APPENDIX

METHODS

Gene-expression quantification by RNASeq

RNA was extracted using RNeasy FFPE Kit (Qiagen, Hilden, Germany). Raw reads were quality-controlled and aligned to the human reference genome (NCBI build 38) using GSNAP version ‘2013-10-10-v2’. Transcript annotation was based on the Ensembl genes database (release 77). To quantify gene-expression levels, the number of reads mapped to the exons of each RefSeq gene was calculated. Samples with low total library size (<5 m reads) or high intergenomic mapping percent (>0.01% of reads mapped to intergenomic region) were removed due to quality-control failure. Raw counts were then normalized to reads per million (RPM) values using a robust library size estimation (DESeq2 version 1.28.1), then log2-transformed after addition of a pseudocount.

TABLE A1. Comparison of Clinical Factors between the GALLIUM ITT and BE Cohorts

	All Samples	
	ITT	**BE**
	(N = 1,401)	**(N = 274)**
Median (range) age, years	58 (23-88)	58 (27-85)
Sex, n (%)		
Male	662 (47.3)	132 (48.2)
Region, n (%)		
Western Europe	717 (51.2)	170 (62)
Eastern Europe	183 (13.1)	55 (20.1)
North America	169 (12.1)	37 (13.5)
Asia	191 (13.6)	0 (0)
Other	141 (10.1)	12 (4.4)
Treatment arm, n (%)		
Obinutuzumab	702 (50.1)	130 (47.4)

Chemotherapy regimen, n (%)		
Bendamustine	827 (59)	187 (68.2)
CHOP	433 (30.9)	69 (25.2)
CVP	141 (10.1)	18 (6.6)
FLIPI		
Low	252 (20.9)	56 (20.4)
Intermediate	448 (37.2)	98 (35.8)
High	504 (41.9)	120 (43.8)
FLIPI2		
Low	106 (9.1)	25 (9.3)
Intermediate	586 (50.2)	136 (50.4)
High	475 (40.7)	109 (40.4)

NOTE. Data are given as n (%) unless otherwise stated.

Abbreviations: BE, biomarker-evaluable; CHOP, cyclophosphamide, doxorubicin, vincristine, prednisone; CVP, cyclophosphamide, vincristine, prednisone; FLIPI, Follicular Lymphoma International Prognostic Index; ITT, intent-to-treat.

TABLE A2. Comparison of the association between clinical factors and choice of chemotherapy

	GALLIUM ITT			GALLIUM RNAseq BE population		
	CHOP/CVP	**Bendamustine**	***P***	**CHOP/CVP**	**Bendamustine**	***P***
Age >80 years	4/21 (19.0)	17/21 (81.0)	.045	0/6 (0.0)	6/6 (100.0)	.21
Sex – male	231/563 (41.0)	332/563 (59.0)	.23	39/132 (29.5)	93/132 (70.5)	.53
Bulky disease	252/525 (48.0)	273/525 (52.0)	.0017	43/111 (38.7)	68/111 (61.3)	.055
Charlson Comorbidity Index ≥1	92/262 (35.1)	170/262 (64.9)	.0049	15/58 (25.9)	43/58 (74.1)	.38
Ethnicity						
Hispanic/Latino	25/54 (46.3)	29/54 (53.7)	–	13/22 (59.1)	9/22 (40.9)	–
Not Hispanic/Latino	440/1067 (41.2)	627/1067 (58.8)	–	65/239 (27.2)	174/239 (72.8)	–
Not reported	32/53 (60.4)	21/53 (39.6)	–	5/8 (62.5)	3/8 (37.5)	–
Unknown	19/28 (67.9)	9/28 (32.1)	.0017	4/5 (80.0)	1/5 (20.0)	.00031
Race						
White	322/968 (33.3)	646/968 (66.7)	< .0001	81/262 (30.9)	181/262 (69.1)	.019
Black/African-American	1/4 (25.0)	3/4 (75.0)	–	0/1 (0.0)	1/1 (100.0)	–

Asian	174/198 (87.9)	24/198 (12.1)	–	0/3 (0.0)	3/3 (100.0)	–
American-Indian/Alaska native	0/1 (0.0)	1/1 (100.0)	–	0/1 (0.0)	1/1 (100.0)	–
Multiple	2/3 (66.7)	1/3 (33.3)	–	0/0 (0)	0/0 (0)	–
Native Hawaiian/other Pacific Islander	1/1 (100.0)	0/1 (0.0)	–	0/0 (0)	0/0 (0)	–
Other	16/27 (59.3)	11/27 (40.7)	–	6/7 (85.7)	1/7 (14.3)	–
Region						
Western Europe	247/581 (42.5)	334/581 (57.5)	< .0001	66/170 (38.8)	104/170 (61.2)	8.10E-05
Eastern Europe	77/157 (49.0)	80/157 (51.0)	–	19/55 (34.5)	36/55 (65.5)	–
North America	13/152 (8.6)	139/152 (91.4)	–	2/37 (5.4)	35/37 (94.6)	–
Asia	173/185 (93.5)	12/185 (6.5)	–	0/0 (0)	0/0 (0)	–
Other	6/127 (4.7)	121/127 (95.3)	–	0/12 (0.0)	12/12 (100.0)	–
Ann Arbor Stage						
I	9/18 (50.0)	9/18 (50.0)	–	2/4 (50.0)	2/4 (50.0)	–
II	37/85 (43.5)	48/85 (56.5)	–	6/14 (42.9)	8/14 (57.1)	–
III	176/417 (42.2)	241/417 (57.8)	–	29/110 (26.4)	81/110 (73.6)	–

IV	293/675 (43.4)	382/675 (56.6)	.91	50/143 (35.0)	93/143 (65.0)	.32
ECOG						
0–1	501/1161 (43.2)	660/1161 (56.8)	–	83/265 (31.3)	182/265 (68.7)	–
2	14/38 (36.8)	24/38 (63.2)	–	4/8 (50.0)	4/8 (50.0)	–
Unknown	1/3 (33.3)	2/3 (66.7)	.7	0/1 (0.0)	1/1 (100.0)	.42
FLIPI						
Low	103/252 (40.9)	149/252 (59.1)	–	16/56 (28.6)	40/56 (71.4)	–
Intermediate	184/447 (41.2)	263/447 (58.8)	–	30/98 (30.6)	68/98 (69.4)	–
High	229/503 (45.5)	274/503 (54.5)	.3	41/120 (34.2)	79/120 (65.8)	.73

Data are given as n (%) unless otherwise stated.

BE, biomarker-evaluable; CHOP, cyclophosphamide, doxorubicin, vincristine, and prednisone; CVP, cyclophosphamide, vincristine, and prednisone; ECOG, Eastern Cooperative Oncology Group; FLIPI, Follicular Lymphoma International Prognostic Index; ITT, intent to treat.

Figure A1. Consort diagram of samples selected for biomarker analysis by RNASeq.

FL, follicular lymphoma; ITT, intent-to-treat; QC, quality control.

*98 samples were excluded due to errors during the RNA library preparation

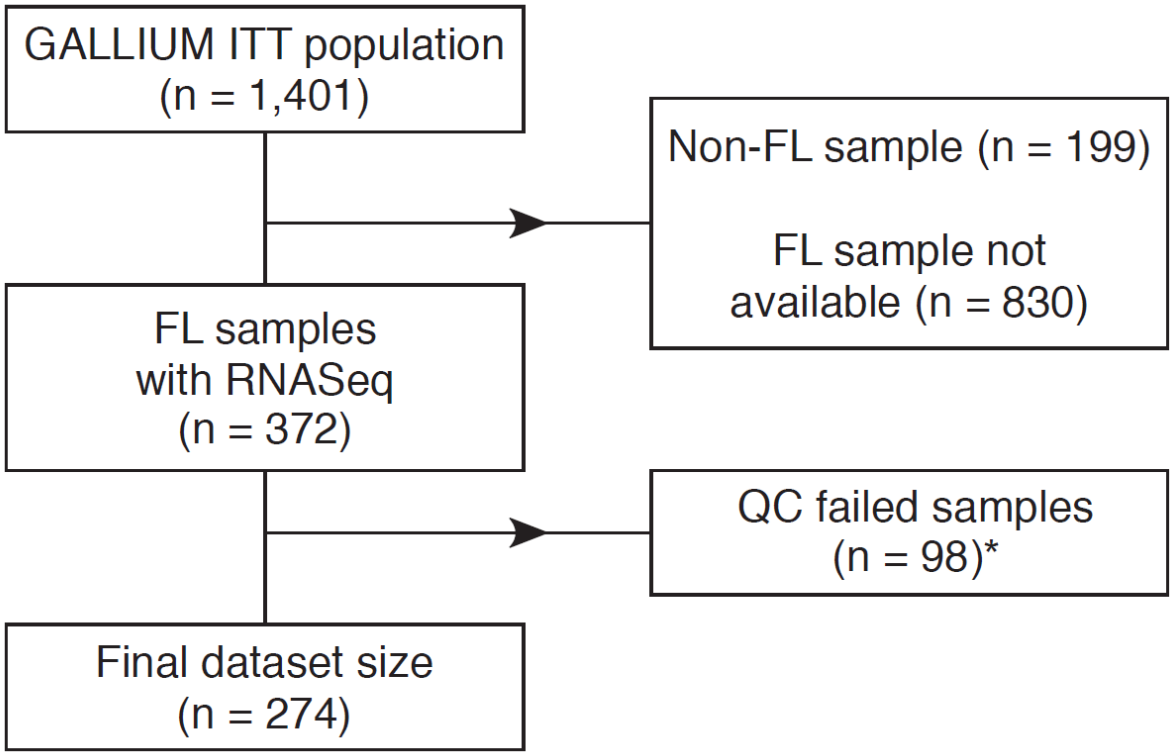

Figure A2. Association of published gene signatures with PFS in GALLIUM.

Summary scores were calculated for five published gene signatures. High risk for the PRIMA 23-gene signature was defined as patients in the top 25th percentile. Other gene signatures were evaluated at three different quartile cut-offs (25th, 50th, and 75th percentiles). Association with PFS was evaluated using a Cox proportional-hazards model in either the total BEP, patients treated with rituximab, or patients treated with obinutuzumab (GA101). HRs and 95% CIs for each comparison are plotted.

BEP, biomarker-evaluable population; CI, confidence interval; HR, hazard ratio; PFS, progression-free survival.