient selection and guide treatment adjustments within these novel therapeutic frameworks.

Limitations of our study include the single-institution nature, retrospective outcome analysis and the relatively small cohort size. Larger multicentre cohorts and prospective studies are needed to validate our results. Additionally, while ctDNA dynamics correlated with treatment response, further studies are required to determine whether pre-emptive treatment modifications based on ctDNA levels can indeed improve outcomes. In addition, we did not have matched germline DNA as a control to filter personal variants or variants related to clonal haematopoiesis. Future studies should incorporate such analyses to improve the accuracy of detecting true lymphoma-derived somatic variants. Standardization of ctDNA analysis remains a key challenge for clinical implementation. Current studies vary in methodology, sequencing depth, variant filtering and sampling time points, which complicates cross-study comparison and clinical interpretation. Harmonized frameworks, such as the recently proposed continuous individualized risk index (CIRI) guidelines, may help establish best practices for assay performance, reporting and quality control.[32]

In conclusion, this study highlights the predictive value of ctDNA in R/R DLBCL treated with salvage chemotherapy. Baseline ctDNA levels, in combination with MTV, can help identify patients at high risk of treatment failure, while ctDNA dynamics offer a real-time measure of treatment response. Given the prognostic value of ctDNA in this study, monitoring of R/R DLBCL patients may serve as a valuable tool for guiding clinical decision-making and optimizing treatment selection.

AUTHOR CONTRIBUTIONS

Y.Z. designed the study, performed experiments, analysed results and wrote the paper. J.B. performed experiments and collected the clinical data. F.M.d.J. performed PET/CT evaluation. N.V. and L.S. created the SNV and MNV analysis pipeline and performed analysis. J.K, A.v.d.B., W.P. and A.D. supervised the study and critically reviewed the manuscript. M.N. designed and supervised the study and critically reviewed the manuscript.

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FUNDING INFORMATION

The authors have nothing to report.

CONFLICT OF INTEREST STATEMENT

The authors declare no competing financial interests.

DATA AVAILABILITY STATEMENT

All materials and raw data are available from the authors upon request for non-commercial research purposes. Contact m.nijland@umcg.nl or y.zhong@umcg.nl.

ETHICS APPROVAL STATEMENT

This study was approved by the Medical Ethics Review Board of the University Medical Center Groningen.

PATIENT CONSENT STATEMENT

Written informed consent was obtained from all participants prior to sample collection.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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