Dose-adjusted EPOCH-R (etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin, and rituximab) in untreated aggressive diffuse large B-cell lymphoma with *MYC* rearrangement: a prospective, multicentre, single-arm phase 2 study

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Summary

Background ***MYC*** **gene rearrangement is present in approximately 10% of aggressive B-cell lymphomas, with half also harbouring a *BCL2* gene rearrangement. Multiple retrospective studies of R-CHOP (rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone or prednisolone) have shown a worse outcome in patients with *MYC* rearrangement (alone or with rearrangement of *BCL2* or *BCL6*, or both) than in patients without *MYC* rearrangement, and suggest improved outcomes after more intensive treatment. We aimed to determine the outcome of dose-adjusted EPOCH-R (etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin, and rituximab; DA-EPOCH-R), an intensive infusional treatment regimen, in untreated aggressive B-cell lymphoma with *MYC* rearrangement.**

Methods **We present the final analysis of a prospective, multicentre, single-arm, phase 2 study of DA-EPOCH-R in patients with untreated aggressive B-cell lymphoma with *MYC* rearrangement. DA-EPOCH-R was scheduled to be administered with CNS prophylaxis for six cycles. Primary endpoints included event-free and overall survival. This study is registered with ClinicalTrials.gov (NCT01092182).**

Findings **53 patients were enrolled, with median age of 61 years (range 29–80; IQR 50–70); 43 (81%) patients had stage III–IV disease and 26 (49%) had high-intermediate or high international prognostic index (IPI) scores. 19 patients had confirmed *MYC* rearrangement alone (single-hit) and 24 also had rearrangement of *BCL2*, *BCL6*, or both (double-hit), with similar characteristics between these two groups. After a median follow-up of 55·6 months (IQR 50·5–61·1), 48-month event-free survival was 71·0% (95% CI 56·5–81·4) and 48-month overall survival was 76·7% (95% CI 62·6–86·1) for all patients. Toxicity included grade 4 neutropenia in 160 (53%) of 301 cycles, grade 4 thrombocytopenia in 40 (13%) cycles, and any grade of fever with neutropenia in 56 (19%) cycles. There were three treatment-related deaths (all infections).**

Interpretation **In this study, DA-EPOCH-R produced durable remission in patients with *MYC*-rearranged aggressive B-cell lymphomas and should be considered for the treatment of these diseases.**

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Introduction

Diffuse large B-cell lymphomas are molecularly heterogeneous and vary in their cell of origin, oncogenic mutations, and deregulated signalling pathways.[1–3] A relatively frequent molecular event is a *MYC* 8q24 rearrangement, often accompanied by translocations involving *BCL2*, *BCL6*, or both, which is present in about 10% of diffuse large B-cell lymphomas.[4,5] These rearrangements have been associated with poor prognosis in multiple retrospective and observational studies of R-CHOP (rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone or pred-nisolone) chemotherapy in diffuse large B-cell lymphoma.[5–11] In one of the first studies to assess the clinical effect of *MYC* rearrangement, patients with tumours that contained a *MYC* rearrangement, some of which also had a *BCL2* rearrangement, had significantly shorter survival following R-CHOP treatment compared with patients without a translocation.[6] Although the presence of rearrangements involving *BCL2* or *BCL6*, or both, might contribute to the poor prognosis, *MYC* rearrangement alone (referred to as single-hit) is also associated with significantly worsened prognosis.[7,8,12,13]

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Research in context

Evidence before this study

We searched PubMed for clinical reports on *MYC*-rearranged aggressive B-cell lymphoma published in English since 2008, using the terms "*MYC* rearranged aggressive B cell lymphomas", "*MYC* rearranged diffuse large B cell lymphomas", and "double and triple hit lymphomas". All of the articles we found were retrospective analyses or reviews of clinical data, and overall they showed a worse prognosis with standard R-CHOP (rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone or prednisolone) chemotherapy compared with non-*MYC* rearranged tumours. The association of *MYC* rearrangement with worsened prognosis led to the establishment of a new category of high-grade B-cell lymphoma with *MYC* rearrangement and translocations of *BCL2* or *BCL6*, or both, termed high-grade B-cell lymphoma double-hit, in the revised 4th edition of the WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. The worse prognosis of these lymphomas with R-CHOP chemotherapy compared with non-*MYC* lymphomas prompted the use of intensive treatments as outlined in the National Comprehensive Cancer Network guidelines (version 4.2018).

We developed a study of DA-EPOCH-R, a dose-intense immunochemotherapy platform, in patients with previously untreated *MYC*-rearranged aggressive B-cell lymphoma, based on the hypothesis that infusional chemotherapy would overcome the adverse prognosis of these highly proliferative lymphomas.

Added value of this study

To our knowledge, this study reports the first prospective information on the treatment of *MYC*-rearranged aggressive B-cell lymphoma. The study provides evidence that most patients, including those with *MYC* rearrangement alone or with additional rearrangements of *BCL2* or *BCL6*, or both, can achieve durable remissions with DA-EPOCH-R treatment.

Implications of all the available evidence

On the basis of these results and those of retrospective studies, regimens such as DA-EPOCH-R should be considered for the treatment of *MYC*-rearranged aggressive B-cell lymphoma. Comparison of DA-EPOCH-R with another intensive regimen is a relevant future research question.

The recognition of *MYC* rearrangement in diffuse large B-cell lymphoma and its association with worsened prognosis led to the establishment of a new category of high-grade B-cell lymphomas (*MYC* rearrangement plus translocation of *BCL2* or *BCL6*, or both; termed high-grade B-cell lymphoma double-hit), in the revised 4th edition of the WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues.[14] Although double-hit high-grade B-cell lymphoma is now recognised as a specific entity, these tumours are pathologically and clinically heterogeneous. *MYC* rearrangement can be found in tumours with morphological features of diffuse large B-cell lymphoma and high-grade B-cell lymphoma not otherwise specified, which are mostly derived from germinal B cells, as well as plasmablastic lymphoma, a post-germinal centre subtype.[4,14]

The poor prognosis for *MYC*-rearranged aggressive B-cell lymphoma with R-CHOP chemotherapy has prompted the use of intensive treatments, such as Burkitt lymphoma regimens and stem-cell trans-plantation, as outlined in the National Comprehensive Cancer Network (NCCN) guidelines (version 4 [2018]).[5,15–17] Because no prospective studies have been done in untreated *MYC*-rearranged aggressive B-cell lym-phoma, there is an unmet medical need to evaluate dose-intense treatment in patients with this type of high-grade B-cell lymphoma. Dose-adjusted EPOCH-R (etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin, and rituximab; DA-EPOCH-R) is a dose-intense immuno-chemotherapy platform in which the continuous infusion of three of its components (etoposide, vincristine, and doxorubicin) and pharmacodynamic dose adjustments might be particularly important in the treatment of highly proliferative lymphomas, including *MYC*-rearranged aggressive B-cell lymphoma and Burkitt lymphoma.[18,19] We hypothesised that DA-EPOCH-R would overcome the negative prognostic effect of *MYC* rearrangement in aggressive B-cell lymphomas, single-hit or double-hit. We were also interested in the effect of international prognostic index (IPI) and age, because older patients often do not tolerate intensive immunochemotherapy and have a worse outcome compared with younger patients.[5]

See Online for appendix

Methods

Study design and participants

We did a prospective, non-randomised phase 2 study in 13 centres in the USA of DA-EPOCH-R in patients with untreated aggressive B-cell lymphoma with de-novo *MYC* rearrangement (including high-grade B-cell lymphoma double-hit, high-grade B-cell lymphoma, high-grade B-cell lymphoma not otherwise specified, diffuse large B-cell lymphoma, and plasmablastic lymphoma) to obtain an estimate of its efficacy. A list of participating sites is provided in the appendix (p 3). Patients were enrolled on a protocol of DA-EPOCH-R that included two separate cohorts: one for *MYC*-rearranged aggressive B-cell lymphoma, and one for Burkitt lymphoma. The two cohorts had independent accrual goals, study objectives, and differences in treatment algorithms, and were combined into a single protocol on the basis of the use of DA-EPOCH-R and overlapping study centres. We planned to report outcomes from the two cohorts separately; results from the Burkitt lymphoma cohort will be reported elsewhere.

To be eligible for participation in the study, all patients had to have a confirmed histological diagnosis of a

MYC-rearranged aggressive B-cell lymphoma. We present participants' histology according to the revised 4th edition of the WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues..[14,20] The histology was confirmed at each participating institution with central review of all histological reports by the National Cancer Institute. Additional eligibility criteria were age 18 years or older, no previous systemic chemotherapy, adequate organ function (serum creatinine <1·5 mg/dL or creatinine clearance >50 mL/min per 1·73 m², total bilirubin <2 mg/dL, absolute neutrophil count >1000 per µL, and platelets >75 000 per µL) unless related to disease, a negative pregnancy test in women of childbearing potential, and a commitment to use contraception. Patients at all disease stages, with any performance status, and with CNS leptomeningeal involvement or HIV infection, were eligible. Pretreatment evaluations were complete blood count and differential, prothrombin time, partial thrombo-plastin time, aspartate transaminase, alanine transaminase, lactate dehydrogenase, alkaline phosphatase, bilirubin, albumin, calcium, phosphate, uric acid, creatinine, electrolytes, glucose, and urinalysis. Viral tests were HIV virus antibody, hepatitis C virus antibody, hepatitis B surface antigen, and serum Epstein-Barr virus load. Serum human chorionic gonadotropin test was required for childbearing women. Additionally, patients received whole-body CT scans, bone marrow aspirate and biopsy, cerebrospinal fluid analysis by flow cytometry and cytology, and (as clinically indicated) brain MRI or CT.[21] Tumour response was assessed according to the revised response criteria for malignant lymphoma.[22] All patients received an interim ^{18}F-fluorodeoxyglucose (^{18}F-FDG) PET scan after two cycles of treatment as a research endpoint. The ^{18}F-FDG PET scans were interpreted by each institution and were not used for medical decisions. The study was approved by the investigational review board of each institution and all patients provided written informed consent.

Procedures

DA-EPOCH-R (starting doses: 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 1–5]; cyclophosphamide 750 mg/m² intravenously [day 5]; prednisone 60 mg/m² twice a day orally [days 1–5]; rituximab 375 mg/m² intravenously [day 1]; and filgrastim 5 mg/kg per day subcutaneously [day 6 until absolute neutrophils >5000 per µL past the nadir]) was administered for six cycles, as previously described, and in the outpatient setting when feasible.[23] We recommended that patients with HIV did not receive antiretroviral therapy during chemotherapy. DA-EPOCH-R was pharmacodynamically dose adjusted on the basis of the neutrophil nadir, which was checked twice weekly. Patients received filgrastim beginning 24 h after the last dose of chemotherapy and continued through the neutrophil nadir until absolute neutrophil recovery, defined as 5000 per µL or higher. Use of pegylated filgrastim was allowed. Bactrim DS (sulfamethoxazole 400 mg and trimethoprim 160 mg) was administered orally twice daily for 3 days per week throughout the trial. Cycles were begun every 21 days providing the absolute neutrophil count was 1000 per µL or greater and platelets were 100 000 per µL or greater. If platelet or neutrophil counts were below these limits, counts were checked daily until recovery and G-CSF (filgrastim) was administered as indicated. Patients without evidence of leptomeningeal involvement received prophylactic intra-thecal methotrexate 12 mg on days 1 and 5 of cycles three to six of DA-EPOCH-R for a total of eight doses. Patients with leptomeningeal involvement received active treatment with methotrexate 12 mg intrathecally or 6 mg via an Ommaya reservoir twice weekly for 4 weeks, then weekly for 6 weeks, and then monthly for 4 months. This study used commercial chemotherapy drugs, which were provided by each institution, and the supplier of the drugs varied by institution on the basis of pharmacy purchasing practices. Filgrastim was provided by Genentech (South San Francisco, CA, USA).

Patients underwent staging of involved sites by CT scan after cycles two and six, every 4 months for 2 years, and then yearly for 3 years until disease progression. ^{18}F-FDG PET scans were done after cycles two and six (if positive after cycle two) and as clinically indicated thereafter. Radiological scans were reviewed at each participating site and were not centrally reviewed.

All patients had confirmation of *MYC* rearrangement by fluorescence in-situ hybridisation (FISH) using (8q24) breakapart probes or conventional cytogenetics, which was done at the participating sites. Rearrangement of *BCL2* was assayed by FISH with probes from the regions *IGH* (14q32) and *BCL2* (18q21), and *BCL6* rearrangement was assayed using (3q27) breakapart probes or cytogenetics, but the results of these did not affect patients' eligibility for inclusion in the study.

Outcomes

The primary study outcomes were progression-free survival (same as event-free survival), event-free survival, and overall survival with analysis in single-hit and double-hit or triple-hit patients (recognised as part of the double-hit category so will be termed double-hit throughout). Event-free survival was determined from the date of enrolment in the study until the date of progression, last documentation of disease at or after the last treatment cycle, death, or last follow-up (whichever occurred first), and overall survival was calculated from the enrolment date until date of death or last follow-up; we used the Kaplan-Meier method for both analyses. Secondary outcomes were assessment of outcomes at interim (after cycle two) ^{18}F-FDG PET-CT scans and toxicity of DA-EPOCH-R. Exploratory analyses for differences in event-free survival and overall survival were assessed according to mutation (single-hit *vs* double-hit or triple-hit lymphomas; termed double-hit), age (<60 years *vs* ≥60 years), IPI, and histology (high-grade B-cell lymphoma

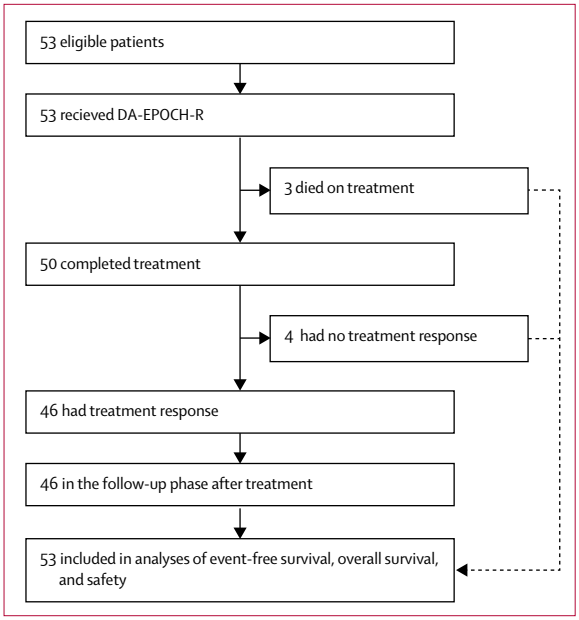

Figure 1: **Trial profile**
The analysis of survival outcome includes all patients enrolled. The analysis of treatment response excludes three patients who died before restaging. DA-EPOCH-R=dose-adjusted etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin, and rituximab.

double-hit or not otherwise specified *vs* diffuse large B-cell lymphoma).

Statistical analysis

Because of the absence of comparator prospective trials, the study endpoint was to estimate event-free and overall survival of DA-EPOCH-R in patients with *MYC*-rearrangement aggressive B-cell lymphoma for a total of 53 patients (prospectively approved for 47 patients). All patients were included for analysis of outcomes and toxicity. The statistical significance of the difference between a pair of Kaplan-Meier curves was determined by an exact log-rank test. All p values are two-tailed and presented without adjustment for multiple comparisons. For baseline characteristics, we used Fisher's exact test for all comparisons, except we used the Cochran-Armitage trend test for comparison of IPI. We did statistical analyses with SAS (version 9.3). The study is registered with ClinicalTrials.gov, number NCT01092182.

Role of the funding source

The Cancer Therapy Evaluation Program provided input into the study design and approved the protocol as the study sponsor. Data management was provided by the Cancer Therapy Support Unit data operations of the Cancer Therapy Evaluation Program. All sites submitted data and responded to queries using the Medidata Rave Clinical Data Management System. The funders were not involved in interpretation of the data or writing of the report. The funders were provided with a copy of the manuscript before submission, but their approval for submission was not required. The corresponding author had full access to all the data and had final responsibility for the decision to submit for publication.

Results

53 patients with *MYC*-rearranged aggressive B-cell lymphoma were enrolled between March 25, 2010, and Feb 20, 2014, and the data were locked on Nov 15, 2017 (figure 1). The 53 participants had a median age of 61 years (range 29–80), 40 (75%) were male, and 43 (81%) had stage III or IV disease; 31 (61%) of the 51 patients with data available had raised lactate dehydrogenase (LDH; table 1). Three (7%) of 44 patients had cerebrospinal fluid involvement at diagnosis and five (9%) of 53 patients were HIV-positive. 26 (49%) patients had high-intermediate or high-risk disease according to their IPI score. Histology results from the 53 patients showed high-grade B-cell lymphoma double-hit or triple-hit in 24 (45%), high-grade B-cell lymphoma not otherwise specified in ten (19%), and diffuse large B-cell lymphoma in 18 (34%) patients.[14] All patients had *MYC* re-arrangement, which included a *BCL2* rearrangement in 22 (42%) and *BCL6* rearrangement in five (16%) of 52 patients with *BCL2 or BCL6* tested, and rearrangement of both *BCL2* and *BLC6* in three (9%) of 32 patients; due to paucity of tissue available, *BCL2* FISH was not done in one patient and *BCL6* FISH was not done in 21 patients. Single-hit lymphoma was confirmed in 19 patients and double-hit lymphoma (including the three patients with re-arrangement of both *BCL2* and *BLC6*) was confirmed in 24 patients. There were no significant differences in clinical characteristics between patients that had single-hit versus double-hit lymphoma, with the exception of worse performance status among those with single-hit disease (p=0·0013; table 1).

In the whole cohort of 53 patients, 39 achieved a complete response and seven achieved a partial response, with the overall proportion of patients achieving a response being 87% (46 of 53 patients). Four patients did not respond to study treatment and three patients could not be evaluated because of infection-related deaths during the study before staging could be done. Three patients had leptomeningeal disease at diagnosis, all of whom died: one from progressive disease, and two from treatment-related infections. Four patients with complete response and one patient with partial response (but without documentation of active disease) underwent autologous bone marrow transplantation (four patients) or allogeneic bone marrow transplantation (one patient) after therapy. Two patients who had complete response received consolidation radiotherapy following DA-EPOCH-R.

The study median potential follow-up was 55·6 months (IQR 50·5–61·1). For all 53 patients, 48-month event-free survival was 71·0% (95% CI 56·5–81·4) and 48-month overall survival was 76·7% (95% CI 62·6–86·1; figure 2). In 32 patients with confirmed single-hit or double-hit lymphoma (table 1), event-free survival at 48 months was

62·7% (95% CI 37·2–80·2) for single-hit versus 73·4% (50·1–87·1) for double-hit (p=0·40), and overall survival was 63·2% (95% CI 38·0–80·4) versus 82·0% (58·8–92·8), respectively (p=0·12), which was not significantly different (figure 2).

We did exploratory analyses of clinical and histological variables. Analysis of 24 double-hit patients by IPI score showed event-free survival at 48 months of 91·7% (95% CI 53·9–98·8) for those with low or low-intermediate IPI (score 0–2) versus 54·5% (22·9–78) for those with high-intermediate or high IPI (score 3–5; p=0·049 for low and low-intermediate *vs* high and high-intermediate), and overall survival at 48 months of 90·9% (95% CI 50·8–98·7) versus 72·2% (37·1–90·3), respectively (p=0·25), indicating that high-risk patients can achieve durable remissions (appendix p 1). When all patients were included in the analysis of outcome by IPI, the results were similar (data not shown). We also analysed the outcome of patients with double-hit lymphoma by age (<60 *vs* ≥60 years) and found no difference, with event-free survival at 48 months of 71·6% (95% CI 35·0–89·9) for those younger than 60 years and 75·0% (40·8–91·2) for those aged 60 years or older (p=0·85), and overall survival at 48 months of 70·7% (95% CI 33·7–89·5) and 91·7% (53·9–98·9), respectively (p=0·18; appendix p 1). When all patients with *MYC* rearrangement were considered, we noted no significant differences by age in event-free or overall survival (data not shown). We also assessed whether histology was associated with outcome. Event-free survival for high-grade B-cell lymphoma double-hit or not otherwise specified at 48 months was 70·8% (95% CI 51·3–83·6), compared with 69·6% (44·5–85·1) for diffuse large B-cell lymphoma (p=0·95); overall survival at 48 months was 77% (95% CI 57·7–88·3) and 75% (50·0–88·7), respectively, with no differences in outcome (appendix p 1) among the 52 patients with these diagnoses.

We did a preplanned analysis to assess whether an interim ^{18}F-FDG PET scan (after cycle two) could identify patients at increased risk of treatment failure with DxCH-R and be used to direct adaptive treatment strategies. In patients with negative (n=17; Deauville 1–3) and positive (n=31; Deauville 4–5) scans, the 48-month event-free survival was 87·4% (95% CI 58·1–96·7) and 64·5% (45·2–78·5; p=0·057) and the overall survival was 87·5% (95% CI 58·6–96·7) and 74·2% (55·0–86·2; p=0·23), respectively (figure 2E, F).

Toxicity was assessed in all 53 patients and for all 301 cycles (table 2). The median (range) dose level administered was 1 (–5 to 6), and the maximum dose-level was 1 in 19 (36%) patients, 2 in 14 (26%) patients, 3 in 14 (26%) patients, 4 in three (6%) patients, 5 in two (4%) patients, and 6 in one (2%) patient. 45 patients received all six cycles of treatment, and three patients received only five cycles of treatment due to concerns about tolerance. Five patients did not complete treatment: due to death during the study in three patients, progressive disease in one patient, and study withdrawal in one patient. Grade 4 neutropenia and thrombocytopenia occurred during 160 (53%) and 40 (13%) cycles, respectively, and fever and neutropenia of any grade occurred in 56 (19%) cycles. Motor neurotoxicity occurred in seven patients (three grade 2, four grade 3), and grade 2 or 3 sensory neurotoxicity occurred in 11 and four patients, respectively. There were three treatment-related deaths, all due to infections: one patient aged 60 years with an IPI score of 3, due to respiratory failure and septic shock; one patient aged 66 years with an IPI

	All *MYC* rearrangement (n=53)	*MYC* rearrangement only (n=19)	*MYC* rearrangement plus rearrangement of *BCL2*, *BCL6*, or both (n=24)	p value*
Total patients	53 (100%)	19/32 (59%)†	24/52 (46%)‡	..
Age (range; IQR)	61 (29–80; 50–70)	63 (36–80; 44–72)	62 (35–76; 54–69)	..
Sex				0·10
Men	40/53 (75%)	16/19 (84%)	14/24 (58%)	..
Women	13/53 (25%)	3/19 (16%)	10/24 (42%)	..
Stage III or IV	43/53 (81%)	14/19 (74%)	20/24 (83%)	0·48
Raised lactate dehydrogenase	31/51 (61%)	11/19 (58%)	14/24 (58%)	1·00
Eastern Cooperative Oncology Group performance status 2–4	11/53 (21%)	13/19 (68%)	4/24 (17%)	0·0013
Two or more extranodal sites	12/53 (23%)	5/19 (26%)	6/24 (25%)	1·00
Bone marrow positive	9/53 (17%)	3/19 (16%)	5/24 (21%)	1·00
Cerebrospinal fluid positive	3/44 (7%)	2/17 (12%)	1/21 (5%)	0·58
International Prognostic Index score				0·76
0–1	12/53 (23%)	4/19 (21%)	6/24 (25%)	..
2	15/53 (28%)	5/19 (26%)	7/24 (29%)	..
3	17/53 (32%)	7/19 (37%)	8/24 (33%)	..
4–5	9/53 (17%)	3/19 (16%)	3/24 (13%)	..
HIV positive	5/53 (9%)	3/19 (16%)	0	0·08
Histology				
HGBL DH	24/53 (45%)	0	24/24 (100%)	..
HGBL NOS	10/53 (19%)	6/19 (32%)	0	..
DLBCL	18/53 (34%)	13/19 (68%)	0	..
Plasmablastic	1/53 (2%)	0	0	..
Translocations				
MYC	53/53 (100%)	19/19 (100%)	24/24 (100%)	..
BCL2	22/52 (42%)	0	22/24 (92%)	..
BCL6	5/32 (16%)	0	5/24 (21%)	..

Data are n/N (%) or median (range; IQR) for 53 patients receiving 301 cycles. HGBL DH=high-grade B-cell lymphoma, double-hit, with *MYC* plus rearrangements of *BCL2*, *BCL6*, or both. HGBL NOS=high-grade B-cell lymphoma, not otherwise specified, with features intermediate between DLBCL and BL, but not harbouring a genetic double hit (synonymous with B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and BL). DA-EPOCH-R=dose-adjusted etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin, and rituximab. DLBCL=diffuse large B-cell lymphoma. BL=Burkitt's lymphoma. FISH=fluorescent in-situ hybridisation. *MYC rearrangement only versus *MYC* rearrangement plus rearrangement of *BCL2*, *BCL6*, or both. †Based on 32 cases with *BCL2* and *BCL6* FISH analysis. ‡Based on 52 cases with FISH analysis of *BCL2*, *BCL6*, or both.

Table 1: **Patient and tumour characteristics**

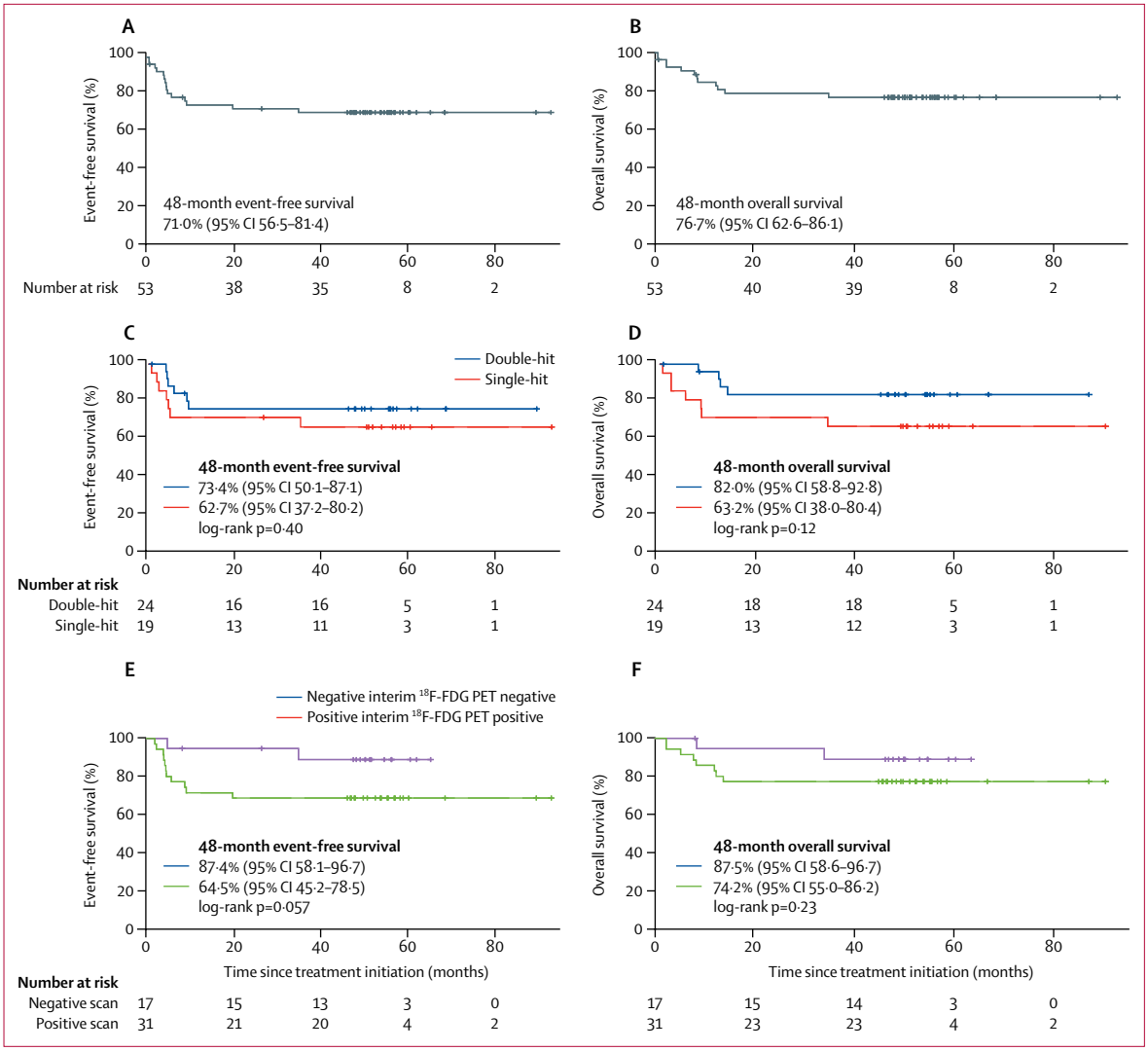

Figure 2: **Kaplan-Meier estimates of event-free and overall survival at 48 months**
Figure shows event-free (A) and overall (B) survival of 53 patients with *MYC*-rearrangement lymphoma; event-free (C) and overall (D) survival of 19 patients with single-hit lymphoma versus 24 patients with double-hit lymphoma; and event-free (E) and overall (F) survival of 17 patients with negative interim ^{18}F-FDG PET scans versus 31 with positive interim ^{18}F-FDG PET scans. ^{18}F-FDG=^{18}F-fluorodeoxyglucose.

score of 4, due to sepsis; and one patient aged 75 years with an IPI score of 5, due to multiorgan failure and sepsis.

Discussion

To our knowledge, this is the first prospective study of chemotherapy in *MYC*-rearranged aggressive B-cell lymphoma. To address an important unmet clinical need,[24] we assessed the outcome of DA-EPOCH-R in a multicentre study of patients with *MYC*-rearrangement aggressive B-cell lymphoma. With a median follow-up of 55·6 months, this study yielded 48-month event-free survival in 71% of participants and overall survival in 77%. Subgroup analyses showed similar results in patients with double-hit lymphomas, with event-free and overall survival at 48 months of 73·4% and 82·0%, respectively. Patients with *MYC* rearrangement alone had a marginally worse outcome compared with patients with double-hit lymphoma. These results are similar to those reported by the Cancer and Leukemia Group B multicentre study of DA-EPOCH-R in de-novo diffuse large B-cell lymphoma,[25] which is consistent with our earlier findings that suggest DA-EPOCH-R obviates the adverse effect of *MYC* rearrangement.[19]

When we initiated this study in 2010, several retrospective studies had identified *MYC* rearrangement as an adverse biomarker for R-CHOP treatment.[5–7] Hence, we chose to include all patients with *MYC* rearrangement, irrespective of secondary hits with *BCL2* or *BCL6*, or both. However, the most recent WHO

classification (revised 4th edition) identified *MYC*-rearranged double-hit tumours (with rearrangement of *BCL2* or *BCL6*, or both) in high-grade B-cell lymphomas as a specific entity on the basis of their poor prognosis with R-CHOP chemotherapy.[14] Despite their exclusion from the WHO classification, patients with single-hit *MYC* rearrangement have an adverse prognosis as well as patients with double-hit.[5,6,8,12,13] Furthermore, the literature remains unclear on the contribution of *BCL2* or *BCL6* rearrangement to the adverse prognosis of single-hit *MYC*-rearranged aggressive B-cell lymphoma. Alternative mechanisms of increased protein production other than rearrangement might also contribute to a poor prognosis with R-CHOP treatment. Notably, a recent retrospective study compared patients with typical double-hit lymphomas to patients with atypical double-hit lymphomas including patients with *MYC* rearrangement and extra copies of *BCL2*, those with *BCL2* rearrangement with extra copies of *MYC*, and those with only extra copies of *MYC* and *BCL2*.[26] In that study, patients with atypical and typical double-hits had a similar 2-year overall survival (54% and 49%, respectively), which was significantly worse than in patients without abnormalities. These results suggest that the alternative mechanisms of expression of *MYC* or *BCL2* could also contribute to a poor outcome.

The outcome of *MYC*-rearranged lymphoma is dependent on IPI score, with all risk groups showing a worse outcome than patients without rearrangement, as shown by findings of a retrospective study of R-CHOP.[5] In our study, 26 (49%) patients had high-intermediate or high-risk disease, 43 (81%) had advanced stage disease, and 27 (50%) were older than 60 years; these characteristics were similar in both patients with single-hit and double-hit lymphomas. When the outcome of patients with double-hit lymphoma was analysed according IPI group, event-free survival at 48 months in those with low or low-intermediate disease was 92% compared with 55% in patients with high-intermediate or high-risk disease. When all patients were included in analysis of outcome according to IPI, similar results were observed. By contrast with reports from treatment with R-CHOP, older patients had similar outcomes to younger ones.[5] Because many patients with *MYC*-rearranged lymphomas are older, and in view of the accepted need for more intensive treatment,[11,16] the absence of an age effect in our study suggests that DA-EPOCH-R could be effective in elderly patients and, unlike more intensive so-called Burkitt-like regimens,[27] has acceptable tolerance (NCCN Guideline 4.2018). We also assessed whether interim ^{18}F-FDG PET scans could identify patients unlikely to achieve durable remissions with DA-EPOCH-R and found that almost all patients with negative ^{18}F-FDG PET scans achieved durable remission, but more than 60% of those with positive scans also had durable remission suggesting that interim ^{18}F-FDG PET scans are not highly specific with DA-EPOCH-R.

	Treatment-related events	Grade 2	Grade 3	Grade 4	Grade 5
Haematological toxicity (% cycles)					
Neutropenia	189	0	29 (10%)	160 (53%)	0
Thrombocytopenia	99	0	59 (20%)	40 (13%)	0
Infection (% cycles)					
Fever and neutropenia	56	0	54 (18%)	2 (1%)	0
Other	40	24 (8%)	15 (5%)	1 (<1%)	0
Gastrointestinal (% cycles)					
Mucositis	44	26 (9%)	18 (6%)	0	0
Constipation	30	30 (10%)	0	0	0
Neurological (% patients)					
Sensory	15	11 (21%)	4 (8%)	0	0
Motor	7	3 (6%)	4 (8%)	0	0
Treatment-related death (% patients)					
Sepsis or organ failure	3	..	..	..	3 (6%)

Toxicity data for 53 patients receiving 301 cycles are shown. DA-EPOCH-R=dose-adjusted etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin, and rituximab.

Table 2: **DA-EPOCH-R toxicity**

Our study has several limitations. Five patients underwent consolidation with bone marrow transplantation (four autologous and one allogeneic) following DA-EPOCH-R treatment in the absence of documented disease. Although these interventions could have altered the disease course, findings from a 2018 retrospective study showed no benefit of autologous transplantation following DA-EPOCH-R in double-hit lymphomas.[28] Patients with single-hit lymphomas had a significantly worse performance status than participants with double-hit lymphoma included in this study, suggesting that patients with double-hit lymphoma could have a worse outcome with DA-EPOCH-R if this characteristic was balanced. The need for specialised tests to detect *MYC* rearrangement is likely to have delayed study enrolment and probably biased accrual towards lower-risk patients in our study.[29] Although this issue also occurs in standard of care, the results from our study might overestimate the benefit of DA-EPOCH-R in an unselected population of patients. Furthermore, because our study did not use central histological review, it is possible that some patients did not have de-novo *MYC*-rearranged aggressive B-cell lymphoma. However, all participants in our study had to have a *MYC* rearrangement, which is the unifying molecular characteristic of these patients. The exploratory analyses of prognostic variables are limited by relatively small numbers and should be interpreted with caution. We observed three treatment-related deaths, which is higher than previously reported by us and other researchers for DA-EPOCH-R, indicating that these patients are at risk of severe toxicity.[23]

Nonetheless, the results from this study suggest that DA-EPOCH-R could improve on the outcome of R-CHOP

in this patient population. One of the early observational studies that assessed the prognostic effect of *MYC* rearrangement evaluated 245 biopsy samples from patients with de-novo diffuse large B-cell lymphoma treated with R-CHOP.[5] In that study, among 35 (14%) patients with *MYC* rearrangement, including 26 (74%) with double-hit *BCL2* rearrangement, 2-year overall survival was 35% compared with 61% for patients without *MYC* rearrangement. A similar result was reported from a retrospective cohort of 135 cases of de-novo diffuse large B-cell lymphoma in which the 5-year overall survival of patients with and without a *MYC* rearrangement was 33% and 72%, respectively.[6] In that series, only 25% of patients with *MYC* rearrangement also harboured a *BCL2* rearrangement. However, not all studies have found that *MYC* rearrangement confers such a poor prognosis following R-CHOP treatment.[12] In a retrospective analysis of 36 patients from a phase 3 randomised study of 14-day versus 21-day R-CHOP, the 2-year overall survival of patients with *MYC* rearrangement was 75% versus 85% in those without rearrangement (p=0·016).[12] In that series, 16 patients with *BCL2*-rearranged double-hit lymphoma had a 2-year overall survival of 63% compared with 84% in patients without rearrangement, which was not statistically different. In this subset analysis, the authors did not report the IPI risk category of patients with double-hit rearrangements, which strongly influence outcome. Furthermore, phase 3 clinical trials can be subject to entry bias toward more favourable patients than the general population, as suggested by the unusually high event-free survival of 85% in patients without *MYC* rearrangement in that study.[12,29] Although we cannot rule out the possibility of bias towards patients with improved prognosis in our study, the distribution of prognostic factors was similar to that of an observational study that showed a 2-year event-free survival of 23% for patients with single-hit *MYC* rearranged treated with R-CHOP compared with 59% for more intensively treated patients.[8] However, a comparison of our results with these retrospective studies of R-CHOP in *MYC*-rearranged aggressive B-cell lymphomas must be tempered by the potential differences in distribution of patients with high-risk IPI scores, and accrual biases.

Several observational studies suggest that intensive treatment is more effective than R-CHOP in *MYC*-rearranged lymphomas. In a single-centre study of 129 patients treated with R-CHOP, DA-EPOCH-R, or R-HyperCVAD/MA (rituximab, hyperfractionated cyclophosphamide, vincristine, doxorubicin, dexamethasone, alternating with cytarabine plus methotrexate), patients who received DA-EPOCH-R had a significantly better 2-year event-free survival (67%) than those who received R-CHOP (25%).[11] In a larger multicentre retrospective study of 311 cases of double-hit lymphoma, patients who received intensive therapy with DA-EPOCH-R, R-CODOX-M/IVAC (cyclophosphamide, vincristine, doxorubicin, cytarabine, methotrexate, ifosfamide, etoposide), or R-HyperCVAD had significantly longer event-free survival (median 21·6 months) than those who received R-CHOP (median 7·8 months).[15] The overall conclusion of these and other studies is that patients with double-hit lymphoma clinically benefit from more intensive treatment.[8–10]

Although the optimal intensive regimen for *MYC*-rearrangement aggressive B-cell lymphoma remains undefined, the favourable outcomes and relative tolerability of DA-EPOCH-R in *MYC*-rearranged lymphoma—as compared with other intensive regimens—has led to its acceptance as one standard (NCCN Guidelines 4.2018).[8–11,16,17] Our single-arm phase 2 study lends support to this treatment option. At the time of writing, the National Cancer Institute National Clinical Trials Network is undertaking a phase 1 trial of DA-EPOCH-R with venetoclax (NCT03036904) in *MYC*-rearranged diffuse large B-cell lymphoma in an effort to further improve outcomes in these tumour types.

Contributors

WHW, KD, and RFL conceived of and designed the study. All authors provided study materials or enrolled patients to the study, and collected and assembled data. WHW and SMS did the primary data analysis and interpretation, and all authors reviewed and commented on the analysis. All authors contributed to writing of the manuscript, gave final approval, and are accountable for all aspects of the work.

Declaration of interests

KD reports support from AbbVie, Adaptive Technologies, Celgene, Amgen, Seattle Genetics, Kite, Janssen, and Karyopharm, outside the submitted work. MAF reports grants and personal fees from Seattle Genetics, Celgene, Takeda, Merck, and BMS, and grants from ADC Therapeutics and Molecular Templates, outside the submitted work. JSA reports personal fees from AbbVie, Celgene, Gilead, Humaningen, Kite Pharma, Juno Therapeutics, Novartis, Verastem, and Merck, outside the submitted work. AN reports grants from National Institutes of Health during the conduct of the study, grants and personal fees from Pharmacyclics, and personal fees from Janssen Global, Medscape, Targeted Oncology, and Prime Oncology outside the submitted work. PFC reports grants from the Eastern Cooperative Oncology Group, during the conduct of the study, grants and personal fees from Genentech, and personal fees from Celgene and Kite Pharmaceutics, outside the submitted work. JWH reports personal fees from AbbVie, outside the submitted work. DJ reports personal fees from Seattle Genetics and Celgene, outside the submitted work. SN owns stock in Pfizer, Bristol Myers, Merck, and Roche. BK reports grants from the National Cancer Institute, during the conduct of the study, and personal fees from Genentech, outside the submitted work. JWF reports support from Bayer and Astellas, outside the submitted work. The other authors declare no competing interests.

Data sharing

Deidentified clinical data will be provided to the Protocol Registration and Results System of ClinicalTrials.gov within 1 year of publication. Information on data sharing can be obtained from the ClinicalTrials.gov website.

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References

1 Alizadeh AA, Eisen MB, Davis RE, et al. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. *Nature* 2000; **403:** 503–11.

2 Rosenwald A, Wright G, Chan WC, et al. The use of molecular profiling to predict survival after chemotherapy for diffuse large-B-cell lymphoma. *N Engl J Med* 2002; **346:** 1937–47.

3 Schmitz R, Wright GW, Huang DW, et al. Genetics and pathogenesis of diffuse large B-cell lymphoma. *N Engl J Med* 2018; **378:** 1396–407.

4 Scott DW, King RL, Staiger AM, et al. High-grade B-cell lymphoma with *MYC* and *BCL2* and/or *BCL6* rearrangements with diffuse large B-cell lymphoma morphology. *Blood* 2018; **131:** 2060–64.

5 Barrans S, Crouch S, Smith A, et al. Rearrangement of *MYC* is associated with poor prognosis in patients with diffuse large B-cell lymphoma treated in the era of rituximab. *J Clin Oncol* 2010; **28:** 3360–65.

6 Savage KJ, Johnson NA, Ben-Neriah S, et al. *MYC* gene rearrangements are associated with a poor prognosis in diffuse large B-cell lymphoma patients treated with R-CHOP chemotherapy. *Blood* 2009; **114:** 3533–37.

7 Niitsu N, Okamoto M, Miura I, Hirano M. Clinical features and prognosis of de novo diffuse large B-cell lymphoma with t(14;18) and 8q24/c-*MYC* translocations. *Leukemia* 2009; **23:** 777–83.

8 Landsburg DJ, Falkiewicz MK, Petrich AM, et al. Sole rearrangement but not amplification of *MYC* is associated with a poor prognosis in patients with diffuse large B cell lymphoma and B cell lymphoma unclassifiable. *Br J Haematol* 2016; **175:** 631–40.

9 Landsburg DJ, Falkiewicz MK, Maly J, et al. Outcomes of patients with double-hit lymphoma who achieve first complete remission. *J Clin Oncol* 2017; **35:** 2260–67.

10 Aukema SM, Siebert R, Schuuring E, et al. Double-hit B-cell lymphomas. *Blood* 2011; **117:** 2319–31.

11 Oki Y, Noorani M, Lin P, et al. Double hit lymphoma: the MD Anderson Cancer Center clinical experience. *Br J Haematol* 2014; **166:** 891–901.

12 Cunningham D, Hawkes EA, Jack A, et al. Rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisolone in patients with newly diagnosed diffuse large B-cell non-Hodgkin lymphoma: a phase 3 comparison of dose intensification with 14-day versus 21-day cycles. *Lancet* 2013; **381:** 1817–26.

13 Kuhnl A, Cunningham D, Counsell N, et al. Outcome of elderly patients with diffuse large B-cell lymphoma treated with R-CHOP: results from the UK NCRI R-CHOP14v21 trial with combined analysis of molecular characteristics with the DSHNHL RICOVER-60 trial. *Ann Oncol* 2017; **28:** 1540–46.

14 Swerdlow SH, Campo E, Pileri SA, et al. The 2016 revision of the World Health Organization classification of lymphoid neoplasms. *Blood* 2016; **127:** 2375–90.

15 Petrich AM, Gandhi M, Jovanovic B, et al. Impact of induction regimen and stem cell transplantation on outcomes in double-hit lymphoma: a multicenter retrospective analysis. *Blood* 2014; **124:** 2354–61.

16 Friedberg JW. How I treat "double hit" lymphoma. *Blood* 2017; **130:** 590–96.

17 Friedberg JW. Double-hit diffuse large B-cell lymphoma. *J Clin Oncol* 2012; **30:** 3439–43.

18 Dunleavy K, Pittaluga S, Shovlin M, et al. Low-intensity therapy in adults with Burkitt's lymphoma. *N Engl J Med* 2013; **369:** 1915–25.

19 Lai C, Roschewski M, Melani C, et al. *MYC* gene rearrangement in diffuse large B-cell lymphoma does not confer a worse prognosis following dose-adjusted EPOCH-R. *Leuk Lymphoma* 2018; **59:** 505–08.

20 Swerdlow SH CE, Harris NL, Campo E, et al. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. 4th edn. Bosman FT JE, Lakhani SR, Ohgaki H, eds. Lyon: International Agency for Research on Cancer, 2008.

21 Hegde U, Filie A, Little RF, et al. High incidence of occult leptomeningeal disease detected by flow cytometry in newly diagnosed aggressive B-cell lymphomas at risk for central nervous system involvement: the role of flow cytometry versus cytology. *Blood* 2005; **105:** 496–502.

22 Cheson BD, Pfistner B, Juweid ME, et al. Revised response criteria for malignant lymphoma. *J Clin Oncol* 2007; **25:** 579–86.

23 Wilson WH, Dunleavy K, Pittaluga S, et al. Phase II study of dose-adjusted EPOCH and rituximab in untreated diffuse large B-cell lymphoma with analysis of germinal center and post-germinal center biomarkers. *J Clin Oncol* 2008; **26:** 2717–24.

24 Nowakowski GS, Blum KA, Kahl BS, et al. Beyond RCHOP: a blueprint for diffuse large B cell lymphoma research. *J Natl Cancer Inst* 2016; **108:** djw257.

25 Wilson WH, Jung SH, Porcu P, et al. A Cancer and Leukemia Group B multi-center study of DA-EPOCH-rituximab in untreated diffuse large B-cell lymphoma with analysis of outcome by molecular subtype. *Haematologica* 2012; **97:** 758–65.

26 Li S, Seegmiller AC, Lin P, et al. B-cell lymphomas with concurrent *MYC* and *BCL2* abnormalities other than translocations behave similarly to *MYC*/*BCL2* double-hit lymphomas. *Mod Pathol* 2015; **28:** 208–17.

27 Mead GM, Sydes MR, Walewski J, et al. An international evaluation of CODOX-M and CODOX-M alternating with IVAC in adult Burkitt's lymphoma: results of United Kingdom Lymphoma Group LY06 study. *Ann Oncol* 2002; **13:** 1264–74.

28 Chen AI, Leonard JT, Okada CY, et al. Outcomes of DA-EPOCH-R induction plus autologous transplant consolidation for double hit lymphoma. *Leuk Lymphoma* 2018; **59:** 1884–89.

29 Maurer MJ, Ghesquieres H, Link BK, et al. Diagnosis-to-treatment interval is an important clinical factor in newly diagnosed diffuse large B-cell lymphoma and has implication for bias in clinical trials. *J Clin Oncol* 2018; **36:** 1603–10.