CORRESPONDENCE

Incidence of single hit Bcl-2 and Bcl-6 rearrangements in DLBCL: the Irish experience

Diffuse large B-cell lymphoma (DLBCL) is the most common non-Hodgkin's lymphoma. While many patients will be cured with Rituximab-CHOP, 30%–50% will either relapse or have disease refractory to treatment.[1] The International Prognostic Index is not sufficiently accurate to stratify these patients into prognostic subgroups, thus gene expression profiling (GEP) and the identification of gene rearrangements (R) involving *Myc*, *Bcl-2* and *Bcl-6* are instead used for risk stratification. GEP or more commonly immunohistochemistry (IHC)-based algorithms are used to stratify patients according to their cell of origin (COO), broadly dividing them into germinal centre B (GCB), and non-GCB subgroups. The latter group incorporates the activated B-cell (ABC) subtype, which is associated with significantly worse outcomes when treated with standard chemotherapy regimens, as compared with the GCB subgroup.[2]

Double hit (DH) lymphomas are characterised by the presence of a *Myc* gene R with a concurrent *Bcl-2* or *Bcl-6* gene R, while triple hit (TH) lymphomas have all three. The rearrangement status of these lymphomas is reflected in the updated WHO classification of lymphoid neoplasms in 2017. DH/TH lymphomas account for 5%–10% of (morphologic) DLBCL.[2] Interestingly, 80% of DH lymphomas have concurrent *Myc* and *Bcl-2* R and are associated with the GCB subtype, while dual translocations of *Myc* and *Bcl-6* are more commonly linked to the ABC phenotype.[3]

Overexpression of c-myc protein (defined as >40% positive neoplastic cells) and BCL2 protein (defined as >50% positive neoplastic cells) has also been shown to be of use in patient stratification; this expression is often independent of the associated gene rearrangements. 33% of DLBCL coexpress BCL2 and MYC proteins—referred to as dual expressors (DE). Contrary to most DH/TH lymphomas, DEs are associated with the ABC subtype of DLBCL. While DE also portend a poor prognosis, their outcomes are nevertheless superior to those of DH/TH lymphomas.[4]

We have previously reported the incidence of single hit (SH) and DH/TH-*Myc* R, DE and MYC protein status in an Irish cohort of (morphologic) DLBCL, not otherwise specified (NOS).[5] Identification of a lower frequency of *Myc* R in the ABC subgroup led our institution to alter the DLBCL fluorescent in situ hybridization (FISH) testing strategy post 2017 to perform a DH panel on all GCB DLBCL, but only test for *Myc* R in the first instance in ABC DLBCL. In this present study, we sought to define the incidence of isolated SH-*Bcl-2* and SH-*Bcl-6* R, and patterns of BCL2 and BCL6 protein expression, within an expanded version of the same Irish (morphologic) DLBCL cohort. Retrospective data were available from 400 (morphologic) DLBCL, NOS cases from St. James's Hospital and Tallaght University Hospital, Dublin between 2013 and 2017. Many cases were referred to these institutions for specialist review and/or FISH testing, thus complete information regarding rearrangement status and protein expression was not available for each case. Incomplete datapoints were omitted from calculations, as reflected in the numbers reported (table 1). COO status for each case was assigned by Hans IHC criteria.

241/366 (66%) of all DLBCLs were BCL2 protein positive. A *Bcl-2* R was present in 93/388 (24%) of cases; of these, 30/65 (46%) were independent SH-*Bcl-2* R, amounting to 30/234 (13%) of all cases with complete rearrangement data available. Not surprisingly, all cases with SH-*Bcl-2* R, where known, were BCL2 protein positive. 27/30 (90%) of SH-*Bcl-2* R cases and 139/240 (58%) of BCL2 protein positive cases were of GCB subtype.

BCL6 protein was expressed in 274/304 (90%) of all DLBCLs. *Bcl-6* R were present in 34/236 (14%) of cases, of which 19/34 (56%) were independent SH-*Bcl-6* R, amounting to 19/234 (8%) of all cases. Of the SH-*Bcl-6* cases, 14/19 (74%) were non-GCB subtypes and 169/273 (62%) of BCL6 protein positive cases were of GCB subtype. All cases with SH-*Bcl-6* R were BCL6 positive.

In this study, we highlight the overexpression of Bcl-2 and Bcl-6 proteins in DLBCL, as has previously been demonstrated.[6 7] We have identified a low but notable rate of isolated SH-*Bcl-2* and SH-*Bcl-6* gene R in all (morphologic) DLBCL cases in this cohort. We also describe a significant association between SH-*Bcl-2* R with the GCB subgroup ($p<0.005$), and a contrasting link between SH-*Bcl-6* R and the non-GCB subtypes ($p<0.005$). BCL6 protein expression was associated with the GCB subtype, consistent with its role in the Hans algorithm, while BCL2 protein expression showed no significant association with either COO subtype.

In 2016, Ye *et al* described SH-*Bcl-6* and SH-*Bcl-2* R in 23.1% and 13.6% of DLBCL cases, respectively; Bellas *et al* reported similar respective rates of 29% and 20%.[7 8] In keeping with our study, they identified a significant association between SH-*Bcl-2* R and the GCB subgroup, and between SH-*Bcl-6* with the ABC subgroup. While Bellas *et al* showed that SH-*Bcl-2* R predict poor overall survival, their prognosis was nevertheless superior to that of concurrent *Myc* and *Bcl-2* R.[8] *Bcl-6* R did not predict poorer survival in DLBCL patients, as interpreted in isolation or in tandem with *Myc* R.[7]

Overexpression of BCL2 and BCL6 proteins has been shown to be more common in the ABC and GCB subtypes,

Table 1 Immunohistochemical and gene rearrangement status in DLBCL NOS cohort

	Bcl-2 R	*Bcl-6* R	SH-*Bcl-2* R	SH-*Bcl-6* R	BCL2 protein	BCL6 protein
All DLBCL NOS	93/388 (24%)	34/236 (14%)	30/234 (13%)	19/234 (8%)	241/366 (66%)	274/304 (90%)
GCB	87/93 (94%)*	19/34 (56%)	27/30 (90%)*	5/19 (26%)	139/240 (58%)	169/273 (62%)
Non-GCB	6/93 (6%)	15/34 (44%)	3/30 (10%)	14/19 (74%)*	101/240 (42%)	104/273 (38%)
SH-*Bcl-2* R	30/65 (46%)	–	–	–	27/27 (100%)*	24/24 (100%)
SH-*Bcl-6* R	–	19/34 (56%)	–	–	7/12 (58%)	15/15 (100%)

Statistical significance: $\chi 2$ test, $p<0.05$.
*$p<0.005$.
Bcl-2, B-cell lymphoma 2; Bcl-6, B-cell lymphoma 6; DLBCL, diffuse large B-cell lymphoma; GCB, germinal centre B-cell; NOS, not otherwise specified; R, rearrangement; SH, single hit.

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respectively.[7 8] In keeping with this, BCL6 positive DLBCLs are associated with superior survival outcomes.[8] Despite the reported link between higher BCL2 expression with the ABC subgroup, its prognostic value is seen only with respect to its expression in the GCB subgroup.[8] However, it has been proposed that BCL2 protein expression is only of prognostic significance when MYC protein is coexpressed in DLBCL as a dual-expressor.[3] Other studies have demonstrated the superiority of using gene rearrangements for prognostication in DLBCL, as compared with protein expression, stating that BCL2 protein expression is not a suitable surrogate marker for the presence of a *Bcl-2* translocation.[6]

Our data highlight the incidence of SH-*Bcl-2* and SH-*Bcl-6* rearrangements in a well-annotated Irish cohort of (morphologic) DLBCL cases and an association between GCB and ABC subtypes respectively, as has been previously shown. While we did not identify a significant association between BCL2 protein status and COO subtypes, we did show that all SH-*Bcl-2* cases overexpressed BCL2 protein and are associated with the better performing GCB group.

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