bjh research paper

NCRI phase II study of CHOP in combination with ofatumumab in induction and maintenance in newly diagnosed Richter syndrome

Toby A. Eyre,[1] Ruth Clifford,[1,2] Adrian Bloor,[3] Lucy Boyle,[4] Corran Roberts,[5] Maite Cabes,[2] Graham P. Collins,[1] Stephen Devereux,[6] George Follows,[7] Christopher P. Fox,[8] John Gribben,[9] Peter Hillmen,[10] Chris S. Hatton,[1] Tim J. Littlewood,[1] Helen McCarthy,[11] Jim Murray,[12] Andrew R. Pettitt,[13,14] Elizabeth Soilleux,[14] Basile Stamatopoulos,[2,15] Sharon B. Love,[5] Andrew Wotherspoon[16] and Anna Schuh[1,2,17,18]

[1]Department of Haematology, Oxford University Hospitals NHS Foundation Trust, Churchill Hospital, Oxford, UK, [2]NIHR BRC Oxford Molecular Diagnostic Centre, Oxford University Hospitals NHS Foundation Trust, John Radcliffe Hospital, Oxford, UK, [3]Department of Haematology, The Christie Hospital NHS Trust, Wimslow Road, Manchester, UK, [4]OCTO – Oncology Clinical Trials Office, Department of Oncology, University of Oxford, Oxford, UK, [5]Centre for Statistics in Medicine, Nuffield Department of Orthopaedics, Rheumatology & Musculoskeletal Sciences, University of Oxford, Oxford, UK, [6]Department of Haematology, Kings College Hospital, London, UK, [7]Department of Haematology, Addenbrooke's Hospital NHS Trust, Cambridge, UK, [8]Department of Haematology, Nottingham University Hospitals NHS Trust, Nottingham, UK, [9]Institute of Cancer, Barts & the London School of Medicine & Dentistry, London, UK, [10]St James's Institute of Oncology; Dena Cohen, University of Leeds, Leeds, UK, [11]Department of Haematology, Royal Bournemouth Hospital, Bournemouth, UK, [12]Department of Haematology, Queen Elizabeth Hospital, Birmingham, UK, [13]Royal Liverpool and Broadgreen University Hospitals NHS Trust, Liverpool, Merseyside, UK, [14]Department of Molecular and Clinical Cancer Medicine, University of Liverpool, Liverpool, UK, [15]Department of Cellular Pathology, Oxford University Hospitals NHS Foundation Trust, John Radcliffe Hospital,

Richter syndrome (RS) is associated with chemotherapy resistance and a poor historical median overall survival (OS) of 8–10 months. We conducted a phase II trial of standard CHOP-21 (cyclophosphamide, doxorubicin, vincristine, prednisolone every 21 d) with ofatumumab induction (Cycle 1: 300 mg day 1, 1000 mg day 8, 1000 mg day 15; Cycles 2–6: 1000 mg day 1) (CHOP-O) followed by 12 months ofatumumab maintenance (1000 mg given 8-weekly for up to six cycles). Forty-three patients were recruited of whom 37 were evaluable. Seventy-three per cent were aged >60 years. Over half of the patients received a fludarabine and cyclophosphamide-based regimen as prior CLL treatment. The overall response rate was 46% (complete response 27%, partial response 19%) at six cycles. The median progression-free survival was 6·2 months (95% confidence interval [CI] 4·9–14·0 months) and median OS was 11·4 months (95% CI 6·4–25·6 months). Treatment-naïve and *TP53*-intact patients had improved outcomes. Fifteen episodes of neutropenic fever and 46 non-neutropenic infections were observed. There were no treatment-related deaths. Seven patients received platinum-containing salvage at progression, with only one patient obtaining an adequate response to proceed to allogeneic transplantation. CHOP-O with ofatumumab maintenance provides minimal benefit beyond CHOP plus rutuximab. Standard immunochemotherapy for RS remains wholly inadequate for unselected RS. Multinational trials incorporating novel agents are urgently needed.

Keywords: Ofatumumab, CHOP, *TP53*, Richter syndrome, Chronic lymphocytic leukaemia.

First published online 5 July 2016
doi: 10.1111/bjh.14177

Oxford, UK, [16]Laboratory of Clinical Cell Therapy, Jules Bordet Institute, Université Libre de Bruxelles (ULB), Brussels, Belgium, [17]Department of Histopathology, The Royal Marsden Hospital, London,UK and [18]Department of Oncology, University of Oxford, Oxford,UK

Received 8 March 2016; accepted for publication 15 April 2016
Correspondence: Professor Anna H. Schuh, Department of Oncology, University of Oxford, Old Road Campus Research Building, Roosevelt Drive, Oxford, OX3 7DQ UK.
E-mail: anna.schuh@oncology.ox.ac.uk
Trial Registration: www.ClinicalTrials.gov NCT01171378
Presented in part as a poster at the European Association of Hematology (EHA) in June 2015, Vienna

B-cell chronic lymphocytic leukaemia (CLL) is the most common indolent chronic lymphoproliferative disease. 2–15% of patients (Rossi *et al*, 2011; 2008; Tsimberidou *et al*, 2006) transform to an invariably aggressive, chemo-resistant high-grade non-Hodgkin lymphoma (NHL) (Morton *et al*, 2006). Broadly, NHL represents the fifth most common cancer worldwide and its incidence continues to increase (Morton *et al*, 2006). Richter syndrome (RS) is a rare complication of CLL, characterized by rapidly growing and/or asymmetrical lymphadenopathy or extranodal fluorodeoxyglucose ([^{18}F]DG) positron-emission-tomography (PET) computerized tomography (CT)-avid masses, new B symptoms, a rapidly rising lactate dehydrogenase (LDH) or new hypercalcaemia in a patient with known CLL. Most RS represent transformation to a clonally-related activated B-cell type (ABC) diffuse large B-cell lymphoma (DLBCL) (90–95%) with a small proportion transforming to Hodgkin lymphoma (Bockorny *et al*, 2012). The long-term overall survival (OS) of de novo DLBCL following the introduction of the anti-CD20 monoclonal antibody rituximab in those fit for an anthracycline-based regimen, typically CHOP (cyclophosphamide, doxorubicin, vincristine, prednisolone) approaches 70% (Coiffier *et al*, 2002).

RS can present in either heavily pre-treated, immunosuppressed patients or in untreated CLL patients (Parikh *et al*, 2013). Patients typically present with a rapidly worsening performance status and often possess therapy-limiting co-morbidities. No randomized trials have been published. Alkylator, anthracycline, platinum and purine analogue chemotherapy have been investigated in RS. Rituximab (R)-CHOP (Coiffier *et al*, 2002), R-hyper-CVXD-MA (fractionated cyclophosphamide, vincristine, liposomal daunorubicin, dexamethasone plus rituximab, alternating with methotrexate and cytarabine with rituximab) (Tsimberidou *et al*, 2003), hyper-CVXD alone (Dabaja *et al*, 2001), FACPGM (fludarabine, cytarabine, cyclophosphamide, cisplatin and GM-CSF) (Tsimberidou *et al*, 2002), OFAR1 and OFAR2 (oxaliplatin, fludarabine, cytarabine, rituximab and pegfilgrastim) (Tsimberidou *et al*, 2008, 2013) regimens have been used. The best response rates are 41% with hyper-CVXD and R-hyper-CVXD-MA and 50% with OFAR1, although responses are short-lived. These regimens are toxic and inappropriate for many elderly patients with RS. The median survival of 8–10 months (Tsimberidou *et al*, 2006; Tadmor *et al*, 2014) from diagnosis has not been bettered in the literature although a recent small cohort of 15 RS patients treated with R-CHOP displayed an overall response rate (ORR) of 67% and a median OS of 21 months (Langerbeins *et al*, 2014). Autologous and allogeneic stem cell transplantation (SCT) are typically reserved for exceptional patients aged less than 65 years with good performance status and chemo-sensitive disease (Parikh *et al*, 2013).

Ofatumumab is a fully human monoclonal IgG anti-CD20 antibody that specifically targets a unique membrane-proximal epitope of CD20 on B-cells with increased affinity and a longer dissociation time compared to rituximab. This mechanism is thought to improve complement-mediated cellular cytotoxicity (Teeling *et al*, 2006; Barth *et al*, 2012). Ofatumumab has the potential to induce B-cell apoptosis independent of TP53 (p53). At the time, its efficacy in CLL refractory to fludarabine and alemtuzumab (a group that commonly possess *TP53*-disruption (Wierda *et al*, 2010) led to its accelerated US Food and Drug Administration (FDA) approval in this indication. In view of the high incidence of

TP53-disruption in DLBCL-RS (Chigrinova *et al*, 2013) and the well documented risk of early relapse, ofatumumab induction (alongside CHOP) and maintenance represented a biologically sound treatment rationale for patients with RS.

Patients and Methods

Study design

The 'CHOP-OR' trial was a single arm, multi-centre, non-randomized phase II National Cancer Research Institute (NCRI) feasibility study in patients with newly diagnosed RS, recruiting from 10 UK centres from April 2011 to December 2014. Forty-three patients were enrolled of whom 37 were ultimately evaluable for objective overall response within the trial. Eligible patients received CHOP-O for six cycles, followed by six cycles of ofatumumab maintenance every eight weeks. The primary objective of the CHOP-OR study was to determine objective response to CHOP-O at week 20 as measured from start of treatment according to the Revised Response Criteria for Malignant Lymphoma (Cheson *et al*, 2007). Response to treatment and disease progression was assessed by the local investigator. Secondary objectives included assessing the feasibility of recruitment, progression-free survival (PFS) and OS, the clinical benefit and changes in patient-reported outcomes (PROs), safety and tolerability. Simon's minimax two-stage design was implemented to calculate the required sample size ($n = 35$) to identify a desired response of the trial drug of 47% with a power of 80% and significance of 20%. For a detailed description of the study design, patient selection, statistical design, interventions and outcome measures, the reader is directed to the open access protocol manuscript (Eyre *et al*, 2015).

Exploratory outcome measures

Given the relative lack of prospective clinical trials performed in RS, there have been few attempts to use clinical features to assess prognostic factors. Two scoring systems have been published. Parikh *et al* (2013) validated a clinical scoring system in a retrospective dataset developed by Tsimberidou *et al* (2006). Adverse risk factors within the scoring system are: 1. Eastern Cooperative Oncology Group (ECOG) performance status (PS) >1, 2. LDH ≥1·5 the upper limit of normal, 3. Platelet count $<100 \times 10^9$/l, 4. Tumour bulk >5 cm, 5. >1 previous lines of chemotherapy for underlying CLL. Patients with a score of 4–5 have a median survival of 0·1 years, compared to those with a score of 0–1 who have a median survival of 1·1 years. A second scoring system by Rossi *et al* (2011) described three factors: 1. ECOG PS >1, 2. Less than complete response (CR) to induction immuno-chemotherapy, 3. Presence of *TP53* disruption (deletion or mutation). In this large retrospective analysis, patients with no risk factors had a 5-year survival of 70%. Those with an ECOG PS >1 had a survival of 0·7 years and those with a performance status ≤1 with *TP53* disruption or less than CR after induction had a median survival of 2 years (Rossi *et al*, 2011). We aimed to further validate both these scoring systems within the CHOP-OR trial.

Immunohistochemistry (IHC), In-situ hybridization (ISH) and Fluorescent In-Situ Hybridization (FISH)

Using the Hans algorithm (Hans *et al*, 2004), the cell of origin was determined by IHC in all patients where feasible, using antibodies from either Novocastra-Leica, Newcastle UK or Dako, Ely, UK on a Bond Max™ immunostaining machine (Novocastra-Leica). In addition, diagnostic formalin-fixed paraffin embedded (FFPE) tissue was analysed for Epstein–Barr virus (EBV) expression by ISH, for EBV-encoded RNA (EBER, Novocastra-Leica) or latent membrane protein-1 (LMP-1, Novocastra-Leica) by IHC both on a Bond Max™ immunostaining machine (Novocastra-Leica). Data on c-Myelocytomatosis (MYC, Novocastra-Leica) tumour expression by IHC (percentage) and/or FISH (Vysis LSI MYC dual colour break-apart rearrangement probes (Abbott Molecular, Maidenhead UK)) was also collected. The median percentage MYC expression was correlated with clinical outcome in a similar fashion to Green *et al* (2012) using a median score cut off.

TP53 *disruption*

Patients were considered to have *TP53* disruption if either a *TP53* deletion or mutation (by FISH or next generation sequencing [NGS] DNA analysis) was found in the CLL clone and/or the DLBCL tumour FFPE tissue. *TP53* mutation status was determined using a TruSeq Custom Amplicon panel (TSCA, Illumina, San Diego, CA, USA) targeting mutational hotspots. Libraries were created from 250 ng of DNA, before undergoing 2 × 150 bp paired-end sequencing on the MiSeq platform (Illumina). Initial alignment and variant calling analysis was performed with the TSCA Workflow of MiSeq Reporter v.2·4 (Illumina) using the default settings. A second alignment followed by variant calling using the Stampy v.1·0·22 (http://www.well.ox.ac.uk/project-stampy) and Platypus v.0·5·1 (http://www.well.ox.ac.uk/platypus) software to specifically identify additional insertions and deletions (InDels) not detected with MiSeq Reporter.

IGHV *gene mutational analysis*

IGHV gene mutational analysis was performed as previously described (Campbell *et al*, 1992), and the sequences were aligned with those in the international ImMunoGeneTics (IMGT) information system database (http://imgt.cines.fr). Sequences with less than 2% deviation from any germline *IGHV* sequence were considered unmutated (Hamblin *et al*, 1999).

All available diagnostic samples underwent central histopathology review by two expert haemato-pathologists who reviewed the cases independently and then together.

FDG-PET

FDG PET-CT has been shown to be useful in differentiating accelerated, 'aggressive' CLL and RS (Bruzzi *et al*, 2006; Falchi *et al*, 2014; Papajík *et al*, 2014). Although FDG PET-CT was not mandated prior to CHOP-O, a number of centres performed a prior PET-CT with the aim to accurately stage patients and to direct a diagnostic biopsy. As such, data on whether a PET-CT was performed, the disease stage and the maximum standardized uptake value (SUVmax) were also collected.

Ethics and Authors Contributions

The institutional review board or ethics committee of each participating institution approved the study protocol, and each patient provided written informed consent before enrolment. The trial sponsor was responsible for data gathering and pharmacovigilance, and to share safety data throughout the study with the corresponding author and an independent trial safety committee. The sponsor and the authors were responsible for data analysis and interpretation. The corresponding author had full access to all the data in the study and all authors approved the manuscript for submission for publication. The corresponding author had final responsibility for the decision to submit the manuscript for full publication.

Results

The CHOP-OR trial was the first UK-wide prospective study in RS and is the largest prospective study performed to date in RS. Between April 2011 and December 2014, 43 patients in total fulfilled eligibility criteria for the trial. Of those, 37 patients were evaluable according to pre-defined criteria (Fig 1; Supplemental Fig 1).

All non-evaluable patients were defined by failure to complete one full cycle of CHOP-O on trial. Table I describes the baseline demographics, clinical characteristics and prior treatments for the 37 evaluable patients. Categorical variables are presented as n (%); continuous variables are presented as mean (range, standard deviation [SD]). Seventy-three per cent of patients were aged over 60 years, and 70% were male gender. Patients received a median of 1 prior therapy (range 0–4) for CLL prior to high-grade transformation with over half of pre-treated patients having received a fludarabine and cyclophosphamide based regimen. Forty-six per cent of patients were CLL treatment-naïve at transformation. *TP53* disruption was noted in 43% of patients either at or already prior to transformation.

The ORR (CR plus partial response [PR]) to CHOP-O induction was 46% (95% CI 29·7–62·2%; CR 27%, PR 19%) at the end of six cycles of induction. Eighteen patients started maintenance, which nine completed. At the time of data censoring, three were receiving ongoing maintenance. The median PFS was 6·2 months (95% CI 4·9–14·0 months), with no convincingly clear plateau on the PFS or OS curves (Fig 2A, 2B). The median OS was 11·4 months (95% CI 6·4–25·6 months), consistent with outcomes described in the literature using R-CHOP and platinum-based regimens (Coiffier *et al*, 2002; Tsimberidou *et al*, 2008, 2013; Parikh *et al*, 2013; Langerbeins *et al*, 2014). Patients received a median number of six courses of CHOP-O (interquartile range 4–6). The reasons for discontinuation of therapy of those evaluable on the trial were: treatment failure ($n = 15$), treatment toxicity or adverse events ($n = 5$), clinician or patient decision ($n = 3$) and death ($n = 2$).

Baseline FDG PET-CT was performed in 42% of enrolled patients, with no clear change of scanning frequency occurring over the recruitment period. The mean SUVmax was 20·8 (range in patients with measurable disease 5·85–69·4). The sites of SUVmax and the pattern of baseline PET CT usage are described in Supplemental Figure 2. One patient who received a PET-CT had a central histopathology review consistent with accelerated CLL rather than RS. The SUVmax in that patient was 10·3. All other patients who received a PET-CT, including the other three patients with SUVmax <10, were assessed by central histopathology as RS.

EBV expression was examined by ISH, IHC or both in 29 of the 37 eligible patients. EBV expression was found in only two patients, where widespread LMP-1 expression was noted. Neither of these two patients had received prior fludarabine-based CLL therapy and one was treatment-naïve. *TP53* status was assessed in 36 of 37 patients. *TP53* was disrupted in 43% of cases by FISH or sequencing analysis. Cell of origin was evaluated in 33 of 37 cases by the Hans algorithm (Hans *et al*, 2004). ABC type DLBCL (25 of 33 evaluable; 76%) was most commonly seen. Eight patients had germinal-centre type DLBCL and 4 patients had inadequate tissue for assessment. MYC expression was assessed in 30 patients by IHC (including two by FISH in addition) and one separate case by FISH alone. All 3 cases were negative by FISH. The median percentage expression was 20% (range 0–70%; mean 21%). This median percentage MYC expression was then used to assess correlations with clinical outcome (<20% versus ≥20%).

The subgroup analysis (Fig 3C-D) aimed to assess the relative prognostic value of the Rossi score, the Tsimberidou score, and the International Prognostic Score (Supplemental Fig 3A). Patients who were treatment-naïve had a significantly superior survival than patients who had received at least one prior line of therapy for the underling CLL (Fig 3A; $P = 0·007$). Five durable remissions of 18 months or more were noted in the treatment-naïve cohort (Fig 3A). The ORR rate of treatment-naïve and pre-treated patients was 52·9% and 40·0% respectively. Patients with *TP53* disruption had a significantly inferior survival than those that did not (Fig 3B;

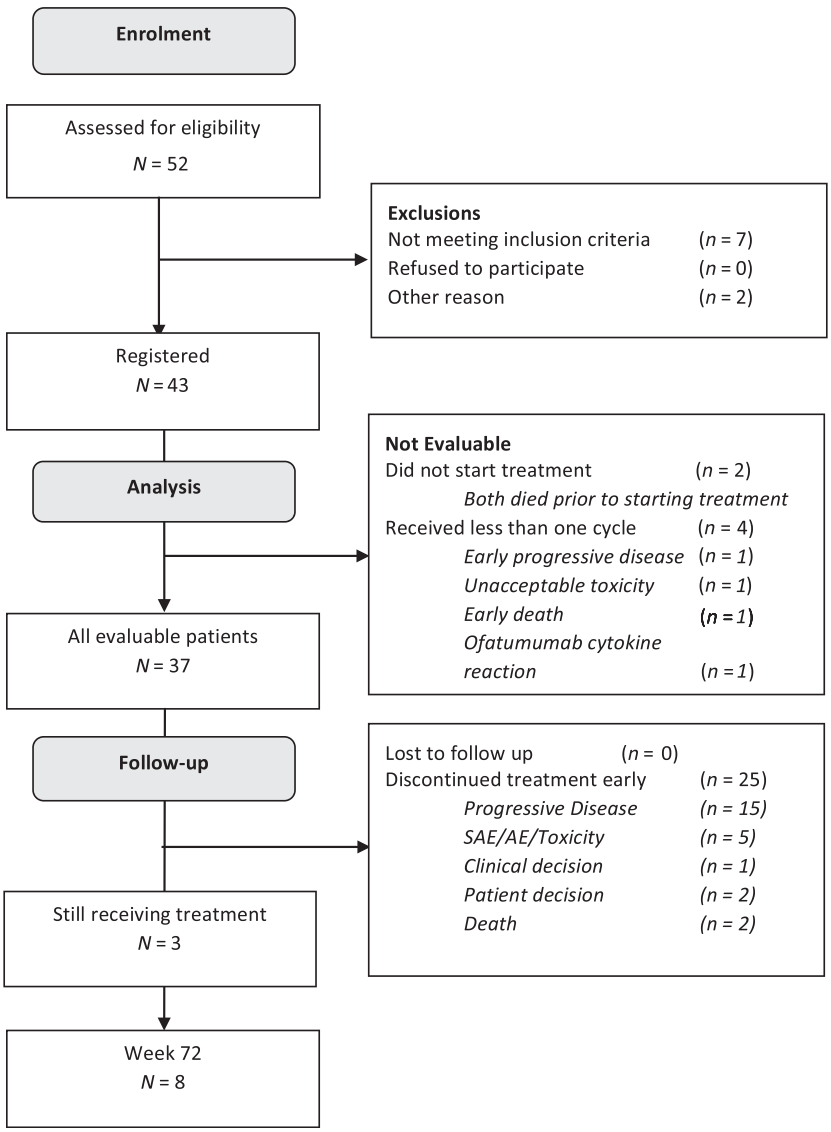

Fig 1. Consort diagram AE, adverse event; SAE, serious adverse event.

$P = 0{\cdot}044$). The cell of origin (supplemental Fig 3B) and MYC status (<20% verses ≥20%) (supplemental Fig 3C) of the DLBCL did not influence survival. The data available for *IGHV* clonality was limited by tissue availability. Of the 20 patients assessable by *IGHV* clonality studies, 17 were evaluable within the trial for outcome data. Of those 17, only 1 patient had DLBCL that was clonally unrelated to their underlying CLL. This patient progressed post-Cycle 5 after a PR at Cycle 4. Six patients had not relapsed at the time of data censoring. Of these 6, the majority ($n = 5$) had both an excellent ECOG PS (0–1) at baseline, and 5 of 6 had no *TP53* disruption. Key baseline characteristics were assessed for their influence on the ORR as the primary outcome measure in a forest plot (Fig 4). There were no clear subgroups that had notably superior responses to CHOP-O induction at the end of Cycle 6.

A retrospective central histopathology review was performed by two expert haematopathologists. Forty of the 43 cases were available for central review, of which 17·5% ($n = 7$) were considered to have features consistent with CLL or accelerated/aggressive CLL and 82·5% ($n = 33$) RS (1 Hodgkin-type, 32 DLBCL-type). No clear difference in survival outcome in those evaluable could be demonstrated according to the histopathology subgroup as defined by central review (Supplemental Fig 3D). All patients were included within the analysis independent of central review status. When patients deemed to have aggressive CLL by central review were excluded from analysis, the survival outcomes were unchanged across all subgroups.

Safety

As predicted for this patient group, CHOP-O caused a significant number of adverse events (AEs; $n = 85$) and serious adverse events (SAEs; $n = 50$). None of these were unexpected. Table II displays a summary of the key grade 3–4 adverse

Table I. Baseline Characteristics

Baseline characteristic	All evaluable patients ($N = 37$) *n* (%)
Age, years; mean (range, SD)	66·2 (43–90, 11·3)
<60 years	10 (27%)
≥60 years	27 (73%)
Gender	
Male	26 (70%)
Female	11 (30%)
ECOG performance status	
0	17 (46%)
1	12 (32%)
2	4 (11%)
3	4 (11%)
No. of extranodal sites	
0	19 (51%)
1	13 (35%)
>1	5 (14%)
Ann Arbour staging	
I	6 (16%)
II	6 (16%)
III	15 (41%)
IV	9 (24%)
Not assessed/documented	1 (3%)
Rai staging	
0	11 (30%)
I	12 (32%)
II	8 (22%)
III	3 (8%)
IV	2 (5%)
Not assessed/documented	1 (3%)
Binet staging	
A	19 (51%)
B	12 (32%)
C	6 (16%)
B-symptoms history	
Yes	22 (59%)
No	15 (41%)
LDH level	
<ULN	13 (35%)
>ULN	24 (65%)
LDH > 1·5 × ULN	11 (30%)
Platelet count	
$<100 \times 10^9$/l	10 (27%)
$\geq 100 \times 10^9$/l	27 (73%)
Beta-2 Microglobulin	
<ULN	14 (38%)
>ULN	18 (49%)
Not assessed/documented	5 (14%)
Bulk in lymph node over 5 cm	
Yes	18 (49%)
No	19 (51%)
TP53 disruption	
Yes	16 (43%)
No	20 (54%)
No data available	1 (3%)
EBV expression	
Positive	2 (7%)
Negative	27 (93%)
Not assessed	8
Cell of origin	
Activated B cell (ABC)	25 (76%)
Germinal centre (GC)	8 (24%)
Undefined as inadequate tissue	4
MYC expression	Median expression 20% (range 0–90%)
<20%	12
≥20%	18
FISH only (negative)	1
Not assessed	6
Previous treatments	
0	17 (46%)
1	12 (32%)
>1	8 (22%)
Previous B-CLL treatments	(Number of patients who received treatment previously)
Fludarabine + cyclophosphamide + rituximab	12
Fludarabine + cyclophosphamide	8
Chlorambucil ± rituximab	5
Alemtuzumab ± Steroid (High dose)	4
Rituximab	2
Other	6
Tsimberidou score (Tsimberidou *et al*, 2006)	
0–1	19 (51%)
2–3	17 (46%)
4–5	1 (3%)
Rossi score (Rossi *et al*, 2011)	
Low risk	6 (16%)
Intermediate risk	23 (62%)
High risk	7 (19%)
Data not available	1 (3%)
International prognostic index (IPI) score	
Low 0–1	10 (27%)
Low/Intermediate 2	10 (27%)
High/Intermediate 3	9 (24%)
High > 4	7 (19%)
Data not available	1 (3%)
Central histopathology review (all 43 enrolled patients)	
Richter (DLBCL type)	33 (82·5%) (29 evaluable)
Not Richter (CLL or accelerated CLL)	7 (17·5%) (6 evaluable)
Tissue unavailable	3 (2 evaluable)

ECOG, Eastern Cooperative Oncology Group; LDH, lactate dehydrogenase; ULN, upper limit of normal; EBV, Epstein-Barr virus; DLBCL, diffuse large B cell lymphoma; CLL, chronic lymphocytic leukaemia; SD, standard deviation.

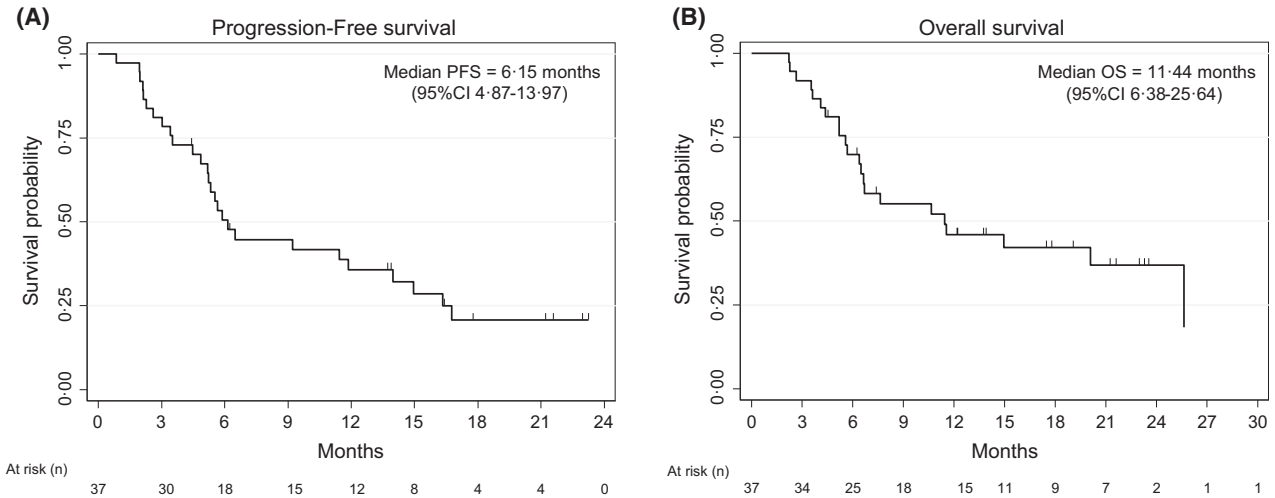

Fig 2. Survival outcomes - A: progression-free survival (PFS). B: overall survival (OS).

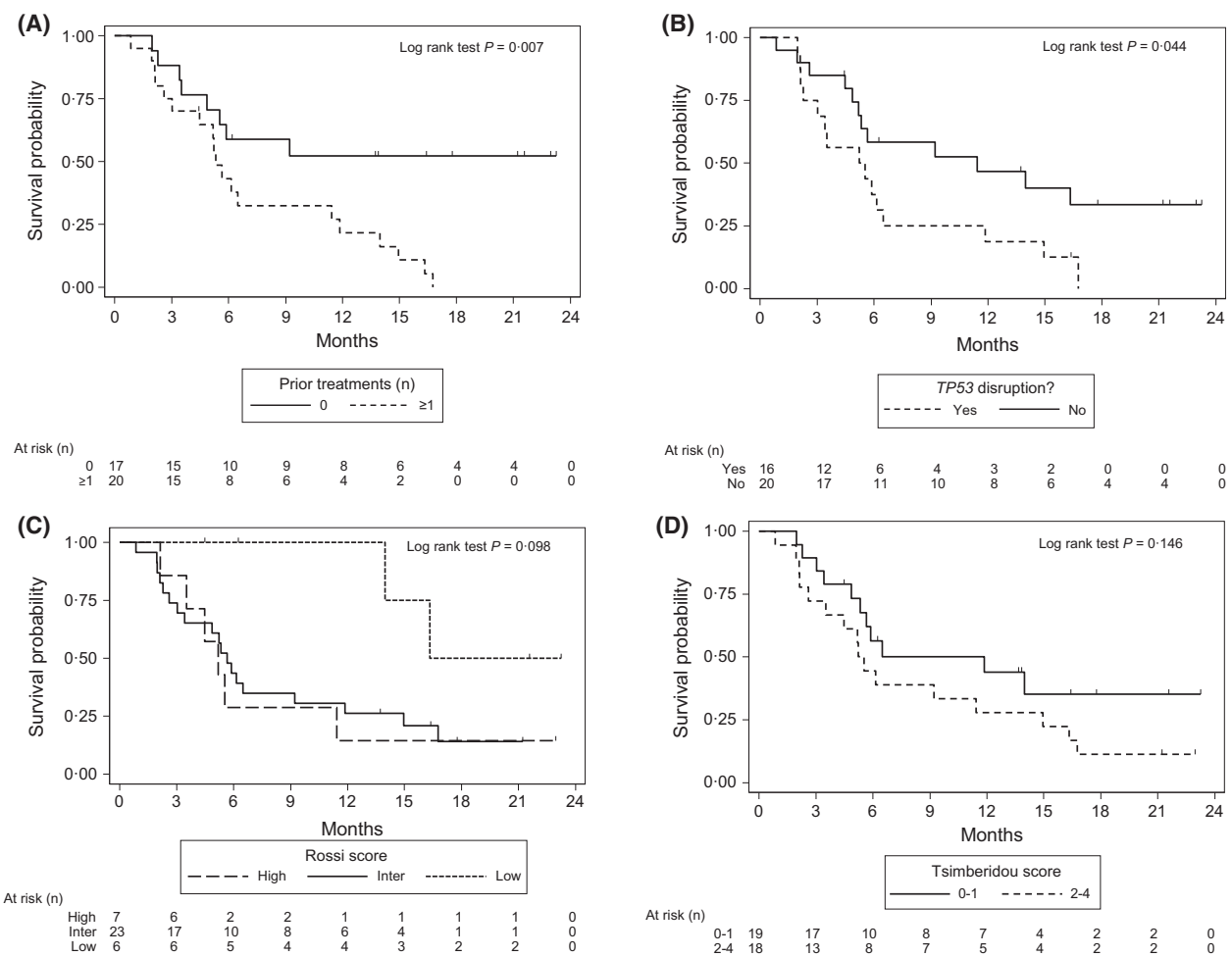

Fig 3. Progression-free survival according to subgroups - A: Number of prior treatments. B: *TP53* disruption. C: Rossi score. D: Tsimberidou score

events reported across all 43 patients. Fifteen episodes of neutropenic fever were reported and grade 3–4 neutropenia was noted on 14 occasions. Forty-six additional non-neutropenic infections were noted, of which 21 were SAEs. A single SAE as the result of an ofatumumab-induced infusion reaction was reported. No treatment-related deaths were reported.

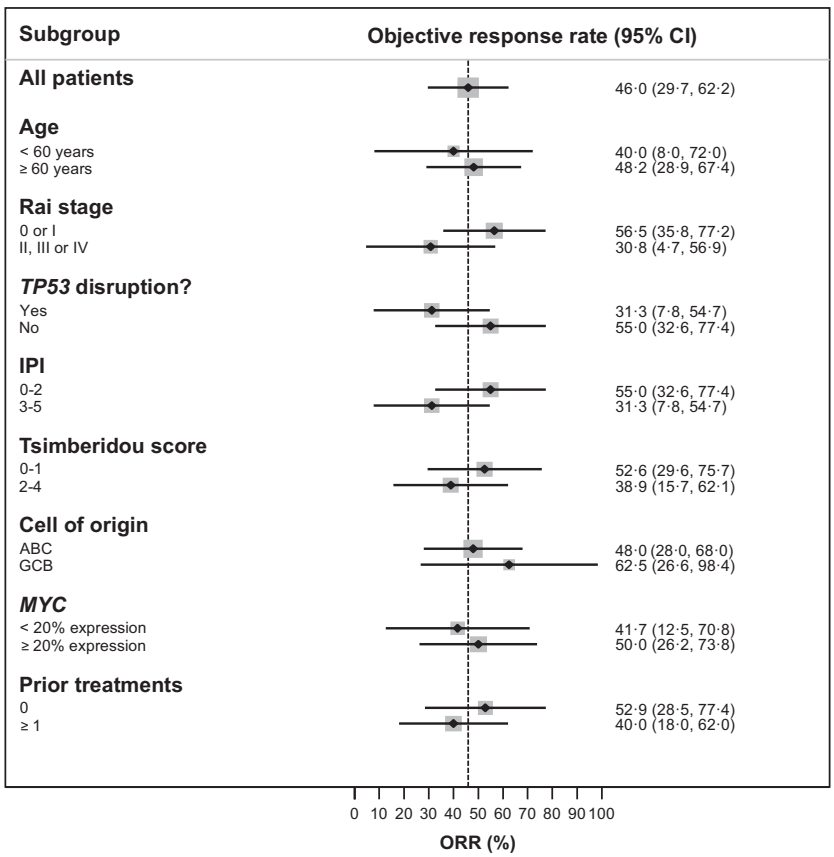

Fig 4. Overall response rate (ORR) at end of cycle 6 CHOP-O induction according to subgroups. The size of grey box is equivalent to patient number. Diamonds indicate the objective response rate value for each specific subgroup and the horizontal lines indicate the 95% confidence interval for that subgroup. IPI, International Prognostic Index; ABC, activated B-cell type; GCB, germinal centre B cell type; 95% CI, 95% confidence interval.

Management at progression and outcome

Seven patients received platinum-containing salvage at progression, and only a single patient (Patient 2, Table III) had sufficiently chemo-responsive disease to receive an allogeneic stem cell transplantation. No patient received an autologous SCT. The outcome in those considered fit enough for platinum-based salvage chemotherapy at relapse post-CHOP-O was generally poor.

Discussion

The successful completion of this trial proves the feasibility of studying novel combinations in a rare disease and shows the importance of productive national collaboration. This study achieved a key secondary end point by showing that it is feasible to study clinico-pathological features of RS and answers important therapy-based questions in clinical trials. Although not compared in a prospective randomized clinical trial, our data shows that CHOP-O does not noticeably improve response and survival outcomes when judged aside historical outcomes after R-CHOP in RS (n = 15, ORR 67%, median PFS = 10 months, OS = 21 months) (Langerbeins *et al*, 2014). A recent retrospective series of 46 patients with newly diagnosed RS (42 of which were DLBCL-subtype) treated with etoposide, vincristine, cyclophosphamide, doxorubicin and rituximab (EPOCH-R) showed limited efficacy with poor PFS (median 3·5 months) and OS (median 5·9 months) (Rogers *et al*, 2015). We demonstrate that survival following relapsed RS is poor. Patients are typically platinum refractory and survived for a median of just over 2 months after relapse.

Although small numbers limited our subgroup analysis, our results were consistent with the prior published data of prognostic scoring systems. Rossi *et al* (2011) demonstrated the prognostic impact of *TP53* disruption in a retrospective series of 86 patients. We demonstrate for the first time within a prospective clinical trial, that patients with RS and *TP53* disruption have an inferior survival to those without *TP53* disruption. The fact that 50% of patients with RS have *TP53* disruption means that novel drugs for this condition need to act independently of TP53.

We also demonstrate for the first time in a prospective clinical trial that patients with treatment-naïve CLL at the time of transformation have a superior outcome compared to those that are previously treated. This is probably related to immunosuppression, prior myelotoxicity and the selection and subsequent transformation of immunochemotherapy-resistant subclones.

Results from PET-CT are consistent with data in the literature that supports the utility of this imaging to exclude RS and direct biopsy. A number of studies have shown that using an SUVmax cut off of 5·0 could detect DLBCL-type

Table II. Grade 3-4 adverse events and relationship to CHOP and/or ofatumumab

AE category and term	AE (SAE) unrelated or unlikely to be related to CHOP or Ofatumumab	AE (SAE) possibly, probably or almost certainly related to CHOP or Ofatumumab	Total AE (SAE)
Blood and lymphatic			
AnaemiaNeutropenia	2 (2)	3 (1)	5 (3)
Thrombocytopenia	0	11 (6)	11 (6)
Febrile Neutropenia	0	2 (0)	2 (0)
	1 (1)	13 (7)	14 (8)
Gastrointestinal			
Abdominal pain	1 (1)	0	1 (1)
Vomiting	0	1 (0)	1 (0)
Other	3 (2)	2 (2)	5 (4)
General disorders			
Fatigue	1 (1)	0	1 (1)
Fever	0	4 (2)	4 (2)
Infusion related reaction	1 (0)	1 (1)	2 (1)
Malaise	1 (1)	0	1 (1)
Infections and infestations			
Pneumonia	4 (3)	3 (1)	7 (4)
Sepsis	2 (1)	7 (7)	7 (7)
Other	2 (1)	6 (5)	8 (6)
Respiratory; thoracic and mediastinal			
Aspiration	1 (1)	0	1 (1)
Bronchiectasis	0	1 (0)	1 (0)
Pleural effusion	1 (1)	0	1 (1)
Skin and subcutaneous tissue			
Hyperhidrosis	0	1 (0)	1 (0)
Rash	0	2 (0)	2 (0)
Urticaria	0	1 (0)	1 (0)
Other	6 (3)	2 (0)	8 (3)
Total Grade 3 or Grade 4 AEs (SAEs)	25 (18)	60 (32)	85 (50)

AE, adverse event; SAE, serious adverse event; CHOP, cyclophosphamide, doxorubicin, vincristine, prednisolone.

Richter transformation with a high sensitivity (91%) and a high negative predictive value (97%) (Bruzzi *et al*, 2006; Papajík *et al*, 2014). This is confirmed by our data showing that the lowest SUVmax was 5·85. EBV detected by staining for LMP-1 or by in situ hybridization of EBER transcripts, has been variably reported (0–15%) in RS (Rossi *et al*, 2008, 2011; Parikh *et al*, 2013). We noted only two cases of EBV expression in the DLBCL FFPE tissue (7%) and neither of these 2 cases were heavily pre-treated. This data suggests a limited role for EBV in the pathogenesis of RS overall.

Unfortunately, only limited numbers of paired samples of sufficient DNA quality were available, but our results showed a lower incidence of clonally unrelated cases than previously described (Rossi *et al*, 2011). Compared to these retrospective studies, our cohort was biased in the sense that only patients fit enough to receive combination chemo-immunotherapy were recruited into the study.

In conclusion, CHOP-O demonstrates an ORR of 46% at the end of induction and a median PFS of just over 6 months. The CHOP-OR study demonstrates the feasibility of undertaking clinical trials in this rare and complex patient group. Importantly, it clearly establishes the modest efficacy and short response duration of CHOP with induction and maintenance ofatumumab in newly diagnosed RS. Although not compared in a prospective randomized clinical trial, we were not able to demonstrate a clear improvement in outcome compared to patients treated with CHOP-R in other phase II trials' although the regimen studied is probably non-inferior to CHOP-R. Our data therefore confirm that standard immunochemotherapy for *bona fide* unselected RS is inadequate.

It is encouraging that novel agents are being investigated in RS. The second generation Bruton Tyrosine Kinase (BTK) inhibitor ACP196 (NCT02029443), the Exportin 1 (XPO1) inhibitor selinexor (NCT02138786), PD1 checkpoint inhibitors (NCT02332980) and the BCL2 targeted DNA inhibitor oligonucleotide PNT2258 (NCT02378038) are all currently in early phase clinical trials. It is the authors' hope that the results of our relatively large national trial will promote further national and international collaborative efforts to investigate novel treatments for RS to improve outcomes for these patients.

Table III. Management of patients fit for ongoing treatment at relapse

Patient	Number of CHOP-O cycles / maintenance	Post CHOP-O therapy	Patient status	Time from salvage to death (d)	Cause of death
Platinum-based salvage treatment					
1	1 CHOP-O then PD	2 cycles R-DHAP	Dead	76	PD
2	4 CHOP-O then PD	2 cycles DHAP to PR then IFRT to CR; consolidated with FMC Allogenic SCT. Died in remission (viral pneumonitis complicating GVHD therapy).	Dead	692	PD
3	4 CHOP-O then PD	2 cycles R-ICE	Dead	144	PD
4	6 CHOP-O then PD	1 cycle R-ESHAP	Dead	57	PD
5	6 CHOP-O, progressed on maintenance O	1 cycle R-ICE - >1 cycle GEM-P- > 1 cycle FMD. No response.	Dead	106	PD
6	4 CHOP-O then PD	1 cycle R-ICE then 1 cycle R-ESHAP. No response.	Dead	37	PD
7	6 CHOP-O, progressed on maintenance O	3 cycles R-ICE ->PD. Selinexor clinical trial ->PD. Started ACP196 trial.	Alive with PD (22 June 2015)	Alive at 35 d	N/A
Other salvage treatment					
8	6 CHOP-O, progressed on maintenance O	Radiotherapy, autologous CD19-specific T-cells with pre-conditioning and IL2 on phase 1 trial, then ibrutinib	Alive (24 April 2015)	Alive at 646 d	N/A
9	4 CHOP-O then PD	Alemtuzumab given pre-SCT; however halted due to CMV reactivation, subsequent PD	Dead	66	PD
10	5 cycles of CHOP-O. Stopped due to O intolerance	Repeat BMAT: 90% CLL with no evidence of RS. Known *TP53* deletion: pulse of dexamethasone then alemtuzumab and HDMP	Dead	145	Traumatic SDH
11	6 CHOP-O, progressed on maintenance O	1 cycle IVE to date	Alive (27 April 2015)	Alive at 24 d	N/A

CHOP-O, cyclophosphamide, doxorubicin, vincristine, prednisolone, ofatumumab; O, ofatumumab (R)-DHAP, (rituximab) dexamethasone, high dose cytarabine, cisplatin; R-ICE, rituximab, ifosfamide, carboplatin, etoposide; R-ESHAP, rituximab, etoposide, high dose cytarabine, methylprednisolone, cisplatin; GEM-P, gemcitabine, cisplatin, methylprednisolone; BEAM, BCNU (carmustine), etoposide, cytarabine, melphalan; FMD, fludarabine, mitoxantrone, dexamethasone, ACP196, acalabrutinib; IL2, interleukin 2; SCT, stem cell transplantation; IVE, ifosfamide, epirubicin, etoposide; FMC, fludarabine, melphalan, alemtuzumab; HDMP: high dose methylprednisolone; CR, complete remission; SD, stable disease; PD, progressive disease; CMV, cytomegalovirus; SDH, subdural haemorrhage; BMAT: bone marrow aspirate and trephine biopsy; GVHD, graft-versus-host disease; IFRT, involved field radiotherapy.

Source of funding

The study is sponsored by the University of Oxford, endorsed by CTAAC and was adopted into the NIHR portfolio. Sites were able to access network (CLRN/NCRN) support. GlaxoSmithKline (GSK) provided Ofatumumab free of charge for use in the trial and additional research grant funding.

Ethical approval

Obtained from the National Research Ethics Service (NRES) Committee South Central – Oxford A. REC reference number: 10/H0604/85. UK CLL BioBank has ethical approval to collect samples from all NCRN associated clinical studies.

Acknowledgements

This work was supported by the Oxford Partnership Comprehensive Biomedical Research Centre with funding from the Department of Health National Institute of Health Research (NIHR) Biomedical Research Centre funding scheme. The views expressed in this publication are those of the authors and not necessarily those of the Department of Health. The CHOP-OR study group acknowledges the support of the National Institute for Health Research, through the National Cancer Research Network. TE is funded by the Julian Starmer-Smith lymphoma fund. GC acknowledges support by the National Institute for Health Research (NIHR) Oxford Biomedical Research Centre Programme. The views expressed are those of the authors and not necessarily those of the funding bodies.

Author contributions

A.S., P.H. and A.P. made substantial contributions to conception and design. A.B., S.D., G.F., C.F., J.G., C.H., H.M.C., J.M., P.H., A.P., G.C. and A.S. provided patients for the study. T.J.L., G.C., T.E., R.C., C.R., B.S. and L.B. were involved in collection and assembly of data. T.E., A.S., R.C., C.R., S.L., E.S. and A.W. were involved in data analysis and interpretation. T.E. drafted the manuscript. All authors were involved in critically revising the manuscript and the final approval.

Conflict of Interest

A.S., T.E., A.B., A.P., G.F., S.D. have received consultancy honoraria from GSK. A.P. and A.S. has received research funding from GSK. P.H., G.F. and S.D. have received speaker fees for GSK.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig S1. Summary of Recruitment.

Fig S2. Results of PET-CT.

Fig S3. Additional survival analyses according to defined subgroups.

References

Barth, M.J., Hernandez-Ilizaliturri, F.J., Mavis, C., Tsai, P.-C., Gibbs, J.F., Deeb, G. & Czuczman, M.S. (2012) Ofatumumab demonstrates activity against rituximab-sensitive and -resistant cell lines, lymphoma xenografts and primary tumour cells from patients with B-cell lymphoma. *Br J Haematol*, **156**, 490–498.

Bockorny, B., Codreanu, I. & Dasanu, C.A. (2012) Hodgkin lymphoma as Richter transformation in chronic lymphocytic leukaemia: a retrospective analysis of world literature. *Br J Haematol*, **156**, 50–66.

Bruzzi, J.F., Macapinlac, H., Tsimberidou, A.M., Truong, M.T., Keating, M.J., Marom, E.M. & Munden, R.F. (2006) Detection of Richter's transformation of chronic lymphocytic leukemia by PET/CT. *J Nucl Med*, **47**, 1267–1273.

Campbell, M.J., Zelenetz, A.D., Levy, S. & Levy, R. (1992) Use of family specific leader region primers for PCR amplification of the human heavy chain variable region gene repertoire. *Mol Immunol*, **29**, 193–203.

Cheson, B.D., Pfistner, B., Juweid, M.E., Gascoyne, R.D., Specht, L., Horning, S.J., Coiffier, B., Fisher, R.I., Hagenbeek, A., Zucca, E., Rosen, S.T., Stroobants, S., Lister, T.A., Hoppe, R.T., Dreyling, M., Tobinai, K., Vose, J.M., Connors, J.M., Federico, M. & Diehl, V. (2007) Revised response criteria for malignant lymphoma. *J Clin Oncol*, **25**, 579–586.

Chigrinova, E., Rinaldi, A., Kwee, I., Rossi, D., Rancoita, P.M.V., Strefford, J.C., Oscier, D., Stamatopoulos, K., Papadaki, T., Berger, F., Young, K.H., Murray, F., Rosenquist, R., Greiner, T.C., Chan, W.C., Orlandi, E.M., Lucioni, M., Marasca, R., Inghirami, G., Ladetto, M., Forconi, F., Cogliatti, S., Votavova, H., Swerdlow, S.H., Stilgenbauer, S., Piris, M.A., Matolcsy, A., Spagnolo, D., Nikitin, E., Zamò, A., Gattei, V., Bhagat, G., Ott, G., Zucca, E., Gaidano, G. & Bertoni, F. (2013) Two main genetic pathways lead to the transformation of chronic lymphocytic leukemia to Richter syndrome. *Blood*, **122**, 2673–2682.

Coiffier, B., Lepage, E., Briere, J., Herbrecht, R., Tilly, H., Bouabdallah, R., Morel, P., Van Den Neste, E., Salles, G., Gaulard, P., Reyes, F., Lederlin, P. & Gisselbrecht, C. (2002) CHOP chemotherapy plus rituximab compared with CHOP alone in elderly patients with diffuse large-B-cell lymphoma. *N Engl J Med*, **346**, 235–242.

Dabaja, B.S., O'Brien, S.M., Kantarjian, H.M., Cortes, J.E., Thomas, D.A., Albitar, M., Schlette, E.S., Faderl, S., Sarris, A., Keating, M.J. & Giles, F.J. (2001) Fractionated cyclophosphamide, vincristine, liposomal daunorubicin (daunoXome), and dexamethasone (hyperCVXD) regimen in Richter's syndrome. *Leuk Lymphoma*, **42**, 329–337.

Eyre, T.A., Clifford, R., Roberts, C., Boyle, L., Francis, A., Schuh, A. & Dutton, S.J. (2015) Single arm NCRI phase II study of CHOP in combination with Ofatumumab in induction and maintenance for patients with newly diagnosed Richter's syndrome. *BMC Cancer*, **15**, 52.

Falchi, L., Keating, M.J., Marom, E.M., Truong, M.T., Schlette, E.J., Sargent, R.L., Trinh, L., Wang, X., Smith, S.C., Jain, N., Estrov, Z., O'Brien, S., Wierda, W.G., Lerner, S. & Ferrajoli, A. (2014) Correlation between FDG/PET, histology, characteristics, and survival in 332 patients with chronic lymphoid leukemia. *Blood*, **123**, 2783–2790.

Green, T.M., Young, K.H., Visco, C., Xu-Monette, Z.Y., Orazi, A., Go, R.S., Nielsen, O., Gadeberg, O.V., Mourits-Andersen, T., Frederiksen, M., Pedersen, L.M. & Moller, M.B. (2012) Immunohistochemical double-hit score is a strong predictor of outcome in patients with diffuse large B-cell lymphoma treated with rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone. *J Clin Oncol*, **30**, 3460–3467.

Hamblin, T.J., Davis, Z., Gardiner, A., Oscier, D.G. & Stevenson, F.K. (1999) Unmutated Ig V (H) genes are associated with a more aggressive form of chronic lymphocytic leukemia. *Blood*, **94**, 1848–1854.

Hans, C.P., Weisenburger, D.D., Greiner, T.C., Gascoyne, R.D., Delabie, J., Ott, G., Müller-Hermelink, H.K., Campo, E., Braziel, R.M., Jaffe, E.S., Pan, Z., Farinha, P., Smith, L.M., Falini, B., Banham, A.H., Rosenwald, A., Staudt, L.M., Connors, J.M., Armitage, J.O. & Chan, W.C. (2004) Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. *Blood*, **103**, 275–282.

Langerbeins, P., Busch, R., Anheier, N., Dürig, J., Bergmann, M., Goebeler, M.-E., Hurtz, H.-J., Stauch, M.B., Stilgenbauer, S., Döhner, H., Fink, A.-M., Cramer, P., Fischer, K., Wendtner, C.-M., Hallek, M. & Eichhorst, B. (2014) Poor efficacy and tolerability of R-CHOP in relapsed/refractory chronic lymphocytic leukemia and Richter transformation. *Am J Hematol*, **89**, 239–243.

Morton, L.M., Wang, S.S., Devesa, S.S., Hartge, P., Weisenburger, D.D. & Linet, M.S. (2006) Lymphoma incidence patterns by WHO subtype in the United States, 1992-2001. *Blood*, **107**, 265–276.

Papajík, T., Mysliveček, M., Urbanová, R., Buriánková, E., Kapitáňová, Z., Procházka, V., Turcsányi, P., Formánek, R., Henzlová, L., Flodr, P., Jarošová, M. & Indrák, K. (2014) 2-[18F]fluoro-2-deoxy-D-glucose positron emission tomography/computed tomography examination in patients with chronic lymphocytic leukemia may reveal Richter transformation. *Leuk Lymphoma.*, **55**, 314–319.

Parikh, SA, Rabe, K.G., Call, T.G., Zent, C.S., Habermann, T.M., Ding, W., Leis, J.F., Schwager, S.M., Hanson, C. a, Macon, W.R., Kay, N.E., Slager, S.L. & Shanafelt, T.D. (2013) Diffuse large B-cell lymphoma (Richter syndrome) in patients with chronic lymphocytic leukaemia (CLL): a cohort study of newly diagnosed patients. *Br J Haematol*, **162**, 774–782.

Rogers, K.A., Salem, G., Stephens, D.M., Andritsos, L.A., Awan, F.T., Byrd, J.C., Flynn, J.M., Maddocks, K.J., Huang, Y., Ruppert, A.S. & Jones, J.A. (2015) A single-institution retrospective cohort study of patients treated with R-EPOCH for Richter's transformation of chronic lymphocytic leukemia. *Blood*, **126**, 2951.

Rossi, D., Cerri, M., Capello, D., Deambrogi, C., Rossi, F.M., Zucchetto, A., De Paoli, L., Cresta, S., Rasi, S., Spina, V., Franceschetti, S., Lunghi, M., Vendramin, C., Bomben, R., Ramponi, A., Monga, G., Conconi, A., Magnani, C., Gattei, V. & Gaidano, G. (2008) Biological and clinical risk factors of chronic lymphocytic leukaemia transformation to Richter syndrome. *Br J Haematol*, **142**, 202–215.

Rossi, D., Spina, V., Deambrogi, C., Rasi, S., Laurenti, L., Stamatopoulos, K., Arcaini, L., Lucioni, M., Rocque, G.B., Xu-Monette, Z.Y., Visco, C., Chang, J., Chigrinova, E., Forconi, F., Marasca, R., Besson, C., Papadaki, T., Paulli, M., Larocca, L.M., Pileri, S.A., Gattei, V., Bertoni, F., Foà, R., Young, K.H. & Gaidano, G. (2011) The genetics of Richter syndrome reveals disease heterogeneity and predicts survival after transformation. *Blood*, **117**, 3391–3401.

Tadmor, T., Shvidel, L., Bairey, O., Goldschmidt, N., Ruchlemer, R., Fineman, R., Rahimi-Levene, N., Herishanu, Y., Yuklea, M., Arad, A., Aviv, A. & Polliack, A. (2014) Richter's transformation to diffuse large B-cell lymphoma: a retrospective study reporting clinical data, outcome, and the benefit of adding rituximab to chemotherapy, from the Israeli CLL Study Group. *Am J Hematol*, **89**, 218–222.

Teeling, J.L., Mackus, W.J.M., Wiegman, L.J.J.M., van den Brakel, J.H.N., Beers, S.A., French, R.R., van Meerten, T., Ebeling, S., Vink, T., Slootstra, J.W., Parren, P.W.H.I., Glennie, M.J. & van de Winkel, J.G.J. (2006) The biological activity of human CD20 monoclonal antibodies is linked to unique epitopes on CD20. *J Immunol*, **177**, 362–371.

Tsimberidou, A.M., O'Brien, S.M., Cortes, J.E., Faderl, S., Andreeff, M., Kantarjian, H.M., Keating, M.J. & Giles, F.J. (2002) Phase II study of fludarabine, cytarabine (Ara-C), cyclophosphamide, cisplatin and GM-CSF (FACPGM) in patients with Richter's syndrome or refractory lymphoproliferative disorders. *Leuk Lymphoma*, **43**, 767–772.

Tsimberidou, A.M., Kantarjian, H.M., Cortes, J., Thomas, D.A., Faderl, S., Garcia-Manero, G., Verstovsek, S., Ferrajoli, A., Wierda, W., Alvarado, Y., O'Brien, S.M., Albitar, M., Keating, M.J. & Giles, F.J. (2003) Fractionated cyclophosphamide, vincristine, liposomal daunorubicin, and dexamethasone plus rituximab and granulocyte-macrophage-colony stimulating factor (GM-CSF) alternating with methotrexate and cytarabine plus rituximab and GM-CSF in patients with Rich. *Cancer*, **97**, 1711–1720.

Tsimberidou, A.-M., O'Brien, S., Khouri, I., Giles, F.J., Kantarjian, H.M., Champlin, R., Wen, S., Do, K.-A., Smith, S.C., Lerner, S., Freireich, E.J. & Keating, M.J. (2006) Clinical outcomes and prognostic factors in patients with Richter's syndrome treated with chemotherapy or chemoimmunotherapy with or without stem-cell transplantation. *J Clin Oncol*, **24**, 2343–2351.

Tsimberidou, A.M., Wierda, W.G., Plunkett, W., Kurzrock, R., O'Brien, S., Wen, S., Ferrajoli, A., Ravandi-Kashani, F., Garcia-Manero, G., Estrov, Z., Kipps, T.J., Brown, J.R., Fiorentino, A., Lerner, S., Kantarjian, H.M. & Keating, M.J. (2008) Phase I-II study of oxaliplatin, fludarabine, cytarabine, and rituximab combination therapy in patients with Richter's syndrome or fludarabine-refractory chronic lymphocytic leukemia. *J Clin Oncol*, **26**, 196–203.

Tsimberidou, A.M., Wierda, W.G., Wen, S., Plunkett, W., O'Brien, S., Kipps, T.J., Jones, J.A., Badoux, X., Kantarjian, H. & Keating, M.J. (2013) Phase I-II clinical trial of oxaliplatin, fludarabine, cytarabine, and rituximab therapy in aggressive relapsed/refractory chronic lymphocytic leukemia or Richter syndrome. *Clin Lymphoma Myeloma Leuk*, **13**, 568–574.

Wierda, W.G., Kipps, T.J., Mayer, J., Stilgenbauer, S., Williams, C.D., Hellmann, A., Robak, T., Furman, R.R., Hillmen, P., Trneny, M., Dyer, M.J.S., Padmanabhan, S., Piotrowska, M., Kozak, T., Chan, G., Davis, R., Losic, N., Wilms, J., Russell, C.A. & Osterborg, A. (2010) Ofatumumab as single-agent CD20 immunotherapy in fludarabine-refractory chronic lymphocytic leukemia. *J Clin Oncol*, **28**, 1749–1755.