Reliable subtype classification of diffuse large B-cell lymphoma samples from GELA LNH2003 trials using the Lymph2Cx gene expression assay

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Supplementary data to

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Supplementary Table 1. Clinical trials included in GELA/LYSA sponsored LNH-2003 program.

aaIPI: age adjusted International Prognostic Index. ACVBP: doxorubicin, cyclophosphamide, vindesine, bleomycin, prednisone, CHOP: cyclophosphamide, doxorubicin, vincristine, and prednisone, R: rituximab, ASCT: autologous stem cell transplantation.

Trial name Clinicaltrial.gov	Number of patients	Condition (age, aaIPI)	Treatment arms	Reference
LNH03-1B NCT00140660	223	<66 years aaIPI = 0	ACVBP +/- R	*Ketterer N et al* *Ann Oncol. 2013;24:1032-7.*
LNH03-2B NCT00140595	379	18-59 years aaIPI = 1	R-CHOP vs R-ACVBP	*Récher C et al* *Lancet. 2011;378:1858-67.*
LNH03-3B NCT00144807	210	18-59 years aaIPI = 2, 3	R-ACVBP + ASCT	*Fitoussi O et al* *Haematologica. 2011;96:1136-43.*
LNH03-6B NCT00144755	602	60-80 years aaIPI > = 1	R-CHOP 14 vs R-CHOP 21	*Delarue R et al,* *Lancet Oncol. 2013;14:525-33.*
LNH03-7B NCT01087424	150	>=80 years	R-miniCHOP	*Peyrade F et al,* *Lancet Oncol. 2011;12:460-8.*
LNH01-5B NCT00135499	*Expected 150*	60-65 years	R-CHOP vs R-ACVBP	*Premature closure*

Supplementary Table 2: Clinical characteristics of the DLBCL patients successfully analyzed with Lymph2Cx assay.

	N= 144 (%)
Clinical trials	
LNH01-5B	8 (5.6)
LNH03-1B	22 (15.3)
LNH03-2B	26 (18.1)
LNH03-3B	20 (13.9)
LNH03-6B	60 (41.7)
LNH03-7B	8 (5.6)
Treatment arm	
ACVBP	10 (6.9)
R-ACVBP+ASCT	6 (4.2)
R-ACVBP+Conso	46 (31.9)
R-CHOP14	28 (19.4)
R-CHOP21	46 (31.9)
R-Mini-CHOP21	8 (5.6)
Age (mean (sd))	59.5 (15.0)
Sex = male (%)	76 (52.8)
Stage (%)	
STAGE 1,2	42 (29.2)
STAGE 3,4	102 (70.8)
LDH >Normal (%)	88 (61.1)
ECOG PS (%)	
0, 1	121 (84)
2, 3	23 (16)
Extra-nodal sites (%)	
0, 1	96 (66.7)
≥2	48 (33.3)
IPI Score (%)	
Low (0, 1)	45 (31.3)
Intermediate (2, 3)	64 (44.4)
High (4,5)	35 (24.3)

Supplementary Table 3. Association of IPI and COO classifications with outcome using Cox models in DLBCL patients.

	PFS HR*	PFS *p-value***	OS HR*	OS *p-value***
IPI				
2,3 vs 0,1	3.48 [1.31-9.25]	0.008	11.49 [1.51-87.48]	0.003
4,5 vs 2,3	2.13 [1.14-3.97]	0.015	3.00 [1.52-5.94]	0.001
Affymetrix COO ABC vs GC	1.68 [0.89-3.17]	0.10	2.04 [0.98-4.26]	0.051
Lymph2Cx COO ABC vs GC	1.43 [0.77-2.67]	0.26	1.55 [0.74-3.22]	0.24

*Hazard ratios (HR) were computed with the coxph function of the R (v3.3) survival package (v2.39.5).

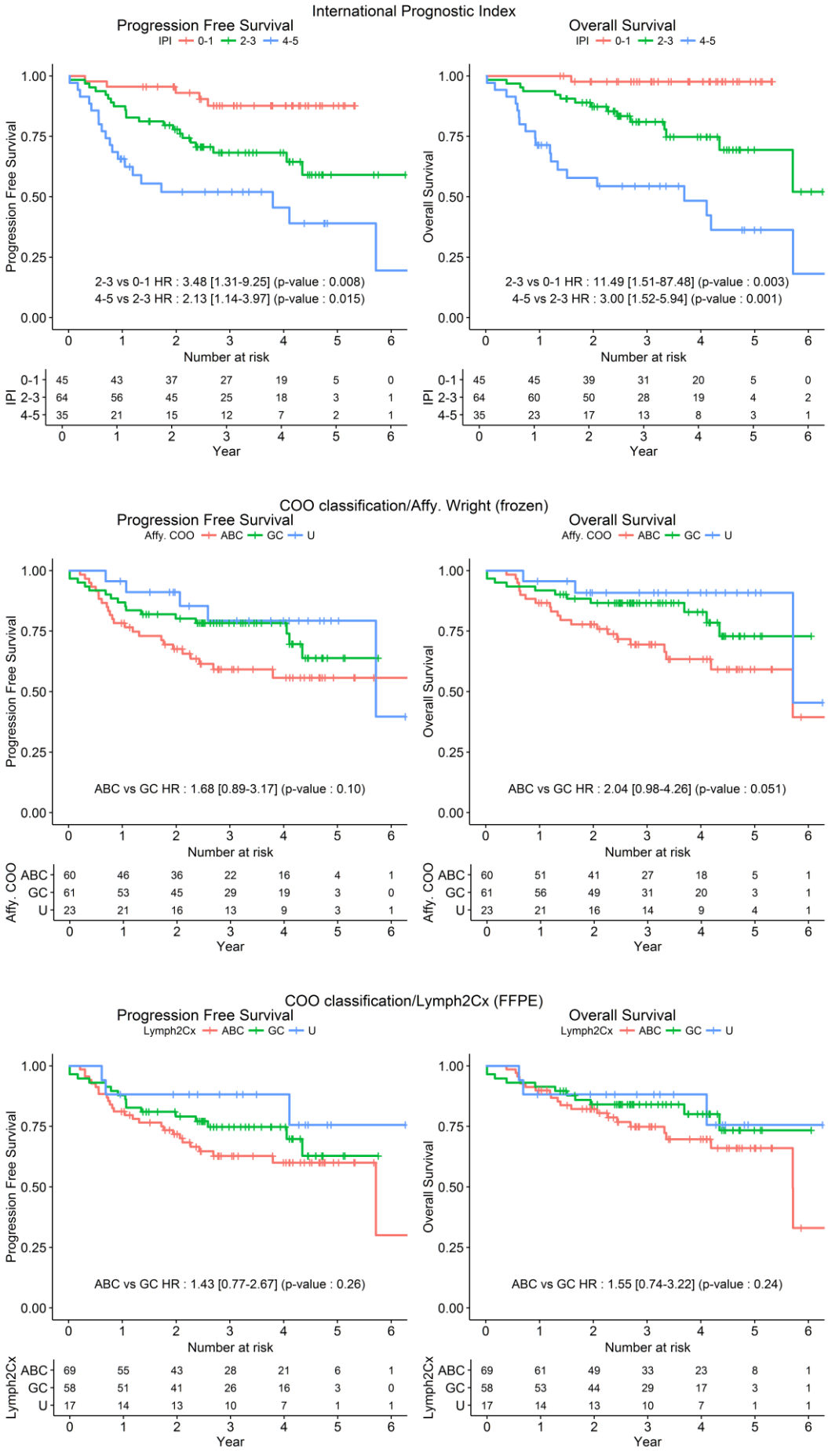
**p-values were computed using the score (logrank) test

Supplementary Table 4. Cases showing discordant classifications

GHEDID	Affymetrix	Lymph2CX	Hans	CD10	BCL6	MUM1	FOXP1
GHE0743A	ABC	Unclassified	noGC	neg	pos	pos	pos
GHE0426A	Unclassified	ABC	noGC	neg	pos	pos	pos
GHE0169A	Unclassified	ABC	noGC	neg	pos	pos	pos
GHE2084A	Unclassified	GC	-	-	-	-	-
GHE0623A	Unclassified	GC	-	-	pos	neg	neg
GHE0523A	Unclassified	ABC	noGC	neg	pos	pos	pos
GHE0997A	Unclassified	ABC	noGC	neg	neg	pos	pos
GHE0140A	Unclassified	GC	noGC	neg	neg	neg	pos
GHE0853A	Unclassified	ABC	noGC	neg	neg	pos	pos
GHE1189A	Unclassified	ABC	GC	pos	-	pos	pos
GHE0632A	Unclassified	GC	-	-	-	-	-
GHE0693A	Unclassified	ABC	-	-	-	-	-
GHE1009A	Unclassified	GC	GC	neg	pos	neg	pos
GHE0049A	Unclassified	ABC	-	-	-	-	0
GHE0463A	Unclassified	ABC	GC	pos	pos	pos	pos
GHE0909A	GC	Unclassified	GC	pos	pos	pos	pos
GHE0414A	GC	Unclassified	noGC	neg	neg	pos	neg
GHE0216A	GC	Unclassified	noGC	neg	pos	pos	neg
GHE1558A	GC	Unclassified	noGC	neg	neg	pos	pos
GHE0371A	GC	Unclassified	GC	pos	pos	pos	pos
GHE0776A	GC	ABC	noGC	neg	neg	pos	pos
GHE0057A	GC	Unclassified	noGC	neg	neg	neg	pos
GHE0219A	GC	Unclassified	noGC	neg	neg	neg	neg

Hans' algorithm was based on CD10, BCL6, and MUM1/IRF4 expression with cutoff levels of 30%. FOXP1 staining was evaluated as positive (variable or strong) versus negative. - : indicates that the data are not available.

Supplementary Figure 1.



Supplementary Figure 2.

Venn diagrams showing the relation between Affymetrix COO (GC *versus* ABC/unclassified), Lymph2Cx COO (GC *versus* ABC/ unclassified) and Hans immuno-histochemical classification based on CD10, BCL6 and MUM1/IRF4 staining in the 118 samples for which the 3 classifications have been established.

On the 118 samples, the three classifiers were concordant for 39 samples classified GC and 55 classified nonGC/ABC/unclassified. On the 24 remaining cases, 7 cases were classified GC by Hans alone, 6 by Affymetrix alone, and 1 by Nanostring alone. Interestingly, 7 cases were classified GC by Affymetrix and Nanostring but not Hans, whereas only 1 and 2 cases were concordant for GC classification between Hans and Nanostring or Affymetrix respectively.