A clinician's guide to double hit lymphomas

Chan Yoon Cheah, Yasuhiro Oki, Jason R. Westin and Francesco Turturro

Department of Lymphoma/Myeloma, University of Texas MD Anderson Cancer Center, Houston, TX, USA

Summary

Double hit lymphomas (DHL) represent a subset of highly aggressive B-cell malignancies characterized by the presence of recurrent cytogenetic rearrangements affecting *MYC* and either *BCL2* and/or *BCL6*. Recent studies have expanded the concept to include MYC/BCL2 protein co-expressing lymphomas. Around 5–10% of diffuse large B-cell lymphomas are 'double hit' using the cytogenetic definition, whilst around 30–40% are MYC/BCL2 protein co-expressing. In this review, we provide a comprehensive overview of this condition written with the practicing clinician in mind, covering the definition and classification, when DHL should be suspected and how to make the diagnosis, the prognostic factors and a detailed discussion of recent evidence regarding optimal therapy. In particular, we discuss choice of induction regimen, the role of central nervous system-directed prophylaxis, stem cell transplantation and relapsing or refractory disease and provide our opinions based on the currently available evidence. Finally, we highlight some of the more exciting therapies currently in development for this highly aggressive disease.

Keywords: double-hit lymphoma, diffuse large B-cell lymphoma, B-cell lymphoma unclassifiable, triple-hit lymphoma, *MYC*.

Recurrent chromosomal translocations are common in patients with B-cell malignancies (Yunis *et al*, 1984; Offit *et al*, 1991). Such translocations frequently juxtapose oncogenes with immunoglobulin loci and can be considered lymphoma-initiating events. Amongst the best studied of these is *MYC;* the prototypic example of an *MYC*-driven lymphoma is rearrangement of chromosome 8q24 in Burkitt lymphoma (BL). When *MYC* rearrangements occur simultaneously with other translocation partners, such as *BCL2* or *BCL6*, the resultant lymphomas have distinctive biology and highly aggressive clinical behaviour and have been termed double hit lymphomas (DHL). Given the rarity and relatively recent awareness of this entity, prospective clinical trials have proven challenging to perform. As such, most existing data is drawn from retrospective studies or subgroup analyses from prospectively treated cohorts of patients with diffuse large B-cell lymphoma (DLBCL). The purpose of this review is to synthesize these data into a comprehensive, clinically focused overview of this challenging group of lymphomas, covering the biology, diagnostic considerations, clinical features, treatment and prognostic factors.

Correspondence: Professor Francesco Turturro, Department of Lymphoma/Myeloma, University of Texas MD Anderson Cancer Center, 1515 Holcombe Blvd, Houston, TX 77030, USA.
E-mail: fturturro@mdanderson.org

MYC biology and its role in lymphomas

MYC is a proto-oncogene that produces the transcription factor MYC (also termed c-Myc). Thus *MYC* regulates around 10% of human genes, with downstream targets influencing cellular proliferation, DNA and protein synthesis and metabolism (Mationg-Kalaw *et al*, 2012). Elevated expression of *MYC* in tumour cells can result from chromosomal translocation, gene amplification, duplication and mutations (Sewastianik *et al*, 2014). *MYC* both directly and indirectly activates *CCND2* and cyclin dependent kinases (CDK) and down-regulates cell cycle inhibitors, promoting the transition from G0 to S phase (Meyer & Penn, 2008). Further, *MYC* drives extensive reprogramming of the microRNA transcriptome, contributing to oncogenesis (Chang *et al*, 2008). *MYC* has indispensable roles in the formation and maintenance of germinal centres (Calado *et al*, 2012). Paradoxically, it had also been observed that elevated levels of *MYC* can result in apoptosis (Murphy *et al*, 2008). Recent data suggested that *MYC* appears to exert cellular effects through amplification of existing transcriptionally active genes, so that its action is contextual (Lin *et al*, 2012; Nie *et al*, 2012). This recognition helps to explain the somewhat contradictory actions described above.

MYC-*expressing lymphomas*

Although *MYC* rearrangements are *sine qua non* in BL, their presence is not specific for this diagnosis. Some studies have described *MYC* rearrangements in other haematological malignancies, including B-cell lymphoma, unclassifiable with features intermediate between DLBCL and BL (BCLU), DLBCL, T-lymphoblastic lymphoma, mantle cell lymphoma, plasmablastic lymphoma, acute lymphoblastic leukaemia, follicular lymphoma and chronic lymphocytic leukaemia, the

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latter two usually as a secondary genetic event in the setting of histological transformation to aggressive lymphoma (Thangavelu *et al*, 1990; Offit *et al*, 1991; Karsan *et al*, 1993). There are key differences in the biology of BL compared with *MYC*-rearranged non-BL. In BL, the translocation partner in around 80% of cases is the immunoglobulin heavy chain locus (*IGH*) on chromosome 14q21, with less common variant translocations involving the immunoglobulin light chain kappa (*IGK*) and lambda (*IGL*) on chromosomes 2p22 and 22q11 respectively (Swerdlow *et al*, 2008). In contrast, in non-BL, the translocation partner is often a non-immunoglobulin gene, such as *PAX5*, *BCL6*, *BCL11A* or *IKZF1* (Bertrand *et al*, 2007; Johnson *et al*, 2009). In BL, *MYC* rearrangements are often the sole abnormality; however, in DLBCL/BCLU, they usually occur as part of a complex karyotype with three or more abnormalities (Boerma *et al*, 2009). Furthermore, the gene expression profile in *MYC*-rearranged DLBCL features genes involved in the nuclear factor kappa-B pathway and anti-apoptotic cascades – whilst in BL, target genes involve cellular proliferation (Hummel *et al*, 2006). As a consequence of these differences, *MYC*-driven non-BL is typically an aggressive entity with poor response to therapy and inferior prognosis. Because BL is relatively rare, in clinical practice *MYC* rearrangements are encountered most frequently in the setting of DLBCL (in which 5–16% of tumours bear *MYC* rearrangements) and BCLU (30–50% of tumours) (Klapper *et al*, 2008; Savage *et al*, 2009; Tibiletti *et al*, 2009).

Defining single, double and triple hit lymphomas

Patients with non-BL and *MYC* rearrangements *without* additional breaks in *BCL2* or *BCL6* are described as single hit lymphoma (SHL). These cases occur less frequently than DHL and evidence regarding the clinical relevance of the finding is mixed. SHL has been reported to confer an adverse prognosis similar to that seen in DHL, although not all studies agree on this point (Savage *et al*, 2009; Barrans *et al*, 2010; Valera *et al*, 2013). The German Molecular Mechanisms in Malignant Lymphoma network studied a series of patients with DHL ($n = 47$) and SHL ($n = 31$) and found similar molecular, morphological and clinical features, with no difference in survival between the two groups (Aukema *et al*, 2014). A Spanish group studied *MYC* abnormalities in 219 patients with DLBCL and found gains (19%), amplifications (2%), SHL (3%) and DHL (4%) (Valera *et al*, 2013). Amplifications, SHL and DHL all had negative impact on survival but *MYC* gains did not. However, in two other studies of patients with DLBCL, *MYC* breaks did not confer adverse prognosis unless *BCL2* breaks were also present (Green *et al*, 2012a; Johnson *et al*, 2012). Further studies are required in larger numbers of uniformly treated patients to resolve this issue.

The term 'double hit' was originally coined to describe lymphomas simultaneously bearing both *MYC* and *BCL2* breaks (Thangavelu *et al*, 1990; Karsan *et al*, 1993). More broadly, the term DHL refers to B-cell lymphomas with multiple activating oncogenes, one of them being *MYC*. *MYC/BCL2* DHL are the most common form of DHL by far, with *MYC/BCL6* DHL and *MYC/BCL2/BCL6* 'triple hit' lymphomas less frequent. In the largest series reported so far, 270 (87%) were *MYC/BCL2*, 16 (5%) were *MYC/BCL6* and 25 (8%) were *MYC/BCL2/BCL6* (Petrich *et al*, 2014). Rarer co-translocations involving genes such as *CCND1 (BCL1)*, *BCL3* and *PAX5* have also been reported (Mitelman *et al*, 2014).

Early publications defined DHL by cytogenetic evidence of translocations, using fluorescence *in situ* hybridization (FISH) (Johnson *et al*, 2009; Tibiletti *et al*, 2009; Tomita *et al*, 2009). DHL so defined is an aggressive disease that is typically chemo-refractory, associated with short survival and poor prognosis independent of the International Prognostic Index (IPI) (Le Gouill *et al*, 2007; Johnson *et al*, 2009; Barrans *et al*, 2010). Furthermore, several groups have demonstrated in large studies of patients with DLBCL that increased expression of both MYC and BCL2 protein by immunohistochemistry (hereafter referred to as MYC/BCL2 co-expressing lymphomas) have inferior survival compared with patients lacking these abnormalities – though their outcome may not be as poor as cytogenetic DHL (Johnson *et al*, 2012; Horn *et al*, 2013; Hu *et al*, 2013; Perry *et al*, 2014). A diagram representing the proposed relationship between DLBCL, BCLU, cytogenetic DHL and MYC/BCL2 co-expressing lymphomas is shown in Fig 1.

When should DHL be suspected, and when and how should patients be tested?

Due to the substantial prognostic and potential therapeutic implications of a diagnosis of DHL, rapid identification of patients is highly desirable. A key question for clinicians treating patients with lymphoma is when further testing with FISH is indicated. This question is commonly encountered in practice, as most patients with DHL have either DLBCL or BCLU, with the former being the most common non-Hodgkin lymphoma (NHL) in Western countries (Swerdlow *et al*, 2008). *MYC* rearrangements are found in up to half of patients with BCLU (Green *et al*, 2012b; Perry *et al*, 2013). *MYC* breaks are also identified as secondary genetic events in patients with indolent lymphoma that undergo histological transformation (Johnson *et al*, 2009; Tomita *et al*, 2009; Snuderl *et al*, 2010). Up to 33% of patients with immunoblastic DLBCL have *MYC* rearrangements (Horn *et al*, 2014). The prevalence of *MYC/BCL2* DHL in DLBCL has been estimated at approximately 5–16% when defined by FISH, but up to one-third of patients with DLBCL are MYC/BCL2 co-expressing (Green *et al*, 2012a; Johnson *et al*, 2012; Horn *et al*, 2013).

An overview of studies that have reported clinical features in patients with DHL is presented in Table I. The median age of onset is 60 years, though patients as young as 17 years and as old as 87 years have been reported. The male/female ratio is approximately 2 (Oki *et al*, 2014; Petrich *et al*, 2014).

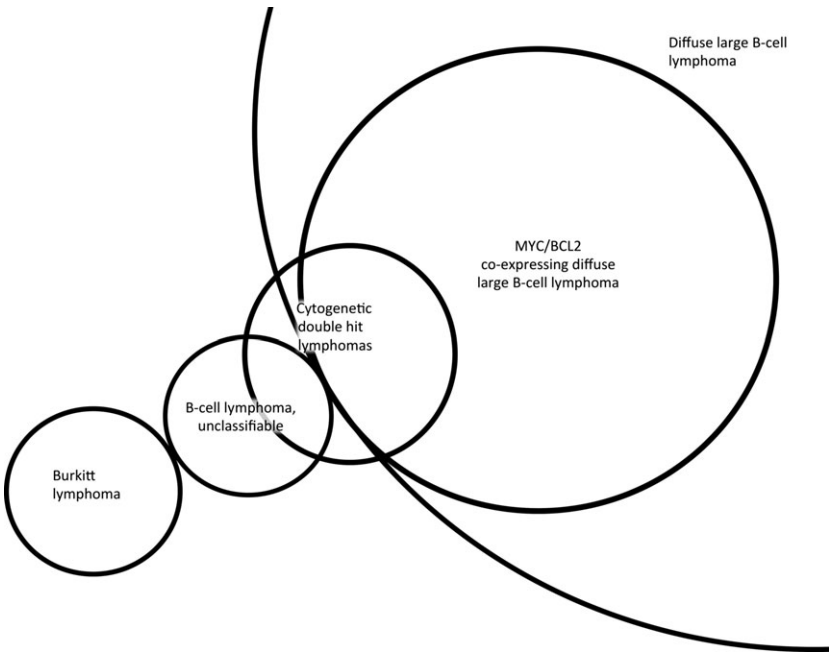

Fig 1. Venn diagram depicting relationship between B-cell lymphoma, unclassifiable, diffuse large B-cell lymphoma, Burkitt lymphoma, protein co-expressing lymphomas and cytogenetic double hit lymphomas.

Histologically transformed indolent lymphomas account for up to 20% of cases. The median Ki67 proliferation index is around 80%, lower than that seen in BL (typically 95–100%). Features of high tumour burden, such as advanced stage, raised serum lactate dehydrogenase (LDH), B symptoms and bone marrow involvement are all common at diagnosis. Unfortunately, these features either alone or in combination are not reliable indicators of underlying DHL. The lack of consistent clinical findings indicating which patients might be harbouring DHL has led some investigators to conclude that all patients with DLBCL should be investigated with FISH for *MYC* breaks (Landsburg *et al*, 2014). However, FISH requires specialized equipment and technical expertise and is time consuming and costly. Nuclear MYC protein staining by immunohistochemistry (hereafter referred to as MYC-IHC) is comparatively cheaper and becoming widely available in routine diagnostic laboratories; reproducibility is high, at least in large academic centres with reporting expertise (Johnson *et al*, 2012). In a resource-constrained environment it is therefore relevant to ask whether MYC-IHC can be used to screen patients for FISH testing, as a commercially available antibody exists (clone Y69 Epitomics, Burlingame, CA, USA). The studies addressing this specific question have shown that *MYC* rearrangements correlate highly with MYC protein expression (Tapia *et al*, 2011; Green *et al*, 2012b; Kluk *et al*, 2012; Horn *et al*, 2013). Horn *et al* (2014) examined the impact of *MYC*, *BCL2* and *BCL6* breaks (by FISH) and protein expression (by IHC) within a subset of patients with DLBCL treated on the RICOVER-60 (Rituximab with CHOP [cyclophosphamide, daunorubicin, Oncovin, prednisone] over 60 Years) study. Of a total of 949 patients, FISH data was available in 442 (47%) and MYC staining by IHC was interpretable in 283 (30%). Amongst *MYC*-rearranged cases, 18/26 (69%) were positive by IHC using a cut-off of ≥40% compared with 67/241 (28%) of non-rearranged cases. In this dataset, using IHC to screen patients for FISH testing, 8 (31%) cases of *MYC/BCL2* DHL would have been missed. In contrast, Green *et al* (2012b) studied 205 DLBCL biopsy specimens by both IHC (using the same antibody) and FISH and determined that ≥70% MYC-IHC+ lymphoma cells was the optimal cut-off, resulting in 100% sensitivity and 93% specificity for the presence of *MYC* breaks. Taken together, these studies demonstrate that MYC-IHC has high sensitivity and potential as a screening test for *MYC* breaks, however the optimal threshold remains to be defined.

Recommendation

All patients with DLBCL should be tested for MYC and BCL2 by IHC, as their presence defines protein co-expressing lymphoma. Ideally, all patients with DLBCL would have FISH testing for *MYC* rearrangements. A compromise would be to use MYC-IHC to screen patients for further testing with FISH. Although the precise cut-off is uncertain, we propose a value of MYC-IHC ≥30% as a trigger to perform FISH, to minimize false negatives. Patients found to have *MYC* rearrangements should have subsequent FISH for *BCL2* and *BCL6* rearrangements. All patients with BCLU, immunoblastic DLBCL and transformed indolent lymphomas should have FISH for *MYC* rearrangements.

Are MYC/BCL2 co-expressing lymphomas the same as FISH-defined MYC/BCL2 DHL?

Patients with DLBCL whose tumours express both MYC/BCL2 co-expressing lymphomas are more common than

Table I. Baseline characteristics of patients with cytogenetic double hit lymphomas in selected studies.

Study	*n* DHL/total (%)	Male (%)	Median age, years	Prior indolent lymphoma	Stage III/IV (%)	LDH >ULN (%)	B symptoms (%)	>1 EN site (%)	CNS+ (%)	BM+ (%)	IPI ≥4 (%)	GCB type (%)	Median ki67, %
Johnson *et al* (2009)	54/54	32 (59)	NR	20 (37)	41 (76)	27 (50)	NR	19 (35)	NR	32 (59)	14 (26)	34 (63)	NR
Niitsu *et al* (2009)	19/394 (4·8)	10 (53)	61	NR	19 (100)*	19 (100)*	11 (58)	17 (89)	4 (21)	16 (84)*	17 (89)	15 (79)	80
Tomita *et al* (2009)	27/27† (100)	15/27 (56)	51	5/23 (22)	22/23 (96)	25/27 (92)	10/22 (45)	15/23 (65)	2/23 (9)	15/23 (65)	20/23 (87)	18/19 (95)	70
Barrans *et al* (2010)	27/303‡ (8·6)	15 (43)	68·5	NR	21 (69)*	NR	NR	NR	NR	NR	10 (30)*	24 (77)*	76
Snuderl *et al* (2010)	20/60 (33)	11 (55)	63*	6 (30)	18 (90)	Med 3·5 × ULN*	NR	NR	4/11 (44)	10/17 (59)*	8 (40)	18 (90)	80
Pedersen *et al* (2012, 2014)	23/228 (10)	11 (48)	64	10 (42)	19 (83)	14/17 (82)	NR	8/17 (47)	NR	4/17 (24)	5 (29)	17/17 (100)*	70
Oki *et al* (2014)	129/129	84 (65)	62	14 (11)	109 (84)	68/99 (69)	NR	63 (49)	5 (4)	54 (42)	28 (26)	107/115 (93)	85
Petrich *et al* (2014)	311/311	181 (67)	60	67 (22)	255 (81)	236 (76)	139 (45)	87 (28)	23 (7)	129 (41)	85 (27)	181/209 (87)	NR

DHL, double-hit lymphoma; LDH, lactate dehydrogenase; ULN, upper limit of normal; EN, extranodal; CNS, central nervous system; BM, bone marrow; NR, not reported; IPI, international prognostic index; med, median; GCB, germinal centre B-cell.

Denominator provided to indicate that some patients were missing data for this variable.

*Denotes statistically different from non-MYC rearranged or non-double hit patients in same study.

†Twenty-three patients had double hit lymphoma, four patients had acute lymphoblastic leukemia.

‡Reports characteristics of *MYC*-rearranged *versus* non-*MYC*-rearranged patients, 27/35 (75%) were *MYC/BCL2*-rearranged.

cytogenetic DHL, as mechanisms other than translocation (such as amplification, mutation and microRNA-dependent mechanisms) can result in over-expression of these proteins (Ott *et al*, 2013). MYC/BCL2 co-expressing lymphomas account for 21–34% of DLBCL; their outcomes appear inferior to non-overexpressing cases but not as poor as cytogenetic MYC/BCL2 DHL (Green *et al*, 2012a,b; Johnson *et al*, 2012; Kluk *et al*, 2012; Hu *et al*, 2013; Valera *et al*, 2013; Fiskvik *et al*, 2014; Perry *et al*, 2014; Yan *et al*, 2014; Zhou *et al*, 2014) (Fig 2). The precise cut-off for a positive result has varied slightly between studies, but ≥40% for MYC-IHC and ≥70% for BCL2-IHC are typical (Green *et al*, 2012a; Johnson *et al*, 2012; Hu *et al*, 2013). Whether overexpression of MYC, BCL2 or BCL6 in isolation is adversely prognostic remains a subject of debate due to inconsistencies in the existing literature. In several studies, over-expression of MYC *or* BCL2 protein has not had a negative impact on survival (Green *et al*, 2012a; Johnson *et al*, 2012; Hu *et al*, 2013), however, in other studies, over-expression of MYC, BCL2 or BCL6 protein was adversely prognostic (Horn *et al*, 2013; Visco *et al*, 2013; Tzankov *et al*, 2014). Further, in contrast to cytogenetic *MYC/BCL2* DHL (which is usually germinal centre B-cell origin) 63–76% of MYC/BCL2 co-expressing lymphomas have activated B-cell origin (Green *et al*, 2012a; Johnson *et al*, 2012; Hu *et al*, 2013).

What are the prognostic factors for patients with DHL?

Patients with cytogenetic *MYC/BCL2* DHL have an aggressive course with poorer responses to standard therapies and shorter progression-free (PFS) and overall survival (OS) compared with *MYC* germline DLBCL, independent from clinical risk factors (Klapper *et al*, 2008; Johnson *et al*, 2009). The IPI has some prognostic relevance in DHL but limited discriminatory power, as elevated LDH, multiple extranodal sites of involvement and advanced stage are common – therefore most are categorized as high-risk (Petrich *et al*, 2014). Several other potential prognostic factors have been explored, however, given almost all reported series have been small, single centre and retrospective, findings have been somewhat inconsistent. Nonetheless, some common themes emerge. Two studies have found DHL patients with a *MYC-IGH* translocation partner (compared with non-immunoglobulin) to be associated with an adverse prognosis (Johnson *et al*, 2009; Pedersen *et al*, 2014). One of these studies also identified BCLU morphology as an adverse prognostic factor, however these patients were also more likely to have non-immunoglobulin translocation partners and marrow involvement (Johnson *et al*, 2009). Oki *et al* (2014) reported the outcomes of 129 patients with cytogenetic DHL treated at MD Anderson Cancer Center (MDACC) and developed a double-hit IPI (DHIPI) using two variables independently significant for adverse OS: performance status ≥2 and bone marrow involvement (Fig 3A). Non-*MYC* translocation (*BCL2*, *BCL6* or triple-hit lymphoma) and morphology (DLBCL or BCLU) had no influence on survival. Interestingly, the limited number of patients with stage I disease ($n = 5$) and grade 3B follicular lymphoma ($n = 2$) were all alive and free from progression at the time of reporting. Patients with stages II–IV did not have significantly different outcomes (Fig 3B) (Oki *et al*, 2014). A large multi-centre retrospective collaboration between 23 US academic centres reporting on 311 patients with cytogenetic DHL found white blood cell count $>10 \times 10^9$/l, LDH ≥ 3× upper limit of normal, stage III/IV and the presence of central nervous system (CNS) involvement to be prognostic (Petrich *et al*, 2014).

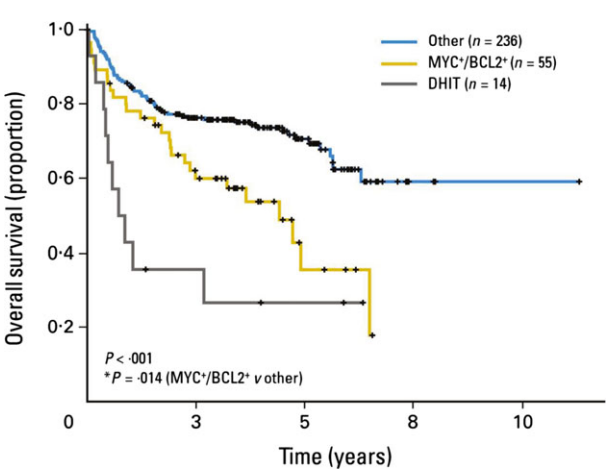

Fig 2. Overall survival of patients with diffuse large B-cell lymphoma and cytogenetic *MYC/BCL2* double hit lymphoma (DHIT, grey), MYC/BCL2 co-expressing lymphoma (yellow) and all remaining cases (blue). Reprinted with permission. © 2012 American Society of Clinical Oncology. All rights reserved: Johnson *et al* (2012).

What is the optimal treatment strategy for patients with DHL?

In this section we describe the currently available evidence informing treatment decisions for patients with DHL. A summary of selected studies is presented in Table II. It is noteworthy that most data are derived from retrospective series and, as such, are unavoidably limited by selection bias. We then provide our opinion on how these data should be translated in to clinical practice.

Choice of induction regimen

Patients with DHL have poor outcomes using standard therapy for DLBCL. In the MDACC series of patients treated with rituximab, cyclophosphamide, doxorubicin, vincristine and prednisolone (R-CHOP), only 23/57 (40%) achieved a complete response (CR). The 2-year PFS and OS rates were 25% and 41%, respectively (Oki *et al*, 2014). These disappointing figures are mirrored in other studies, with

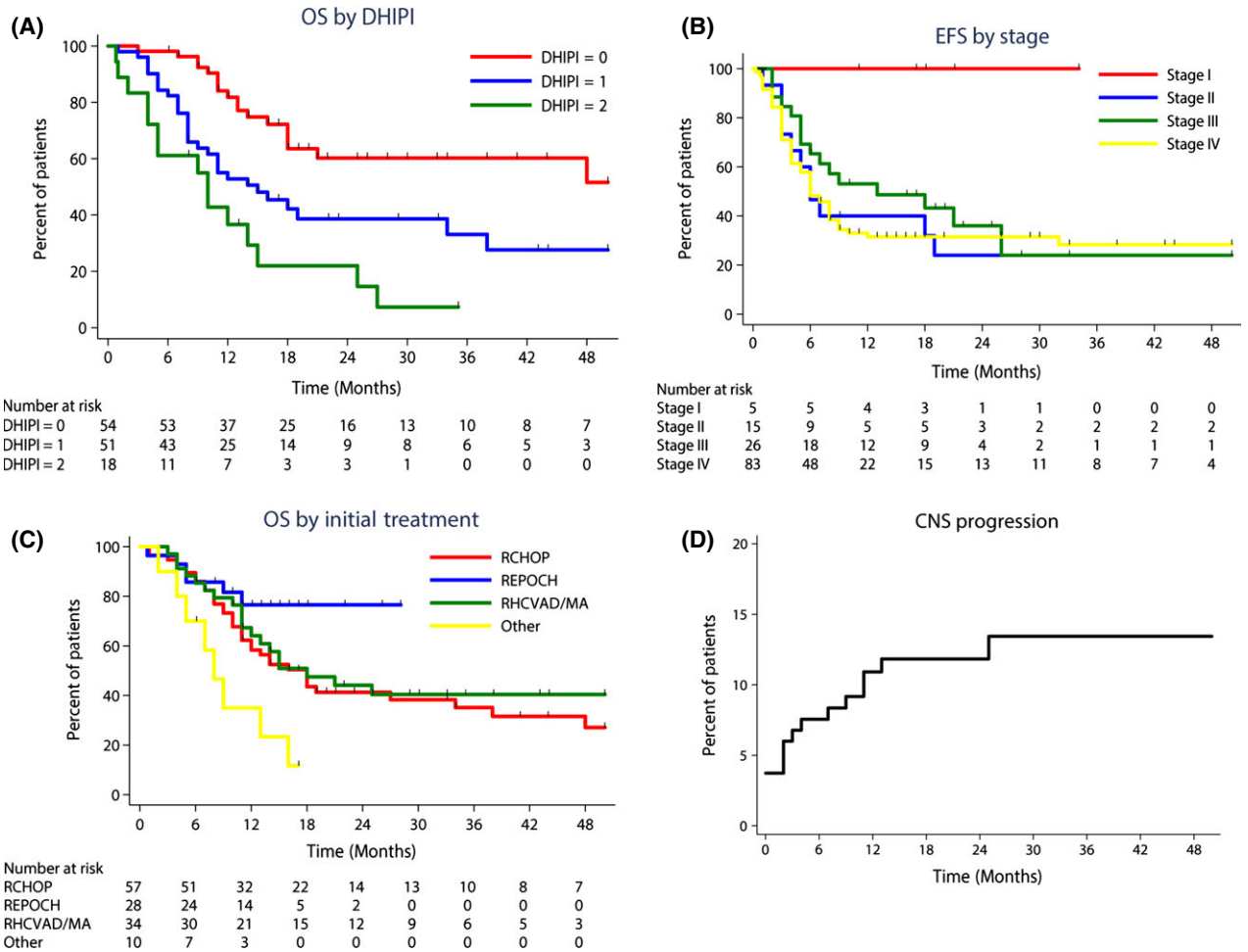

Fig 3. (A) Overall survival by DHIPI amongst patients with cytogenetic DHL (B) Event-free survival by Ann Arbor stage (C) Overall survival by induction regimen (D) Time to CNS progression, all from the in the MD Anderson Series. Reproduced from Oki *et al* (2014). DHIPI, double hit international prognostic index; DHL, double-hit lymphoma; OS, overall survival; EFS, event-free survival; R, rituximab; CHOP, cyclophosphamide, doxorubicin, vincristine, prednisone; EPOCH, etoposide, prednisone, doxorubicin, cyclophosphamide, prednisone; HCVAD/MA, hyper-fractionated cyclophosphamide, doxorubicin, vincristine, dexamethasone, methotrexate, cytarabine; CNS, central nervous system.

most patients dying of disease within 2 years of diagnosis (Le Gouill *et al*, 2007; Klapper *et al*, 2008; Johnson *et al*, 2009; Niitsu *et al*, 2009; Barrans *et al*, 2010; Snuderl *et al*, 2010; Green *et al*, 2012a; Hu *et al*, 2013; Pedersen *et al*, 2014; Perry *et al*, 2014; Yan *et al*, 2014; Zhou *et al*, 2014). The *MYC*-driven nature and propensity for CNS involvement of these tumours has prompted some clinicians to use the intensified regimens successful in BL, such as hyper-fractionated cyclophosphamide, doxorubicin, vincristine, dexamethasone, methotrexate, cytarabine with rituximab (R-HCVAD/MA) and cyclophosphamide, vincristine, doxorubicin, methotrexate, ifosfamide, etoposide, cytarabine with rituximab (R-CODOXMIVAC) as induction. No prospective, comparative data are available; however in the MDACC series, 23/34 (68%) patients treated with R-HCVAD/MA achieved a CR, superior to the 40% CR rate observed with R-CHOP ($P = 0{\cdot}017$) (Oki *et al*, 2014). However, this did not result in a significant improvement in subsequent disease control compared with R-CHOP, possibly due to the limited number of patients treated; hazard ratios (HR) for event-free survival (EFS) and OS were 0·61 [95% confidence interval (CI) 0·36–1·05, $P = 0{\cdot}074$] and 0·67 (95% CI 0·31–1·21, $P = 0{\cdot}30$) respectively. Patients treated with dose-adjusted rituximab, etoposide, prednisone, vincristine, cyclophosphamide and doxorubicin (DA-EPOCH-R) in the MDACC series appeared to achieve superior EFS [HR 0·37 (95% CI 0·18–0·77, $P = 0{\cdot}008$)] and trend toward improved OS [HR 0·47 (95% CI 0·19–1·14, $P = 0{\cdot}096$)] (Oki *et al*, 2014). The OS of patients with DHL by induction regimen is shown in Fig 3C. The large US multi-centre study included more patients treated with intensive regimens and found patients treated with R-CODOXMIVAC ($n = 41$), DA-EPOCH-R ($n = 57$) and R-HCVAD/MA ($n = 38$) all had superior PFS compared with patients treated with R-CHOP (median 21·6 vs. 7·8 months, $P = 0{\cdot}001$). An exploratory multivariate analysis, which included 10 prognostic factors by univariate analysis and

Table II. Outcomes for patients with double hit lymphoma according the induction regimen in selected studies.

Study	*n* (DHL)	DHL identification method	Induction	CR, %	Outcome
Johnson *et al* (2009)	54	FISH	CHOP (*n* = 23)	NR	Median OS 4·8 months
			R-CHOP (11)		Median OS 14·4 months
			HD chemo ± ASCT (6)		Median OS 3·6 months
			Palliation (14)		Median OS 1·2 months
Johnson *et al* (2012)	14	FISH	R-CHOP	NR	5-year OS 27%, PFS 18%
	55	IHC	R-CHOP		5-year OS 36%, PFS 32%
Oki *et al* (2014)	129	FISH	R-CHOP (57)	40	2-year OS 41%, EFS 25%
			R-EPOCH (28)	68†	2-year OS 76%, PFS 67%†
			R-HCVAD/MA (34)	68†	2-year OS 44%, EFS 32%
			Other (10)	60	2-year OS <10%, OS <12%
Petrich *et al* (2014)	311	FISH	R-CHOP (100)	48*	Median PFS 7·8 months
			R-EPOCH (64)	65*,†	Pooled median PFS 21·6 months†
			R-HCVAD/MA (65)	55*	
			R-CODOXMIVAC (42)	40*	
			R-ICE (9)	NR	NR
			Other (31)	30*	NR
Green *et al* (2012a)	11	FISH	R-CHOP	NR	3-year OS 46%, PFS 46%
	54	IHC	R-CHOP	70	3-year OS 43%, PFS 39%
Hu *et al* (2013)	10	FISH	R-CHOP	NR	Median OS *c.* 21 months*, PFS *c.* 15 months*
	157	IHC	R-CHOP		5-year OS 30%, PFS 27%
Horn *et al* (2013)	21	FISH‡	R-CHOP	NR	3-year OS 36%, EFS 38%

DHL, double-hit lymphoma; FISH, fluorescence *in situ* hybridization; IHC, immunohistochemistry; R, rituximab; CHOP, cyclophosphamide, doxorubicin, vincristine, prednisone; HD chemo, high dose chemotherapy; ASCT, autologous stem cell transplantation; EPOCH, etoposide, prednisone, doxorubicin, cyclophosphamide, prednisone; HCVAD/MA, hyper-fractionated cyclophosphamide, doxorubicin, vincristine, dexamethasone, methotrexate, cytarabine; CODOXMIVAC, cyclophosphamide, vincristine, doxorubicin, methotrexate, ifosfamide, etoposide, cytarabine; ICE, ifosfamide, carboplatin, etoposide; OS, overall survival; PFS, progression free survival; EFS, event free survival.

*Estimated from graph, precise figure not available.

†Statistically superior to R-CHOP in the same study.

‡Although detailed analysis of immunohistochemistry was provided in this paper, precise data on MYC/BCL2 co-expressing cases was not provided.

induction regimen, found that use of intensive induction improved OS compared with R-CHOP [HR = 0·53 (95% CI 0·29–0·98, *P* = 0·042] (Petrich *et al*, 2014). A meta-analysis of 401 patients also reported PFS advantage in favour of DA-EPOCH-R (*n* = 91) over R-CHOP (*n* = 180) (HR 0·64, 95% CI 0·42–0·92). In this analysis, R-HCVAD or R-CODOXMIVAC induction (combined *n* = 130) were not associated with prolongation of PFS, and no induction regimens were associated with improved OS (Howlett *et al*, 2014). Preliminary results from a prospective multicentre phase II study in 52 patients with *MYC*-rearranged DLBCL/BCLU (including 14 with *BCL2*-rearrangements) showed that, after a median follow up of 14 months, the PFS was an encouraging 87% (Dunleavy *et al*, 2014).

Recommendation

Because of the suboptimal outcomes of patients with DHL, all patients should be encouraged to enrol on prospective clinical trials where possible. Outside this setting, we recommend patients with DHL and suitable performance status and organ function receive dose adjusted DA-EPOCH-R. Patients unfit for intensive induction therapies and ineligible for trials may be treated with R-CHOP, though the probability of disease control and survival beyond 2 years is minimal.

What is the risk of CNS involvement in patients with DHL?

Cytogenetic DHL appears to have a propensity for CNS involvement. Early, smaller series suggested the risk was markedly increased (Kanungo *et al*, 2006; Le Gouill *et al*, 2007; Snuderl *et al*, 2010). However, in the two largest retrospective series (Oki *et al*, 2014; Petrich *et al*, 2014), 4–7% of patients had CNS involvement at baseline. In the MDACC series, the 3-year cumulative risk of CNS involvement was 13%; with the only predictive factor identified being DHIPI score [HR 2·14 (95% CI 1·08–4·22), *P* = 0·029] (Fig 3D). Amongst CNS-negative patients at diagnosis, those who received intrathecal methotrexate experienced a lower rate of

CNS progression (3-year incidence 5% vs. 15%, $P = 0{\cdot}017$) (Oki *et al*, 2014). Petrich *et al* (2014) did not specifically report on rates of CNS progression, but patients who received CNS-directed prophylaxis had improvement in OS. However, both analyses are limited by their retrospective nature and potential for bias. Savage *et al* (2014) studied 447 patients with DLBCL; 131 (29%) were MYC/BCL2 co-expressing and this finding was a significant independent predictor of CNS involvement (HR 3·76, $P = 0{\cdot}007$). Furthermore, the 2-year CNS-progression risk in patients with MYC/BCL2 co-expressing lymphomas and IPI 2–3 and 4–5 was 12·6% and 17·2% respectively, significantly higher than patients not co-expressing these proteins (Savage *et al*, 2014).

Recommendation

Patients with cytogenetic DHL should receive CNS-directed prophylaxis as part of their induction therapy. Our current policy is to give intrathecal methotrexate once per cycle. In DLBCL, the addition of 2–4 cycles of systemic high dose methotrexate at the completion of chemoimmunotherapy lowers risk of CNS-progression further; this could be considered in patients not receiving this as part of induction therapy (Cheah *et al*, 2014; Ferreri *et al*, 2014). Patients with MYC/BCL2 co-expressing lymphoma and IPI ≥2 should receive also CNS-directed prophylaxis (Savage *et al*, 2014).

Role of transplant

The role of autologous (auSCT) and/or allogeneic (alloSCT) stem cell transplantation in DHL remains unclear. It should be noted that the data addressing this issue are limited by guarantee-time bias in favour of patients surviving long enough to receive stem cell transplantation. In the MDACC series, 26 (20%) of patients had frontline transplantation, including three patients who received alloSCT. Most of this cohort comprised patients receiving induction with DA-EPOCH-R ($n = 14$, 54%). Despite these patients being selected by virtue of achieving an initial response to induction, and having adequate organ function and performance status, frontline transplant was not associated with a statistically significant prolongation of either EFS or OS (Oki *et al*, 2014). The US multi-centre collaboration also examined the impact of high dose therapy and stem cell rescue in patients in CR after induction therapy; they also reported no significant improvement in OS for those who received transplantation ($n = 39$: auSCT 28, alloSCT 11) compared with those observed ($n = 112$) ($P = 0{\cdot}14$) (Petrich *et al*, 2014). A subset analysis of 16 patients with MYC/BCL2 protein co-expressing lymphoma in the Southwestern Oncology Group (SWOG) S9704 study (in which patients with aggressive NHL received induction with CHOP ± R with randomization to auSCT or observation) was presented. After a median 127 months of follow up, auSCT was associated with a median PFS of 41 vs. 11 months in patients not consolidated. Only three patients in this study had *MYC/BCL2* cytogenetic DHL and their outcomes were poor (Puvvadda *et al*, 2014).

Recommendation

Acknowledging the limitations of the data, auSCT may be considered for patients with cytogenetic DHL and protein co-expressing lymphoma in CR following induction therapy, ideally in the setting of a prospective clinical trial. AlloSCT is currently unsupported by data in DHL, and is not recommended.

Role of radiation

Given that patients with DHL usually present with advanced stage disease and therapeutic failure is usually due to primary chemo-refractoriness or systemic disease progression, radiotherapy has a limited role. The empiric use of radiation may be reasonable in patients with advanced stage disease in selected circumstances, such as spinal cord compression, symptom palliation, hypermetabolic residual masses following completion of chemotherapy or sanctuary sites, such as the testis. In the MDACC series 12 patients (9%) received radiation as part of their initial therapy and the impact on outcome was not assessed due to the limited numbers of patients.

Relapsed and refractory disease

With currently available therapies, the outcome of patients with DHL who either fail to respond to induction or progress after initial response is particularly bleak. Amongst 79 such patients in the MDACC series, the 12-month post-progression survival was 20%, with only two patients (3%) remaining alive beyond 2 years (Oki *et al*, 2014). Petrich

Table III. New drugs in development with potential activity in *MYC*-driven and double hit lymphomas.

Class of drug	Examples	References	Phase	*n*	Population	ORR
Selective inhibitor of nuclear export (SINE)	Selinexor (KPT-330)	Gutierrez *et al* (2014)	I	28	R/R NHL	25%
BH3-mimetic	ABT-199 (GDC-0199)	Davids *et al* (2014)	I/II	44	R/R NHL	44%
BET bromodomain inhibitors	GSK525762	NCT01943851	I	*	R/R haematological cancers	*
	CPI-0610	NCT01949883	I	*	R/R NHL	*

ORR, objective response rate; R/R, relapsed/refractory; NHL, non-Hodgkin lymphomas.
*Indicates preliminary findings not reported at the time of writing.

et al (2014) reported that, amongst patients who received salvage therapy – typically rituximab, ifosfamide, carboplatin and etoposide (R-ICE) – the median post-progression survival was 17 months.

Recommendation

Given the disastrous consequences of progression, we recommend patients with DHL who wish to receive further therapy be enrolled into prospective investigational protocols evaluating novel agents with either sound preclinical rationale or demonstrated activity in *MYC*-driven lymphomas. In clinically appropriate patients, use of a non-cross resistant regimen, such as rituximab, dexamethasone, cytarabine and cisplatin (R-DHAP) or R-ICE (in etoposide-naïve patients) may be considered to bridge patients to enrolment on clinical trials or as part of an active palliative approach.

Novel approaches

Given that most patients with DHL will die rapidly from their disease, development of new and effective agents is a significant unmet medical need. *MYC*-driven lymphomas are the subject of much research attention, with several candidate therapies in early phase clinical trials. Selinexor (KPT-330) is a first-in-class selective inhibitor of nuclear export which binds to the nuclear export protein XPO1, forcing nuclear retention and activation of tumour suppressor proteins (Parikh *et al*, 2014). It has shown promising preclinical activity in a range of haematological cancers (Etchin *et al*, 2013; Walker *et al*, 2013; Zhong *et al*, 2014). Preliminary phase I data in patients with relapsed lymphomas (including four patients with DHL) suggest promising single agent activity with negligible toxicity (Gutierrez *et al*, 2014). This drug was recently designated orphan drug status by the US Food and Drug Administration and further efficacy studies in this promising compound are anticipated with interest. BET bromodomain inhibitors have been reported to interfere with *MYC*-inducing cell differentiation, cell cycle inhibition and pro-apoptotic activity (Delmore *et al*, 2011). Several compounds in this class have shown preclinical activity and are in early clinical development (Filippakopoulos & Knapp, 2014). The anti-apoptotic protein BCL2 mediates chemoresistance in lymphoma cells, and is a rational target in DHL. ABT-199 is a small molecule, orally administered BH3-mimetic that has shown promising single-agent activity in heavily pre-treated NHL (Davids *et al*, 2014). ABT-199 is currently being explored with bendamustine and rituximab in a phase I study of patients with relapsed NHL, including patients with double hit lymphoma (NCT01594229). Lenalidomide and ibrutinib have activity in DLBCL, but studies have not specified the MYC/BCL2 status of patients treated (Witzig *et al*, 2011; Zinzani *et al*, 2011; Vose *et al*, 2013; Wang *et al*, 2013; Vitolo *et al*, 2014; Younes *et al*, 2014). A summary of selected investigational agents holding promise in double hit lymphomas is presented in Table III.

Conclusion

DHL remains a challenging problem for clinicians and patients alike. Clinicians should be proactive in requesting FISH for *MYC* breaks in patients with DLBCL, BCLU and transformed indolent lymphomas as a timely diagnosis to facilitate the use of DA-R-EPOCH and CNS-directed prophylaxis. Ultimately, only patient and physician participation in ongoing clinical trial efforts will result in improvement in outcomes for patients with this highly aggressive form of NHL.

Contributions

CYC performed the literature review and wrote the first draft of the manuscript. FT designed the research and co-wrote the manuscript. YO and JRW reviewed and co-wrote the manuscript.

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