WILEY

Frontiers in Flow Cytometry™ is for researchers across the globe looking for an opportunity to share and learn about current developments in flow cytometry. This 24 hour virtual event will feature keynote presentations by industry colleagues, webinars, demos, live networking opportunities and more.

Key topics include:

- Spectral and conventional flow cytometry
- Immunophenotyping and Standardization
- Panel design and optimization
- Cancer Biology and Auto-immune Diseases
- Infectious diseases
- Advances in flow cytometry technology

Register Now

This event is sponsored by ThermoFisher SCIENTIFIC

Received: 13 September 2020 | Revised: 5 December 2020 | Accepted: 8 December 2020

DOI: 10.1002/ajh.26068

RESEARCH ARTICLE

AJH WILEY

First-line treatment of double-hit and triple-hit lymphomas: Survival and tolerance data from a retrospective multicenter French study

Marie-Charlotte Laude[1] | Laure Lebras[2] | Pierre Sesques[3] | Herve Ghesquieres[3] | Simon Favre[4] | Krimo Bouabdallah[4] | Carolyne Croizier[5] | Romain Guieze[5] | Laurianne Drieu La Rochelle[6] | Emmanuel Gyan[6] | Roza Chin[7] | Thérèse Aurran-Schleinitz[7] | Amira Marouf[8] | Bénédicte Deau-Fischer[8] | Paul Coppo[9] | Sandrine Malot[9] | Xavier Roussel[10] | Adrien Chauchet[11] | Marianne Schwarz[12] | Charles Bescond[13] | Thierry Lamy de la Chapelle[14] | Lucile Bussot[15] | Sylvain Carras[16] | Bénédicte Burlet[17] | Cédric Rossi[18] | Adrien Daniel[19] | Franck Morschhauser[20] | Fabien Subtil[21] | Anne-Sophie Michallet[2]

[1]Claude Bernard University, Lyon, France

[2]Department of Hematology and Medical Oncology, Centre Léon Bérard, Lyon, France

[3]Department of Hematology, Hospices Civils de Lyon, Lyon, France

[4]Department of Hematology and Cell Therapy, University Hospital of Bordeaux, Bordeaux, France

[5]Department of Hematology and Cell Therapy, Estaing University Hospital, Clermont-Ferrand, France

[6]Department of Hematology and Cell Therapy, University Hospital of Tours, Tours, France

[7]Department of Hemato-Oncology, Institut Paoli Calmette, Marseille, France

[8]Department of Hematology, Cochin Hospital, Paris, France

[9]Department of Hematology and French Reference Center for Thrombotic Microangiopathies, Saint-Antoine Hospital, Paris, France

[10]University of France-Comté, Besançon, France

[11]Department of Hematology, University Hospital of Besançon, Besançon, France

[12]University of Angers, Angers, France

[13]Department of Hematology, University Hospital of Angers, Angers, France

[14]Department of Clinical Hematology, University Hospital of Rennes, Rennes, France

[15]University of Grenoble, Grenoble, France

[16]Department of Hematology, University Hospital of Grenobles Alpes, Grenoble, France

[17]University of Dijon, Dijon, France

[18]Department of Clinical Hematology, University Hospital of Dijon, Dijon, France

[19]University of Lille, Lille, France

[20]Department of Hematology, University Hospital of Lille, Lille, France

[21]Department of Biostatistics, Hospices Civils de Lyon, Lyon, France

Correspondence
Marie-Charlotte Laude, Department of Hematology and Medical Oncology, 28 rue Laennec, 69008 Lyon, France.
Email: mariecharlotte.laude@gmail.com

Abstract

Historically, double or triple hit lymphoma (DHL and THL) have poor outcomes with conventional chemotherapy, but there is currently no guideline. We report the French

experience in managing DHL and THL in first line using collective data on both survival and tolerance. All consecutive patients with newly diagnosis of large B-cell lymphoma with *MYC*, *BCL2*, and/or *BCL6* rearrangements, as determined by FISH between January 2013 and April 2019 were included. Based on the eligibility criteria, 160 patients were selected among the 184 patients identified. With a median follow-up of 32 months, 2- and 4-year progression free survival (PFS) rates were 40% and 28% with R-CHOP compared with 57% and 52% with intensive chemotherapy ($P = .063$). There was no difference in overall survival (OS). For advanced stages, PFS was significantly longer with intensive chemotherapy than with R-CHOP ($P = .029$). There was no impact of autologous stem cell transplantation among patient in remission. For patients with central nervous system (CNS) involvement, the 2-year PFS and OS rate was 21% and 39%, vs 57% and 75% without CNS disease ($P = .007$ and $P < .001$). By multivariate analysis, elevated IPI score and CNS disease were strongly and independently associated with a poorer survival, whereas treatment was not significantly associated with OS. This is the largest series reporting the treatment of DHL and THL in Europe. The PFS was significantly longer with an intensive regimen for advanced stage, but no difference in OS, supporting the need for a prospective randomized trial.

1 | INTRODUCTION

Diffuse large B-cell lymphoma (DLBCL) is the most frequent non-Hodgkin lymphoma (40%), with an estimated incidence of 5071 new cases per year in France (INCa 2018 data).[1]

Knowledge about the genetic and molecular characteristics of DLBCL has increased over the last decade, leading to a single category for higher-risk anomalies being recognized in the World Health Organization (WHO) 2016 classification: high-grade B-cell lymphoma with *MYC* and *BCL2* and/or *BCL6* rearrangements, so called double-hit or triple-hit lymphoma (DHL and THL).[2] This denomination, which now includes all histological subtypes of large B-cell lymphoma with a rearrangement of *MYC*, *BCL2* and/or *BCL6*, allows a purely molecular definition of a high-risk lymphoma for the first time.

Both DHLs and THLs have an incidence of 8%-10% among diagnoses of de novo DLBCL, with a higher frequency (20%) in transformed indolent B-cell lymphoma.[3,4]

Patients with DHL and THL experience high rates of early treatment failure and relapse or death, as indicated in the steep initial drops in both overall survival (OS) and progression-free survival (PFS) curves. These relapsed or refractory (R/R) patients have a median OS following relapse of 8.6 months,[5] despite undergoing intensive salvage therapy including autologous stem-cell transplantation (ASCT).[6] The Lunenburg Lymphoma Biomarker Consortium[7] recently showed a time-dependent effect with a negative prognosis for DHL with *MYC* rearrangement in the first 2 years after diagnosis. After 2 years, this effect seems to disappear, even for DHL/THL patients. For this reason, achieving response to first-line chemotherapy is essential for improving long-term survival.[8]

Controversies persist over the choice of first-line treatment for these lymphomas, in order to achieve complete remission. Very few studies on the subject exist, most of which are retrospective, and providing only limited information to determine which appropriate intensive regimen to use. Patients with DHL and THL are known to have very poor outcomes with conventional chemotherapy, such as R-CHOP (rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone).[9,10] Several retrospective studies have shown improved PFS but not OS after intensive chemotherapy, compared to R-CHOP.[5,11,12] Currently there is only one prospective study, reported by Dunlevy et al., showing favorable survival after DA-R-EPOCH (dose-adjusted rituximab, etoposide, prednisolone, vincristine, cyclophosphamide, and doxorubicin) than historical data. However, this study was not randomized and did not correspond to the current expectation of prospective study.[13]

To our knowledge, our study is the largest work reporting the treatment of DHL and THL in Europe. In addition, previous studies have not included safety data in this population, which is often elderly and has aggressive presentation at diagnosis.

Therefore, through this retrospective multicenter study, we present the French experience of managing DHL/THL in the first line, using collective data on both survival and tolerance.

2 | METHODS

2.1 | Patients

We conducted a multicenter retrospective study of patients with previously untreated DHL and THL. Therefore DHL and THL were defined as large B-cell lymphoma with *MYC*, *BCL2*, and/or *BCL6* rearrangements, as determined by fluorescence in situ hybridization (FISH).

We collected data from all consecutive patients meeting the inclusion criteria (n = 184), from 14 French academic medical centers between January 2013 and April 2019. All patients diagnosed after May 2016 were routinely screened for MYC and then for BCL2 and BCL6 if present (60.3% of the entire cohort). The others were tested based on aggressive morphological features (cell morphology, proliferation index, mainly) or aggressive clinical presentation. We identified 66 HGBL-DH/TH among the 4593 DLBCL biopsies tested from January 2013 to May 2016 (1.4%) and 118 of the 3398 DLBCL biopsies from June 2016 to April 2019 (3.5%).

Inclusion criteria included patients aged >18 years, with large B-cell lymphoma (de novo or transformed), and with FISH available at diagnosis that showed *MYC*, *BCL2*, and/or *BCL6* rearrangements. Patients were excluded if they had received prior treatment for an indolent lymphoma, had a histology of grade 1-2 and 3a follicular lymphoma, had abnormalities involving *MYC* genes other than rearrangement (copy gain, amplification, aberrant somatic mutation), when no treatment or follow-up data were available, and when there was immediate palliative care.

All cases corresponded to HGBL-DH/TH category with DLBCL morphology in the WHO 2016 classification. The cell-of-origin (COO) was defined according to Hans algorithm. In all departments of pathology and cytogenetics, FISH for *MYC* was performed using a dual-color break-apart probe (Vysis Abbott, ZytoLight, Kreatech or MetaSystems probes).

All data were retrospectively recorded from medical records. Patients initially treated with R-CHOP and then escalated to more intensive chemotherapy after receiving FISH results were classified in the intensive chemotherapy group. Patients whose treatment had to be reduced for toxicity were left in the intensive treatment group.

The following grade 3-4 adverse events have been investigated: neutropenia, thrombocytopenia, anemia, need of transfusion, febrile neutropenia, mucositis, nausea, diarrhea, constipation, neurological toxicity, infections, interruption of treatment and treatment-related death. These data were collected from summaries at every successive cycle of treatment, hospital visits between cycle, and from weekly ambulatory blood test. Toxicity was assessed using the Common Terminology Criteria for Adverse Events (CTCAE Version 5.0). Response assessment was performed on a PET-CT at the end of induction according to the 2014 Lugano classification.

All cases were reviewed by an expert hematological center, as part of the Lymphopath network. This network is certified by the French National Cancer Institute, which has aimed to review all newly diagnosed lymphomas in France since 2010.[14] This study was performed according to the principles of the Declaration of Helsinki. Our patient database was approved by the local authority for the protection of privacy and personal data in clinical research (N° R201-004-075).

2.2 | Statistical analysis

Quantitative variables were described as median and first and third quartiles (Q1-Q3); qualitative variables were described as frequency and percentage of each modality (excluding missing data from percentages). Comparison of proportions between groups was performed using the chi-square test; comparisons adjusted for age (≤65 vs >65 years old) were performed using logistic regression.

Progression free survival (PFS) was defined as the time interval from the date of diagnosis to either disease progression, relapse disease, last follow-up, or death. Overall survival (OS) was defined as the time interval from the date of diagnosis to last follow-up or death from any cause. Both PFS and OS were described using Kaplan-Meier curves and summarized by median survival or percent survival at different time points, with the associated 95% confidence interval (95% CI); comparisons between groups were performed using the log-rank test. Multivariate analyses of OS were conducted using Cox models: factors with a *P* value less <.2 in univariate analyses were included in the multivariate model (excluding factors with excessive correlation), then a backward selection approach was used (coercing the treatment in the analysis).

A *P* value <.05 was considered statistically significant. Analyses were performed using the R software, version 3.6.1.

3 | RESULTS

3.1 | Patients characteristics

Based on the eligibility criteria, 160 patients were included. Of these, three patients were removed from the analysis because they received a treatment other than those being studied (two patients with weekly rituximab and one with rituximab-cyclophosphamide alone), and one patient died prior to the initiation of treatment.

Baseline characteristics, for all patients and divided by conventional and intensive chemotherapy, are listed in Table 1. Median age at diagnosis was 62.5 years (22 to 87 years), with male predominance (63.5%). Most patients presented with high tumor burden and aggressive behavior, as shown by an Ann Arbor stage III-IV (90.3%), high lactate dehydrogenase (LDH) level (78.1%), Ki67 ≥ 70% (85.0%), and international prognostic index (IPI) score 3-5 (74.0%). Of the 156 patients that were analyzed, 30 (19.2%) presented a history or discovery of low-grade lymphoma. Central nervous system (CNS) involvement was present in 14 of the 130 patients who were tested by lumbar puncture (10.8%). Forty-five patients (41.3%) had a high-risk CNS IPI score (4-6).

A total of 93 patients had DHL with *MYC* and *BCL2* rearrangements (59.6%), 34 had DHL with *MYC* and *BCL6* rearrangements (21.8%), and 29 had THL (18.6%).

3.2 | Treatments

Note, R-CHOP was the most common regimen used (n = 99, 63.5%) (Table S1, online supplementary material). Fifty-seven patients (36.5%) received chemotherapy that was considered to be intensive: 14 (8.9%) with DA-R-EPOCH (dose adjusted, rituximab, etoposide, prednisone, vincristine, cyclophosphamide, and doxorubicin), 16 (10.3%) with R-ACVBP (rituximab, doxorubicin, cyclophosphamide,

TABLE 1 Baseline patient characteristics (n = 156)

Treatment	R-CHOP-LIKE n = 99	Intensive chemotherapy n = 57	Total n = 156
Age, y			
Median (Q1-Q3)	66.00 (60-73)	58.00 (46-64)	62.50 (56-70)
≤65	49 (49.5%)	46 (80.7%)	95 (60.9%)
Male	63 (63.6%)	36 (63.2%)	99 (63.5%)
B symptom	31 (32.0%)	22 (39.3%)	53 (34.6%)
ECOG PS			
0-1	63 (66.3%)	45 (81.8%)	108 (72.0%)
≥ 2	32 (33.7%)	10 (18.2%)	42 (28.0%)
Ann Arbor stage			
I-II	14 (14.1%)	1 (1.8%)	15 (9.7%)
III-IV	85 (85.9%)	55 (98.2%)	140 (90.3%)
HIV-positive	3 (3.0%)	4 (7.0%)	7 (4.5%)
Extranodal sites >1			
Yes	43 (44.3%)	18 (31.6%)	61 (39.6%)
No	54 (55.7%)	39 (68.4%)	93 (60.4%)
Bone marrow involvement			
Yes	21 (36.2%)	19 (43.2%)	40 (39.2%)
No	37 (63.8%)	25 (56.8%)	62 (60.8%)
CNS involvement			
Yes	6 (7.9%)	8 (14.8%)	14 (10.8%)
No	70 (92.1%)	46 (85.2%)	116 (89.2%)
WBC			
≤ ULN	56 (76.7%)	36 (76.6%)	92 (76.7%)
> ULN	17 (23.3%)	11 (23.4%)	28 (23.3%)
LDH			
≤ ULN	18 (18.9%)	15 (26.8%)	(21.9%)
> ULN	77 (81.1%)	41 (73.2%)	118 (78.1%)
IPI score			
0-2	23 (25.0%)	15 (27.8%)	38 (26.0%)
3-5	69 (75.0%)	39 (72.2%)	108 (74.0%)
CNS IPI			
Low risk	14 (19.7%)	5 (13.2%)	19 (17.4%)
Intermediate risk	25 (35.2%)	20 (52.6%)	45 (41.3%)
High risk	32 (45.1%)	13 (34.2%)	45 (41.3%)
COO			
Non-GCB	22 (24.4%)	5 (11.4%)	27 (20.1%)
GCB	68 (75.6%)	39 (88.6%)	107 (79.9%)
Ki67 ≥ 70%	76 (80.9%)	43 (93.5%)	119 (85.0%)
Ki67 ≥ 90%	28 (38.9%)	18 (46.2%)	46 (41.4%)
Prior low-grade lymphoma			
Yes	17 (17.2%)	13 (22.8%)	30 (19.2%)
No	82 (82.8%)	44 (77.2%)	126 (80.8%)
Overexpression of *cMYC* (>40%)			
Yes	67 (80.7%)	45 (88.2%)	112 (83.6%)
No	16 (19.3%)	6 (11.8%)	22 (16.4%)
Overexpression of *BCL2* (>50%)			
Yes	75 (79.8%)	39 (73.6%)	114 (77.6%)
No	19 (20.2%)	14 (26.4%)	33 (22.4%)
Overexpression of *BCL6* (>30%)			
Yes	71 (74.7%)	41 (75.9%)	112 (75.2%)
No	24 (25.3%)	13 (24.1%)	37 (24.8%)
DHL *MYC/BCL2*	60 (60.6%)	33 (57.9%)	93 (59.6%)
DHL *MYC/BCL6*	23 (23.2%)	11 (19.3%)	34 (21.8%)
THL *MYC/BCL2/BCL6*	16 (16.2%)	13 (22.8%)	29 (18.6%)

Note: Missing data: ECOG PS (n = 6), Ann Arbor stage (n = 12), Extranodal sites >1 (n = 2), Bone marrow involvement (n = 54), CNS involvement (n = 26), WBC (n = 36), LDH (n = 5), IPI score (n = 10), CNS IPI (n = 47), COO (n = 22), Ki67 70% (n = 16), Ki67 90% (n = 45), Overexpression of *MYC* (n = 22), Overexpression of *BCL2* (n = 9), and Overexpression of *BCL6* (n = 7).

Abbreviations: BCLU, B-cell lymphoma unclassifiable; CNS, central nervous system; COO, cell-of-origin; DHL, double-hit lymphoma; DLBCL, diffuse large B-cell lymphoma; ECOG, Eastern Cooperative Oncology Group; GCB, germinal center B cell; HGBCL, high-grade B-cell lymphoma; IPI, international prognostic score; LDH, lactate dehydrogenase; PS, performance status; THL, triple-hit lymphoma; ULN, upper limit of normal; WBC, white blood cell.

vindesine, bleomycin, and prednisone), and 27 (17.3%) with R-COPADEM (rituximab, cyclophosphamide, vincristine, prednisone, doxorubicin, and methotrexate).

Twenty-eight patients received consolidation with therapeutic intensification and ASCT, including 25 (89.3%) in first complete remission. There was more ASCT in the R-ACVBP (75.0%) and R-COPADEM (25.9%) groups, compared to the R-CHOP (8.1%) and DA-R-EPOCH (7.1%) groups. The conditioning regimen for frontline ASCT was BEAM for 18 patients (BICNU, etoposide, cytarabine, and melphalan), TEAM for six (thiotepa, etoposide, cytarabine, and melphalan) and BAM for three (busulfan, cytarabine and melphalan).

So, R-DHAOx (rituximab, dexamethasone, high-dose cytarabine, and oxaliplatin) was the main salvage therapy used (61.7%). Four patients (2.6%) received second-line autologous transplantation. Despite the completion of a second line, 79.2% of patients had progressive disease following these treatments, showing the high failure rate on the second line.

3.3 | Outcomes

Figure S1 shows survival in the entire cohort. With a median follow-up of 32 months (range 28-39 months), the median PFS was

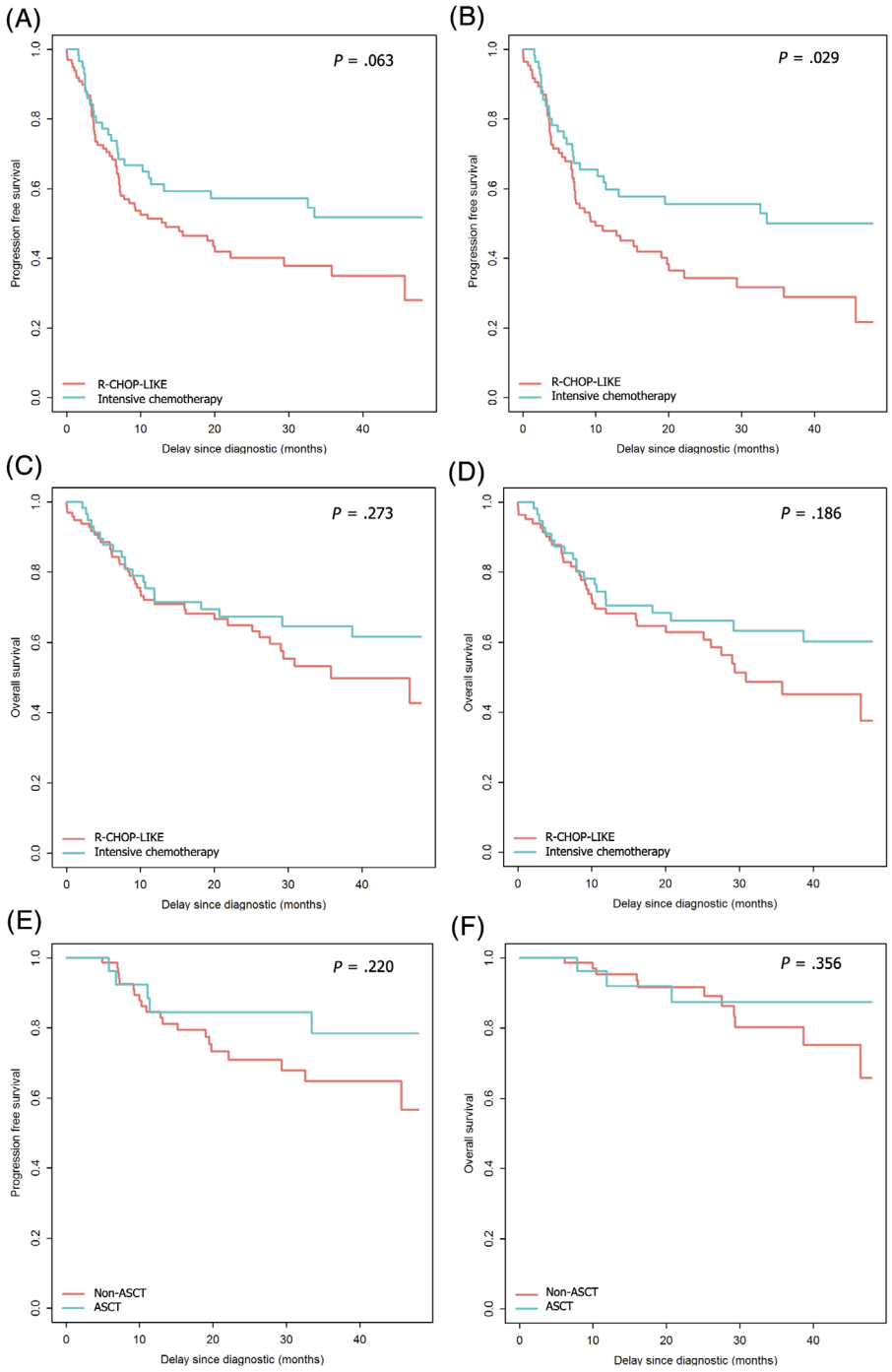

FIGURE 1 Survival according to front-line treatment. A, Progression-free survival by R-CHOP-like or intensive chemotherapy for all stages (n = 156); B, Progression-free survival by R-CHOP-like or intensive chemotherapy for advanced stage (III/IV) (n = 140); C, Overall survival by R-CHOP-like or intensive chemotherapy for all stage D, Overall survival by R-CHOP-like or intensive chemotherapy for advanced stage (III/IV); E, Progression-free survival and overall survival F, by use of autologous stem-cell transplantation [Color figure can be viewed at wileyonlinelibrary.com]

19.5 months, and the median OS was not achieved. Two-year and four-year PFS rates for all patients were 47% (95% CI 39-55) and 39% (95% CI 30-50), respectively. Two- and 4-years OS rates were 66% (95% CI 58-74) and 52% (95% CI 42-63), respectively. There was no missing data.

The overall response rate (ORR), which included complete response (CR) and partial response (PR), was 78.9%. Ninety-four patients achieved first remission after induction therapy (60.2%), consisting of 59.8% with R-CHOP and 68.4% with intensive chemotherapy (P = .375). After adjusting for age (≤65 and > 65 years), there was no difference in CR between groups (P = .604). Among intensive chemotherapy, seven patients (50.0%) were in CR after DA-R-EPOCH, 14 (87.5%) after R-ACVBP, and 18 (66.7%) after R-COPADEM.

Figure 1A shows the PFS rate and Figure 1C shows the OS rate by induction regimen for the entire cohort. There was no statistically significant difference in survival after treatment with R-CHOP or intensive chemotherapy. However, there was a trend toward longer PFS after intensive first-line chemotherapy. Two-year and four-year PFS rates were 40% (95% CI 31-52) and 28% (95% CI 16-68) with R-CHOP compared with 57% (95% CI 46-72) and 52% (95% CI 40-68) with intensive chemotherapy (P = .063).

When we analyzed only the advanced stage (III/IV Ann Arbor stage), progression-free survival was significantly longer with intensive chemotherapy with a two-year and four-year PFS rates of 56% and 50% compared to 34% and 22% with RCHOP, respectively (P = .029) (Figure 1B). There was no difference in OS in advanced stage (Figure 1D).

Among patients older than 65 years (50 in the R-CHOP group and 11 in the intensive chemotherapy group), there was no difference in progression-free survival with a two-year PFS rates of 35% in the R-CHOP group compared to 46% in the intensive chemotherapy group (P = .383) (data not shown).

Both PFS and OS were similar among patients in CR after front-line therapy, regardless of whether they received consolidation ASCT (P = .220 and P = .356, respectively). Median survival was not reached for either (Figure 1E,F). Notably, there was more ASCT with intensive chemotherapy (n = 18) than with R-CHOP (n = 8), which can be explained by an older median age of patients in the R-CHOP group than intensive chemotherapy.

Despite few available data, which do not allow for statistical testing, a comparison of intensive regimes revealed a trend for prolonged survival with R-ACVBP and R-COPADEM while DA-R-EPOCH showed comparable survival outcomes to R-CHOP (Figures S2A and S2B).

There was no statistically significant difference in PFS for patients with a history of low-grade lymphoma (known or discovered at diagnosis) with a two-year PFS rate of 43% vs 47% for patient without low-grade (P = .439). However, the two-year OS rate was 49% for patient with low-grade vs 69% for the others (P = .096) (data not shown).

Also, PFS and OS were similar across the type of second hit (*BCL2* and/or *BCL6*) that was associated with *MYC* (data not shown).

Fourteen patients presented with CNS involvement at diagnosis (10.8%). Among them, PFS and OS were significantly inferior than for patients that were free of CNS disease (Figure 2A,B), with a 2-year PFS and OS rate of 21% and 39% for patient with CNS involvement, vs 57% and 75% without CNS disease (P = .007 and P < .001). Of the 116 patients that were free of CNS involvement, 76 (65%) received CNS prophylaxis, including 68 (83%) with intrathecal methotrexate and 41 (50%) with intravenous methotrexate. The majority received both intravenous and intrathecal methotrexate. The 2-year PFS rate was 64% with prophylaxis vs 47% without prophylaxis (P = .079). There was no difference on the OS. In an exploratory analysis, there

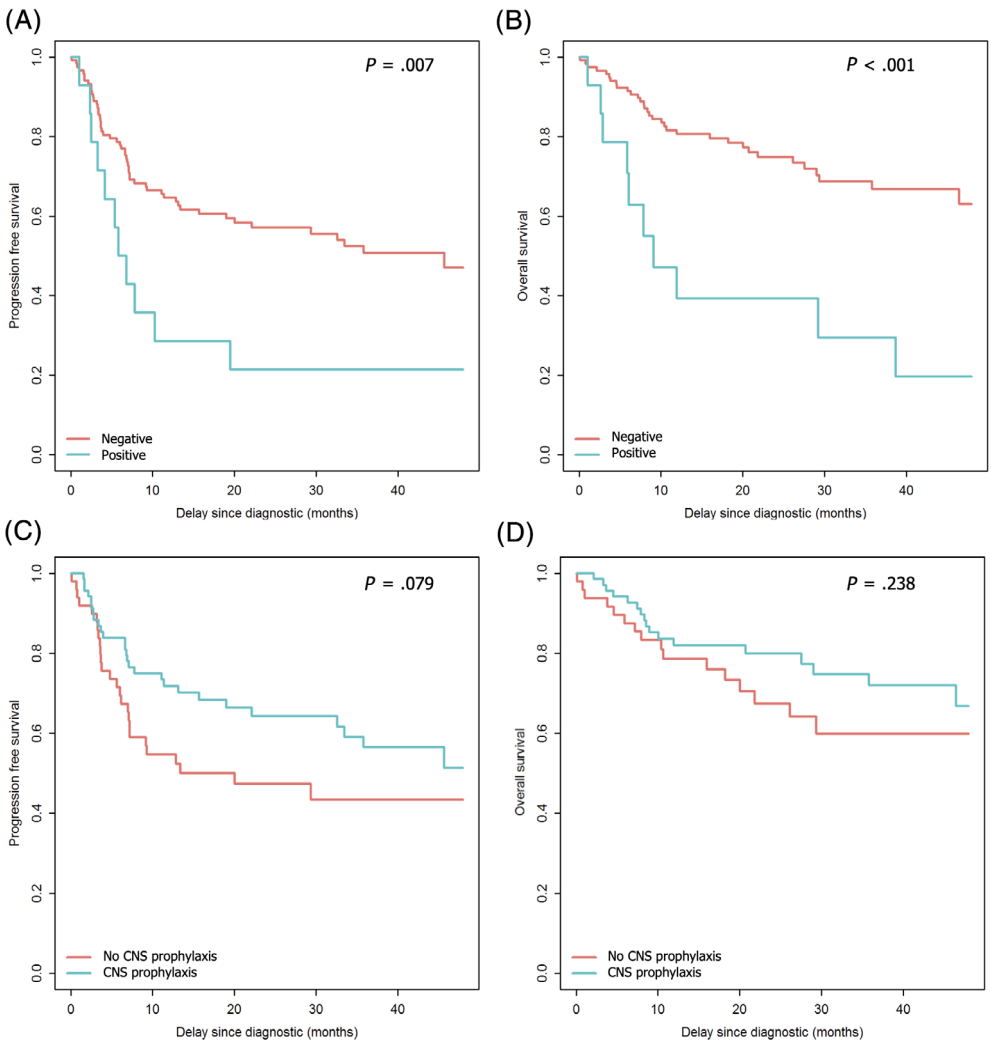

FIGURE 2 Survival according to central nervous system involvement (n = 130). A, Progression-free survival by CNS involvement B, Overall survival by CNS involvement C, Progression-free survival by CNS prophylaxis in patient free of CNS disease D, Overall survival by CNS prophylaxis in patient free of CNS disease [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 2 Treatment-related toxicities

Treatment	R-CHOP-LIKE n = 99	DA-R-EPOCH n = 14	R-ACVPB n = 16	R-COPADEM n = 27	*P* value After adjusting for age
Neutropenia grade 3-4	28 (39.4%)	9 (64.3%)	11 (78.6%)	18 (81.8%)	<.001
	n = 71	n = 14	n = 14	n = 22	
Thrombocytopenia grade 3-4	10 (14.1%)	2 (16.7%)	1 (7.1%)	12 (54.5%)	.001
	n = 71	n = 12	n = 14	n = 22	
Anemia grade 3-4	11 (16.4%)	3 (23.1%)	2 (14.3%)	14 (66.7%)	<.001
	n = 67	n = 13	n = 14	n = 21	
Transfusion of red blood cells	25 (34.2%)	4 (28.6%)	2 (14.3%)	16 (80.0%)	<.001
	n = 73	n = 14	n = 14	n = 20	
Transfusion of platelet	11 (15.1%)	2 (14.3%)	0 (0.0%)	16 (72.7%)	<.001
	n = 73	n = 14	n = 14	n = 22	
Febrile neutropenia grade 3-4	18 (24.7%)	8 (57.1%)	10 (71.4%)	17 (77.3%)	<.001
	n = 73	n = 14	n = 14	n = 22	
Mucitis grade 3-4	4 (5.7%)	2 (15.4%)	5 (35.7%)	8 (40.0%)	.001
	n = 70	n = 13	n = 14	n = 20	
Nausea grade 3-4	0 (0.0%)	0 (0.0%)	2 (16.7%)	1 (6.2%)	.029
	n = 66	n = 12	n = 12	n = 16	
Diarrhea grade 3-4	2 (2.9%)	0 (0.0%)	0 (0.0%)	3 (17.6%)	.083
	n = 70	n = 11	n = 12	n = 17	
Constipation grade 3-4	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	–
	n = 70	n = 11	n = 13	n = 17	
Neurological toxicity grade 3-4	1 (1.4%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	–
	n = 71	n = 12	n = 13	n = 18	
Infections grade 3-4	5 (7.0%)	2 (14.3%)	4 (28.6%)	1 (5.0%)	.141
	n = 71	n = 14	n = 14	n = 20	
Interruption of treatment toxicity	1 (1.0%)	0 (0.0%)	3 (18.8%)	3 (11.1%)	.009
	n = 99	n = 14	n = 16	N = 27	
Treatment-related death	7 (7.1%)	0 (0.0%)	0 (0.0%)	1 (3.7%)	–
	n = 99	n = 14	n = 16	n = 27	

was no significant difference in PFS and OS in these patients free of CNS involvement based on first-line treatment, with a two-year PFS rate of 47% in the R-CHOP group compared to 63% in the intensive chemotherapy group (P = .168). Therefore, 5.1% of patients presented a brain relapse (eight patients), of whom half (2.5%) were without CNS involvement at diagnosis, including 0.9% who had a prophylaxis.

There were 31 primary refractory patients with a poor median OS at relapse of 8.6 months (Figure S3A). When taking R/R disease into account, median OS at relapse was 16 months. There was no difference in survival after relapse according to the first-line regimen received (Figure S3B).

3.4 | Toxicities

The main grade 3-4 toxicities are listed in Table 2. Toxicity data related to ASCT were excluded. Adjusting on age, there were significantly more hematological toxicities in the intensive chemotherapy group of patients who had a greater need for transfusion (P < .001) and more febrile neutropenia (P < .001). There were six discontinuations due to toxicity in the intensive chemotherapy group, three in the R-ACVBP group, and three in the R-COPADEM group (P = .009). Of the eight treatment-related toxic deaths reported, seven were in the R-CHOP group, including three gastro-intestinal bleeding, a lysis syndrome, a multi-organ failure, a febrile neutropenia and a progressive multifocal leukoencephalopathy. Age did not explain greater toxicities in the intensive group.

3.5 | Univariate/multivariate analysis and composite score

Univariate and multivariate analysis for progression-free survival and overall survival are listed in Tables S2,S3 and S4,S5, respectively.

The variable that affected PFS and OS on univariate analysis was incorporated into the MVA. We added the variable of treatment (intensive chemotherapy vs R-CHOP). Treatment was not associated with progression-free survival in multivariate analysis while it was in univariate analysis. As expected, an elevated IPI score and CNS involvement were considered strong adverse factors for OS (hazard ratio [HR] = 6.32, 95% CI 1.43-28.02, P = .015 and HR = 2.60, 95% CI 1.31-5.16, P = .007, respectively), whereas age > 65 years and intensive treatment were not.

The percentage of patients that responded to chemotherapy without major toxicity was calculated with a composite score that considered the following toxicities: neutropenia, anemia, aplasia, mucositis, nausea, and early toxic death. The 88 pieces of tolerance data that were available for R-CHOP and the 49 pieces of data for intensive chemotherapy revealed that 22 patients (27.2%) had a response without major toxicity after R-CHOP, compared to three (6.1%) after intensive chemotherapy (P = .007).

4 | DISCUSSION

There is currently no recognized standard of care for DHL and THL. To our knowledge, this study is the largest to address the treatment of DHL and THL in a real-life study of a cohort-based population in Europe. We showed that a majority of these lymphomas remain treated with R-CHOP. Thus, PFS was significatively longer with an intensive treatment such as DA-R-EPOCH, R-ACVBP or R-COPADEM compared to R-CHOP for advanced stage (Ann Arbor III/IV) (P = .029). For all cohorts, including localized stage, there was only a trend to a longer PFS, and a difference may have emerged with a larger cohort of intensive treatment (P = .063). Although an intensive treatment appears to delay relapse, overall survival is not improved despite 32 months of proper follow-up.

Several retrospective studies have supported that survival may be improved by intensive immunochemotherapy. The largest study published was conducted by Petrich et al.[12] which included 311 patients with a median follow-up of 23 months. They showed that intensive induction regimens were associated with improved response rate and PFS (21.6 vs 7.8 months). There was no difference in OS. However, the characteristics of the patients at diagnosis were not reported according to the treatment group, which makes comparison difficult. It should also be noted that the majority of patients (71%) presented with a good general condition, suggested by a performance status (PS) between zero and one, with a median age of 60 years, which may raise the question of a selection bias of patients able to tolerate intensive chemotherapy. The experience of the MD Anderson, reported by Oki et al.[11] showed similar superiority of DA-R-EPOCH in terms of ORR and PFS, but not OS (n = 129). The study conducted by Landsburg et al. also showed that three-year PFS was shorter with R-CHOP compared to intensive chemotherapy (respectively 56% vs 88%, P = .002), suggesting more relapse in the R-CHOP group (n = 159). However, this study included only patients in first complete remission and was not initially designed to investigate induction therapy.

We observed a trend toward longer survival (PFS and OS) in our series compared to previous studies, regardless of the regimen used, with two-year and four-year OS of 66% and 52%. This may be explained because we limited case selection to the last 5 years (until April 2019), with a median year of diagnosis in 2016, to ensure routine FISH technique. This aim to reduce the historical selection bias of the most aggressive cases by the treating physician or interpreting hematopathologist, according to aggressive clinical behavior or high-grade pathologic features, respectively. On the other hand, this may result in a bias with a dilution of the most aggressive cases over time. Frosh et al. showed that DHL identified with a routine FISH technique resulted in longer patient survival than DHL identified by selective FISH.[15] Such generalization of FISH technique however raises the question of the management of localized cases of DHL and THL (Stage I/II Ann Arbor). A recent study reported by Torka et al. on 40 localized DHL showed that there was no benefit of using intensive chemotherapy over R-CHOP.[16] Of the 15 patients with a localized stage in our study, 14 were treated with R-CHOP, among which 10 had a durable CR. This may constitute a bias favoring better survival with R-CHOP. In an exploratory analysis, we explored the prognostic role of advanced stage, and we showed a significantly longer PFS with intensive chemotherapy.

Consolidative ASCT for patients in first complete remission (CR1) was not associated with improved survival, as 2-year OS was 92% vs 87% (P = .356). These data are similar to those found by Landsburg et al.[5] thereby confirming the better prognosis of patients with a CR after the first line.

Patients with CNS involvement at diagnosis face a very poor prognosis, with 4-year OS of 20%, compared to 63% for those without CNS disease (P < .001). This also appears to be a strong independent adverse factor on OS in multivariate analysis, with a hazard ratio (HR) of 2.585 (P = .007). For patients with no proven CNS disease at diagnosis, the use of methotrexate prophylaxis (intrathecal and/or intravenous) seems to improve progression-free survival (P = .079). To note, 45 patients had a high-risk CNS IPI score (41.3%).

By multivariate analysis, the two factors having the strongest impact on survival were elevated IPI score at diagnosis and CNS disease. These data are similar to those of Petrich, who developed a prognostic score based on these factors.

The data published so far did not include tolerance or toxicity data for this population. Here, we have reported a composite score that showed more CRs without toxicities for R-CHOP, compared to intensive chemotherapy. Six patients had to be downgraded to a less-intensive regimen, due to poor tolerance. Even after adjusting for age, toxicities remained more important in the intensive group. These results were expected. The higher toxic deaths in the R-CHOP group correspond to earlier deaths and can be explained by a discreetly older population in poorer general condition at diagnosis in the R-CHOP group.

The survival of patients with R/R DHL and THL remains very poor, with a median OS at relapse of 16 months in our study and did not seem to depend on the front-line regimen used. These results are similar to those of Landsburg et al., who reported a median OS of

8.6 months. Furthermore, Petrich highlights a non-significant difference in OS with or without salvage treatment.

Our interpretation about these R/R patients is that classical salvage therapy by immunochemotherapy, even when intensified with ASCT,[6] is not the solution. It is essential to look at alternatives to conventional chemotherapy. Currently, patients with *MYC/BCL2* rearrangements that are diagnosed in the USA can be included in study NCT0398448, which randomizes DA-R-EPOCH chemotherapy with or without venetoclax. There is also promising data with targeted therapies, such as BET inhibitors. Finally, despite the low numbers in this category, the results presented on CAR-T cells are very encouraging for DHL and THL.[17-20]

The main limitation of this study, due to its retrospective nature, is the lack of randomization, which is even more important when an intensive treatment is compared to another. We observed that patients were younger in the intensive chemotherapy group (81% ≤65 years vs 50% in the R-CHOP group) and in greater condition (82% of ECOG-PS ≤1 vs 66%), but the IPI score integrating these two factors remained balanced between the two groups. This may represent a selection bias, especially when considering that there was no significant difference in progression-free survival when we analyzed only patients older than 65 years, and that treatment was not associated with progression-free survival in multivariate analysis while it was in univariate analysis. It should also be noted that the proportion of HGBL-DH/TH among the DLBCL biopsies tested is lower than expected (3.5%). This could be explained by the slow implementation of routine testing and by the fact that the numbers known so far were mostly from clinical trials.

In addition, our series does not include some prognostic pathology data, such as *MYC* translocation partner. This is explained by the FISH break-apart technique,[21,22] which is mainly used in routine centers, without cytogenetics. Only six pieces of data in the 160 cases were available, thanks to conventional karyotype. After the recruitment of patients in this study was completed, the dual-fusion technique has started to become widely used in France. This will provide additional data in the coming years. Two interesting studies, reported by Copie-Bergman and more recently by the Lunenburg Lymphoma Biomarker Consortium, have shown that only patients in whom *MYC* is translocated to an immunoglobulin (IG) partner have poorer survival.[7,23]

Recent data have highlighted additional techniques to identify high-risk DLBCL beyond the single character of DHL or THL as *TP53* alterations and combinations of genomic sequencing and gene expression profile (GEP).[24] Indeed, *TP53* mutation or protein overexpression of *P53* in immunohistochemistry appears to have an additional negative independent effect on survival in THL and DHL.[25] Data are now beginning to emerge, but existing data do not allow us to include additional elements in this study.

Finally, it remains difficult to answer the question of the benefit of therapeutic intensification for these patients. Despite some consistency between the studies published in the literature and our own, the retrospective nature of the data remains problematic. Although R-CHOP appears to be inadequate, especially for advanced stage, the best treatment regimen, if one exists, remains unknown. The construction of prospective randomized clinical trials designed for this particular population is necessary and could probably concentrate rather on the incorporation of new targeted agents than on the intensification to improve outcomes. Alternatives, such as CAR-T cells, should also be considered as soon as possible for R/R patients.

We confirm that the most important prognostic factor for these lymphomas is to obtain a CR with the first line. Adverse prognostic factors that need to be considered for the therapeutic decision are elevated IPI score and CNS disease.

ACKNOWLEDGEMENTS

We thank all authors for collecting data, discussion and input into the manuscript. We warmly thank Prof. Traverse-Glehen Alexandra and Prof. Laurent Camille for extracting the necessary data quickly.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Marie-Charlotte Laude, Laure Lebras and Anne-Sophie Michallet designed the study, acquired, analyzed, interpreted the data and wrote the manuscript. Fabien Subtil conducted the statistical analysis. All authors contributed provisions of study materials or patients and collection and assembly of data. All authors reviewed the manuscript and approved the final version of the manuscript. All authors had full access to all study data and the corresponding author had final responsibility for the decision to submit for publication.

ETHICS APPROVAL STATEMENT

Approved by the local authority for the protection of privacy and personal data in clinical research (N° R201-004-075).

DATA AVAILABILITY STATEMENT

Author elects to not share data

ORCID

Marie-Charlotte Laude https://orcid.org/0000-0001-9967-706X
Pierre Sesques https://orcid.org/0000-0001-8264-822X
Anne-Sophie Michallet https://orcid.org/0000-0002-4256-8126

REFERENCES

1. Le Guyader-Peyrou S, Defossez G, Dantony E, et al. Rapport - Volume 2 Hémopathies malignes - Estimations nationales de l'incidence et de la mortalité par cancer en France métropolitaine entre 1990 et 2018 - Juillet 2019 - Ref: RAHMINCNAT19. https://www.e-cancer.fr/Expertises-et-publications/Catalogue-des-publications/Rapport-Volume-2-Hemopathies-malignes-Estimations-nationales-de-l-incidence-et-de-la-mortalite-par-cancer-en-France-metropolitaine-entre-1990-et-2018. Accessed July 5, 2019.
2. Swerdlow SH, Campo E, Pileri SA, et al. The 2016 revision of the World Health Organization classification of lymphoid neoplasms. *Blood*. 2016;127:2375-2390.
3. Scott DW, King RL, Staiger AM, et al. High-grade B-cell lymphoma with MYC and BCL2 and/or BCL6 rearrangements with diffuse large B-cell lymphoma morphology. *Blood*. 2018;131:2060-2064.

4. Pedersen MØ, Gang AO, Poulsen TS, et al. Double-hit BCL2/MYC translocations in a consecutive cohort of patients with large B-cell lymphoma - a single centre's experience. *Eur J Haematol*. 2012;89:63-71.
5. Landsburg DJ, Falkiewicz MK, Maly J, et al. Outcomes of patients with double-hit lymphoma who achieve first complete remission. *J Clin Oncol*. 2017;35:2260-2267.
6. Herrera AF, Mei M, Low L, et al. Relapsed or refractory double-expressor and double-hit lymphomas have inferior progression-free survival after autologous stem-cell transplantation. *J Clin Oncol*. 2017;35:24-31.
7. Rosenwald A, Bens S, Advani R, et al. Prognostic significance of MYC rearrangement and translocation partner in diffuse large B-cell lymphoma: a study by the Lunenburg Lymphoma Biomarker Consortium. *J Clin Oncol*. 2019;37:3359-3368.
8. Cohen JB, Geyer SM, Lozanski G, et al. Complete response to induction therapy in patients with Myc-positive and double-hit non-Hodgkin lymphoma is associated with prolonged progression-free survival. *Cancer*. 2014;120:1677-1685.
9. Barrans S, Crouch S, Smith A, et al. Rearrangement of MYC is associated with poor prognosis in patients with diffuse large B-cell lymphoma treated in the era of rituximab. *J Clin Oncol*. 2010;28:3360-3365.
10. Savage KJ, Johnson NA, Ben-Neriah S, et al. MYC gene rearrangements are associated with a poor prognosis in diffuse large B-cell lymphoma patients treated with R-CHOP chemotherapy. *Blood*. 2009;114:3533-3537.
11. Oki Y, Noorani M, Lin P, et al. Double hit lymphoma: the MD Anderson Cancer Center clinical experience. *Br J Haematol*. 2014;166:891-901.
12. Petrich AM, Gandhi M, Jovanovic B, et al. Impact of induction regimen and stem cell transplantation on outcomes in double-hit lymphoma: a multicenter retrospective analysis. *Blood*. 2014;124:2354-2361.
13. 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:e609-e617.
14. Laurent C, Baron M, Amara N, et al. Impact of expert pathologic review of Lymphoma diagnosis: study of patients from the French Lymphopath network. *J Clin Oncol*. 2017;35:2008-2017.
15. Frosch ZAK, Nasta SD, Schuster SJ, et al. Outcomes for double hit Lymphoma patients identified via routine vs selective testing for MYC rearrangement. *Blood*. 2019;134:1607-1607.
16. Torka P, Kothari SK, Sundaram S, et al. Outcomes of patients with limited-stage aggressive large B-cell lymphoma with high-risk cytogenetics. *Blood Adv*. 2020;4:253-262.
17. Sesques P, Ferrant E, Safar V, et al. Commercial anti-CD19 CAR T cell therapy for patients with relapsed/refractory aggressive B cell lymphoma in a European center. *Am J Hematol*. 2020;95:1324–1333.
18. Schuster SJ, Bishop MR, Tam CS, et al. Tisagenlecleucel in adult relapsed or refractory diffuse large B-cell lymphoma. *N Engl J Med*. 2019;380:45-56.
19. Locke FL, Ghobadi A, Jacobson CA, et al. Long-term safety and activity of axicabtagene ciloleucel in refractory large B-cell lymphoma (ZUMA-1): a single-arm, multicentre, phase 1-2 trial. *Lancet Oncol*. 2019;20:31-42.
20. Abramson JS, Palomba ML, Gordon LI, et al. Pivotal safety and efficacy results from transcend NHL 001, a multicenter phase 1 study of lisocabtagene maraleucel (liso-cel) in relapsed/refractory (R/R) large B cell lymphomas. *Blood*. 2019;134:241-241.
21. Nguyen L, Papenhausen P, Shao H. The role of c-MYC in B-cell lymphomas: diagnostic and molecular aspects. *Genes (Basel)*. 2017;8:116.
22. Ventura RA, Martin-Subero JI, Jones M, et al. FISH analysis for the detection of lymphoma-associated chromosomal abnormalities in routine paraffin-embedded tissue. *J Mol Diagn*. 2006;8:141-151.
23. Copie-Bergman C, Cuillière-Dartigues P, Baia M, et al. MYC-IG rearrangements are negative predictors of survival in DLBCL patients treated with immunochemotherapy: a GELA/LYSA study. *Blood*. 2015;126:2466-2474.
24. Frosch ZAK, Landsburg DJ. Molecular risk stratification in aggressive B-cell lymphomas. *J Clin Oncol*. 2020;38:2014-2017.
25. Wang XJ. P53 expression correlates with poorer survival and augments the negative prognostic effect of MYC rearrangement, expression or concurrent MYC/BCL2 expression in diffuse large B-cell lymphoma. *Mod Pathol*. 2017;30:194-203.

SUPPORTING INFORMATION

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

How to cite this article: Laude M-C, Lebras L, Sesques P, et al. First-line treatment of double-hit and triple-hit lymphomas: Survival and tolerance data from a retrospective multicenter French study. *Am J Hematol*. 2021;96:302–311. https://doi.org/10.1002/ajh.26068