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Cell-of-Origin in Diffuse Large B-cell Lymphoma: findings from the UK's population-based Haematological Malignancy Research Network

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Diffuse large B-cell lymphoma (DLBCL) is the commonest haematological malignancy, accounting for approximately half of all aggressive B-cell lymphomas. Around 80% of patients present with DLBCL not otherwise specified (NOS); which, although potentially curable with combination therapy (R-CHOP), comprises a biologically heterogeneous group that varies widely in terms of clinical characteristics and prognostic factors. The classification of DLBCL NOS into germinal centre B-cell (GCB) and activated B-cell (ABC) using gene-expression profiling (GEP) provided a milestone in the understanding of DLBCL pathogenesis; cell-of-origin (COO) is now incorporated into the latest WHO classification, and is a requirement for entry into most contemporary clinical trials (Swerdlow *et al*, 2017). More recently, in pursuit of molecular based approaches to the differentiation of Burkitt lymphoma from DLBCL, further subdivisions that include 'Burkitt-like' or 'high-grade' gene expression profiles have emerged (Sha *et al*, 2015; Dave *et al*, 2006; Hummel *et al*, 2006).

Set within the UK's population-based Haematological Malignancy Research Network (www.hmrn.org), and utilizing both established and potentially extended classifications, the findings reported on here are from the largest real-world DLBCL GEP series assembled to date. Full details of HMRN's methods can be found elsewhere (Smith *et al*, 2015, 2018). Importantly, initiated in September 2004, and tracking all patients newly diagnosed with a haematological malignancy until death, all diagnoses across HMRN's 14 hospitals (catchment population ~ 4 million) are made by specialist haematopathologists at a single integrated haematopathology laboratory – the Haematological Malignancy Diagnostic Service (www.hmds.info).

The present report includes data on 2100 patients (≥18 years) newly diagnosed with de novo DLBCL-NOS (ICD-O3, 9680; excluding primary CNS) between 1st September 2004 and 31st August 2016; all of whom were treated with curative intent and were followed-up for mortality through UK-wide national systems until 31st March 2018. Of these, 674 (32.1%) had suitable material available for GEP; which was carried out at HMDS on RNA extracted from formalin fixed paraffin embedded (FFPE) pre-treatment biopsies using the Illumina WG-DASL platform and the "DLBCL automatic classifier" (DAC) to classify COO (Care *et al*, 2013). The same methods (Barrans *et al*, 2012; Care *et al*, 2013) were applied in the recent REMoDL-B Phase III trial, ISRCTN51837425) (Davies *et al*, 2015). Cases were further subdivided to include a molecular high grade (MHG) class using a transcriptomic classifier, originally developed to identify Burkitt lymphoma-like gene expression signatures (Sha *et al*, 2015).

The demographic and clinical characteristics of the 674 patients with GEP data are distributed by COO group in Table I; data on the total cohort (n=2100) are presented on the left. Albeit younger (median age 66.3 years *versus* 68.0 years, P<0.05), the presenting characteristics of patients in the COO study group are broadly similar to those of the cohort as a whole. Furthermore, in both groups around 93% of patients were treated with R-CHOP, and 2-3% with CODOX-M based chemotherapies. Survival of patients in the COO study group was, however, significantly better than in the cohort as a whole; the 5-year overall survivals (OS) being 68.3% and 62.8% (P<0.05) respectively, and relative survivals (RS), which take into account the underlying age-specific and sex-specific mortality in the population as a whole, were 72.3% *versus* 77.0% (P<0.05).

The standard 3-group classifier assigned 369 (54.7%) patients to GCB,184 (27.3%) to ABC, and 121 (18.0%) were unclassified. As in other series (Scott *et al*, 2015), patients in the GCB group were significantly (P<0.05) younger (median age 65.7 years), had better survival (5-year OS 75.0%), and were more likely to have a *MYC* gene rearrangement (*MYC*-R, 12.2%) than those in the ABC group (median age 70.0 years, 5-year OS 53.9 years, *MYC*-R 5.0%); the remaining prognostic characteristics in the two groups are comparable.

Burkitt lymphoma displays germinal centre B-cell gene expression characteristics (Swerdlow *et al*, 2017); accordingly it is perhaps not surprising that members of the MHG subgroup were, almost exclusively, identified as GCB by the 3-group classifier (43/46). Separation of these cases widened the survival disparity between the ABC and GCB groups (Fig 1); the 5-year OS being 78.8%, 54.3%, 45.5% and 69.9% in the GCB, ABC, MHG, and UNC groups respectively. Indeed, the survival of patients in the MHG group is substantially worse than that of those remaining in the GCB group (P<0.001), and significantly worse than those classified as ABC (P<0.05); these differences holding when the hazard ratios were adjusted for other prognostic factors. Consistent with their poor survival, the cancer stage of MHG classified patients was more likely to be III/IV (MHG 81.8% *versus* GCB 60.9%, P<0.05) (Table 1). It is also notable that the overall survival curve of the MHG subgroup shows a striking similarity to that of Burkitt lymphoma (Supplementary Figure 1), with both curves falling steeply before flattening around 2 years after diagnosis.

The intrinsic relationship between *MYC*-R and Burkitt lymphoma is reflected in the dramatic excess of *MYC*-R in the MHG subgroup. As is evident from Table 1, in the course of subsequent investigations to exclude Burkitt lymphoma, a greater proportion of MHG cases were assessed for MYC-R; these were, in turn, significantly more likely to be positive than the remaining members of the GCB class (21/42 versus 17/256, P<0.001). Additionally, among those with *MYC*-R, MHG cases were marginally more likely than those that remained

in the GCB group to be double or triple hit (*MYC*-R together with *BCL2* and/or *BCL6 rearranged*), 19/21 (90.5%) compared with 13/17 (76.5%) respectively, but the difference is not statistically significant. Hence, while MHG encompasses many of the double or triple hit lymphomas in the series, it is important to note that the GEP based grouping both subdivides double/triple hit lymphomas, and extends the number of cases identified as biologically aggressive.

In conclusion, our findings confirm the heterogeneity of DLBCL NOS; demonstrating the prognostic strength of GEP in the real-world setting and supporting its use in the routine diagnostic process. The discrimination of a poor-risk molecular high-grade (MHG) group from the conventional COO classes potentially provides the foundation for the development of future trials aimed at improving outcome for these patients.

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Author Contributions

ER and DP are responsible for the paper; ER, AS, SC, and DP designed the study; DP, AS, and SL managed the data; SB, CB, and RT oversaw laboratory aspects; DP, SL, and SC carried out the statistical analysis; DW, CS and RT developed the classifier; CB, RT and RP provided clinical input; all authors contributed to writing the paper and reviewed the manuscript prior to submission.

Supporting Information

Fig S1 De novo molecular high grade (MHG) diffuse large B-cell cell lymphoma NOS and Burkitt lymphoma overall survival curves (median age at diagnosis); patients treated with curative intent, Haematological Malignancy Research Network (HMRN) diagnoses 2004-2015

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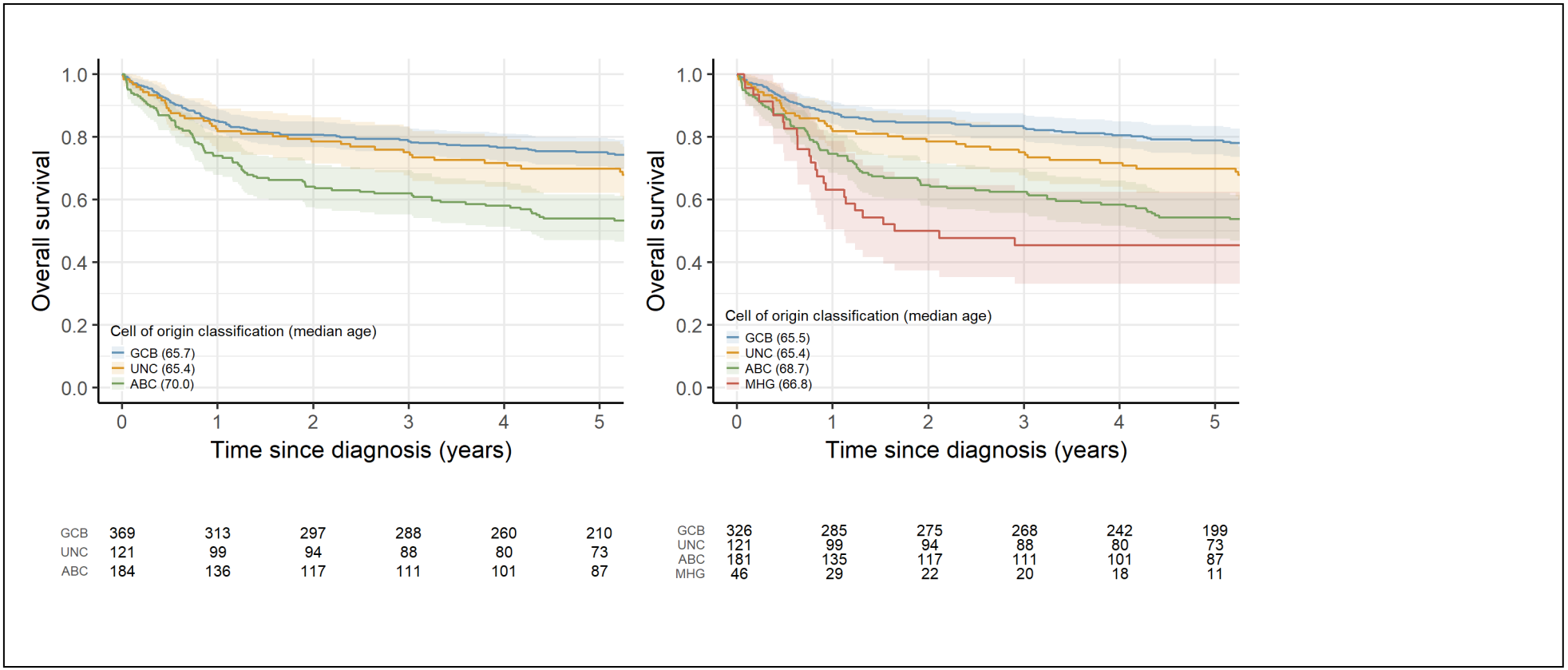
Table I De novo diffuse large B-cell cell lymphoma (DLBCL) NOS (ICD-O3 9680/3) distributed by patient and tumour characteristics; patients treated with curative intent, Haematological Malignancy Research Network (HMRN) diagnoses 2004-2015

	Source Cohort	**Study Cohort: molecular subtypes**							
		Total Patients	Classic 3-group cell-of-origin (COO) stratification			Refined 4-group cell-of-origin (COO) stratification			
			GCB	ABC	Unclassified	GCB	ABC	MHG	Unclassified
Number of patients	2100	674	369	184	121	326	181	46	121
Gender									
Males (%)	1130 (53.8)	365 (54.2)	194 (52.6)	102 (55.4)	69 (57.0)	168 (51.5)	102 (56.4)	26 (56.5)	69 (57.0)
Age (years)									
Median (range)	68.0 (18.9-91.7)	66.3 (20.0-89.0)	65.7 (20.0-85.8)	70.0 (30.9-89.0)	65.4 (25.8-85.8)	65.5 (20.0-85.8)	68.7 (30.9-89.0)	66.8 (34.8-84.9)	65.4 (25.8-85.8)
≥ 60 (%)	1511 (72.0)	457 (67.8)	240 (65.0)	137 (74.5)	80 (66.1)	211 (64.7)	134 (74.0)	32 (69.6)	80 (66.1)
Stage (%)									
I/II	783 (40.9)	236 (37.0)	128 (37.0)	73 (41.0)	35 (31.0)	120 (39.3)	73 (41.7)	8 (18.2)	35 (31.0)
III/IV	1132 (59.1)	401 (63.0)	218 (63.0)	105 (59.0)	78 (69.0)	185 (60.7)	102 (58.3)	36 (81.8)	78 (69.0)
Not fully staged	185	37	23	6	8	21	6	2	8
ECOG (%)									
0/1	1631 (78.5)	530 (79.8)	286 (79.0)	148 (80.9)	96 (80.7)	256 (80.3)	146 (81.1)	32 (69.6)	96 (80.7)
≥2	447 (21.5)	134 (20.2)	76 (21.0)	35 (19.1)	23 (19.3)	63 (19.7)	34 (18.9)	14 (30.4)	23 (19.3)
Missing	22	10	7	1	2	7	1	0	2
IPI (%)									
Low (0/1)	472 (29.0)	150 (27.6)	80 (27.1)	36 (24.2)	34 (34.0)	77 (29.5)	36 (24.3)	3 (8.6)	34 (34.0)
Intermediate (2-3)	809 (49.7)	294 (54.0)	165 (56.0)	81 (54.3)	48 (48.0)	146 (55.9)	81 (54.8)	19 (54.3)	48 (48.0)
High (4-5)	347 (21.3)	100 (18.4)	50 (16.9)	32 (21.5)	18 (18.0)	38 (14.6)	31 (20.9)	13 (37.1)	18 (18.0)
Not calculable	472	130	74	35	21	65	33	11	21
MYC ± *BCL2* and/or *BCL6* rearrangement (%)									
MYC-R negative	1294 (88.5)	469 (90.0)	259 (87.8)	133 (95.0)	77 (89.5)	239 (93.4)	132 (93.4)	21 (50.0)	77 (89.5)
MYC-R positive	168 (11.5)	52 (10.0)	36 (12.2)	7 (5.0)	9 (10.5)	17 (6.6)	5 (3.6)	21 (50.0)	9 (10.5)
- Single hit	50 (3.4)	13 (2.5)	4 (1.4)	5 (3.6)	4 (4.7)	4 (1.6)	3 (2.2)	2 (4.8)	4 (4.7)
- Double/triple hit	113 (7.7)	37 (7.1)	32 (10.8)	1 (0.7)	4 (4.7)	13 (5.1)	1 (0.7)	19 (45.2)	4 (4.7)
- *BCL2* and/or *BCL6* not done	5 (0.3)	2 (0.4)	0	1 (0.7)	1(1.1)	0	1 (0.7)	0	1 (1.1)
Missing	638	153	74	44	35	70	44	4	35
Chemotherapy (%)									
CHOP-R	1957 (93.2)	629 (93.3)	346 (93.8)	173 (94.0)	110 (90.9)	308 (94.5)	170 (93.9)	41 (89.1)	110 (90.9)
CODOX-M based	50 (2.4)	18 (2.7)	13 (3.5)	1 (0.5)	4 (3.3)	10 (3.1)	1 (0.6)	3 (6.5)	4 (3.3)
5-year survival (%)									
Overall (OS)	63.0	68.3	75.0	53.9	69.9	78.8	54.3	45.5	69.9
Relative (RS)	72.3	77.0	82.7	62.1	79.7	86.6	62.6	48.0	79.7

Figure 1 De novo diffuse large B-cell cell lymphoma (DLBCL) NOS overall survival stratified by cell of origin (median age at diagnosis); patients treated with curative intent, Haematological Malignancy Research Network (HMRN) diagnoses 2004-2015



Supplementary Figure: De novo molecular high grade (MHG) diffuse large B-cell cell lymphoma NOS and Burkitt lymphoma overall survival curves (median age at diagnosis); patients treated with curative intent, Haematological Malignancy Research Network (HMRN) diagnoses 2004-2015