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Post-treatment circulating tumor DNA in large B-cell lymphoma with an immunoglobulin-based assay: real-world outcomes

*Patrick Gould,[1,2] *Devyn Coskey,[1] Xin Ma,[1] Shikun Wang,[1] Sonia Godbole,[3] David Bond,[3] Andrew Ip,[4] Snegha Ananth,[5] Shaunak Varma,[6] Emily Ayers,[6] Rachel Faden,[7] Cassandra Gillespie,[7] Basem William,[7] Ryan Jacobs,[8] Bei Hu,[8] Jake Lattin,[9] David Russler-Germain,[9] Michele Stanchina,[10] Ashley Rose,[10] Michael Randall,[11] Michael Spinner,[11] Vidiya Srikakulapu,[12] Praveen Ramakrishnan Geethakumari,[12] Alan Skarbnik,[13] David W. Chitty,[14] Mazie Tsang,[15] Cassandra Duarte,[16] Ajay Major,[16] Allison M. Bock,[17] Kelsey Baron,[17] Brian Hess,[18] Seda Tolu,[1] Barbara Pro,[1] Supriya Vaidya,[1] Jennifer E. Amengual,[1] Ran Reshef,[1] Hua-Jay J. Cherng[1#]

[1]Columbia University Irving Medical Center, New York, NY; [2]Memorial Sloan Kettering Cancer Center, New York, NY; [3]Ohio State University, Columbus, OH; [4]Hackensack University Medical Center, Hackensack, NJ; [5]University of Nebraska, Omaha, NE; [6]University of Virginia, Charlottesville, VA; [7]OhioHealth, Columbus, OH; [8]Levine Cancer Institute, Atrium Health Wake Forest, Charlotte, NC; [9]Washington University in St. Louis, St. Louis, MO; [10]University of Miami, Miami, FL; [11]University of California San Francisco, San Francisco, CA; [12]University of Texas, Southwestern, Dallas, TX; [13]Novant Health Cancer Institute, Charlotte, NC; [14]Northwell Health, Lake Success, NY; [15]Mayo Clinic Arizona, Phoenix, AZ; [16]University of Colorado, Aurora, CO; [17]University of Utah, Salt Lake City, UT; [18]Medical University of South Carolina, Charleston, SC

*P. G. and D. C contributed equally to this study

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[#]Corresponding author: Hua-Jay J. Cherng, MD

Email: hjc2157@cumc.columbia.edu

Phone #: 212-305-0591

Address: 177 Fort Washington Avenue, 6GN-Rm 435
New York, NY, 10032

Data Sharing Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Authorship Contributions

P.G collected and analyzed data and wrote the manuscript. D.C collected and analyzed data. X.M and S.W analyzed data. S.G, D.B, A.I, S.A, S.V, E.A, R.F, C.G, B.W, R.J, B.Hu, J.L, D.R-G, M.Stanchina, A.R, M.R, M.Spinner, V.S., P.R.G, A.S, D.C, M.T, C.D, A.M, A.M.B, K.B, and B.Hess collected data and cared for patients. S.T., B.P., S.V., J.E.A., and R.R cared for patients. H-J.J.C designed the study, collected and analyzed data, and wrote the manuscript.

Disclosure of Conflicts of Interest

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Abstract

Measurable residual disease (MRD) analysis using circulating tumor DNA (ctDNA) is a non-invasive method of response assessment in large B-cell lymphoma (LBCL), but data supporting its real-world feasibility and performance are needed. We conducted a multicenter, retrospective study of patients with LBCL assessed in real time with a commercially available, immunoglobulin rearrangement-targeted ctDNA-MRD assay (clonoSEQ®). Our primary objective was to assess post-treatment MRD after immunochemotherapy or chimeric antigen receptor T-cell (CAR-T) therapy. Median time from sample collection to MRD result was 9 days. Following frontline treatment (N=102), 12-month progression-free survival (PFS) was 15% versus 85% for detectable versus undetectable MRD (HR 14.5, p<0.001). Detectable MRD was associated with worse PFS for patients with a positive positron emission tomography (PET) scan (HR 4.9, p=0.003). Following CAR-T (N=48), 7 patients with longitudinal testing post-infusion converted from detectable to undetectable MRD by a median 81 days post-infusion without intervention. Detectable MRD by a day 100 landmark after CAR-T was associated with significantly worse 12-month PFS (35% versus 78%, HR 5.0, p<0.001). Detectable MRD was associated with worse PFS for patients with negative PET (HR 4.5, p=0.036). MRD remained associated with PFS after adjustment for PET response, international prognostic index, and histology in immunochemotherapy (p<0.001) and CAR-T settings (p=0.002). Following frontline therapy, MRD demonstrated 64% sensitivity, 96% specificity, 82% positive predictive value, and 90% negative predictive value for progression. Following CAR-T, corresponding values were 63%, 82%, 71%, and 76%, respectively. Real-time ctDNA-MRD assessment is feasible and adds prognostic value after curative-intent LBCL treatment.

Introduction

Large B-cell lymphoma (LBCL) is often curable with frontline immunochemotherapy or chimeric antigen receptor T-cell therapy (CAR-T).[1–6] However, a significant fraction of patients will experience relapsed or refractory disease. Conventional response assessment depends on positron emission tomography/computed tomography (PET/CT) scans,[7] but 20% of patients in an end-of-treatment (EOT) complete metabolic response (CMR) will relapse after frontline therapy, and PET/CT positivity can be non-specific for residual disease.[8–11] Following CAR-T, 40% of patients with a CMR on PET/CT one month after infusion will relapse, whereas up to a third of patients with a partial metabolic response (PMR) will convert to a CMR at 3 months.[12–14] PET/CT assessment remains imperfect and novel tools are needed to better prognosticate patients.

Measurable residual disease (MRD) analysis has emerged as a non-invasive method of response assessment in lymphomas.[15] B-cell malignancies harbor clonal immunoglobulin (Ig) genes due to VDJ recombination and somatic hypermutation. Identifying the enriched “clonotype” from baseline tumor tissue allows for its monitoring from patient samples for MRD detection (clonoSEQ®) by Ig high throughput sequencing (IgHTS) targeting the unique rearrangement. For diffuse LBCL (DLBCL), the commercial assay sequences circulating tumor DNA (ctDNA) isolated from plasma due to greater disease burden compared to the circulating cellular fraction.[16] This is in contrast to the cellular assay approved for other disease indications.[17]

ctDNA detection by IgHTS in LBCL has been evaluated in clinical studies using prospectively collected samples from patients receiving frontline treatment.[16,18–20] Interim undetectable MRD during treatment was prognostic,[18] but in a study of atezolizumab consolidation only a tenth of relapses were predicted by detectable EOT MRD.[20] Post-treatment MRD surveillance demonstrated greater specificity than imaging[16,18] but poor sensitivity.[19] The National

Comprehensive Cancer Network assigns a category 2B recommendation to ctDNA-MRD assessment at EOT with an assay with a detection limit of <1 part per million in cases of equivocal PET/CT,[21] though real-world data are limited supporting the applicability of IgHTS here or establishing its sensitivity for ctDNA at EOT. After CAR-T,[22,23] MRD detection was associated with poor outcomes and risk-stratified patients with positive PET/CT, though specificity varied across post-infusion timepoints and CAR-T product.

IgHTS is not approved by the U.S. Food and Drug Administration for DLBCL or other LBCLs, though it received Medicare coverage and has been commercially available for routine use for real-time MRD assessment since July 2022. As data are limited regarding its performance, and to inform best use-cases, we performed the first multicenter, retrospective study of real-world patients with LBCL profiled with IgHTS. The primary objective of this study was to evaluate the prognostic utility of post-treatment ctDNA-MRD assessment with a real-time commercial IgHTS assay after curative intent treatment for LBCL.

Methods

Patients and MRD assessments

We conducted an Institutional Review Board-approved, multicenter, retrospective study of LBCL patients treated at 17 U.S. centers (Table S1) profiled with the commercial clonoSEQ ctDNA assay. Inclusion criteria included pathologically confirmed LBCL (including DLBCL not otherwise specified [NOS], high grade B-cell lymphoma [HGBCL], primary mediastinal B-cell lymphoma [PMBCL], transformed indolent lymphomas, and post-transplant lymphoproliferative disorder [PTLD], DLBCL subtype).

Genomic DNA extraction for clonality identification (ID) was performed on formalin-fixed paraffin-embedded slides. Patients with failure of clonality ID from tissue or with successful clonality ID but missing MRD assessments were included for descriptive purposes. MRD assessments must have been performed on cell-free DNA (cfDNA) from plasma and been ordered through the Adaptive Biotechnologies diagnostic portal. MRD assessments on genomic cellular DNA or ordered after March 2025 were excluded from analysis. MRD assessments were performed at the treating clinician's discretion. Data cutoff was 10/31/2025.

The primary analysis focused on patients assessed for MRD status after curative intent combination immunochemotherapy or CAR-T (standard-of-care or investigational product). A descriptive cohort of all patients profiled by IgHTS inclusive of any treatments and MRD timing was analyzed for assay-specific metrics. Providers were surveyed on how each MRD result affected clinical decision making (Table S2).

The primary endpoint was progression-free survival (PFS) by post-treatment MRD in the frontline and CAR-T cohorts. For the CAR-T cohort, a day 100 landmark analysis was conducted in which patients were categorized according to their last MRD assessment between days 28 and 100 post-infusion (±10-day window), excluding patients that progressed before day 100. MRD undetectable was defined as no (0) residual clonal sequences detected and MRD detectable as any residual (>0) clonal sequences. PET/CT response was categorized using Lugano 2014 classification[7] by investigator assessment without central review, with a Deauville five-point score (5PS) of 1 to 3 corresponding to CMR (negative) and 4 to 5 corresponding to non-CMR (positive).

Statistical analysis

Patient, disease, and assay characteristics were summarized using descriptive statistics and percentages calculated excluding missing data. Continuous variables were described using the

median, range, and interquartile range. Characteristics were compared between subgroups with Fisher's exact test or Wilcoxon rank-sum tests. PFS was defined as time from EOT (day 1 of the final cycle of frontline treatment) or from day of CAR-T infusion to progression of LBCL or death from any cause without progression. Overall survival (OS) was defined as time from EOT or CAR-T infusion to death from any cause. Time-to-event outcomes were analyzed using the Kaplan-Meier method, and associations with PFS and OS were evaluated using univariable and multivariable Cox proportional hazards regression.

The paired sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of MRD and PET/CT for progression (excluding death without progression) as a dichotomous outcome were calculated, excluding patients with less than 6 months follow-up without progression. Sensitivity and specificity were compared with the pairwise exact McNemar's test. Differences in PPV and NPV were estimated using nonparametric bootstrap resampling of paired observations (2,000 replicates) with two-sided p-values derived from the bootstrap distributions. Statistical analysis was performed using R version 4.5.0 (R Core Team, Vienna, Austria). Additional methodological details are provided in the supplemental data.

Results

Assay characteristics

Our analysis included 354 unique patients; disposition for primary analyses is described in Figure S1. Of the entire cohort, 16 (5%) experienced clonality ID failure, most commonly due to inadequate tissue (7, 44%) or no dominant sequences identified (7, 44%). The median turnaround time from tissue receipt to clonality ID result was 10 days (range 3-41, interquartile range [IQR] 9-13). Clonal Ig loci identified at baseline were most commonly heavy chain (IgH) and kappa light chain (IgK) in 178 (54%) patients followed by IgH only in 67 (20%) patients and IgH, IgK, and

lambda light chain (IgL) in 55 (17%) patients. Clonality ID characteristics are described in Table S3.

There were 551 total IgHTS MRD tracking assessments; the median turnaround time from collection to result was 9 days (range 5-97, IQR 8-17), including instances where samples were collected and stored before clonality ID was completed. Whole blood was collected in Streck cfDNA stabilizing tubes (74%) or EDTA tubes (26%), and the median plasma volume extracted was 1.2 mL (0.5-7.1). A total of 168 (30%) samples were MRD detectable. Detectable clonal loci were most commonly IgH and IgK in 63 (38%) cases followed by IgH only in 48 (29%) and IgK only in 36 (21%); 50 (30%) of MRD-detected samples lost at least one Ig loci type compared to clonality ID. **Table 1** summarizes the MRD assay characteristics. Investigators were surveyed for how each MRD assessment affected clinical management. Responses were provided for 478 unique assessments (87%), and 116/478 (24%) of assessments were incorporated into clinical decision making (Tables S4, S5).

Frontline cohort characteristics

There were 102 patients treated with frontline therapy with EOT MRD testing. Most (N=66, 65%) were older than 60, 82 (84%) had stage III/IV disease, 65 (68%) had elevated lactate dehydrogenase, 18 (18%) had ECOG performance status of ≥2, and 31 (30%) had ≥2 extranodal sites. The international prognostic index (IPI) was high (4-5) in 21 (22%) of patients. Histology was DLBCL NOS in 69 (68%), HGBCL in 11 (11%), transformed follicular lymphoma in 10 (10%), PTLD in 5 (5%), and other histologies in 7 (7%) patients. Thirty-one (37%) had non-germinal center B-cell (GCB) cell of origin (COO) and 12 (13%) had rearrangements of *MYC* and *BCL2*. Treatment was standard dose in 55 (54%), intensive in 33 (32%), reduced dose in 9 (9%), and other treatments in 5 (5%) of patients; 4 patients did not receive anthracycline (**Table 2**).

The EOT MRD assessment occurred a median 35 days after day 1 of the final cycle (range -5-68) and was MRD detectable in 20 (20%) of cases. Nine (9%) patients did not have clonal IgH rearrangements from baseline clonality ID. EOT PET/CT demonstrated CMR in 65 (68%), PMR in 18 (19%), progressive disease in 13 (14%), and was missing in 6 patients. Patients with detectable EOT MRD were more likely to be younger than 60 (p=0.04), have GCB COO (p=0.034), and demonstrate positive EOT PET/CT (p<0.001). Collection tube was not associated with MRD (p=0.78). Characteristics from the frontline cohort are summarized in **Table 2**.

EOT MRD is prognostic after frontline therapy

After a median follow-up of 13.5 months from EOT, the 12-month PFS and OS rates for the entire frontline cohort were 71% and 86%, respectively. A total of 28 patients (28%) experienced a PFS event, 2/28 (7%) of whom died without prior progression; 14/26 (54%) progressions were biopsy-confirmed. Three patients experienced CNS-only progression at 1.0, 1.1, and 3.3 months, 2 of whom were MRD undetectable at EOT. Detectable MRD at EOT was associated with significantly worse PFS (hazard ratio [HR] 14.5, 95% CI 6.7-31.7, p<0.001) compared to undetectable EOT MRD with 12-month PFS rates of 15% versus 85%, respectively (**Figure 1A**). EOT MRD status was still prognostic for PFS after excluding the 13 patients with progressive disease on EOT PET/CT (HR 10.0, 95% CI 3.0-33.8, p<0.001, Figure S2). A positive EOT PET/CT was also associated with significantly worse PFS (HR 12.8, 95% CI 4.8-34.5, p<0.001) compared to negative EOT PET/CT with 12-month PFS rates of 38% versus 92%, respectively (**Figure 1B**). EOT MRD and PET/CT were also prognostic for OS (Figure S3).

There was only one patient with a negative PET/CT who had detectable EOT MRD and they were progression-free at 12 months; they had a low MRD level (1 residual sequence/mL) and no longitudinal testing. The 12-month PFS rate for the other 64 patients with negative PET/CT who had undetectable EOT MRD was 92%. For the 31 patients with a positive EOT PET/CT, detectable

EOT MRD was associated with worse PFS (HR 4.9, 95% CI 1.7-13.9, p=0.003) compared with undetectable EOT MRD with 12-month PFS rates of 13% versus 65%, respectively (**Figure 1C**). Of these 31 patients, the Deauville 5PS was 4 for 15 patients (50%), 5 for 15 patients (50%), and missing for one. Detectable EOT MRD was associated with worse PFS for Deauville 5PS of 4 (HR 7.1, 95% CI 1.0-52.9, p=0.054, Figure S4A) but not 5 (HR 0.6, 95% CI 0.2-2.3, p=0.46, Figure S4B).

Univariable Cox regression models associating characteristics with PFS are displayed in Table S6. In addition to EOT MRD and PET/CT, non-DLBCL NOS histology (p<0.001) and intensive frontline treatment (p=0.039) were associated with worse PFS. A multivariable model adjusted for EOT MRD status, EOT PET/CT response, histology, and IPI 4-5 demonstrated that PFS was significantly associated with all covariates including detectable EOT MRD (HR 6.9, 95% CI 2.3-21.3, p<0.001) and positive EOT PET/CT (HR 4.6, 95% CI 1.3-15.5, p=0.015) (**Figure 1D**). A swimmer's plot depicting longitudinal ctDNA dynamics and progression outcomes in 26 patients with subsequent MRD assessment after EOT is displayed in Figure S5. Of the 9 patients without baseline clonal IgH rearrangements, 2 had detectable EOT MRD; one had fluctuating MRD between 2-16 sequences/mL but remained progression free at 16 months. The other had an EOT MRD quantity of 65 sequences/mL but became undetectable by day 70 and remained progression free at 12.5 months.

Diagnostic performance of MRD and PET/CT in the frontline setting

Sensitivity and specificity of EOT MRD for a progression event after frontline therapy were 64% and 96%; PPV and NPV were 82% and 90%, respectively. Sensitivity and specificity of EOT PET/CT were 86% and 85%; PPV and NPV were 63% and 95%, respectively. EOT MRD demonstrated higher specificity (p=0.022) and PPV (p=0.039) while EOT PET/CT demonstrated numerically higher sensitivity (p=0.063) and higher NPV (p=0.017) **(Figure 2A-B)**. There were no

significant differences in sensitivity (67% versus 64%, p=1) or specificity (97% versus 94%, p=0.62) of early (<35 days) versus late (≥35 days) EOT MRD assessment. The sensitivity and specificity of EOT MRD for the 10 patients with transformed follicular lymphoma were 57% and 100%, respectively.

CAR-T cohort characteristics

There were 48 patients treated with CAR-T with day 100 landmark MRD assessment; another 2 were excluded due to progression prior to day 100. Twenty-eight (58%) were older than 60, 29 (66%) had stage III/IV disease, 28 (58%) had elevated lactate dehydrogenase, 6 (13%) had ECOG performance status ≥ 2, and 13 (28%) had ≥2 extranodal sites at time of CAR-T; IPI was 4-5 in 3 (6%) patients. Histology was DLBCL NOS in 33 (69%), transformed follicular lymphoma in 9 (19%), HGBCL in 3 (6%), PMBCL in 2 (4%), and PTLD in one (2%) patient. Fourteen patients (39%) had non-GCB COO and 6 (13%) had rearrangements of *MYC and BCL2*. The costimulatory domain in the CAR construct was 4-1BB in 23 (48%) and CD28 in 25 (52%), and 5 (10%) received an investigational CAR-T product (**Table 3**).

The first post-infusion MRD assessment occurred a median 34 days (28–106) after CAR-T infusion and demonstrated detectable MRD in 21 (44%). One (2%) patient did not have clonal IgH rearrangements from baseline clonality ID. Post-infusion PET/CT response was CMR in 32 (68%), PMR in 12 (26%), and progressive disease in 3 (6%), and missing in 1. The MRD detectable rate by day 100 was 18/48 (38%) and was associated with elevated LDH (p=0.007), higher IPI (p=0.007), and positive PET/CT (p=0.01) but not collection tube (p=0.074). Characteristics from the CAR-T cohort are summarized in **Table 3.**

MRD is prognostic after CAR-T

After a median follow-up of 13.1 months from cell infusion, the 12-month PFS and OS rates for the entire CAR-T cohort were 62% and 80%, respectively. A total of 19 (40%) patients experienced a PFS event, none experiencing death without prior progression; 11/19 (58%) progressions were biopsy-confirmed. Two patients experienced CNS-only progression at 7.7 and 8.2 months, and both were MRD undetectable by day 100. Detectable MRD by day 100 was associated with significantly worse PFS (HR 5.0, 95% CI 2.0-13.0, p<0.001) compared to undetectable MRD with 12-month PFS rates of 35% versus 78%, respectively (**Figure 3A**). Detectable MRD by day 100 was still prognostic for PFS after excluding the 3 patients with progressive disease on PET/CT (HR 4.0, 95% CI 1.5-10.8, p=0.007, Figure S6). Detectable MRD on the first available assessment from the early post-infusion period (days 28-59) was only associated with numerically worse PFS (HR 2.9, 95% CI 0.9-9.1, p=0.074, Figure S7A) compared to undetectable MRD. Conversely, detectable MRD on the last available assessment from the late post-infusion period (days 60-100) was associated with significantly worse PFS (HR 5.3, 95% CI 1.8-15.1, p=0.002, Figure S7B) compared to undetectable MRD. Positive PET/CT was also associated with significantly worse PFS (HR 4.6, 95% CI 1.8-11.5, p=0.001) compared to negative PET/CT, with 12-month PFS rates of 27% versus 79%, respectively (**Figure 3B**). Day 100 MRD and PET/CT were also prognostic for OS (Figure S8).

For the 32 patients with negative PET/CT, detectable MRD by day 100 was associated with worse PFS (HR 4.5, 95% CI 1.1-18.3, p=0.036) compared to undetectable MRD, with 12-month PFS rates of 54% versus 86%, respectively (**Figure 3C**). For the 15 patients with a positive PET/CT, detectable MRD by day 100 trended towards worse PFS (HR 2.8, 95% CI 0.7-10.7, p=0.14) compared to undetectable MRD, with 12-month PFS rates of 13% versus 40%, respectively (**Figure 3D**). Of these 15 patients, Deauville 5PS was 4 for 4 patients (31%), 5 for 9 patients (69%), and missing for 2. Detectable MRD by day 100 was not associated with PFS with Deauville 5PS of 4 (Figure S9A) or 5 (Figure S9B), though sample sizes were small.

Univariable Cox regression models associating characteristics with PFS are displayed in Table S7. In addition to day 100 MRD and PET/CT, ECOG PS ≥2 (p=0.012), IPI 4-5 (p=0.005), and receiving a 4-1BB-based product (p=0.034) were associated with worse PFS. A multivariable model adjusted for day 100 MRD status, PET/CT response, histology, and IPI 4-5 demonstrated that PFS was worse for detectable MRD by day 100 (HR 4.7 95% CI 1.8-12.5, p=0.002) and trended towards worse for positive PET/CT (HR 2.6 95% CI 0.9-7.0, p=0.07, **Figure 3E**). A swimmer's plot depicting longitudinal ctDNA dynamics and progression outcomes in 27 patients with subsequent MRD assessment is displayed in Figure S10**.** Of 11 initially with detectable MRD, 7/11 (64%) became undetectable without intervention by a median 81 days post-infusion (40-126). These 7 patients had low quantitative MRD levels (median 1 sequence/mL, range <1-9) on their first post-infusion assessment. Of these 7, 5 patients remained progression free at last follow-up. One experienced molecular recurrence on day 127 prior to clinical progression, while another without subsequent MRD testing experienced late progression at 15 months.

Diagnostic performance of MRD and PET/CT in the CAR-T setting

Sensitivity and specificity of day 100 MRD for a progression event after CAR-T were 63% and 82%; PPV and NPV were 71% and 76%, respectively. Sensitivity and specificity of PET were 58% and 85%; PPV and NPV were 73% and 73%, respectively. Day 100 MRD and PET/CT had similar sensitivity (p=1), specificity (p=1), PPV (p=0.85), and NPV (p=0.73) (**Figure 4A-B**). Sensitivity and specificity of MRD by the first assessment from the early post-infusion period for progression were 58% and 67%; PPV and NPV were 50% and 74%, respectively. The corresponding test performance values were 60%, 88%, 82%, and 70% when using the last assessment from the late post-infusion period. The sensitivity and specificity of day 100 MRD for the 9 patients with transformed follicular lymphoma were 100% and 71%, respectively.

Discussion

In this multicenter retrospective analysis, post-treatment ctDNA-MRD status by a commercial IgHTS assay was strongly associated with PFS in patients with LBCL treated with frontline immunochemotherapy and CAR-T and was an independent prognostic marker when paired with conventional PET/CT. Post-treatment outcomes were poor with detectable MRD; 12-month PFS rates were 15% after frontline therapy and 35% after CAR-T. Conversely, undetectable MRD post-treatment was associated with encouraging 12-month PFS rates of 85% after frontline therapy and 78% after CAR-T. This is the first study to demonstrate the prognostic utility of MRD by a commercial IgHTS assay from a single EOT timepoint for newly diagnosed LBCL and confirms the findings from prospective CAR-T studies.[22,23] Real-world ctDNA-MRD assessment in LBCL using IgHTS was feasible; the success rate of clonality ID from tumor tissue in this cohort was high at 96%. With a median MRD result turnaround of 9 days, it is possible to incorporate its assessment into real-time clinical decision making, acknowledging the need for archival tissue to be submitted in advance.

The sensitivity and NPV of MRD by IgHTS for progression were numerically lower than that of PET/CT after frontline therapy (64% and 90% versus 86% and 95%, respectively) and similar after CAR-T (63% and 76% versus 58% and 73%); notably, the sensitivity/NPV of PET/CT was higher than expected after frontline therapy. There was no additive prognostic value of MRD among patients with a CMR after frontline treatment, though MRD by day 100 did help stratify patients with a CMR after CAR-T. Patients with undetectable MRD and a concurrent positive PET/CT still experienced suboptimal outcomes with 12-month PFS rates of 65% after immunochemotherapy and 40% after CAR-T, suggestive of a substantial false negative rate and the need for vigilance for disease relapse. MRD was also not useful for detecting CNS-only relapses. Sensitivity of IgHTS in low ctDNA-input settings could be limited due to the assay

methodology only tracking a single tumor-specific signal, despite optimal sample conditions (fresh collection and processing). While the analytic performance of IgHTS for genomic DNA has been described,[24] no validation studies determining the limit of detection or analytical sensitivity from cfDNA are published. It was not possible to perform this analysis with dilution series for this study. In March 2025, the commercial assay was updated to optimize cfDNA extraction and accommodate increased plasma volume to increase sensitivity, however MRD assessments after this change were excluded from this analysis due to insufficient follow-up.

Due to the high specificity and PPV of MRD over PET/CT after frontline therapy (96% and 82% versus 85% and 63%, respectively), IgHTS may best be viewed as a “rule-in” test to use in conjunction with conventional imaging in that setting. Detectable MRD was associated with worse outcomes after frontline therapy with concurrent positive PET/CT. The high specificity suggests that MRD-guided consolidation should be evaluated prospectively; one trial is enrolling older patients with DLBCL who have detectable EOT MRD by IgHTS after frontline therapy to receive mosunetuzumab (GOLD, NCT06828991).

Specificity and PPV of MRD by day 100 after CAR-T was similar to that of PET/CT (82% and 71% versus 85% and 73%, respectively). These metrics improve over time due to the potential for persistent CAR T-mediated antitumor activity and delayed MRD clearance. Specificity was 67% by the first assessment between days 28-59 and 88% by the last assessment between days 60-100; clearance was even observed after day 100. Dean and colleagues noted that metabolic tumor volume correlated better with ctDNA quantity by 3 months post CAR-T infusion compared to one.[25] Prior work has suggested that due to lower clonal diversity of light chain loci, there may be impaired specificity of IgHTS for patients tracked with light chain rearrangements,[26] but the majority of patients (93%) had baseline heavy chain rearrangements. The only cases descriptive

of clinical specificity were 2 patients without baseline IgH clonotypes that had detectable EOT MRD after frontline therapy but did not progress.

This study has several limitations. Real-time MRD assessment can only be performed once baseline clonality ID is completed. Though quick to result once tissue was submitted, there can be significant delays in tissue procurement, particularly if biopsies were performed at other institutions. There was inherent heterogeneity related to patient selection, as the clinician's decision to perform a post-treatment MRD assessment was influenced by pre-test perception of individual patient risk. The results reflect clinician-biased testing rather than universal screening practices, and direct comparison to studies with prospective sample collection from unselected populations is difficult. There was also survivorship bias as patients must have had an MRD assessment before progression. Due to the unblinded nature of MRD testing, real-time results likely influenced investigator-assessed, unblinded PET/CT interpretation, particularly in cases without confirmatory biopsy. Undetectable MRD could bias clinicians to consider paired PET/CTs as negative, and vice versa. This study included patients treated with different frontline regimens and with variant LBCL histologies including transformed follicular lymphoma, though adjusting for histology did not change the association between MRD and PFS. The timing of post-treatment MRD assessment was not uniform, which limits the generalizability of the findings, particularly after CAR-T; however, excluding early MRD assessments prior to day 60 after CAR-T led to similar test performance as the primary day 100 landmark analysis. As longitudinal monitoring was not universally performed, the benefit of long-term surveillance and lead times before clinical relapse were not assessed. Lastly, the modest follow-up time could overestimate the sensitivity and underestimate the specificity of diagnostic tests if late relapses are not captured.

Two smaller studies evaluating investigational immunoglobulin-targeting ctDNA-MRD assays in newly diagnosed DLBCL have demonstrated similar findings, though they were also able to

perform clonality ID from peripheral blood.[27,28] Notably, though Wang and colleagues did not find additive sensitivity of MRD over PET/CT,[28] Soscia and colleagues were able to significantly stratify outcomes by MRD in patients with CMR on EOT PET/CT.[27] Other assays targeting multiple tumor-specific signals have also been investigated in LBCL. Phased variant enrichment and detection sequencing is an ultrasensitive MRD assay late in development that identifies multiple mutations co-occurring on the same ctDNA molecule.[29,30] Three clinical studies have demonstrated prognostic value of phased variant-informed MRD at EOT after frontline treatment,[31–33] and prospective studies investigating treatment consolidation for detectable EOT MRD (ALPHA3, NCT06500273)[34] or de-escalation for undetectable mid-treatment MRD (SHORTEN-ctDNA, NCT06693830)[35] are underway. A tumor-informed multiplex PCR targeted sequencing assay is commercially available (Signatera™) and uses whole exome/genome sequencing to design a tumor-specific panel; real-world retrospective studies evaluating ctDNA dynamics have demonstrated its utility in risk stratifying patients.[36,37]

As multiple ctDNA-MRD assays become available for routine clinical use, data-driven guidelines are needed to advise clinicians on best practices with respect to MRD. Based on the data presented, the authors first suggest that MRD assessment should be repeated to monitor for molecular clearance in cases of low level MRD after CAR-T (<10 residual clonal sequences) in the absence of clinical relapse. Secondly, IgHTS ctDNA-MRD may not be sufficient to rule out residual disease with a single undetectable result, so MRD undetectable patients should continue routine surveillance and/or repeat MRD testing. Lastly, though no universal interventions can be recommended without prospective studies, for select patients with detectable MRD and positive PET/CT where confirmatory biopsy is not feasible and/or MRD quantity is rising, clinicians could consider those patients as having residual disease.

In summary, post-treatment MRD assessment with IgHTS after curative intent frontline immunochemotherapy or CAR-T cell therapy for LBCL was independently prognostic for outcomes in this multicenter retrospective study. Real-time MRD evaluation in LBCL is now feasible in the clinic, and future work is needed to inform on how best to utilize this response biomarker.

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Table 1. Testing characteristics of all MRD assessments

Variable (N=551)	*N (%)*
Turnaround time (N=550)	
Median (IQR) [range], days	9 (8-17) [5-97]
Plasma volume	
Median (IQR) [range], mL	1.2 (1.1-1.3) [0.5-7.1]
Collection tube type (N=544)	
EDTA	144 (26)
Streck	400 (74)
Limit of detection, residual clonal sequences per mL plasma (N=526)	
<1	86 (16)
1	74 (14)
2	349 (66)
≥ 3	17 (3)
Timing of Sample[1]	
Pre-Treatment	32 (6)
Interim	74 (13)
Post-treatment	156 (28)
Longitudinal	289 (53)
MRD status	
Undetectable	383 (70)
Detectable	168 (30)
Unique dominant sequences in MRD-detectable samples (N=168)	
1	51 (30)
2	50 (30)
≥ 3	67 (40)
Clonal Ig loci present in MRD-detectable samples (N=168)	
IgH, IgK, and IgL	14 (8)
IgH and IgK	63 (38)
IgH and IgL	2 (1)
Lost IgK from baseline	1
IgK and IgL	3 (2)
Lost IgH from baseline	1
IgH only	48 (29)
Lost IgK from baseline	13
Lost IgK and IgL from baseline	3
Lost IgL from baseline	2
IgK only	36 (21)
Lost IgH from baseline	20
Lost IgH and IgL from baseline	7
Lost IgL from baseline	1
IgL only	2 (1)
Lost IgH and IgK from baseline	2

IQR, Inter-quartile range; EDTA, Ethylenediaminetetraacetic acid; MRD, Measurable residual disease; Ig, immunoglobulin; IgH, immunoglobulin heavy chain; IgK/IgL, immunoglobulin light chain (kappa or lambda)

[1]Timing of sample categorization:
Pre-treatment: collected up to 4 weeks before start of frontline treatment, chimeric antigen receptor T-cell therapy (CAR-T)
Interim: after pre-treatment but before post-treatment sample

Post-treatment: Between 5 days before to 70 days after day 1 of the final cycle of frontline treatment or between 28 to 105 days after CAR-T infusion ~~or ASCT~~
Longitudinal: sample collected at any other time

Table 2. Characteristics of frontline treatment cohort

Variable	All patients (N=102) N (%)	EOT MRD negative (N=82)	EOT MRD positive (N=20)	p-value
Male sex	61 (60%)	48 (59%)	13 (65%)	0.60
Age (median (IQR) [range], years)	66 (53-74) [23, 97]	68 (55-75) [23-97]	57 (48-69) [31-90]	0.083
Age greater than 60	66 (65%)	57 (70%)	9 (45%)	**0.040**
Stage III-IV (N=98)	82 (84%)	66 (84%)	16 (84%)	1.0
LDH above upper limit of normal (N=96)	65 (68%)	52 (65%)	13 (81%)	0.20
ECOG PS ≥2 (N=99)	18 (18%)	15 (19%)	3 (17%)	1.0
≥2 extranodal sites	31 (30%)	25 (30%)	6 (30%)	0.97
IPI (N=94)				0.98
0-1	11 (11%)	9 (11%)	2 (13%)	
2	29 (31%)	25 (32%)	4 (27%)	
3	33 (35%)	27 (34%)	6 (40%)	
4-5	21 (22%)	18 (23%)	3 (20%)	
Bulky disease ≥7.5 cm	35 (43%)	26 (32%)	99 (45%)	0.26
De novo secondary CNS lymphoma	3 (3%)	3 (4%)	0 (0%)	1.0
Histologic subtype				0.050
DLBCL NOS	69 (68%)	59 (72%)	10 (50%)	
HGBCL	11 (11%)	9 (11%)	2 (10%)	
PTLD	5 (5%)	2 (2%)	3 (15%)	
tFL	10 (10%)	6 (7%)	4 (20%)	
Other[1]	7 (7%)	6 (7%)	1 (5%)	
Cell of origin (N=83)				**0.034**
Germinal center B-cell	52 (63%)	39 (57%)	13 (87%)	
Non germinal center B-cell	31 (37%)	29 (43%)	2 (13%)	
Double Expressor Lymphoma (N=89)	25 (28%)	18 (26%)	7 (37%)	0.34
MYC and *BCL2* rearranged (N=96)	12 (13%)	11 (14%)	1 (5%)	0.45
HIV Positive (N=101)	4 (4%)	2 (3%)	2 (10%)	0.18
Type of Front-Line Treatment				0.45
Standard[2]	55 (54%)	47 (57%)	8 (40%)	
Intensive[3]	33 (32%)	24 (29%)	9 (45%)	
R-miniCHOP	9 (9%)	7 (9%)	2 (10%)	
Other[4]	5 (5%)	4 (5%)	1 (5%)	
Intrathecal chemotherapy prophylaxis	29 (28%)	21 (26%)	8 (40%)	0.20
High-dose methotrexate prophylaxis	14 (14%)	14 (17%)	0 (0%)	0.066
Sample tube				0.78
EDTA	24 (24%)	20 (24%)	4 (20%)	
Streck	78 (76%)	62 (76%)	16 (80%)	
Time from EOT to MRD test (median (IQR) [range], days)	35 (11-45) [-5--68]	34 (9-44) [-5-68]	38 (17-50) [-3-60]	0.45
MRD positive Residual clonal sequences/cc plasma if MRD positive (median (IQR) [range])	20 (20%) 83 (7-448) [0.4-2,287]	- -	- -	- -
Baseline clonal Ig loci (N=101)				1.0
IgH present	92 (91%)	74 (91%)	18 (90%)	
IgH absent	9 (9%)	7 (9%)	2 (10%)	
Limit of detection (residual clonal sequences/cc plasma)				0.80
<1	24 (24%)	19 (23%)	5 (25%)	
1	21 (21%)	16 (20%)	5 (25%)	
2	49 (48%)	41 (50%)	8 (40%)	
≥3	8 (8%)	6 (7%)	2 (10%)	
EOT PET/CT response (N=96)				**<0.001**
CMR	65 (68%)	64 (81%)	1 (6%)	
PMR	18 (19%)	13 (16%)	5 (29%)	
PD	13 (14%)	2 (3%)	11 (65%)	

IQR, Inter-quartile range; LDH, lactate dehydrogenase; ECOG PS, Eastern Cooperative Group Performance Status; IPI, international prognostic index; CNS, central nervous system; DLBCL NOS, diffuse large B-cell lymphoma not otherwise specified; HGBCL, high grade B-cell lymphoma; PTLD, post-transplant lymphoproliferative disorder; tFL, transformed follicular lymphoma; EDTA, Ethylenediaminetetraacetic acid; EOT, end-of-treatment; MRD, measurable residual disease; Ig, immunoglobulin; IgH, immunoglobulin heavy chain; CMR, complete metabolic response; PMR, partial metabolic response; PD, progressive disease; PET/CT, positron emission tomography/computed tomography

[1]Includes 2 cases of primary mediastinal B-cell lymphoma, 2 EBV+ DLBCL, 1 intravascular DLBCL, 1 T-cell histiocyte-rich LBCL, and 1 unknown subtype
[2]Includes R-CHOP or R-pola-CHP
[3]Includes DA-EPOCH-R, R-CODOX-M/R-IVAC, and R-HCVAD
[4]Includes R-pola (1), R-GDP (1), R-CEOP (2), and not available (1)

Table 3. Characteristics of CAR-T cohort

Variable	All patients (N=48) N (%)	D100 MRD undetectable (N=30)	D100 MRD detectable (N=18)	p-value
Male sex	32 (67%)	21 (70%)	11 (61%)	0.53
Age at time of treatment (median (IQR) [range], years)	62 (50-72) [18-91]	62 (50-70) [18-83]	62 (46-76) [25-91]	0.98
Age greater than 60	28 (58%)	17 (57%)	11 (61%)	0.76
Stage III-IV (N=44)	29 (66%)	16 (59%)	13 (76%)	0.24
LDH above upper limit of normal	28 (58%)	13 (43%)	15 (83%)	**0.007**
ECOG PS ≥2	6 (13%)	3 (10%)	3 (17%)	0.66
≥2 extranodal sites (N=46)	13 (28%)	6 (21%)	7 (41%)	0.18
IPI (N=42)				**0.007**
0-1	10 (23%)	10 (38%)	0 (0%)	
2	16 (38%)	10 (38%)	6 (38%)	
3	13 (31%)	5 (19%)	8 (50%)	
4-5	3 (7%)	1 (4%)	2 (13%)	
Bulky disease ≥7.5 cm	11 (23%)	5 (17%)	6 (33%)	0.29
Prior CNS disease	7 (15%)	5 (17%)	2 (11%)	0.70
Histologic subtype				0.51
DLBCL NOS	33 (69%)	21 (70%)	12 (678%)	
HGBCL	3 (6%)	3 (10%)	0 (0%)	
PTLD	1 (2%)	0 (0%)	1 (6%)	
tFL	9 (19%)	5 (17%)	4 (22%)	
PMBCL	2 (4%)	1 (3%)	1 (6%)	
Cell of origin[1] (N=36)				0.97
Germinal center B-cell	22 (61%)	14 (61%)	8 (62%)	
Non germinal center B-cell	14 (39%)	9 (39%)	5 (38%)	
Double Expressor Lymphoma (N=46)	24 (52%)	14 (48%)	10 (59%)	0.49
MYC and *BCL2* rearranged (N=47)	6 (13%)	2 (7%)	4 (22%)	0.19
Received bridging therapy	42 (88%)	25 (83%)	17 (94%)	0.39
Cell therapy product				0.72
Axicabtagene cioleucel	24 (50%)	16 (53%)	8 (44%)	
Lisocabtagene maraleucel	17 (35%)	11 (37%)	6 (33%)	
Tisagenlecleucel	2 (4%)	1 (3%)	1 (6%)	
Investigational product	5 (10%)	2 (7%)	3 (17%)	
Co-stimulatory domain of product				0.82
4-1BB	23 (48%)	14 (47%)	9 (50%)	
CD28	25 (52%)	16 (53%)	9 (50%)	
Sample tube				0.074
EDTA	11 (23%)	4 (13%)	7 (39%)	
Streck	37 (77%)	26 (87%)	11 (61%)	
Baseline clonal Ig loci				
IgH present	47 (98)	29 (97)	18 (100)	1.0
IgH absent	1 (2)	1 (3)	0 (0)	
Time from cell infusion to first MRD test (median (IQR) [range], days)	34 (29-59) [28-106]	34 (29-49) [28-105]	34 (27-77) [28-106]	0.70
Residual clonal sequences/cc plasma if MRD detectable (median (IQR) [range])	3 (0.7-5.0) [0.7-3223]	-	-	-
Limit of detection (residual clonal sequences/cc plasma)				0.72
<1	7 (15%)	4 (13%)	3 (17%)	
1	4 (8%)	2 (7%)	2 (11%)	
2	33 (69%)	22 (73%)	1 (61%)	
≥3	4 (8%)	2 (7%)	2 (11%)	
PET/CT response (N=47)				**0.010**
CMR	32 (68%)	24 (83%)	8 (44%)	
PMR	12 (26%)	5 (17%)	7 (39%)	
PD	3 (6%)	0 (0%)	3 (17%)	

CAR-T, chimeric antigen receptor T-cell; D100, day 100; IQR, Inter-quartile range; LDH, lactate dehydrogenase; ECOG PS, Eastern Cooperative Group Performance Status; IPI, international prognostic index; CNS, central nervous system; DLBCL NOS, diffuse large B-cell lymphoma not otherwise specified; HGBCL, high grade B-cell lymphoma; PTLD, post-transplant lymphoproliferative disorder; tFL, transformed follicular lymphoma; PMBCL, primary mediastinal B-cell lymphoma; CMR, complete metabolic response; PMR, partial metabolic response; SD, stable disease; PD, progressive disease; EDTA, Ethylenediaminetetraacetic acid; MRD, measurable residual disease; Ig, immunoglobulin; IgH, immunoglobulin heavy chain; PET/CT, positron emission tomography/computed tomography

[1]Excluding tFL and PMBCL

Figure Legends

Figure 1. EOT MRD status is prognostic for PFS after frontline therapy. A) PFS for the entire frontline cohort stratified by EOT MRD detectable (median 1.1 months, 17/20 events) versus undetectable (median NR, 11/82 events). B) PFS for the entire frontline cohort stratified by EOT PET/CT positive (median 2.4 months, 19/31 events) versus negative (median NR, 5/65 events). C) PFS for 31 patients with positive PET/CT stratified by EOT MRD detectable (median 1.2 months, 14/16 events) versus undetectable (median NR, 5/15 events). D) Multivariable Cox regression for PFS for the frontline cohort adjusted for non-DLBCL NOS histology, IPI, EOT PET/CT response, and EOT MRD status.

EOT, end-of-treatment; MRD, measurable residual disease; PFS, progression free survival; NR, not reached; PET/CT, positron emission tomography/computed tomography; DLBCL NOS, diffuse large B-cell lymphoma not otherwise specified; IPI, international prognostic index

Figure 2. Paired comparison of diagnostic test performance between EOT MRD status and EOT PET/CT response after frontline treatment. A) Paired dot (slope) plot comparing sensitivity, specificity, PPV, and NPV with associated p-values. Each point represents the estimate of a given performance metric, with lines connecting paired estimates to illustrate within-metric differences between MRD and PET/CT. B) Table comparison of test performance metrics and associated p-values; sensitivity and specificity were compared with the pairwise exact McNemar's test, while PPV and NPV were compared using nonparametric bootstrap resampling of paired observations (2,000 replicates).

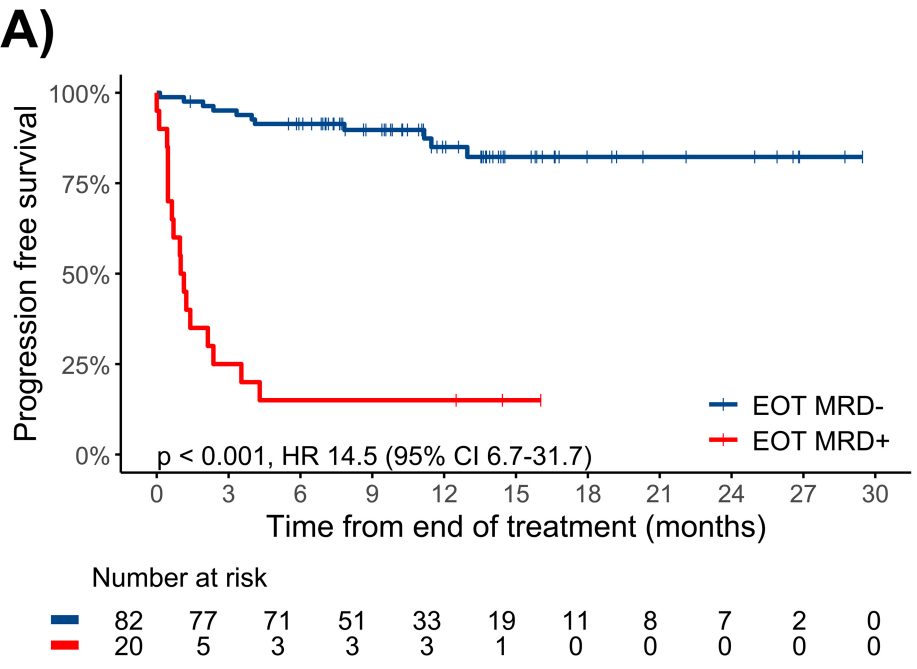
EOT, end-of-treatment; MRD, measurable residual disease; PET/CT, positron emission tomography/computed tomography; PPV, positive predictive value; NPV, negative predictive value

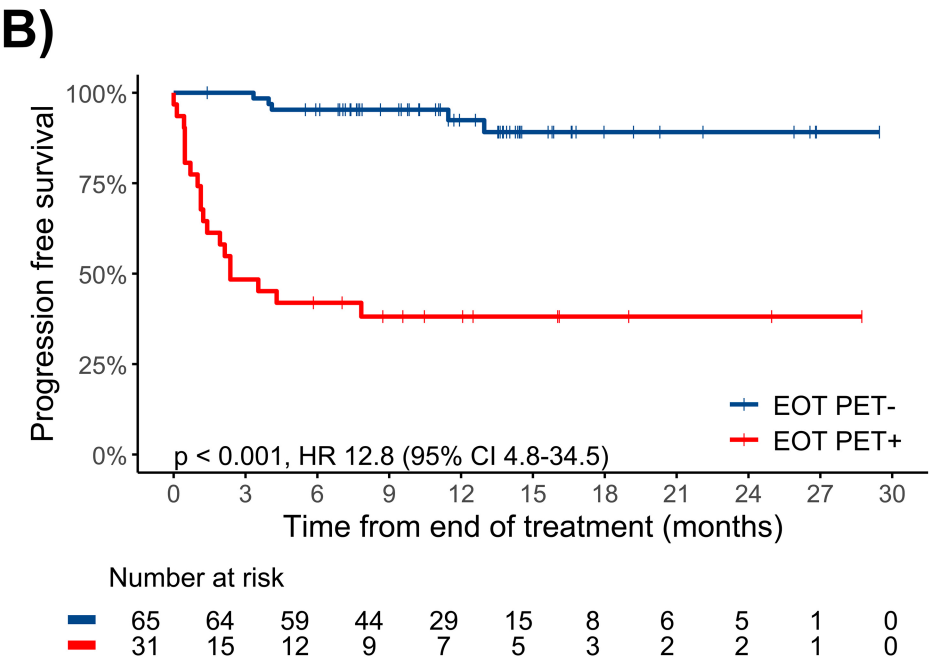
Figure 3. Day 100 MRD status is prognostic for PFS after CAR-T cell therapy. A) PFS for the entire CAR-T cohort stratified by day 100 landmark MRD detectable (median 5.1 months, 12/18 events) versus undetectable (median NR, 7/30 events). B) PFS for the entire CAR-T cohort stratified by PET/CT positive (median 4.7 months, 11/15 events) versus negative (median NR, 8/32 events). C) PFS for 32 patients with a negative PET/CT stratified by day 100 landmark MRD detectable (median 6.6 months, 2/5 events) versus undetectable (median NR, 6/27 events). D) PFS for 15 patients with positive PET/CT stratified by day 100 landmark MRD detectable (median 3.8 months, 8/9 events) versus undetectable (median 8.2 months, 3/6 events). E) Multivariable Cox regression for PFS for the CAR-T cohort adjusted for DLBCL NOS histology, IPI, ~~EOT~~ PET/CT response, and day 100 MRD status.

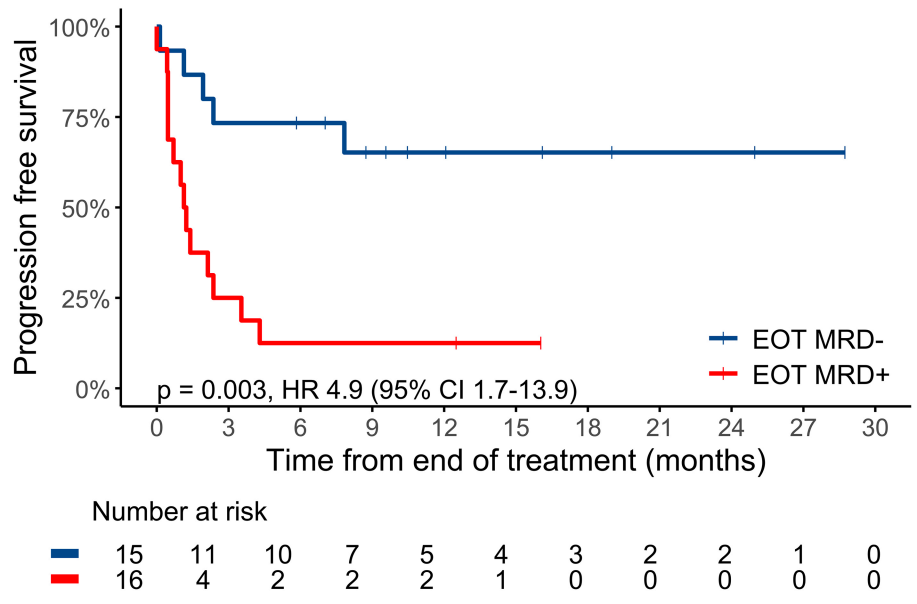
MRD, measurable residual disease; PFS, progression free survival; CAR-T, chimeric antigen receptor T-cell; NR, not reached; PET/CT, positron emission tomography/computed tomography; CMR, complete metabolic response; DLBCL NOS, diffuse large B-cell lymphoma not otherwise specified, IPI, international prognostic index

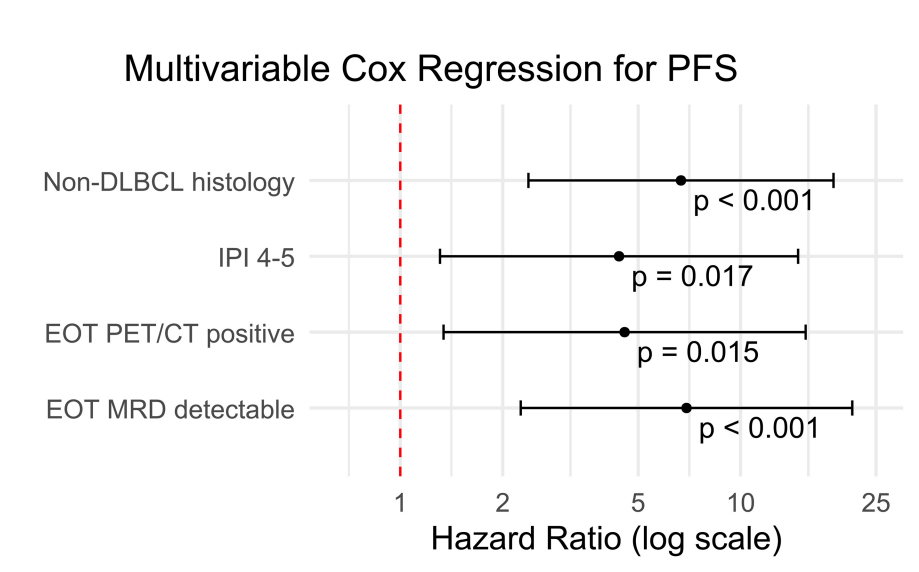
Figure 4. Paired comparison of diagnostic test performance between day 100 MRD status and PET/CT response after CAR-T. A) Paired dot (slope) plot comparing sensitivity, specificity, PPV, and NPV with associated p-values. Each point represents the estimate of a given performance metric, with lines connecting paired estimates to illustrate within-metric differences between MRD and PET/CT. B) Table comparison of test performance metrics and associated p-values; sensitivity and specificity were compared with the pairwise exact McNemar's test, while PPV and NPV were compared using nonparametric bootstrap resampling of paired observations (2,000 replicates).

MRD, measurable residual disease; PET/CT, positron emission tomography/computed tomography; PPV, positive predictive value; NPV, negative predictive value, CAR-T chimeric antigen receptor T-cell therapy

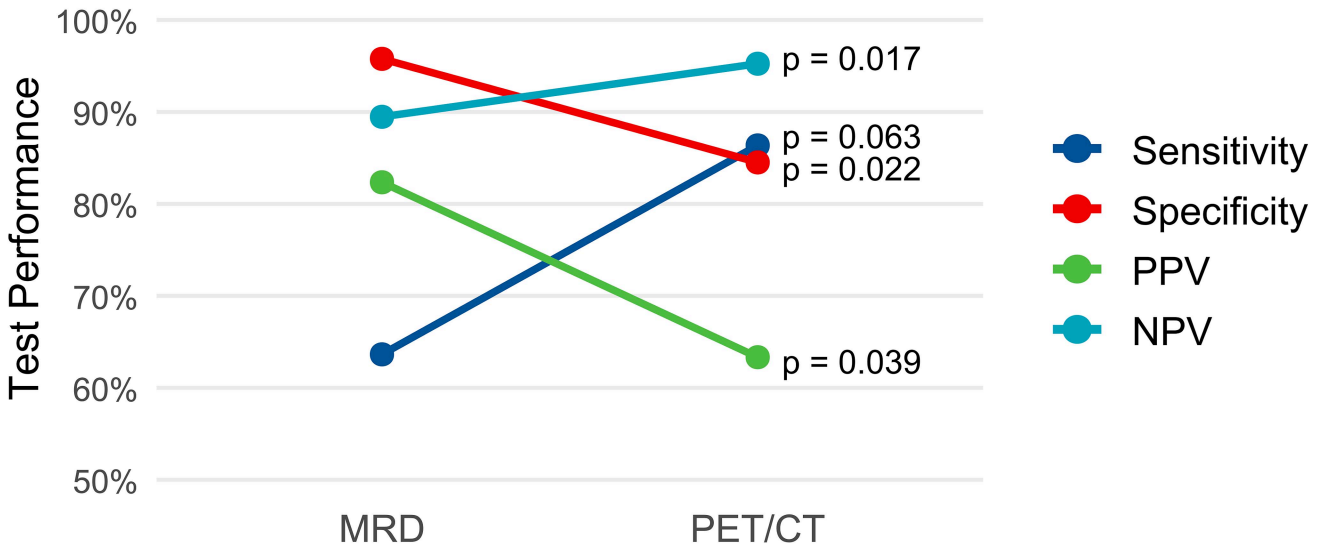

A)
Progression free survival
100%
75%
50%
25%
0%
p < 0.001, HR 14.5 (95% CI 6.7-31.7)
EOT MRD-
EOT MRD+
0 3 6 9 12 15 18 21 24 27 30
Time from end of treatment (months)
Number at risk
82 77 71 51 33 19 11 8 7 2 0
20 5 3 3 3 1 0 0 0 0 0


B)
Progression free survival
100%
75%
50%
25%
0%
p < 0.001, HR 12.8 (95% CI 4.8-34.5)
EOT PET-
EOT PET+
0 3 6 9 12 15 18 21 24 27 30
Time from end of treatment (months)
Number at risk
65 64 59 44 29 15 8 6 5 1 0
31 15 12 9 7 5 3 2 2 1 0


C)
Progression free survival
100%
75%
50%
25%
0%
p = 0.003, HR 4.9 (95% CI 1.7-13.9)
EOT MRD-
EOT MRD+
0 3 6 9 12 15 18 21 24 27 30
Time from end of treatment (months)
Number at risk
15 11 10 7 5 4 3 2 2 1 0
16 4 2 2 2 1 0 0 0 0 0

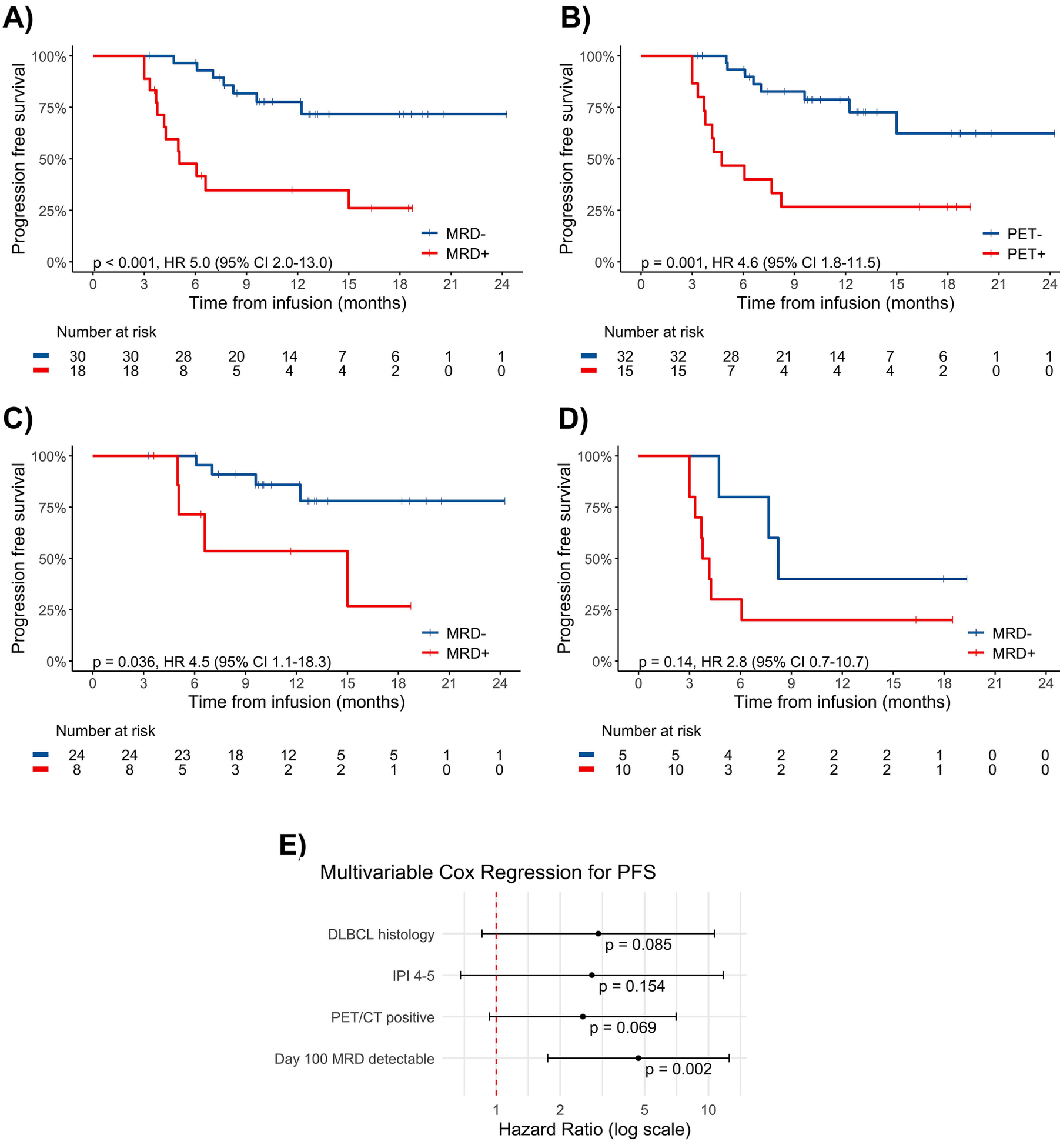

D)
Multivariable Cox Regression for PFS
Non-DLBCL histology
p < 0.001
IPI 4-5
p = 0.017
EOT PET/CT positive
p = 0.015
EOT MRD detectable
p < 0.001
1 2 5 10 25
Hazard Ratio (log scale)

A)

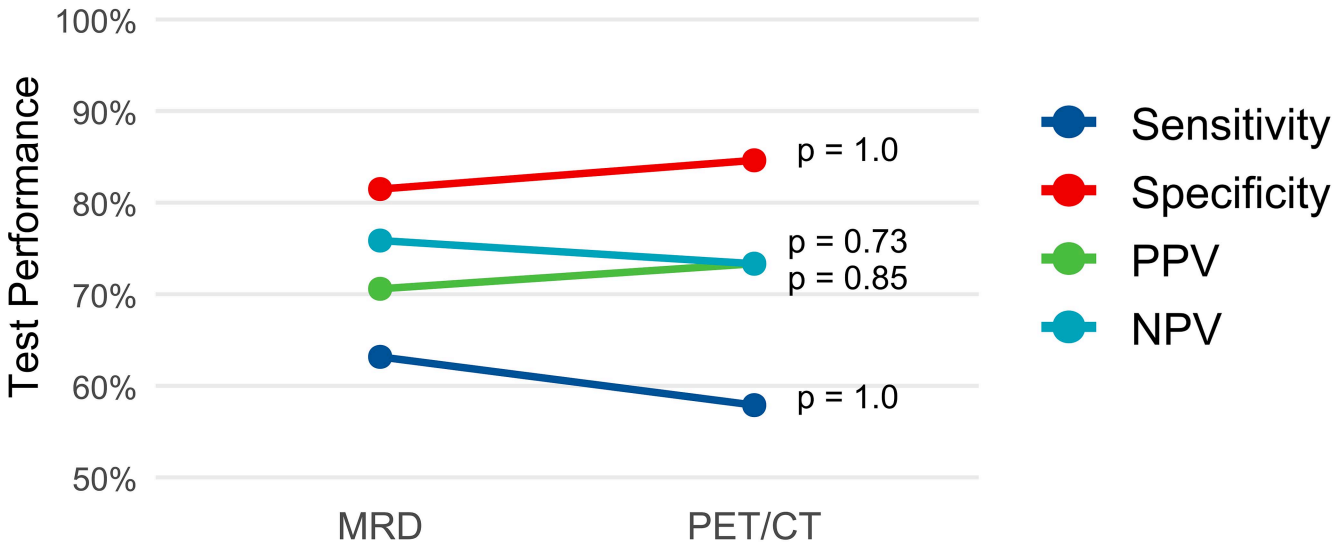


B)

Test performance	EOT MRD	EOT PET/CT	p-value
Sensitivity	64% (95% CI 41-83%)	86% (95% CI 65-97%)	0.063
Specificity	96% (95% CI 88-99%)	85% (95% CI 74-92%)	0.022
PPV	82% (95% CI 57-96%)	63% (95% CI 44-80%)	0.039
NPV	90% (95% CI 80-95%)	95% (95% CI 87-99%)	0.017


A)
Progression free survival
100%
75%
50%
25%
0%
MRD-
MRD+
p < 0.001, HR 5.0 (95% CI 2.0-13.0)
0 3 6 9 12 15 18 21 24
Time from infusion (months)
Number at risk
30 30 28 20 14 7 6 1 1
18 18 8 5 4 4 2 0 0
B)
Progression free survival
100%
75%
50%
25%
0%
PET-
PET+
p = 0.001, HR 4.6 (95% CI 1.8-11.5)
0 3 6 9 12 15 18 21 24
Time from infusion (months)
Number at risk
32 32 28 21 14 7 6 1 1
15 15 7 4 4 4 2 0 0
C)
Progression free survival
100%
75%
50%
25%
0%
MRD-
MRD+
p = 0.036, HR 4.5 (95% CI 1.1-18.3)
0 3 6 9 12 15 18 21 24
Time from infusion (months)
Number at risk
24 24 23 18 12 5 5 1 1
8 8 5 3 2 2 1 0 0
D)
Progression free survival
100%
75%
50%
25%
0%
MRD-
MRD+
p = 0.14, HR 2.8 (95% CI 0.7-10.7)
0 3 6 9 12 15 18 21 24
Time from infusion (months)
Number at risk
5 5 4 2 2 2 1 0 0
10 10 3 2 2 2 1 0 0
E)
Multivariable Cox Regression for PFS
DLBCL histology
p = 0.085
IPI 4-5
p = 0.154
PET/CT positive
p = 0.069
Day 100 MRD detectable
p = 0.002
1 2 5 10
Hazard Ratio (log scale)

A)



B)

Test performance	Day 100 MRD	PET/CT	p-value
Sensitivity	63% (95% CI 38-84%)	58% (95% CI 33-80%)	1.0
Specificity	82% (95% CI 62-94%)	85% (95% CI 65-96%)	1.0
PPV	71% (95% CI 44-90%)	73% (95% CI 45-92%)	0.85
NPV	76% (95% CI 56-90%)	73% (95% CI 54-88%)	0.73

Supplementary methods

Sample criteria and assessment timing

Patients were included in the primary survival analysis if they had primary refractory disease but excluded if MRD was only assessed after progression. For the frontline cohort, an MRD assessment was categorized as EOT if performed between 5 days before to 70 days after day 1 of the final cycle of frontline treatment. MRD timing after frontline treatment was dichotomized as “early” or “late” based on the median time to MRD assessment. The first MRD assessment in the EOT window was used in case of multiple tests. For the CAR-T cohort, the PET/CT scan performed closest to the landmark day 100 MRD assessment was used for comparative analyses when multiple were available during the window. Sensitivity analyses were performed where patients were categorized according to their first MRD assessment between days 28-59 (early) and according to their last MRD assessment between days 60-100 (late), when available.

Frontline treatment categorization

Frontline regimens were categorized as standard dose (rituximab, cyclophosphamide, doxorubicin, prednisone, and vincristine [R-CHOP] or polatuzumab vedotin [R-pola-CHP]), intensive (infusional or cytarabine-containing), reduced-dose (R-miniCHOP), or other.

Cell-of-origin determination

Cell of origin was determined by Hans algorithm and limited to patients with DLBCL NOS, PTLD (DLBCL subtype), and HGBCL.

Table S1. List of Participating Centers

Columbia University Irving Medical Center
Ohio State University
Hackensack University Medical Center
University of Nebraska
University of Virginia
OhioHealth
Levine Cancer Institute, Atrium Health Wake Forest
Washington University in St. Louis
University of Miami
University of California San Francisco
University of Texas, Southwestern
Novant Health Cancer Institute
Northwell Health
Mayo Clinic Arizona
University of Colorado
University of Utah
Medical University of South Carolina

Table S2. Questionnaire on how individual MRD assessment affected clinical decision making

• Any non-treatment decision (i.e. re: biopsy, monitoring) made based on this result? (Y/N) • Explain non-treatment decision (free text)
• Any treatment decision made based on this result? (Y/N) • Explain treatment decision (free text)

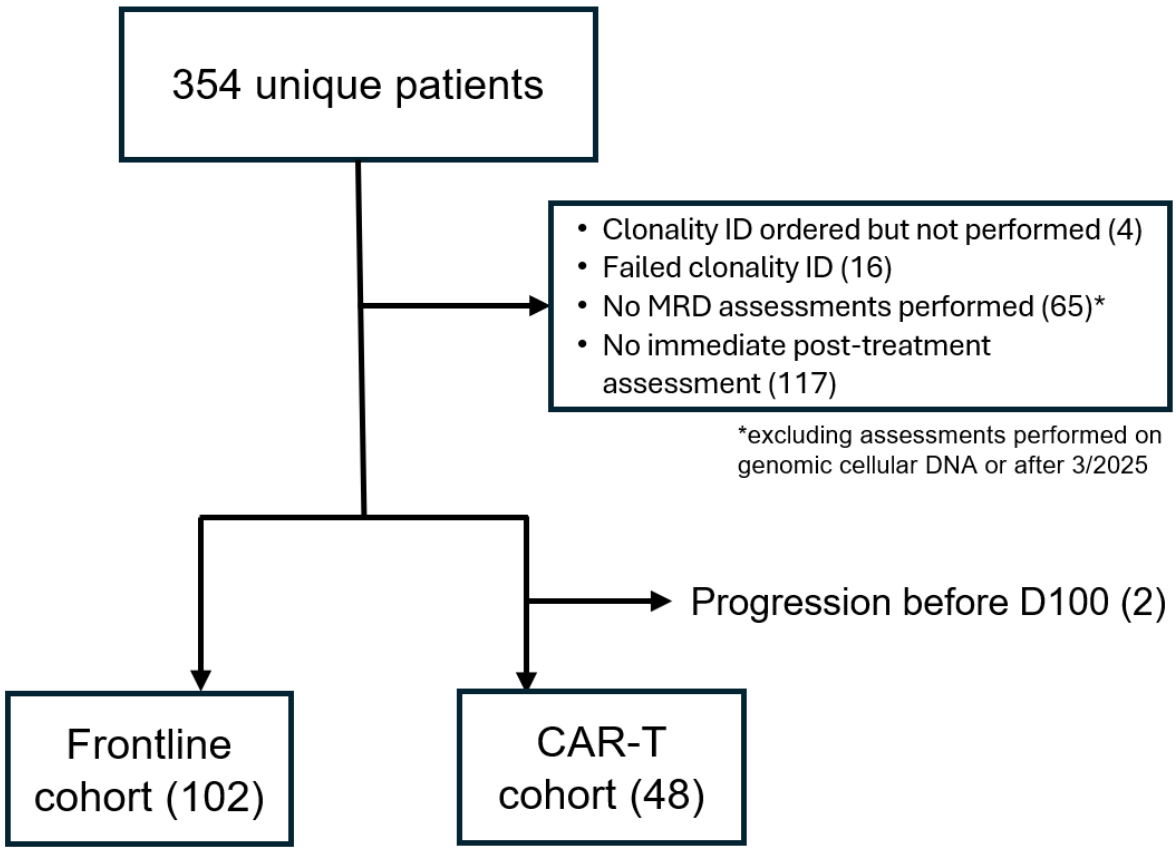


Figure S1. CONSORT flow diagram depicting patient disposition between the entire descriptive cohort and the primary frontline and CAR-T cohorts

Table S3. Characteristics from baseline clonality ID

Clonality ID variables (N=350)	**N (%)**
Clonality ID failure	16 (5)
Reason (N=16)	
Inadequate tissue sample not meeting quality control minimum	7 (44)
No dominant sequence identified (polyclonal)	7 (44)
Unknown	2 (13)
Clonality ID turnaround time (N=334)	
Median (IQR) [range], days	10 (9-13) [3-41]
Baseline clonal immunoglobulin loci present (N=330)	
IgH, IgK, and IgL	55 (17)
IgH and IgK	178 (54)
IgH and IgL	6 (2)
IgK and IgL	3 (1)
IgH only	67 (20)
IgK only	20 (6)
IgL only	1 (0.3)
Number of unique baseline clonal sequences (N=334)	
Median (IQR) [range], sequences	3 (2-4) [1-13]

Table S4. Summary of investigator responses explaining how MRD assessments influenced clinical decision making, divided by MRD status

Clinical decision supported by MRD assessment	**MRD-detected (N=28)**	**MRD-undetected[1] (N=88)**
Additional diagnostic evaluation	8 (29%)	-
More intensive MRD surveillance	7 (25%)	-
New anti-lymphoma treatment	13 (46%)	-
Avoid biopsy	-	47 (53%)
Stop surveillance imaging	-	28 (32%)
Continue current management	-	13 (15%)

[1]Inclusive of MRD-detected tests with a decreasing MRD quantity
MRD, measurable residual disease

Table S5. Description of how MRD-detected assessments supported change in anti-lymphoma treatment

Category (N=13)	N (%)	Summary of management details
Corroborated other clinical evidence of progression of disease (imaging and/or biopsy)	5 (38%)	• ID 215 – "change treatment" • ID 218 – "proceeded to 6th line treatment, Epcoritamab" • ID 223 – "started 2nd line treatment, Epcoritamab" • ID 223 – "referred for CAR-T versus ASCT" • ID 240 – "changed treatment after primary refractory as bridge to CAR-T"
Corroborated imaging evidence of progression of disease with non-diagnostic biopsy	2 (15%)	• ID 49 – "mixed response post CAR-T, biopsy indeterminant, started subsequent therapy when MRD detected" • ID 142 – "biopsy necrotic, proceeded to CAR-T for concerns for refractory disease"
Suggested progression despite remission on imaging	2 (15%)	• ID 65 – "prepared for CAR-T despite CR due to rising MRD" • ID 85 – "Proceed to CAR-T despite borderline CR on PET scan"
Supported consolidation with partial response on imaging	2 (15%)	• ID 100 – "recommended systemic consolidation after CAR-T, patient declined" • ID 194 – "radiation to spleen after ASCT"
Supported consolidation with negative imaging	1 (8%)	• ID 165 – "referral to radiation oncology for ISRT"
Corroborated imaging evidence of progression of disease with unfeasible biopsy	1 (8%)	• ID 9 – "brain MRI with findings concerning for CNS progression, started on Zanubrutinib when detected MRD"

MRD, measurable residual disease; CAR-T, chimeric antigen receptor T-cell; ASCT, autologous stem cell transplant; CR, complete response; PET, position emission tomography; ISRT, involved site radiation therapy; MRI, magnetic resonance imaging; CNS, central nervous system

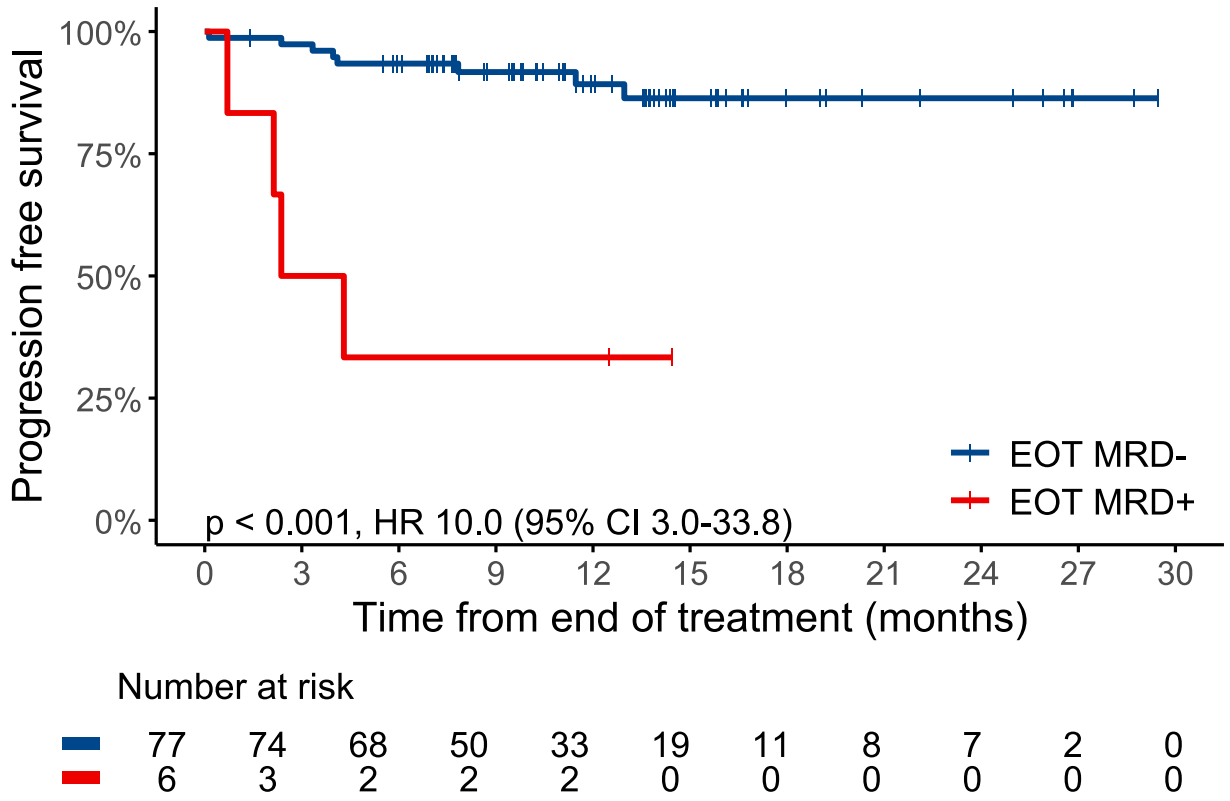


Figure S2. Progression free survival in the frontline cohort stratified by end-of-treatment (EOT) measurable residual disease (MRD) status excluding patients with progressive disease on EOT positron emission tomography/computed tomography assessment

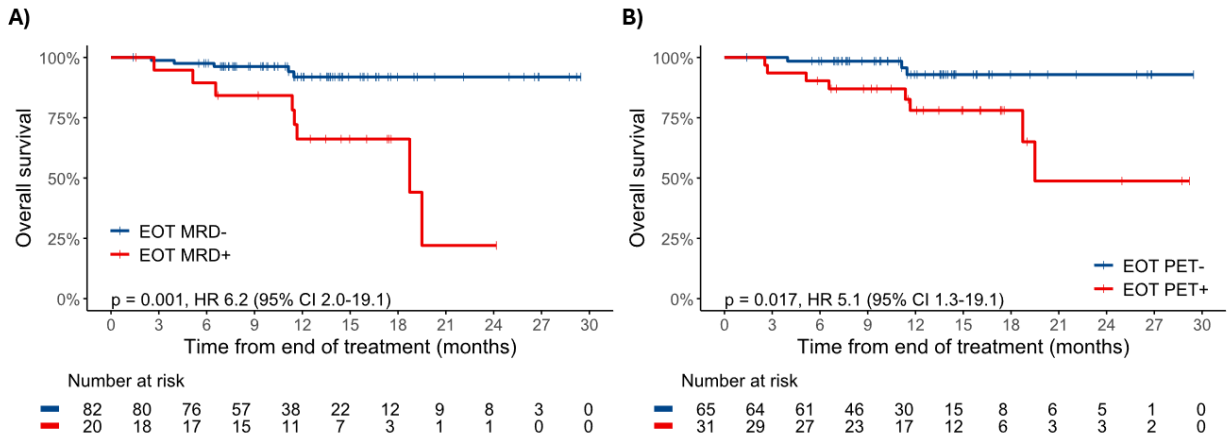


Figure S3. Overall survival in the frontline cohort stratified by end-of-treatment (EOT) measurable residual disease (MRD) status (A) and EOT positron emission tomography/computed tomography (PET/CT) (B)

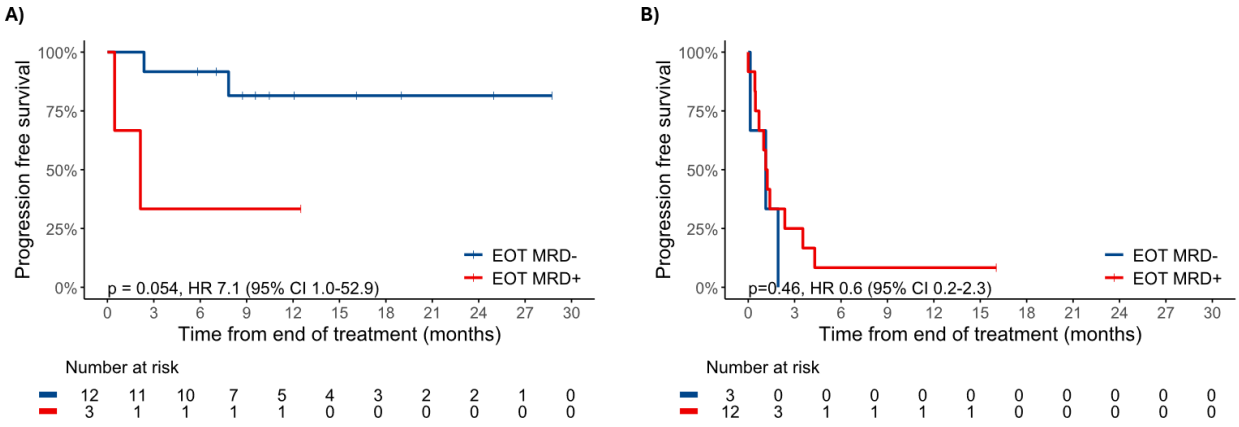


Figure S4. Progression free-survival of patients with a positive end-of-treatment (EOT) positron emission tomography/computed tomography scan from the frontline cohort with a Deauville five-point score of 4 (A) or 5 (B) stratified by EOT measurable residual disease (MRD) status.

Table S6. Univariable Cox Regression Models for PFS in Frontline Cohort

Characteristic	**HR**	**95% CI**	**p-value**
Age > 60	0.56	0.27, 1.18	0.13
Stage III/IV	0.83	0.31, 2.19	0.7
LDH elevated	1.44	0.57, 3.66	0.4
ECOG PS	0.63	0.19, 2.11	0.5
2 or more extranodal sites	1.27	0.59, 2.75	0.5
IPI 4-5	1.55	0.60, 3.99	0.4
Bulky disease ≥7.5 cm	1.87	0.89, 3.92	0.10
De novo secondary CNS lymphoma	1.31	0.18, 9.69	0.8
Non-DLBCL histology	4.31	2.00, 9.27	<0.001
MYC and *BCL2* rearranged	1.16	0.40, 3.35	0.8
Non-germinal center B-cell cell of origin	0.41	0.14, 1.26	0.12
First-Line Treatment			
Standard	—	—	—
Intensive	2.33	1.04, 5.21	0.039
R-miniCHOP	1.16	0.26, 5.24	0.8
Other	2.58	0.57, 11.8	0.2
MRD detectable	14.5	6.66, 31.7	<0.001
Positive EOT PET/CT	12.8	4.75, 34,5	<0.001

PFS, progression-free survival; HR, hazard ratio; CI, confidence interval; LDH, lactate dehydrogenase; ECOG PS, Eastern Cooperative Oncology Group performance status; IPI, international prognostic index; CNS, central nervous system; DLBCL, diffuse large B-cell lymphoma; MRD, measurable residual disease; EOT, end-of-treatment; PET/CT, positron emission tomography/computed tomography

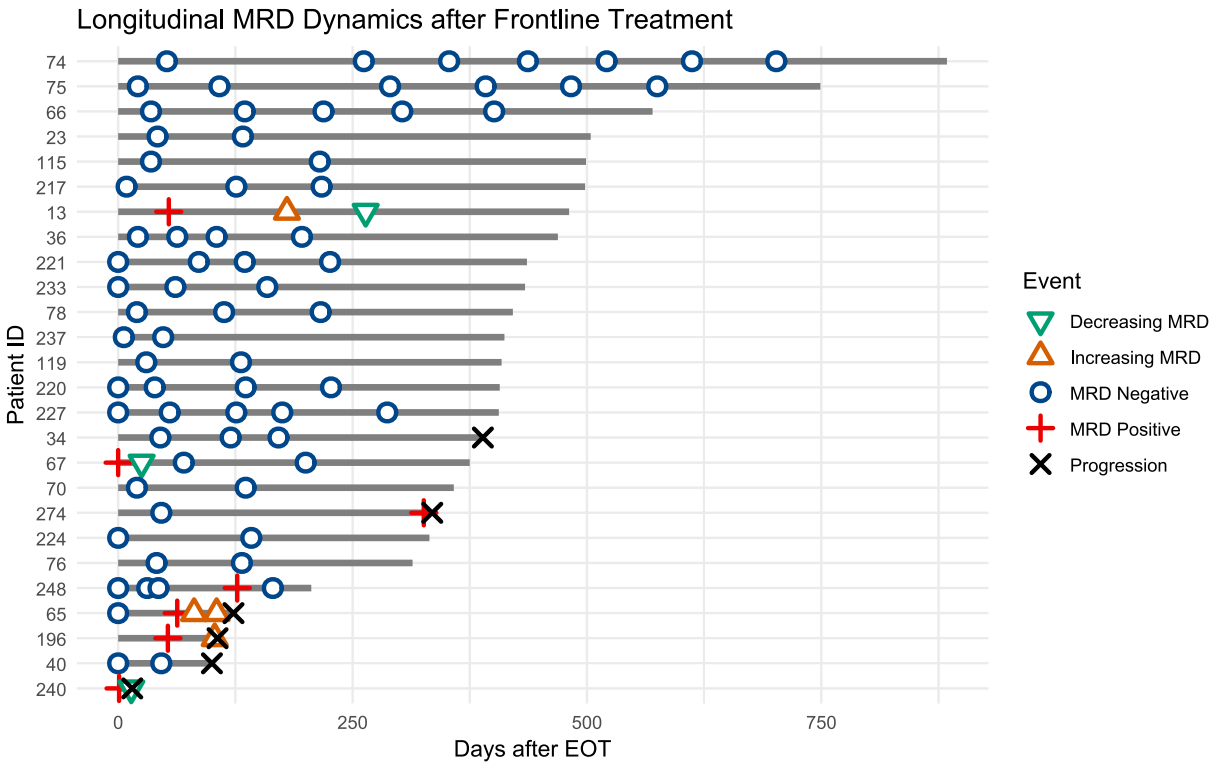


Figure S5. Swimmer's plot for the frontline cohort, highlighting longitudinal measurable residual disease (MRD) dynamics. MRD positive (red cross) refers to the first MRD positive test after treatment. Decreasing or increasing MRD (green or orange triangles) are relative to the MRD level of the prior test. 22 patients were EOT MRD negative: on longitudinal assessment, 17 were persistently MRD negative without progression, 2 were persistently MRD negative but progressed 54 and 218 days after their last MRD assessment, 2 became MRD positive 9 and 60 days before progression, and one was transiently MRD positive on one assessment without progression. 4 patients were EOT MRD positive: 2 were persistently MRD positive and progressed, one became MRD negative after 70 days without progression, and one had fluctuating MRD levels without progression.

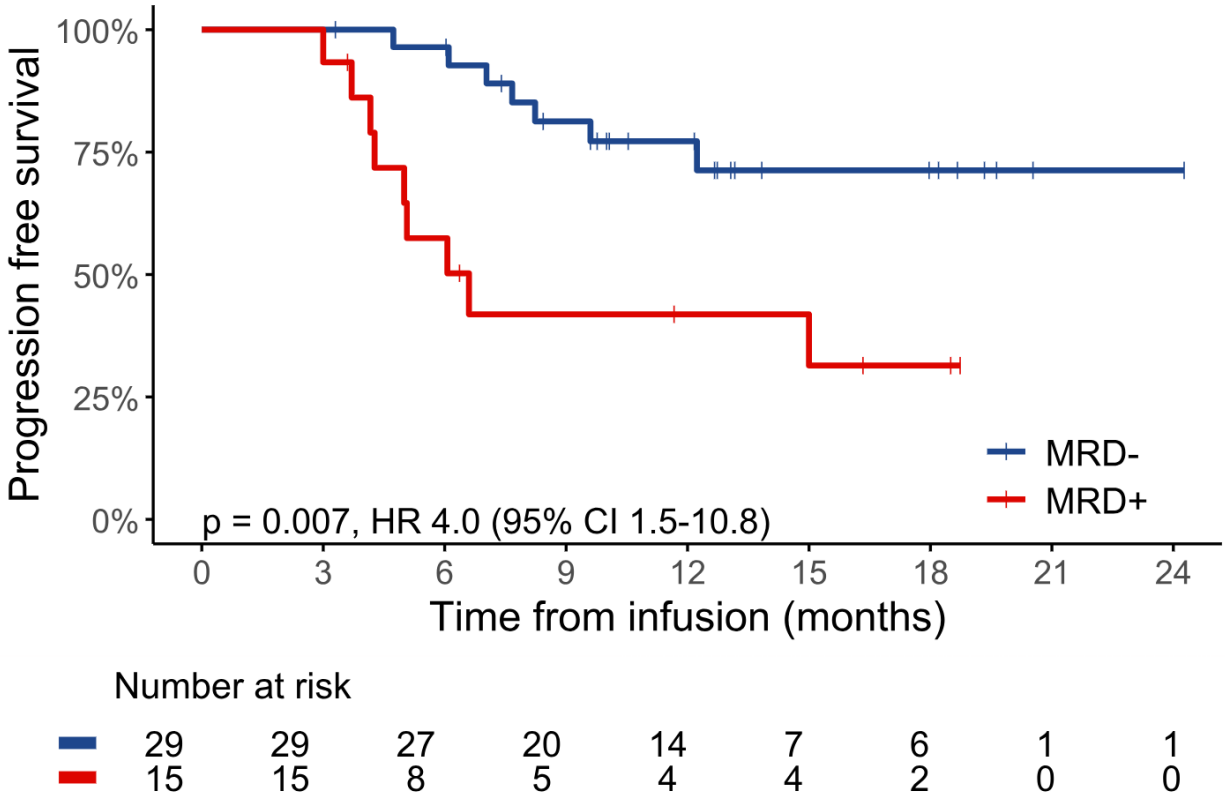


Figure S6. Progression free survival in the CAR-T cohort stratified by day 100 measurable residual disease status, excluding patients with progressive disease on positron emission tomography/computed tomography assessment

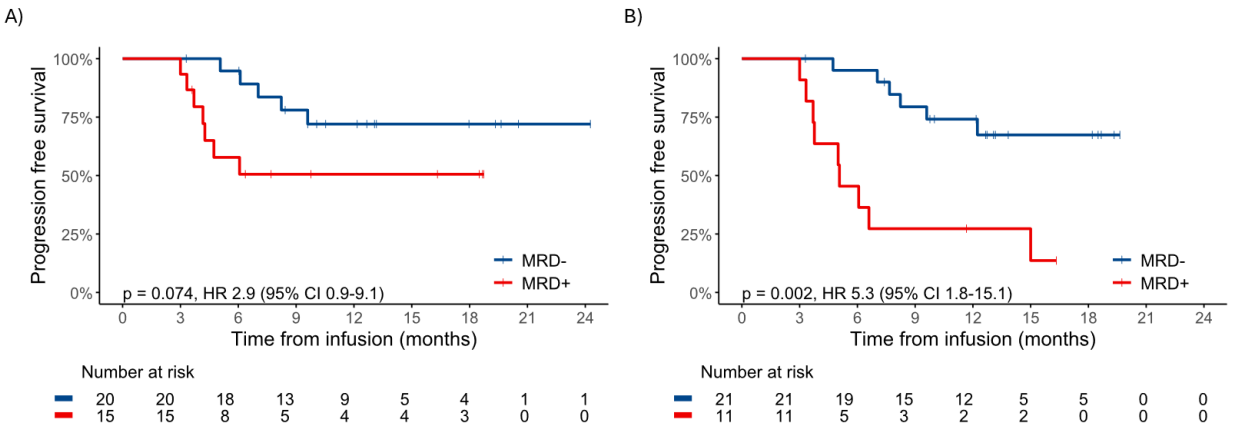


Figure S7. Progression free survival in the CAR-T cohort, stratified by (A) measurable residual disease status by the first assessment during the early post-infusion period (days 28-59) when available, and by (B) measurable residual disease status by the last assessment during the late post-infusion period (days 60-100) when available.

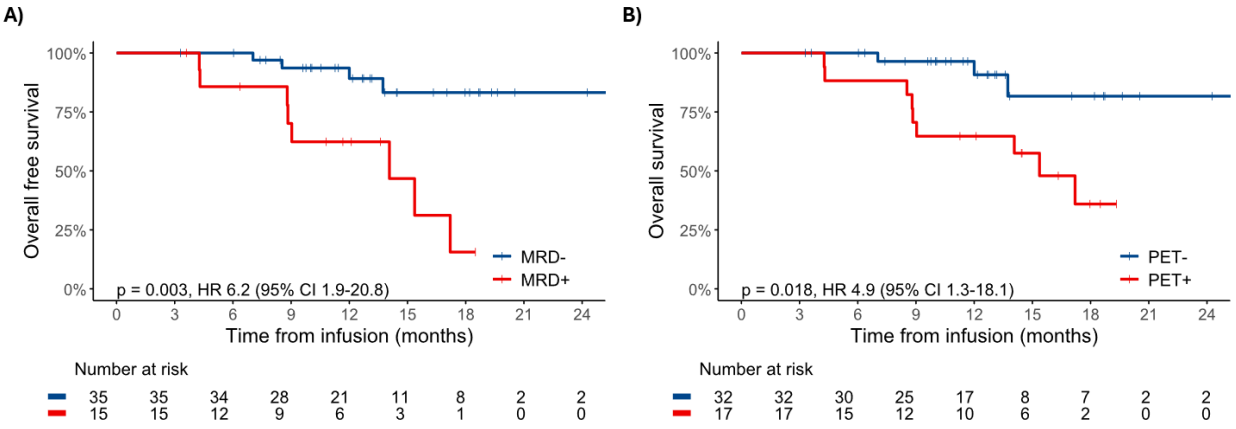


Figure S8. Overall survival in the CAR-T cohort, stratified by day 100 measurable residual disease status (A) and positron emission tomography/computed tomography (PET/CT) response (B).

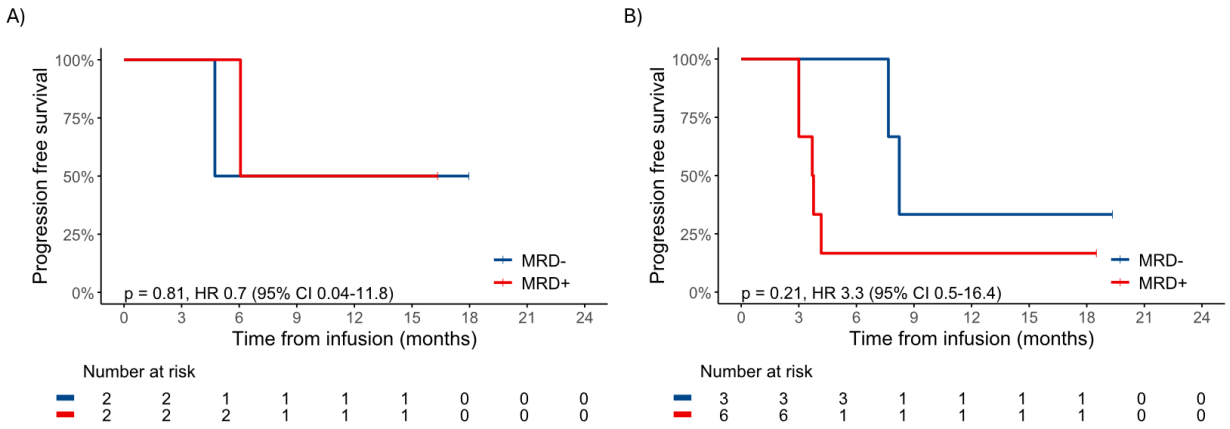


Figure S9. Progression free-survival of patients with a positive positron emission tomography/computed tomography scan from the CAR-T cohort with a Deauville five-point score of 4 (A) or 5 (B) stratified by day 100 measurable residual disease (MRD) status.

Table S7. Univariable Cox Regression Models for PFS in CAR-T Cohort

Characteristic	HR	95% CI	p-value
Age >60	1.82	0.69, 4.79	0.23
Stage III/IV	2.32	0.77, 7.00	0.14
LDH elevated	2.12	0.76, 5.89	0.15
ECOG PS 2 or greater	3.94	1.36, 11.5	0.012
2 or more extranodal sites	2.24	0.90, 5.58	0.084
IPI 4-5	6.18	1.73, 22.1	0.005
Bulky disease ≥7.5 cm	1.83	0.69, 4.85	0.22
Prior CNS disease	1.58	0.52, 4.77	0.42
Non-DLBCL histology	0.34	0.10, 1.18	0.088
MYC and *BCL2* rearranged	1.60	0.47, 5.51	0.45
Non-germinal center B-cell cell of origin	1.64	0.63, 4.26	0.31
Refractory disease prior to CAR-T	1.71	0.61, 4.82	0.31
Bridging therapy	3.44	0.46, 25.8	0.23
4-1BB co-stimulatory domain	2.85	1.08, 7.51	0.034
MRD detectable at first early assessment (d28-59)	2.87	0.90, 9.11	0.074
MRD detectable at last late assessment (d60-100)	5.28	1.84, 15.1	0.002
MRD detectable up to day 100 landmark	5.03	1.95, 13.0	<0.001
Positive PET/CT	4.58	1.83, 11.5	0.001

PFS, progression-free survival; CAR-T, chimeric antigen receptor T-cell; HR, hazard ratio; CI, confidence interval; LDH, lactate dehydrogenase; ECOG PS, Eastern Cooperative Oncology Group performance status; IPI, international prognostic index; CNS, central nervous system; DLBCL, diffuse large B-cell lymphoma; PET/CT, positron emission tomography/computer tomography

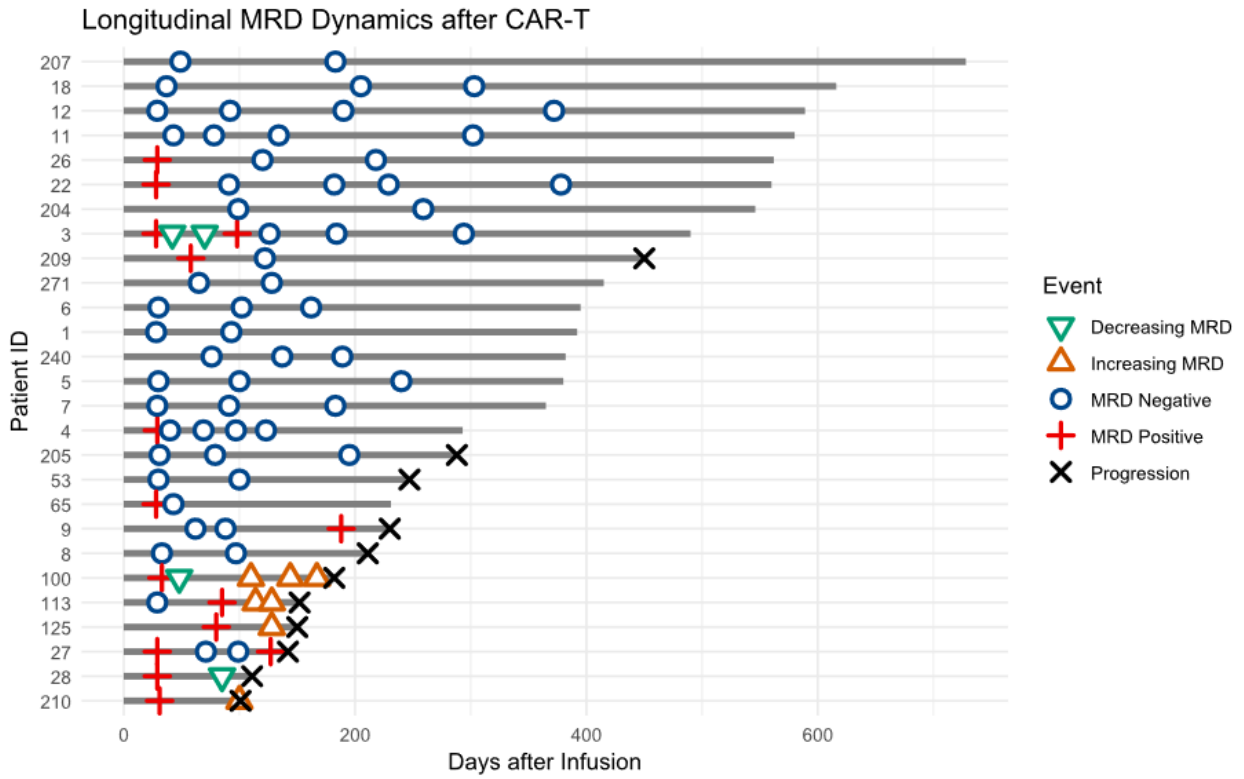


Figure S10. Swimmer's plot for the CAR-T cohort, highlighting longitudinal measurable residual disease (MRD) dynamics. MRD positive (red cross) refers to the first MRD positive test after treatment. Decreasing or increasing MRD (green or orange triangles) are relative to the MRD level of the prior test. 16 patients were MRD negative on the first post-infusion assessment: on longitudinal assessment, 11 were persistently MRD negative without progression, 3 were persistently MRD negative but progressed 93, 114, and 147 days after their last MRD assessment, and two patients became MRD positive 42 and 67 days before progression. 11 patients were MRD positive on their first post-infusion assessment: 5 patients became MRD negative without progression, 4 patients were persistently MRD positive and all progressed, one patient became transiently MRD negative but becoming MRD positive again 15 days before progression, and one patient became MRD negative but progressed 328 days after their last MRD assessment.