DA-EPOCH-R combined with high-dose methotrexate in patients with newly diagnosed stage II-IV CD5-positive diffuse large B-cell lymphoma: a single-arm, open-label, phase II study

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Online Supplementary Information

DA-EPOCH-R combined with high-dose methotrexate in patients with newly diagnosed stage II-IV CD5-positive diffuse large B-cell lymphoma: a single-arm, open-label, phase 2 study

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The following sections are included:

Online Supplementary Methods

Study design and participants

Inclusion criteria for stages

Because we had found that patients with newly diagnosed stage I CD5-positive diffuse large B-cell lymphoma (CD5+ DLBCL) showed significantly superior progression-free survival (PFS) to those with stage II-IV CD5+ DLBCL in our previous retrospective study (2-year PFS: stage I, 86%; stage II-IV, 51%; *P* = 0.0026) (*Online Supplementary Figure S1*), we enrolled only patients with Ann Arbor stage II to IV disease to this study.

Treatment

Dose-adjusted (DA)-EPOCH-R/ high-dose methotrexate (HD-MTX) regimen

Starting doses of DA-EPOCH-R (level 1) consisted of rituximab 375 mg/m² intravenously (day 1); etoposide 50 mg/m² per day, doxorubicin 10 mg/m² per day, and vincristine 0.4 mg/m² per day, all infused for 96 h (days 2–5); cyclophosphamide 750 mg/m² intravenously (day 6); prednisolone 60 mg/m² twice a day orally (days 2–6); and G-CSF 75-100 μg per day subcutaneously/intravenously [day 7 until absolute neutrophil count (ANC) >5,000 /μL past the nadir].[1-3]

Dose adjustment was done according to original reports.[1-3] The maximum dose level of EPOCH was level 6. The next cycle of DA-EPOCH-R was initiated after 21 days providing ANC count was ≥1,000 /μL and platelets were ≥$10x10^4$ /μL.

The first cycle of HD-MTX after 21 days of the fourth cycle of DA-EPOCH-R was started providing white blood cell (WBC) count ≥2,000 /μL, platelets ≥$10x10^4$ /μL, and serum creatinine <2.0 mg/dL. If the WBC count was <2,000 /μL on day 1 or the previous

day of a cycle of HD-MTX, the cycle of HD-MTX was postponed and G-CSF was administered. The dose of MTX was determined according to creatinine clearance examined within 7 days before day 1 of both the first and the second cycles of HD-MTX (80%, creatinine clearance 60-79 mL/min; 60%, creatinine clearance 50-59 mL/min; 0%, creatinine clearance <50 mL/min). In patients who experienced toxicity defining as febrile neutropenia, grade 3 hepatotoxicity, and grade 3 infections during the first cycle of HD-MTX, the dose of MTX was reduced to 60% in the second cycle.

DLBCL subtyping

Immunohistochemistry and methods of Lymph2Cx assay

Immunohistochemical staining using antibodies against CD5, CD20, CyclinD1, CD10, BCL6, MUM1, MYC, and BCL2, and in situ hybridization for Epstein-Barr virus-encoded small RNA-1 (EBER) were performed at the central pathology office using formalin-fixed paraffin-embedded (FFPE) sections. All cases were classified into morphologic variants of CD5+ DLBCL as previously reported.[4] For the Lymph2Cx assay, total RNA was extracted from FFPE tissues using RNeasy FFPE extraction kit (Qiagen, Hilden, Germany) after treatment with Deparaffinization Solution (Qiagen). We determined the amount of input RNA (542.5 ± 413.4 ng) for subsequent nCounter assay at 100 ng of RNA ≥300 nt. Input RNA was deposited onto a glass cartridge of the nCounter Prep Station using the “high sensitivity” setting on the nCounter Prep Station (NanoString Technologies, Seattle, WA, USA). Cell-of-origin assignment was performed using the expression levels of 20 genes as described by Scott et al.[5]

Endpoints

Primary endpoint

It is known that there is no significant difference in the complete response (CR) rate between CD5+ DLBCL and CD5-negative (CD5-) DLBCL.[6] On the other hand, disease progression/relapse is more frequent in CD5+ DLBCL than in CD5- DLBCL.[7] In the rituximab-era, the prognosis of patients with DLBCL who experienced relapse within 1 year after diagnosis or were refractory to the first-line therapy is extremely poor.[8] To achieve early disease control as an initial step to improve the prognosis of patients with CD5+ DLBCL, we selected 2-year PFS as the primary endpoint of this study. The 2-year central nervous system relapse rate was assessed as a secondary endpoint because of the rarity of the disease and the feasibility of this clinical trial.

Statistical analysis

Definition of PFS and overall survival

PFS was defined as the time from date of registration to the earliest date of progression, relapse, or death from any cause. Overall survival was defined as the time from date of registration to the date of death as a result of any cause.

Calculation of the threshold 2-year PFS

The threshold 2-year PFS was calculated using a data set of our previous retrospective study including 337 patients with CD5+ DLBCL (*Online Supplementary Figure S1*). In this analysis, PFS was calculated from the date at diagnosis. In 149 patients with newly diagnosed stage II-IV CD5+ DLBCL who received rituximab-containing chemotherapy and had available follow-up data, the 2-year PFS was 51% and it was selected as the threshold 2-year PFS of this trial.

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8. Gisselbrecht C, Glass B, Mounier N, et al: Salvage regimens with autologous transplantation for relapsed large B-cell lymphoma in the rituximab era. J Clin Oncol 2010;28(27):4184-4190.

Supplementary Table S1. Patient selection criteria

Inclusion criteria

(1) Histologically confirmed CD5-positive DLBCL according to the 2008 WHO classification. Patients with primary DLBCL of the CNS or having a history of lymphoproliferative disorders are excluded from this study.

(2) Confirmed CD20-positive and CD5-positive by immunohistochemistry and/or flow cytometry.

(3) Clinical stage: II, III, or IV diagnosed by the latest imaging study within 28 days before registration. Clinical stage is diagnosed using CT scanning. PET or PET/CT only is insufficient as a reason for upstaging.

(4) Lymphoma cell count in peripheral blood 14 days before registration ≥10,000/mm^3

(5) Age: 20 to 75 years

(6) PS (ECOG): 0-3

(7) No clinical symptoms of CNS involvement (CSF examination and brain MRI are not mandatory.)

(8) Measurable lesion present on a CT scan; at least 1 lesion/node with a long axis of >1.5 cm

(9) No prior chemotherapy, radiotherapy, or antibody therapy

1) Patients who have undergone a surgical operation are eligible if a measurable region is present.

2) Patients who have received corticosteroids alone are eligible for this study, but those under treatment must be discontinued before registration.

(10) Patients with sufficient hematopoietic (except for cases with bone marrow involvement or HPS), hepatic, renal, cardiac, and pulmonary function (and fulfilling the following eligibility criteria.). Laboratory data should be obtained within 14 days before registration. Echocardiography should be performed within 3 months.

i) WBC ≥ 3,000 /mm^3

ii) ANC ≥ 1,200 /mm^3

iii) Platelet count ≥ 7.5 x 10^4 /mm^3

For cases with bone marrow involvement or HPS, platelet count must be ≥ 5.0 x 10^4/mm^3.

iv) AST≤ upper normal limit x 5

v) ALT≤ upper normal limit x 5

vi) Total bilirubin ≤ 2.0 mg/dl

vii) Serum creatinine ≤ 1.5 mg/dl

viii) No ischemic change, atrial fibrillation or ventricular arrhythmia requiring treatment by ECG

ix) Left ventricular ejection fraction ≥ 50%

x) O2 saturation (SaO_2) ≥ 90% (under room air)

(11) Patient's written informed consent before registration.

Exclusion criteria

(1) History of angle-closure glaucoma

(2) Uncontrollable diabetes mellitus despite insulin therapy

(3) Uncontrollable hypertension

(4) Pleural effusion or ascites, except for small amounts

(5) Coronary artery disease under treatment; cardiomyopathy, heart failure, or arrhythmia treated with antiarrhythmic drugs

(6) HBs antigen positive

(7) HCV antibody positive

(8) HIV antibody positive

(9) Accompanying interstitial pneumonitis or pulmonary fibrosis (all apparently diagnosed by a chest X-ray)

(10) Severe infection

(11) Liver cirrhosis, either biopsy proven or clinically diagnosed

(12) Other active malignancies: overlapping cancer or asynchronous cancer within 5 years. Carcinoma in situ, intra mucosal cancers, and other equivalent lesions are not included for active double cancer; history of lymphoma, myelodysplastic syndrome, or leukemia

(13) Pregnancy, possible pregnancy, or breastfeeding

(14) Severe psychosis

(15) Systemic corticosteroid therapy

(16) Consideration as ineligible by attending physicians for other reasons

Supplementary Table S2. Characteristics of the patients who experienced CNS relapse (n=4)

Patient No.	Age	Sex	Diagnosis	COO	Stage	Site(s) of extranodal involvement	IPI	CNS-IPI	Protocol treatment	Site(s) of involvement at relapse	Time to CNS relapse, yrs	OS, yrs, Outcome
1	39	M	High grade B NOS	ABC	II	Orbit, ethmoid sinus	Low	Low	Discontinued after 2 cycle of DA-EPOCH-R	Leptomeninges	0.1	1.0, DOD
2	78	M	Primary testicular DLBCL	ABC	II	Testis	Low	Low	Completed	Brachial plexus, bone marrow, brain parenchyma	1.3	1.7, DOD
3	57	F	DLBCL	ABC	III	None	LI	Intermediate	Discontinued after the first R due to grade 4 TLS Switched to R-hyper CVAD/MA	Spinal cord	0.9	2.3, DOD
4	62	F	High grade B NOS	ABC	IV	Subcutaneous tissue, skeletal muscle	High	High	Discontinued after 4 cycle of DA-EPOCH-R	Brain parenchyma	0.2	0.5, DOD

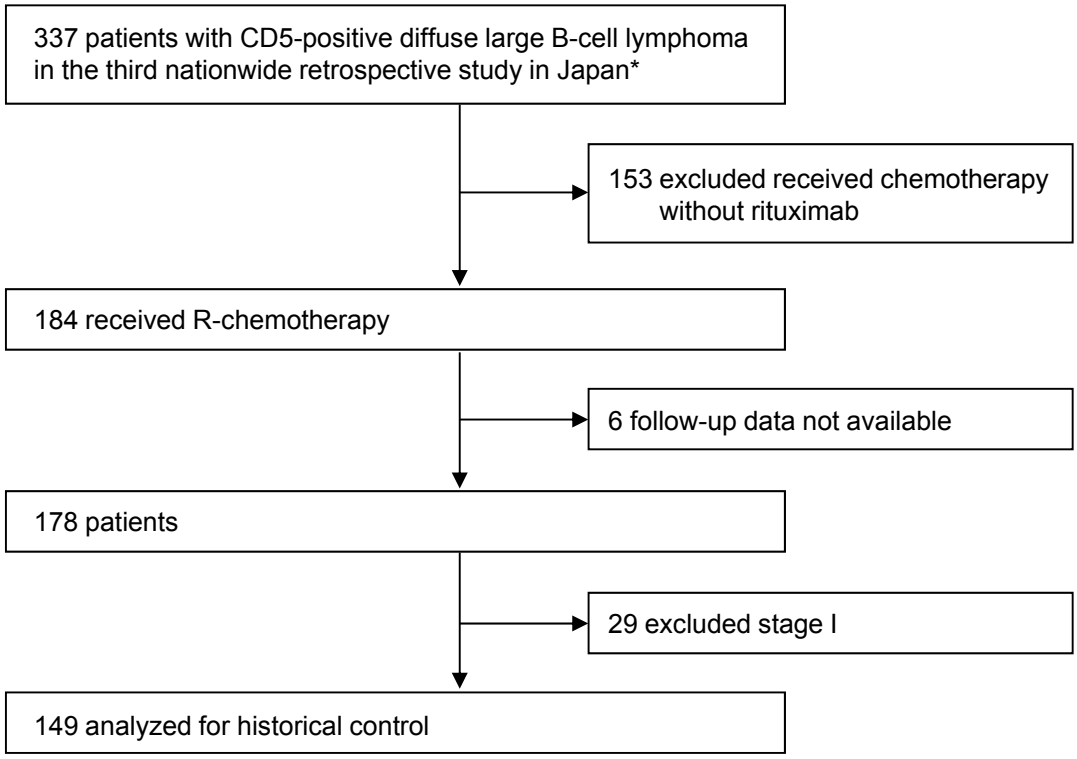
ABC, activated B-cell-like; CNS, central nervous system; COO, cell-of-origin; DA-EPOCH, dose-adjusted etoposide, vincristine, doxorubicin, cyclophosphamide, and prednisone; DOD, died of disease; High grade B NOS, High grade B-cell lymphoma, not otherwise specified; Hyper CVAD/MA, hyper fractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone alternating with methotrexate and cytarabine; IPI, International Prognostic Index; LI, Low-intermediate; OS, overall survival; R, rituximab; TLS, tumor lysis syndrome; yrs, years.

Supplementary Table S3. Hematological and non-hematological toxicities in all 357 cycles of DA-EPOCH-R chemotherapy

	Grade 3	Grade 4
Hematological adverse event		
Neutropenia	39 (11%)	278 (78%)
Leukopenia	60 (17%)	252 (71%)
Thrombocytopenia	67 (19%)	27 (8%)
Anemia	122 (34%)	12 (3%)
Febrile neutropenia	82 (23%)	—
Non-hematological adverse event		
Blood bilirubin increased	2 (1%)	0
AST increased	2 (1%)	0
ALT increased	8 (2%)	0
Hyperglycemia*	1 (0.3%)	0
Hyponatremia	8 (2%)	0
Hyperkalemia	0	1 (0.3%)
Hypokalemia	8 (2%)	0
Hypocalcaemia	1 (0.3%)	8 (2%)
Cardiac disorders	0	0
Constipation	5 (1%)	0
Ileus	2 (1%)	0
Nausea	1 (0.3%)	0
Vomiting	1 (0.3%)	0
Infection	7 (2%)	0
Allergic reaction	0	0
Tumor lysis syndrome	2 (1%)	1 (0.3%)
Peripheral motor neuropathy	3 (1%)	0
Peripheral sensory neuropathy	5 (1%)	0
Pneumonitis	1 (0.3%)	0
Others	19 (1%)	0

AST, aspartate aminotransferase; ALT, alanine aminotransferase.
*Examined in 333 cycles.

A



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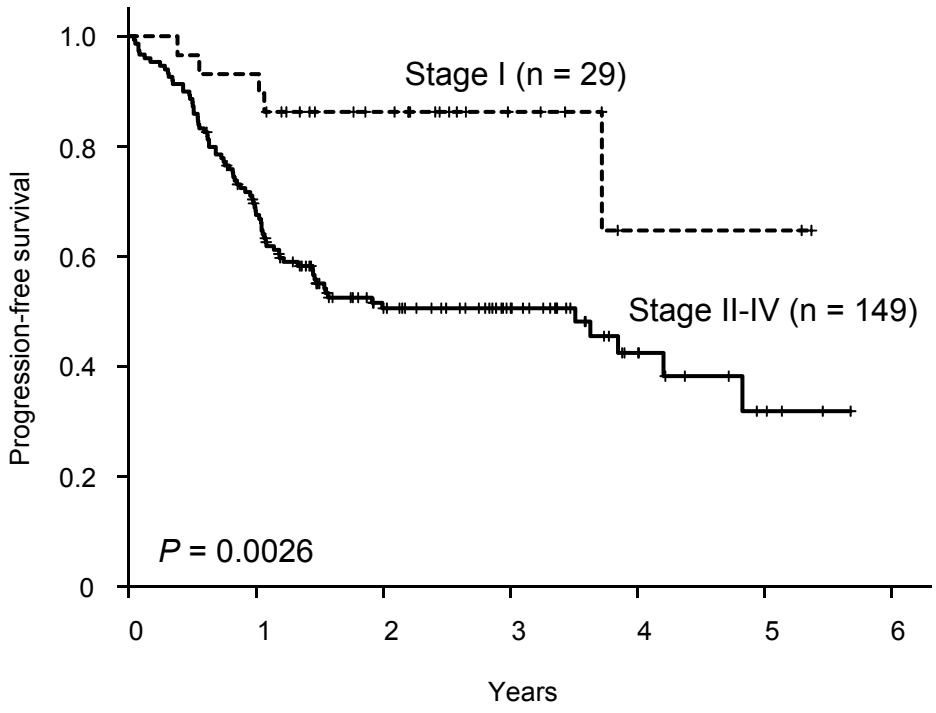
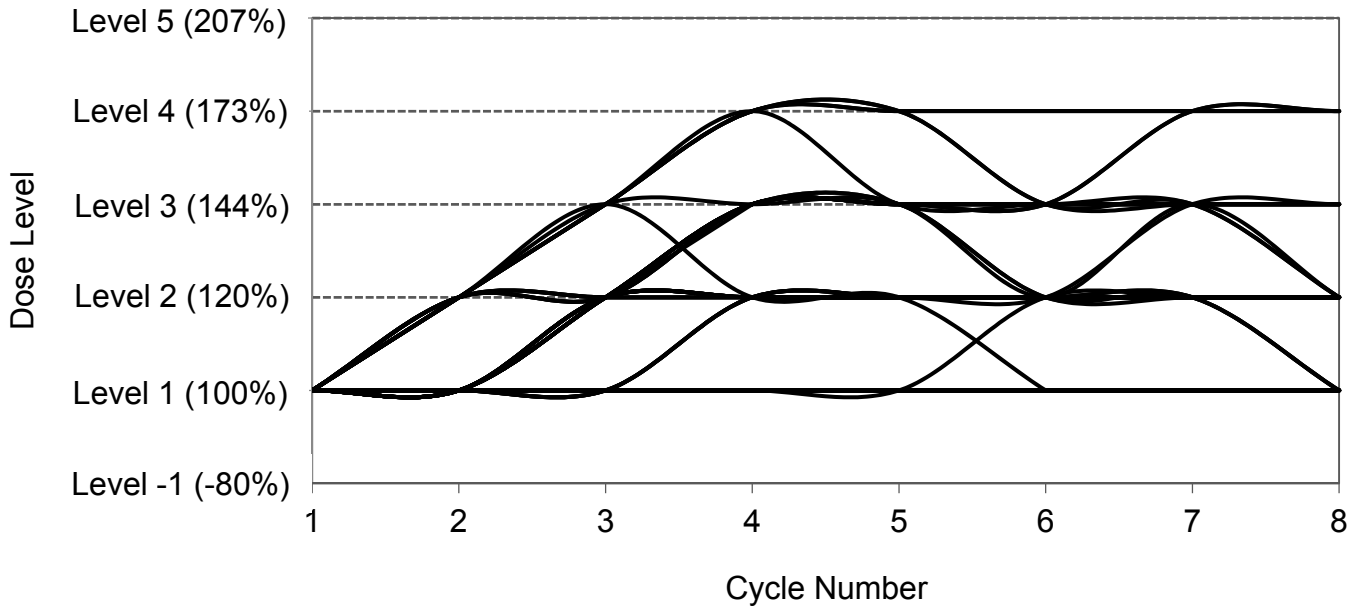
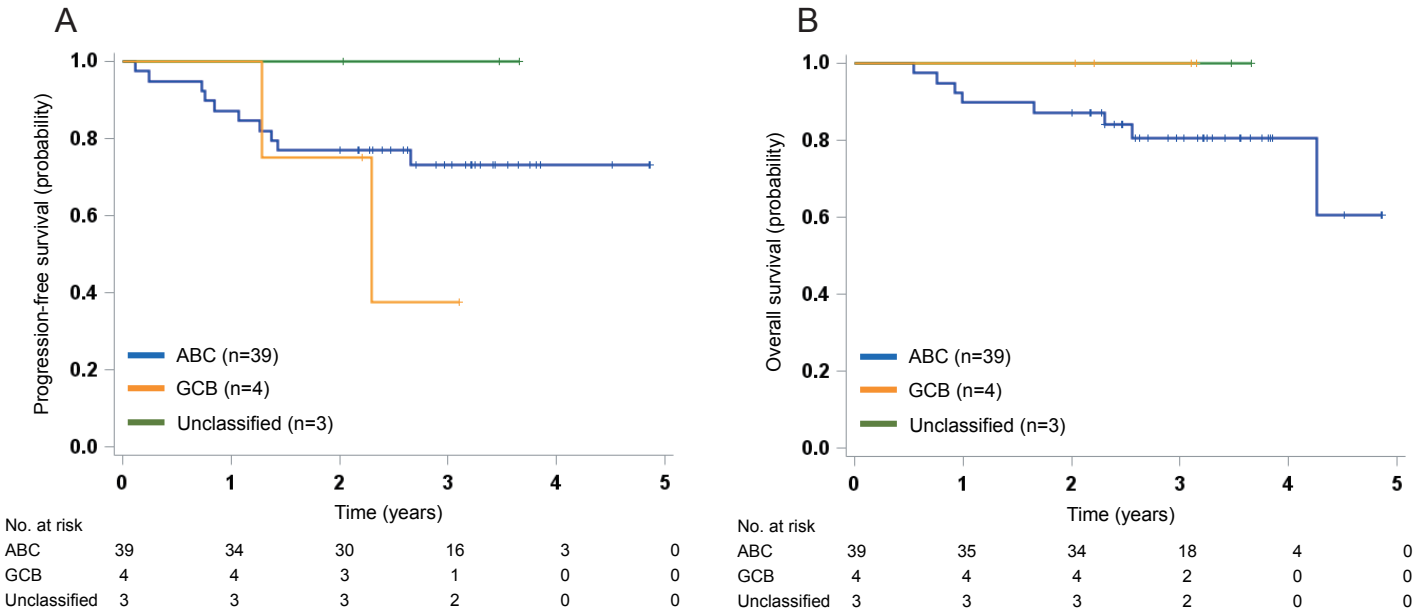


Supplementary Figure S1. The rationale for the historical control. (A) Patient selection (*Miyazaki, et al. Ann Oncol 2009.[8]). (B) Progression-free survival of the third nationwide retrospective study in Japan. Figure shows progression-free survival of 29 patients with stage I versus 149 patients with stage II-IV CD5-positive diffuse large B-cell lymphoma.



Supplementary Figure S2. Dose-adjustment map of etoposide, prednisone, vincristine, cyclophosphamide, and doxorubicin with rituximab (EPOCH-R).



Supplementary Figure S3. Survival according to COO categories. (A) PFS according to COO categories. (B) OS according to COO categories.

CD5+ DLBCL Treatment Study Group
Supported in part by grants-in-aid from Japan Agency for Medical Research and Development, AMED
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A Phase II trial of DA-EPOCH And Rituximab with HD-MTX therapy for newly-diagnosed DLBCL with CD5 expression (PEARL5 study)

Protocol Ver. 2.3

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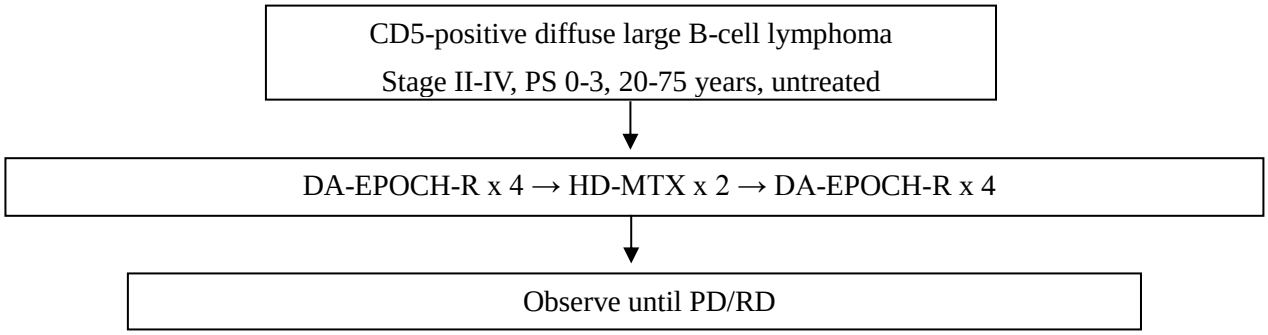
5/31/2012	v1.0
6/25/2012	v1.1
6/28/2012	v1.2
11/1/2012	v1.3
2/18/2014	v2.0
10/30/2014	v2.1
1/14/2015	v2.2
3/28/2017	v2.3

Note: This protocol was originally written in Japanese. For the purpose of review, the following parts of the original protocol were translated into English.

- Selection of patients, including both eligibility and ineligibility criteria
- Schema and treatment plan, including administration schedule
- Rules for dose modification
- Measurement of treatment effect including response criteria, definitions of response and survival, and methods of measurement
- Reasons for early cessation of trial therapy
- Objectives and entire statistical section (including endpoints)

0. Overview

0.1 Study scheme



0.2 Objective

To explore a more effective treatment for newly diagnosed stage II-IV CD5-positive diffuse large B-cell lymphoma (DLBCL) according to the WHO classification, we conduct a phase II study of dose-adjusted (DA)-EPOCH-R (etoposide, prednisolone, vincristine, cyclophosphamide, doxorubicin, rituximab) combined with high dose (HD)- methotrexate (MTX) and evaluate efficacy and safety of this treatment.

Primary endpoint: 2-year progression-free survival

Secondary endpoints: complete response rate, overall response rate, overall survival, 2-year central nervous system relapse rate, and toxicity

0.3 Patient selection criteria

Patients who fulfill all the following criteria and not are eligible.

(1) Histologically confirmed CD5-positive diffuse large B-cell lymphoma according to the 2008 WHO classification. Patients with a history of lymphoproliferative disorder are excluded from this study.
(2) Confirmed CD20-positive and CD5-positive by immunohistochemistry and/or flow cytometry.
(3) Clinical stage ("4.2."): II, III, or IV diagnosed by the latest imaging study within 28 days before registration. Clinical stage is diagnosed using CT scan. PET or PET/CT only is insufficient as a reason of up-stage.
(4) Lymphoma cell count in peripheral blood 14 days before registration $\geq 10{,}000/mm^3$
(5) Age: 20 to 75 years old
(6) PS (ECOG): 0-3
(7) No clinical symptoms of CNS involvement (CSF examination and brain MRI are not mandatory.)
(8) Measureable lesion present
(9) No prior chemotherapy, radiotherapy, and antibody therapy
(10) Preserved organ function
(11) Written informed consent

0.4 Protocol treatment

Treatment should start within 7 days from registration.

DA-EPOCH-R/HD-MTX therapy

Four cycles of DA-EPOCH-R followed by 2 cycles of HD-MTX and 4 additional cycles of DA-EPOCH-R were planned as the protocol treatment.

1) DA-EPOCH-R therapy (level 1)

Drug	Dose	Route	Treatment Days
Rituximab (RTX)	375 mg/m^2	IV	day 1*
Etoposide (ETP)	50 mg/m^2/day**	CIV	day 2,3,4,5 (96 hours)
Doxorubicin (DOX)	10 mg/m^2/day**	CIV	day 2,3,4,5 (96 hours)
Vincristine (VCR)	0.4 mg/m^2/day	CIV	day 2,3,4,5 (96 hours)
Cyclophosphamide (CPA)	750 mg/m^2**	CIV	day 6
Prednisolone (PSL)	60 mg/m^2/day	PO/IV	day 2,3,4,5,6
G-CSF	See below***	SC/IV	day 7 – ANC > 5,000/mm^3

*, Rituximab was administered on day 1 (day -1 or day 2) of EPOCH chemotherapy. Patients at risk of tumor lysis syndrome will administrated RTX on day8 in the first cycle at the investigator's discretion.
**, Starting dose of EPOCH (level 1). Measurement of ANC nadir based on twice weekly CBC only (3 days apart). Only use twice weekly CBC for dose-adjustment, even if additional CBC's are obtained.
***, The dose of G-CSF shall be administered in accordance with the national health insurance coverage rule.

- If Nadir ANC ≥ 500 /mm^3 on all measurements: ↑ 1 dose level above last cycle
- If Nadir ANC < 500 /mm^3 on 1 or 2 measurements: Same dose level as last cycle
- If Nadir ANC < 500 /mm^3 ≥ 3 measurements: ↓ 1 dose level below last cycle

Or

- If nadir platelet < 25,000 /mm^3** on 1 measurement: ↓ 1 dose level below last cycle.

2) HD-MTX therapy

Drug	Dose	Route	Treatment Days
Methotrexate (MTX)	3.5 g/m^2	IV (2 hours)	day 1
Calcium folinate	24 mg x4/day*	IV/PO	day 2,3,4

*, Started from the time-point of 24 hours after the MTX initiation.

0.5 Projected sample size and study period

Projected sample size：45

Registration period：3.5 years

Follow-up period：5 years

Total of study period：8.5 years

0.6 Inquiry

Eligibility criteria, treatment modification criteria, and issues needed for clinical considerations: The Study Coordinator (front cover)

Registration procedure and how to input electronic data capture (EDC): The Registration Office (17.5)

Adverse event reporting: The Study Coordinator & the Principal Investigator (front cover, 17.3 17.4) → Data and Safety Monitoring Board (17.9)

Contents

1. Objective

To explore a more effective treatment for newly diagnosed stage II-IV CD5-positive diffuse large B-cell lymphoma (DLBCL) according to the WHO classification, we will conduct a phase II study of dose-adjusted (DA)-EPOCH-R (etoposide, prednisolone, vincristine, cyclophosphamide, doxorubicin, and rituximab) combined with high-dose (HD)-methotrexate (MTX) and will evaluate the efficacy and safety of this treatment.

Primary endpoint: 2-year progression-free survival

Secondary endpoints: complete response rate, overall response rate, overall survival, 2-year central nervous system relapse rate, and toxicity

2. Background

2.1 Study population

2.2 Rationale for the study plan

2.3 Study design

2.3.1 Rationale for the study design

2.3.2 Projected subsequent studies

2.3.3 Rationale for the endpoint setup

There is no significant difference in the complete response (CR) rate between CD5+ DLBCL and CD5- DLBCL, although this conclusion is based on observations before the introduction of rituximab (RTX)[3]. In the third nationwide observational study, the CR rate in 184 patients who received RTX-containing chemotherapy as first-line therapy was 80%[5]. In the same study, the CR rate in 149 patients with stage II-IV disease who received a RTX-containing regimen and whose follow-up data were available was 77% (115/149). On the other hand, disease progression/relapse is more frequent in CD5+ DLBCL than in CD5- DLBCL. In the third nationwide study, the 2-year PFS rate in the RTX-containing chemotherapy group was 56% (unpublished data). In the RTX era, the prognosis of patients with CD5+ DLBCL who experienced relapse within 1 year after diagnosis or were refractory to the first-line therapy has been extremely poor[14]. To achieve early disease control of CD5+ DLBCL, 2-year PFS was selected as the primary endpoint of this study. Overall survival (OS) and a CNS relapse rate will be analyzed as secondary endpoints of this study.

2.3.4 Expected patient accrual

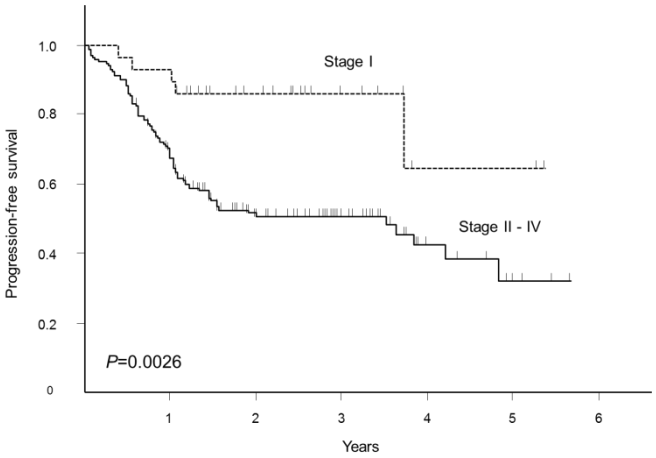
2.3.5 Clinical hypothesis and rationale for the sample size

The main clinical hypothesis of this study is that the protocol treatment is regarded as a promising treatment for CD5+ DLBCL if the 2-year PFS rate by DA-EPOCH-R/HD-MTX is superior to the historical control of 51% (see “2.1.2 Rationale of patient selection”).

The primary analysis is performed using the data of “all eligible patients” by the Kaplan-Meier method. The 2-year PFS rate is defined as an estimated value at 2 years on a PFS curve. For the interval estimation, the 90% confidence intervals (CIs) are calculated using Greenwood’s formula and analyzed as to whether the

lower 90% CI of the 2-year PFS exceeds the threshold of 51%.

The projected sample size of this study is 45. The expected 2-year PFS rate for the protocol treatment is estimated to be 70%. The threshold 2-year PFS rate is defined as 51% in the historical control of 149 patients with newly diagnosed stage II-IV CD5+ DLBCL among 178 patients who received RTX-containing chemotherapy and had available follow-up data in the third nationwide survey. Based on this setting with a statistical power of 80% and an alpha error of 5%, the number of patients required for this study is calculated to be 41 by using a binominal method. Considering 10% ineligible or lost-to-follow-up cases, the projected sample size of this study is calculated to be 45.



2.4 Summary of the expected benefit and risk

2.5 Ancillary analysis

The ancillary analysis of this study comprises immunohistochemistry, gene expression profiling by means of nCounter™ Analysis System, and other methods using FFPE tumor samples from patients before the protocol treatment. In the ancillary analysis, subtype-specific biomarkers of CD5+ DLBCL and predictive biomarkers of DA-EPOCH-R/HD-MTX will be exploratorily analyzed.

The study protocol of the ancillary analysis is prepared by the members of the ancillary analysis committee (17.10 ancillary analysis committee). After an approval of the institutional review board (IRB) in participating institutes, the ancillary analysis will be initiated.

3. Drug information

4. Criteria for evaluation

4.1 Histologic diagnosis criteria

For histologic criteria, the 2008 WHO classification[1] is used.

The disease eligible for the present study is boxed below.

【Variants, subgroups, and subtypes/entities of DLBCL in the 2008 WHO classification】

DLBCL, not otherwise specified (NOS)
- Common morphologic variants
  - Centroblastic, Immunoblastic, Anaplastic
- Rare morphologic variants
- Molecular subgroups

Germinal center B-cell-like (GCB), Activated B-cell-like (ABC)

Immunohistochemical subgroups

CD5-positive DLBCL, GCB, non-GCB

DLBCL subtypes

T-cell/histiocyte-rich large B-cell lymphoma

Primary DLBCL of the CNS

Primary cutaneous DLBCL, leg type

EBV positive DLBCL of the elderly

Other lymphomas of large B cells

Primary mediastinal (thymic) large B-cell lymphoma

Intravascular large B-cell lymphoma

DLBCL associated with chronic inflammation

Lymphomatous granulomatosis

ALK-positive large B-cell lymphoma

Large B-cell lymphoma arising in HHV8-associated multicentric Castleman disease

Primary effusion lymphoma

Borderline cases

B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and Burkitt lymphoma

B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and classical Hodgkin lymphoma

4.2 Staging criteria

4.2.1 Stage

For staging, the AJCC (American Joint Committee on Cancer) Cancer Staging Manual 6th Edition[15] is used. AJCC uses the staging criteria based on the Ann-Arbor classification[16].

Stage

Stage I: involvement of a single lymph node region (I); or localized involvement of a single extralymphatic organ or site in the absence of any lymph node involvement (I_E).

Stage II: involvement of 2 or more lymph node regions on the same side of the diaphragm (II); or localized involvement of a single extralymphatic organ or site in association with regional lymph node involvement with or without involvement of other lymph node regions on the same side of the diaphragm (II_E).

Stage III: involvement of lymph node regions on both sides of the diaphragm (III), which also may be accompanied by extralymphatic extension in association with adjacent lymph node involvement (III_E) or by involvement of the spleen (III_S) or both ($III_{S, E}$).

Stage IV: diffuse or disseminated involvement of one or more extralymphatic organs, with or without associated lymph node involvement; or isolated extralymphatic organ involvement in the absence of adjacent regional lymph node involvement, but in conjunction with disease in distant site(s).

A and B symptoms

All stages are divided into A and B as defined below. The B designation is given with any of the following symptoms:

1) Unexplained fever with a body temperature above 38 °C.
2) Drenching night sweats.
3) Unexplained loss of more than 10% of body weight in the 6 months before diagnosis.

4.2.2 Prognostic factors and risk groups of the International Prognostic Index

International Prognostic Index (IPI)

Parameter	Adverse prognostic factor
Age	> 60 years
Serum LDH level	> upper limit of normal
PS	2 - 4
Stage	III or IV
Sites of extranodal involvement	> 1

There are four risk groups of the IPI according to the number of adverse prognostic factors.

IPI, 0 or 1; Low risk, 2; Low-intermediate risk, 3; High-intermediate risk, 4 or 5; High risk

4.3 Definition of the criteria for evaluations and laboratory tests

4.3.1 Absolute neutrophil count (ANC)

Total counts of neutrophilic band and segmental forms (/mm^3)

4.3.2 CD5-positive lymphoma cells

Lymphoma cells that are diagnosed as CD5-positive in the flow-cytometric analysis and/or immunohistochemistry using tissue samples of lymphomatous involvement.

There is no cut-off of the positivity of CD5 expression in this study. The expression of CD5 in lymphoma cells should be evaluated with the results of the analysis for the expression of other CD antigens.

4.3.3 Lymphoma cell count in peripheral blood

Total counts of white blood cells (/mm^3) x the proportion of lymphoma cells in peripheral blood at microscopy (%) = lymphoma cell count in peripheral blood (/mm^3)

5. Patient selection criteria

Patients who fulfill all of the following inclusion criteria and do not satisfy any of the following exclusion

criteria are regarded as eligible patients.

5.1 Inclusion criteria

(2) Histologically confirmed CD5-positive DLBCL according to the 2008 WHO classification. Patients with primary DLBCL of the CNS or having a history of lymphoproliferative disorders are excluded from this study.

(2) Confirmed CD20-positive and CD5-positive by immunohistochemistry and/or flow cytometry.

(3) Clinical stage ("4.2."): II, III, or IV diagnosed by the latest imaging study within 28 days before registration. Clinical stage is diagnosed using CT scanning. PET or PET/CT only is insufficient as a reason for upstaging.

(4) Lymphoma cell count in peripheral blood 14 days before registration $\geq$ 10,000/mm^3

(5) Age: 20 to 75 years

(6) PS (ECOG): 0-3

(7) No clinical symptoms of CNS involvement (CSF examination and brain MRI are not mandatory.)

(8) Measurable lesion present on a CT scan; at least 1 lesion/node with a long axis of >1.5 cm

(9) No prior chemotherapy, radiotherapy, or antibody therapy

1) Patients who have undergone a surgical operation are eligible if a measurable region is present.

2) Patients who have received corticosteroids alone are eligible for this study, but those under treatment must be discontinued before registration.

(10) Patients with sufficient hematopoietic (except for cases with bone marrow involvement or HPS), hepatic, renal, cardiac, and pulmonary function (and fulfilling the following eligibility criteria.). Laboratory data should be obtained within 14 days before registration. Echocardiography should be performed within 3 months.

i) WBC $\geq$ 3,000 /mm^3

ii) ANC $\geq$ 1,200 /mm^3

iii) Platelet count $\geq$ 7.5 x 10^4 /mm^3

For cases with bone marrow involvement or HPS, platelet count must be $\geq$ 5.0 x 10^4/mm^3.

iv) AST $\leq$ upper normal limit x 5

v) ALT $\leq$ upper normal limit x 5

vi) Total bilirubin $\leq$ 2.0 mg/dl

vii) Serum creatinine $\leq$ 1.5 mg/dl

viii) No ischemic change, atrial fibrillation or ventricular arrhythmia requiring treatment by ECG

ix) Left ventricular ejection fraction $\geq$ 50%

x) O_2 saturation (SaO2) $\geq$ 90% (under room air)

(11) Patient's written informed consent before registration.

5.2 Exclusion criteria

(1) History of angle-closure glaucoma

(2) Uncontrollable diabetes mellitus despite insulin therapy
(3) Uncontrollable hypertension
(4) Pleural effusion or ascites, except for small amounts
(5) Coronary artery disease under treatment; cardiomyopathy, heart failure, or arrhythmia treated with antiarrhythmic drugs
(6) HBs antigen positive
(7) HCV antibody positive
(8) HIV antibody positive
(9) Accompanying interstitial pneumonitis or pulmonary fibrosis (all apparently diagnosed by a chest X-ray)
(10) Severe infection
(11) Liver cirrhosis, either biopsy proven or clinically diagnosed
(12) Other active malignancies: overlapping cancer or asynchronous cancer within 5 years. Carcinoma in situ, intramucosal cancers, and other equivalent lesions are not included for active double cancer; history of lymphoma, myelodysplastic syndrome, or leukemia
(13) Pregnancy, possible pregnancy, or breastfeeding
(14) Severe psychosis
(15) Systemic corticosteroid therapy
(16) Consideration as ineligible by attending physicians for other reasons

6. Registration

7. Treatment plan and dosage modification

7.1 Treatment plan (DA-EPOCH-R/HD-MTX)

1) Four cycles of DA-EPOCH-R followed by 2 cycles of HD-MTX and 4 additional cycles of DA-EPOCH-R are planned as the protocol treatment.
2) Treatment should start within 7 days from registration.
3) Protocol completion is defined as the day when the dosing of PSL on day 5 in the eighth cycle of DA-EPOCH-R therapy is completed.
4) After the completion of the protocol treatment, subjects who obtained complete remission will be followed up without treatment (e.g., radiation therapy, maintenance therapy of RTX, high-dose chemotherapy with autologous stem cell transplantation, interferon therapy, and another chemotherapy) until disease progression.

(1) DA-EPOCH-R therapy

Drug	Dose	Route	Treatment Days
Rituximab (RTX)	375 mg/m^2	IV	day 1*
Etoposide (ETP)	50 mg/m^2/day**	CIV	day 2, 3, 4, 5 (96 hours)

Doxorubicin (DOX)	10 mg/m^2/day**	CIV	day 2, 3,4, 5 (96 hours)
Vincristine (VCR)	0.4 mg/m^2/day	CIV	day 2, 3, 4, 5 (96 hours)
Cyclophosphamide (CPA)	750 mg/m^2**	CIV	day 6
Prednisolone (PSL)	60 mg/m^2/day	PO/IV	day 2, 3, 4, 5, 6
G-CSF	See below***	SC/IV	day 7 – ANC > 5,000/mm^3

*, RTX was administered on day 1 (day 1 or day 2) of EPOCH chemotherapy. Patients at risk of tumor lysis syndrome will administered RTX on day 8 in the first cycle at the investigator's discretion.

**, Starting dose of EPOCH (level 1). Measurement of ANC nadir based on twice weekly CBC only (3 days apart). Only use twice weekly CBC for dose-adjustment, even if additional CBCs are obtained.

***, The dose of G-CSF shall be administered in accordance with the national health insurance coverage rule.

- If Nadir ANC ≥ 500 /mm^3 on all measurements: ↑ 1 dose level above last cycle
- If Nadir ANC < 500 /mm^3 on 1 or 2 measurements: Same dose level as last cycle
- If Nadir ANC < 500 /mm^3 ≥ 3 measurements: ↓ 1 dose level below last cycle

Or

- If nadir platelet < 25,000 /mm^3** on 1 measurement: ↓ 1 dose level below last cycle.

Table of doses per level for adjusted agents:

	Drug Doses per Dose Levels							
Drugs	-2	-1	1	2	3	4	5	6
ETP (mg/m^2/day)	50	50	50	60	72	86.4	103.7	124.4
DOX (mg/m^2/day)	10	10	10	12	14.4	17.3	20.7	24.8
CPA (mg/m^2/day)	480	600	750	900	1080	1296	1555	1866

1) Repeat cycles every 3 weeks. A starting dose level of EPOCH is level 1.
2) The maximum dose level of EPOCH is level 6.
3) For the dose of agents defined by the body surface area, RTX, ETP, CPA should be cut off by the 10 mg/body unit, DOX by the 1 mg/body unit, VCR by the 0.1 mg/body unit, and PSL by the 5 mg/body unit.
4) For patients whose body weight (BW) has changed after the protocol treatment, the drug doses should not be modified.
5) RTX administration
   a) The first administration of RTX requires hospitalization and careful monitoring. Patients at risk for tumor lysis syndrome will be administered RTX on day 8 in the first cycle at the investigator's discretion.
   b) RTX for clinical use should be stored in a cool and dark place before the administration of RTX. Do not freeze. If the RTX has been frozen, do not use it.
   c) The antibody should be diluted into a final volume of 0.9% sodium chloride or 5% dextrose in water

for a final concentration of 1 mg/ml. RTX will be administered as an intravenous infusion.

d) Oral premedication [(1), (2)] will be administered 30 min prior to starting each infusion of RTX. The use of a corticosteroid may be considered at the onset of the infusion reaction [(3), (4)].

Example)

(1) Acetaminophen 400 mg

(2) Antihistamine: chlorpheniramine maleate 2 mg or diphenhydramine hydrochloride 30 mg

(3) Hydrocortisone 100 m IV or DIV

(4) Only the first cycle of cases with a very high tumor burden: dexamethasone (6.6 mg/vial) 2-5 vials

e) The initial dose rate during a patient's first RTX infusion should be 25 mg/25 ml/hr. In the absence of an infusion reaction at 25 mg/25 ml/hr for 1 hour, the dose rate may be increased by 100 mg/100 ml/hr. The dose rate may be increased if tolerated by 100 mg/100 ml/hr increments every 1 hour to a maximum rate of 200 mg/hr. Finally, passing the 0.9% sodium chloride solution is recommended in order to administer all the RTX in the route.

f) During the intravenous infusion of RTX, efforts will be made to prevent severe nonhematologic toxicity (infusion reactions) in patients by pretreatment with steroid preparations as designated below.

Examples:

(1) Hydrocortisone (100 mg) IV or DIV

(2) Methylprednisolone (40 mg) IV or DIV

g) According to the grade of nonhematologic adverse events (according to CTCAE v 4.0), the infusion rate is changed as described below, and the abovementioned supportive regimen is performed.

Grade 2: Slow down the infusion rate at the onset or observe the condition as is, and when recovering to Grade 1, increase the infusion rate to the next step shown in e). Supportive therapy is enforced as necessary. If patients do not recover to Grade 1, the RTX infusion will be stopped temporarily, and supportive therapy will be enforced and restart when the patients are recovered to Grade 1.

Grade 3: Temporarily discontinue the infusion and perform supportive therapy, and when recovering to Grade 1, start again from 25 mg/25 ml/hr and raise the infusion rate as described in e).

h) If the administration was well tolerated during a patient's first treatment with RTX, the initial dose rate during subsequent treatment cycles may be increased to 100 mg/hr.

i) Patients at risk for tumor lysis will receive allopurinol 300 mg daily for up to 3 weeks following the administration of the first cycle of therapy. Additional measures such as hospitalization with aggressive IV hydration and rasburicase will be used.

6) ETP, DOX and VCR will be diluted in 500 mL 0.9% sodium chloride and administered as a 96-hour continuous IV infusion. ETP will be adjusted to a concentration of 0.4 mg/ml or less to prevent crystal precipitation. The chemotherapy will be administered with a suitable infusion pump via a central venous access device. Both catheter infection and thrombus formation will be monitored.

7) CPA will be diluted in 0.9% sodium chloride and administered intravenously. (Example; (1) 500 mL 0.9%

sodium chloride at 2.5 hours, (2) 250 mL 0.9% sodium chloride at 1.5 hours and (3) 100 mL 0.9% sodium chloride at 10-60 min). The total volume of infusion on the day of CPA administration will be at least 2 L/m^2/day, and maintaining a good state of hydration and good urine flow during and after drug administration is necessary. The urinary pH should be kept higher than 7.0 by adding 7% sodium bicarbonate 20-40 mL for every 500 mL volume of drip and be checked three times a day. If a patient will be administered levels ≥ 5 of DA-EPOCH-R, mesna should be given for the prevention of cystitis.

8) If the oral administration of prednisolone is difficult, the same amount of intravenous prednisolone is administered intravenously.
9) For patients who develop prednisolone withdrawal symptoms, PSL can be tapered after day 5.
10) Patients with diabetes should be carefully monitored for their blood sugar levels during the prednisolone period and should be adequately medicated.

(2) HD-MTX therapy

Drug	Dose	Route	Treatment Days
Methotrexate (MTX)	3.5 g/m^2	IV (2 hours)	day 1
Calcium folinate	24 mg x 4/day*	IV/PO	day 2, 3, 4

*, Started from the time-point of 24 hours after the MTX initiation.

1) HD-MTX therapy should be planned to be repeated twice with an interval of 14 days.
2) Methotrexate should be diluted in 500 mL of 5% glucose.
3) For the dose of agents defined by the body surface area, MTX should be cut off at 100 mg/body.
4) Urine alkalization is required to avoid the delay of methotrexate excretion. The urinary pH should be kept higher than 7.0 by adding 7% sodium bicarbonate 20-40 mL for every 500 mL volume of drip or by the oral intake of baking soda 3.0 g/day. Acetazolamide is recommended for diuretics, and acidifying agents such as furosemide, ethacrynic acid, or thiazides should be avoided, if possible.
5) **[Important]:** From day 1 to day 5, the following agents should not be given because they interfere with the concentration of serum methotrexate levels: Nonsteroid anti-inflammatory agents such as salicylic acid, sulfonamides, tetracycline, chloramphenicol, phenytoin, barbiturates, sulfamethoxazole-trimethoprim, sodium piperacillin, and proton pump inhibitors.
6) Calcium folinate (leucovorin) should be started from the time-point of 24 hours after the MTX initiation (22 hours after the completion of MTX). Check the serum MTX concentrations at the time-point of 48 hours and 72 hours after the MTX initiation and confirm them to be lower than 1 x 10^{-6} M and 1 x 10^{-7} M, respectively.
7) If the serum methotrexate concentration exceeds the level of 1 x 10^{-6} M at 48 hours or 1 × 10^{-7} M at 72 hours, hydration, urine alkalization and leucovorin administration must be continued until the concentration falls below the threshold. For SAEs due to a continuously high methotrexate concentration, such as myelosuppression, hepatic or renal dysfunction, long lasting stomatitis, diarrhea, melena, etc., a massive dose of calcium folinate should be given.

8) Other ancillary therapy with HD-MTX, similar to that of DA-EPOCH-R, was received.

7.2 Criteria for premature treatment withdrawal and protocol completion

7.2.1 Definition of protocol completion

Completion of the 4 cycles of DA-EPOCH-R followed by 2 cycles of HD-MTX and 4 additional cycles of DA-EPOCH-R is defined as the protocol completion.

The day of protocol completion is defined as the last day of drug administration of DA-EPOCH-R therapy.

7.2.2 Premature treatment withdrawal

Patients should be removed from the study under the following conditions:

1) The protocol therapy is regarded as ineffective.

Disease progression while on treatment.

2) The protocol therapy cannot be continued due to adverse reactions.

(1) Any Grade 4 nonhematologic toxicity according to NCI-CTCAE.
having no application to the following cases:

a) Malignancy-associated hypercalcemia

b) Hypokalemia due to transfusion or a drug

c) Hyponatremia due to transfusion or a drug (including SIADH)

d) Sepsis without severe symptoms

(2) Delay of the next cycle of chemotherapy more than 3 weeks due to toxicity.

(3) Termination of the study according to the definition of treatment modification ("7.3.").

(4) Physician's judgment of termination due to adverse reactions that are not listed in the treatment modification criteria.

3) Patients choose to withdraw because of reasons associated with adverse reactions.

- If the association with adverse reactions cannot denied, use this category.

4) Patients choose to withdraw because of reasons unrelated to adverse reactions.

- This category is only applicable for cases apparently unrelated to adverse reactions, such as relocation of the patient or his/her family to a different home.

5) Death occurs during the protocol therapy.

- Any death before the judgment of protocol termination by other reasons.

6) Others

- Rapid disease progression before the initiation of study chemotherapy
- Emergence of protocol violation.
- Change in diagnosis after registration.

The day of study termination is defined as the day of death for 5), but as the day of decision for other cases.

7.3 Definition of treatment modification

7.3.1 Criteria for the initiation of DA-EPOCH-R therapy

7.3.1.1. Criteria for the initiation of RTX

Patients should be treated with next cycle of RTX after fulfilling the following criteria:

(1) Three weeks or more have passed after the prior cycle of DA-EPOCH-R.

(2) Two weeks or more have passed after the second cycle of HD-MTX.

7.3.1.2. Criteria for the initiation of next cycle of DA-EPOCH therapy

Patients should be treated with next cycle of DA-EPOCH therapy after fulfilling the following criteria. G-CSF may be started for ANC < 1000/mm^3 and stopped 24 hours before treatment. If ANC count reaches higher than 1,000/mm^3 after the administration of G-CSF, the next cycle will be started two days after the day of administration of G-CSF. If the postponed days are 8 days or more, ↓ 1 dose level below the last cycle.
If the patient does not fulfill the criteria, wait until recovery ("7.3."). If recovery is not seen after 21 days of the prior therapy (21 days after day 22 of prior cycle), the study therapy will be terminated.

(Important) The following recovery is achieved on the day of or on one or two days before the next cycle of DA-EPOCH therapy. An early start of the next cycle of therapy until 2 days (1 cycle minimum 19 days) is acceptable.

1) ANC ≥ 1,000/mm^3
2) Platelet ≥ 10 x 10^4/mm^3
3) Patient does not have any other symptoms or complications not suitable for the initiation of DA-EPOCH.

7.3.2 Dosage modification of DA-EPOCH-R therapy ("7.1.")

The dosage administration amount after next cycle is defined as follows. Only one level is lowered even if it violates two or more dosage reduction criteria. The fourth and fifth cycles of EPOCH are administered at the same dose level.

- If Nadir ANC ≥ 500/mm^3 on all measurements: ↑ 1 dose level above last cycle
- If Nadir ANC < 500/mm^3 on 1 or 2 measurements: Same dose level as last cycle
- If Nadir ANC < 500/mm^3 ≥ 3 measurements: ↓ 1 dose level below last cycle

Or

If Nadir platelet < 25,000/mm^3** on 1 measurement: ↓ 1 dose level below last cycle.

Table of doses per level for adjusted agents:

	Drug Doses per Dose Levels							
Drug	-2	-1	1	2	3	4	5	6
ETP (mg/m^2/day)	50	50	50	60	72	86.4	103.7	124.4

DOX (mg/m^2/day)	10	10	10	12	14.4	17.3	20.7	24.8
CPA (mg/m^2/day)	480	600	750	900	1080	1296	1555	1866

7.3.3 Criteria for dose reduction of DA-EPOCH-R

If the following adverse events are observed during DA-EPOCH-R therapy, reduce the dose of drugs in the next cycle.

If more than one toxicities are observed, use the strictest criteria.

1) Neurotoxicity due to VCR

If at least one of the following adverse events is observed, reduce the dose of VCR to 75% in the next cycle. If the toxicity is recovered to ≤ Grade 1, increase the dose of VCR to 100% in the next cycle. If the toxicity remains Grade 2 despite dose reduction, use the same dose of VCR in the next cycle.

Peripheral motor neuropathy Grade 2	Moderate symptoms; limiting instrumental ADL
Ileus Grade 2	Symptomatic; altered GI function; bowel rest indicated

If at least one of the following adverse events is observed, reduce the dose of VCR in the following cycles to 50%. The use of vinca alkaloids other than VCR is not permitted. If the toxicity recovers to ≤ Grade 2, increase the dose of VCR to 75% in the next cycle. If the toxicity remains Grade 3 despite dose reduction, use the same dose of VCR in the next cycle.

Peripheral sensory neuropathy Grade 3	Severe symptoms; limiting self care ADL
Ileus Grade 3	Severely altered GI function; TPN indicated

If the following toxicity is observed, reduce the dose of VCR in the following cycles to 25%. The use of vinca alkaloids other than VCR is not permitted.

Peripheral motor neuropathy Grade 3	Severe symptoms; limiting self care ADL; assistive device indicated

If at least one of the following toxicities is observed, terminate the administration of VCR.

Peripheral sensory neuropathy Grade 4	Life-threatening consequences; urgent intervention indicated
Peripheral motor neuropathy Grade 4	Life-threatening consequences; urgent intervention indicated
Ileus Grade 4	Life-threatening consequences; urgent intervention indicated

2) Hepatotoxicity due to VCR

· In case of 2.0 mg/dl < T-Bil ≤ 3.0 mg/dl on day 1 or the previous day of the next cycle of DA-EPOCH-

R, reduce the dose of VCR to 75%.

- In case of T-Bil > 3.0 mg/dl on day 1 or the previous day of the next cycle of DA-EPOCH-R, reduce the dose of VCR to 50%.

3) Gastrointestinal ulcer or neuropsychiatric symptoms due to prednisone

In case of the following toxicities, the administration of prednisone should be terminated.

- Grade ≥ 2 gastric or duodenal ulceration confirmed by upper GI radiography or endoscopy despite the prophylactic use of H2 blockers or proton pump inhibitors
- Steroid-induced psychosis due to prednisone that needs a major tranquilizer, antidepressant or antimanic after initiating DA-EPOCH-R

4) Hematuria due to CPA

In case of Grade 3 hematuria (gross hematuria; transfusion, IV medications or hospitalization indicated; elective endoscopic, radiologic or operative intervention indicated), terminate the administration of CPA.

5) Criteria for dose reduction of RTX

The dose of RTX will not be reduced.

7.3.4 Criteria for the initiation of HD-MTX therapy

Each cycle of HD-MTX will be initiated after fulfilling all of the following conditions.

(1) Three weeks from day 1 of the fourth cycle of DA-EPOCH-R or two weeks from day 1 of the first cycle of HD-MTX has passed. Early initiation within 2 days is acceptable.

(2) The following recovery is achieved on the day of or the day before HD-MTX therapy.

(2)-1. WBC ≥ 2,000/mm^3

(2)-2. Platelet ≥ 10 x 10^4/mm^3

(2)-3. AST, ALT ≤ upper normal limit X 5

(2)-4. Total bilirubin ≤ 2.0 mg/dl

(2)-5. Serum creatinine ≤ 2.0 mg/dl

(3) CCr ≥ 50 ml/min in the examination within 7 days before day 1 of HD-MTX

(In case of CCr < 80 ml/min, reduce the dose of MTX according to the criteria described in 7.3.6.)

(4) No moderate - massive body cavity fluid

(5) No other toxicities or symptoms that are suitable for the initiation of HD-MTX.

7.3.5 Criteria for restarting or postponing HD-MTX therapy

1) If the WBC count is < 2,000/mm^3 on day 1 or the previous day of a cycle of HD-MTX, postpone the cycle of HD-MTX and use G-CSF. The cycle will be able to start after the day when WBC ≥ 2,000/mm^3 and ≥ 2 days after the last day of G-CSF.

2) If a patient does not fulfill the criteria for the initiation of HD-MTX because of body cavity fluid or > 14

days after the day of scheduled second cycle, HD-MTX will be terminated and will move to DA-EPOCH-R therapy.

7.3.6 Criteria for dose reduction and termination of HD-MTX

For patients with CCr < 80 mL/min before the first cycle of HD-MTX or those who experienced the following toxicities during the first cycle of HD-MTX, the dose of MTX in the second cycle of HD-MTX should be reduced according to the following criteria.

<u>If more than one toxicities are observed, use the strictest criteria.</u>

1) Dose reduction due to hematologic toxicity

Grade 3 or 4 thrombocytopenia (platelet count < 5 x 10^4 /mm^3)

- Reduce the dose of MTX to 80% in the second cycle.

2) Dose reduction or termination due to nonhematologic toxicity

(1) Dose reduction due to infection

For patients who experience at least one of the following toxicities during the first cycle of HD-MTX, reduce the dose of MTX to 60% in the second cycle.

① febrile neutropenia, ② Grade 3 adverse events in the category of "infections and infestations" in CTCAEver4.0

(2) Dose reduction due to hepatotoxicity

For patients who experience Grade 3 hepatotoxicity related to MTX during the first cycle of HD-MTX, reduce the dose of MTX to 60% in the second cycle.

(3) Dose reduction due to nephrotoxicity

The dose of MTX is determined according to CCr examined within 1 week before day 1 of both the first and the second cycles of HD-MTX. CCr (ml/min) is calculated using Cockcroft-Gault's formula*.

CCr ≥ 80: 100%
60 ≤ CCr < 80: 80%
50 ≤ CCr < 60: 60%
CCr < 50: 0%

*Cockcroft-Gault's formula

Male: Ccr = {(140-age) x BW (kg)}/{72 x serum creatinine (mg/dl)}

Female: Ccr = 0.85 x {(140-age) x BW (kg)}/{72 x serum creatinine (mg/dl)}

(4) Termination due to renal insufficiency, persistently elevated serum concentrations of MTX, and MTX-related mucositis

In case of at least one of the following conditions, terminate HD-MTX therapy and initiate DA-EPOCH-R as soon as possible after fulfilling the criteria for the initiation of DA-EPOCH-R.

・Renal insufficiency that does not satisfy the criteria for the initiation of HD-MTX (serum Cre ≥ 2.0

mg/dl or CCr < 50 mL/min) and that persists > 14 days after preplanned day 1 of the next cycle

・Prolonged elevated serum concentration of MTX or requirement of an additional or elevated dose of folinate calcium in the first cycle of HD-MTX that an attending doctor considers the termination of HD-MTX.

・Mucosal damage such as bleeding colitis, GI ulcer, and severe stomatitis in the first cycle of HD-MTX

7.4 Concomitant treatment and supportive therapy

7.4.1 Recommended concomitant treatment and supportive therapy

1) For anemia with hemoglobin less than 8.0 g/dl, transfuse RBCs and keep the hemoglobin level at 8.0 g/dl or higher.
2) Transfuse platelets to keep the platelet count more than 1 x $10^4/mm^3$.
3) For patients with a history of upper gastrointestinal ulcers, use a histamine H2-blocker or a proton pump inhibitor if needed.
4) For nausea and/or vomiting, use antiemetic agents according to the Japan Society of Clinical Oncology clinical practice guidelines for antiemesis in oncology. cf. In the guidelines, EPOCH chemotherapy is regarded as a regimen with a high emetic risk, and MTX is regarded as a drug of moderate emetic risk.
5) For neutropenic fever, obtain clinical sample(s) for bacterial examination, and then use adequate antibiotics as soon as possible according to the guidelines.
6) To prevent pneumocystis pneumonitis due to lymphocytopenia induced by RTX, the prophylactic use of sulfamethoxazole-trimethoprim is recommended. Do not use sulfamethoxazole-trimethoprim on days 1-5 of HD-MTX.
7) To prevent herpes zoster, the prophylactic use of acyclovir (200 mg/day) is recommended from the fourth cycle of DA-EPOCH-R to at least 6 months after the completion of the protocol treatment.
8) For a fever that is resistant to antibiotics, use antifungal agents and take into consideration the interstitial pneumonitis or cytomegalovirus infection.
9) For HBV carriers, start the use of entecavir approximately 1 week before the initiation of the protocol treatment.
10) For severe oral ulcers, diarrhea or bloody stools, consider the use of leucovorin. Patients with severe mucositis are recommended to gargle with 100 mL of water supplemented with leucovorin 15 mg, and then drink it several times a day.
11) Patients who are HBs antigen negative and HBc antibody positive and/or HBs antibody positive should be managed according to the JSH Guidelines for the Management of Hepatitis B Virus infection. (Omission)

7.4.2 Acceptable concomitant treatment and supportive therapy

For adverse drug reactions due to RTX, the following supportive therapy may be considered.

・Hydrocortisone for severe allergic reactions (e.g., bronchospasm)

7.4.3 Unacceptable concomitant treatment and supportive therapy

The following treatments are prohibited in this protocol:

1. The use of any antineoplastic agents not defined in the protocol (including interferons).
2. Any radiotherapy exceeding the field or dose permitted in this protocol.

7.5 Additional treatment

After the completion of the protocol treatment, patients in CR will be followed without any posttreatment until relapse. There is no limitation on poststudy treatments for nonresponders or withdrawals due to adverse events.

8. Expected adverse reactions

9. CRITERIA FOR EVALUATION, LABORATORY TESTS, AND THE EVALUATION SCHEDULE

9.1 Check list before registration

9.1.1 Initial work-up before registration

1) Histopathologic examination of biopsy specimen
   (1) Pathologic diagnosis by the WHO Classification
   (2) Flow-cytometric or immunohistochemical confirmations of the expression of CD5 and CD20.
2) Clinical staging
   (1) Present history and physical examinations
   Presence/absence of B symptoms, past history, PS (performance status), blood pressure, sites of involvement (sites and number of lymph nodes and/or extranodal involvement), size of target lesions (bi-dimensionally), site and size of the largest mass
   (2) Chest X-ray examination
   (3) CT of the brain, neck, chest, abdomen, and pelvic region.
   (4) Bone marrow biopsy or aspiration.
   (5) Complete and differential blood counts including hemoglobin, WBCs, neutrophils (ANC, see 4.3.1), lymphocytes and platelets.
   (6) GI endoscopy.
   Upper GI endoscopy. For patients with a suspicion of lower GI tract involvement, lower GI endoscopy is performed. Biopsy is necessary for confirming lymphomatous involvement.
   (7) PET or PET/CT
   PET or PET/CT alone is not sufficient for upstaging. Physical examination, CT, MRI, echo, endoscopy, biopsy, and other examinations are needed for upstaging.
   * Pretreatment PET is strongly recommended, but not mandatory.
   (8) Bone X-ray, Bone scintigraphy

For patients with a suspicion of bone involvement (e.g., bone pain), a bone X-ray should be performed. For patients whose bone involvement is not confirmed by bone X-ray, either bone scintigraphy or MRI should be considered.

(9) CNS examinations: For patients with focal symptoms such as headache, nausea, paralysis or sensory disturbance, or those with psychiatry symptoms, neck stiffness, or neurologic abnormalities, suspect CNS involvement and check both CSF and MRI examinations. (When the MRI cannot immediately be performed, a brain CT scan instead is permitted.)

3) Risk factors of the IPI.

(1) Age (2) serum LDH (3) PS (4) clinical stage (5) extranodal sites (4.2.2)

4) **Assessment of organ function**

(1) Blood chemistry:

TP, Alb, Total bilirubin, AST/GOT, ALT/GPT, LDH, ALP, Na (sodium), K, Ca, BUN, Cre, and blood sugar

(2) Serum examination:

CRP (C-reactive protein), IgG, IgA, IgM

(3) Urine examination:

Sugar, proteinuria (qualitative), and occult bleeding

(4) ECG (electro cardiogram)

(5) Echo cardiogram and ejection fraction

(6) Oxygen saturation (SpO2)

5) **Viral antigen/antibody**

(1) HBs antigen, HBs antibody, HBc antibody, HBe antibody

Patients who are positive for at least one of the following: HBs antibody, HBc antibody, and HBe antibody, HBs antigen, HBe antigen, HBs antibody, HBc antibody, and HBe antibody should be checked during the evaluation of safety after the initiation of the protocol treatment.

(2) HCV antigen

(3) HIV antigen (after informed consent). This test can be omitted if the patient does not agree to be checked.

(4) HTLV-I antigen (mandatory in Japan)

Complete and differential blood count, blood chemistry, serological tests, and arterial blood gas (or oxygen saturation), and electrocardiograms should be examined within 14 days before registration (excluding the day of registration). Echocardiography should be examined within 12 weeks before registration, and the other work-up should be examined within 28 days.

9.1.2 Biopsy for diagnosis

9.2 Laboratory tests and clinical evaluations during the protocol treatment

9.2.1 Laboratory parameters to be monitored twice a week during DA-EPOCH-R

Blood cell count: ANC, platelet count

9.2.2 Laboratory parameters to be monitored at least once a week during DA-EPOCH-R

Blood cell count: hemoglobin, leukocyte count, lymphocyte count

Blood chemistry: serum albumin, total bilirubin, AST/GOT, ALT/GPT, creatinine, Na (sodium), K, Ca, blood sugar

Serum examination: CRP

9.2.3 Safety evaluation to be monitored at least once before and within every cycle (CTCAEver4.0)

Blood and lymphatic system disorders：Anemia, Febrile neutropenia

Cardiac disorders：Acute coronary syndrome, Atrial fibrillation, Conduction disorders, Heart failure、Myocardial infarction, Supraventricular tachycardia, Ventricular arrhythmia, Ventricular tachycardia

Gastrointestinal disorders：Constipation, Diarrhea, Duodenal ulcer, Gastric ulcer, Ileus, Mucositis oral, Nausea, Vomiting

General disorders and administration site conditions：Edema (with parts), Fatigue, Fever, Injection related reaction

Immune system disorders：Allergic reaction

Infections and infestations：G3-4 Infections and infections-other, specify

Investigations：AST increased, ALT increased, Alkaline phosphatase increased, Blood bilirubin increased, Creatinine increased, Lymphocyte count decreased, Neutrophil count decreased, Platelet count decreased, Weight loss, White blood cell decreased

Metabolism and nutrition disorders：Anorexia, Dehydration, Hypercalcemia, Hyperglycemia, Hypokalemia, Hyponatremia, Tumor lysis syndrome

Neoplasm benign, malignant and unspecified：Treatment related secondary malignancy

Nervous system disorders：Peripheral motor neuropathy, Peripheral sensory neuropathy

Renal and urinary disorders：Hematuria

Respiratory, thoracic and mediastinal disorders：Dyspnea, Hypoxia, Pneumonitis, Pulmonary fibrosis

Skin and subcutaneous tissue disorders：Alopecia

Vascular disorders：Phlebitis

9.2.4 Evaluation of efficacy and safety after the fourth cycle of DA-EPOCH-R

(1) Blood chemistry:

TP, Alb, total bilirubin, AST/GOT, ALT/GPT, LDH, ALP, Na (sodium), K, Ca, BUN, creatinine, and blood sugar

(2) Serum examination:

CRP, IgG, IgA, IgM

(3) Urine examination:

Sugar, proteinuria (qualitative), and occult bleeding

(4) ECG (electrocardiogram)

(5) Oxygen saturation (SpO2)

(6) Chest X-ray examination

(7) CT of the brain, neck, chest, abdomen, and pelvic regions

9.3 Laboratory test and clinical evaluation after the treatment completion

9.3.1 Evaluation of efficacy at the treatment completion/withdrawal (restaging)

The following examinations should be performed as soon as possible for cases with premature termination, and from the sixth to the eighth week (from day 36 to 56) of Day 1 of the eighth cycle of DA-EPOCH-R therapy for those with protocol completion. The efficacy of treatment will be evaluated according to the criteria described in the section “12.1 Definition of response”. This procedure is called as “restaging)”.

(1) CT scan of the brain, neck, chest, abdomen, and pelvic regions

(2) Examination for the sites of involvement before treatment (bone marrow examination, GI survey, bone X-ray, etc.)

(3) PET or PET/CT

9.3.2 Safety evaluation after the completion of the protocol treatment

Within 2 years after chemotherapy, the existence and severity of adverse reactions should be monitored and recorded at least once two months.

9.4 Study calendar

Cycle (DA-EPOCH) (MTX)		1			2			3			4			1		2		5			6			7			8				
Week	pre	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28		32
＜Therapy＞																															
Rituximab		○			○			○			○							○			○			○			○				
EPOCH		○			○			○			○							○			○			○			○				
MTX														○		○															
＜General status＞																															
Body weight	○				○			○			○			○		○		○			○			○			○				
PS*	○	△	△	△	○	△	△	○	△	△	○	△	△	○	△	○	△	○	△	△	○	△	△	○	△	△	○	△	△		○
Physical examination	○	△	△	△	○	△	△	○	△	△	○	△	△	○	△	○	△	○	△	△	○	△	△	○	△	△	○	△	△		○
Fever	○	△	△	△	○	△	△	○	△	△	○	△	△	○	△	○	△	○	△	△	○	△	△	○	△	△	○	△	△		○
Toxicity*		△	△	△	○	△	△	○	△	△	○	△	△	○	△	○	△	○	△	△	○	△	△	○	△	△	○	△	△		○
＜Laboratory tests＞																															
Cell count(ANC, platelet count)	○	◎	◎	◎	◎	◎	◎	◎	◎	◎	◎	◎	◎	○	○	○	○	◎	◎	◎	◎	◎	◎	◎	◎	◎	◎	◎	◎		○
Cell count (Hb、leukocyte count、lmphocyte count)	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○		○
Blood chemistory・CRP	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○		○
IgG, IgA, IgM	○												○																		○
HBV**	○												○																		○
Viral test (except HBV)	○																														
Urine	○												○																		○
24 hours CCr	○												○																		
SpO2	○												○																		
＜others＞																															
Chest X-ray	○												○																		○
Electro cardiogram (ECG)	○												○																		○
Echo cardiogram (ejection fraction)	○																														○
Whole body CT	○												○																		○
Bone marrow・others#	○																														○
PET, PET/CT##	○																														○

***** To be monitored at least once before and within every cycle.

****** For patients who are positive for at least one of the following: HBs antibody, HBc antibody, and HBe antibody in the baseline examination, HBs antigen, HBe antigen, HBs antibody, HBc antibody, and HBe antibody should be examined.

If lymphomatous involvement is detected by bone marrow examination or GI endoscopy in staging, the same examinations are needed.

Not mandatory at registration.

10. Data collection

11. Adverse event reporting

12. Response evaluation and endpoint definition

12.1 Methods for response evaluation

The response evaluation of tumor shrinkage for patients who have measurable lesions was determined according to the report by the Revised International Working Group Criteria[18, 19] (JCOG version).

12.1.1 Methods for response assessment

(1)Baseline evaluation (refer to 12.1.4.) is used as a comparative in response assessment criteria and is based on the clinical evaluation and examination prior to registration. However, data collected after registration and prior to the initiation of treatment is used as the baseline measure if the results are different from the data collected prior to registration.

(2)Based on "re-staging after treatment (refer to 9.3.1. Assessment of efficacy at the time of discontinuation or completion of the treatment (re-staging))", overall response in patients who completed a treatment course (refer to 12.1.6. Response assessment criteria) is determined 6-8 weeks (days 36-56) after the initiation of the last course of DA-EPOCH-R. Overall response is evaluated based on 1) the target lesion, non-target nodal lesion, and non-target extranodal lesion, 2) bone marrow infiltration, and 3) presence of a new site of disease identified on positron emission tomography (PET) or positron emission tomography/computed tomography (PET/CT), and is classified as complete response (CR), partial response (PR), stable disease (SD), progressive disease (PD), or not evaluable (NE). Response assessment is performed on the day that the last examination for response assessment is performed, and not on the day that the data is entered into the case report form (CRF).

(3)The size of the target lesion (refer to 12.1.3.) is determined based on CT image by measuring the maximum diameter (long axis) and maximum width perpendicular to the diameter (short axis). The product of the greatest diameters and the sum of the products of the greatest diameters (refer to 12.1.5.) are entered into the clinical records and eCRF.

(4)For patients whose treatment course was discontinued for reasons other than "progression of the primary disease" or "death during the treatment course", re-staging is performed at the time of discontinuation if possible. Re-staging is not required for patients whose treatment course was discontinued due to clinical disease progression that occurred before or after the time of "re-staging after treatment".

(5)Patients are assessed on the following variables during the course of treatment to determine whether the treatment should be continued (except when changes in the treatment course are needed due to the occurrence of adverse events): self-reported and apparent symptoms, abnormal test results, physical examination, and imaging. PD, defined based on the response assessment, does not always lead to discontinuation of the treatment.

(6)If a patient achieved CR at the time of discontinuation or completion of the treatment, the patient is followed without treatment and "follow-up re-staging" is performed according to "9.3.2. Assessment of safety following completion of the treatment".

(7)Overall response is used to calculate CR and response rates, and is determined as follows. Overall response determined at the time of "re-staging after treatment" is used for patients who completed the treatment course. If "re-staging after treatment" is performed in patients whose treatment was discontinued for reasons other than disease progression or death, overall response determined at the time of discontinuation is considered. If response assessment was not performed after discontinuation of treatment, overall response is defined as NE. Overall response for patients whose treatment was discontinued due to disease progression or death from the primary disease is considered PD.

12.1.2 Definition of a lymph node lesion

- Lymphoma is defined as a lesion with cellular infiltration based on the clinical diagnosis at baseline according to the CT cross-sectional image, positive finding on PET (or PET/CT), or cytology and histological diagnosis.
- The original criteria by Cheson et al. define "lymph node mass" as multiple lymph nodes adhered as one mass. However, it can be difficult to determine whether a mass represents one lymphoma or adhesion of multiple lymphomas at baseline. Thus, we will not differentiate mass from a single lesion. If lymphoma comprised of adhesion of multiple lymph nodes exists, its long axis should be measured especially during the pre-treatment assessment by considering the mass as one lesion. If lymphoma comprised of adhesion is divided into multiple nodes following the initiation of treatment, the sum of the products of the greatest diameters is calculated by adding the sum of the products of the greatest diameters for all isolated lymph nodes.

12.1.3 Definition of a measurable lesion

A lesion is considered measurable if it meets all three criteria listed below:

1) The lesion is a nodal lesion or a nodal mass in extranodal organs (extranodal lesion) defined as lymphoma on CT;
2) Its short and long axes are measurable on CT cross section;
3) Its long axis is ≥1.5 cm on CT cross section.

- A lesion is considered unmeasurable if at least one of the criteria is not met.
- CT films are used to measure the diameter of the tumor. Coronal or sagittal images based on 3D-reconstuction are not used for craniocaudal measurements (MRI is not used for measurement).
- For the purpose of staging, lesions that no longer exist due to biopsy or surgery are still considered as the sites of disease. However, such lesions will not be considered as measurable. (Maintain a record on CRF pre-treatment report about lesions that no longer exist due to biopsy.)

12.1.4 Pre-treatment evaluation (baseline evaluation)

1) CT

- Assess the location and size of a lesion based on neck, chest, abdominal, pelvic, and inguinal CT (≤1 cm slice thickness). Contract CT is used in principle; however, non-contrast CT may be performed for patients who have a history of hypersensitivity to contract agents. CT should include the ocular region if a patient had tumor lesions in the eye appendage prior to the initiation of treatment. CT should include the brachial, epitrochlear, or popliteal lymph nodes (see Appendix 5) if any of these nodes are palpable and is diagnosed as lymphoma and if these nodes are considered the target lesion. The same slice thickness is used for all CT images.
- The same procedure is performed at the time of re-staging.

2) PET or PET/CT

- Fludeoxyglucose (FDG) uptake is assessed using PET or PET/CT.
- The following physiological uptake of FDG should be considered to ensure accurate assessment of disease-associated FDG uptake:
  1: Organs with high FDG uptake: brain, kidneys and bladder (due to urinary excretion)
  2: Organs with varying levels of FDG uptake: salivary glands, tonsils, pharyngeal and hilar lymph nodes (generally symmetrical), heart, great vessels, liver, stomach, spleen, large intestine, testis, muscle
  3: Organs in which FDG uptake may occur: thyroid, breast, uterus, ovary, bone marrow, thymus, brown fat
- When bone marrow infiltration is suspected based on FDG uptake in the bone marrow, the diagnosis should not made until either bone marrow aspiration or biopsy is performed to confirm bone marrow infiltration. However, the data on FDG uptake in the bone marrow should be entered in the clinical records to evaluate the role of PET for disease staging and response assessment in the future.
- Majority of the subjects in this trial are expected to show uptake in the lesion either by PET or PET/CT. However, some patients may be negative in pre-treatment PET or PET/CT.
- If pre-treatment PET is not performed, the patient is considered PET positive.

3) Selection of the target lesions

- Among the measurable lesions (both nodal and extranodal), a maximum of 6 lesions are selected as target lesions based on the selection criteria below. If there are more than 6 measurable lesions, only 6 are selected. If there are fewer than 6 measurable lesions, all of the lesions are selected.
- Measurable lesions (both nodal and extranodal) are selected (up to 6 lesions) in the order of the length of the long axis or in the order of the length of the short axis if all lesions have the same long axis lengths. In the CRF, record the lesions from TL1 to TL6 in the cranio-caudal order.
- Measure the lengths of the long and short axes for all target lesions and calculate the product of the two axes (products of the greatest diameters, cm^2). Similarly, the sum of the products of the greatest diameters for all lesions is defined as the “sum of the products of the greatest diameters (refer to

12.1.5.)".

4) Evaluation of the non-target lesions

- Lymphomas that were either unmeasurable or were not selected as target lesions are considered as non-target lesions.
- Non-target lesions are classified into nodal non-target lesion and extranodal non-target lesion. If a lesion is a lymphoma <1.5 cm in the long axis, it is also considered nodal non-target lesion.
- For nodal non-target lesions, the presence and absence of lymphoma is evaluated and recorded based on the lymph node regions (see Appendix 5). Nodal masses in the spleen that were not selected as target lesions are considered nodal non-target lesions.
- Presence of an extranodal target lesion is evaluated and recorded for each extranodal organ. Nodal masses in the liver and kidneys that were not selected as target lesions are considered extranodal non-target lesions.

5) Evaluation of bone marrow infiltration

- Based on bone marrow aspiration or biopsy, bone marrow infiltration is categorized as follows:
  (1) Positive: Malignancy such as apparent infiltration of atypical cells and/or structural abnormalities.
  (2) Negative: Little or no aggregation of lymphocytes
- Even when a small number of B cell clones are detected by flow cytometry (less than 2% of monocytes), a patient is considered negative for bone marrow infiltration if tumor infiltration is not observed histologically.
- Even if FDG uptake is detected in the spine by PET or PET/CT, a patient is considered negative for bone marrow infiltration if the result of bone marrow aspiration or biopsy is negative.

6) Evaluation of gastrointestinal lesions

- If any lesions (e.g. ulcerative or polypoid lesions) that may have infiltration are identified by endoscopy, perform biopsy to examine the presence of lymphoma infiltration. If a lesion was diagnosed as lymphoma, consider it as extranodal non-target lesion.

12.1.5 Response assessment by lesions

In this trial, treatment response is assessed once at the time of "re-staging after treatment".

1) Rate of reduction and increase of the sum of the products of the greatest diameters (SPD) of the target lesion

Rate of reduction and increase of SPD of the target lesion is calculated as follows:

$$\text{Rate of SPD reduction (if reduced)} = \frac{\text{Pre-treatment SPD} - \text{SPD at the time of assessment}}{Pre-treatment\ SPD}\ x\ 100$$

$$\text{Rate of SPD increase (if increased)} = \frac{\text{SPD at the time of assessment} - \text{Pre-treatmetn SPD}}{Pre-treatment\ SPD}\ x\ 100$$

2) Assessment of PET and PET/CT findings

- It is difficult to determine the normal threshold of standard uptake values (SUV) as it is affected by various factors including the physical status, time to imaging, blood glucose level, image processing method, partial volume affect, and setting of the region of interest. Thus, PET and PET/CT images are assessed visually to determine positivity.
- For organs with physiological FDG uptake (refer to 12.1.4.), the presence of lymphoma is not determined solely based on the FDG uptake. When an uptake is suspected of lymphoma, further examinations, such as palpation, CT, MRI, ultrasound, endoscopy, and biopsy, are performed to confirm the presence of lymphoma. Biopsy is performed by endoscopy for all suspected gastrointestinal lesions. If other imaging modalities are not sufficient to confirm the presence or absence of remaining lesions, an additional biopsy is performed to confirm.
- If an uptake is observed in regions other than where physiological uptake is expected, the uptake may be a false-positive caused by various pathological conditions, such as inflammatory disease, non-malignant tumor, and bone marrow hyperplasia, due to chemotherapy or administration of granulocyte-colony stimulating factor (G-CSF). When a false-positive result is suspected, further examinations, such as palpation, CT, MRI, ultrasound, endoscopy, and biopsy, are performed to confirm the presence of lymphoma.
- If a bulky mass was identified in the mediastinum at baseline and CT revealed that the tumor remained following treatment, there is as much as a 40% chance that the result is a false-positive on PET due to thymic hyperplasia, non-specific inflammation of the mediastinal lymph nodes, and pneumonia [18,20,21]. Thus, if a positive finding was noted on PET or PET/CT based on FDG uptake at the time of re-staging after the treatment, the data should be examined carefully by considering the possible false-positive findings to determine whether additional treatments are required. If a patient is to be followed up without treatment, the patient should be monitored carefully by CT to examine any changes to the size of the tumor during the follow-up visits.

Given the criteria listed above, PET and PET/CT findings for response assessment are classified as follows:

1: PET negative; No lesions observed by PET or PET/CT

2: PET positive; Lesions are observed by PET or PET/CT

3) Evaluation of nodal target and non-target lesions

Based on the evaluation, all nodal target and non-target lesions are classified as follows:

1: Nodal target lesion

[If PET positive before treatment]

- Normal: PET negative; negative histological or cytology findings if performed.

[If PET negative before treatment]

- Normal: None of the lymph nodes are ≥1.5 cm in the long axis.

2: Nodal non-target lesion

[If PET positive before treatment]

- Normal: PET negative; negative histological or cytology findings if performed.
- No increase in size: Presence of at least one nodal non-target lesion that has not normalized, and no apparent increase of nodal non-target lesions compared to pre-treatment. Apparent increase in size is roughly defined as more than 50% increase in the long axis; however, measurement is not required.
- Increase in size: Apparent increase of nodal non-target lesions compared to pre-treatment
- Not evaluable: Presence of unmeasurable lymph node regions.

[If PET negative before treatment]

- Normal: All nodal non-target lesions that were ≥1.5 cm in the long axis at baseline have become <1.5 cm in the long axis, and all nodal non-target lesions that were <1.5 cm in the long axis at baseline have become <1.0 cm in the long axis; negative histological or cytology findings if performed.
- No increase in size: Presence of at least one nodal non-target lesion that has not normalized, and no apparent increase of nodal non-target lesions compared to pre-treatment. Apparent increase in size is roughly defined as more than 50% increase in the long axis; however, measurement is not required.
- Increase in size: Apparent increase of nodal non-target lesions compared to pre-treatment
- Not evaluable: Presence of unmeasurable lymph node regions.

4) Evaluation of extranodal target and non-target lesions

1: Extranodal target lesion

[If PET positive before treatment]

- Disappearance of disease: PET-negative; negative histological or cytology findings if performed.

[If PET negative before treatment]

- Disappearance of disease: All extranodal non-target lesions disappeared or extranodal non-target lesions did not exist at the time of baseline evaluation.

2: Extranodal non-target lesion

[If PET positive before treatment]

- Disappearance of disease: PET-negative; negative histological or cytology findings if performed.
- No increase in size: Presence of at least one extranodal non-target lesion, and no apparent increase of extranodal non-target lesions compared to pre-treatment. Apparent increase in size is roughly defined as more than 50% increase in the long axis; however, measurement is not required. If a lesion identified endoscopically has not worsened based on visual

inspection, determination of lymphoma infiltration by biopsy is not required.

- Increase in size: Apparent increase of extranodal non-target lesions compared to pre-treatment.
- Not evaluable: Presence of unmeasurable extranodal non-target lesions.

[If PET negative before treatment]

- Disappearance of disease: All extranodal non-target lesions disappeared or extranodal non-target lesions did not exist at the time of baseline evaluation. A lesion identified endoscopically has been eliminated and no infiltration of lymphoma is identified on biopsy, or polypoid lesions and ulcerative scars identified endoscopically remain while no infiltration of lymphoma is identified on biopsy.
- No increase in size: Presence of at least one extranodal non-target lesion, and no apparent increase of extranodal non-target lesions compared to pre-treatment. Apparent increase in size is roughly defined as more than 50% increase in the long axis; however, measurement is not required. If a lesion identified endoscopically has not worsened based on visual inspection, determination of lymphoma infiltration by biopsy is not required.
- Increase in size: Apparent increase of extranodal non-target lesions compared to pre-treatment.
- Not evaluable: Presence of unmeasurable extranodal non-target lesions.
- Bone marrow infiltration will not be considered as extranodal non-target lesion.
- If a gastrointestinal lesion is present at baseline, perform endoscopy only if other examinations collectively suggest that the patient may have achieved CR.

5) Evaluation of bone marrow infiltration

Evaluation of bone marrow infiltration by bone marrow aspiration or biopsy is performed following initiation of treatment only if a patient is PET-positive at baseline and is expected to achieve CR if the PET finding is negative. Evaluation is based on the same criteria as the baseline assessment (positive and negative). Bone marrow infiltration will also be evaluated if infiltration is suspected; this is based on observations such as development of cytopenia that was not expected according to his/her clinical course, and appearance of atypical cells suspected of lymphoma in the peripheral blood.

6) Evaluation of new sites of disease

Positive finding of a new site of disease is defined if a lesion is identified at a new site that was not previously identified at baseline. New site of disease is not determined solely based on FDG uptake in PET or PET/CT; further examinations, such as palpation, CT, MRI, ultrasound, endoscopy, and biopsy, are performed to confirm the presence of a new lesion.

12.1.6 Assessment criteria for overall response

Overall response is determined at the time of “re-staging after treatment (9.3.1.) based on the

combinations of the following variables as shown in the table below:

(1) Assessment of PET or PET/CT
(2) Assessment of the nodal target lesion
(3) Assessment of the extranodal target lesion
(4) Assessment of the SPD of the target lesion
(5) Assessment of the nodal non-target lesion
(6) Assessment of the extranodal non-target lesion
(7) Assessment of bone marrow infiltration
(8) Assessment of new sites of disease

Overall response assessment criteria

<table>
<tr><td colspan="2" rowspan="2">Overall response</td><td colspan="2">SPD of the target lesion</td><td colspan="2">Non-target lesion</td><td rowspan="2">Bone marrow infiltration</td><td rowspan="2">New sites of disease</td></tr>
<tr><td>Nodal</td><td>Extranodal</td><td>Nodal</td><td>Extranodal</td></tr>
<tr><td>1</td><td>CR</td><td colspan="4">PET positive before treatment, PET negative after treatment (no remaining tumor; CT required)</td><td>Negative</td><td>No</td></tr>
<tr><td rowspan="2">1’</td><td rowspan="2">CR</td><td colspan="4">(If PET negative before treatment)</td><td rowspan="2">Negative</td><td rowspan="2">No</td></tr>
<tr><td>Normal</td><td>Disappeared</td><td>Normal</td><td>Disappeared</td></tr>
<tr><td>2</td><td>PR</td><td colspan="4">PET positive before treatment, PET negative after treatment (no remaining tumor; CT required)</td><td>Positive</td><td>No</td></tr>
<tr><td rowspan="2">2’</td><td rowspan="2">PR</td><td colspan="4">(If PET positive before and after treatment)</td><td rowspan="2">Positive or negative</td><td rowspan="2">No</td></tr>
<tr><td>Normal</td><td>Disappeared</td><td>Normal</td><td>Disappeared</td></tr>
<tr><td colspan="8">PET negative and has not achieved CR before treatment</td></tr>
<tr><td>2’’</td><td>PR</td><td colspan="2">≥50% reduction</td><td>Normal or no increase</td><td>Disappearance or no increase</td><td>Positive or negative</td><td>No</td></tr>
<tr><td>3</td><td>SD</td><td colspan="2"><50% reduction and <50% increase</td><td>Normal or no increase</td><td>Disappearance or no increase</td><td>Positive or negative</td><td>No</td></tr>
<tr><td>4</td><td>PD</td><td colspan="2">≥50% increase in SPD</td><td>Increase</td><td>Increase</td><td>Became positive</td><td>Yes</td></tr>
</table>

- If classified as PD in any of the categories, the patient is considered as having PD.
- If any of the variables cannot be evaluated, the overall response is “not evaluable”.

Note 1: Progression is defined as worsening of the primary disease determined clinically based on medical consult, physical examination, and blood tests, and is distinct from PD used to determine the efficacy in the response assessment. Change of treatment regimen due to worsening of the disease is based on disease progression. If PD is determined to be the same as progression, next treatment is considered.

Note 2: Recurrence is defined as progression after achieving CR. The table shown above will not be used to determine recurrence.

Note 3: PET is performed for all patients in response assessment (re-staging after treatment). PET is not required for re-staging during the follow-up period.

Note 4: CT is required even if PET or PET/CT findings are negative. (Measurement of target lesion is not required.)

Note 5: Bone marrow is evaluated only when a patient is PET-positive at baseline and is expected to achieve CR if PET finding is negative.

12.2 Definition of analysis objectives

Subjected populations for analysis are defined below:

· "All eligible patients" are used for analysis of th endpoint of efficacy in the final analysis.

· "All treated patients" are used for analysis of the endpoints of safety (toxicities and adverse events).

12.2.1 All registered patients

All registered patients are defined as those registered according to the maneuver defined in the section "6.1. Registration guideline", excluding those with duplicated registration or misregistration.

12.2.2 All eligible patients

All eligible patients are defined as those registered, excluding "ineligible patients" determined by analyses by the committee for this study.

12.2.3 Eligible patients by central diagnosis

Eligible patients by central diagnosis are defined as those who are eligible, excluding patients who are diagnosed as ineligible in the central pathology review.

12.2.4 All treated patients

All treated patients are defined as those registered and receiving all or a part of the protocol treatment.

12.3 Definition of endpoints

12.3.1 Two-year progression-free survival rate (2-year PFS)

Progression-free survival is defined as the time from the date of registration to the earliest date of

progression, relapse, or death from any cause.

1) Progression" and "relapse" include both progression that can be confirmed by imaging studies such as CT scan and progression of the disease (clinical progression) that cannot be confirmed by imaging studies. During the follow-up period, an abnormal laboratory test alone (e.g., elevated serum LDH level) is insufficient for the diagnosis of progression or relapse. Progression or relapse should be diagnosed based on imaging studies (e.g., CT scan) or clinically based on disease progression.
2) For patients alive and progression/relapse-free, PFS will be censored on the last day of progression/relapse-free (the latest date among the following: the date of survey for patients in hospital and the date of the latest visit for out-of-hospital patients).
3) When protocol treatment is terminated because of toxicity or patient refusal, the patients are still regarded as progression-free (neither PFS event nor censored).
4) The diagnosis of progression/relapse should be confirmed not only by PET but also by other additional examinations such as CT, MRI, X-ray, or biopsy. When progression is diagnosed by imaging, the date of the event is not the date of "suspicion of progression in the image" but the date of "confirmation of progression". When diagnosed clinically without imaging, the date of the event is the date of clinical diagnosis of progression.
5) In the case that recurrence or new occurrence is diagnosed by pathological diagnosis of a biopsy specimen, the date of the event is the date when diagnosed clinically and the date of biopsy performed when diagnosed by pathological diagnosis of the biopsy specimen.
6) Second malignancy is regarded as neither an event nor censored and as progression-free until progression or death is observed.

The 2-year PFS rate is calculated using the Kaplan-Meier method. The 90% and 95% CIs are calculated using Greenwood's formula.

12.3.2 Complete response (CR) rate

The CR rate is defined as a ratio of patients in CR at the time of protocol completion divided by all eligible patients (cf. "12.2.2 All eligible patients"). The CI is calculated by using a binominal method.

12.3.3 Overall response rate (ORR)

ORR is defined as a ratio of patients with an effect of CR or PR at the time of protocol completion divided by all eligible patients. The CI is calculated by using a binominal method.

12.3.4 Overall survival (OS)

OS is defined as the period from the date of registration to the date of death from any cause. OS is calculated using the data of all eligible patients. In living patients, OS is censored at the date of last follow-up. The date of last follow-up is the day when an attending physician has confirmed the patient's survival by a

medical examination or telephone call. In case of loss to follow-up, the patient is censored on the last day when the patient's survival is confirmed.

12.3.5 Two-year CNS relapse rate

The two-year CNS relapse rate is defined as the ratio of patients who experience CNS progression/relapse within 2 years after registration divided by all eligible patients. The CI is calculated by using a binominal method.

12.3.6 Proportion of severe adverse events (reactions)

In all treated patients, frequencies of the worst grade of the following adverse events (toxicity) are documented according to CTCAEver4.0.

Clinical laboratory test：White blood cell decreased, Neutrophil count decreased, Lymphocyte count decreased, Platelet count decreased, Weight loss, Blood bilirubin increased, AST or ALT increased, Creatinine increased
General disorders and administration site conditions：Fever, Edema face, Edema limbs, Edema trunk
Immune system disorders：Allergic reaction, Anaphylaxis
Skin and subcutaneous tissue disorders：Alopecia
Gastrointestinal disorders：Constipation, Diarrhea, Nausea, Mucositis oral, Vomiting, Ileus
Metabolism and nutrition disorders：Anorexia, Dehydration, Hypoalbuminemia, Hyperglycemia, Hyponatremia, Hyper/Hypocalcemia
Infections and infestations：Infections and infestations-other, specify, Sepsis
Blood and lymphatic system disorders：Anemia, Febrile neutropenia
Nervous system disorders：Peripheral sensory neuropathy
Renal and urinary disorders：Hematuria
Respiratory, thoracic, and mediastinal disorders：Dyspnea, Hypoxia, Pneumonitis
Vascular disorders：Hypotension

Adverse events (toxicity) other than those listed above are reported by EDC only when Grade 3 or more adverse events except for hematologic toxicity are observed. The incidence of these adverse events is not calculated unless the number of specific adverse events is particularly large.

13. Statistical considerations

13.1 Primary analysis and rationale

The primary endpoint of this study is the 2-year PFS. The CR rate of CD5+ DLBCL is comparable to that of CD5- DLBCL, although this conclusion is based on observations before the introduction of RTX[3]. In the RTX era, the prognosis of patients with CD5+ DLBCL who experience relapse within 1 year after diagnosis or are refractory to the first-line therapy is extremely poor[14]. To achieve early disease control of CD5+ DLBCL, 2-year PFS was selected as the primary endpoint of this study.

The main purpose of this study is to evaluate whether the 2-year PFS rate of the protocol treatment (DA-EPOCH-R/HD-MTX) significantly exceeds a threshold 2-year PFS rate of 51% by conventional treatment. The null hypothesis in the primary analysis is that the 2-year PFS rate of DA-EPOCH-R/HD-MTX is the same as that of conventional therapy (e.g., R-CHOP chemotherapy and other standard chemotherapies for aggressive lymphoma).

The 2-y PFS rate is calculated using the Kaplan-Meier method. The 90% and 95% CIs are calculated using Greenwood's formula. If a lower level of the 90% CI of the 2-year PFS rate of DA-EPOCH-R/HD-MTX exceeds the threshold 2-year PFS rate of 51%, the null hypothesis is dismissed with a one-sided significance level of 5%. The primary analysis is performed on all eligible patients.

For reference, 2-year PFS rates in all registered patients and in eligible patients by central diagnosis are also analyzed.

If the 2-year PFS of DA-EPOCH-R/HD-MTX significantly exceeds that of the historical control, DA-EPOCH-R/HD-MTX is regarded as an effective treatment. Moreover, the use of DA-EPOCH-R/HD-MTX in clinical practice will be recommended, and a subsequent clinical study of a new chemotherapeutic regimen of DA-EPOCH-R/HD-MTX combined with a new agent will be conducted. If the 2-year PFS of DA-EPOCH-R/HD-MTX does not exceed that of the historical control, DA-EPOCH-R/HD-MTX will be regarded as an ineffective treatment.

13.2 Sample size, registration period, and follow-up

The number of the patients needed for this study is 45. The registration period is 3 years and 6 months. The follow-up period is 5 years after the registration.

13.3 Analysis of secondary endpoints

13.3.1 Safety

Because the number of patients who have received DA-EPOCH-R/HD-MTX is small, all Grade 3 or higher adverse events and their incidence are recorded and analyzed. It is anticipated that the total incidence of Grade 3 or 4 adverse events due to DA-EPOCH-R/HD-MTX will not considerably exceed that for R-CHOP alone and HD-MTX alone.

13.3.2 Efficacy

Secondary endpoints regarding efficacy are the CR rate, ORR, OS, and 2-year CNS relapse rate. Among

these endpoints, CR rate and ORR will be analyzed after the confirmation of data on the CR rate and ORR of all registered patients. The OS and 2-year CNS relapse rate can only be analyzed in the primary analysis. All eligible patients but also all registered patients for comparison.

13.4 Final analysis

After the end of the follow-up period, data will be confirmed by the final examination, and then the final analysis regarding all endpoints will be carried out. Until the final analysis, analyses of the primary and secondary endpoints regarding efficacy should be performed except in cases stipulated in this protocol or under approval by the data and safety monitoring committee. The results of the final analysis will be discussed in the study group, and the study coordinator will write up the final report. The study will be terminated after approval of the final report by the principal investigator.

14. Ethical considerations

15. Monitoring

16. Special instructions

17. Organization

18. Publication policy

19. References

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20. Appendix

A phase II study of dose-adjusted EPOCH-R/HD-MTX therapy for untreated CD5-positive diffuse large B-cell lymphoma

(A Phase II trial of DA-EPOCH and Rituximab with HD-MTX therapy for newly-diagnosed DLBCL with CD5 expression

Abbreviation: PEARL5 study)

Plan for statistical analysis

Version 2.0

Study representative

Date: 12/25/2017

Mie University Graduate School of Medicine

Department of Hematology and Oncology

Motoko Yamaguchi 山口 素子 (Signature in Japanese)

Person responsible for statistical analysis

Date: 12/25/2017

Osaka University Hospital

Department of Medical Innovation, Data Coordinating Center

Tomomi Yamada 山田 知美 (Signature in Japanese)

Revision history

Version	Date of preparation	Person responsible for preparation	Main change
1.0	November 17, 2017	Tomomi Yamada	Prepared based on v2.3 of the protocol
2.0	December 25, 2017	Tomomi Yamada	Contents of core member meeting were reflected.

Table of contents

1. Purpose

The purpose of this plan for statistical analysis is to present the details of statistical methods to be used in a "phase II trial of DA-EPOCH and rituximab with HD-MTX therapy for newly-diagnosed DLBCL with CD5 expression (PEARL5 study)"(this study) before data fixation.

2. Outline of this study

The outline of this study is shown below.

Purpose	To explore a more effective treatment for newly diagnosed stage II-IV CD5-positive diffuse large B-cell lymphoma (DLBCL) according to the WHO classification, we conduct a phase II study of dose-adjusted (DA)-EPOCH-R (etoposide, prednisolone, vincristine, cyclophosphamide, doxorubicin, rituximab) combined with high dose (HD)- methotrexate (MTX) and evaluate efficacy and safety of this treatment.
Study design	A prospective, multicenter, cooperative, single-arm phase II study
Disease	CD5-positive diffuse large B-cell lymphoma
Selection criteria	(1) Histologically confirmed CD5-positive diffuse large B-cell lymphoma according to the 2008 WHO classification (2) Confirmed CD20-positive and CD5-positive by immunohistochemistry and/or flow cytometry (3) Ann Arbor stage: II, III, or IV (4) Lymphoma cell count in peripheral blood 14 days before registration >= 10,000/mm3 (5) Age: 20 to 75 years old (6) PS (ECOG): 0-3 (7) No clinical symptoms of CNS involvement (8) Measureable lesion present (9) No prior chemotherapy, radiotherapy, and antibody therapy (10) Adequate organ function (11) Written informed consent

Exclusion criteria	(1) History of angle-closure glaucoma (2) Uncontrollable diabetes mellitus in spite of insulin therapy (3) Uncontrollable hypertension (4) Pleural effusion or ascites except for those with little amount (5) Coronary artery disease under treatment; cardiomyopathy, heart failure, or arrhythmia treated with anti-arrhythmic (6) HBs antigen positive (7) HCV antibody positive (8) HIV antibody positive (9) Interstitial pneumonia, pulmonary fibrosis (10) Severe infection (11) Liver cirrhosis (12) Other active malignancies; history of lymphoma, myelodysplastic syndrome, or leukemia (13) Pregnant, possible pregnant, or breastfeeding woman (14) Severe psychosis (15) Under systemic corticosteroid therapy (16) Considered as ineligible by attending physicians for other reasons
Test treatment	DA-EPOCH-R/HD-MTX therapy After performing 4 courses of DA-EPOCH-R therapy at 3-week intervals, 2 courses of HD-MTX therapy will be conducted at 2-week intervals. Subsequently, 4 courses of DA-EPOCH-R therapy will be performed again at 3-week intervals.
Contraindicated drugs and therapies	(1) Administration of anticancer drugs other than those included in the protocol treatment regimen (including interferon) (2) Radiotherapy at an irradiation field/radiation dose that is not permissible in the protocol (3) From Day 1 until Day 5: Drugs that influence the blood concentration of MTX: non-steroidal anti-inflammatory drugs, such as salicylic acid, sulfonamide preparations, tetracycline, chloramphenicol, phenytoin, barbiturate derivatives, sulfamethoxazole/trimethoprim, piperacillin sodium, and proton pump inhibitors
Criteria for the discontinuation of protocol treatment	(1) Patients in whom protocol treatment was evaluated as ineffective (2) Those in whom protocol treatment cannot be continued due to adverse events

	(3) Cases in which patients wish to discontinue protocol treatment because its association with adverse events cannot be ruled out (4) Cases in which patients wish to discontinue protocol treatment because its association with adverse events can be ruled out (5) Death during protocol treatment (6) Others, including exacerbation before the start of treatment after registration
Observation/examination schedule	See Appendix.
Primary endpoint	Two-year progression-free survival rate
Secondary endpoint	Complete response rate, response rate, overall survival, 2-year central-nervous-system recurrence rate, incidence of adverse events
Target number of subjects to be registered	45 subjects
Registration period	Registration period: 3.5 years (July 25, 2012, to November 7, 2015)
Study period	July 25, 2012, to November 7, 2017

Observation/examination schedule

Course (DA-EPOCH) (MTX)		1			2			3			4			1		2		5			6			7			8				
Week	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28		32
<Treatment>																															
Rituximab		○			○			○			○							○			○			○			○				
EPOCH		○			○			○			○							○			○			○			○				
MTX														○		○															
<General condition>																															
Body weight	○				○			○			○			○		○		○			○			○			○				
PS*	○	△	△	△	○	△	△	○	△	△	○	△	△	○	△	○	△	○	△	△	○	△	△	○	△	△	○	△	△		○
Physical findings*	○	△	△	△	○	△	△	○	△	△	○	△	△	○	△	○	△	○	△	△	○	△	△	○	△	△	○	△	△		○
Fever*	○	△	△	△	○	△	△	○	△	△	○	△	△	○	△	○	△	○	△	△	○	△	△	○	△	△	○	△	△		○
Evaluation of adverse events*		△	△	△	○	△	△	○	△	△	○	△	△	○	△	○	△	○	△	△	○	△	△	○	△	△	○	△	△		○
<Clinical examination>																															
Blood count (ANC, platelet count)	○	◎	◎	◎	◎	◎	◎	◎	◎	◎	◎	◎	◎	○	○	○	○	◎	◎	◎	◎	◎	◎	◎	◎	◎	◎	◎	◎		○
Blood count (Hb, leukocyte count, lymphocyte count)	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○		○
Blood biochemistry • CRP	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○		○
IgG, IgA, IgM	○												○																		○
HBV**	○												○																		○
Virus test (other than HBV)	○																														
Urinalysis	○												○																		○
24-hour CCr	○												○																		
SpO2	○												○																		
<Others>																															

Thoracic Xp	○												○																	○
ECG	○												○																	○
Cardiac EF	○																													○
Systemic CT	○												○																	○
Bone marrow • others #	○																													○
PET, PET/CT##	○																													○

***** These parameters should be evaluated at least once immediately before each course and during inter-course periods.

****** In HBs-, HBc-, or HBe-antibody-positive patients on pre-registration examination, the HBs antigen, HBe antigen, HBs antibody, HBc antibody, and HBe antibody levels must be measured.

In patients with lesions on bone marrow biopsy (puncture) or endoscopy for stage determination, the same examination as conducted at the time of stage determination must always be performed.

Execution before registration is not necessary. Patients in whom neither PET nor PET/CT was performed before registration are regarded as PET-positive. However, PET for re-staging after the start of protocol treatment is necessary.

3. Population to be analyzed

3.1 Definition

Of all registered patients, a population will be regarded as all registered subjects, excluding duplicated registrations and mis-registrations. A population to be analyzed in this study is defined below:

1) All eligible subjects

   Those excluding ineligible subjects from all registered subjects are defined as all eligible subjects.

2) Central-pathological-diagnosis-based eligible subjects

   Those excluding ineligible subjects on central pathological diagnosis from all eligible subjects are defined as central-pathological-diagnosis-based eligible subjects.

3) All treated subjects

Of all registered subjects, those in whom a portion of protocol treatment or all contents were completed are defined as all treated subjects.

A population to be analyzed for the efficacy will consist of all eligible subjects. A population to be analyzed for the safety will consist of all treated subjects. However, when evaluating the primary endpoint, all registered subjects and central-pathological-diagnosis-based eligible subjects will also be analyzed for reference.

4. Analytical items and methods

4.1 Details of the subjects

Of all registered subjects, the number of those belonging to each population to be analyzed, number of those who were not adopted as a member of a population to be analyzed, number of those in whom the protocol treatment was completed, and number of those in whom the protocol treatment was discontinued will be presented. Furthermore, a list of subjects who were adopted/excluded as a member of each population to be analyzed will be shown. Concerning excluded subjects, the reasons will be described. Concerning subjects in whom the protocol treatment was discontinued, the reasons will be listed.

4.2 Subject characteristics and outline of test treatment

4.2.1 Subject characteristics

In all eligible and all treated subjects, the main characteristics (age at the time of informed consent signature, sex, medical history, stage, general condition, serum LDH level, extranodal lesions, IPI classification, CNS-IPI classification, and cell-of-origin (COO) classification) will be summarized using descriptive statistics.

Concerning continuous variables, the median and interquartile range will be calculated. Concerning category variables, the frequency and rate will be calculated. On subsequent analyses, descriptive statistics, as described above, will also be used when summarizing the distribution.

4.2.2 Number of prognostic factors and classification of risk groups

In all eligible subjects, risk groups will be specified based on the International Prognostic Index (Table 1). The subjects will be classified into 4 risk groups based on the number of prognostic factors (0 or 1: low risk, 2: low-intermediate risk, 3: high-intermediate risk, 4 or 5: high risk).

Table 1: International Prognostic Index (IPI)

IPI-based prognostic factor	Predictive value for an unfavorable prognosis
Age	≥61 years
Serum LDH	> the upper limit of the institutional reference range
PS	2 to 4
Stage	III or IV
Number of extranodal involvement	≥2

Furthermore, the subjects will be classified based on the CNS-IPI (Table 2).

Table 2: Central Nervous System International Prognostic Index (CNS-IPI)

CNS-IPI-based prognostic factor	Predictive value for an unfavorable prognosis
Age	≥61 years
Serum LDH	> the upper limit of the institutional reference range
PS	2 to 4
Stage	III or IV
Number of extranodal involvement	≥2
Extranodal sites of involvement	Kidney or adrenal involvement is present.

(0 or 1: low risk, 2 or 3: low-intermediate risk, 4 or 6: high risk)

4.2.3 Outline of test treatment

In all eligible and all treated subjects, data on drugs, doses, and administration methods in each course will be collected. Furthermore, the frequency and rate of a ≤1-grade/≥2-grade increase in the dose level will be calculated.

4.3 Primary endpoint

A null hypothesis is established as "the 2-year progression-free survival rate after test treatment is below 51% of the historical control value (threshold 2-year progression-free survival rate)". In all eligible subjects, the 2-year progression-free survival rate will be estimated using the Kaplan-Meier method, and its 90% confidence interval will be calculated using Greenwood's formula. If the lower limit of the 90% confidence interval of the 2-year progression-free survival rate after test treatment exceeds the threshold (51%), the null hypothesis will be rejected, with a one-sided significance level of 5%.

As a reference, the 95% confidence interval of the 2-year progression-free survival rate will be calculated. All registered subjects, as well as central-pathological-diagnosis-based eligible subjects, will also be analyzed.

4.4 Secondary endpoints

Secondary endpoints will be evaluated in all eligible subjects. However, as a reference, the results will be compared with those in all registered subjects. The day of registration will be regarded as a baseline.

1) Complete response rate

The number and rate of patients evaluated as achieving a complete response and exact 95% confidence interval (two-sided) will be calculated.

2) Response rate

The number and rate of patients evaluated as achieving a response and exact 95% confidence interval (two-sided) will be calculated.

3) Overall survival

The overall survival rate will be estimated using the Kaplan-Meier method. The estimated 2- and 5-year survival rates and 90%/95% confidence intervals (two-sided) will be calculated using Greenwood's formula.

4) Two-year central-nervous-system recurrence rate

The number of patients with central-nervous-system recurrence after 2 years will be calculated, and the incidence will be estimated using the Kaplan-Meier method. The estimated 2-year incidence and 90%/95% confidence intervals (two-sided) will be calculated using Greenwood's formula.

5) Incidence of adverse events

The number of grade 3 or higher toxic events, as well as the number and rate of patients with such events, will be calculated.

4.5 Summary of adverse events

A list of adverse events in each subject (event, date of appearance, date of outcome, duration, outcome, severity, causal relationship, management of adverse events) is presented. Furthermore, the number of patients with event appearance, number of episodes, and incidence will be totalized with respect to adverse events and severity. With respect to the severity, 1 highest-grade episode will be adopted. The duration of an adverse event is defined as "date of outcome – date of appearance + 1" (unit: days). Serious adverse events and reactions will be similarly totalized.

4.6 Examination of prognostic factors

Concerning the 2-year progression-free survival rate, overall survival rate, and 2-year central-nervous-system recurrence rate, Kaplan-Meier curves for each category of the IPI (low risk / low-intermediate risk or high-intermediate risk / high risk), CNS-IPI (high risk/ intermediate risk / low risk), and COO (ABC / GCB / Unclassified) will be presented. Log-rank tests will be conducted. A p-value of 0.1 (two-sided) is regarded as significant.

4.7 Unit and number of digits

In tables, the input values of both the unit and number of digits will be expressed. However, in totalization tables, the unit and number of digits will be expressed in accordance with the following regulations. Furthermore, the rate (%) will be expressed to the first decimal place. However, the reference number of digits refers to the number of digits described in a database definition document.

Median, interquartile range, confidence interval: reference number of digits +1 digit

Concerning derivation variables (items requiring calculation), rounding-off during calculation should be avoided, and values should be rounded off to a prescribed number of digits immediately before presenting them in tables and lists.

4.8 Missing values

Missing values will not be complemented.

5 Software to be used

For statistical analysis, SAS version 9.3 or higher software will be used.

6 Storage and management of documents

Documents related to statistical analysis in this study should be stored and managed according to the standard operating procedure established separately.