The Journal of Pathology: Clinical Research
J Pathol Clin Res November 2021; **7:** 604–615
Published online 9 August 2021 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/cjp2.223

ORIGINAL ARTICLE

c-MYC and p53 expression highlight starry-sky pattern as a favourable prognostic feature in R-CHOP-treated diffuse large B-cell lymphoma

Antonin Bouroumeau[1,2,3], Lucile Bussot[4], Thierry Bonnefoix[2,5], Cyril Fournier[6,7], Caroline Chapusot[8], Olivier Casasnovas[9], Laurent Martin[8], Anne McLeer[1,2], Edwige Col[1], Laurence David-Boudet[1], Christine Lefebvre[10], Caroline Algrin[11], Tatiana Raskovalova[10], Marie-Christine Jacob[10], Claire Vettier[10], Simon Chevalier[10], Mary B Callanan[6,7], Rémy Gressin[4], Anouk Emadali[2,5] and Hervé Sartelet[1,12]*

[1]*Department of Pathology, Grenoble-Alpes University Hospital, Grenoble, France*
[2]*Institute for Advanced Biosciences, INSERM U1209/CNRS UMR 5309/Grenoble-Alpes University, Grenoble, France*
[3]*Division of Clinical Pathology, Diagnostic Department, Hôpitaux Universitaires Genève, Geneva, Switzerland*
[4]*Department of Clinical Hematology, Grenoble-Alpes University Hospital, Grenoble, France*
[5]*Pole Recherche, Grenoble-Alpes University Hospital, Grenoble, France*
[6]*University of Bourgogne, INSERM 1231, Dijon, France*
[7]*Unit for Innovation in Genetics and Epigenetics in Oncology, Dijon University Hospital, Dijon, France*
[8]*Department of Pathology, Dijon University Hospital, Dijon, France*
[9]*Department of Clinical Hematology, Dijon University Hospital, Dijon, France*
[10]*Hematology, Oncogenetics and Immunology Unit, Grenoble-Alpes University Hospital, Grenoble, France*
[11]*Institut Daniel Hollard, Grenoble, France*
[12]*Department of Pathology, Nancy Regional University Hospital, Nancy, France*

**Correspondence to: Hervé Sartelet, Department of Pathology, Nancy Regional University Hospital, Nancy, France. E-mail: h.sartelet@chru-nancy.fr*

Abstract

Diffuse large B-cell lymphoma (DLBCL) is a clinically heterogeneous entity, in which the first-line treatment currently consists of an immuno-chemotherapy regimen (R-CHOP). However, around 30% of patients will not respond or will relapse. Overexpression of c-MYC or p53 is frequently found in DLBCL, but an association with prognosis remains controversial, as for other biomarkers previously linked with DLBCL aggressivity (CD5, CD23, or BCL2). The aim of this study was to explore the expression of these biomarkers and their correlation with outcome, clinical, or pathological features in a DLBCL cohort. Immunohistochemical (c-MYC, p53, BCL2, CD5, and CD23), morphological ('starry-sky' pattern [SSP]), targeted gene panel sequencing by next-generation sequencing (NGS), and fluorescence *in situ* hybridisation analyses were performed on tissue microarray blocks for a retrospective cohort of 94 R-CHOP-treated *de novo* DLBCL. In univariate analyses, p53 overexpression ($p53^{high}$) was associated with unfavourable outcome ($p = 0.04$) and with c-MYC overexpression ($p = 0.01$), whereas c-MYC overexpression was linked with an SSP ($p = 0.004$), but only tended towards an inferior prognosis ($p = 0.06$). Presence of a starry-sky morphology was found to be correlated with better survival in $p53^{high}$ DLBCL ($p = 0.03$) and/or c-MYC-positive DLBCL ($p = 0.002$). Furthermore, NGS data revealed that these three variables were associated with somatic mutations (*PIM1*, *TNFRSF14*, *FOXO1*, and *B2M*) involved in B-cell proliferation, survival, metabolism, and immune signalling. Taken together, these results show that the SSP pattern seems to be a protective factor in high-risk DLBCL subgroups and highlight cell death as a built-in failsafe mechanism to control tumour growth.

Keywords: DLBCL; R-CHOP; p53; prognostic; starry-sky; c-MYC; protective; factor

Received 15 October 2020; Revised 16 April 2021; Accepted 19 April 2021

No conflicts of interest were declared.

© 2021 The Authors. *The Journal of Pathology: Clinical Research* published by The Pathological Society of Great Britain and Ireland and John Wiley & Sons, Ltd.
This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

Introduction

Diffuse large B-cell lymphoma (DLBCL) is a clinically heterogeneous entity, accounting for around 30% of all lymphomas [1] and is the most common haematological malignancy in Western countries [2,3]. DLBCL is also among the most aggressive lymphoid neoplasms, with a 5-year overall survival (OS) of around 50% [4]. Currently, a significant proportion of patients will still not have a primary response or will relapse with the standard of care, R-CHOP (rituximab, a humanised monoclonal CD20 antibody plus cyclophosphamide, doxorubicin hydrochloride, vincristine sulphate, and prednisolone). Although outcomes in DLBCL have improved with this immunochemotherapy [5,6], around 10–15% of patients exhibit primary refractory disease and an additional 20–25% relapse, usually within the first 2 years [5], highlighting the limits of R-CHOP and the need for new predictive biomarkers that could guide individualised risk-adapted therapy [7]. Currently, the only robust prognostic factor is the International Prognostic Index (IPI) score [8], revised in the era of R-CHOP [9] (r-IPI), which segregates patients into different outcome groups.

Advances in molecular genetics have confirmed the biological heterogeneity of DLBCL [10,11] through the identification of several alterations leading to oncogene activation and modulation of tumour suppressor activity [12,13]. Patterns of gene expression reminiscent of germinal centre B cell (GCB group) and activated B cell (ABC group) allowed the definition of the 'cell of origin' DLBCL classification, the GCB subtype being associated with better prognosis [10]. New entities have also been described such as high-grade B-cell lymphoma (HGBL), encompassing HGBL double-hit (DHL)/triple-hit lymphomas (THL) and HGBL, NOS (not otherwise specified). HGBL DHL or THL are defined by *MYC* and *BCL2* and/or *BCL6* rearrangements, whereas HGBL, NOS presents a blastoid morphology or an intermediate aspect between DLBCL, NOS, and Burkitt lymphomas and lacking DHL or THL [14,15]. More recently, different molecular DLBCL subgroups associated with outcomes have been uncovered based on mutational profiling [16,17]. However, although these investigations have shed light on the biological heterogeneity of DLBCL and revealed new potential therapeutic targets, they failed to precisely discriminate very high-risk DLBCL patients.

Immunohistochemistry (IHC) is routinely used to classify DLBCL as GCB or non-GCB using Hans' algorithm [18] and to evaluate expression of several biomarkers associated with DLBCL aggressivity, including c-MYC, BCL2, p53, CD5, or CD23. Of note, c-MYC, BCL2, and BCL6 overexpression may occur independent of *MYC*, *BCL2*, or *BCL6* gene rearrangement [19].

MYC is a proto-oncogene encoding the transcription factor c-MYC controlling cellular processes such as cell cycle and cell growth [20]. Its oncogenic activation has been described in Burkitt lymphomas and in 10% of DLBCL [19,21]. It has been proven that *MYC* rearrangement has an adverse effect on survival in R-CHOP-treated DLBCL, especially if the partner is an immunoglobulin (Ig) gene [22]. Although c-MYC protein expression is found in 30–50% of DLBCL (according to standard cut-off values) [23], its survival significance is still controversial even if it seems to correlate with poorer outcomes in the rituximab era [19,23–25]. *BCL2* is an anti-apoptotic gene encoding the BCL2 protein which is overexpressed in approximately 50% of DLBCL [26] and has been a well-recognised prognostic factor in the pre-rituximab era [27,28]. Nowadays, the addition of rituximab can mitigate the adverse effects of BCL2 expression, especially in the ABC subtype [29,30]. Furthermore, double-expressor lymphoma (DEL), defined by co-expression of c-MYC and BCL2, is also associated with a poor prognostic effect but with a stronger negative impact than BCL2 or c-MYC overexpression taken individually [14,31]. The protein p53 (or TP53) is encoded by the gene *TP53* (also known as *p53*), a well-known tumour suppressor mediating several crucial cell functions such as cell cycle arrest, apoptosis, or DNA reparation [32]. Thus, *p53* mutations (gain or loss of function) are found in many cancers, including in 20% of DLBCL [13], and confer inferior survival [33,34]. Protein overexpression is more common (30–40% of DLBCL are p53-positive, depending on the positivity threshold) and is also predictive of poorer prognosis in R-CHOP-treated patients [33,35]. Moreover, some studies showed that p53 overexpression enhances c-MYC-related adverse effects in DLBCL, identifying a high risk of relapse subgroup of patients co-expressing c-MYC and p53 [34,36]. Other IHC biomarkers can also be used, such as CD5 and CD23, which are positive in 5–10% of DLBCL [37,38] but their significance still remains controversial. On one hand, CD23 is expressed on naïve B cells and follicular dendritic cells and its overexpression has been associated with unfavourable outcomes in DLBCL [38,39]. On the other hand, CD5 is expressed on normal T cells but as it is also positive in other lymphoid malignancies (chronic lymphocytic leukaemia and Mantle cell lymphoma), further analyses (Cyclin D1 and SOX11) are needed to exclude these differential diagnoses. *De novo* CD5-positive DLBCL seems to be associated with adverse clinical and

© 2021 The Authors. *The Journal of Pathology: Clinical Research* published by The Pathological Society of Great Britain and Ireland and John Wiley & Sons, Ltd.

biological features [40] and with poorer OS and progression-free survival (PFS) after chemotherapy compared to CD5-negative DLBCL [37,41].

Finally, some DLBCL have been shown to exhibit a morphological 'starry-sky' pattern (SSP). SSP, also found in Burkitt lymphoma, is defined by the presence of tingible-body macrophages (appearing as 'stars' because they contain cellular debris within their cytoplasm) intermingled with a 'sky' of tumour cells, and is a reflection of a high mitotic rate and a high degree of apoptosis [42]. Macrophage proliferation in SSP has been described as promoted by lymphoma cell apoptosis [43].

The aim of the present study was to explore, in a retrospective cohort of R-CHOP-treated DLBCL, the correlation between SSP and expression of several biomarkers (c-MYC, p53, BCL2, CD5, and CD23) with outcome, clinical, pathological, and molecular features.

Materials and methods

Patient selection

Ninety-four patients were included in a monocentric retrospective study in Grenoble, France (Centre Hospitalier Universitaire Grenoble Alpes and Institut Daniel Hollard, Department of Hematology) from January 2011 to December 2018. All patients had signed an institutional consent form (reference AC-2014-2094 no. 9). Each case was reviewed to ensure compliance with the following inclusion criteria. DLBCL tumours had to be *de novo*, and all patients with a history of other lymphoma and relapses were excluded. Primitive cerebral DLBCL, DLBCL leg-type, HGBL, and primary mediastinal B-cell lymphoma were discarded because of their particular pathological and biological presentations. Treatment had to be R-CHOP in the first line (at least 3 cycles). Tissues had to be formalin-fixed paraffin-embedded Alcohol, Formalin, and Acetic acid (AFA) fixation was excluded to avoid IHC bias) and enough residual material had to be available to perform tissue microarray (TMA) construction (needle biopsies were not included). Biological and clinical data were collected for each patient, including gender, age, IPI score, Ann Arbor stage, serum lactate dehydrogenase level as well as detailed treatment and long-term survival data. DLBCL were classified according to the WHO classification with the GCB or non-GCB status using Hans' algorithm. Finally, a morphological review was conducted to search for an SSP for each case.

TMA construction

Five TMA blocks were built as described previously [44]. TMAs were composed of four 0.6 mm cores from each patient's samples and non-tumour samples were also added as internal positive controls (normal appendix and testis).

Morphological analysis

TMA tissue spots were reviewed by a morphological analysis (haematoxylin–eosin–saffron-stained section) to exclude those with an unrepresentative aspect (fibrotic or necrotic changes) and to ensure that every case showed a morphology compatible with a DLBCL, NOS.

Immunohistochemistry

IHC analysis was performed on 4-µm thick sections cut from TMA blocks. A fully automated (baking, deparaffinisation, antigen retrieval, IHC, and counterstaining) BenchMark ULTRA (Roche Diagnostics, Meylan, France) was used for all antibodies tested. Nine antibodies were used for each TMA: c-MYC, BCL2, p53, CD5, CD23, MUM-1, Cyclin D1, SOX11, and Ki67 (see supplementary material, Table S1) and a routinely used procedure was followed. For c-MYC IHC, the UltraView™ Universal DAB Detection Kit (Ventana Medical Systems Inc., Tucson, AZ, USA) was used to increase the staining intensity. The cut-off for c-MYC positivity was more than 40% of tumour cells showing immunoreactivity, whereas thresholds for BCL2, p53, CD5, and CD23 were more than 50%, in accordance with previously established cut-offs [31,33,37,38]. For Ki67, results were rendered semi-quantitatively on a scale from 1 to 4+, according to the percentage of positive cells (1+ [1–25%], 2+ [26–50%], 3+ [51–75%], and 4+ [76–100%]) [18]. IHC staining was scored 'blindly' by two pathologists (AB and HS) and discordant cases were subjected to a joint proof reading.

Antibodies against MUM1, Cyclin D1, and SOX11 were used to exclude DLBCL with *IRF4* rearrangement and differential diagnoses for all *de novo* CD5-positive DLBCL.

EBER *in situ* hybridisation

To identify EBV-positive DLCBL, NOS, we performed Epstein-Barr virus-encoded small RNA (EBER) *in situ* hybridisation (ISH) on deparaffinised 4-µm thick sections from TMA blocks using a BenchMark ULTRA automated tissue stainer, a fluorescein-conjugated EBER oligonucleotide probe (Roche Diagnostics), and an INFORM iView Blue ISH detection kit (Roche

© 2021 The Authors. *The Journal of Pathology: Clinical Research* published by The Pathological Society of Great Britain and Ireland and John Wiley & Sons, Ltd.

Diagnostics). Slides were counterstained using Red Counterstain II (Roche Diagnostics). Slides were assessed by two pathologists (AB and HS) and 80% of EBER-positive tumour cells was the cut-off applied to consider a DLBCL EBV-positive [1].

Fluorescence *in situ* hybridisation

To be sure of the absence of HGBL DHL/THL in our cohort, interphase fluorescence *in situ* hybridisation (FISH) was performed using 4-µm thick TMA sections. All samples were tested using *MYC*, *BCL2*, and *BCL6* break-apart FISH using the ZytoLight® SPEC *MYC* Dual Color Break Apart Probe, the ZytoLight® SPEC *BCL2* Dual Color Break Apart Probe, and the ZytoLight® SPEC *BCL6* Dual Color Break Apart Probe (ZytoVision, Bremerhaven, Germany), respectively. In addition, a Vysis LSI *IGH/MYC/CEP* 8 Tri-Color Dual Fusion Probe Kit (Abbott Laboratories, Abbott Park, IL, USA) was used to determine if *IGH* was the partner gene of *MYC* rearrangement. At least 100 tumour nuclei were analysed for each sample. Nuclei were counterstained with DAPI/Vectashield® (Vector Laboratories, Burlingame, CA, USA) and were analysed using a Leica CytoVision GSL10 FISH fluorescence capture system® (Leica, Nanterre, France) under a ×63 oil immersion objective. Signals were counted using the CytoVision imaging system® (Leica). A cut-off value of 10% of the cells showing rearrangement was selected to confirm rearrangement for a specimen.

Targeted NGS

Four 0.6-mm FFPE core biopsies were used per case at the same time as TMAs were built. DNA was extracted using a QIAsymphony DSP DNA Mini Kit (Qiagen, Hilden, Germany). DNA quality was assessed by TapeStation analysis and quantified for next-generation sequencing (NGS) by fluorometry (ThermoFisher Scientific, Waltham, MA, USA). Targeted sequencing was performed by a capture approach covering 51 genes that present mutations in DLBCL, follicular lymphoma, and chronic lymphocytic leukaemia [45]. Libraries were prepared by standard procedures using 50 ng of DNA and a KAPA HyperPlus kit (Roche Diagnostics). Libraries were purified, quantified, and barcoded before being sequenced using a NextSeq 500/550 Mid Output Kit V2 (300 cycles) kit and a NextSeq 550 Illumina sequencer (Illumina, San Diego, CA, USA). Somatic mutation calling was performed using an in-house sequence alignment, variant caller, and annotation pipeline. Somatic mutations that were present at >5% variant allele frequency and that affected protein coding regions (non-synonymous) or exon splice site junctions were retained for analysis. All somatic variants were reviewed manually for specificity and pathogenicity.

Table 1. Cohort description – clinical characteristics.

Parameters	(Range, *95% CI*) [Proportion]
Median age at diagnosis (years)	67 (29–94, *62–68*)
>60 years	70.0% [66/94]
Gender	
Male	56.3% [53/94]
Female	43.7% [41/94]
Median duration of treatment (days)	122 [13; 214]
Median follow-up time (months)	41.3 (2–93, *29.0–53.6*)
OS	62.2% (*49.4–72.4*)
PFS	51.4% (*36.2–64.1*)
Number of relapsed patients	39% [37/94]
Ann Arbor staging classification (diagnosis)	
I (single LN involved)	11.8% [11/94]
II (two or more LN ipsilateral to the diaphragm)	20.2% [19/94]
III (LN on both sides of the diaphragm)	22.3% [21/94]
IV (Involvement; extralymphatic organs or tissues)	45.7% [43/94]
LDH > upper limit of normal	74.5% [70/94]
R-IPI class (diagnosis)	
Very low risk: 0	7.4% [7/94]
Low risk: 1–2	43.6% [41/94]
High risk: 3–5	49.0% [46/94]

LDH, lactate dehydrogenase; LN, lymph node.

Table 2. Cohort description – pathological characteristics.

Parameters	Cohort (proportion)
DLBCL subtype (Hans' algorithm)	
GCB	36.2% (34/94)
Non-GCB	63.8% (60/94)
SSP	29.8% (28/94)
CD5	
Positive	8.5% (8/94)
Negative	91.5% (86/94)
CD23	
Positive	10.6% (10/94)
Negative	89.4% (84/94)
c-MYC	
Positive	31.9% (30/94)
Negative	68.1% (64/94)
BCL2	
Positive	60.6% (57/94)
Negative	39.4% (37/94)
DEL (c-MYC+ and BCL2+)	21.3% (20/94)
p53	
$p53^{high}$ (≥50%)	40.0% (36/90)
$p53^{low}$ (<50%)	60.0% (54/90)
Proliferation index (Ki67)	
Ki67 = 4+	52.1% (49/94)
Ki67 < 4+	47.9% (45/94)
Presence of *MYC* rearrangement (by FISH)	5.8% (5/86)
Presence of an *MYC-IgH* rearrangement (by FISH)	3.5% (3/86)

© 2021 The Authors. *The Journal of Pathology: Clinical Research* published by The Pathological Society of Great Britain and Ireland and John Wiley & Sons, Ltd.

Statistics

OS and PFS were assessed by Kaplan–Meier analyses and Cox regression. The starting point for time-to-event analysis was the date of the pathological diagnosis. Observational intervals of patients without any event at the time of the last follow-up or at 7.5 years after diagnosis were censored. The proportional hazards assumption was checked on the basis of Schoenfeld residuals after Cox model fitting. The concordance probability, which is defined as the probability that predictions and outcomes are concordant, was provided by Harrell's *C* and Gönen and Heller's concordance coefficients.

All statistical analyses were performed using Stata 16.1 (Stata Corporation, College Station, TX, USA) and R statistical software 3.5.0 (The R Foundation for Statistical Computing, Vienna, Austria). All statistical tests were two-sided, and the level for significance was set at $p < 0.05$.

Results

Cohort clinical characteristics

A total of 94 patients were included; the main clinical characteristics are presented in Table 1. In brief, within this cohort, the gender distribution was balanced with a slight predominance of male patients (56.3%) and a median age at diagnosis of 67 years. The average time

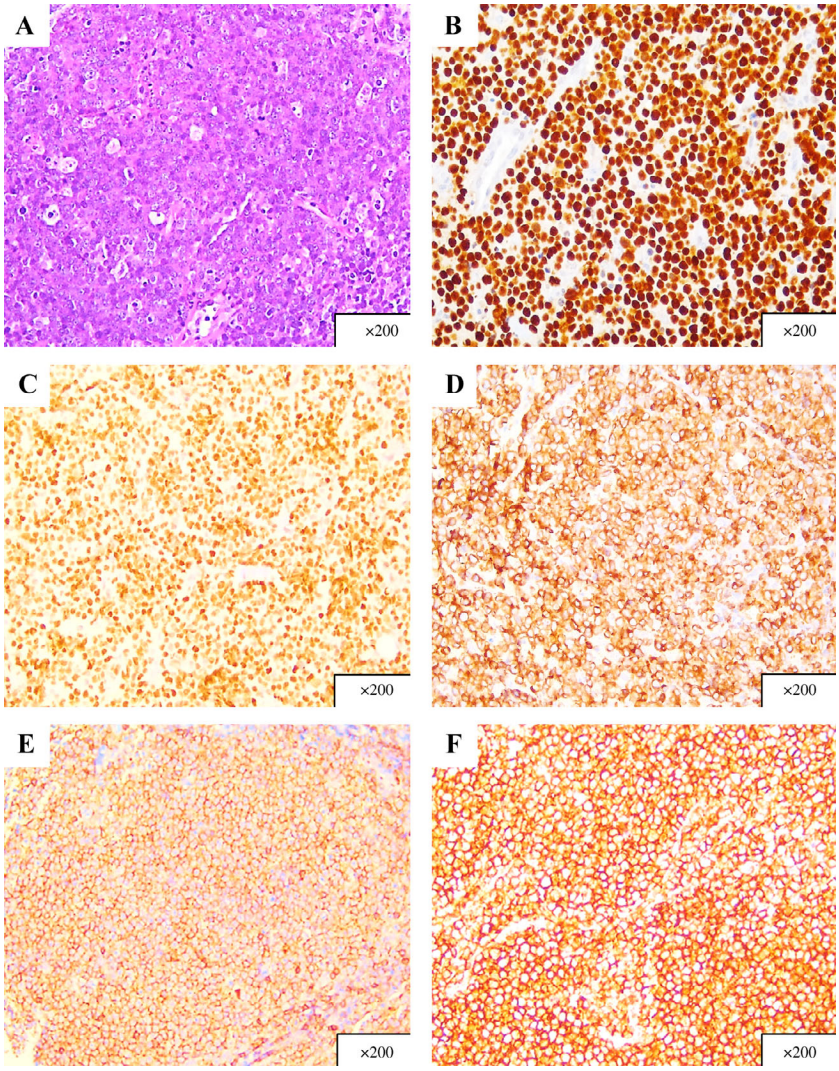

Figure 1. SSP and IHC biomarkers in R-CHOP-treated DLBCL. (A) SSP is characterised by the presence of tingible-body macrophages compounded with tumour cells. IHC positivity was defined by a strong nuclear staining for (B) p53 and (C) c-MYC or a diffuse cytoplasmic/membranous staining for (D) BCL2, (E) CD5, and (F) CD23.

© 2021 The Authors. *The Journal of Pathology: Clinical Research* published by The Pathological Society of Great Britain and Ireland and John Wiley & Sons, Ltd.

of long-term follow-up was over 3 years (41 months). Around half of the patients presented an advanced stage DLBCL (Ann Arbor stage IV in 45.7% and a 3–5 IPI score in 49.0%).

Cohort pathological features

The main pathological characteristics are summarised in Table 2. Following morphological assessment, all DLBCL showed a typical morphology. No case of HGBL, NOS was identified. SSP was seen in 29.8% of cases (Figure 1A). Using Hans' algorithm, more non-GCB DLBCL were observed than GCB (63.8 versus 36.2%). Regarding IHC analyses, p53 was found positive ($p53^{high}$) in 40.0% of DLBCL (Figure 1B), c-MYC was expressed in around one-third of cases (30/94, Figure 1C), and BCL2 was overexpressed in 57 patients (60.6%, Figure 1D). Patients with DEL accounted for nearly one-quarter of this cohort (21.3%). CD5 expression was observed in 8 cases (8.5%, Figure 1E) and 10 patients were CD23 positive (10.6%, Figure 1F). Of note, all CD5-positive DLBCL were negative for Cyclin D1 and SOX11 and no MUM1 overexpression compatible with an *IRF4* rearrangement was observed. Using Ki67 staining, more than half of the cases showed a 4+ proliferation index (52.1%). Only one patient provided a positive EBER ISH result.

FISH analyses revealed five patients had *MYC* rearrangement (Figure 2A,B), including three cases of *MYC-IgH* rearrangement (Figure 2C,D), and these five patients with *MYC* rearrangement were c-MYC-positive by IHC. We did not find any HGBL DHL/THL in our cohort.

Cohort validation

As expected, this cohort shows the inferior outcome associated with the non-GCB subtype compared to GCB DLBCL ($p = 0.005$, see supplementary material, Figure S1A). Prognostic impact of the R-IPI class was also confirmed with a better outcome for patients with IPI = 0 compared to patients with IPI = 1–2 or

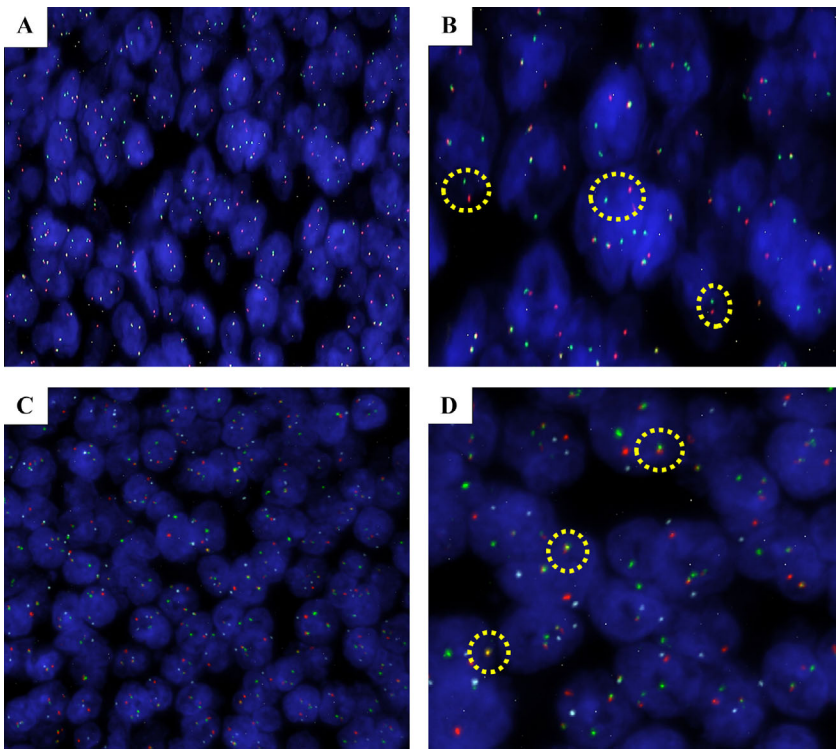

Figure 2. Illustration of a DLBCL with an *IgH-MYC* rearrangement by FISH. (A) This case presented an *MYC* rearrangement, defined by a split between the red and the green signals (ZytoLight® SPEC *MYC* Dual Color Break Apart Probe), better visualised (B, yellow circles) at greater magnification. (C) Here, the partner gene of *MYC* rearrangement was *IgH* (Vysis LSI *IGH/MYC/CEP* 8 Tri-Color Dual Fusion Probe Kit), as illustrated in the bottom panels (D, yellow circles) with double-colour fusion signals.

© 2021 The Authors. *The Journal of Pathology: Clinical Research* published by The Pathological Society of Great Britain and Ireland and John Wiley & Sons, Ltd.

IPI = 3–5 (p = 0.0008, see supplementary material, Figure S1B).

Impact of c-MYC-positive DLBCL on clinical features and outcomes

Overexpression of c-MYC was significantly correlated with IPI score higher than 2 (odds ratio [OR] = 3.4, p = 0.01), with SSP (OR = 3.9, p = 0.004), and with 4+ proliferation index (OR = 7.4, p = 0.0005). DLBCL with c-MYC overexpression tended to have a worse prognosis (p = 0.06, Figure 3A), whereas SSP did not impact survival in our cohort (p = 0.38, see supplementary material, Figure S2A). However, c-MYC-positive DLBCL without SSP defined a subgroup of patients with inferior survival compared to those showing SSP (for OS, p = 0.002, Figure 3B, and for PFS, p = 0.001, see supplementary material, Figure S2B). In c-MYC-negative DLBCL subgroup, SSP did not impact survival.

Impact of p53^{high} DLBCL on clinical features and outcomes

In univariate survival analysis, p53^{high} DLBCL presented poorer outcome (p = 0.04, Figure 3C). This p53 prognostic impact was even stronger when DLBCL did not present SSP (p = 0.03, Figure 3D). Moreover, in the present study, p53 overexpression was linked with c-MYC overexpression (OR = 3.4, p = 0.01) but not with SSP.

SSP DLBCL morphology was also able to segregate two prognostic groups within p53^{high} and c-MYC-positive DLBCL (p = 0.004, see supplementary material, Figure S2E). As for c-MYC, SSP was not correlated with survival in p53^{low} DLBCL subgroup.

Impact of other biomarkers on clinical features and outcomes

None of the other biomarkers tested showed an association with outcome (DEL, BCL2-positive DLBCL, CD23-positive DLBCL, CD5-positive DLBCL, and

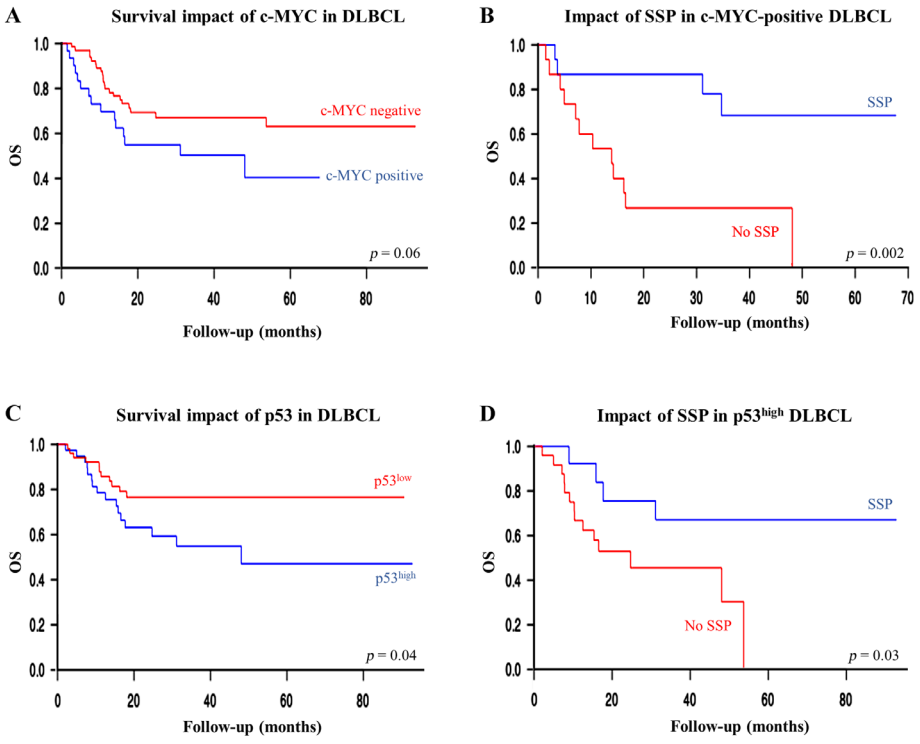

Figure 3. Survival impact of c-MYC and p53, depending on SSP. (A) Kaplan–Meier analysis of OS for DLBCL c-MYC-positive (blue curve) or c-MYC-negative (red curve). (B) Kaplan–Meier analysis of OS for DLBCL c-MYC-positive, depending on SSP. (C) Kaplan–Meier analysis of OS for DLBCL p53^{high} (blue curve) or p53^{low} (red curve). (D) Kaplan–Meier analysis of OS for DLBCL p53^{high}, depending on SSP.

© 2021 The Authors. *The Journal of Pathology: Clinical Research* published by The Pathological Society of Great Britain and Ireland and John Wiley & Sons, Ltd.

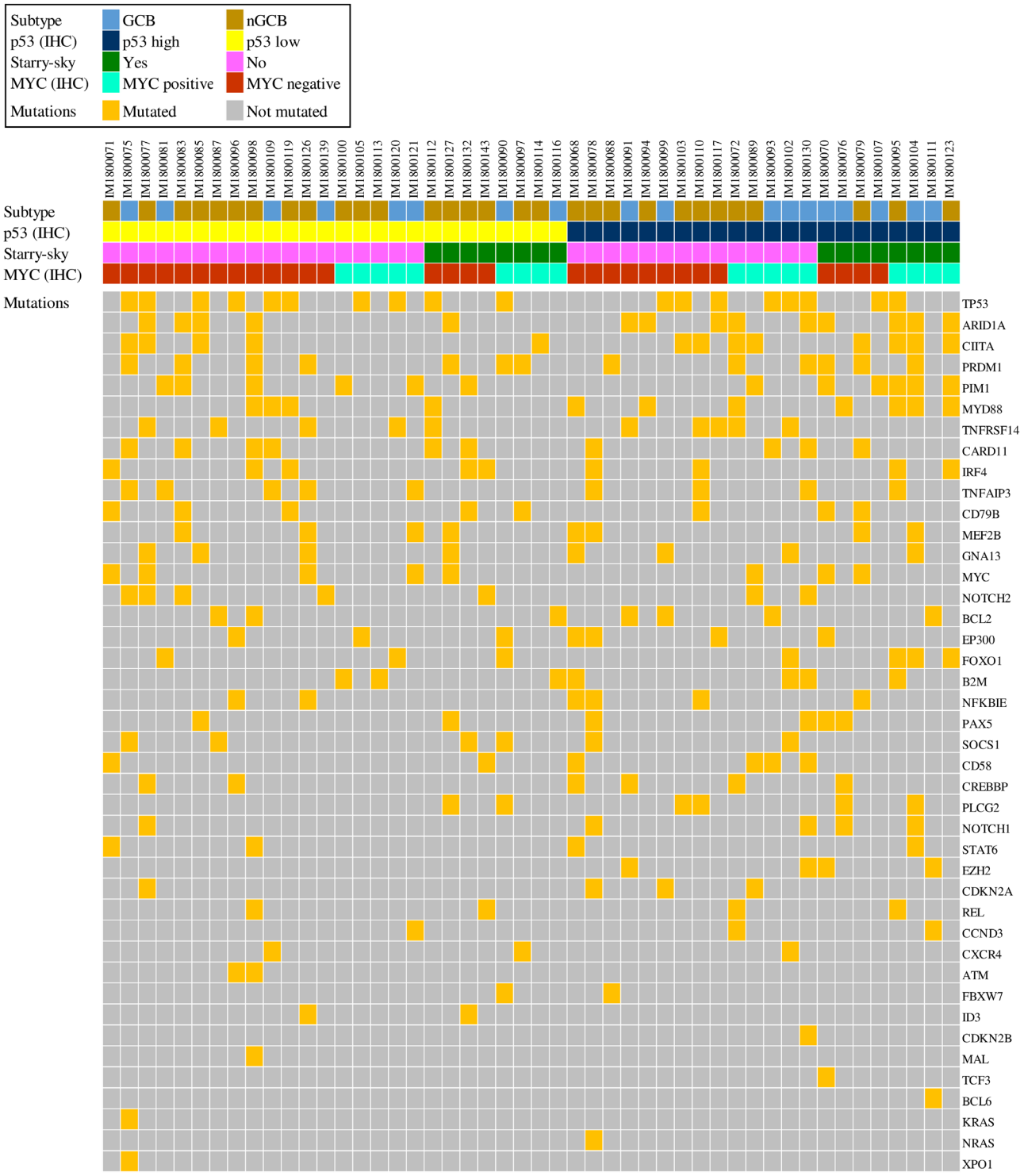

Figure 4. NGS analysis of 48 DLBCL using a 51 gene lymphoma panel.

DLBCL with a 4+ proliferation index). We noted that SSP was more frequently found in DEL (OR = 4.1, $p = 0.01$) and 4+ proliferation index DLBCL (OR = 3.2, $p = 0.02$). As with c-MYC-positive and $p53^{high}$ DLBCL, the presence of SSP defined a subgroup of patients with better survival in DEL

© 2021 The Authors. *The Journal of Pathology: Clinical Research* published by The Pathological Society of Great Britain and Ireland and John Wiley & Sons, Ltd.

($p = 0.001$, see supplementary material, Figure S2C) and in DLBCL with a 4+ proliferation index ($p = 0.03$, see supplementary material, Figure S2D).

Correlation with NGS data

NGS lymphopanel sequencing was performed for 48 DLBCL and correlated with p53 and c-MYC expression and DLBCL morphology (Figure 4). The most frequently mutated gene was *TP53* (16/38, 42.1%) and mutation profiles were broadly in line with GCB versus ABC patterns. c-MYC overexpression seemed enriched for *B2M* and *FOXO1* mutations (6 of 18 cases in c-MYC-positive DLBCL compared to 1 of 30 cases in c-MYC-negative DLBCL, OR = 8.2, $p = 0.004$).

SSP DLBCL was not significantly associated with any specific mutational profile. Nevertheless, some degree of correlation was observed between DLBCL without SSP and *TNFRSF14* mutations, without reaching significance threshold (9 of 32 cases, OR = 3.1, $p = 0.07$). *TNFRSF14* mutations were significantly linked with $p53^{high}$ DLBCL without SSP (5 of 14 cases, OR = 3.7, $p = 0.05$).

Finally, in the $p53^{high}$ subgroup, 5 of 8 cases with SSP DLBCL showed *PIM1* mutations compared to 1 of 14 DLBCL without this pattern (OR = 7.8, $p = 0.005$).

Discussion

The aim of this study was to assess the impact of different biomarkers on survival in R-CHOP-treated DLBCL. To achieve this evaluation, we established a retrospective cohort representative of *de novo* DLBCL in term of baseline characteristics, including inferior outcomes for patients with a high IPI score and a non-GCB subtype, both known as robust prognostic factors in DLBCL [7,18].

c-MYC protein overexpression was found in one-third of the cases, in agreement with previously reported data [23,24]. In univariate analysis, this overexpression showed only a weak trend with survival but some studies have drawn similar conclusions [31,46]. The prognostic impact of c-MYC expression still remains controversial in DLBCL. This could be explained by the absence of consensus in IHC cut-off positivity but also by a lack of treatment homogeneity in the cohort resulting in a substantial percentage of patients treated not only by R-CHOP. Furthermore, supporting previous reports, c-MYC-positive DLBCL in this study showed a strong interaction with a high IPI score [24,25] and a high proliferation index [19,31].

c-MYC overexpression was significantly associated with the presence of SSP, which is defined here by the presence, even focal, of tingible-body macrophages intermingled with tumour cells. In our work, to counterbalance the potential interpretation bias in evaluating the presence of SSP, we took four DLBCL samples per case to maximise tumour representativeness on the TMAs. In the literature, a correlation of *c-MYC*/SSP was once described but this was only between SSP and *c-MYC* gene rearrangement [47]. Moreover, a statistical analysis confirmed that these two factors could stratify DLBCL patients into prognostically relevant subgroups. These data strongly suggest that the adverse effect of c-MYC expression on survival could be outbalanced by SSP, which is correlated with a high apoptotic rate in the tumour [43], indicating that a high degree of apoptosis is a protective factor in c-MYC-driven DLBCL. This is consistent with findings in experimental oncogenesis where loss of pro-apoptotic signalling via p53 favours outgrowth of c-MYC overexpressing B-cell tumour clones [48]. Our data suggest that immune checkpoint escape (*B2M*) or *FOXO1* signalling might also confer selective advantages for outgrowth of c-MYC overexpressing B-cell clones. Indeed, *FOXO1* loss-of-function mutations are reported to confer poor prognosis in low-risk IPI DLBCL [49]. The association with MYC expression has not been previously reported.

Regarding p53 expression, in accordance with previous works [34], the $p53^{high}$ DLBCL subgroup was associated with c-MYC overexpression and with unfavourable outcomes. Moreover, the prognostic impact of p53 expression was even stronger in combination with SSP. As for c-MYC, $p53^{high}$ DLBCL without SSP had worse outcomes. The same applies for both c-MYC-positive and $p53^{high}$ cases confirming apoptosis as a protective factor in both p53 and c-MYC-driven lymphomas. This could be related to the well-known role of p53 in cell survival [50] and by upregulation of p53 expression by c-MYC [51].

Overall, SSP has a clear protective impact on $p53^{high}$ and c-MYC-positive DLBCL but the link between these three variables could be strengthened by a complementary multivariate analysis on a larger cohort.

In this cohort, NGS data revealed that p53, c-MYC, and SSP were linked with a specific mutational profile including several cell survival genes, such as *FOXO1*, *B2M*, *TNFRSF14*, and *PIM1*.

c-MYC-positive DLBCL presented a significant enrichment for *FOXO1* and *B2M* mutations. *FOXO1*

© 2021 The Authors. *The Journal of Pathology: Clinical Research* published by The Pathological Society of Great Britain and Ireland and John Wiley & Sons, Ltd.

is known to play a key role in homeostatic control of B-cell survival and proliferation in particular in the germinal centre reaction where *MYC* and *p53* are also key and in metabolic signalling [49,52,53]. Mutations in *FOXO1*, therefore considered as tumour suppressors, are also associated with decreased OS in c-MYC-driven DLBCL [48,49,53]. However, *B2M* mutations completely blocked the *B2M*-mediated apoptosis programme [54], representing another independent pathway by which MYC-driven DLBCL could escape apoptosis.

Interestingly, we found that $p53^{high}$ DLBCL without SSP had a higher frequency of *TNFRSF14* mutations which have been associated with unfavourable prognosis in follicular lymphomas [55]. In physiological conditions, the TNFRSF14 receptor encoded by this gene has a key role in cell survival and homeostasis of T and B cells, while in lymphoma, loss of function has been implicated in shaping the tumour microenvironment [54,56]. It is considered as a tumour suppressor gene, inducing apoptosis and immune response against tumour cells [56]. Conversely, as well as driving cell autonomous proliferation, *TNFRSF14* mutation seems to create a tumour-supportive microenvironment and promote tumourigenesis [57]. Furthermore, it was shown that restoration of TNFRSF14 suppresses tumour growth in xenograft models, thus highlighting this receptor as an interesting therapeutic target in lymphoma [57]. *TNFRSF14* mutation frequency in $p53^{high}$ DLBCL without SSP suggests that this gene is a major player in the anti-tumour immune response and apoptosis in p53-driven DLBCL.

Lastly, *PIM1* mutations were significantly linked with $p53^{high}$ DLBCL with SSP. This gene has a well-characterised role in cell survival and is seen as a proto-oncogene acting synergistically with genes encoding proteins involved in cell death, especially p53 [58,59]. *PIM1* oncogenic effects are effective only if tumour cells can bypass p53-dependent apoptosis by inhibiting p53 expression [59]. Oncogenic *PIM1* also cooperates with MYC signalling to enhance oncogenic signalling in lymphoma which is known to select for *p53* loss-of-function events. Thus, we make the assumption that, by maintaining p53 overexpression which induces high cell death level (with SSP as a consequence), cells are protecting themselves from *PIM1* oncogenic mutations.

DEL accounted for 22.7% of this cohort, a ratio in agreement with other studies [14,31]. Here, DEL was associated with SSP, which had not previously been described. DEL without SSP presented poorer outcomes than DEL with this morphology, strengthening the association between activation of apoptotic pathways and improved survival. 60.8% of DLBCL cases were BCL2-positive [29,31] but they were not associated with inferior outcomes, consistent with a large number of studies on R-CHOP-treated DLBCL [29,30]. These data strongly suggest that the adverse effect of BCL2 overexpression on DLBCL outcome could be mitigated by the addition of rituximab as previously reported [29,30].

A high proliferation index (Ki67 = 4+) was found in more than half of the patients but was not correlated with outcome in our cohort. The question of this parameter as an independent and robust prognostic factor in R-CHOP-treated DLBCL is still controversial and a topic of debate. Some studies have shown that it predicts a worse survival in this lymphoma [60] but other works have concluded the opposite [31,61].

Finally, we found expected proportions of *de novo* CD5-positive (9 cases) and CD23-positive DLBCL (10 cases) [37,38] but they were not associated with outcomes. However, these results should be taken with caution because of the limited number of positive cases.

In conclusion, on a representative cohort of DLBCL uniformly treated by R-CHOP as the first-line therapy, only p53 overexpression was associated with poorer outcomes in univariate analysis. However, c-MYC and p53 overexpression was associated with improved survival when combined with SSP, which is a morphological pattern found in lymphomas with a high rate of apoptosis [43]. Thus, persistence of a high rate of apoptosis seems to have a protective impact when c-MYC and/or p53 are overexpressed. Although requiring confirmation in a larger cohort, this pro-apoptotic environment, controlled by several key genes including *MYC*, *p53*, *FOXO1*, *B2M*, *TNFRSF14*, and *PIM1*, could therefore indicate a key tumour suppressor pathway. Taken together, these results show an apoptosis-mediated network in DLBCL which impacts outcome, plays a tumour-suppressive role, and when lost presents potential for therapeutic intervention.

Author contributions statement

AB, HS, AE and TB conceived and carried out experiments and statistics. LB and RG collected clinical data. AM performed FISH analysis. EC and LD-B performed IHC and ISH assessments. MBC and CF produced and interpreted NGS data. The other authors helped build the cohort. All authors were involved in writing the paper and had final approval of the submitted and published versions.

© 2021 The Authors. *The Journal of Pathology: Clinical Research* published by The Pathological Society of Great Britain and Ireland and John Wiley & Sons, Ltd.

References

1. 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.
2. Teras LR, DeSantis CE, Cerhan JR, *et al*. 2016 US lymphoid malignancy statistics by World Health Organization subtypes. *CA Cancer J Clin* 2016; **66:** 443–459.
3. 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.
4. Smith A, Crouch S, Lax S, *et al*. Lymphoma incidence, survival and prevalence 2004–2014: sub-type analyses from the UK's Haematological Malignancy Research Network. *Br J Cancer* 2015; **112:** 1575–1584.
5. Coiffier B, Thieblemont C, Van Den Neste E, *et al*. Long-term outcome of patients in the LNH-98.5 trial, the first randomized study comparing rituximab-CHOP to standard CHOP chemotherapy in DLBCL patients: a study by the Groupe d'Etudes des Lymphomes de l'Adulte. *Blood* 2010; **116:** 2040–2045.
6. Pfreundschuh M, Kuhnt E, Trümper L, *et al*. CHOP-like chemotherapy with or without rituximab in young patients with good-prognosis diffuse large-B-cell lymphoma: 6-year results of an open-label randomised study of the MabThera International Trial (MInT) Group. *Lancet Oncol* 2011; **12:** 1013–1022.
7. Sehn LH, Gascoyne RD. Diffuse large B-cell lymphoma: optimizing outcome in the context of clinical and biologic heterogeneity. *Blood* 2015; **125:** 22–32.
8. International Non-Hodgkin's Lymphoma Prognostic Factors Project. A predictive model for aggressive non-Hodgkin's lymphoma. *N Engl J Med* 1993; **329:** 987–994.
9. Sehn LH, Berry B, Chhanabhai M, *et al*. The revised International Prognostic Index (R-IPI) is a better predictor of outcome than the standard IPI for patients with diffuse large B-cell lymphoma treated with R-CHOP. *Blood* 2007; **109:** 1857–1861.
10. Alizadeh AA, Eisen MB, Davis RE, *et al*. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. *Nature* 2000; **403:** 503–511.
11. Rosenwald A, Wright G, Chan WC, *et al*. The use of molecular profiling to predict survival after chemotherapy for diffuse large-B-cell lymphoma. *N Engl J Med* 2002; **346:** 1937–1947.
12. Lenz G, Wright GW, Emre NCT, *et al*. Molecular subtypes of diffuse large B-cell lymphoma arise by distinct genetic pathways. *Proc Natl Acad Sci U S A* 2008; **105:** 13520–13525.
13. Pasqualucci L, Dalla-Favera R. Genetics of diffuse large B-cell lymphoma. *Blood* 2018; **131:** 2307–2319.
14. Rosenthal A, Younes A. High grade B-cell lymphoma with rearrangements of MYC and BCL2 and/or BCL6: double hit and triple hit lymphomas and double expressing lymphoma. *Blood Rev* 2017; **31:** 37–42.
15. Aukema SM, Siebert R, Schuuring E, *et al*. Double-hit B-cell lymphomas. *Blood* 2011; **117:** 2319–2331.
16. 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:** 679–690.
17. Schmitz R, Wright GW, Huang DW, *et al*. Genetics and pathogenesis of diffuse large B-cell lymphoma. *N Engl J Med* 2018; **378:** 1396–1407.
18. Hans CP, Weisenburger DD, Greiner TC, *et al*. Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. *Blood* 2004; **103:** 275–282.
19. Horn H, Ziepert M, Becher C, *et al*. MYC status in concert with BCL2 and BCL6 expression predicts outcome in diffuse large B-cell lymphoma. *Blood* 2013; **121:** 2253–2263.
20. Nguyen L, Papenhausen P, Shao H. The role of c-MYC in B-cell lymphomas: diagnostic and molecular aspects. *Genes (Basel)* 2017; **8:** 116.
21. 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.
22. 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.
23. Karube K, Campo E. MYC alterations in diffuse large B-cell lymphomas. *Semin Hematol* 2015; **52:** 97–106.
24. Valera A, López-Guillermo A, Cardesa-Salzmann T, *et al*. MYC protein expression and genetic alterations have prognostic impact in patients with diffuse large B-cell lymphoma treated with immunochemotherapy. *Haematologica* 2013; **98:** 1554–1562.
25. Zhou M, Wang J, Ouyang J, *et al*. MYC protein expression is associated with poor prognosis in diffuse large B cell lymphoma patients treated with RCHOP chemotherapy. *Tumor Biol* 2014; **35:** 6757–6762.
26. Vaidya R, Witzig TE. Prognostic factors for diffuse large B-cell lymphoma in the R(X)CHOP era. *Ann Oncol* 2014; **25:** 2124–2133.
27. Gascoyne RD, Adomat SA, Krajewski S, *et al*. Prognostic significance of Bcl-2 protein expression and Bcl-2 gene rearrangement in diffuse aggressive non-Hodgkin's lymphoma. *Blood* 1997; **90:** 244–251.
28. Hermine O, Haioun C, Lepage E, *et al*. Prognostic significance of bcl-2 protein expression in aggressive non-Hodgkin's lymphoma. Groupe d'Etude des Lymphomes de l'Adulte (GELA). *Blood* 1996; **87:** 265–272.
29. Iqbal J, Meyer PN, Smith LM, *et al*. BCL2 predicts survival in germinal center B-cell–like diffuse large B-cell lymphoma treated with CHOP-like therapy and rituximab. *Clin Cancer Res* 2011; **17:** 7785–7795.
30. Mounier N, Briere J, Gisselbrecht C, *et al*. Rituximab plus CHOP (R-CHOP) overcomes bcl-2–associated resistance to chemotherapy in elderly patients with diffuse large B-cell lymphoma (DLBCL). *Blood* 2003; **101:** 4279–4284.
31. 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:** 3452–3459.
32. Zilfou JT, Lowe SW. Tumor suppressive functions of p53. *Cold Spring Harb Perspect Biol* 2009; **1:** a001883.

© 2021 The Authors. *The Journal of Pathology: Clinical Research* published by The Pathological Society of Great Britain and Ireland and John Wiley & Sons, Ltd.

33. 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:** 3986–3996.
34. Wang XJ, Jeffrey Medeiros L, Bueso-Ramos CE, *et al*. 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.
35. 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:** 3088–3098.
36. Xie Y, Bulbul MA, Ji L, *et al*. p53 expression is a strong marker of inferior survival in de novo diffuse large B-cell lymphoma and may have enhanced negative effect with MYC coexpression: a single institutional clinicopathologic study. *Am J Clin Pathol* 2014; **141:** 593–604.
37. Zhao P, Li L, Zhou S, *et al*. CD5 expression correlates with inferior survival and enhances the negative effect of p53 overexpression in diffuse large B-cell lymphoma. *Hematol Oncol* 2019; **37:** 360–367.
38. Linderoth J, Jerkeman M, Cavallin-Ståhl E, *et al*. Immunohistochemical expression of CD23 and CD40 may identify prognostically favorable subgroups of diffuse large B-cell lymphoma: a Nordic Lymphoma Group Study. *Clin Cancer Res* 2003; **9:** 722–728.
39. Linderoth J, Ehinger M, Jerkeman M, *et al*. CD40 expression identifies a prognostically favourable subgroup of diffuse large B-cell lymphoma. *Leuk Lymphoma* 2007; **48:** 1774–1779.
40. Jain P, Fayad LE, Rosenwald A, *et al*. Recent advances in de novo CD5 + diffuse large B cell lymphoma. *Am J Hematol* 2013; **88:** 798–802.
41. Xu-Monette ZY, Tu M, Jabbar KJ, *et al*. Clinical and biological significance of de novo CD5+ diffuse large B-cell lymphoma in Western countries. *Oncotarget* 2015; **6:** 5615–5633.
42. Chuang S-S, Ye H, Du M-Q, *et al*. Histopathology and immunohistochemistry in distinguishing Burkitt lymphoma from diffuse large B-cell lymphoma with very high proliferation index and with or without a starry-sky pattern: a comparative study with EBER and FISH. *Am J Clin Pathol* 2007; **128:** 558–564.
43. Ford CA, Petrova S, Pound JD, *et al*. Oncogenic properties of apoptotic tumor cells in aggressive B cell lymphoma. *Curr Biol* 2015; **25:** 577–588.
44. Kampf C, Olsson I, Ryberg U, *et al*. Production of tissue microarrays, immunohistochemistry staining and digitalization within the human protein atlas. *J Vis Exp* 2012; **63:** 3620.
45. Dubois S, Viailly P-J, Mareschal S, *et al*. Next-generation sequencing in diffuse large B-cell lymphoma highlights molecular divergence and therapeutic opportunities: a LYSA study. *Clin Cancer Res* 2016; **22:** 2919–2928.
46. 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:** 3460–3467.
47. Oliveira CC, Maciel-Guerra H, Kucko L, *et al*. Double-hit lymphomas: clinical, morphological, immunohistochemical and cytogenetic study in a series of Brazilian patients with high-grade non-Hodgkin lymphoma. *Diagn Pathol* 2017; **12:** 3.
48. Vecchio E, Fiume G, Correnti S, *et al*. Insights about MYC and apoptosis in B-lymphomagenesis: an update from murine models. *Int J Mol Sci* 2020; **21:** 4265.
49. Trinh DL, Scott DW, Morin RD, *et al*. Analysis of FOXO1 mutations in diffuse large B-cell lymphoma. *Blood* 2013; **121:** 3666–3674.
50. Sakamuro D, Eviner V, Elliott KJ, *et al*. c-Myc induces apoptosis in epithelial cells by both p53-dependent and p53-independent mechanisms. *Oncogene* 1995; **11:** 2411–2418.
51. Klapproth K, Wirth T. Advances in the understanding of MYC-induced lymphomagenesis. *Br J Haematol* 2010; **149:** 484–497.
52. Miao Y, Medeiros LJ, Li Y, *et al*. Genetic alterations and their clinical implications in DLBCL. *Nat Rev Clin Oncol* 2019; **16:** 634–652.
53. Gehringer F, Weissinger SE, Swier LJ, *et al*. FOXO1 confers maintenance of the dark zone proliferation and survival program and can be pharmacologically targeted in Burkitt lymphoma. *Cancers* 2019; **11:** 1427.
54. Mori M, Terui Y, Ikeda M, *et al*. Beta(2)-microglobulin identified as an apoptosis-inducing factor and its characterization. *Blood* 1999; **94:** 2744–2753.
55. Cheung K-JJ, Johnson NA, Affleck JG, *et al*. Acquired TNFRSF14 mutations in follicular lymphoma are associated with worse prognosis. *Cancer Res* 2010; **70:** 9166–9174.
56. Zhu Y-D, Lu M-Y. Increased expression of TNFRSF14 indicates good prognosis and inhibits bladder cancer proliferation by promoting apoptosis. *Mol Med Rep* 2018; **18:** 3403–3410.
57. Boice M, Salloum D, Mourcin F, *et al*. Loss of the HVEM tumor suppressor in lymphoma and restoration by modified CAR-T cells. *Cell* 2016; **167:** 405–418.e13.
58. Magnuson NS, Wang Z, Ding G, *et al*. Why target PIM1 for cancer diagnosis and treatment? *Future Oncol* 2010; **6:** 1461–1478.
59. Zemskova M, Lilly MB, Lin Y-W, *et al*. p53-dependent induction of prostate cancer cell senescence by the PIM1 protein kinase. *Mol Cancer Res* 2010; **8:** 1126–1141.
60. He X, Chen Z, Fu T, *et al*. Ki-67 is a valuable prognostic predictor of lymphoma but its utility varies in lymphoma subtypes: evidence from a systematic meta-analysis. *BMC Cancer* 2014; **14:** 153.
61. Koh YW, Hwang HS, Park C-S, *et al*. Prognostic effect of Ki-67 expression in rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone-treated diffuse large B-cell lymphoma is limited to non-germinal center B-cell-like subtype in late-elderly patients. *Leuk Lymphoma* 2015; **56:** 2630–2636.

SUPPLEMENTARY MATERIAL ONLINE

Figure S1. Cohort validation

Figure S2. Prognostic impact of SSP alone or in combination with other parameters

Table S1. Antibodies used

© 2021 The Authors. *The Journal of Pathology: Clinical Research* published by The Pathological Society of Great Britain and Ireland and John Wiley & Sons, Ltd.