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Review Article

Treatment of Diffuse Large B-Cell Lymphoma

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Diffuse large B-cell lymphoma (DLBCL) comprises a heterogeneous group with pathophysiological, genetic and clinical features. Many patients can be cured with R-CHOP therapy, which is the current standard regimen. Despite recent progress in improving patient survival, the 40% survival of DLBCL patients remains poor. Therefore, the most important issue for patients with DLBCL remains the development of a new front-line therapy. Several studies have reported that intensified chemotherapy with dose-adjusted EPOCH-R or R-ACVBP was superior to R-CHOP. Gene expression profiling has identified two distinct forms of DLBCL: activated B cell-like (ABC) and germinal center B-cell-like (GCB) types. ABC DLBCL exhibits a worse prognosis than GCB DLBCL by molecular diagnosis after R-CHOP therapy. Next-generation sequencing has identified unique oncogenic mechanisms and genetic complexity, which has provided rational therapeutic targets. There are also a number of biomarkers, including CD5, and prognostic factors. Efforts to distinguish many biomarkers will be crucial for individualized treatment in the future. 〔*J Clin Exp Hematop 56(2):79-88, 2016*〕

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INTRODUCTION

Diffuse large B-cell lymphoma (DLBCL) is aggressive lymphoma and accounts for approximately 30% of all malignant lymphomas. DLBCL includes a heterogeneous group of lymphomas with distinct pathophysiological, genetic, and clinical features. The 2008 World Health Organization (WHO) classification divides DLBCL into a variety of subtypes, subgroups, and a not otherwise specified group. Patients present with nodal and extranodal disease.[1] Approximately 40% of the patients have involvement of extranodal sites, the most common of which is the gastrointestinal tract.[2] Except for certain subtypes and disease with particular extranodal involvement, the current standard regimen of DLBCL is R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone), which achieves a cure in many patients. In clinical practice, the treatment decision is also determined based on various factors such as the stage, age, presence of bulky disease, and International Prognostic Index (IPI).[3] IPI is the most commonly-used model for predicting survival in aggressive lymphoma. Five clinical factors including age, lactate dehydrogenase (LDH), Eastern Cooperative Oncology Group (ECOG) performance status (PS), stage, and number of extranodal sites, recognize 4 risk groups in newly diagnosed DLBCL patients.

Despite recent progress in improving prognosis, the outcome of nearly 40% of DLBCL patients remains poor. Gene expression profiling and next generation sequencing have identified specific signal pathways, unique oncogenic mechanisms, and genetic complexity, which have provided rational therapeutic targets. This review describes the first-line treatment of DLBCL in the rituximab era and focuses on recent clinical trials and therapeutic development regarding each biomarker and genetic mutations.

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STAGE BASED APPROACH

Localized DLBCL

The development of therapy for limited-stage DLBCL has focused on CHOP followed by involved-field radiotherapy (IFRT). Representative evidence for the first-line therapy of localized DLBCL is based on two clinical trials performed by the Southwest Oncology Group (SWOG) and Groupe d'Etudes des Lymphomes de l'Adulte (GELA). In the pre-rituximab era, a randomized trial by SWOG reported that 3 cycles of CHOP followed by IFRT [40-55 gray (Gy)] was less toxic and showed significantly better progression free survival (PFS) and overall survival (OS) than 8 cycles of CHOP in aggressive lymphoma patients with stage I to non-bulky II disease (S8736).[4] The long-term outcome was

statistically similar in both groups. GELA also revealed that CHOP plus radiotherapy (RT) did not provide any advantage over CHOP alone for the treatment of low-risk, localized, aggressive lymphoma in elderly patients.[5] In the rituximab era, a phase II trial (S0014)[6] with a matched historical control (S8736) was conducted in CD20-positive aggressive lymphoma patients with 0-1 risk factors of stage-modified IPI [non-bulky II stage, over 60 years old, PS 2-4, and higher serum LDH level] treated with 3 cycles of R-CHOP 21 (+ 1 cycle of R) followed by IFRT (40-46 Gy). The R-CHOP21 group had an improved 4-year PFS (76% vs. 63%) and 4-year OS (92%, 88%) compared with the CHOP group, with a median follow-up period of 5.3 years. However, either the PFS or OS curve shows a continuing pattern of relapse without a plateau. In limited stage disease, the addition of rituximab to CHOP has also been suggested to improve the outcome, but it may result in a smaller incremental benefit.

There are no large clinical trials using chemotherapy for patients with localized DLBCL only. In a randomized trial from 18 participating countries, a total of 824 young patients with DLBCL from 18 to 60 years of age with an age-adjusted international prognostic index (aa-IPI) score 0-1 were randomized to 6 cycles of R-CHOP(-like)14 therapy or 6 cycles of R-CHOP(-like)14 therapy (MInT).[7] Chemotherapy was followed by RT (30-40 Gy) for disease involving bulky mass over 5 cm. Overall, 72% of the patients had limited stage. The addition of R to CHOP was associated with a better outcome (3-year event-free survival [EFS] and OS). An updated analysis of the 72-mouths (mon) follow-up revealed that R-chemotherapy was significantly better than chemotherapy alone in the 6-year EFS (74.3% vs. 55.8%, $p < 0.0001$) and 5-year OS (90.1% vs. 80.0%, $p = 0.005$).[8] For all of these reasons, the standard therapy of non-bulky localized DLBCL has been considered either 3 cycles of R-CHOP followed by IFRT or 6-8 cycles of R-CHOP. These patients have a favorable prognosis; however, the necessity of IFRT, optimum total radiation dose, optimum number of cycles of R-CHOP, and biological differences are uncertain. In clinical practice, patients with localized DLBCL are administered treatment based on the individual patient profile (e.g., sites of disease, pathological legion, age, organ function).

Advanced DLBCL

Standard of care for advanced DLBCL is R-CHOP chemotherapy (Table 1). It is based on the finding that the addition of rituximab to CHOP improved the outcome compared to CHOP. GELA conducted a phase III randomized trial in DLBCL patients with stage II-IV, 60-80 years of age, and PS 0-2, who were treated with 8 cycles of R-CHOP 21 (administered every 21 days) or 8 cycles of CHOP21.[9] R-CHOP21 improved the complete response (CR) rate (76% vs. 63%), 2-year EFS (57% vs. 38%) and 2-year OS (70% vs. 57%) compared to CHOP21. The updated analysis of the 10-year follow-up revealed that 10-year OS was significantly better with R-CHOP (43.5% vs. 27.6%).[10] In the pre-rituximab era, the German High Grade Non Hodgkin Lymphoma Study Group (DSHNHL) reported the results of a phase III trial and demonstrated that OS with six cycles of CHOP14, in which a dose-dense CHOP regimen was administered every 14 days under human recombinant granulocyte colony stimulating factor support, was superior to that with six cycles of CHOP21 in aggressive lymphoma patients 60 years and older.[11] Therefore, this group conducted their clinical study as a matter of the policy based on the decision that CHOP14 was a standard regimen in older patients with DLBCL. In the rituximab era, DSHNHL reported the results of a randomized 2x2 factorial trial of 6 vs. 8 cycles of CHOP14 with or without 8 cycles of R in CD20-positive B-cell lymphoma patients (80% of the patients; DLBCL) from 61 to 80 years of age with stage I-IV disease (RICOVER-60).[12] Patients treated with 6 cycles of R-CHOP14 followed by 2 cycles of R (-2R) had a significantly better EFS, PFS, and OS than did those treated with 6 cycles of CHOP14. There was no significant difference in outcome between 8 cycles of R-CHOP14 and 6 cycles of R-CHOP-2R. Toxicity in patients with 8 cycles of R-CHOP14 increased more markedly than in those who received 6 cycles of R-CHOP14. Thus, the optimal number of cycles of chemotherapy was considered to be six in older patients treated with R-CHOP14.

The results of two, large, randomized, phase III trials to verify the effect of R-CHOP14 against R-CHOP21 were revealed. In the United Kingdom - National Cancer Research Institute trial,[13] a total of 1,080 untreated DLBCL patients 18 years and older with stage I-IV disease were randomized to 6 cycles of R-CHOP14 or 8 cycles of R-CHOP21, with a median follow-up period of 46 mon. There were no significant differences in the CR rate, 2-year PFS, or OS (82.7% in R-CHOP14 vs. 80.8% in R-CHOP21; $p = 0.3763$). In the LNH03-6B trial[14] by GELA, a total of 602 untreated DLBCL patients from 60 to 80 years of age with aa-IPI score of 1-3 were randomized to 8 cycles of R-CHOP14 or 8 cycles of R-CHOP21, with a median follow-up period of 56 mon. Similarly, there were no significant differences in the CR rate, 2-year EFS, PFS, or OS (69% in R-CHOP14 vs. 72% in R-CHOP21; $p = 0.7486$). The rates of hematological toxicity (grade 3/4 neutropenia and febrile neutropenia) and infection were higher in the patients treated with R-CHOP21, whereas the rate of grade 3/4 thrombocytopenia and the frequency of red blood cell transfusion were higher in the patients treated with R-CHOP14. Therefore, R-CHOP21 was defined as a standard chemotherapy; however, it remains unclear whether 6 or 8 is the optimal number of cycles for R-CHOP21.

In the rituximab era, a dose-intensive treatment regimen

Table 1. Key randomized trials in advanced diffuse large B-cell lymphoma in rituximab era

Study [Reference]	Number	Age	Stage	IPI	Regimen	Response	Outcome
LNH98-5 [9]	399	60-80	II-IV	IPI 0-5	R-CHOP21 x8 vs CHOP21 x8	CR: 76% vs 63%	2-yr EFS (57% vs 38%) 2-yr OS (70% vs 57%)
RICOVER-60 [12]	1,222	61-80	I-IV	IPI 1-5	CHOP14 x6 vs CHOP14 x8 vs R-CHOP14 x6 vs R-CHOP14 x8	CR: 68% vs 72% vs. 78% vs 76%	3-yr OS (67.7% vs 66.0% vs 78.1% vs 72.5%)
CALGB 9793/ ECOG-SWOG 4494 [62]	632	> 60	I-IV	aaIPI 0-5	R-CHOP21 x6-8 vs CHOP21 x6-8 +/- maintenance R	ORR: 77% vs 76%	3-yr FFS (52% vs 39%) 3-yr OS (67% vs 58%)
MInT [8]	824	18-60	II-IV	aaIPI 0-1	R-CHOP(-like) x6 vs CHOP(-like) x6	CR: 86% vs 68%	6-yr EFS (74.8% vs 55.8%) 6-yr OS (90.1% vs 80.0%)
LNH03-2B [15]	380	18-59	I-IV	aaIPI 0-1	R-ACVBP x8 vs R-CHOP21 x8	ORR: 90.3% vs 88.5%	3-yr EFS (80.9% vs 66.7%) 3-yr PFS (86.8% vs 73.4%) 3-yr OS (92.2% vs 83.8%)
UK NCRI [13]	1,080	≥ 18	I-IV	IPI 0-5	R-CHOP14 x6 (+R x2) vs R-CHOP21 x8	CR: 58% vs 63%	2-yr FFS (75% vs 75%) 2-yr OS (81% vs 83%)
LNH-03-6B [14]	602	60-80	I-IV	aaIPI 1-3	R-CHOP14 x8 vs R-CHOP21 x8	CR: 71% vs 74%	3-yr EFS (56% vs 60%) 3-yr OS (69% vs 72%)

IPI, International Prognostic Index; CR, complete response; EFS, event-free survival; OS, overall survival; yr, year; ORR, overall response rate; aaIPI, age-adjsted International Prognostic Index; FFS, failure-free survival; R-CHOP, rituximab, cyclophosphamide, doxorubicin, vincristine, and prodnisone

has been attempted for younger high-risk DLBCL patients. The GELA conducted a randomized trial in a total of 380 patients with DLBCL from 18 to 59 years of age with aa-IPI score of 0-1 comparing R-ACVBP with R-CHOP (LNH03-2B).[15] Overall, 44% of the patients had limited-stage disease. The R-ACVBP group had an improved 3-year EFS (81% vs. 67%) and 3-year OS (92% vs. 84%) compared with the R-CHOP group, with a median follow-up period of 44 mon. However, due to the severe toxicity, this regimen seems not to be chosen in routine clinical practice. The value of high-dose chemotherapy followed by autologous stem-cell transplantation (ASCT) as a first-line therapy for younger patients with DLBCL has remained controversial in several randomized trials. SWOG conducted a phase III randomized trial (S9704) in DLBCL patients 15-65 years of age with an aa-IPI score of 2-3 by comparing 5 cycles of CHOP±R followed by 1 cycle of CHOP±R and ASCT with 3 cycles of CHOP±R.[16] The ASCT group showed a significantly better 2-year PFS than did the CHOP±R group (69% vs. 55%, $p = 0.005$). In contrast, there was no significant difference in the 2-year OS (74%, 71%, $p = 0.30$). ASCT provided a therapeutic benefit (PFS, OS) only for those patients with a high risk based on the aa-IPI.

Elderly male patients had significantly lower R serum levels and worse outcomes in RICOVER-60.[17] Subsequently, a phase II trial by DSHNHL reported that an increased dose of R (500 instead of 375 mg/m^2) eliminated this risk in elderly male patients (SEXIE-R-CHOP-14). In the rituximab era, several studies of elderly DLBCL patients treated with CHOP combined with early dose-dense applications of R have been conducted by DSHNHL (DENSE-R-14, SMARTE-R-CHOP-14).[18]

CELL-OF-ORIGIN BASED APPROACH

GCB and ABC categorization

Gene expression profiling has identified two distinct forms of DLBCL: ABC and GCB types, which were classified with the focus on the normal cellular counterpart of lymphoma, that is, GCB DLBCL appears to be derived from germinal center B cells and ABC DLBCL may be derived from a post–germinal center B-cell (cell-of-origin; COO).[19] These forms were defined as molecular subgroups in the 2008 WHO classification. ABC DLBCL shows a more activated phenotype that is characterized by increased activity of the nuclear factor κB (NF-κB) pathway[20-22] and a worse prognosis than GCB DLBCL. The 5-year PFS rate was 40% in ABC DLBCL and 74% in GCB DLBCL with R-CHOP.[23] Therefore, therapeutic targets for ABC DLBCL have been

explored principally because ABC DLBCL has a poor prognosis. To look for more effective therapeutic strategies, research attention has been promoted with a central focus on the molecular biology of ABC DLBCL regarding its pathogenesis, signal pathways, and gene mutations. In particular, both modifications of conventional R-CHOP chemotherapy itself and the addition R-CHOP to novel agents which have adequate rationale have been considered (Table 2).

Conventional chemotherapy

The National Cancer Institute conducted a phase II trial of a total of 72 untreated DLBCL patients 18 years and older with stage II-IV disease treated with dose-adjusted (DA)-EPOCH-R (rituximab, etoposide, cyclophosphamide, doxorubicin, vincristine, and prednisone) therapy, which was an infusion regimen.[24] The CR rate achieved was 94%. With a median follow-up period of 54 mon, the 5-year PFS and OS were 79% and 80%, respectively. Likewise, a phase II trial by the Cancer and Leukemia Group B (CALGB) of a total of 69 untreated DLBCL patients 18 years and older with stage II-IV disease treated with DA-EPOCH-R revealed that the CR rate, 5-year EFS, and 5-year OS were 84%, 75%, and 84%, respectively (CALGB 50103).[25] This regimen showed a promising approach compared with the published data of R-CHOP and suggested a most relevant therapy for GCB DLBCL. Moreover, there was no significant difference in the PFS between GCB and non-GCB DLBCL from the results of the National Cancer Institute trial, and this regimen has received much attention as a treatment for non-GCB DLBCL. A randomized phase III trial comparing DA-EPOCH-R and R-CHOP (NCT01092182, Alliance 50303) is ongoing in the United States and its resulting conclusions, including a subgroup analysis of COO, are awaited. Among remaining trials, the LNH03-2B trial by GELA reported that the survival benefit related to R-ACVBP over R-CHOP is partly linked to improved survival among patients with non-GCB DLBCL.[26]

PROTEASOME INHIBITOR

Bortezomib is a proteasome inhibitor that affects the degradation of phosphorylated IκBa, which is involved in activating the NF-κB pathway. This mechanism resulted in the anti-tumor activity of ABC DLBCL. A phase II trial of patients with relapsed/refractory DLBCL who received DA-EPOCH with bortezomib reported that the overall response rate (ORR) of 12 patients with ABC DLBCL was

Table 2. Cell-of-origin based therapy and outcome in diffuse large B-cell lymphoma

Regimen [Reference]	DLBCL	Phase	Number	Stage	Response (ABC/non-GCB vs GCB)	Outcome (ABC/non-GCB vs GCB)
R-CHOP [23]	Untreated		233	II-IV		3-yr PFS: 40% vs 74%
DA-EPOCH-R [24]	Untreated	II	71	II-IV		5-yr PFS: 56% vs 79% 5-yr OS: 64% vs 84%
DA-EPOCH-R [25]	Untreated	II	69	II-IV		5-yr TTP: 67% vs 100% 5-yr EFS: 58% vs 94% 5-yr OS: 68% vs 94%
Bortezomib + DA-EPOCH [27]	Relapse/Refractory	II	27		ORR: 83% vs 13% CR: 42% vs 6%	median OS: 10.8 mon vs 3.4 mon
Lanalidomide [29]	Relapse/Refractory	Retrospective	40	I-IV	ORR: 52.9% vs 8.7% CR: 29.4% vs 4.3%	median PFS: 6.2 mon vs 1.7 mon median OS: 14 mon vs 13 mon
Lanalidomide + R-CHOP [31]	Untreated	II	32	II-IV	ORR: 88% vs 88% CR: 88% vs 81%	2-yr EFS: 74% vs 61% 2-yr PFS: 81% vs 71% 2-yr OS: 94% vs 88%
Lanalidomide + R-CHOP [30]	Untreated	II	64	II-IV		2-yr PFS: 60% vs 59% 2-yr OS: 83% vs 75%
Ibrutinib [25]	Relapse/Refractory	I/II	58		ORR: 37% vs 5% CR: 16% vs 0%	median PFS: 2.02 mon vs 1.31 mon median OS: 10.32 mon vs 3.35 mon
Ibrutinib + R-CHOP [34]	Untreated	Ib	11	Bulky I-IV	ORR: 100% vs 100% CR: 100% vs 71%	

DLBCL, diffuse large B-cell lymphoma; ABC, activated B-cell like; GCB, germinal center B-cell like; yr, year; mon, months; R-CHOP, rituximab, cyclophosphamide, doxorubicin, vincristine, and prodnisone; DA-EPOCH, dose-adjusted etoposide, vincristine, doxorubicin, cyclophosphamide and predonisone; PFS, progression-free survival; OS, overall survival; TTP, time to progression survival; EFS, event-free survival; ORR, overall response rate; CR, complete response rate

significantly higher than that of 15 patients with GCB DLBCL (83% vs. 13%, $p = 0.004$).[27] The median OS of patients with ABC DLBCL also showed a significantly better prognosis compared with the OS of those with GCB DLBCL (10.8 mon vs. 3.4 mon, $p = 0.026$). There was no significant difference in outcome between 14 patients with GCB DLBCL and 18 patients with non-GCB DLBCL when treated with R-CHOP plus bortezomib (BR-CHOP).[28] A phase III randomized trial has been examined in untreated DLBCL comparing BR-CHOP with R-CHOP. This trial is unique in the identification of ABC/GCB DLBCL by real-time gene expression profiling analysis (NCT01324596).

Immunomodulatory drugs

Lenalidomide, an immunomodulatory drug, has a complex mechanism of action associated with immune modulation and anti-angiogenesis. This drug inhibits NF-κB activity *in vitro* and also exerts significant anti-tumor activity. A retrospective study of patients with relapsed/refractory DLBCL who received single-agent lenalidomide revealed that the ORR of 17 patients with non-GCB DLBCL was significantly better than that of 23 patients with GCB DLBCL (52.9% vs. 8.7%, $p = 0.006$).[29] Lenalidomide combined with R-CHOP (R2-CHOP) can be safely administered and is suggested to contribute to the improvement of non-GCB DLBCL, particularly in outcome. The British Columbia Cancer Agency (BCCA) and colleagues reported that the non-GCB and GCB subtypes had a similar outcome in stage II-IV DLBCL treated with 6 cycles of R2-CHOP.[30] The Fondazione Italiana Linfomi group also reported that there was no difference in 2-year PFS (81% vs. 71%; $p = 0.705$) and OS (94% vs. 88%; $p = 0.58$) in patients with stage II-IV disease, 60-80 years of age, treated with 6 cycles of R2-CHOP between the non-GCB type and GCB type, with a median follow-up in survivors of 28 mon (REAL07).[31] Several clinical trials, such as phase III trials comparing R-CHOP and R2CHOP and trials consisting of lenalidomide maintenance therapy, have been conducted on untreated DLBCL.

BTK inhibitors

Mutations of CD79B and CD79A, which are subunit components of the B-cell receptor (BCR), cause chronic activation of BCR signaling and result in the activation of the NF-κB pathway.[32] Therefore, new therapeutic agents for the key enzyme and critical protein of BCR signaling have been developed to treat ABC DLBCL. Ibrutinib, Bruton's tyrosine kinase (BTK) inhibitor, has been shown to be effective in treating several types of B-cell lymphomas driven by the activation of BCR, such as chronic lymphocytic leukemia, mantle cell lymphoma and ABC DLBCL.[33] A phase I study of ibrutinib with R-CHOP reported good tolerance and an ORR of 100% in 18 patients with DLBCL. All non-GCB DLBCL patients (n = 4) achieved a CR; therefore, ibrutinib is expected to be a promising selective agent for non-GCB DLBCL.[34] The results of a phase I/II study in patients with relapsed/refractory DLBCL treated with ibrutinib alone revealed the relationship between mutations and ORR in detail.[35] In total, 71% of patients with mutant *CD79B*, 34% of patients with wild-type *CD79B*, and 80% of patients with both mutant *CD79B*/mutant *MYD88* responded, whereas patients with wild-type *CD79B* and mutant *MYD88*, wild-type *CARD11*, and wild-type *TNFAIP3* did not respond. The international, randomized, phase III trial of R-CHOP with or without ibrutinib for untreated non-GCB DLBCL classified by Hans's method is registered (NCT018557502).

SUBTYPES REQUIRING SPECIFIC CONSIDERATIONS

MYC rearrangement/double hit lymphoma (Table 3)

DLBCL with a *MYC* rearrangement (MYC^+ DLBCL) comprises 4 to 14% of DLBCL cases,[36,37] and more patients are diagnosed with MYC^+ DLBCL than with Burkitt's lymphoma. Barrans S *et al*. reported that the OS was significantly worse for patients with a *MYC* rearrangement treated with R-CHOP.[37] MYC^+ DLBCL had a relatively high incidence of central nervous system (CNS) relapse compared with MYC^- DLBCL.[37] Savage KJ *et al*. also revealed that MYC^+ DLBCL treated with R-CHOP had a significantly worse outcome than DLBCL without a *MYC* rearrangement. The 5-year OS rate was 33% in MYC^+ DLBCL patients and 72% in patients with DLBCL without a *MYC* rearrangement (MYC^- DLBCL).[38] MYC^+ DLBCL with an additional rearrangement of *BCL2* (or, alternatively, *BCL6*) had a worse prognosis.[39,40] B-cell lymphoma with multiple chromosome breakpoints (*MYC*, *BCL2*, *BCL6*) is described as "double-hit lymphoma (DHL)". The 2008 WHO classification[1] divides these diseases into B-cell lymphoma, unclassifiable, and with features intermediate between DLBCL and Burkitt's lymphoma (intermediate DLBCL/BL); however, a retrospective analysis of 52 patients with DHL classified these as 29 cases of intermediate DLBCL/BL, 19 cases of DLBCL, and 4 cases of others.[40] Due to a morphologic spectrum in which many cases have some resemblance to Burkitt's lymphoma and some are more like DLBCL, DHL was not able to be diagnosed by histology alone.[40] Moreover, the morphologic features in DHL do not have prognostic implications. Despite the clinical features of aggressiveness, DHL is classified as GCB type by immunohistochemistry. Fluorescence *in situ* hybridization (FISH) or chromosomal studies are needed for accurate diagnosis of DHL. To make a diagnosis of DHL, dual protein expression of MYC and BCL2 should be

Table 3. Therapy and outcome in DLBCL with MYC rearrangement and double hit lymphoma

Authors [Reference]	MYC+ DLBCL/DHL	Number	Regimen*	Outcome	Comment
Barrans *et al*. [37]	MYC+ DLBCL	35 [26 with t(14;18), 10 with BCL6 translocation, and 7 with triple translocation]	R-CHOP	2-yr OS: 35%	MYC- DLBCL 2-yr OS: 61%
Johnson *et al*. [36]	DHL	54	R-CHOP (11) CHOP (23) HD-chemo (6)	R-CHOP vs CHOP vs HD-chemo (median OS: 1.40 yr vs 0.42 yr vs 0.26 yr)	
Savage *et al*. [38]	MYC+ DLBCL	12 (3 with BCL2 translocaion)	R-CHOP	5-yr PFS: 31% 5-yr OS: 33%	MYC- DLBCL (5-yr PFS: 66%, 5-yr OS: 72%)
Snuderl *et al*. [39]	DHL	20	R-CHOP + M (6), R-CHOP (3), R-EPOCH + M (3), CODOX-M/R-IVAC (3) R-ICE + M/auto SCT (1), CHOP (1) Pariative (1), Not known (1)	median OS: 4.5 mon	CNS involvement 45%
Li *et al*. [40]	DHL	52	R-CHOP (19) R-Hyper CVAD (23) CODOX (1), other (1)	median OS 18.6 mon 1-yr OS 58%	
Petrich *et al*. [44]	DHL	311	R-CHOP (100), R-Hyper CVAD (65) DA-EPOCH-R (64), R-CODOX-M/IVAC (42) other (24), R-ICE (9) (83 with consolidative SCT)	median PFS 10.9 mon median OS 21.9 mon 2-yr PFS: 40% 2-yr OS: 49%	R-CHOP vs intensive chemo median PFS (7.8 mon vs 21.6 mon) OS was similar with and without consolidative SCT
Oki *et al*. [63]	DHL	129	R-CHOP (57), R-EPOCH (28) R-Hyper CVAD/MA (34), other (10)	R-CHOP vs R-EPOCH vs R-hyper CVAD vs other (2-yr OS: 41% vs 70% vs 44% vs 44%)	

MYC+ DLBCL, diffuse large B-cell lymphoma with MYC rearrangement; MYC- DLBCL, DLBCL without MYC rearrangement; yr, year; OS, overall survival; DHL, double hit lymphoma; R, rituximab; CHOP, cyclophosphamide, doxorubicin, vincristine, and prodnisone; HD-chemo, high-dose chemotherapy; PFS, progression-free survival; CNS, central nerve system; EPOCH, etoposide, vincristine, doxorubicin, cyclophosphamide and predonisone; M, methotrexate; CODOX-M/IVAC, cyclophosphamide, vincristine, doxorubicin, methotrexate, ifosfamide, etoposide and cytarabine; ICE, ifosfamide, carboplatin and etoposide; auto SCT, autologous stem cell transplantaion; mon, months; DA, dose-adjusted; Hyper CVAD/MA, hyper fractionated cyclophosphamide, vincristine, doxorubicin, dexamethasone alternating with methotrexate and cytarabine
*The number in the parentheses indicates the number of patients treated with each regimen.

performed by immunohistochemistry. DLBCL with MYC/BCL2 double-expression was associated with a poor prognosis.[41-43] However, double-expressor DLBCL accounts for 29% of DLBCL[42] and requires careful attention to distinguish this from "true" DHL.

The prognosis of DHL is markedly poor with R-CHOP. Moreover, it remains unclear which intensive therapy, such as a Burkitt's-type regimen and HDC/ASCT, may offer a benefit.[44] First-line treatment with R-EPOCH significantly reduced the risk of progression compared with R-CHOP in a meta-analysis.[45] Several phase II clinical trials recruiting patients with DHL treated with R-EPOCH therapy are ongoing. Therapeutic novel agents for DHL, such as a bcl-2 inhibitor (ABT-199)[46] and a suppressor of MYC expression (JQ1),[47] are currently in clinical trials. In the next WHO classification, DHL may be categorized as another entity. In clinical practice, adequate identification of DHL with cytogenetic studies is of great clinical importance.

Intravascular large B-cell lymphoma

Intravascular large B-cell lymphoma (IVLBCL) is one of subtype of DLBCL in the 2008 WHO classification and is pathologically characterized by selective growth of large tumor cells in the lumina of small vessels of systemic organs. IVLBCL is a rare disease, and hence, there have been no reports about results of prospective clinical trials. A retrospective study suggested a survival benefit with the addition of R to chemotherapy for IVLBCL; the 2-year OS for 49 patients treated with R-chemotherapy was significantly higher than that of 57 patients treated with chemotherapy alone (66% vs. 46%; $p = 0.02$).[48] In IVLBCL patients without CNS involvement at initial diagnosis, the risk of CNS recurrence at 3 years was 25% with a median follow-up in

survivors of 39 mon.[49] At present, a phase II trial of R-CHOP combined with high-dose-methotrexate (HD-MTX) and intrathecal administration of MTX to prevent CNS relapse for untreated IVLBCL is ongoing in Japan (UMIN000005707).

CD5

CD5-positive ($CD5^+$) DLBCL accounts for 10% of DLBCL cases and is associated with many clinical characteristics, such as elderly onset, advanced stage at diagnosis, elevated LDH level, and frequent involvement of extranodal sites.[50,51] This immunohistochemical subgroup of DLBCL is not otherwise specified in the 2008 WHO classification. By gene expression profiling, most patients with $CD5^+$ DLBCL are classified as ABC DLBCL.[52] Immunohistochemically, 82% of $CD5^+$ DLBCL cases are classified as the non-GCB type, and the BCL2 protein is positive in more than 70% of the patients. Despite the use of rituximab, the OS of patients with $CD5^+$ DLBCL remains poor. The reason for this is that this disease is also characterized by a high incidence (13%) of CNS relapse compared with an incidence of approximately 5% for the all patients with DLBCL, even in the rituximab era.[53] Moreover, 83% of the patients who experienced CNS relapse had brain parenchymal disease. More than 60% of patients with $CD5^+$ DLBCL are older than 65 years at diagnosis and would therefore not be eligible for high-dose chemotherapy. DA-EPOCH-R was beneficial for Bcl-2 positive tumors, and its neurologic and cardiac toxicities are mild; therefore, it may be better suited for the elderly. Thus, we designed a new therapeutic strategy of DA-EPOCH-R combined with HD-MTX therapy, which is commonly used in the treatment of primary DLBCL of the CNS. This approach is potentially more effective than intrathecal administration of MTX to prevent CNS relapse of $CD5^+$ DLBCL, particularly for brain parenchymal disease. The results of a phase II study of DA-EPOCH-R/HD-MTX for newly diagnosed $CD5^+$ DLBCL (PEARL5 trial) are awaited (UMIN000008507).

Extranodal involvement

The incidence of CNS relapse in DLBCL has been reported to be 5% or less.[54-56] Several clinical parameters, such as an elevated serum LDH level and extranodal or bone marrow involvement, have been established as risk factors for CNS relapse in DLBCL.[57-59] Testicular involvement of DLBCL is associated with a particularly high risk of CNS relapse. A phase II study by the International Extranodal Lymphoma Study Group (IELSG) reported that R-CHOP with intrathecal administration of MTX followed by contralateral testicular irradiation could reduce the incidence of CNS relapse in testicular DLBCL.[60] It remains undetermined whether patients who have involvement of extranodal sites other than the testis require CNS prophylaxis. Of the 3,840 untreated DLBCL patients who were treated with R-CHOP in the nine consecutive prospective trials of the DSHNHL, 292 patients had skeletal involvement that was associated with a poor prognosis.[61] Additional RT to the skeletal sites improved the prognosis of the patients with skeletal involvement. Chemotherapy with RT may be considered standard care for patients with skeletal involvement. Prospective clinical trials based on therapeutic targets for each extranodal DLBCL are needed.

CONCLUSIONS

R-CHOP-based treatment has dramatically improved the prognosis of DLBCL over the past 20 years. High-risk patients who do not achieve adequate clinical results are needed to provide clues to the underlying causes of the disease. Chemotherapy combined with new therapeutic candidates is expected to lead to a breakthrough. In the future, therapeutic strategies of DLBCL, in terms of an individual biomarker algorithm, including immunohistochemistry, gene expression profiling, and cytogenetic analysis, are inevitable.

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DISCLOSURE STATEMENT

The author declares that there are no conflicts of interest regarding the present paper.

REFERENCES

1 WHO Classification of Tumours, Tumours of Haematopoietic and Lymphoid Tissues. Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, *et al*. (eds): 4th ed, Lyon, IARC, 2008
2 Harris NL, Jaffe ES, Stein H, Banks PM, Chan JK, *et al*.: A revised European-American classification of lymphoid neoplasms: a proposal from the International Lymphoma Study Group. Blood 84:1361-1392, 1994
3 A predictive model for aggressive non-Hodgkin's lymphoma. The International Non-Hodgkin's Lymphoma Prognostic Factors Project. N Engl J Med 329:987-994, 1993
4 Miller TP, Dahlberg S, Cassady JR, Adelstein DJ, Spier CM, *et al*.: Chemotherapy alone compared with chemotherapy plus radiotherapy for localized intermediate- and high-grade non-Hodgkin's lymphoma. N Engl J Med 339:21-26, 1998
5 Bonnet C, Fillet G, Mounier N, Ganem G, Molina TJ, *et al*.: CHOP alone compared with CHOP plus radiotherapy for

localized aggressive lymphoma in elderly patients: a study by the Groupe d'Etude des Lymphomes de l'Adulte. J Clin Oncol 25:787-792, 2007
6 Persky DO, Unger JM, Spier CM, Stea B, LeBlanc M, *et al*.: Phase II study of rituximab plus three cycles of CHOP and involved-field radiotherapy for patients with limited-stage aggressive B-cell lymphoma: Southwest Oncology Group study 0014. J Clin Oncol 26:2258-2263, 2008
7 Pfreundschuh M, Trümper L, Osterborg A, Pettengell R, Trneny M, *et al*.: CHOP-like chemotherapy plus rituximab versus CHOP-like chemotherapy alone in young patients with good-prognosis diffuse large-B-cell lymphoma: a randomised controlled trial by the MabThera International Trial (MInT) Group. Lancet Oncol 7:379-391, 2006
8 Pfreundschuh M, Kuhnt E, Trümper L, Osterborg A, Trneny M, *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 12:1013-1022, 2011
9 Coiffier B, Lepage E, Briere J, Herbrecht R, Tilly H, *et al*.: CHOP chemotherapy plus rituximab compared with CHOP alone in elderly patients with diffuse large-B-cell lymphoma. N Engl J Med 346:235-242, 2002
10 Coiffier B, Thieblemont C, Van Den Neste E, Lepeu G, Plantier I, *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 116:2040-2045, 2010
11 Pfreundschuh M, Trümper L, Kloess M, Schmits R, Feller AC, *et al*.: Two-weekly or 3-weekly CHOP chemotherapy with or without etoposide for the treatment of elderly patients with aggressive lymphomas: results of the NHL-B2 trial of the DSHNHL. Blood 104:634-641, 2004
12 Pfreundschuh M, Schubert J, Ziepert M, Schmits R, Mohren M, *et al*.: Six versus eight cycles of bi-weekly CHOP-14 with or without rituximab in elderly patients with aggressive $CD20^+$ B-cell lymphomas: a randomised controlled trial (RICOVER-60). Lancet Oncol 9:105-116, 2008
13 Cunningham D, Hawkes EA, Jack A, Qian W, Smith P, *et al*.: Rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisolone in patients with newly diagnosed diffuse large B-cell non-Hodgkin lymphoma: a phase 3 comparison of dose intensification with 14-day versus 21-day cycles. Lancet 381:1817-1826, 2013
14 Delarue R, Tilly H, Mounier N, Petrella T, Salles G, *et al*.: Dose-dense rituximab-CHOP compared with standard rituximab-CHOP in elderly patients with diffuse large B-cell lymphoma (the LNH03-6B study): a randomised phase 3 trial. Lancet Oncol 14:525-533, 2013
15 Récher C, Coiffier B, Haioun C, Molina TJ, Fermé C, *et al*.: Intensified chemotherapy with ACVBP plus rituximab versus standard CHOP plus rituximab for the treatment of diffuse large B-cell lymphoma (LNH03-2B): an open-label randomised phase 3 trial. Lancet 378:1858-1867, 2011
16 Stiff PJ, Unger JM, Cook JR, Constine LS, Couban S, *et al*.: Autologous transplantation as consolidation for aggressive non-Hodgkin's lymphoma. N Engl J Med 369:1681-1690, 2013
17 Pfreundschuh M, Müller C, Zeynalova S, Kuhnt E, Wiesen MH, *et al*.: Suboptimal dosing of rituximab in male and female patients with DLBCL. Blood 123:640-646, 2014
18 Pfreundschuh M, Poeschel V, Zeynalova S, Hänel M, Held G, *et al*.: Optimization of rituximab for the treatment of diffuse large B-cell lymphoma (II): extended rituximab exposure time in the SMARTE-R-CHOP-14 trial of the german high-grade non-Hodgkin lymphoma study group. J Clin Oncol 32:4127-4133, 2014
19 Alizadeh AA, Eisen MB, Davis RE, Ma C, Lossos IS, *et al*.: Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. Nature 403:503-511, 2000
20 Lenz G, Davis RE, Ngo VN, Lam L, George TC, *et al*.: Oncogenic CARD11 mutations in human diffuse large B cell lymphoma. Science 319:1676-1679, 2008
21 Compagno M, Lim WK, Grunn A, Nandula SV, Brahmachary M, *et al*.: Mutations of multiple genes cause deregulation of NF-κB in diffuse large B-cell lymphoma. Nature 459:717-721, 2009
22 Ngo VN, Young RM, Schmitz R, Jhavar S, Xiao W, *et al*.: Oncogenically active MYD88 mutations in human lymphoma. Nature 470:115-119, 2011
23 Lenz G, Wright G, Dave SS, Xiao W, Powell J, *et al*.: Stromal gene signatures in large-B-cell lymphomas. N Engl J Med 359:2313-2323, 2008
24 Wilson WH, Dunleavy K, Pittaluga S, Hegde U, Grant N, *et al*.: Phase II study of dose-adjusted EPOCH and rituximab in untreated diffuse large B-cell lymphoma with analysis of germinal center and post-germinal center biomarkers. J Clin Oncol 26:2717-2724, 2008
25 Wilson WH, Jung SH, Porcu P, Hurd D, Johnson J, *et al*.: A Cancer and Leukemia Group B multi-center study of DA-EPOCH-rituximab in untreated diffuse large B-cell lymphoma with analysis of outcome by molecular subtype. Haematologica 97:758-765, 2012
26 Molina TJ, Canioni D, Copie-Bergman C, Recher C, Brière J, *et al*.: Young patients with non-germinal center B-cell-like diffuse large B-cell lymphoma benefit from intensified chemotherapy with ACVBP plus rituximab compared with CHOP plus rituximab: analysis of data from the Groupe d'Etudes des Lymphomes de l'Adulte/lymphoma study association phase III trial LNH 03-2B. J Clin Oncol 32:3996-4003, 2014
27 Dunleavy K, Pittaluga S, Czuczman MS, Dave SS, Wright G, *et al*.: Differential efficacy of bortezomib plus chemotherapy within molecular subtypes of diffuse large B-cell lymphoma. Blood 113:6069-6076, 2009
28 Ruan J, Martin P, Furman RR, Lee SM, Cheung K, *et al*.:

Bortezomib plus CHOP-rituximab for previously untreated diffuse large B-cell lymphoma and mantle cell lymphoma. J Clin Oncol 29:690-697, 2011

29 Hernandez-Ilizaliturri FJ, Deeb G, Zinzani PL, Pileri SA, Malik F, *et al*.: Higher response to lenalidomide in relapsed/refractory diffuse large B-cell lymphoma in nongerminal center B-cell-like than in germinal center B-cell-like phenotype. Cancer 117:5058-5066, 2011

30 Nowakowski GS, LaPlant B, Macon WR, Reeder CB, Foran JM, *et al*.: Lenalidomide combined with R-CHOP overcomes negative prognostic impact of non-germinal center B-cell phenotype in newly diagnosed diffuse large B-Cell lymphoma: a phase II study. J Clin Oncol 33:251-257, 2015

31 Vitolo U, Chiappella A, Franceschetti S, Carella AM, Baldi I, *et al*.: Lenalidomide plus R-CHOP21 in elderly patients with untreated diffuse large B-cell lymphoma: results of the REAL07 open-label, multicentre, phase 2 trial. Lancet Oncol 15:730-737, 2014

32 Davis RE, Ngo VN, Lenz G, Tolar P, Young RM, *et al*.: Chronic active B-cell-receptor signalling in diffuse large B-cell lymphoma. Nature 463:88-92, 2010

33 Aalipour A, Advani RH: Bruton's tyrosine kinase inhibitors and their clinical potential in the treatment of B-cell malignancies: focus on ibrutinib. Ther Adv Hematol 5:121-133, 2014

34 Younes A, Thieblemont C, Morschhauser F, Flinn I, Friedberg JW, *et al*.: Combination of ibrutinib with rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) for treatment-naive patients with CD20-positive B-cell non-Hodgkin lymphoma: a non-randomised, phase 1b study. Lancet Oncol 15:1019-1026, 2014

35 Wilson WH, Young RM, Schmitz R, Yang Y, Pittaluga S, *et al*.: Targeting B cell receptor signaling with ibrutinib in diffuse large B cell lymphoma. Nat Med 21:922-926, 2015

36 Johnson NA, Savage KJ, Ludkovski O, Ben-Neriah S, Woods R, *et al*.: Lymphomas with concurrent BCL2 and MYC translocations: the critical factors associated with survival. Blood 114: 2273-2279, 2009

37 Barrans S, Crouch S, Smith A, Turner K, Owen R, *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 28:3360-3365, 2010

38 Savage KJ, Johnson NA, Ben-Neriah S, Connors JM, Sehn LH, *et al*.: MYC gene rearrangements are associated with a poor prognosis in diffuse large B-cell lymphoma patients treated with R-CHOP chemotherapy. Blood 114:3533-3537, 2009

39 Snuderl M, Kolman OK, Chen YB, Hsu JJ, Ackerman AM, *et al*.: B-cell lymphomas with concurrent IGH-BCL2 and MYC rearrangements are aggressive neoplasms with clinical and pathologic features distinct from Burkitt lymphoma and diffuse large B-cell lymphoma. Am J Surg Pathol 34:327-340, 2010

40 Li S, Lin P, Fayad LE, Lennon PA, Miranda RN, *et al*.: B-cell lymphomas with MYC/8q24 rearrangements and IGH@BCL2/t(14;18)(q32;q21): an aggressive disease with heterogeneous histology, germinal center B-cell immunophenotype and poor outcome. Mod Pathol 25:145-156, 2012

41 Johnson NA, Slack GW, Savage KJ, Connors JM, Ben-Neriah S, *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 30:3452-3459, 2012

42 Green TM, Young KH, Visco C, Xu-Monette ZY, Orazi A, *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 30:3460-3467, 2012

43 Hu S, Xu-Monette ZY, Tzankov A, Green T, Wu L, *et al*.: MYC/BCL2 protein coexpression contributes to the inferior survival of activated B-cell subtype of diffuse large B-cell lymphoma and demonstrates high-risk gene expression signatures: a report from The International DLBCL Rituximab-CHOP Consortium Program. Blood 121:4021-4031, 2013

44 Petrich AM, Gandhi M, Jovanovic B, Castillo JJ, Rajguru S, *et al*.: Impact of induction regimen and stem cell transplantation on outcomes in double-hit lymphoma: a multicenter retrospective analysis. Blood 124:2354-2361, 2014

45 Howlett C, Snedecor SJ, Landsburg DJ, Svoboda J, Chong EA, *et al*.: Front-line, dose-escalated immunochemotherapy is associated with a significant progression-free survival advantage in patients with double-hit lymphomas: a systematic review and meta-analysis. Br J Haematol 170:504-514, 2015

46 Li L, Pongtornpipat P, Tiutan T, Kendrick SL, Park S, *et al*.: Synergistic induction of apoptosis in high-risk DLBCL by BCL2 inhibition with ABT-199 combined with pharmacologic loss of MCL1. Leukemia 29:1702-1712, 2015

47 Cinar M, Rosenfelt F, Rokhsar S, Lopategui J, Pillai R, *et al*.: Concurrent inhibition of MYC and BCL2 is a potentially effective treatment strategy for double hit and triple hit B-cell lymphomas. Leuk Res 39:730-738, 2015

48 Shimada K, Matsue K, Yamamoto K, Murase T, Ichikawa N, *et al*.: Retrospective analysis of intravascular large B-cell lymphoma treated with rituximab-containing chemotherapy as reported by the IVL study group in Japan. J Clin Oncol 26:3189-3195, 2008

49 Shimada K, Murase T, Matsue K, Okamoto M, Ichikawa N, *et al*.: Central nervous system involvement in intravascular large B-cell lymphoma: a retrospective analysis of 109 patients. Cancer Sci 101:1480-1486, 2010

50 Yamaguchi M, Seto M, Okamoto M, Ichinohasama R, Nakamura N, *et al*.: *De novo* $CD5^+$ diffuse large B-cell lymphoma: a clinicopathologic study of 109 patients. Blood 99:815-821, 2002

51 Yamaguchi M, Nakamura N, Suzuki R, Kagami Y, Okamoto M, *et al*.: *De novo* $CD5^+$ diffuse large B-cell lymphoma: results of a detailed clinicopathological review in 120 patients. Haematologica 93:1195-1202, 2008

52 Miyazaki K, Yamaguchi M, Imai H, Kobayashi K, Tamaru S, *et*

al.: Gene expression profiling of diffuse large B-Cell lymphomas supervised by CD5 expression. Int J Hematol 102:188-194, 2015

53 Miyazaki K, Yamaguchi M, Suzuki R, Kobayashi Y, Maeshima AM, *et al*.: CD5-positive diffuse large B-cell lymphoma: a retrospective study in 337 patients treated by chemotherapy with or without rituximab. Ann Oncol 22:1601-1607, 2011

54 Hollender A, Kvaloy S, Nome O, Skovlund E, Lote K, *et al*.: Central nervous system involvement following diagnosis of non-Hodgkin's lymphoma: a risk model. Ann Oncol 13:1099-1107, 2002

55 Feugier P, Virion JM, Tilly H, Haioun C, Marit G, *et al*.: Incidence and risk factors for central nervous system occurrence in elderly patients with diffuse large-B-cell lymphoma: influence of rituximab. Ann Oncol 15:129-133, 2004

56 Tilly H, Lepage E, Coiffier B, Blanc M, Herbrecht R, *et al*.: Intensive conventional chemotherapy (ACVBP regimen) compared with standard CHOP for poor-prognosis aggressive non-Hodgkin lymphoma. Blood 102:4284-4289, 2003

57 Bernstein SH, Unger JM, Leblanc M, Friedberg J, Miller TP, *et al*.: Natural history of CNS relapse in patients with aggressive non-Hodgkin's lymphoma: a 20-year follow-up analysis of SWOG 8516 -- the Southwest Oncology Group. J Clin Oncol 27:114-119, 2009

58 Boehme V, Zeynalova S, Kloess M, Loeffler M, Kaiser U, *et al*.: Incidence and risk factors of central nervous system recurrence in aggressive lymphoma--a survey of 1693 patients treated in protocols of the German High-Grade Non-Hodgkin's Lymphoma Study Group (DSHNHL). Ann Oncol 18:149-157, 2007

59 Tomita N, Yokoyama M, Yamamoto W, Watanabe R, Shimazu Y, *et al*.: Central nervous system event in patients with diffuse large B-cell lymphoma in the rituximab era. Cancer Sci 103:245-251, 2012

60 Vitolo U, Chiappella A, Ferreri AJ, Martelli M, Baldi I, *et al*.: First-line treatment for primary testicular diffuse large B-cell lymphoma with rituximab-CHOP, CNS prophylaxis, and contralateral testis irradiation: final results of an international phase II trial. J Clin Oncol 29:2766-2772, 2011

61 Held G, Zeynalova S, Murawski N, Ziepert M, Kempf B, *et al*.: Impact of rituximab and radiotherapy on outcome of patients with aggressive B-cell lymphoma and skeletal involvement. J Clin Oncol 31:4115-4122, 2013

62 Habermann TM, Weller EA, Morrison VA, Gascoyne RD, Cassileth PA, *et al*.: Rituximab-CHOP versus CHOP alone or with maintenance rituximab in older patients with diffuse large B-cell lymphoma. J Clin Oncol 24:3121-3127, 2006

63 Oki Y, Noorani M, Lin P, Davis RE, Neelapu SS, *et al*.: Double hit lymphoma: the MD Anderson Cancer Center clinical experience. Br J Haematol 166:891-901, 2014