

This is a repository copy of *Cell-of-origin in diffuse large B-cell lymphoma: findings from the UK's population-based Haematological Malignancy Research Network*.

White Rose Research Online URL for this paper:

<https://eprints.whiterose.ac.uk/id/eprint/140697/>

Version: Accepted Version

Article:

Painter, Daniel Edwin orcid.org/0000-0002-3936-7569, Barrans, Sharon, Lacy, Stuart Edward orcid.org/0000-0002-8570-7528 et al. (8 more authors) (2018) Cell-of-origin in diffuse large B-cell lymphoma: findings from the UK's population-based Haematological Malignancy Research Network. *British Journal of Haematology*. ISSN: 0007-1048

<https://doi.org/10.1111/bjh.15619>

Reuse

Items deposited in White Rose Research Online are protected by copyright, with all rights reserved unless indicated otherwise. They may be downloaded and/or printed for private study, or other acts as permitted by national copyright laws. The publisher or other rights holders may allow further reproduction and re-use of the full text version. This is indicated by the licence information on the White Rose Research Online record for the item.

Takedown

If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing eprints@whiterose.ac.uk including the URL of the record and the reason for the withdrawal request.

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

Daniel Painter¹, Sharon Barrans², Stuart Lacy¹, Alexandra Smith¹, Simon Crouch¹, David Westhead³, Chulin Sha³, Russell Patmore⁴, Reuben Tooze⁵, Cathy Burton², Eve Roman¹

Eve Roman is the corresponding author – eve.roman@york.ac.uk

¹Epidemiology & Cancer Statistics Group, Department of Health Sciences, University of York, York, UK

²Haematological Malignancy Diagnostic Service, St James's University Hospital, Leeds, UK

³Bioinformatics Group, Institute of Molecular and Cellular Biology, University of Leeds, UK

⁴Queen's Centre for Oncology and Haematology, Hull and East Yorkshire Hospitals, Cottingham, UK

⁵Section of Experimental Haematology, University of Leeds, Leeds, UK

Word count:

Text = 997; Tables= 1; Figure =1; Supplementary = 1 Figure

Key words: Gene-expression; prognostic factors; survival, epidemiology

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.

Acknowledgments

HMRN is funded by Bloodwise (grant number 15037) and receives support from NHS clinical and administrative staff across the study catchment area.

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 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

References

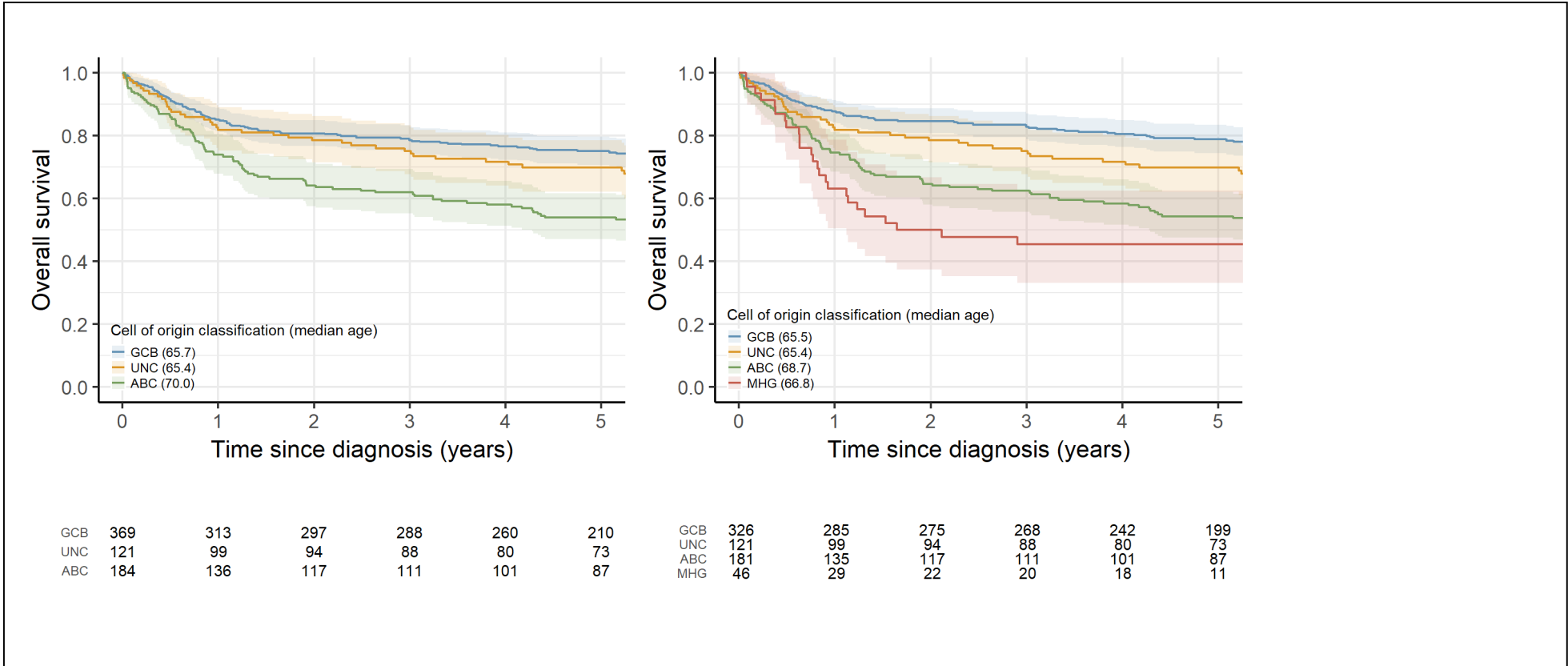
- Barrans, S.L., Crouch, S., Care, M.A., Worrillow, L., Smith, A., Patmore, R., Westhead, D.R., Tooze, R., Roman, E. & Jack, A.S. (2012) Whole genome expression profiling based on paraffin embedded tissue can be used to classify diffuse large B-cell lymphoma and predict clinical outcome. *British Journal of Haematology*, **159**, 441–453.
- Care, M.A., Barrans, S., Worrillow, L., Jack, A., Westhead, D.R. & Tooze, R.M. (2013) A microarray platform-independent classification tool for cell of origin class allows

- comparative analysis of gene expression in diffuse large B-cell lymphoma. *PloS One*, **8**, e55895.
- Dave, S.S., Fu, K., Wright, G.W., Lam, L.T., Kluin, P., Boerma, E.-J., Greiner, T.C., Weisenburger, D.D., Rosenwald, A., Ott, G., Müller-Hermelink, H.-K., Gascoyne, R.D., Delabie, J., Rimsza, L.M., Brazier, R.M., Grogan, T.M., Campo, E., Jaffe, E.S., Dave, B.J., Sanger, W., et al (2006) Molecular diagnosis of Burkitt's lymphoma. *The New England Journal of Medicine*, **354**, 2431–2442.
- Davies, A.J., Caddy, J., Maishman, T., Barrans, S., Mamot, C., Care, M., Pocock, C., Stanton, L., Hamid, D., Pugh, K., McMillan, A., Fields, P., Kruger, A., Jack, A. & Johnson, P.W.M. (2015) A Prospective Randomised Trial of Targeted Therapy for Diffuse Large B-Cell Lymphoma (DLBCL) Based upon Real-Time Gene Expression Profiling: The Remodl-B Study of the UK NCRI and SAKK Lymphoma Groups (ISRCTN51837425). *Blood*, **126**, 812–812.
- Hummel, M., Bentink, S., Berger, H., Klapper, W., Wessendorf, S., Barth, T.F.E., Bernd, H.-W., Cogliatti, S.B., Dierlamm, J., Feller, A.C., Hansmann, M.-L., Haralambieva, E., Harder, L., Hasenclever, D., Kühn, M., Lenze, D., Lichter, P., Martin-Subero, J.I., Möller, P., Müller-Hermelink, H.-K., et al (2006) A biologic definition of Burkitt's lymphoma from transcriptional and genomic profiling. *The New England Journal of Medicine*, **354**, 2419–2430.
- Scott, D.W., Mottok, A., Ennishi, D., Wright, G.W., Farinha, P., Ben-Neriah, S., Kridel, R., Barry, G.S., Hother, C., Abrisqueta, P., Boyle, M., Meissner, B., Telenius, A., Savage, K.J., Sehn, L.H., Slack, G.W., Steidl, C., Staudt, L.M., Connors, J.M., Rimsza, L.M., et al (2015) Prognostic Significance of Diffuse Large B-Cell Lymphoma Cell of Origin Determined by Digital Gene Expression in Formalin-Fixed Paraffin-Embedded Tissue Biopsies. *Journal of Clinical Oncology*, **33**, 2848–2856.
- Sha, C., Barrans, S., Care, M.A., Cunningham, D., Toozé, R.M., Jack, A. & Westhead, D.R. (2015) Transferring genomics to the clinic: distinguishing Burkitt and diffuse large B cell lymphomas. *Genome Medicine*, **7**, 64.
- Smith, A., Crouch, S., Howell, D., Burton, C., Patmore, R. & Roman, E. (2015) Impact of age and socioeconomic status on treatment and survival from aggressive lymphoma: a UK population-based study of diffuse large B-cell lymphoma. *Cancer Epidemiology*, **39**, 1103–1112.
- Smith, A., Howell, D., Crouch, S., Painter, D., Blase, J., Wang, H.-I., Hewison, A., Bagguley, T., Appleton, S., Kinsey, S., Burton, C., Patmore, R. & Roman, E. (2018) Cohort Profile: The Haematological Malignancy Research Network (HMRN); a UK population-based patient cohort. *International Journal of Epidemiology*.
- Swerdlow, S.H., Harris, N.L., Jaffe, E.S., Pileri, S.A., Stein, H. & Thiele, J. eds. (2017) WHO classification of tumours of haematopoietic and lymphoid tissues Revised 4th edition. Lyon: International Agency for Research on Cancer.

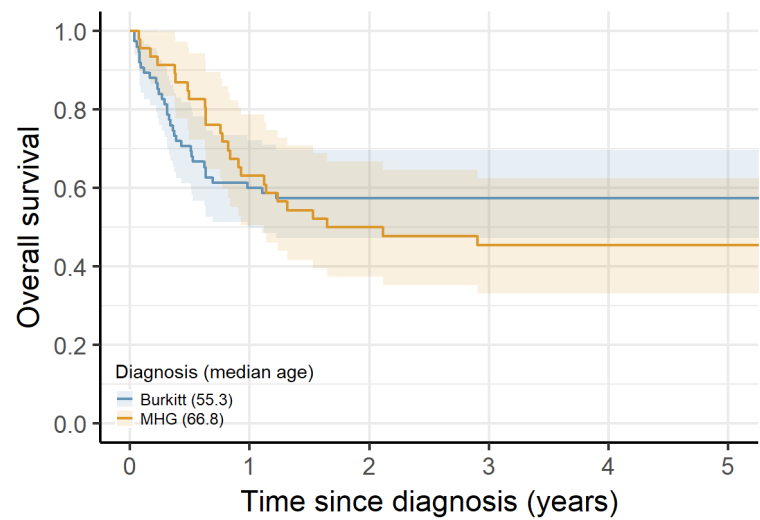
Table I De novo diffuse large B-cell cell lymphoma (DLBCL) NOS (ICD-O3 9680/3) distributed by patient and tumour characteristics; patients treated with curative intent, Haematological Malignancy Research Network (HMRN) diagnoses 2004-2015

		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



Burkitt	75	45	43	40	34	31
MHG	46	29	22	20	18	11