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Remission Assessment by Circulating Tumor DNA in Large B-Cell Lymphoma

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ABSTRACT

PURPOSE Large B-cell lymphomas (LBCLs) are curable, but patients with residual disease after therapy invariably experience progression. Ultrasensitive methods to detect circulating tumor DNA (ctDNA) as minimal residual disease (MRD) may improve the determination of remission.

METHODS We integrated data from five prospective studies of frontline anthracycline-based chemotherapy in patients with LBCL. Tumor-specific phased variants were identified from pretreatment samples and monitored at landmark time points. Serial plasma specimens were blindly analyzed for detectable ctDNA as MRD. MRD status was compared with conventional response criteria for prognosis of progression-free survival (PFS).

RESULTS We studied ctDNA-MRD in 137 patients by monitoring 409 plasma specimens over time. Detectable ctDNA rates decreased during therapy with 55% and 78% of patients achieving undetectable ctDNA after two cycles and at the end of therapy, respectively. After a median follow-up of 37 months, the 2-year PFS for patients with detectable versus undetectable ctDNA after two cycles was 67% versus 96% (P = .0025; hazard ratio [HR], 6.9) and after therapy was 29% versus 97% (P < .0001; HR, 28.7), respectively. Ninety-two (94%) patients with undetectable ctDNA at the end of therapy remained alive without progression, while 19 (68%) patients with detectable ctDNA progressed or died. MRD status at the end of therapy had greater prognostic utility than conventional lymphoma response criteria using positron emission tomography (PET) scans (HR, 3.6 for positive PET and 28.3 for detectable ctDNA).

CONCLUSION Ultrasensitive ctDNA detection after frontline LBCL therapy is more prognostic than conventional radiographic response criteria. A refined definition of remission with ctDNA-MRD may improve clinical and psychological outcomes for patients with LBCL.

ACCOMPANYING CONTENT

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Data Supplement

Protocol

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INTRODUCTION

Large B-cell lymphomas (LBCLs) are curable malignancies, but patients with residual disease after therapy will invariably progress. The standard for determining remission relies on [^{18}F]fluorodeoxyglucose (^{18}F-FDG)-positron emission tomography (PET) scans,[1] but imaging tests cannot detect disease at the molecular level, and nearly 20% of patients with negative PET scans at the end of therapy will experience relapse.[2,3] Furthermore, PET avidity is not lymphoma-specific and persistent metabolic activity after therapy often does not represent residual disease, representing a high false-positive rate.[4,5] These limitations of PET scans lead to unnecessary interventions including confirmatory tissue biopsies and repeat PET scans that adversely affect patients and delay salvage treatment.[6,7] To overcome these limitations, the interpretation of PET scans in lymphoma has evolved from a qualitative visual method to a five-point Deauville score for response assessment standardization.[1,8-10] Yet, the positive predictive value of PET scans with standardization at the end of therapy is only approximately 50%.[11-13] Guidelines caution against treatment based solely on residual metabolic activity.[1]

Although LBCLs lack circulating tumor cells making flow cytometry–based minimal residual disease (MRD) noninformative, analysis of circulating tumor DNA (ctDNA) can detect MRD in patients without clinically or radiographically

CONTEXT

Key Objective

Can ultrasensitive methods to detect circulating tumor DNA (ctDNA) as minimal residual disease (MRD) improve remission determination after frontline therapy for large B-cell lymphoma (LBCL)?

Knowledge Generated

One hundred and thirty-seven patients with untreated LBCL had plasma samples serially collected during therapy and analyzed for ctDNA. The detection of ctDNA after two cycles and after therapy was highly prognostic. At the end of therapy, ctDNA was more prognostic than conventional lymphoma response criteria using positron emission tomography scans.

Relevance *(C. Craddock)*

Detection of ctDNA is an important and widely applicable new prognostic biomarker in patients with newly diagnosed LBCL. Future prospective studies will be important to establish the value of ctDNA measurement in guiding therapeutic strategies—both treatment intensification and de-escalation.*

*Relevance section written by *JCO* Associate Editor Charles Craddock, MD.

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evident disease during and after therapy.[14] Early studies of ctDNA in LBCL established the prognostic utility of baseline levels and dynamic changes after one or two cycles of chemotherapy but did not firmly establish ctDNA as a reliable MRD detection method at the end of therapy.[15,16] This affirms the technical challenge of ctDNA detection at low tumor volumes and underscores the need for ultrasensitive detection methods.[17] Newer ctDNA methods address this limitation by analyzing multiple somatic variants present on the same cell-free DNA (cfDNA) molecule, known as phased variants, through Phased Variant Enrichment and Detection by Sequencing (Figs 1A and 1B).[18] Compared with first-generation MRD assays, this approach reduces the background error rate by tracking multiple alterations, thereby allowing for ctDNA detection of 1 in 10^6 cfDNA molecules.[19] We hypothesized that the improved analytical sensitivity of tracking phased variants in ctDNA would enable detection of MRD at end of therapy for LBCL including in patients deemed in remission by conventional lymphoma response criteria.

METHODS

Patients and Treatment

Patients age 18 years and older with previously untreated LBCL were treated on five prospective clinical studies that delivered four to six cycles of anthracycline-based immunochemotherapy with curative intent. Both de novo LBCL and those that transformed from indolent lymphoma without previous therapy were eligible. High-grade B-cell lymphomas (ie, having *MYC* translocation with either *BCL2* and/or *BCL6* translocations) and primary mediastinal B-cell lymphoma (PMBL) were eligible. Patients with a history of documented previous indolent lymphomas had no previous systemic chemotherapy for their low-grade disease. Four trials incorporated novel agents including acalabrutinib, lenalidomide, obinutuzumab, polatuzumab, and/or tafasitamab. Two trials remain ongoing, while three have published results.[20-22] All studies were registered at ClinicalTrials.gov (NCT04002947; NCT00398177; NCT02529852; NCT04231877; NCT04134936). Each study was approved by local institutional review boards with patients providing informed consent, and each study was conducted in accordance with the principles of the Declaration of Helsinki.

This integrated analysis was conducted under a secondary research use protocol receiving an exempt research determination from a central institutional review board (Data Supplement, online only). The primary objective was to assess the prognostic utility of MRD at the end of frontline therapy, with secondary objectives focused on prognostic value at other treatment milestones during therapy. Included patients had samples from both pretreatment and a follow-up time point (cycle 2, day 1; cycle 3, day 1; and/or end of therapy). Patients who remained alive without progression were included if they had at least 12 months of follow-up beyond the end of therapy. Additional details are provided in the Data Supplement.

Specimen Collection and Analysis of Circulating Tumor DNA

Plasma samples were collected at baseline, interim time points, and at the end of therapy. Pretreatment tumor or plasma specimens along with a source of germline DNA were used to identify somatic phased variants, and cfDNA was profiled centrally at Foresight Diagnostics Clinical Laboratory Improvement Amendments–certified laboratory (Aurora, CO) or Stanford University (Stanford, CA) blinded to clinical outcomes. MRD was considered detectable when ctDNA signal exceeded a predetermined analytical detection threshold, with an analytical limit of detection of 0.7 parts

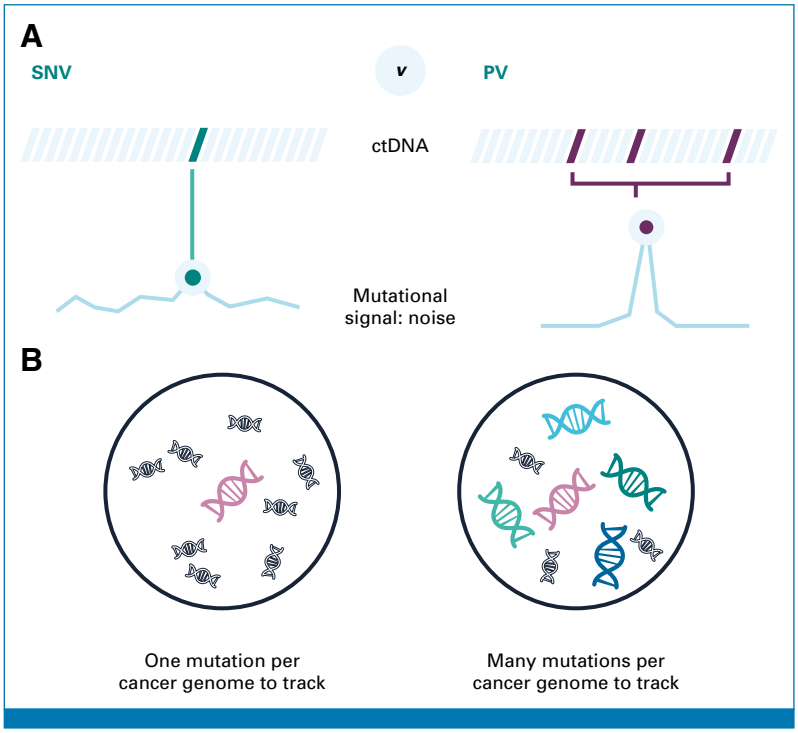

FIG 1. PhasED-Seq. (A) The figure depicts the key features of phased variants. As opposed to single-nucleotide variants, phased variants are multiple alterations that can be observed together on a single DNA molecule. The concordant observation of multiple alterations simultaneously significantly lowers the background error profile for MRD detection. (B) PhasED-Seq uses phased variants to track many phased variants simultaneously. Since the amount of cell-free DNA in plasma is limited, this is essential to enable detection to the parts per million range from a standard blood plasma collection. ctDNA, circulating tumor DNA; MRD, minimal residual disease; PhasED-Seq, Phased Variant Enrichment and Detection by Sequencing; PV, phased variants; SNV, single-nucleotide variants.

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per million and a corresponding false-positive rate of <1% for samples tested at Foresight Diagnostics.[18,19]

PET-CT Scan Interpretation

Diagnostic ^{18}FDG PET-computed tomography (CT) scans were performed after completion of therapy and interpreted by nuclear medicine radiologists per local institutional standards, with Deauville score of 4 or 5 considered positive for residual disease, and scores 1, 2, or 3 considered negative. Progression was defined by either biopsy-proven disease or persistent positivity on repeat PET-CT imaging on the basis of institutional standards.

Statistical Analysis

Progression-free survival (PFS), freedom from progression (FFP), and overall survival were calculated from treatment initiation until event or last follow-up. Patients who died from nonlymphoma causes were censored for FFP. Time-to-event variables were visualized using the Kaplan-Meier method, implemented with the "*survival*" package in R. Cox proportional hazards regression was used to assess the prognostic impact of risk factors. In a sensitivity analysis, samples were classified as positive if the measured allelic fraction exceeded the thresholds of 10^{-4} or 10^{-6} mutant molecules. Additional details are provided in the Data Supplement.

RESULTS

Patient Characteristics and Plasma Samples

Among 163 patients, 137 (84%) had available samples and met inclusion criteria (Fig 2). Baseline patient and disease characteristics are summarized in Table 1, including female sex in 64 (47%), median age of 62 (range, 21-85) years, stage III or IV disease in 104 (76%), and a high-intermediate or high International Prognostic Index (IPI) score in 66 (48%). LBCL subtypes included 111 (81%) diffuse LBCL, 19 (14%) high-grade B-cell lymphoma, and 7 (5%) PMBL. Of all 137 patients, 61 (45%) were genotyped from pretreatment tumor and 76 (55%) genotyped from pretreatment plasma (Data Supplement, Table S1). A total of 409 plasma samples were

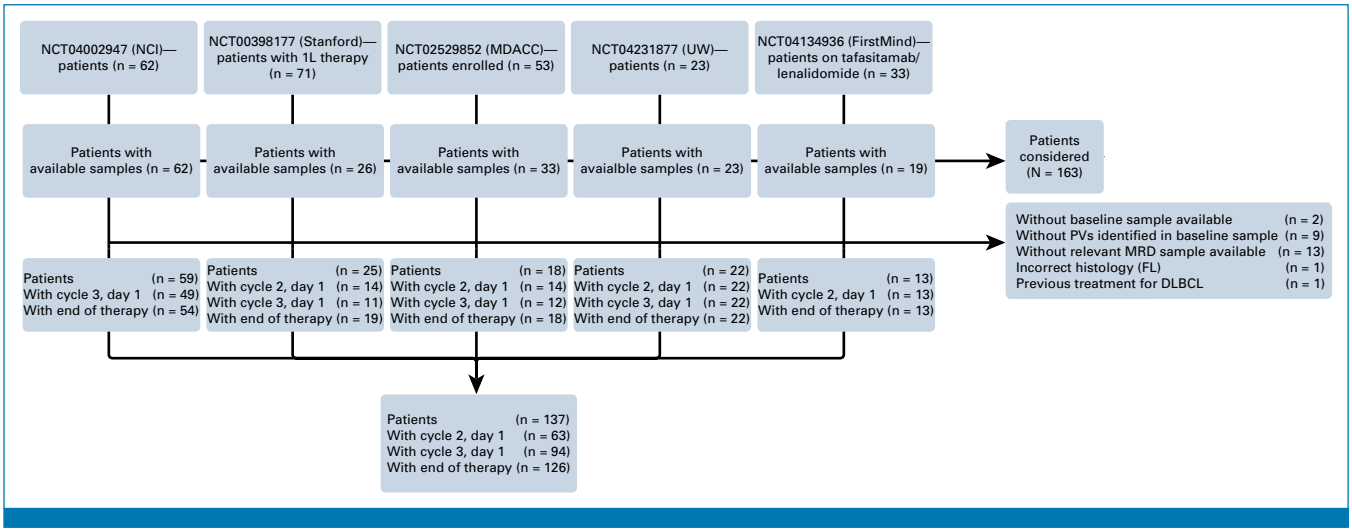

FIG 2. Flow diagram for pooled cohort inclusion. The diagram summarizes the enrollment and sample availability across five prospective clinical trials included in the pooled ctDNA-MRD analysis. A total of 163 patients were initially considered. Patients were excluded because of absence of a baseline sample (n = 2), failure to identify phased variants (n = 9), lack of post-treatment plasma samples (n = 13), incorrect histologic diagnosis (n = 1), or previous systemic therapy (n = 1), resulting in a final evaluable cohort of 137 patients. Sample availability at MRD landmark time points (cycle 2 day 1, cycle 3 day 1, and end of therapy) is shown by trial. ctDNA-MRD, circulating tumor DNA-minimal residual disease; FL, follicular lymphoma; MDACC, University of Texas MD Anderson Cancer Center; MRD, minimal residual disease; NCI, National Cancer Institute; PV, phased variants; UW, University of Washington.

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tracked at landmark time points including baseline in 126 (92%) patients, after cycle 1 in 63 (46%), after cycle 2 in 94 (69%), and at the end of therapy in 126 (92%). Among 22 patients who met progression criteria, 13 (59%) had biopsy-confirmed disease and nine (41%) progression events were determined by unequivocal changes on repeat imaging.

Prognostic Utility of Phased Variants as MRD

cfDNA was profiled in 94 (69%) patients at Foresight Diagnostics and 43 (31%) patients at Stanford. Multivariable analysis showed that neither the trial cohort nor the testing site affected the prognostic utility of MRD (hazard ratio [HR], 25.7 and 36.4 at Foresight Diagnostics and Stanford, respectively; Data Supplement, Figs S1 and S2).

We first explored the prognostic utility of MRD at landmark time points throughout therapy. As expected, ctDNA levels declined rapidly, and the frequency of undetectable MRD increased throughout therapy (Fig 3A). After one cycle of therapy, 25% of patients had undetectable MRD, increasing to 55% after two cycles, and 78% at the end of therapy (Fig 3B). The achievement of undetectable MRD was prognostic after two cycles and at the end of therapy (Data Supplement, Fig S3A). Although undetectable MRD after one cycle of therapy was not prognostic as a binary variable (P = .46; HR, 1.6), patients with undetectable MRD after two cycles had a 2-year PFS of 96% compared with 67% for patients with detectable MRD (P = .0025; HR, 6.9; Data Supplement, Fig S3B). MRD status at end of therapy was the best prognostic landmark for MRD assessment, with undetectable MRD heralding a 2-year PFS of 97%, as contrasted to 29% for those with detectable MRD (Fig 4A, Data Supplement, Fig S3B; P < .001; HR, 28.7). After a median follow-up of 36.6 (95% CI, 33.8 to 40.5) months, 92 (94%) patients with undetectable MRD at the end of therapy remained alive without disease.

Overall, 25 patients experienced progression or death after therapy. Six (24%) of these patients had undetectable MRD at the end of therapy, of whom only three developed recurrent lymphoma, while the other three patients died without evidence of lymphoma. Of the three lymphoma recurrences in patients with undetectable MRD, one patient had an isolated lymphoma relapse in the CNS 8 months after therapy. Another patient had a biopsy-proven lymphoma 10 years after initial therapy; in this case, the cell-of-origin was discordant between initial and subsequent diagnoses, suggesting a second primary lymphoma but the relapse tumor was not available to resolve the clonal relationship.[23] The third patient experienced biopsy-proven disease relapse 4 months after therapy. The remaining 19 (76%) patients who experienced progression or death after therapy had detectable MRD at the end of therapy. Of these, 18 (95%) patients experienced relapse or death at a median of 2.7 months (range, 0-17.5) after therapy, and one (5%) patient died in remission 20 months after therapy.

Next, we explored the impact of various alternative analytical thresholds on MRD detection at the end of therapy as part of a sensitivity analysis. Using an analytic threshold of 1:10^4 cfDNA molecules, 10 (40%) patients who experienced

TABLE 1. Patient Characteristics

Patient Characteristic	N = 137, n/N (%)
Female	64/137 (47)
Age, years, median (min-max)	62 (21-85)
Histology	
DLBCL	111/137 (81)
HGBCL	19/137 (14)
PMBCL	7/137 (5.1)
Stage	
I/II	33/137 (24)
III/IV	104/137 (76)
IPI	
0-1	35/137 (26)
2	36/137 (26)
3	46/137 (34)
4-5	20/137 (15)
Cell of origin	
GCB	75/119 (63)
Non-GCB	44/119 (37)
Genotyping sample	
cfDNA	76/137 (55)
Tumor	61/137 (45)
C2D1	63/137 (46)
C3D1	94/137 (69)
EOT	126/137 (92)

Abbreviations: C2D1, cycle 2 day 1; C3D1, cycle 3 day 1; cfDNA, cell-free DNA; DLBCL, diffuse large B-cell lymphoma; EOT, end of treatment; GCB, germinal center B cell-like; HGBCL, high-grade B-cell lymphoma; IPI, International Prognostic Index; PMBL, primary mediastinal B-cell lymphoma.

progression or death after therapy had detectable MRD at the end of therapy, whereas applying an analytic threshold of 1:10^6 cfDNA molecules, 19 (76%) patients who later experienced progression or death had detectable MRD at the end of therapy. When evaluating 2-year PFS, sensitivity increased from 45% for the 10^{-4} threshold to 86% for the 10^{-6} threshold (Fig 4C). The negative predictive value (NPV) improved from 89% for the 10^{-4} threshold to 97% for the 10^{-6} threshold. Additional diagnostic metrics, including C-index and diagnostic accuracy, also favored the 10^{-6} threshold (Data Supplement, Fig S4). Ultrasensitive analytical thresholds translated to improved outcome prognostication at the end of therapy (HR, 28.7 *v* 15.2; $P < .0001$; Data Supplement, Fig S3C).

Prognostic Utility of MRD With Discordant PET Scans

We next evaluated the prognostic significance of MRD along with PET scans. Among 125 cases, patients with a negative PET scan at the end of therapy had a 2-year PFS of 88% (95% CI, 82% to 95%) compared with 61% (95% CI, 46% to 82%) for patients with a positive PET scan ($P = .0017$; HR, 3.6; Fig 5A). Notably, MRD status independently risk-stratified patients, regardless of PET scan results. Among 96 patients with a negative PET scan at the end of therapy, 13 (13.5%) had detectable MRD. The 2-year PFS for these patients was 31% (95% CI, 13% to 65%) compared with 98% (95% CI, 96% to 100%) for patients with undetectable MRD and a negative PET scan ($P < .001$; HR, 31.4; Fig 5C). MRD status at the end of therapy also risk-stratified patients with a positive PET scan; of 29 (23%) patients with a positive PET scan at the end of therapy, only 15 (52%) had detectable MRD. For patients with a positive PET scan and detectable MRD, the 2-year PFS was 30% (95% CI, 13% to 67%) compared with 93% (95% CI, 80% to 100%) for patients with a positive PET scan and undetectable MRD ($P = .009$; HR, 15.6; Fig 5D). On multivariable analysis of MRD status, PET response, IPI, cell-of-origin, and histologic type of LBCL, only MRD status was prognostic (Fig 5E, Data Supplement, Fig S5). Considered separately, MRD remained prognostic for both diffuse large

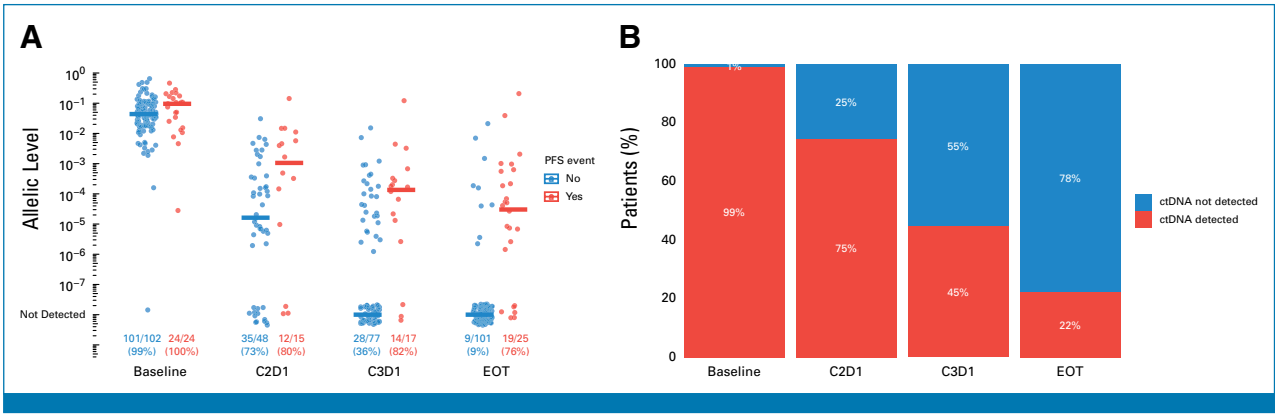

FIG 3. ctDNA kinetics during treatment. (A) Dot plot illustrating variant allelic levels across treatment courses, stratified by PFS status during the follow-up period—red indicates patients who experienced a PFS event, and blue represents those who remained event-free. Variant allelic level is expressed as the fraction of molecules harboring lymphoma-specific phased variants among all informative molecules analyzed. Horizontal lines indicate median values within each group. Patients with undetectable ctDNA are annotated, and the proportion of patients with undetectable ctDNA is displayed at the bottom of the plot. (B) Bar graph depicting the percentage of patients with undetectable ctDNA at each profiled time point during treatment. C2D1, cycle 2 day 1; C3D1, cycle 3 day 1; ctDNA, circulating tumor DNA; EOT, end of treatment; PFS, progression-free survival.

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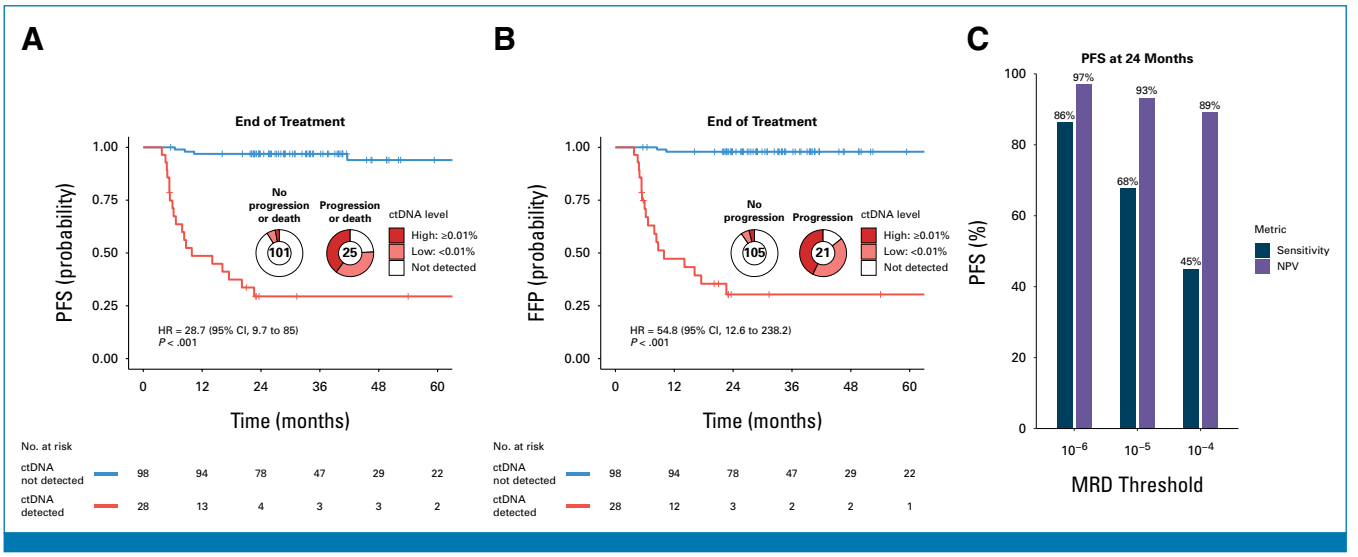

FIG 4. Stratification and performance metrics of end of therapy ctDNA profiling. (A) Kaplan-Meier curve for PFS stratified by ctDNA detection status at the end of therapy. HR and *P* value from Cox proportional hazards regression are shown. Donut plots display the proportion of patients with detectable ctDNA by PFS status, distinguishing cases with relatively high ctDNA burden (≥0.01%, ie, ≥10^{-4}) in dark red from cases with lower ctDNA levels (<0.01%, ie, <10^{-4}) in light red. (B) Kaplan-Meier curve for FFP stratified by ctDNA detection status at the EOT. HR and *P* value from Cox proportional hazards regression are shown. Donut plots display the proportion of patients with detectable ctDNA by FFP status, distinguishing cases with relatively high ctDNA burden (≥0.01%, ie, ≥10^{-4}) in dark red from cases with lower ctDNA levels (<0.01%, ie, <10^{-4}) in light red. (C) Sensitivity (blue) and NPV (purple) of end-of-treatment ctDNA profiling for predicting 24-month PFS, evaluated across analytical thresholds ranging from 10^{-6} to 10^{-4}. ctDNA, circulating tumor DNA; EOT, end of treatment; FFP, freedom from progression; HR, hazard ratio; MRD, minimal residual disease; NPV, negative predictive value; PFS, progression-free survival.

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B-cell lymphoma, not otherwise specified, and high-grade histologies, demonstrating the utility of this approach across LBCLs (Data Supplement, Figs S5A and S5B).

When considering MRD status, PET scans did not independently risk-stratify outcomes. Among 97 patients with undetectable MRD, 14 (14%) had a positive PET scan at the end of therapy. No significant difference was observed in the 2-year PFS for these patients (93% [95% CI, 80% to 100%]) when compared with those with a negative PET scan (99% [95% CI, 96% to 100%]; $P = .83$; HR, 1.3; Data Supplement, Fig S5C). Conversely, among 28 patients with detectable MRD at the end of therapy, 13 (46%) had a negative PET scan. Nevertheless, the 2-year PFS was similar for those with detectable MRD and negative PET scans (29% [95% CI, 13% to 65%]) compared with those with positive PET scans (30% [95% CI, 13% to 67%]; $P = .38$; HR, 1.5; Data Supplement, Fig S5D).

Clinical Outcomes After Detectable MRD at the End of Therapy

To better understand the clinical implications of residual ctDNA after therapy, we performed an unblinded analysis of all 28 patients with detectable ctDNA at the end of therapy. After a median follow-up of 23.7 months, 19 (68%) of these patients experienced progression or death. However, nine (32%) did not recur during the follow-up period (Data Supplement, Fig S6). Two (22%) of these patients had very high residual ctDNA concentrations at the end of therapy and received radiation therapy to PET-avid lesions (Data Supplement, Fig S6C). The ctDNA concentration in the remaining seven patients ranged from 3.6×10^{-6} to 8.8×10^{-4}, which was similar to patients with detectable MRD who experienced progression or death ($P = .28$). Notably, additional surveillance samples beyond the end of therapy were available from three patients, which demonstrated later clearance of ctDNA on serial testing (Data Supplement, Fig S7).

DISCUSSION

These results show that MRD using ultrasensitive ctDNA detection after frontline therapy for LBCL risk-stratifies patients for treatment failure. By tracking phased variants in cfDNA, we observed that MRD assessment at the end of therapy anticipated the majority of future lymphoma events. Detectable ctDNA identified patients at high risk for relapse regardless of PET scan results, and foreshadowed recurrences up to 18 months later while nearly all patients with undetectable ctDNA maintained remission.

Our results illustrate the critical importance of ultrasensitive analytic thresholds targeting parts per million for MRD detection in LBCL, as a reduced analytical sensitivity of 1 in 10^4 inaccurately classified nearly half of future progression

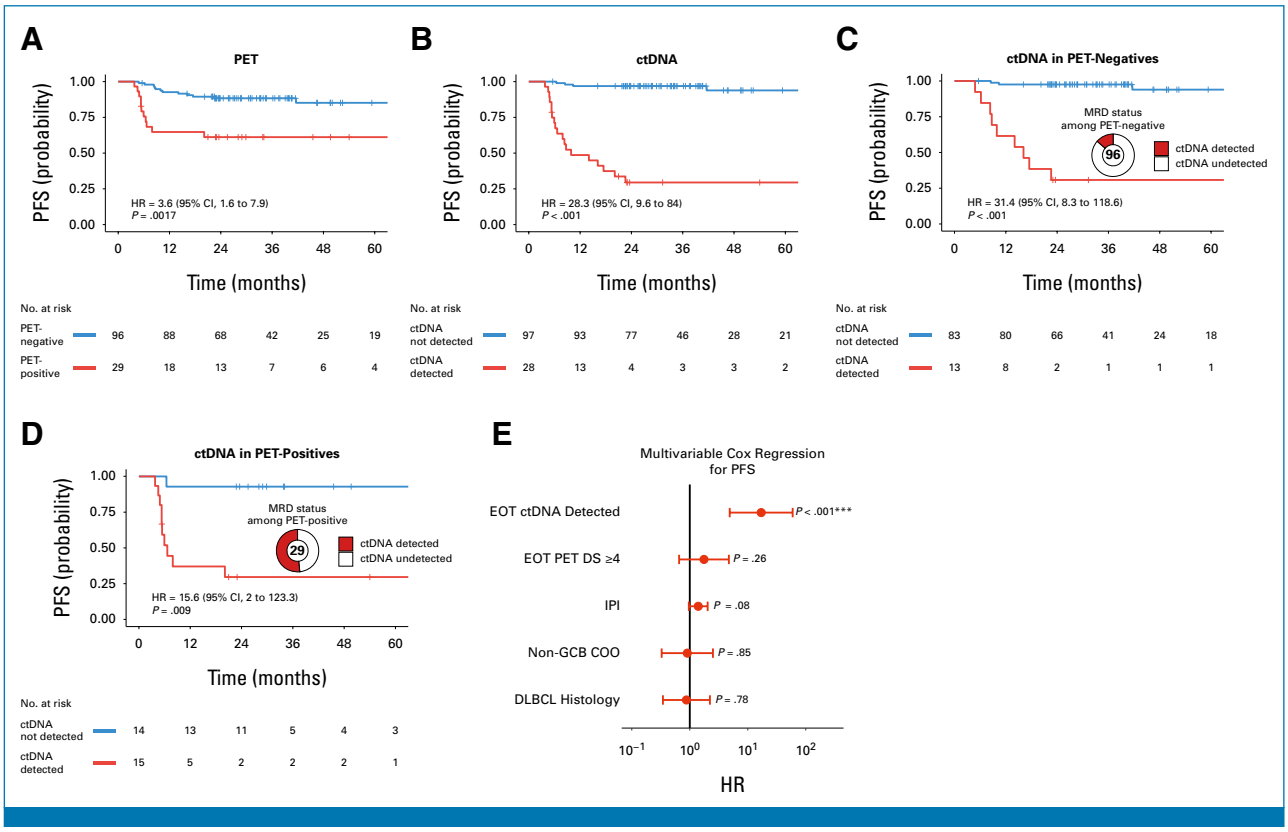

FIG 5. Prognostic value of EOT ctDNA detection compared with PET-CT and clinical risk factors. (A) Kaplan-Meier curve for PFS stratified by EOT PET-CT status in patients eligible for analysis of both modalities. HRs and *P* values from Cox proportional hazards regression are displayed. (B) Kaplan-Meier curve for PFS stratified by EOT ctDNA detection status in the same patient cohort. HRs and *P* values from Cox proportional hazards regression are shown. (C) Kaplan-Meier curve for PFS stratified by EOT ctDNA detection status within the PET-negative subset. HR and *P* value from Cox proportional hazards regression are shown. The donut plot illustrates the proportion of patients with detectable ctDNA within the PET-negative subset. (D) Kaplan-Meier curve for PFS stratified by EOT ctDNA detection status within the PET-positive subset. HR and *P* value from Cox proportional hazards regression are shown. The donut plot illustrates the proportion of patients with detectable ctDNA within the PET-positive subset. (E) Forest plot showing HRs, 95% CIs, and *P* values derived from multivariable Cox proportional hazards regression for PFS, incorporating EOT ctDNA detection, EOT PET-CT, IPI, COO, and histology subtype (ie, DLBCL *v* PMBL or HGBL) as covariates. COO, cell-of-origin; ctDNA, circulating tumor DNA; CT, computed tomography; DLBCL, diffuse Large B-cell lymphoma; EOT, end of treatment; GCB, germinal center B cell-like; HGBL, high-grade B-cell lymphoma; HR, hazard ratio; IPI, International Prognostic Index; PET, positron emission tomography; PFS, progression-free survival; PMBL, primary mediastinal B-cell lymphoma.

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events. Other ctDNA detection methods that do not have analytical limits of detection in the parts-per-million range, or lower, are less likely to predict future relapse when tested at the end of therapy because levels of residual ctDNA are very low. This is further substantiated by the improved sensitivity, positive predictive value, NPV, and C-index, with a threshold of 1 in 10^6.

We also show that MRD status at the end of therapy improved upon the prognostic value of PET scans, the cornerstone of current response criteria. Residual metabolic activity often does not represent active disease, so guidelines urge clinicians to adjudicate positive results with tissue biopsies before salvage therapy. This practice, which is necessary to prevent overtreatment, adds procedural risks and health care costs that could be avoided with precise tools. An accurate MRD test can inform clinical decision making in patients with both positive and negative PET scans. For patients with a positive PET scan, detectable ctDNA would confirm active lymphoma and avoid invasive tissue biopsies. A discordant result of undetectable ctDNA would suggest a false-positive PET scan and reduce unnecessary treatment-related toxicity, psychological hardship, and health care costs.[24,25] Indeed, the National Comprehensive Cancer Network guidelines include ultrasensitive ctDNA as an alternative to tissue biopsies to adjudicate a positive PET scan after therapy.[26]

In patients with a negative PET scan, undetectable ctDNA-MRD would confirm a very low likelihood of relapse and obviate the need for surveillance imaging and its antecedent risks of radiation.[27-30] Our data suggest that tracking MRD

with ultrasensitive ctDNA methods enhances the definition of remission, and the goal of curative-intent therapy for LBCL should be achievement of undetectable MRD.

Our results indicate that detectable ctDNA at the end of therapy, even in the absence of a concordant PET result, conveys a high risk of relapse. Consolidation in response to a detectable MRD result has precedent in acute lymphoblastic leukemias but the clinical utility of this approach has not been demonstrated in LBCL, and well-designed clinical trials will be required to define the overall risk versus benefit of early intervention before clinical relapse. This question is being tested in a randomized, pivotal trial in LBCL (ALPHA3; ClinicalTrials.gov identifier: NCT06500273), wherein consolidation with allogeneic CAR T cells is being compared with observation in patients with LBCL and detectable MRD at the end of therapy.

Conversely, as a demonstration of a de-escalation approach, given the high NPV of the assay, abbreviation of therapy after early achievement of undetectable MRD is being studied (ClinicalTrials.gov identifier: NCT06693830). Indeed, the consistency between mid-treatment ctDNA-MRD clearance and end-of-treatment results reinforces the clinical relevance of early response. Among 56 patients who achieved undetectable ctDNA after one or two cycles of therapy and had an end-of-therapy sample, 98% (55/56) maintained undetectable ctDNA after therapy (Data Supplement, Fig S6), suggesting early clearance is often durable and may serve as a surrogate of treatment efficacy.

Our study has several limitations. Although detectable MRD at the end of therapy conveyed a high risk of relapse, this was not universal, raising questions as to the cause of this finding. The exploratory nature of our study may have influenced the observed results. For example, although most recurrences occur within 2 years, the duration of clinical follow-up was relatively short in relation to long-term outcomes, potentially missing late relapses.[31] Additionally, since our study included patients with transformed lymphomas, an unrecognized indolent counterpart may cause relapses to be delayed. By contrast, nascent immune mechanisms that could potentiate delayed clearance of ctDNA might provide an alternative explanation. These possibilities warrant further study, although our interpretation here is hampered by a limited availability of serial samples. Nonetheless, we described examples of late clearance of ctDNA without additional intervention and surveillance ctDNA monitoring should be the focus of future trials.

A small proportion of patients (5.5%) could not be evaluated because of insufficient phased variants for MRD tracking. This limitation was observed primarily in cases where plasma genotyping was applied and in cases with low disease burden highlighting the utility of a tissue-informed approach in some cases. The optionality to use either a plasma or tissue sample as the source of PV identification will allow further flexibility and applicability in the clinic. Our study did include patients receiving novel therapies built on the backbone of standard chemoimmunotherapy. We consider inclusion of a variety of therapies is a strength, since the utility of ctDNA will be most impactful if it is broadly applicable across treatment approaches, especially given the evolving therapeutic landscape.

Finally, practical barriers to widespread use of MRD in LBCL remain. These include the need for rapid turnaround times, widely available testing, and standardization of collection. To validate and expand upon our findings, clinical trials in LBCL should collect plasma at baseline, during therapy, and at the end of therapy since MRD may ultimately prove to be a useful surrogate end point for accelerated analyses of long-term outcomes in LBCL and other lymphoma subtypes, as has been established in other hematologic malignancies.[32-34]

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DISCLAIMER

The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

EQUAL CONTRIBUTION

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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Deidentified clinical data will be provided to the Protocol Registration and Results System of ClinicalTrials.gov within 1 year of publication. Information on data sharing may be obtained from ClinicalTrials.gov website.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Remission Assessment by Circulating Tumor DNA in Large B-Cell Lymphoma

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