Received: 29 November 2024 | Accepted: 16 March 2025

DOI: 10.1002/hem3.70139

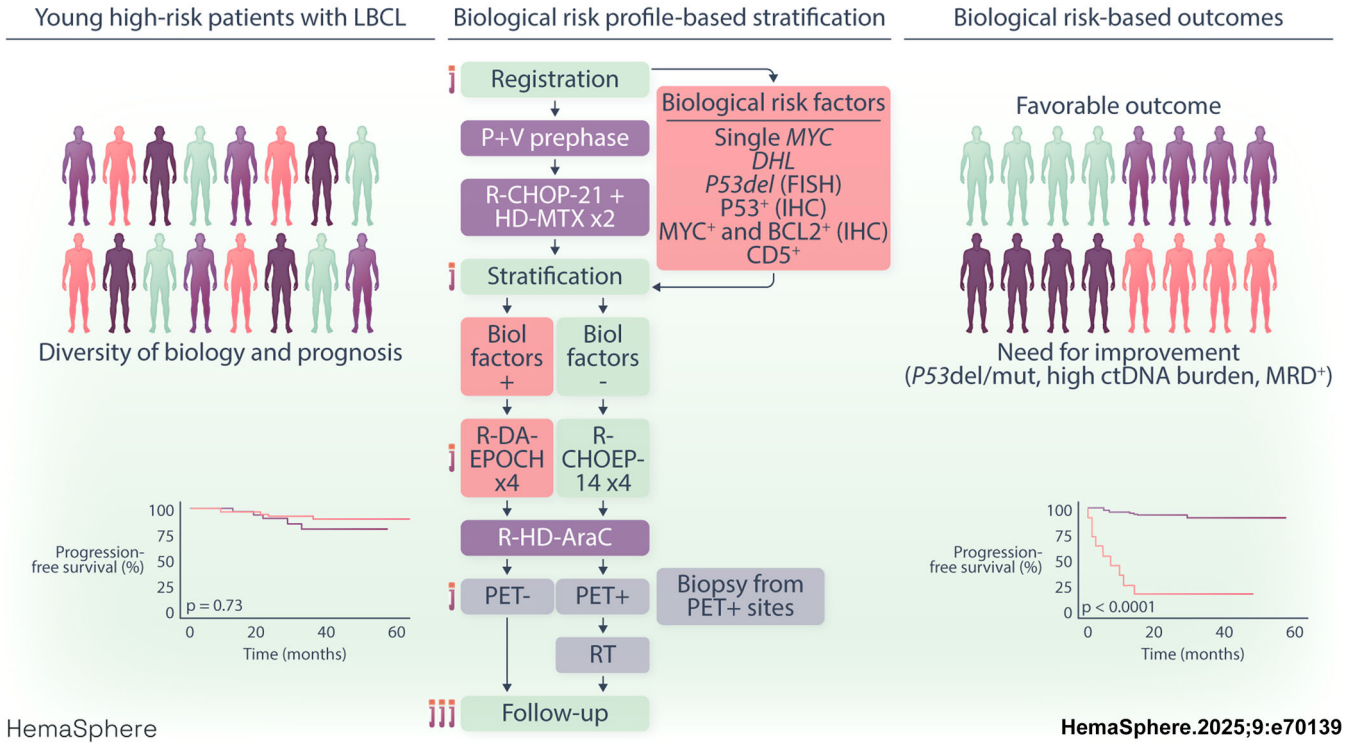
HemaSphere eha

ARTICLE

Biomarker-adapted treatment in high-risk large B-cell lymphoma

Sirpa Leppä[1,2] | Leo Meriranta[1,2] | Maare Arffman[1,2] | Judit Jørgensen[3] | Marja-Liisa Karjalainen-Lindsberg[4] | Klaus Beiske[5,6] | Mette Pedersen[7,8] | Kristina Drott[9] | Annika Pasanen[1,2] | Kristiina Karihtala[1,2] | Susanna Mannisto[1,2] | Bente Wold[10] | Marianne Brodtkorb[10] | Unn-Merete Fagerli[11,12] | Thomas Stauffer Larsen[13] | Lars Munksgaard[14] | Kaisa Sunela[15] | Øystein Fluge[16] | Sirkku Jyrkkiö[17] | Peter Brown[18] | Harald Holte[10,19]

Graphical Abstract



Received: 29 November 2024 | Accepted: 16 March 2025

DOI: 10.1002/hem3.70139

ARTICLE

HemaSphere eha

Biomarker-adapted treatment in high-risk large B-cell lymphoma

Sirpa Leppä[1,2] | Leo Meriranta[1,2] | Maare Arffman[1,2] | Judit Jørgensen[3] | Marja-Liisa Karjalainen-Lindsberg[4] | Klaus Beiske[5,6] | Mette Pedersen[7,8] | Kristina Drott[9] | Annika Pasanen[1,2] | Kristiina Karihtala[1,2] | Susanna Mannisto[1,2] | Bente Wold[10] | Marianne Brodtkorb[10] | Unn-Merete Fagerli[11,12] | Thomas Stauffer Larsen[13] | Lars Munksgaard[14] | Kaisa Sunela[15] | Øystein Fluge[16] | Sirkku Jyrkkiö[17] | Peter Brown[18] | Harald Holte[10,19]

Correspondence: Sirpa Leppä (sirpa.leppa@helsinki.fi)

Abstract

Survival rates for patients with high-risk large B-cell lymphoma (LBCL), particularly those with biological risk factors, remain inadequate. We conducted a biomarker-driven phase II trial involving 123 high-risk patients aged 18–64 with LBCL. Based on their biological risk profiles, patients received either R-CHOEP-14 (without risk factors) or DA-EPOCH-R-based regimens (with risk factors). Biological high-risk factors included *C-MYC* translocation, *C-MYC* and *BCL2* co-translocation, *17p/TP53* deletion, co-expression of MYC and BCL2, and P53 and/or CD5 immunopositivity. Additionally, we evaluated circulating tumor DNA (ctDNA) kinetics during therapy. Sixty-one patients (50%) were classified into biologically high-risk group. Three-year failure-free survival and overall survival rates for the entire study population were 79% and 88%, respectively. DA-EPOCH-R did not improve survival compared to our previous trial, where patients with the same biological risk factor criteria received R-CHOEP-14-based therapy. High pretreatment ctDNA levels, *17p/TP53* deletion, and *TP53* mutations were associated with worse outcomes. In contrast, ctDNA negativity at the end of therapy (EOT) was indicative of a cure and effectively addressed false residual PET positivity. The findings demonstrate promising survival for high-risk LBCL patients, aside from those with *TP53* aberrations, high ctDNA levels, and/or EOT ctDNA positivity.

INTRODUCTION

Large B-cell lymphomas (LBCLs) encompass a diverse group of aggressive lymphoid cancers.[1,2] The most prevalent form, comprising over 80% of all LBCLs, is diffuse LBCL not otherwise specified (DLBCL NOS), which is further categorized into germinal center B-cell (GCB) and activated B-cell (ABC) subtypes. Around 10% of LBCLs are high-grade B-cell lymphomas (HGBLs) with *MYC* and *BCL2* gene rearrangements, also known as double-hit lymphomas (DHLs).

[1]Department of Oncology, Helsinki University Hospital Comprehensive Cancer Centre, Helsinki, Finland
[2]Research Programs Unit and iCAN Flagship, University of Helsinki, Helsinki, Finland
[3]Department of Hematology, Aarhus University Hospital, Aarhus, Denmark
[4]Department of Pathology, Helsinki University Hospital, Helsinki, Finland
[5]Department of Pathology, Oslo University Hospital, Oslo, Norway
[6]Institute of Clinical Medicine, Medical Faculty, University of Oslo, Oslo, Norway
[7]Department of Pathology, Zealand University Hospital, Roskilde, Denmark
[8]Department of Clinical Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark
[9]Department of Oncology, Skane University Hospital, Lund, Sweden
[10]Department of Oncology, Oslo University Hospital, Oslo, Norway
[11]Department of Oncology, St. Olavs Hospital, Trondheim, Norway
[12]Institute of Clinical and Molecular Medicine, NTNU, Trondheim, Norway
[13]Department of Hematology, Odense University Hospital, Odense, Denmark
[14]Department of Hematology, Zealand University Hospital, Roskilde, Denmark
[15]Department of Oncology, Tampere University Hospital, Tampere, Finland
[16]Department of Oncology, Haukeland University Hospital, Bergen, Norway
[17]Department of Oncology, Turku University Hospital, Turku, Finland
[18]Department of Hematology, Rigshospitalet, Copenhagen, Denmark
[19]KG Jebsen Centre for B-cell malignancies, Oslo, Norway

Primary LBCLs have the potential to be cured with rituximab, cyclophosphamide, doxorubicin, and prednisone (R-CHOP) or other similar immunochemotherapy regimens, even in advanced stages and across all age groups. However, 30%–40% of patients relapse and die from lymphoma.[3] Since the advent of rituximab, efforts to enhance R-CHOP immunochemotherapy through subtype-targeted or risk-adapted strategies have not significantly improved overall survival (OS). For instance, the Intergroup phase III trial showed that dose-adjusted etoposide, doxorubicin, cyclophosphamide, vincristine, prednisone, and rituximab (DA-EPOCH-R) was not superior to R-CHOP-21 and was associated with increased toxicity.[4] Similarly, rituximab combined with hyperfractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone (R-HCVAD) alternating with rituximab, high-dose methotrexate, and cytarabine (R-MA) did not outperform R-CHOP in terms of survival due to high treatment-related mortality.[5] In the phase III POLARIX study, polatuzumab vedotin, an antibody-drug conjugate targeting CD79b, combined with rituximab, cyclophosphamide, doxorubicin, and prednisone (Pola-R-CHP), improved progression-free survival (PFS) but not OS compared to R-CHOP.[6]

For young, clinically high-risk DLBCL patients, an optimal therapy has not been established. Comparisons of high-dose therapy and autologous stem cell transplantation (HDT-ASCT) with conventional chemotherapy have not convincingly favored HDT-ASCT.[7] Likewise, R-Mega-CHOEP has not been superior to R-CHOEP-14 and carries significantly more toxicity.[8] However, Nordic population-based studies have suggested that adding etoposide to the R-CHOP-14 regimen improves OS among young, high-risk patients.[9,10] Additionally, results from previous Nordic Lymphoma Group (NLG) phase II studies targeting young, clinically high-risk LBCL patients demonstrated 70%–90% PFS and OS in response to R-CHOEP-14 combined with systemic central nervous system (CNS) targeted therapy.[11,12] However, biological risk was not considered in any of these studies.

Survival among patients with biologically high-risk LBCL, particularly those with *BCL2* and *MYC* translocations (DHLs), co-expression of BCL2 and MYC, CD5 positivity, and *TP53* aberrations, remains suboptimal when treated with R-CHOP-like immunochemotherapy.[13–23] This trial aimed to evaluate the feasibility and effectiveness of a biologically risk-adapted intensified treatment strategy for young patients with high-risk LBCL.

METHODS

Study design and participants

We conducted an open-label, single-arm, phase 2 trial (NLG-LBC-06; Bio-CHIC) across 14 hospital sites in Denmark, Finland, Norway, and Sweden. Eligible patients were 18–64 years old with previously untreated, histologically confirmed CD20 + LBCL or follicular lymphoma (FL) grade 3B based on the WHO 2016 Lymphoma Classification.[24] The following LBCL entities or variants were allowed: DLBCL NOS, ALK-positive LBCL, intravascular LBCL, T-cell/histiocyte-rich LBCL, HGBL without or with *MYC* and *BCL2* or *BCL6* rearrangements (double hit, DH), or with *MYC* and *BCL2* and *BCL6* rearrangements (triple hit, TH), and DLBCL with previously undiagnosed concurrent small cell infiltration in bone marrow, lymph node, or extranodal site. Primary mediastinal B-cell lymphoma, post-transplantation lymphoma, transformed lymphoma after previously diagnosed indolent lymphoma and primary CNS lymphoma were ineligible.

Patients had to present WHO performance status <4 without clinical, radiological, or cytological signs of CNS involvement, while occult cerebrospinal fluid (CSF) involvement (flow cytometry+/cytology−) was allowed. Patients had to present at least stage II disease with age-adjusted International Prognostic Index (aaIPI) of 2 or 3, and/or specific risk factors for CNS recurrence defined by more than one extranodal site, testicular lymphoma, stage IIE and higher, paranasal sinus and orbital lymphoma with the destruction of bone, or large cell infiltration of the bone marrow. Organ function had to be adequate, allowing the planned treatment schedule. Additional details on inclusion and exclusion criteria, and study procedures are provided in the Supporting Information.

Biological high risk was defined as the presence of at least one of the following factors: *C-MYC* translocation, DH, *17p/TP53* deletion by fluorescent in situ hybridization (FISH), or immunohistochemically (IHC)-defined co-expression of MYC and BCL2 (double protein expression, DPE), IHC-defined p53 and/or CD5 positivity. The biological low-risk group did not have any of the above-listed factors.

The historical comparator cohort consisted of young (18–64 years), clinically high-risk LBCL patients treated in the Nordic LBC-05 trial with similar inclusion criteria.[12]

This study was conducted by the Guidelines on Good Clinical Practice from the International Conference on Harmonization and the principles of the Declaration of Helsinki. The protocol was approved by the medical agencies and ethics committees in Finland, Denmark, Norway, and Sweden, and the trial was registered at ClinicalTrials.gov (number NCT01325194). All patients signed informed consent before study participation.

Pathology and stratification

Patients were included in the study based on a histological diagnosis from the local pathologists. After inclusion, diagnostic tissue samples were forwarded to the National Pathology Review representatives (MP, KB, M-LK-L) for the confirmation of the diagnosis, further subclassification into the two immunohistochemically defined subgroups of germinal center B-cell (GCB) type and non-germinal center B-cell (non-GCB) type according to Hans algorithm,[25] and stratification to biological risk groups. IHC stainings and FISH analyses with break-apart probes for *c-MYC*/8q24, *BCL2*/18q21, and *BCL6*/3q27 translocations, and a probe for *17p/TP53* deletion were performed according to routine diagnostic procedures.

In the LBC-05 comparator cohort,[12] the biological high-risk group was determined retrospectively.

ctDNA analysis

Plasma samples were collected at multiple time points during the therapy and follow-up (Figure 1A). Whole blood was used as a matched control. Cell-free DNA (cfDNA) extraction was performed as previously described.[26] Purified cfDNA was constructed to sequencing libraries using IDT xGEN Duplex Seq adapters (Integrated DNA Technologies, Coralville, Iowa, US). Target enrichment was performed using biotinylated probes for genomic targets covering common lymphoma driver genes, regions of somatic hypermutations, and immunoglobulin loci, and captured libraries were sequenced on the Novaseq 6000 (Illumina) instrument. Diagnostic tumor tissue-derived DNA was used for variant calling together with pretreatment circulating tumor DNA (ctDNA) when available. The minimal residual disease (MRD) test was based on duplex sequencing and was calibrated to 95% specificity using cfDNA from healthy donor samples (n = 10). A detailed methodology is provided in the Supporting Information.

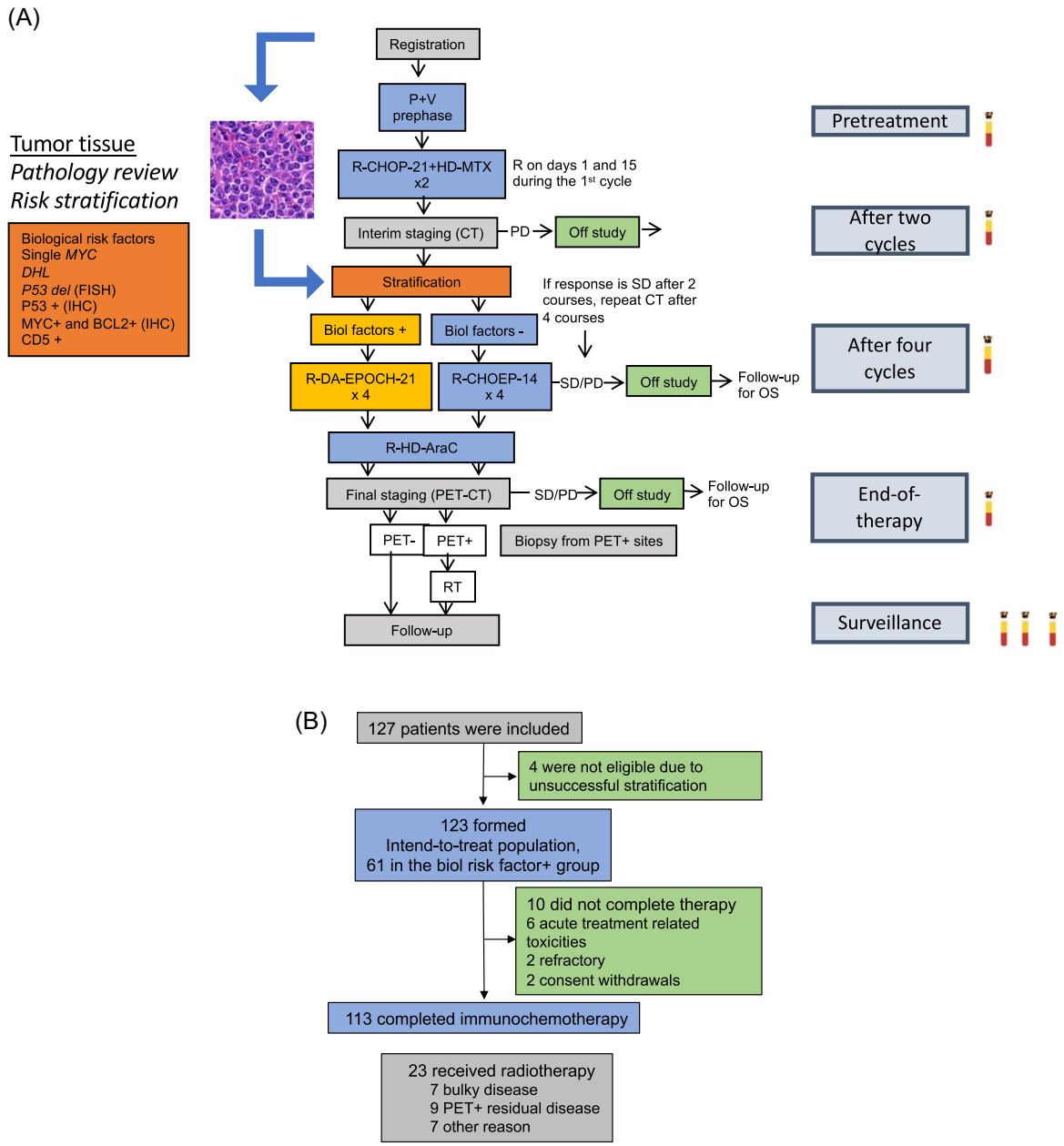


FIGURE 1 **Study schema. (A)** Trial profile and sampling schedule. **(B)** Patient disposition. CT, computer tomography; DHL, double hit lymphoma; IHC, immunohistochemistry; OS, overall survival; P, prednisone; PD, progressive disease; PET, positron emission tomography; RT, radiotherapy; SD, stable disease; V, vincristine.

Treatment

Treatment consisted of a prephase (prednisone 100 mg x 1 for 3–6 days, vincristine 1,0 mg), seven chemotherapy courses, and eight doses of rituximab (Figure 1A). After a prephase, all patients received two courses of R-CHOP-21 with interpolated HD-Mtx 3000 mg/m^2 on day 15 with one additional dose of rituximab on day 15 during course 1. Depending on the biological risk factors, the treatment was continued with either four courses of R-CHOEP-14 (no risk factors; R-CHOP-14 and etoposide; Table S1), or four DA-EPOCH-R courses in three-week intervals (Table S1). The rationale for selecting DA-EPOCH-R for patients with biological risk factors included evidence of less tumor resistance with prolonged exposure times, less cardiac toxicity with prolonged doxorubicin administration, and maximization of dose intensity by pharmacodynamic dose adjustment based on each cycle's neutrophil nadir. Additionally, all patients received one course of R-HD-cytarabine 12 g/m^2 (in patients aged 59 years or younger) or 8 g/m^2 (in patients 60–64 years) divided into 4 separate doses during days 1 and 2. Radiotherapy was given according to the local guidelines and was not considered an event for failure-free survival (FFS) or PFS.

The relative dose (RD) for each chemotherapeutic agent was the ratio of dose received to protocol dose. The RD intensity (RDI) was the RD times stipulated protocol time divided by elapsed time for a given patient.

In the historical comparator group, there was no prospective stratification according to biological risk factors, and all patients were treated with the same systemic immunochemotherapy regimen as the biological low-risk group. In addition, they received liposomal

cytarabine intrathecally in courses 1, 3, and 5.[12] Detailed information on treatment is provided in the Supporting Information.

Outcomes

The primary endpoint was 3-year FFS of the patients with biological risk factors compared to a similar biological risk profile of patients from the previous NLG-LBC-05 study.[12] FFS was defined as an interval between the registration date and the date of documented progression or lack of response, first relapse, death from any reason, or discontinuation/change of therapy because of toxicity, whichever occurred first. Secondary hematologic malignancies (leukemia and MDS) were considered events in this category. Otherwise, patients were censored at the last date they were known to be alive. For patients not responding at any time point on study treatment, FFS was defined as 1 day.

Of the secondary endpoints, OS was defined as the interval between the registration date and death from any cause, and PFS was defined as the period between the registration date and lymphoma progression or death from any cause. Time to CNS relapse was defined as the interval between the registration date and the date of documented CNS progression. Other secondary endpoints were response rate, toxicity, and molecular correlates for survival. Exploratory analyses included variables associated with outcome, including predefined biological risk factors in the tumor tissue and ctDNA burden at baseline and end of therapy (EOT).

Statistical analyses

The study aimed to assess FFS for all patients included in the trial and separately for those with and without biological risk factors. Three-year FFS rate of the patients with biological risk factors compared to similar patients from the previous Nordic study was a preplanned primary objective.

At the time of study design, 3-year FFS for biological high-risk LBCL patients with aaIPI ≥ 2 after standard therapy was estimated to be no better than 50%, based on historical data.[11] One-third of the patients were estimated to have biological high-risk profiles. If a total of 120 patients were included, approximately 40 patients would be in the biological high-risk group. We hypothesized that 3-year FFS for this group is 75%, and thus the standard error of the estimate is the square root of $0.75 \times 0.25/40 = 0.068$. When we further hypothesized two groups, one with standard therapy and the other with new protocol treatment with 40 biological high-risk patients in each group, the probability for detecting a difference in FFS of 0.25 (0.5 vs. 0.75) with one-sided alfa = 0.05 was 0.75 (=beta). It was anticipated that the patient accrual (120 eligible and evaluable patients) would require 3 years.

Descriptive statistics were used to summarize patient demographics and baseline characteristics. Survival rates were estimated using the Kaplan–Meier method. Clinical and tumor-related factors were analyzed by chi-square tests or non-parametric trend tests for response rates, log-rank tests, and the Cox proportional hazards multivariate analysis for survival. Statistical analyses were performed with SPSS v. 25.0 (IBM, Armonk, NY, USA) or in the R environment (version ≥ 3.6.1). Probability values below 0.05 were considered statistically significant. All comparisons and all comparative tests were two-tailed.

RESULTS

Patient demographics and characteristics

We recruited 127 previously untreated patients aged 18–64 years between August 2017 and January 2021. After a central pathology review, four cases were excluded due to unsuccessful stratification, leaving 123 evaluable patients in the intent-to-treat population (Figure 1B). The distribution of patients according to baseline demographics is presented in Table 1. The clinical characteristics were consistent with those typical of high-risk LBCL. The median age of the patients was 55 years (range: 19–64). Notably, 84 patients (68%) presented with stage IV disease, 72 patients (59%) reported B-symptoms, 107 patients (87%) had elevated lactate dehydrogenase (LDH) levels, and 46 patients (37%) exhibited bulky lesions (>7.5 cm). Twelve patients (9%) with low aaIPI were included per protocol due to site-specific risk factors for CNS relapse. Adverse clinical risk factors were not more prevalent in the biological high-risk group; in fact, a higher proportion of patients with poor performance status was found in the biological low-risk group ($p = 0.016$). Otherwise, there were no significant differences in baseline characteristics between the biologically low- and high-risk groups (Table S2).

TABLE 1 Patient and tumor characteristics.

Baseline demographic	*n* (%)
Age (years), median (range)	55 (19–64)
Sex	
Male	70 (57)
Female	53 (43)
PS ECOG > 1	35 (28)
Stage	
I–II	12 (10)
III	27 (22)
IV	84 (68)
B-symptoms	72 (59)
LDH□	107 (87)
aaIPI	
0–1	12 (9)
2	76 (62)
3	35 (29)
Bulky disease	46 (37)
DLBCL NOS	102 (83)
GCB	47 (46)
Non-GCB	54 (53)
Unclassified	1 (1)
DHL/THL	14 (11)
TCRBCL	4 (3.3)
FL 3B	3 (2.4)
Biol high risk+ (All)	61 (50)
MYC-SH	20 (16)
MYC-BCL2 DH	11 (8.9)
MYC-BCL6 DH	1 (0.8)
MYC-BCL2-BCL6 TH	2 (1.6)
17p/p53 del/not available	19 (17)/4
MYC-BCL2 DPE	39 (32)
TP53 IHC+	17 (14)
CD5+	8 (7)

Most of the patients were diagnosed with DLBCL NOS ($n = 102$; 83%). According to the Hans algorithm, 47 patients (46%) were classified as germinal center B (GCB), while 54 patients (53%) were identified as non-GCB DLBCLs; 1 patient (1%) could not be classified. Additionally, 14 patients (11%) had HGBL with DH or TH characteristics. Translocations involving c-MYC on chromosome 8q24 were detected in 20 patients (16%), and *17p/TP53* deletions were found in 19 samples (17%). p53+, CD5+, and BCL2/MYC DPE were observed in 17 (14%), 8 (7%), and 39 (32%) samples, respectively. Overall, 61 patients (50%) exhibited biological high-risk profiles (Table 1). The number and distribution of biological risk factors per patient (median 2, range 1–5) are shown in Figure S1.

Treatment outcomes

Patient disposition is shown in Figure 1B. A total of 10 patients discontinued treatment due to various reasons: toxicity ($n = 6$), refractory disease ($n = 2$), or withdrawal of consent ($n = 2$). Most patients ($n = 113$; 92%) completed all treatment courses. The median duration of the first and second cycles was 21 days. HD-Mtx was not given to five (4.1%) and six (4.9%) patients in the first and second cycles due to adverse events. The second and third cycles were delayed after previous HD-Mtx by over 7 days in four (3.4%) and 6 (5.3%) patients. The maximum dose level (DL) achieved with DA-EPOCH-R was DL1 in 22 (37.3%), DL2 in 18 (30.5%), DL3 in 13 (22%), and DL4 in 6 (10.2%) patients. The RDI based on all courses given was high in the R-CHOEP arm (Table 2), and the RDI reductions were mainly due to a mean prolonged treatment duration of 6 days. The RDI was somewhat lower in the DA-EPOCH-R arm, mainly due to the low proportion of patients achieving the highest DL.

Local radiotherapy was administered to 25 patients, prompted by a bulky lesion at diagnosis ($n = 7$), a PET-positive lesion at the end of immunochemotherapy ($n = 9$), or unspecified reasons ($n = 7$).

The fraction of patients with reported grade 3–4 toxic effects and treatment failures is summarized in Table 3. Six patients discontinued the study due to toxicity, which included one case of cerebral hemorrhage and one gastric perforation. Notably, no patients died from treatment-related toxicities.

Fifteen patients discontinued the study due to disease progression. Five patients had primary refractory disease, while 10 experienced lymphoma progression during follow-up, including two CNS events: one patient with isolated CNS progression and another with combined systemic and CNS progression. Ten patients succumbed to lymphoma, and one died from an unrelated disease.

At the time of analysis, with a median follow-up of 37 months (range: 1–63), the 3-year FFS, PFS, and OS rates were 79%, 84%, and 88%, respectively (Figure 2A). Clinical risk factors, including the age-adjusted International Prognostic Index (aaIPI) groups 2 and 3, were not associated with survival (Figures 2B and S2).

Impact of biological risk factors on outcome

In the biologically high-risk group, the 3-year FFS rate was 76%. Based on a preplanned historical comparison and after adjusting for age and the aaIPI, the FFS for the biological high-risk group was comparable to that of biological high-risk patients treated with the R-CHOEP-14 regimen in our previous LBC-05 trial (hazard ratio [HR], 0.89; 95% confidence interval [CI], 0.38–1.71; $p = 0.58$; Figure 2C).[12] Similarly, there was no significant difference in PFS or OS rates between the biologically high-risk subgroups and the overall populations of the LBC-05 and LBC-06 studies (Figures 2D and S3A–D). However, when biological high-risk patients were further stratified by aaIPI, a trend toward improved outcomes was observed in a subgroup of patients with aaIPI 3 who were treated with the DA-EPOCH-R regimen within the LBC-06 trial (Figure 2E,F). The baseline demographics of the LBC-05 comparator cohort are shown in Table S2.

TABLE 2 Number of treatment cycles given, relative dose (RD) and relative dose intensity (RDI) for DA-EPOCH and R-CHOEP, and RD for HD-Mtx and HD-Ara-C.

Number of cycles	All	Biologically high risk DA-EPOCH-R	Biologically low risk R-CHOEP-14
1	0 (0)	0 (0)	0 (0)
2	4 (3.3)	2 (3.3)	2 (3.2)
3	2 (1.6)	0 (0)	2 (3.2)
4	1 (0.8)	1 (1.6)	0 (0)
5	1 (0.8)	1 (1.6)	0 (0)
6	2 (1.6)	1 (1.6)	1 (1.6)
7	113 (91.9)	56 (91.8)	57 (91.9)
RD/RDI (mean, %)			
Mtx	95	95	96
Median (min–max)	100 (50–100)	100 (50–100)	100 (50–100)
Cyclophosphamide	94/89	88/85	100/93
Median (min–max)	100 (57/100)/ 91 (50–109)	87 (57/100)/ 85 (60–109)	100 (75–100)/ 95 (50–105)
Doxorubicin	94/89	87/85	100/93
Median (min–max)	100 (61–100)/ 91 (50–109)	88 (61–100)/ 85 (61–109)	100 (75–100)/ 95 (50–105)
Vincristine	85/81	84/82	86/80
Median (min–max)	91 (15–107)/ 86 (14–105)	91 (15–100)/ 87 (14–103)	95 (33–107)/ 85 (26–105)
Etoposide	83/78	80/76	86/80
Median (min–max)	90 (0–108)/ 83 (0–105)	82 (0–104)/ 79 (0–105)	100 (0–108)/ 90 (0–103)
Ara-C	88	90	87
Median (min–max)	100 (0–100)	100 (0–100)	100 (0–100)

There were no differences in survival rates between the biological high- and low-risk groups in the LBC-06 cohort (Figure S2D,E). Neither was the cell of origin in the DLBCL NOS patients associated with survival (Figure S2F). When the prognostic impact of various predefined biological risk factors was evaluated across the entire study population, *17p/TP53* deletion emerged as the only marker significantly associated with worse outcomes (Figure 3A). CD5 positivity, p53 positivity, DPE status, and DHL status did not show a correlation with survival (Figures 3B and S2G–I). In Cox regression analysis accounting for age and aaIPI, *17p/TP53* deletion remained a significant risk factor for both progression and death (Figure 3C).

Pretreatment ctDNA uncovers hidden heterogeneity and predicts survival

We used baseline plasma samples to investigate the association between ctDNA burden, clinical characteristics, and survival outcomes (Figures 4 and S3). The overall landscape of mutated driver genes in plasma was found to be heterogeneous, similar to that observed at the tissue level (Figure 4A). Pretreatment ctDNA levels varied widely across the entire study population and among the distinct aaIPI subgroups (Figure 4A,B).

TABLE 3 Toxicity and treatment failures.

Event	All N = 123 (%)		Biologically high risk n = 61 (%)		Biologically low risk n = 62 (%)	
Adverse event grade	3	4	3	4	3	4
Hematological[a]						
Anemia	58 (47)	4 (3.3)	25 (41)	1 (1.6)	33 (54)	3 (4.9)
Neutropenia	13 (11)	75 (61)	4 (6.6)	44 (72)	9 (15)	31 (50)
Trombocytopenia	17 (14)	56 (45)	11 (18)	26 (43)	6 (9.7)	30 (48)
Infection	48 (39)	2 (1.6)	26 (43)	2 (3.3)	22 (25)	0 (0.0)
Gastrointestinal	11 (8.9)	3 (2.4)	6 (9.8)	1 (1.6)	5 (8.1)	2 (3.2)
Hepatic	9 (7.3)	0 (0.0)	2 (3.3)	0 (0.0)	7 (11)	0 (0.0)
Renal	3 (2.4)	0 (0.0)	2 (3.3)	0 (0.0)	1 (1.6)	0 (0.0)
Cardiac	4[b] (3.3)	1 (0.8)	0 (0.0)	0 (0.0)	4[b] (6.5)	1 (1.6)
Tromboembolism	10 (8.1)	0 (0.0)	5 (8.1)	0 (0.0)	5 (8.1)	0 (0.0)
Treatment failure due to toxicity	6 (4.9)		2 (3.3)		4 (6.4)	
Cerebral hemorrhage	1 (0.8)		0 (0.0)		1 (1.6)	
Gastric perforation	1 (0.8)		0 (0.0)		1 (1.6)	
Unspecified	4 (3.3)		2 (3.3)		2 (3.2)	
Treatment failure due to progression	15 (12)		10 (16)		5 (8.1)	
Primary refractory lymphoma	5 (4.1)		4 (6.6)		1 (1.6)	
Later progression	10 (8.3)		6 (9.8)		4 (6.5)	
Previous nodal site	7 (5.7)		4 (6.6)		3 (4.8)	
Previous EN site	5 (4.1)		5 (8.2)		0 (0)	
New EN site	1 (0.8)		0 (0)		1 (1.6)	
CNS progression	1 (0.8)		1 (1.6)		0 (0)	
Systemic and CNS progression	1 (0.8)		0 (0)		1 (1.6)	
Death (other disease)	1 (0.8)		0 (0)		1 (1.6)	
Consent withdrawn	4 (3.3)		3 (5.0)		1 (1.6)	

[a]Hematological toxicity is based on nadir values.

[b]Atrial fibrillation (gr 2).

Although no significant difference in ctDNA burden was observed between the low and high biological risk groups (Figure 4C), a high pretreatment ctDNA burden was associated with advanced disease stage, elevated LDH levels, and high aaIPI scores (Figures 4B and S3A,B), correlating with worse PFS and OS (Figures 4D and S3C). Furthermore, *TP53* mutations in ctDNA were associated with poor PFS and OS (Figures 4E and S3D).

In comparing cases with distinct *TP53* aberrations, 16 (84%) cases exhibiting *TP53* deletions in tumor tissue also displayed *TP53* mutations in the ctDNA. In addition, there were 17 more cases with TP53 mutations in the ctDNA, of which 10 were in the predefined low-risk group. These patients demonstrated worse outcomes than other low-risk patients (Figure S3E,F).

We identified various genetic subgroups of DLBCL from tumor tissue and/or ctDNA (Figure S4A).[27] However, in this population, these genetic subgroups did not show any association with survival (Figure S4B,C).

ctDNA-based MRD test complements PET in response evaluation

The responses are summarized in Table S3. Among the 112 patients who underwent PET-CT at the end of immunochemotherapy, 88 (79%) achieved a metabolic complete remission (mCR), while 19 (17%) had a metabolic partial remission (mPR) and 5 metabolic progression (mPD). Notably, only 2 out of 7 tissue biopsies (27%) from PET-positive lesions revealed refractory lymphoma, and only 5 out of 24 (21%) cases of PET-positive residual disease progressed within the following 6 months of follow-up (Figure 5A and Table S3).

The outcomes for patients with Deauville Score (DS) 4 were comparable to those with DS 1–3, further underscoring the necessity of confirming refractory disease histologically by tissue biopsy. Among the nine patients who received radiotherapy for localized PET-positive residual lesions, seven were classified as DS 4 and two as DS 5, which may have contributed to more favorable outcomes. The specificity, sensitivity, positive predictive value, and negative predictive value of the PET imaging were 80%, 45%, 22%, and 92%, respectively.

Early clearance of ctDNA levels to MRD negativity after two treatment cycles was associated with favorable outcomes (Figures 5B,C and S5A). Although this conversion to molecular remission improved in most patients during subsequent treatment cycles, those who remained ctDNA positive at the EOT experienced very poor outcomes (Figures 5D and S5B).

Among the 90 patients with available MRD and PET data at the end of treatment, 6 out of 19 PET-positive patients (32%) experienced progression. In comparison, the ctDNA-based MRD test was

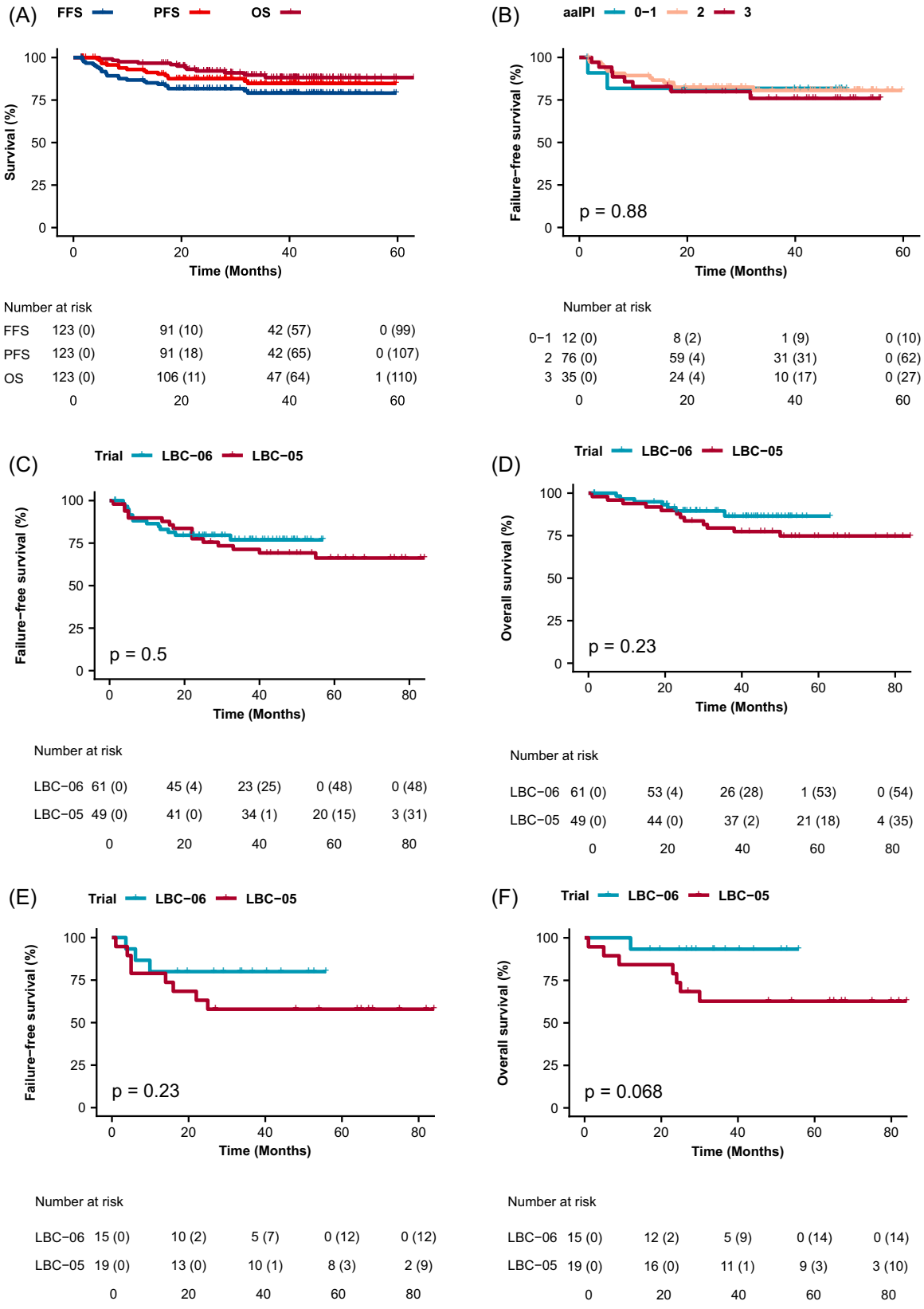


FIGURE 2 **Kaplan–Meier survival estimates.** **(A)** FFS, PFS, and OS rates in the LBC-06 trial. **(B)** FFS according to aaIPI in the LBC-06 trial. **(C)** Comparison of FFS rates of biological high-risk groups in the LBC-06 versus LBC-05 trials. **(D)** Comparison of OS rates of biological high-risk groups in the LBC-06 versus LBC-05 trials. **(E)** Comparison of FFS rates in patients with biological high-risk and aaIPI3 in the LBC-06 versus LBC-05 trials. **(F)** Comparison of OS rates in patients with biological high-risk and aaIPI3 in the LBC-06 versus LBC-05 trials.

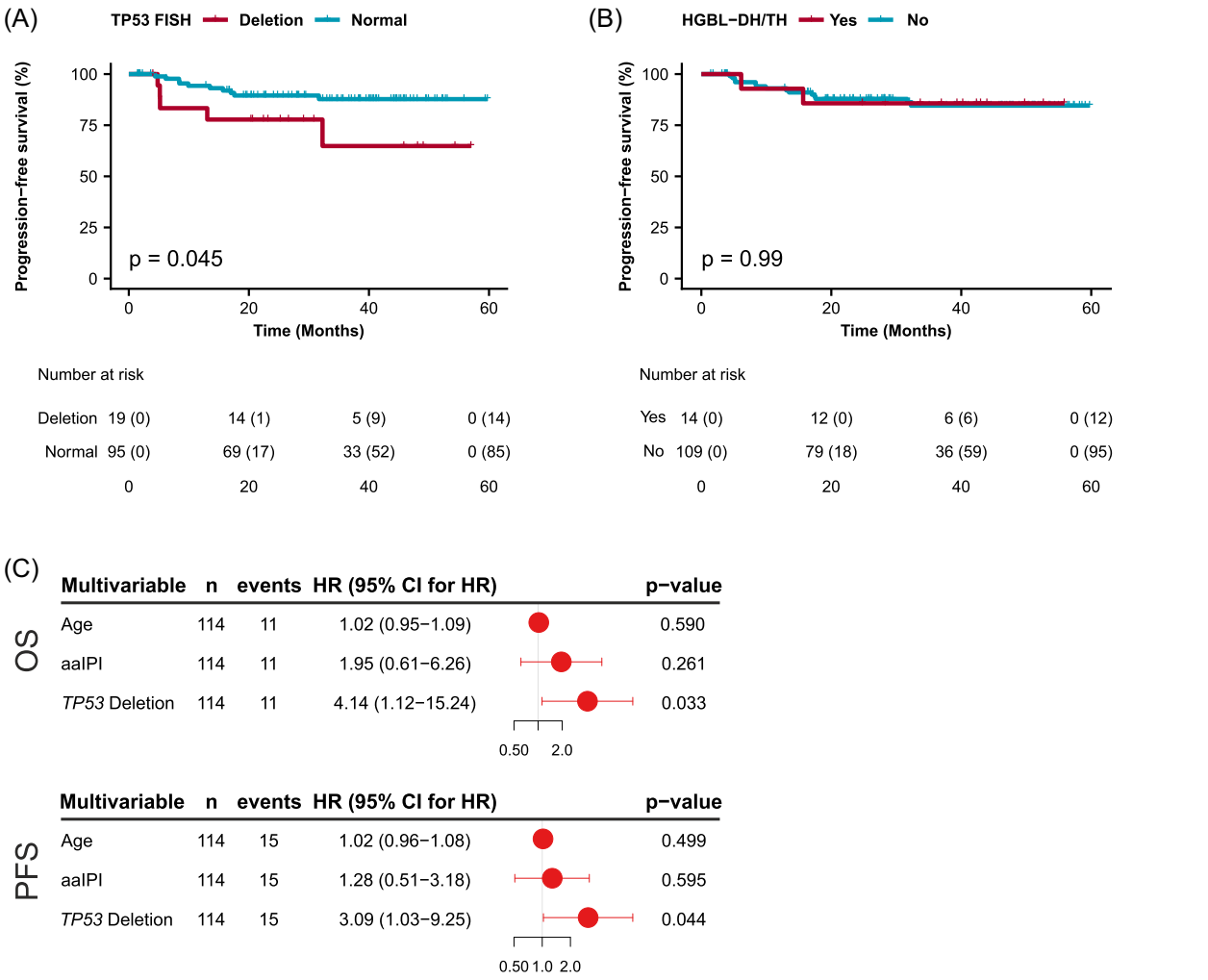


FIGURE 3 **Kaplan–Meier estimates of survival and Cox multivariable analysis. (A)** PFS according to *TP53* deletion status. **(B)** PFS according to HGBL-DH status. **(C)** Cox multivariable analyses on OS and PFS including age, aaIPI, and *TP53* deletion as covariates.

positive for 8 out of 12 patients (67%) who progressed. Of the 71 PET-negative patients, 6 progressed, and MRD was detectable in 3 of these cases (50%). The specificity, sensitivity, positive predictive value, and negative predictive value of the MRD test for progression were 98%, 56%, 82%, and 92%, respectively.

DISCUSSION

To our knowledge, this is the first LBCL trial to use a biological risk profile to guide treatment intensity. Our findings demonstrate that stratification based on biological risk factors is feasible in a prospective, multicenter trial and that intensified DA-EPOCH-R-based immunochemotherapy shows promising efficacy and manageable toxicity in young patients with clinical and biological high-risk LBCL. The 3-year FFS rate was 79%, exceeding the 75% target, which we had anticipated. PFS and OS rates were 84% and 90%, respectively.

In recent years, HGBL with DH or TH characteristics has emerged as a robust adverse risk factor in patients with LBCL and also a justification for more intensive chemotherapy. In contrast, intensification based on other biological risk factors can be questioned. However, based on available data at the time of the trial design and, in particular, the results from the Nordic LBC-04 trial cohort,[11] where combined genetic abnormalities in *myc*, *bcl2*, and *p53* genes and their increased protein levels translated to dismal survival in response to an intensified immunochemotherapy,[28] the selected biological factors appeared to be a justified combination to form a biological high-risk group. Interestingly, we found no significant differences in outcomes based on the predefined individual biological risk factors, except for *17p/p53* deletion. This suggests that intensified treatment may mitigate the adverse impact of these factors on survival, including DH and TH, indicating broader applicability for high-risk patients. In addition to *17p/p53* deletion, a high ctDNA burden and *TP53* mutations in baseline plasma samples were associated with poor survival. Additionally, *TP53* mutations in ctDNA were observed in patients designated as biologically low-risk based on their tumor tissue findings, further clarifying any potential stratification failures.

When we designed and initiated this study in 2017, the results of the randomized Alliance study (DA-EPOCH-R vs. R-CHOP) had not yet been published. However, existing phase II studies demonstrated high and durable responses to DA-EPOCH-R in patients with aggressive lymphomas, including Burkitt lymphoma, *MYC*-rearranged lymphomas, and primary mediastinal B-cell lymphomas,[29–31] and a meta-analysis showed that DA-EPOCH-R reduced the risk of progression compared with R-CHOP.[32] Together, the results indicated that DA-EPOCH-R was a justified immunochemotherapy backbone in

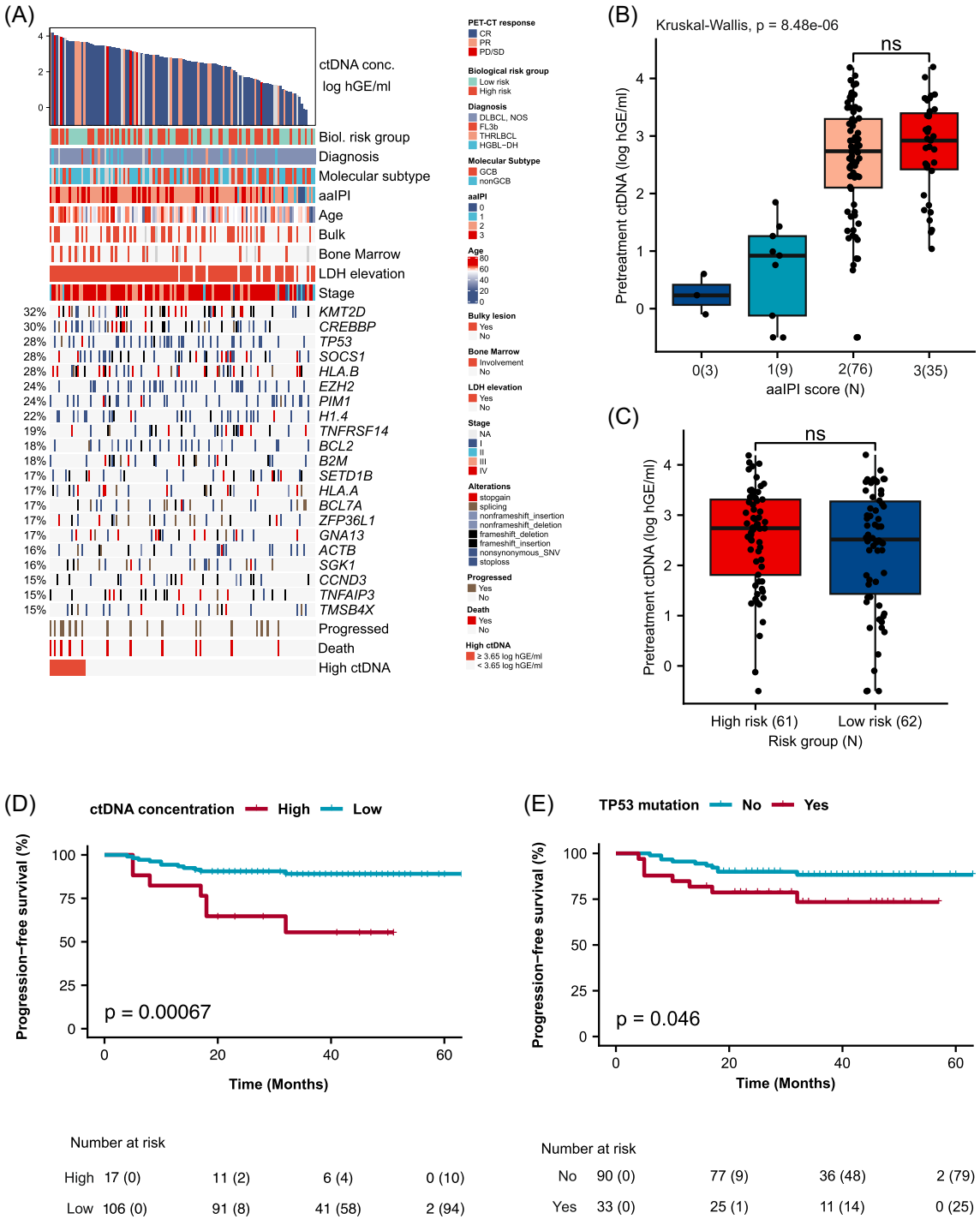


FIGURE 4 **Baseline ctDNA burden and TP53 mutations. (A)** Oncoprint of the coding driver mutation landscape according to pretreatment ctDNA concentration. Columns represent individual patients, and rows represent different clinical variables or driver genes. Genes mutated in ≥15% of the patients included, and the percentages are indicated. **(B)** Pretreatment ctDNA concentrations (log hGE/mL) according to aaIPI scores. **(C)** Pretreatment ctDNA concentrations (log hGE/mL) in the biological high- and low-risk groups. **(D)** PFS of patients with high (≥3.60 hGE/mL) versus low (<3.60 hGE/mL) pretreatment ctDNA concentration. **(E)** PFS of patients with or without *TP53* mutations detected from ctDNA. Subclonal mutations were excluded from the analysis.

patients with biologically high-risk LBCL. Our results appear to compare favorably with other phase II studies assessing intensified treatments in biologically high-risk LBCL despite the inherent limitations of cross-study comparisons. In a study involving 53 patients with *MYC*-rearranged LBCL, including 24 DHLs, the 4-year EFS and OS rates were 71% and 77%, respectively.[29] Similarly, a study combining DA-EPOCH-R with HD-Mtx in 47 patients with CD5 + DLBCL reported 5-year PFS and OS rates of 72% and 79%.[33,34] Our results also showed favorable outcomes compared to R-CODOX/IVAC and R-ACVBP in clinically defined high-risk patients.[35,36] In a recent retrospective study comparing R-CHOEP-14 with R-pola-CHP, 2-year PFS was 72% for R-CHOEP and 75% for Pola-R-CHP, while

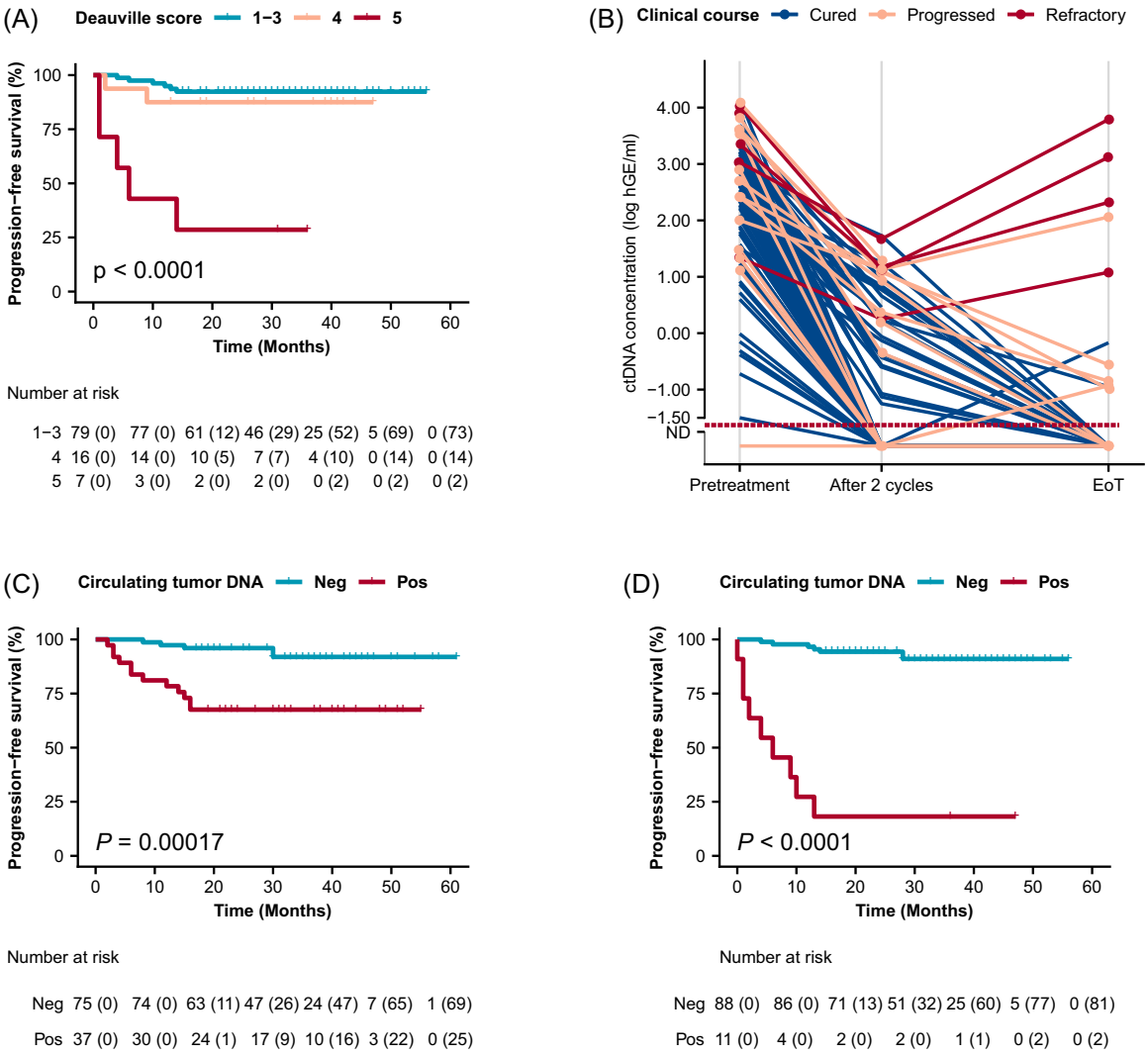


FIGURE 5 **Prognostic impact of PET and MRD. (A)** PFS according to end-of-therapy PET. **(B)** Dynamics of ctDNA burden during therapy. **(C)** PFS according to ctDNA-based molecular response after two courses of therapy. **(D)** PFS according to ctDNA-based molecular response at the EOT.

OS was 88% for both regimens.[37] While OS rates were comparable, PFS was better in our study, suggesting the potential favorable impact of HD-Mtx and HD-AraC on treatment outcomes.

A limitation of this trial is the absence of a randomized comparator, especially given the failure of DA-EPOCH-R and other promising regimens to demonstrate a survival benefit over R-CHOP in phase III trials.[38–40] To contextualize our clinical findings, we performed a preplanned historical comparison with a cohort of patients exhibiting similar biological high-risk profiles from the previous Nordic LBC-05 prospective trial. We found no significant differences in survival rates between the two studies. Both regimens could also overcome the adverse impact of DHL on survival. However, in a subgroup of patients with biological risk factors and aaIPI 3, there was a trend toward better survival following the DA-EPOCH-R-based regimen. We conclude that intensified immunochemotherapy, whether via DA-EPOCH-R or R-CHOEP-14 combined with early HD-Mtx and HD-cytarabine as consolidation therapy, offers favorable outcomes for patients with biologically high-risk LBCL. Given that the exploratory analyses of biological risk factors in our previous study are constrained by small sample sizes and were performed retrospectively, the results also suggest that in a subgroup of ultrahigh-risk patients, treatment with DA-EPOCH-R may lead to improved outcomes over the R-CHOEP-14 backbone.

The benefit of HD-Mtx as CNS prophylaxis in LBCLs remains controversial. While the results from several prospective trials suggest that aggressive systemic therapies utilizing drugs that penetrate the blood–brain barrier (BBB) may reduce CNS relapses in younger high-risk patients,[11,12,41] recent large retrospective analyses have failed to confirm this benefit.[42,43] There is also no consensus on the optimal timing of CNS prophylaxis. In a recent large retrospective study, end-of-therapy HD-Mtx did not increase the risk of CNS relapse compared with intercalated delivery and caused fewer delays to R-CHOP therapy.[44] In contrast, shifting HD-Mtx to the beginning of the therapy in our previous trial translated to improved FFS, PFS, and a lower number of CNS events.[12] Consistent with our previous trial, we observed no significant delays to treatment cycles following early administered HD-Mtx and only two CNS events in this study, resulting in a CNS recurrence rate of 1.6% over 3 years. Together with our earlier studies,[11,12] these results suggest that young high-risk patients may benefit from incorporating BBB-penetrating HD-Mtx and HD-cytarabine into their immunochemotherapy regimens.

Most patients (91%) with negative PET scans (DS 1–3) achieved long-term remission; in contrast, only 21% of PET-positive patients (DS 4-5) experienced progression within the following six months of follow-up. The similarity in outcomes between patients with DS 4 and those with negative PET scans underscores the importance of histologically confirming residual PET-positive lesions, as well as the potentially beneficial impact of consolidative radiotherapy on survival.

When we evaluated the relationship between ctDNA clearance (MRD negativity) and EOT PET-based response assessments, we found that patients who were MRD-negative at the EOT had a significantly lower risk of recurrence, whereas nearly all MRD-positive patients progressed. The high positive and negative predictive values of MRD compared to EOT PET in this and prior studies[26,45] highlight the need for prospective trials to explore how ctDNA dynamics can guide treatment decisions. While it is currently unclear which is the best method to assess ctDNA and its independence from imaging technologies, it is clear that ctDNA offers opportunities for better risk stratification and response evaluation. For example, low pretreatment ctDNA burden and early MRD negativity may help to guide treatment de-escalation. Conversely, high pretreatment ctDNA levels and MRD positivity at later time points could be used to guide treatment intensification, change of therapy, or consolidation with novel agents.

In conclusion, this study demonstrates that stratifying patients based on biological risk factors in a prospective, multicenter trial is feasible and effective. Intensified immunochemotherapy, incorporating early administration of HD-Mtx and R-HD-cytarabine consolidation, is a successful regimen for biologically high-risk LBCL. Notably, *TP53* aberrations and ctDNA burden were identified as predictors of poor outcomes. Additionally, our findings indicate that ctDNA analysis can complement PET imaging in response evaluation. We see this trial as an advancement in developing more effective, biomarker-driven treatment strategies for high-risk LBCL. Incorporating ctDNA analysis to assess baseline characteristics and molecular responses throughout treatment could enhance diagnostics, offer more sensitive methods for predicting treatment response compared to PET, and inform necessary adjustments in therapy.

ACKNOWLEDGMENTS

We thank all participating study sites for patient enrollment and sample collection, Knut Liestøl for statistical advice, Laura Hakala for study coordination, the staff at the Clinical Trial Office in Aarhus, Denmark, for trial management, and Anne Aarnio for technical assistance. Open access publishing facilitated by Helsingin yliopisto, as part of the Wiley - FinELib agreement.

AUTHOR CONTRIBUTIONS

Conceptualization: Sirpa Leppä, Judit Jørgensen, Kristina Drott, Øystein Fluge, Sirkku Jyrkkiö, Peter Brown, and Harald Holte. Project administration: Sirpa Leppä, Judit Jørgensen, Kristina Drott, and Harald Holte. Investigation: Sirpa Leppä, Judit Jørgensen, Marja-Liisa Karjalainen-Lindsberg, Klaus Beiske, Mette Pedersen, Kristina Drott, Øystein Fluge, Sirkku Jyrkkiö, Peter Brown, and Harald Holte. Resources: Sirpa Leppä, Judit Jørgensen, Kristina Drott, Annika Pasanen, Kristiina Karihtala, Susanna Mannisto, Susanna Mannisto, Marianne Brodtkorb, Unn-Merete Fagerli, Thomas Stauffer Larsen, Leo Meriranta, Kaisa Sunela, Øystein Fluge, Sirkku Jyrkkiö, Peter Brown, and Harald Holte. Formal analysis: Sirpa Leppä, Leo Meriranta, and Maare Arffman. Visualization: Sirpa Leppä, Leo Meriranta, and Maare Arffman. Data interpretation: All authors. Funding acquisition: Sirpa Leppä. Writing—original draft preparation: Sirpa Leppä. Writing—review and editing: All authors. Final approval of manuscript: All authors.

CONFLICT OF INTEREST STATEMENT

S.L.*: AbbVie: consultancy; Genmab: consultancy, research funding; Gilead: consultancy; Incyte: consultancy; Novartis: research funding; Roche: consultancy, honoraria, research funding; SOBI: consultancy, honoraria; Bayer: research funding; BMS/Celgene: research funding; Hutchmed: research funding. J.J.*: BMS: consultancy; Gilead: consultancy; Incyte: consultancy; Novartis: consultancy; Roche: consultancy. H.H.*: Genmab: honoraria, safety committee; Gilead: honoraria, advisory board; Incyte: honoraria, advisory board; Nordic Nanovector: honoraria, safety committee: Novartis: honoraria, advisory board; Takeda: honoraria, advisory board. Other authors declare no competing interests. *All outside of the submitted work.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The study was conducted by the Guidelines on Good Clinical Practice from the International Conference on Harmonization and the principles of the Declaration of Helsinki. The protocol was approved by the medical agencies and ethics committees in Finland, Denmark, Norway, and Sweden, and the trial was registered at ClinicalTrials.gov (number NCT01325194). All patients signed informed consent forms before participating in the study.

FUNDING

This study was funded by the Research Council of Finland, Finnish Cancer Organizations, iCAN Flagship, Nordic Cancer Union, Southern Finland Regional Cancer Center (FICAN South), Sigrid Juselius Foundation, and Helsinki and Uusimaa Hospital District. Open access was funded by Helsinki University Library.

ORCID

Sirpa Leppä http://orcid.org/0000-0002-8265-511X
Leo Meriranta https://orcid.org/0000-0002-6378-5381
Maare Arffman https://orcid.org/0009-0004-1837-1587
Harald Holte https://orcid.org/0000-0001-9799-9428

SUPPORTING INFORMATION

Additional supporting information can be found in the online version of this article.

REFERENCES

1. Campo E, Jaffe ES, Cook JR, et al. The International Consensus Classification of Mature Lymphoid Neoplasms: a report from the Clinical Advisory Committee. *Blood*. 2022;140(11):1229-1253.
2. Alaggio R, Amador C, Anagnostopoulos I, et al. The 5th edition of the World Health Organization classification of haematolymphoid tumours: lymphoid neoplasms. *Leukemia*. 2022;36(7):1720-1748.
3. Sehn LH, Salles G. Diffuse large B-cell lymphoma. *N Engl J Med*. 2021;384(9):842-858.
4. Bartlett NL, Wilson WH, Jung SH, et al. Dose-adjusted EPOCH-R compared with R-CHOP as frontline therapy for diffuse large B-cell lymphoma: clinical outcomes of the Phase III Intergroup Trial Alliance/CALGB 50303. *J Clin Oncol*. 2019;37(21):1790-1799.
5. Oki Y, Westin JR, Vega F, et al. Prospective phase II study of rituximab with alternating cycles of hyper-CVAD and high-dose methotrexate with cytarabine for young patients with high-risk diffuse large B-cell lymphoma. *Br J Haematol*. 2013;163(5):611-620.

6. Tilly H, Morschhauser F, Sehn LH, et al. Polatuzumab vedotin in previously untreated diffuse large B-cell lymphoma. *N Engl J Med*. 2022;386(4):351-363.
7. Greb A, Bohlius J, Trelle S, et al. High-dose chemotherapy with autologous stem cell support in first-line treatment of aggressive non-Hodgkin lymphoma - results of a comprehensive meta-analysis. *Cancer Treat Rev*. 2007;33(4):338-346.
8. Schmitz N, Nickelsen M, Ziepert M, et al. Conventional chemotherapy (CHOEP-14) with rituximab or high-dose chemotherapy (MegaCHOEP) with rituximab for young, high-risk patients with aggressive B-cell lymphoma: an open-label, randomised, phase 3 trial (DSHNHL 2002-1). *Lancet Oncol*. 2012;13(12):1250-1259.
9. Gang AO, Strøm C, Pedersen M, et al. R-CHOEP-14 improves overall survival in young high-risk patients with diffuse large B-cell lymphoma compared with R-CHOP-14. A population-based investigation from the Danish Lymphoma Group. *Ann Oncol*. 2012;23(1):147-153.
10. Wästerlid T, Hartman L, Székely E, Jerkeman M. Impact on survival of addition of etoposide to primary chemotherapy in diffuse large B-cell lymphoma: a Swedish Lymphoma Registry study. *Hematol Oncol*. 2017;35(2):151-157.
11. Holte H, Leppä S, Björkholm M, et al. Dose-densified chemoimmunotherapy followed by systemic central nervous system prophylaxis for younger high-risk diffuse large B-cell/follicular grade 3 lymphoma patients: results of a phase II Nordic Lymphoma Group study. *Ann Oncol*. 2013;24(5):1385-1392.
12. Leppä S, Jørgensen J, Tierens A, et al. Patients with high-risk DLBCL benefit from dose-dense immunochemotherapy combined with early systemic CNS prophylaxis. *Blood Adv*. 2020;4(9):1906-1915.
13. Miyazaki K, Yamaguchi M, Suzuki R, et al. CD5-positive diffuse large B-cell lymphoma: a retrospective study in 337 patients treated by chemotherapy with or without rituximab. *Ann Oncol*. 2011;22(7):1601-1607.
14. Young KH, Leroy K, Møller MB, et al. Structural profiles of TP53 gene mutations predict clinical outcome in diffuse large B-cell lymphoma: an international collaborative study. *Blood*. 2008;112(8):3088-3098.
15. Xu-Monette ZY, Wu L, Visco C, et al. Mutational profile and prognostic significance of TP53 in diffuse large B-cell lymphoma patients treated with R-CHOP: report from an International DLBCL Rituximab-CHOP Consortium Program Study. *Blood*. 2012;120(19):3986-3996.
16. Zenz T, Kreuz M, Fuge M, et al. TP53 mutation and survival in aggressive B cell lymphoma. *Int J Cancer*. 2017;141(7):1381-1388.
17. Chapuy B, Stewart C, Dunford AJ, et al. Molecular subtypes of diffuse large B cell lymphoma are associated with distinct pathogenic mechanisms and outcomes. *Nat Med*. 2018;24(5):679-690.
18. Dodero A, Guidetti A, Marino F, et al. Dose-adjusted EPOCH and rituximab for the treatment of double expressor and double-hit diffuse large B-cell lymphoma: impact of TP53 mutations on clinical outcome. *Haematologica*. 2022;107(5):1153-1162.
19. Johnson NA, Savage KJ, Ludkovski O, et al. Lymphomas with concurrent BCL2 and MYC translocations: the critical factors associated with survival. *Blood*. 2009;114(11):2273-2279.
20. Green TM, Young KH, Visco C, et al. Immunohistochemical double-hit score is a strong predictor of outcome in patients with diffuse large B-cell lymphoma treated with rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone. *J Clin Oncol*. 2012;30(28):3460-3467.
21. Johnson NA, Slack GW, Savage KJ, et al. Concurrent expression of MYC and BCL2 in diffuse large B-cell lymphoma treated with rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone. *J Clin Oncol*. 2012;30(28):3452-3459.
22. Staiger AM, Ziepert M, Horn H, et al. Clinical impact of the cell-of-origin classification and the MYC/BCL2 dual expresser status in diffuse large B-cell lymphoma treated within prospective clinical trials of the German High-Grade Non-Hodgkin's Lymphoma Study Group. *J Clin Oncol*. 2017;35(22):2515-2526.
23. Meriranta L, Pasanen A, Alkodsi A, Haukka J, Karjalainen-Lindsberg ML, Leppä S. Molecular background delineates outcome of double protein expressor diffuse large B-cell lymphoma. *Blood Adv*. 2020;4(15):3742-3753.
24. Swerdlow SH, Campo E, Pileri SA, et al. The 2016 revision of the World Health Organization classification of lymphoid neoplasms. *Blood*. 2016;127(20):2375-2390.
25. Hans CP. Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. *Blood*. 2004;103(1):275-282.
26. Meriranta L, Alkodsi A, Pasanen A, et al. Molecular features encoded in the ctDNA reveal heterogeneity and predict outcome in high-risk aggressive B-cell lymphoma. *Blood*. 2022;139(12):1863-1877.
27. Wright GW, Huang DW, Phelan JD, et al. A probabilistic classification tool for genetic subtypes of diffuse large B cell lymphoma with therapeutic implications. *Cancer Cell*. 2020;37(4):551-568.e14.
28. Fiskvik I, Beiske K, Delabie J, et al. Combining MYC, BCL2 and TP53 gene and protein expression alterations improves risk stratification in diffuse large B-cell lymphoma. *Leuk Lymphoma*. 2015;56(6):1742-1749.
29. Dunleavy K, Fanale MA, Abramson JS, et al. Dose-adjusted EPOCH-R (etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin, and rituximab) in untreated aggressive diffuse large B-cell lymphoma with MYC rearrangement: a prospective, multicentre, single-arm phase 2 study. *Lancet Haematol*. 2018;5(12):e609-e617.
30. Dunleavy K, Pittaluga S, Maeda LS, et al. Dose-adjusted EPOCH-rituximab therapy in primary mediastinal B-cell lymphoma. *N Engl J Med*. 2013;368(15):1408-1416.
31. Dunleavy K, Pittaluga S, Shovlin M, et al. Low-intensity therapy in adults with Burkitt's lymphoma. *N Engl J Med*. 2013;369(20):1915-1925.
32. Howlett C, Snedecor SJ, Landsburg DJ, et al. Front-line, dose-escalated immunochemotherapy is associated with a significant progression-free survival advantage in patients with double-hit lymphomas: a systematic review and meta-analysis. *Br J Haematol*. 2015;170(4):504-514.
33. Miyazaki K, Asano N, Yamada T, et al. DA-EPOCH-R combined with high-dose methotrexate in patients with newly diagnosed stage II-IV CD5-positive diffuse large B-cell lymphoma: a single-arm, open-label, phase II study. *Haematologica*. 2020;105(9):2308-2315.
34. Miyazaki K, Sakai R, Iwaki N, et al. Five-year follow-up of a phase II study of DA-EPOCH-R with high-dose MTX in CD5-positive DLBCL. *Cancer Sci*. 2023;114(6):2689-2691.
35. Fitoussi O, Belhadj K, Mounier N, et al. Survival impact of rituximab combined with ACVBP and upfront consolidation autotransplantation in high-risk diffuse large B-cell lymphoma for GELA. *Haematologica*. 2011;96(8):1136-1143.
36. McMillan AK, Phillips EH, Kirkwood AA, et al. Favourable outcomes for high-risk diffuse large B-cell lymphoma (IPI 3-5) treated with front-line R-CODOX-M/R-IVAC chemotherapy: results of a phase 2 UK NCRI trial. *Ann Oncol*. 2020;31(9):1251-1259.
37. Lenz G, Tilly H, Ziepert M, et al. Pola-R-CHP or R-CHOEP for first-line therapy of younger patients with high-risk diffuse large B-cell lymphoma: a retrospective comparison of two randomized phase 3 trials. *Leukemia*. 2024;38(12):2709-2711.
38. Crump M, Leppä S, Fayad L, et al. Randomized, double-blind, phase III trial of Enzastaurin versus placebo in patients achieving remission after first-line therapy for high-risk diffuse large B-cell lymphoma. *J Clin Oncol*. 2016;34(21):2484-2492.
39. Younes A, Sehn LH, Johnson P, et al. Randomized phase III trial of ibrutinib and rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone in non-germinal center B-cell diffuse large B-cell lymphoma. *J Clin Oncol*. 2019;37(15):1285-1295.

40. Nowakowski GS, Chiappella A, Gascoyne RD, et al. ROBUST: a phase III study of lenalidomide plus R-CHOP versus placebo plus R-CHOP in previously untreated patients with ABC-type diffuse large B-cell lymphoma. *J Clin Oncol*. 2021;39(12):1317-1328.
41. Thieblemont C, Altmann B, Frontzek F, et al. Central nervous system relapse in younger patients with diffuse large B-cell lymphoma: a LYSA and GLA/DSHNHL analysis. *Blood Adv*. 2023;7(15):3968-3977.
42. Lewis KL, Jakobsen LH, Villa D, et al. High-dose methotrexate as CNS prophylaxis in high-risk aggressive B-cell lymphoma. *J Clin Oncol*. 2023;41(35):5376-5387.
43. Puckrin R, El Darsa H, Ghosh S, Peters A, Owen C, Stewart D. Ineffectiveness of high-dose methotrexate for prevention of CNS relapse in diffuse large B-cell lymphoma. *Am J Hematol*. 2021;96(7):764-771.
44. Wilson MR, Eyre TA, Kirkwood AA, et al. Timing of high-dose methotrexate CNS prophylaxis in DLBCL: a multicenter international analysis of 1384 patients. *Blood*. 2022;139(16):2499-2511.
45. Kurtz DM, Soo J, Co Ting Keh L, et al. Enhanced detection of minimal residual disease by targeted sequencing of phased variants in circulating tumor DNA. *Nat Biotechnol*. 2021;39(12):1537-1547.