Supplement for Scott *et al*

Patient Cohorts

The 1228 biopsies were drawn from a population registry at the BCCA and 3 clinical trials: the MegaCHOEP[1] and RICOVER-60[2] trials from the German High-Grade non-Hodgkin Lymphoma Study Group and E1412 trial from the US Intergroup. The number of biopsies and entry criteria for each patient cohort is shown in **Supplemental Table 3**. The studies were approved by the University of British Columbia/BC Cancer Agency Research Ethics Board. Investigations were performed on biopsies from the RICOVER-60, MegaCHOEP and E1412 trials after obtaining written consent to participate in the clinical trial and the accompanying scientific investigations.

Fluorescence *in situ* hybridization

Fluorescence *in situ* hybridization (FISH) was performed on tissue microarrays comprising diagnostic biopsies from the population registry-based cohort from the BCCA [ref] (n = 327) and the MegaCHOEP (n = 84) and RICOVER-60 (n = 381) trials and unstained sections for a prospectively collected population registry-based cohort from the BCCA (n = 171) and the US Intergroup E1412 trial (n = 265). FISH was performed in research environments with the exception of the E1412 trial, where the FISH was performed by the Mayo Clinical Core Laboratory. FISH probe sets were Vysis LSI dual color break-apart. In 37 cases at the BCCA, due to results that were uninterpretable with the Vysis LSI probes for *MYC*, break-apart probes from DAKO were used. In 5 cases from the E1412 study, where Vysis break-apart assay results were not available, *MYC-IGH* dual fusion FISH assay results were used.

In biopsies from the MegaCHOEP and RICOVER-60 trials at least 100 nuclei were examined and the thresholds for positivity were 15%, 13% and 12% for *MYC*, *BCL2* and *BCL6*, respectively. At the BCCA at least 200 nuclei were examined and the threshold for considering a biopsy positive for break-apart was greater than 5% of nuclei. However, the lowest number of nuclei with break-apart signal for *MYC*, *BCL2* and *BCL6* in biopsies consider to be break-apart positive were 22%, 14% and 17%, respectively. For E1412, thresholds for positivity were ≥ 7%, 9% and 6% for *MYC*, *BCL2*, and *BCL6* break-apart assays, respectively. For *MYC-IGH* dual fusion FISH the threshold was ≥ 5%.

Immunohistochemistry

Immunohistochemistry stains were performed using the antibodies shown in **Supplemental Table 4**.

Thresholds of MYC Immunohistochemistry

MYC IHC in percentage increments (as opposed to positive vs negative) was available for 905 biopsies. Receiver operating characteristic (ROC) curves are shown for (A) *MYC* rearrangement, (B) HGBL-DH/TH with *BCL2* rearrangement and (C) HGBL-DH/TH in **Supplemental Figure 1**. The area under the curve of the ROC curves are 0.740, 0.737 and 0.74, respectively. These curves demonstrate that there is no MYC IHC threshold (apart from 0%) that will detect all biopsies with *MYC* rearrangement.

	HGBL-DH/TH with *BCL2* rearrangement	**HGBL-DH with *BCL6* rearrangement**	**Total HGBL-DH/TH**	**Total Cohort**
COO – GCB by Lymph2Cx %	99	43	88	50
COO – GCB/UNC by Lymph2Cx %	100	50	92	62
COO – GCB by Hans IHC %	99	57	92	51
MYC IHC + %	80	87	80	48
BCL2 IHC + %	95	60	89	68
DPE %	75	53	70	34
CD10 IHC + %	95	50	87	40
BCL6 IHC + %	96	79	93	85
MUM1 IHC %	88	53	82	44

Supplemental Table 1: Cell-of-origin and immunohistochemistry of the HGBL-DH/TH groups and the total cohort.

Abbreviations: COO: cell-of-origin; GCB: germinal centre B-cell-like subtype; UNC: unclassified; IHC: immunohistochemistry

		HGBL-DH/TH with *BCL2* rearrangement				HGBL-DH with *BCL6* rearrangement				Total HGBL-DH/TH			
Characteristic	**Frequency – n (%)**	**Sens**	**PPV**	**Spec**	**NPV**	**Sens**	**PPV**	**Spec**	**NPV**	**Sens**	**PPV**	**Spec**	**NPV**
MYC IHC (≥ 40%)	519 (48)	0.80	0.11	0.54	0.97	0.87	0.03	0.52	1.00	0.80	0.13	0.55	0.97
MYC IHC+/Lymph2Cx GCB	198 (18)	0.77	0.25	0.86	0.98	0.43	0.03	0.82	0.99	0.70	0.28	0.86	0.97
MYC IHC+/Lymph2Cx GCB and UNC	256 (24)	0.78	0.20	0.80	0.98	0.50	0.03	0.77	0.99	0.73	0.23	0.80	0.97
MYC IHC+/Hans GCB	223 (20)	0.79	0.25	0.84	0.98	0.57	0.04	0.81	0.99	0.74	0.28	0.85	0.98
BCL2 IHC (≥ 50%)	802 (68)	0.95	0.09	0.34	0.99	0.60	0.01	0.32	0.98	0.89	0.11	0.34	0.97
BCL2 IHC+/ Lymph2Cx GCB	307 (27)	0.93	0.21	0.77	0.99	0.27	0.01	0.73	0.99	0.80	0.22	0.77	0.98
BCL2 IHC+/ Lymph2Cx GCB and UNC	385 (34)	0.94	0.17	0.70	0.99	0.33	0.01	0.66	0.99	0.83	0.18	0.70	0.98
BCL2 IHC+/Hans GCB	367 (31)	0.94	0.20	0.74	0.99	0.29	0.01	0.69	0.99	0.84	0.21	0.74	0.98
CD10 IHC (≥ 30%)	475 (40)	0.95	0.16	0.64	0.99	0.50	0.01	0.61	0.99	0.87	0.17	0.64	0.98
BCL6 IHC (≥ 30%)	989 (85)	0.96	0.07	0.15	0.98	0.79	0.01	0.15	0.98	0.93	0.09	0.15	0.96
MUM1IHC (< 30%)	517 (44)	0.88	0.13	0.60	0.99	0.47	0.01	0.57	0.99	0.82	0.15	0.60	0.97

Supplemental Table 2: Test characteristics for strategies to screen tumors of DLBCL morphology for FISH testing.

Sens: sensitivity; PPV: positive predictive value; Spec: specificity; NPV: negative predictive value; COO: cell-of-origin; GCB: germinal centre B-cell-like DLBCL; UNC: unclassified DLBCL; DPE: dual protein expresser of MYC and BCL2 by immunohistochemistry; IHC: immunohistochemistry.

	BCCA Cohort 1	BCCA Cohort 2	MegaCHOEP	RICOVER-60	E1412
No. of Biopsies	327	171	84	381	265
Material for FISH	Tissue microarray	Unstained sections	Tissue microarray	Tissue microarray	Unstained sections
Biopsy type	Incision/Excision	Incision/Excision	Incision/Excision	Incision/Excision	Incision/Excision/ Core needle
Stage	All	All	All*	All	II with bulky disease and III/IV
Age	≥16	≥16	18-60	61-80	≥18
Entry Criteria	Treated with R-CHOP and had a matched fresh frozen biopsy	Treatment with curative intent	Must have 2-3 of the following: Stage III/IV, ECOG PS 2-3, elevated LDH	IPI ≥ 2 and ECOG PS 0-2	ECOG PS 0-2
MYC rearranged %	14.7	15.2	11.9	10.2	10.2
HGBL-DH/TH with *BCL2* rearrangement %	8.0	9.9	7.1	5.2	4.2
HGBL-DH with *BCL6* rearrangement %	1.2	1.2	1.2	1.0	1.5
Total HGBL-DH/TH %	9.2	11.1	8.3	6.2	5.7
Lymph2Cx Available %	99.1	100	88.1	74.0	93.6
Lymph2Cx COO GCB/UNC/ABC %	55.4/11.8/32.8	50.3/12.3/37.4	55.4/13.5/31.1	41.5/14.5/44.0	50.8/9.7/39.5
Hans IHC available %	99.1	100	92.3	94.0	97.7
Hans COO GCB/non-GCB %	57.6/42.4	54.4/45.6	52.6/47.4	46.1/53.9	49.0/51.0
MYC IHC available %	98.8	100	90.5	68.5	93.6
MYC IHC ≥ 40% %	42.4	52.6	52.6	46.4	52.8
BCL2 IHC available	98.2	100	89.3	96.1	95.8
BCL2 IHC ≥ 50% %	65.7	74.9	69.3	66.9	65.4
DPE available %	99.1	100	91.7	78.2	98.1
DPE +ve %	31.5	43.9	39.0	28.4	34.2
HGBL-DH/TH with *BCL2* rearrangement DPE–ve %	28	17.6	33.3	23.1	30

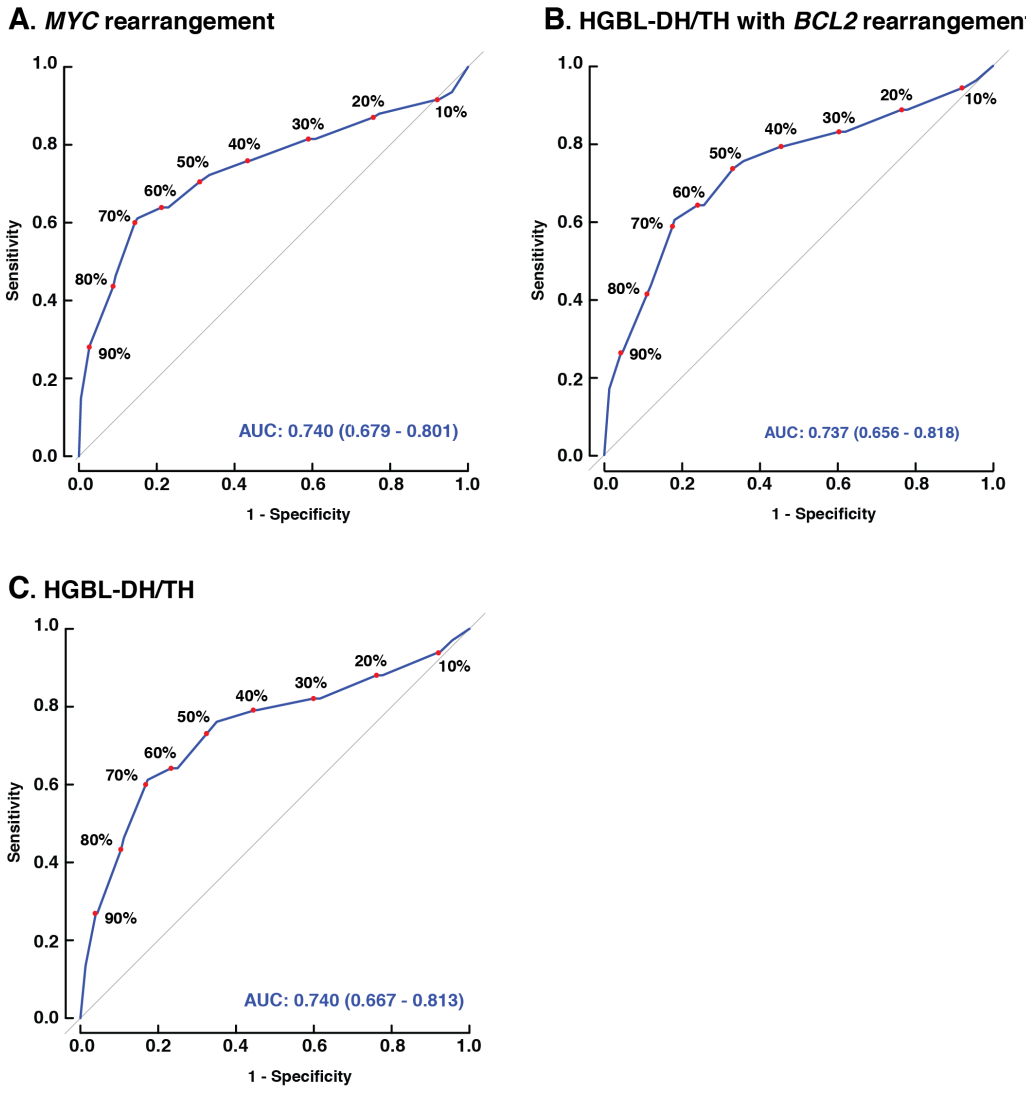
Supplemental Table 3: Basic characteristics of the cohort populations. For full entry criteria for the clinical trials please see refs[1,2] and clinicaltrials.gov

*The vast majority of patients had stage III/IV disease

Abbreviations: COO: cell-of-origin; GCB: germinal centre B-cell-like subtype; UNC: unclassified; ABC: activated B-cell-like subtype; IHC: immunohistochemistry; DPE: dual protein expresser

	BCCA	MegaCHOEP/RICOVER-60	E1412
MYC	Y69	Y69	EP121 (Y69)
BCL2	124	M0887	124
CD10	56C6	NCL-CD10-270	56C6
BCL6	LN22	P6-B6-p	PG-B6-p
MUM1/IRF4	Mum1p	Mum1P	Mum1P

Supplemental Table 4: Antibody clones used in immunohistochemistry studies



Supplemental Figure 1: ROC curves for MYC Immunohistochemistry. Red dots are shown at thresholds of the percentage of tumor cells positive for MYC staining.

References:

1. Schmitz N, Nickelsen M, Ziepert M, et al. Conventional chemotherapy (CHOEP-14) with rituximab or high-dose chemotherapy (MegaCHOEP) with rituximab for young, high-risk patients with aggressive B-cell lymphoma: an open-label, randomised, phase 3 trial (DSHNHL 2002-1). Lancet Oncol 2012; 13(12): 1250-9.

2. Pfreundschuh M, Schubert J, Ziepert M, et al. Six versus eight cycles of bi-weekly CHOP-14 with or without rituximab in elderly patients with aggressive CD20+ B-cell lymphomas: a randomised controlled trial (RICOVER-60). Lancet Oncol 2008; 9: 105-16.